Title: A variation in the infant oxytocin receptor gene modulates infant hippocampal volumes in association with sex and prenatal maternal anxiety

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Abstract

Genetic variants in the oxytocin receptor (OTR) have been linked to distinct social phenotypes, psychiatric disorders and brain volume alterations in adults. However, to date, it is unknown how OTR genotype shapes prenatal brain development and whether it interacts with maternal prenatal environmental risk factors on infant brain volumes.

In 105 Finnish mother-infant dyads (44 female, 11-54 days old), the association of offspring OTR genotype rs53576 and its interaction with prenatal maternal anxiety (revised Symptom Checklist 90, gestational weeks 14, 24, 34) on infant bilateral amygdalar, hippocampal and caudate volumes were probed.

A sex-specific main effect of rs53576 on infant left hippocampal volumes was observed. In boys compared to girls, left hippocampal volumes were significantly larger in GG-homozygotes compared to A-allele carriers. Furthermore, genotype rs53576 and prenatal maternal anxiety significantly interacted on right hippocampal volumes irrespective of sex. Higher maternal anxiety was associated both with larger hippocampal volumes in A-allele carriers than GG-homozygotes, and, though statistically weak, also with smaller right caudate volumes in GG-homozygotes than A-allele carriers.

Our study results suggest that OTR genotype enhances hippocampal neurogenesis in male GG-homozygotes. Further, prenatal maternal anxiety might induce brain alterations that render GG-homozygotes compared to A-allele carriers more vulnerable to depression.

1. Introduction

The neuropeptide oxytocin (OT) has received much attention for its prominent role in human socio-emotional behavior and cognition (Carter 2007; Bos 2017; Bosch and Young 2017), as well as for its implication in psychiatric disorder susceptibility (Ebstein et al. 2012; Cataldo et al. 2018). OT is produced in the paraventricular and supraoptic nuclei of the hypothalamus and is released in the peripheral tissues and the central nervous system upon reproductive or stressful stimuli (Gimpl and Fahrenholz 2001). OT exerts its effects by binding to the oxytocin receptor (OTR). The distribution and number of the OTR is subject to major changes during ontogeny, and is profoundly regulated by gonadal steroids (Gimpl and Fahrenholz 2001); in the adult brain, OTRs are located in brain areas such as the amygdala, hypothalamus, hippocampus and striatum among others (Boccia et al. 2013; Uhrig et al. 2016). OT enhances attention to social cues and heightens their salience by linking them to mesolimbic dopamine pathways (Insel and Young 2001; Bartz et al. 2011; Skuse and Gallagher 2011). OT is also regarded as a key modulator of anxiety-related behavior and autonomic functions, modulating amygdala activity in fear acquisition and extinction (Yoshida et al. 2009; Bartz et al. 2011; Knobloch et al. 2012; Neumann and Slattery 2016).

Individual differences in the availability of OT and OTR convey variability in human social behavior and mental health (Uhrig et al. 2016; Bos 2017; Cataldo et al. 2018). Important sources for these individual differences are genetic variants and/or effects of fetal programming, among other factors (Bos 2017). In rodents, genetic polymorphisms of OTR account for most alterations in the expression of OTR (Bosch and Young 2017). In humans, genetic OTR variants have been associated with distinct social phenotypes (e.g., with regard to emotional dysregulation or parental sensitivity) and susceptibility for psychiatric disorders (e.g., depression, autism and eating disorders) (Ebstein et al. 2012;

Li et al. 2015; Cataldo et al. 2018; Lee et al. 2018). The genetic single nucleotide polymorphism (SNP) rs53576 (G/A) is one of the most researched genetic variants in the human OTR gene (Li et al. 2015; Bos 2017). This SNP, located in the third intron of the OTR gene, is of unknown functionality, but has been associated with lower OTR expression in the hippocampus and in striatal regions, most pronounced in the caudate, in GG-homozygotes compared to A-allele carriers (GTEx database; gtexportal.org, accessed at April 08, 2020). Interestingly, in the prairie vole, lower densities of striatal OTR binding rendered individuals less resilient to adverse caregiving experiences (Bosch and Young 2017). Several studies suggest that GG-homozygotes of rs53576 are more sensitive to social cues and social stress (Rodrigues et al. 2009; Tost et al. 2010; Krueger et al. 2012; Norman et al. 2012; Li et al. 2015; McQuaid et al. 2015) and display an increased preference for social stimuli after OT administration while opposite effects have been observed in A-allele carriers (Marsh et al. 2012). GG-homozygotes have also been associated with increased susceptibility to environmental influences, both adverse and beneficial [differential susceptibility, (Belsky et al. 2009)], affecting emotion processing and the risk for psychopathologies such as depression and anxiety (Bradley et al. 2011; McQuaid et al. 2013; Hostinar et al. 2014; Schneider-Hassloff et al. 2016; Cataldo et al. 2018).

Neuroimaging studies in adults have reported that GG-homozygotes of rs53576 compared to A-allele carriers, irrespective of sex, exhibit a stronger activation of the amygdala in response to emotionally salient social stimuli (Tost et al. 2010) and display larger bilateral temporal pole and right hippocampal volumes (Schneider-Hassloff et al. 2016). A genotype-by-sex interaction has been observed for right amygdalar volumes: Male GG-homozygotes of rs53576 compared to male A-allele carriers showed smaller amygdalar volumes (Tost et al. 2010) (but see also conflicting findings in Schneider-Hassloff et al.

2016). Smaller amygdalar volumes have been associated with stronger reward dependence (Tost et al. 2010). It has been put forward that structural alterations in the right hippocampus might be implicated in increased social memory formation in GG-homozygotes (Insausti and Amaral 2012; Schneider-Hassloff et al. 2016). OT has been proven to increase the signal-to-noise ratio in hippocampal neural activity (Mühlethaler et al. 1984; Owen et al. 2013) and to stimulate hippocampal neurogenesis (Leuner et al. 2012). However, to date, it is unknown how the brain structural alterations associated with the genetic OTR variant rs53576 evolve during ontogeny.

