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# Oxytocin receptor gene and depressive symptoms associated with physiological reactivity to infant crying

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**Both the oxytocin receptor (OXTR) gene and depressive symptoms have been associated with parenting behaviour. The OXTR GG genotype has been suggested to be related to more sensitive parenting, whereas depressive symptoms may affect sensitivity negatively. We examined the role of OXTR and the influence of depressive symptoms in explaining differences in physiological reactivity to infant crying. Heart rate responses of 40 healthy females without children (age 19–47 years, randomly selected half of twin pairs) were measured during the presentation of three episodes of infant cry sounds. Participants with the presumably more efficient variant of the oxytonergic system gene (OXTR GG) had more pronounced physiological reactivity to repeated cry sounds, except when they showed more symptoms of depression. Results were replicated in the second half of the twin sample. This is the first study to suggest effects of OXTR genotype on physiological reactivity to infant crying. Depressive symptoms may however suppress the effect of the OXTR GG genotype.**

**Keywords:** oxytocin receptor gene; depression; physiological reactivity; infant crying; parenting

## INTRODUCTION

The infant cry is a signal of distress evolved to elicit parental proximity and caregiving (Bowlby, 1969/1982, Zeifman, 2001). Infant crying evokes physiological reactions in adults that are associated with prompt caregiving responses (Del Vecchio *et al.*, 2009). Genetic factors have been shown to contribute to individual differences in physiological reactivity to infant crying (D.Out *et al.*, submitted for publication). More specifically, the oxytocin receptor (OXTR) may be involved in explaining the variance in maternal sensitivity (Feldman *et al.*, 2007; Bakermans-Kranenburg and Van IJzendoorn, 2008), defined as the ability to accurately perceive children's signals and to respond in an adequate and prompt way (Ainsworth *et al.*, 1978). Maternal sensitivity might be associated with more pronounced physiological reactivity to infant crying, which in turn may be related to OXTR genotype. At the same time, depressive symptoms are expected to decrease physiological reactivity to infant crying, since depressed mothers have more often been found to be less responsive to their infant's signals than non-depressed mothers (Murray *et al.*, 1996; Murray and Cooper, 1997; Donovan *et al.*, 1998; Schuetze and Zeskind, 2001). In this study, we examined the role of the OXTR gene and depressive symptoms in explaining differences in physiological reactivity to infant crying.

Infant crying provides information about the infant's health and the intensity of distress through the acoustics of the cry sound (Murray, 1979; Gustafson *et al.*, 2000). Cries produce autonomic arousal in adults, such as elevated heart rate (HR) and skin conductance (Frodi *et al.*, 1978; Wiesenfeld *et al.*, 1981; Frodi and Lamb, 1980; Frodi, 1985; Crowe and Zeskind, 1992), and this state of arousal leads to a quick response to the infant in order to terminate the cry (Del Vecchio *et al.*, 2009). In a behavioral genetic study, D.Out *et al.* (submitted for publication) showed that adults' cardiac activity increased during repeated infant distress signals; they reasoned that adults become increasingly sensitized to these signals. In addition, variance in adults' cardiac reactivity to cry sounds was shown to be explained by genetic factors, making some adults physiologically more reactive to cry stimuli than others. Bakermans-Kranenburg and Van IJzendoorn (2008) found higher levels of sensitive responsiveness to toddlers in parents with the OXTR rs53576 GG genotype, the potentially more effective variant of the oxytonergic system gene, than in parents with OXTR AA and AG genotypes. The link between OXTR gene and parenting has been suggested as a promising direction for future research into parenting (Taylor, 2008). The role of oxytocin in parenting has been demonstrated in several animal studies (see for a review Carter, 1998). A recent study involving human mothers showed that oxytocin levels across pregnancy and the postpartum period were positively related to

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sensitive parenting in the first month after birth (Feldman *et al.*, 2007). Oxytocin might play a role in the synchrony of interaction, defined as the matching of behavior, affective states and biological rhythms between parent and child (Feldman, 2007).