Moreover, little is known about prenatal programming effects on the OT system (Bos 2017). The intrauterine development is vulnerable to environmental risk factors, such as maternal psychological distress and anxiety, shaping neurodevelopmental, affective and behavioral outcomes of the offspring over the lifespan (Bock et al. 2015; Entringer et al. 2015; O'Donnell and Meaney 2017). In prenatally stressed rats, significantly less OT mRNA in the paraventricular nucleus, and increased OTR binding in the central amygdala have been detected (Lee et al. 2007). In humans, higher prenatal stress has not consistently been related to epigenetic modifications of OTR (Cecil et al. 2014; King et al. 2017; Rijlaarsdam et al. 2017; Cardenas et al. 2019). However, one study has probed whether infant OTR genotype moderates prenatal effects on brain development and reported that fetal prenatal testosterone levels which are influenced by prenatal maternal stress (Ward and Weisz 1980; Barrett and Swan 2015), interacted with the genetic OTR variant rs53576 on offspring cognitive empathy in men, but not women (Weisman et al. 2015). Hence, there is some evidence that genetic OTR variants might moderate prenatal programming effects on brain development, potentially in a sexually dimorphic manner, but this topic is still underinvestigated. Consequently, it is not yet known how offspring's genetically determined sensitivity for OT (rs53576) might interact with prenatal maternal

anxiety, a prenatal risk factor, on offspring brain structural development. Maternal endocrine alterations due to prenatal anxiety are known to affect the endocrine milieu of the developing fetus (Bock et al. 2015). Mother and fetus are connected via the maternal-placental-fetal axis, and maternal hormones such as OT can cross the placenta (Malek et al. 1996; Entringer et al. 2015). Anxiety is regarded as a potent stimulator of peripheral and central OT release (Neumann and Slattery 2016). Hence, it is conceivable that a genetic variant in the offspring OTR gene might interact with maternal anxiety and shape fetal brain development.

With the current study, we aimed to investigate whether rs53576 is associated with brain structural alterations in the neonatal brain. We chose the amygdala, hippocampus and caudate as structures of interest. We hypothesized that rs53576 might be associated with larger right hippocampal volumes in GG-homozygotes (Schneider-Hassloff et al. 2016), and with larger right amygdalar volumes in male GG-homozygotes (Tost et al. 2010). Second, we wanted to explore whether offspring rs53576 genotype interacts with prenatal maternal anxiety on infant brain structure. We expected GG-homozygotes to be more susceptible to effects of maternal anxiety than A-allele carriers in limbic and striatal volumes.

2. Methods

2.1 Subjects

Participants were mother-infant-dyads recruited from the FinnBrain Birth Cohort Study [www.finnbrain.fi] (Karlsson et al. 2018). Neuroimaging data were collected from 189 infants at the age of one to eight weeks after birth. Inclusion criteria for neuroimaging were gestational age at birth ≥35 weeks and birth weight >1500g. Exclusion criteria were previously diagnosed CNS anomalies or abnormal findings in a previous MRI scan. Written

informed consent was obtained from all parent(s). The study was conducted according to the Declaration of Helsinki and was reviewed and approved by the Ethics Committee of the Hospital District of Southwest Finland (ETMK:31/180/2011). Of the 189 participants, 64 were excluded due to failed MRI scanning or motion artifacts in the MR images. Of the remaining 125 infants, 19 were excluded because of missing genetic data (no GWAS performed (*n*=15) or drop-out in quality control (*n*=4)). One more subject was excluded due to missing maternal SCL questionnaire data as described below. In the final sample, we included 105 mother-infant-dyads (infants: 41.9% female, age after birth [days]: *M*= 26.1, *SD*= 7.2, range= 11-54; mothers' age (at term) [years]: *M*= 29.7 (*SD*= 4.6, range=19-41)).

2.2 Measures and Procedures

2.2.1 Maternal prenatal anxiety

We administered the anxiety subscale of the revised Symptom Checklist 90 (SCL-90-R) (Derogatis 1983; Holi et al. 1998) at gestational weeks (gwk) 14, 24 and 34 to assess general maternal anxiety during pregnancy. Missing values (at maximum 3 items per time point) were imputed with the mean value of the existing ones. If no SCL questionnaire data were available for one or two of the three time points, data were imputed by the MissForest method (gwk14: *n*=2, gwk24: *n*=1, gwk34: *n*=6) (Stekhoven and Bühlmann 2012). One mother did not provide any SCL questionnaire data and this mother-infant-dyad was therefore excluded from the analyses. The individual sum scores of all three time points were added up to form an individual total SCL sum score [SCL Sum; computed for the individual subject (i) as follows: SCL Sum (i) = SCL gwk14 (i) + SCL gwk24 (i) + SCL gwk34 (i)]. Additionally, the individual sum scores of each trimester (SCL gwk14, SCL gwk24, SCL gwk34) were investigated in *post hoc* analyses. We observed outliers (> 3*SD*)

(gwk14: *n*=1, gwk24: *n*=1, gwk34: *n*=2; Sum: *n*=1) and excluded them in control analyses (see 2.2.6).

2.2.2 Other maternal and infant variables

Maternal prenatal depressive symptoms were assessed with the Finnish version of the Edinburgh Postnatal Depression Scale (EPDS) (Cox, J. Holden, J. Sagovsky 1987) at gwk 14, 24 and 34. Missing values at each time point (at maximum 3 items per time point) were imputed with the mean value of the existing ones, and individual sum scores (EPDS Sum), adding up the individual EPDS sum scores of gwk 14, gwk 24 and gwk 34 [EPDS Sum (i) = EPDS gwk14 (i) + EPDS gwk24 (i) + EPDS gwk34 (i)], were computed for depressive symptoms during pregnancy. The following maternal variables were assessed via mothers' self-report at gwk 14 and/or 34: maternal education, maternal age, maternal prenatal medication, and prenatal alcohol, nicotine and illicit drug consumption. Obstetric data were retrieved from the Finnish Medical Birth Register of the National Institute for Health and Welfare (http://www.thl.fi) and included maternal prepregnancy body mass index (BMI, missings: *n*=2), and infant birth weight (missing: *n*=1), gestational weeks and Apgar scores (missing: *n*=1, Apgar = appearance, pulse, grimace, activity and respiration). We dichotomized maternal medication use as assessed at gwk14 [selective serotonin reuptake inhibitors (SSRI) and/or other CNS affecting medication (yes/no, missings: *n*= 4), thyroxine (yes/no, missings: *n*= 4), corticosteroids (yes/no, missings: *n*= 4)] and alcohol, nicotine and/or illicit drug exposure (yes/no, missings: *n*= 8). Education was trichotomized [low: high school or vocational education (<12 years), middle: (career) college (12-15 years), high: university (+15 years), missings: N=2]. In the final sample, 12 infants had a record of mild asphyxia (missings: N=2), and these infants were excluded in control analyses (see 2.2.6). Missing data of the control variables were imputed by the MissForest method (Stekhoven and Bühlmann 2012).

2.2.3 MRI acquisition

A detailed description of the MRI acquisition protocol is provided in a previous publication by the same research team (Lehtola et al. 2019). Participants were scanned with a Siemens Magnetom Verio 3T scanner (Siemens Medical Solutions, Erlangen, Germany) during natural sleep. More details are provided in the Supplementary Information (SI; SI-1).

2.2.4 Assessment of structure volumes

The volumes of the left and right amygdalae, hippocampi and caudate nuclei were assessed for each subject via label-fusion-based methods. These methods depend on achieving good registrations between the subjects and the entries in a library of templates. This is increasingly difficult to achieve the further the templates are from the subjects in terms of similarity. Thus, we constructed a template library based on the subjects in this study. We first constructed a population specific base infant template (Fonov et al. 2011). Then we warped that template to the 21 subjects that best represented the morphological variation in the sample, and manually labeled the structures of interest in each, based on the methods established by Perlaki et al (2017) and Hashempour et al. (2019). Then, from these 21 manual segmentations, we created consensus segmentation labels on the base infant template via voxel-wise majority vote. We then constructed two libraries of warped versions of the labeled template - one for the hippocampus and amygdala, and one for the caudate - such that each library best represented the morphological variation in the sample for those structures. Those libraries were then used to label the individual brains via label-fusion-based labeling methods (Coupé et al. 2011; Weier et al. 2014; Lewis et al. 2019). Finally, we calculated the volume of each structure from its label. The details of this approach are described in SI (SI-2).

2.2.5 Genetic analyses

An umbilical cord blood sample was drawn from each newborn at birth. DNA samples were extracted according to standard procedures at the National Institute for Health and Welfare and genotyped with Illumina Infinium PsychArray and Illumina Infinium Global Screening Array at the Estonian Genome Centre. Quality control (QC) was performed with PLINK 1.9 (www.cog-genomics.org/plink/1.9/) (Chang et al. 2015). Markers were removed for missingness (>5%) and Hardy-Weinberg equilibrium (p-value < 1 x 10^{-6}). Individuals were checked for missing genotypes (>5%), relatedness (identical by descent calculation, PI_HAT>0.2) and population stratification (multidimensional scaling). Genotyped data was pre-phased with Eagle v2.4 (Loh et al. 2016) and imputed with Beagle v4.1 (Browning and Browning 2016) using the population-specific SISu v2 whole-genome sequencing data as imputation reference panel.

In our sample, 41 infants were GG-homozygotes of rs53576 (G/A), 50 were AG allele carriers and 14 were AA homozygotes. The allele frequencies did not significantly deviate from Hardy-Weinberg equilibrium. We combined the genotype groups AG and AA, adopting the same grouping strategy as reported by others (Rodrigues et al. 2009; Bradley et al. 2011; Schneider-Hassloff et al. 2016).

2.2.6 Statistical analyses

Statistical analyses were performed using R 3.4.4 and R 3.6.3 (R Core Team 2016) (http://www.r-project.org/). Packages in use were “psych” (Revelle 2018), “nortest” (Gross and Ligges 2015), “ggplot2” (Wickham 2009), and “car” (Fox and Weisberg 2011) among others.

Infant age after birth at MRI scan time, gestational weeks at birth, total brain volume and infant sex were included as control variables in all analyses (if not included as predictor).

We further assessed the association of additional control variables (namely, infant birth weight, infant Apgar scores, maternal education, maternal BMI, prenatal maternal depressive symptoms, maternal prenatal medication, and maternal prenatal alcohol, nicotine and/or illicit drug exposure) with predictors and outcomes. Control variables that were significantly related to either one of the predictors or outcomes were also included into the analyses. As a result, we used eight covariates in total: Infant age after birth at MRI scan time, gestational weeks at birth, total brain volume, infant sex, Apgar scores, prenatal maternal depressive symptoms, and maternal medication (2 variables: SSRI / CNS affecting, corticosteroids).

First, standard multiple linear regression analyses were performed to probe the association of the selected bilateral subcortical volumes with 1) individual genotype (rs53576) (model G), 2) a genotype-by-sex interaction, 3) prenatal maternal general anxiety (SCL) and 4) a SCL-by-sex interaction, each in separate analyses. Second, we investigated 1) the interaction of rs53576 genotype with SCL (model GxE) and 2) the interaction of rs53576 genotype, SCL and sex on subcortical volumes in standard multiple linear regression analyses. The type of interaction was investigated by use of the R package “LEGIT” (Jolicoeur-Martineau 2019) to elucidate whether the interaction better fits a differential susceptibility model or a diathesis-stress model, and the Bayesian information criterion (BIC) is given.