Whereas the OXTR GG genotype may increase maternal sensitivity to infants' distress signals, depressive symptoms have been associated with poor mother-infant interaction. Depressed mothers have difficulty providing appropriate social responses during interactions with their infant (Murray *et al.*, 1996) and exhibit fewer affectionate contact behaviors towards their infants (Feldman and Eidelman, 2003, 2004). They are less able to track their infant's activities and physically protect them from potential hazards (Gelfand and Teti, 1990). Murray *et al.* (1993) found that speech of depressed mothers expressed more negative affect and was less focused on the infant. In addition, depressive symptoms are associated with reduced sensitivity to infants' distress signals (Donovan *et al.*, 1998). Depressed mothers rated cries as less perceptually salient and as less likely to elicit active caregiving responses, especially at times of greater distress (Schuetze and Zeskind, 2001). Not only does clinical depression affect interaction patterns, mild depression and even experimentally induced maternal depressed mood were found to reduce responsivity (Zekoski *et al.*, 1986; Bettes, 1988).

In this study, we examine the role of the OXTR gene and the influence of depressive symptoms in physiological reactivity to infant crying. In an experimental paradigm, adults' HR responses were measured during the presentation of three sets of cry sounds varying in pitch. We hypothesized that participants with the OXTR GG genotype would display more pronounced HR reactivity across the three cry episodes than participants with the OXTR AA and AG genotypes, unless they showed more symptoms of depression that may dampen their reactivity.

## METHODS

### Participants

Participants for this study were selected from a larger study investigating the genetic susceptibility to sensitive and harsh caregiving and physiological reactivity in response to infant crying (D.Out *et al.*, submitted for publication). The original sample consisted of 50 male and 134 female twin pairs who were recruited using the municipal registers of five cities in the western region of the Netherlands, through advertisements and via a website. Zygosity was determined on the basis of questionnaires (Magnus *et al.*, 1983) and additional genetic analysis of selected polymorphisms. Twin pairs were selected to take part in a future neuroimaging study if they were monozygotic females without children of their own, in good health and without hearing impairments. This resulted in 40 twin pairs for whom the OXTR gene was determined. The mean age of the selected participants was 27.05 years (s.d. = 7.55, range 19–47). The majority of the participants were born in the Netherlands and from Caucasian origin (87.5%). Their mean educational level was 3.58 (s.d.= 0.77) on a scale ranging from 1 (elementary school) to 7 (bachelor's or master's degree). Permission for this study was obtained from the local ethics committee and all participants gave informed consent.

### Procedure

The twin pairs were invited for a lab session lasting about 3 h. They were tested individually in two quiet rooms. The lab session started with several cognitive assessments. Following these assessments and a short break an electrocardiographic device was fitted and their physiological responses were measured during the remaining session. After a 1-h interview, the cry perception task was administered, which lasted about 30 min. The participants were told that they would hear infant crying through headphones, and that they had to complete rating scales online during the task. At the end of the lab session, participants completed an anxiety questionnaire. They were asked to complete a questionnaire on depressive symptoms at home and returned it by mail within on average 2 weeks.

### Measures

#### *Cry perception task*

The cry perception task was administered on a laptop using E-Prime software (Version 1.1; Psychology Software Tools, Inc., PA, USA). Cry sounds were derived from the spontaneous crying of a healthy 2-day-old, full birth weight and full-term female infant while she was in a supine position in a bassinette, midway between scheduled feedings. The cry was recorded at a sampling rate of 44.1 kHz using a directional microphone held ~20 cm from the infant's mouth. A 10-s portion of the sustained period of crying, containing seven expiratory sounds, was selected for presentation. The durations and peak fundamental frequencies (peak $F_0$) of each expiratory component were determined from a digital sound spectrographic display. The frequency of the peak $F_0$ was obtained from the power spectrum resulting from a fast Fourier transform (FFT) of the 25 ms point at which the fundamental frequency reached its highest point in the expiratory sound. The seven cry expiratory sounds had durations with a mean of 1.055 s (range: 0.545–1.899 s) and a mean peak $F_0$ of 452.6 Hz (range: 425.2–515.6 Hz). The peak $F_0$ of the entire cry was $515 \pm 15$ Hz. Two new 10-s cry stimuli were created by digitally increasing the original cry by ~200 and 400 Hz, respectively, resulting in two new 10-s cry sounds with an overall peak $F_0$ of 714.5 Hz (700-Hz Cry) and 895.8 Hz (900-Hz Cry). Changes in the Peak $F_0$ of these two cries were made with comparable changes in the harmonic structures of the seven cry expirations across the entire 10-s cry sound segment while holding the temporal components constant.