We performed following control analyses of significant results: First, we repeated the multiple regression analyses, adding all the control variables to the model that have not been significantly associated with predictors or outcome, namely, infant birth weight, maternal education, maternal BMI, maternal prenatal medication (thyroxine), and maternal prenatal alcohol, nicotine and/or illicit drug exposure. Second, we excluded infants with mild asphyxia at birth (*n*=12, resulting sample: *n*= 93). For the interaction analyses with

SCL as predictor, we additionally performed two more control analyses: 1) We excluded SCL outliers (> 3*SD*) (SCL Sum: N=1; resulting sample: *n*= 104), and 2) we split the sample into two groups with low and high SCL values (<=20% quantile and ≥80% quantile) and performed control analyses with this dichotomous SCL variable.

We report the estimates, their standard errors and p-values of significant predictors. The significance threshold was set to $p<0.05$. We report the 95% confidence interval (CI) of the β-values of significant predictors and hypothesized significant effects (confint function in R). As the study is exploratory in nature we did not control for the error rate related to multiple comparisons.

3. Results

3.1 Demographic overview

Demographic characteristics of the sample are presented in Table 1. No significant sex differences were observed for the genotype rs53576 or SCL scores. Higher prenatal maternal general anxiety was significantly associated with prenatal maternal SSRI and/or other CNS affecting medication intake (SCL gwk14: t= -2.2, p= 0.030; SCL gwk24: t= -3.1, p= 0.003, SCL gwk34: t= -2.3, p= 0.023, SCL Sum: t= -2.8, p= 0.006), higher prenatal maternal depression (SCL gwk14: r= 0.74, p< 0.001; SCL gwk24: r= 0.75, p< 0.001; SCL gwk34: r= 0.65, p< 0.001; SCL Sum: r= 0.79, p< 0.001) and lower infant Apgar scores (SCL gwk24: r= -0.19, p=0.049). Female infants showed significantly smaller bilateral hippocampal and right amygdalar volumes than males, but also significantly smaller total brain volumes (Table 1). After controlling for infant age, gestational weeks at birth and total brain volume, significant sex differences were only observed for right amygdalar volumes (p= 0.027, all other p> 0.26).

3.2 Genotype rs53576 and subcortical volumes

In multiple linear regression analyses with genotype as predictor, no significant main genotype effects on bilateral amygdalar, hippocampal or caudate volumes were found (Table 2, Figure 2). Bilateral hippocampal volumes were larger in GG-homozygotes compared to A-allele carriers (Figure 1), but not significantly so.

Investigating sexually dimorphic effects of rs53576, we observed a significant interaction of genotype and sex on left hippocampal volumes (Table 2, Figure 1). This effect remained significant in one of the control analyses, including all covariates, but failed to pass the significance threshold after exclusion of infants with asphyxia (p= 0.077). *Post hoc* multiple linear regression analyses showed that GG-homozygotes compared to A-allele carriers displayed larger left hippocampal volumes in boys [non-significant in the main analysis ($\beta \pm SE$= -56.20 ± 28.63, p= 0.055) and the control analyses (p>0.085)], and no association was observed in girls ($\beta \pm SE$= 21.76 ± 32.38, p= 0.506). No sexually dimorphic association of rs53576 with right amygdalar volumes was found (Figure 2).

3.3 Prenatal maternal general anxiety and subcortical volumes

In multiple linear regression analyses with SCL Sum scores as predictor, we observed no significant main effects of SCL Sum scores on subcortical volumes (Table 3) nor did we find significant sexually dimorphic main effects of SCL Sum scores (Table 3).

3.4 Genotype-by-environment interaction effects on subcortical volumes

We found a significant interaction effect of rs53576 and SCL Sum scores on right hippocampal volumes (Table 4, Figure 3). A-allele carriers compared to GG-homozygotes exhibited a significantly more positive association between SCL Sum scores and right hippocampal volumes. This interaction effect remained significant in all control analyses, except after exclusion of infants with asphyxia (p= 0.074). The interaction was best described with a strong diathesis-stress model (BIC= 1268.7). *Post hoc* multiple linear regression analyses yielded no association between SCL Sum scores and right

hippocampal volumes in GG-homozygotes (*β* ± *SE*= -2.63 ± 2.71, *p*= 0.340), but a weak positive association in A-allele carriers [*β* ± *SE*= 2.48 ± 1.28, *p*= 0.057, that was significant in one of the control analyses, including all control variables (*p*= 0.013).

We also observed that A-allele carriers compared to GG-homozygotes showed a more positive association between SCL Sum scores and right caudate volumes. This interaction effect showed a trend for significance (*p*= 0.063, Table 4) in the main analysis and was significant in two of the control analyses (after exclusion of infants with asphyxia: *β* ± *SE*= 5.79 ± 2.79, *p*= 0.041; and after exclusion of the SCL Sum outlier: *β* ± *SE*= 5.18 ± 2.45, *p*= 0.037). The association between SCL Sum scores and right caudate volumes was more negative in GG-homozygotes compared to A-allele carriers. This interaction effect was best described as a strong diathesis-stress effect (BIC= 1346.6). *Post hoc* analyses yielded no significant associations of right caudate volumes with SCL Sum scores, neither in the main nor in the control analyses, for GG-homozygotes (*β* ± *SE*= -4.39 ± 4.26, *p*= 0.311) or A-allele carriers (*β* ± *SE*= -0.75 ± 2.00, *p*= 0.708).

No further significant interaction effects of rs53576 and SCL Sum scores were found. We also did not observe significant sexually dimorphic interaction effects (Table 4).

3.4.1 Post hoc analyses with SCL scores of gwk14, gwk24 and gwk34

In additional *post hoc* analyses for right hippocampal volumes, investigating the SCL scores of gwk14, gwk24 and gwk34 separately, we found significant interactions of genotype with SCL gwk14 scores (*β* ± *SE*= 12.78 ± 4.55, *p*= 0.006, significant in all control analyses), but not with SCL gwk24 scores (*β* ± *SE*= 6.72 ± 3.96, *p*= 0.093) or SCL gwk34 scores (*β* ± *SE*= 7.31 ± 5.01, *p*= 0.148). *Post hoc* multiple linear regression analyses showed no association between SCL gwk14 scores and right hippocampal volumes in GG-

homozygotes (β ± *SE*= -5.97 ± 5.61, p= 0.296), but a positive association in A-allele carriers (β ± *SE*= 7.18 ± 3.51, p= 0.046).

By contrast, for right caudate volumes, significant interactions between genotype and SCL gwk24 scores (β ± *SE*= 13.30 ± 5.74, p= 0.023, significant in all control analyses, and partially between genotype and SCL gwk34 scores [β ± *SE*= 14.18 ± 7.24, p= 0.053, significant after exclusion of infants with asphyxia (p= 0.045) and with the dichotomous SCL variable (p= 0.012)] but not with SCL gwk14 scores (β ± *SE*= 4.83 ± 6.96, p= 0.489) were observed. *Post hoc* multiple linear regression analyses showed a negative association between SCL gwk24 scores and right caudate volumes in GG-homozygotes (β ± *SE*= -27.69 ± 11.76, p= 0.025), and no association in A-allele carriers (β ± *SE*= -2.64 ± 4.16, p= 0.528). No significant associations were observed with SCL gwk34 scores in GG-homozygotes or A-allele carriers (all $p > 0.4$).

4. Discussion

With this study, we probed the association of an offspring genetic OTR variant, rs53576, with infant bilateral amygdalar, hippocampal and caudate volumes. We further investigated whether offspring genotype interacts with prenatal maternal anxiety or infant sex on infant subcortical volumes.

We observed no main genotype effect on any of these bilateral subcortical volumes, but we did observe a sexually dimorphic effect on left hippocampal volumes: In boys compared to girls, left hippocampal volumes were larger in GG-homozygotes compared to A-allele carriers.

Our analyses revealed no main effect of SCL scores on infant subcortical volumes, but we detected that genotype and SCL scores interacted on right hippocampal volumes. SCL Sum scores were positively associated with right hippocampal volumes in A-allele carriers,

but not in GG-homozygotes. No evidence for a differential susceptibility was found, but the interaction resembled a diathesis-stress effect, and was mainly driven by the weak positive association between SCL scores and right hippocampal volumes in A-allele carriers. *Post hoc* analyses showed that this GxE effect was strongest for SCL scores of the early second trimester (gwk14).

Moreover, our analyses yielded an interaction effect of genotype and SCL Sum scores on right caudate volumes, which was non-significant in the main analysis, but significant, albeit weakly, in some control analyses. SCL Sum scores were more negatively associated with right caudate volumes in GG-homozygotes compared to A-allele carriers. This interaction effect also resembled a diathesis-stress effect, and was more driven by a negative association between SCL scores and right caudate volumes in GG-homozygotes than A-allele carriers. The GxE effect was strongest for SCL scores of the late second trimester (gwk24). Taken together, our data provide some evidence that higher maternal prenatal anxiety is associated with larger right hippocampal volumes in infant A-allele carriers and smaller right caudate volumes in infant GG-homozygotes. As discussed in more detail below, maternal prenatal anxiety might thereby enhance resilience to depression in A-allele carriers by increasing hippocampal volumes, but could also reduce resilience in GG-homozygotes by decreasing caudate volumes.

In adults, GG-homozygotes of rs53576 have been associated with larger right hippocampal volumes, irrespective of sex (Schneider-Hassloff et al. 2016). In our study, infant GG-homozygotes displayed larger right hippocampal mean volumes, but this effect was weak and statistically non-significant. It is conceivable that genotype-related right hippocampal volume alterations evolve postnatally; hence, more pronounced genotype effects on right hippocampal volumes might be visible only later in development. The

hippocampus shows substantial neurogenesis postnatally (Insausti and Amaral 2012) and reaches its maximum size by the age of 2 to 3 years (Van Petten 2004). The growth of the right hippocampus is even more protracted in girls and continues until adulthood (Giedd et al. 1996; Van Petten 2004). However, we observed a significant sexually-dimorphic genotype effect on left hippocampal volumes, with larger hippocampal volumes in GG-homozygous males. Thereby, our study provides some evidence for larger hippocampal volumes in GG-homozygous infants. The sex-specific finding dovetails to some extent with studies in adults showing that males are more affected by rs53576 genotype with regard to amygdalar and hypothalamic volumes and with regard to fetal prenatal testosterone effects on cognitive empathy (Tost et al. 2010; Weisman et al. 2015). OTR as well as the hippocampus are both profoundly regulated by steroid receptors (Gimpl and Fahrenholz 2001; Sheppard et al. 2019). Hippocampal synaptic and dendritic spine density and neurogenesis are modulated by estrogen in both males and females and by androgen steroids in young male rodents (Duarte-Guterman et al. 2019; Sheppard et al. 2019). In adult male rats ventral hippocampal neurogenesis has been increased by OT administration (Leuner et al. 2012). The effects of OT on hippocampal neurogenesis were independent of glucocorticoids, but its mechanisms are not yet known and might involve OTR-dependent or OTR-independent pathways (Leuner et al. 2012). Taking into account the OT-related findings in rats (Leuner et al. 2012), we assume that larger hippocampal volumes in GG-homozygous males compared to A-allele carriers might be related to enhanced hippocampal neurogenesis stimulated by OT. Considering the lower hippocampal OTR density in GG-homozygous adults, the mechanisms by which rs53576 might act on male infant hippocampal volumes remain elusive. Larger hippocampal volumes are regarded as a protective factor in general (Leuner et al. 2012), given that chronic stress has been linked to hippocampal atrophy (Vyas et al. 2002) and smaller

hippocampal volumes have consistently been related to disorders such as depression (Arnone et al. 2012) and Alzheimer's (Juottonen et al. 1999); still, the association between hippocampal volume and memory function is complex and age-dependent (Van Petten 2004).

Contrary to our expectations, no sex-specific genotype effect on amygdalar volumes has been detected. Findings in adults have been inconsistent (Tost et al. 2010; Schneider-Hassloff et al. 2016), There is also some evidence from rodent studies on prenatal stress that amygdalar volume alterations might emerge postnatally (Kraszpulski et al. 2006).

However, in our study, right hippocampal volumes were significantly more positively associated with prenatal maternal anxiety in A-allele carriers compared to GG-homozygotes. Anxiety stimulates the release of both peripheral and central OT (Neumann and Slattery 2016). Partly conflicting empirical findings on anxiety-related peripheral OT have been attributed to the questionable validity of OT data in several studies (Neumann and Slattery 2016). OT is able to pass the placenta (Malek et al. 1996), and – to a lower extent - the blood-brain barrier (Kang and Park 2000; Gimpl and Fahrenholz 2001). Maternal peripheral OT might stimulate offsprings' peripheral and central OT pathways directly and/or indirectly. We assume that maternal prenatal anxiety enhances offspring hippocampal neurogenesis in A-allele carriers, but not GG-homozygotes. As larger hippocampal volumes are regarded as a protective factor (Leuner et al. 2012), our data suggest that maternal prenatal anxiety stimulates protective mechanisms in the right hippocampus of A-allele carriers, but not of GG-homozygotes. The association with maternal anxiety was strongest for gwk14, i.e., a time when hippocampal anlage and fissure have been formed (gwk 9 and 10), hippocampal fields appear (gwk 15-19), hippocampal apoptosis in pyramidal cells is comparably high (gwk 14-27) and non-pyramidal cells start to appear and to increase (gwk 15) (Ashwell and Mai 2012; Insausti

and Amaral 2012). Additional exploratory analyses in our study revealed (data not shown) that SCL gwk14 scores also significantly interacted with genotype on left hippocampal volumes comparable to the GxE interaction effect observed on right hippocampal volumes lending further support to our findings on right hippocampal volumes.

Interestingly, a different pattern was observed for right caudate volumes. Higher maternal anxiety was associated with smaller caudate volumes in GG-homozygotes compared to A-allele carriers. The interaction was stronger for maternal anxiety in the late second and third trimester. Maternal prenatal anxiety in the late second trimester (gwk 24) was negatively associated with right caudate volumes in GG-homzygotes, but no association was yielded in A-allele carriers. The dorsal striatum can first be identified in the fetal brain around gwk 9 comparably to the hippocampus, but the caudate starts to develop a few weeks later than the hippocampus, and glial cells in the caudate appear from gwk17 on (Ashwell and Mai 2012). This relatively delayed developmental pattern of caudate might explain why caudate volumes are affected by maternal anxiety later in pregnancy. In adults, smaller caudate volumes have consistently been related to major depressive disorders (Arnone et al. 2012; Bora et al. 2012). The association of higher maternal prenatal anxiety with smaller caudate volumes in GG-homozygotes, but not A-allele carriers, might indicate that maternal prenatal anxiety increases the risk for developing depression only in GG-homozygotes. Altogether, our data suggest that prenatal maternal anxiety induces genotype-specific brain alterations that could be interpreted as protective in A-allele carriers and vulnerability-enhancing in GG-homozygotes. This dovetails with findings in adults showing a higher prevalence of depression after the experience of childhood maltreatment in GG-homozygotes compared to A-allele carriers (McQuaid et al. 2013), and a higher incidence of depression in (predominantly female) GG-homozygotes (Costa et al. 2009).

Limitations

Several limitations of our study have to be acknowledged. Our study was exploratory and hence lacked a correction for multiple comparisons. The sample size was moderately large, and regarded as sufficient for investigating genetic main effects in neuroimaging studies (e.g., Krug et al., 2010). However, due to the challenges of imaging neonates the sample size of this study was limited and smaller compared to similar studies in adults. A replication in another sample would be desirable (Button et al. 2013).

Moreover, the functionality of the investigated genetic variant in the OTR is unknown which limits the interpretation of the results. We also did not have information about epigenetic modifications in the OTR gene at hand; epigenetic modifications are partly influenced by genotype and affect OTR expression (Reiner et al. 2015; Ziegler et al. 2015).

Conclusions

In our study, the genetic OTR variant rs53576 was associated with significantly larger left hippocampal volumes in GG-homozygotes compared to A-allele carriers in boys, but not girls suggesting a sex-specific genotype effect on hippocampal neurogenesis in infants. Prenatal maternal anxiety modulated infant right hippocampal volumes positively in A-allele carriers and – to a weaker extent - right caudate volumes negatively in GG-homozygotes which might render GG-homozygotes compared to A-allele carriers more vulnerable to depression.

Conflicts of interest

none

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Table 1: The mean scores (M) and standard deviations (SD) or frequencies, respectively, are listed for prenatal maternal EPDS scores and non-imputed control variables (missings as described in the methods section), for the whole sample and for girls (N= 44) and boys (N= 61) separately. In the right column p-values for sex differences in the sample, assessed with t-tests without covariates (see 3.1 for brain volume analyses with control variables), are listed.

Variable	Whole sample	Boys	Girls	p
M±SD (range)				
Child's age [days]	26.1±7.2 (11-54)	26.9±7.6 (11-43)	24.9±6.5 (14-54)	0.153
Gestational weeks	39.8±1.1 (36.3-42.1)	39.7±1.0 (37.6-41.9)	40.0±1.2 (36.3-42.1)	0.208
Birth weight [g]	3481.1±418.8 (2530-4700)	3536.6±438.2 (2720-4700)	3402.3±380.8 (2530-4340)	0.108
Apgar, 5 min	9.0±1.0 (4-10)	8.8±1.1 (4-10)	9.2±0.5 (8-10)	0.011*
Left amygdala [mm^3]	267.1±37.8	272.3±41.8	259.8±30.5	0.093
Right amygdala [mm^3]	266.3±39.3	277.1±39.6	251.3±34.0	<0.001*
Left hippocampus [mm^3]	767.8±116.1	793.0±121.7	732.9±98.9	0.008*
Right hippocampus [mm^3]	768.4±111.2	786.9±109.5	742.6±109.7	0.044*
Left caudate [mm^3]	1395.6±142.8	1409.4±149.5	1376.4±132.3	0.244
Right caudate [mm^3]	1448.7±145.6	1456.7±138.8	1437.5±155.6	0.508
Total brain volume [mm^3]	621791.5±46752.9	633575.3±45810.4	605454.8±43454.8	0.002*
SCL (gwk 14)	3.63±4.58 (0-19)	3.59±4.30 (0-16)	3.68±4.99 (0-19)	0.921
SCL (gwk 24)	4.61±5.47 (0-28)	4.87±5.06 (0-19)	4.24±6.04 (0-28)	0.562
SCL (gwk 34)	3.47±4.09 (0-19)	3.77±3.67 (0-12)	3.05±4.61 (0-19)	0.377
SCL Sum	11.70±12.86 (0-54)	12.23±11.89 (0-41.34)	10.97±14.22 (0-54)	0.623
EPDS Sum	16.65±13.74 (0.00-63.95)	17.51±13.88 (0.00-57.48)	15.45±13.62 (1.00-63.95)	0.451
Prenatal maternal BMI	24.3±4.2 (17.5-38.4)	24.7±4.4 (18.0-38.4)	23.7±3.9 (17.5-35.0)	0.213
Frequencies				
Genotype rs53576 (GG / A)	41/64	23/38	18/26	0.740
Prenatal alcohol, nicotine and/or illicit drug consumption (no/yes)	64/33	37/21	27/12	0.579
Prenatal medication – SSRI and other CNS affecting drugs (no/yes)	92/9	52/7	40/2	0.217
Prenatal medication – thyroxine (no/yes)	93/8	53/6	40/2	0.321
Prenatal medication – corticosteroids (no/yes)	95/6	56/3	39/3	0.666

Maternal education (low/middle/high)	31/30/42	18/16/26	13/14/16	0.768

*p<0.05

Abbr.: BMI= body mass index; CNS= central nervous system, EPDS= Edinburgh Postnatal Depression Scale, SCL= prenatal maternal general anxiety (Symptom Check List 90-R), SSRI= selective serotonin reuptake inhibitor

Table 2: The main effect of rs53576 and its interaction with sex on amygdalar, hippocampal and caudate volumes are shown as assessed in multiple regression analyses (estimates (β) with standard error (SE) and p-values).

	Main effect of rs53576		Rs53576 x sex	
	β ± SE	p	β ± SE	p
R Amygdala	-4.32±6.81	0.528	-0.68±13.38	0.960 95% CI [-27.24,25.88]
L Amygdala	-6.25±6.53	0.341	-11.69±12.76	0.362
R Hippocampus	-18.90±19.23 95% CI [-57.08,19.28]	0.328	-43.73±37.49	0.246
L Hippocampus	-21.51±20.64	0.300	-85.77±39.54	0.033* 95% CI [-164.27,-7.26]
R Caudate	13.61±28.07	0.629	-8.92±55.10	0.872
L Caudate	-1.71±28.21	0.952	-21.07±55.34	0.704

*p<0.05

Table 3: The main effect of prenatal maternal general anxiety (SCL Sum) and its interaction with sex on amygdalar, hippocampal and caudate volumes are shown (estimates (β) with standard error (SE) and p-values).

	Main effect of SCL Sum		SCL Sum x sex	
	β ± SE	p	β ± SE	p
R Amygdala	0.59±0.41	0.152	-0.70±0.52	0.179
L Amygdala	0.06±0.40	0.884	-0.54±0.50	0.283
R Hippocampus	1.95±1.15	0.093	0.22±1.47	0.883
L Hippocampus	1.69±1.24	0.175	1.44±1.58	0.365
R Caudate	-1.56±1.69	0.359	-0.45±2.16	0.837
L Caudate	-0.74±1.70	0.664	0.80±2.18	0.716

*p<0.05

Table 4: The interaction effects of rs53576 genotype and SCL Sum scores on bilateral amygdalar, hippocampal and caudate volumes are shown as assessed in multiple regression analyses (estimates (β) with standard error (SE) and p-values).

	Rs53576 x SCL Sum		Rs53576 x SCL Sum x Sex	
	β ± SE	p	β ± SE	p
R Amygdala	0.64 ±0.60	0.291	-0.75±1.31	0.570
L Amygdala	0.14±0.59	0.814	0.92±1.28	0.471
R Hippocampus	3.62±1.66	0.032* 95% CI [0.32,6.92]	-1.77±3.61	0.624
L Hippocampus	2.00±1.83	0.276	-0.94±3.87	0.809
R Caudate	4.65±2.47	0.063 95% CI [-0.25,9.56]	-3.62±5.40	0.504
L Caudate	2.20±2.53	0.387	-4.07±5.52	0.463

*$p<0.05$

Figure 1: Genotype rs53576 and hippocampal volumes.

A GG-homozygotes of rs53576 showed insignificantly larger infant bilateral hippocampal volumes compared to A-allele carriers (right: β= -18.90±19.23, p= 0.328, left: β= -21.51±20.64, p= 0.300). **B** The association between rs53576 and infant left hippocampal volumes was sexually dimorphic (β= -85.77±39.54, p= 0.033). The residuals of hippocampal volumes, controlling for infant age, gestational weeks and TBV and partly sex, are depicted for GG-homozygotes and A-allele carriers.

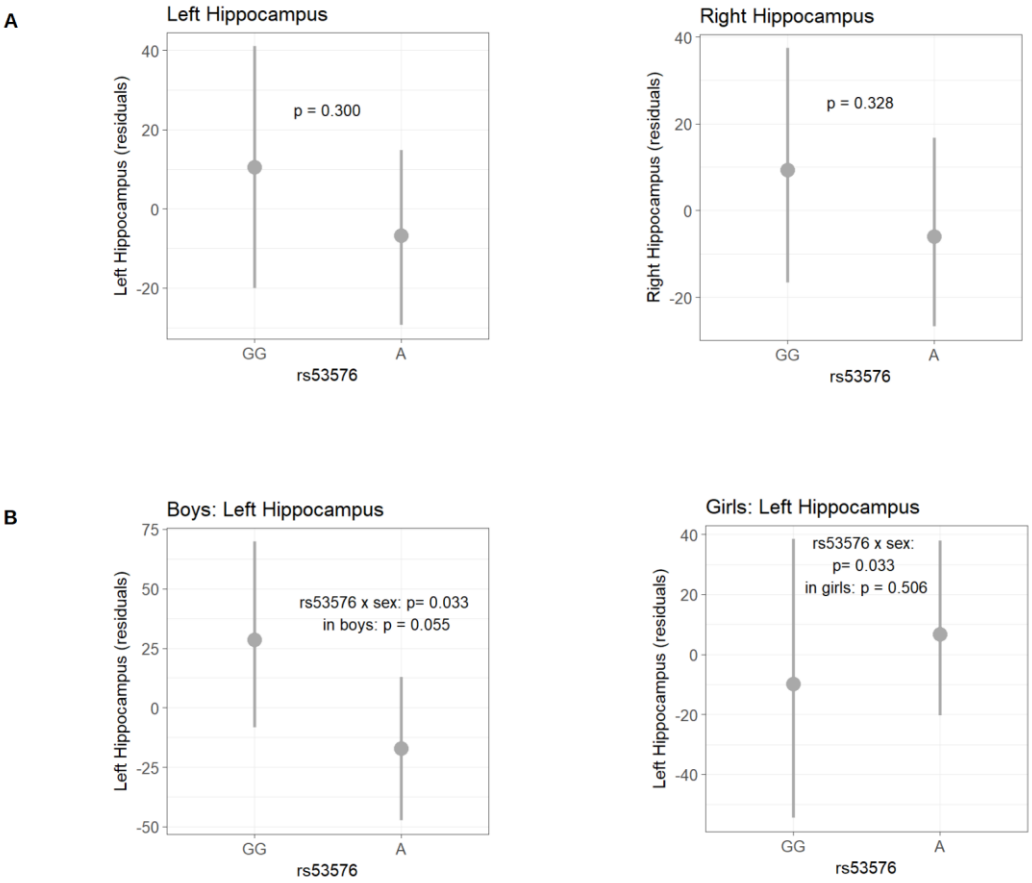

Figure 2: Genotype rs53576 and right amygdalar volumes.

No effect of genotype on right amygdalar volumes was observed (β= -4.32±6.81, p= 0.528), and no sexually dimorphic associations were found (β= -0.68±13.38, p= 0.960). The residuals of amygdalar volumes, controlling for infant age, gestational weeks and TBV and partly sex, are depicted for GG-homozygotes and A-allele carriers.

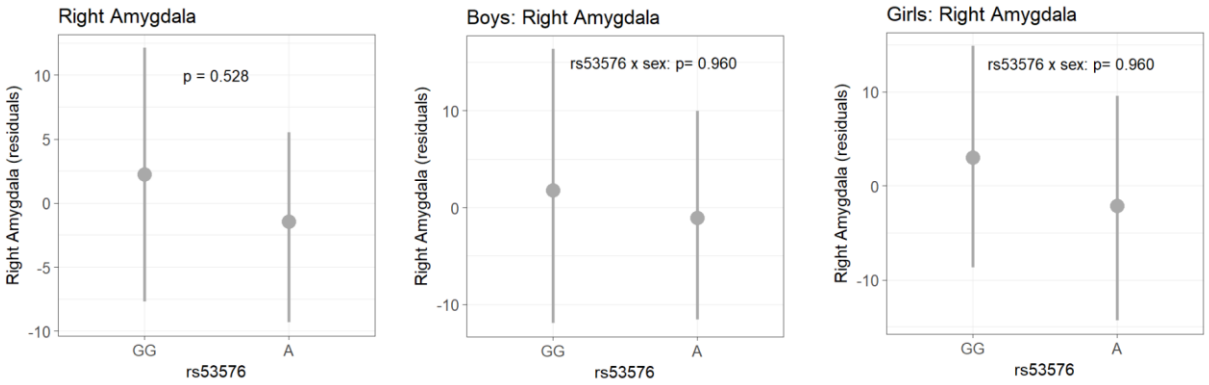

Figure 3: The interaction between genotype rs53576 and SCL Sum scores on right hippocampal and caudate volumes.

A significant GxE interaction effect was observed on right hippocampal (β= 3.62±1.66, p= 0.032) volumes. The GxE interaction effect on right caudate volumes was insignificant in the main analysis (β= 4.65±2.47, p= 0.063), but significant in some control analyses.

The residuals of hippocampal and caudate volumes, controlling for infant age, gestational weeks, TBV and sex, are depicted for GG-homozygotes and A-allele carriers, as well as the regression lines and their 95% confidence intervals.