Participants listened to the cry stimuli that were presented on a constant volume through Sennheiser HD202

headphones. The task started with a baseline condition during which participants were instructed to relax and look at three landscape photographs for six minutes in total. The cry paradigm consisted of two parts. Participants started each part with a practice trial during which the 500-Hz cry was presented. After the practice trial the cry stimuli were presented in three cycles or *episodes* (each consisting of the 500-, 700- and 900-Hz stimuli). The order of presentation was random within each cycle. During the first part of the paradigm, the presentation of each stimulus was followed by the collection of a saliva sample which took about one minute. Participants also rated their perception of the cry on four rating scales. During the second part of the task, participants rated their intended caregiving responses to each cry sound on seven rating scales. No saliva samples were collected in this part. Here, we only report on cardiac reactivity during the first part of the cry paradigm. The second part was not included in the present analyses since the procedure was different: the intertrial intervals between the stimuli were shorter and varied across persons.

### Depressive symptoms

Participants completed the Dutch version of the Center for Epidemiological Studies Depression Scale (CES-D) (Radloff, 1977). The scale consists of 20 items and measures mood, somatic symptoms and interpersonal relationships within the last week on a four-point scale based on frequency of occurrence. This scale was found to have high test–retest reliability ($r = 0.51$) and high internal consistency ($\alpha = 0.85$ for community samples; Radloff, 1977). The internal consistency in our sample was 0.88.

### Anxiety

The Dutch version of the State-Trait Anxiety Inventory (STAI) (Spielberger, 1983) was used to measure anxiety. The STAI is a 40-item self-report questionnaire that differentiates between the temporary condition of state anxiety and the longstanding quality of trait anxiety. The STAI trait and state both have high test–retest reliability ($r = 0.88$, $r = 0.70$, respectively) and high internal consistency ($\alpha > 0.89$; Barnes *et al.*, 2002). In our sample, the internal consistencies for both STAI trait and state were 0.91.

### Cardiac activity

HR was recorded with the Ambulatory Monitoring System (VU-AMS5fs; TD-FPP, Vrije Universiteit, Amsterdam, the Netherlands). The electrocardiogram (ECG) signal was recorded continuously using three disposable pre-gelled Ag–Agcl electrodes (ConMed, New York, USA) that were placed below the right collar bone 4 cm to the right of the sternum, 4 cm under the left nipple and at the lateral right side. The full ECG signal was stored at a 16-bit sampling rate. HR responses were synchronized to the cry sounds using a marker button on the AMS device. The experimenter pushed the button two seconds before the stimulus was presented, leaving markers that allowed for accurate labeling of each cry sound.

Mean HR was calculated by peak-detection of the R-wave via a Matlab script (version 7.6.0; MathWorks, MA, USA). The resulting interbeat intervals were visually inspected by a rater who was unaware of genotype status; in case of irregularities, peak-detection of the raw signal was repeated after using a 5–50 Hz zero-phase shifting bandpass filter. Mean HR was calculated for each baseline period and each individual cry episode, resulting in a series of mean HRs for each person. These values were standardized and series from which one or more mean HRs were out of the $-3$ to $+3$ range were winsorized (Tabachnik and Fidell, 2001), ensuring that differences within individual series that were characteristic for an individual reactivity pattern remained intact. Baseline levels of HR were averaged across the baseline periods. HR levels during the presentation of the cry stimuli were aggregated across each cry pitch as well as across each of the three cry episodes.

### Genotyping

Buccal swabs from the mothers were collected in lysis buffer (100mM NaCl, 10mM EDTA, 10mM Tris pH 8, 0.1 mg ml$^{-1}$ proteinase K and 0.5% w/v SDS) until further processing. Genomic DNA was isolated from the samples using the Chemagic buccal swab kit on a Chemagen Module I workstation (Chemagen Biopolymer Technologie AG, Baesweiler, Germany). DNA concentrations were measured using the Quant-iT DNA Assay kit (Invitrogen, Breda, the Netherlands). The average yield was 4 µg of genomic DNA per buccal swab sample. The region of interest from the OXTR gene (OXTR rs53576) was amplified by polymerase chain reaction (PCR) using a forward primer (5′-GCCCACCATGCTCTCCACATC-3′) and a reverse primer (5′-GCTGGACTCAGGAGGAATAGGGAC-3′). Typical PCR reactions contained between 10 and 100 ng genomic DNA template, 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% dimethyl sufoxide with 0.3 U of BioThermAB polymerase (GeneCraft, Munster, Germany) in a total volume of 30 µl using the following cycling conditions: initial denaturation step of 3 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 60°C, 1 min at 72°C and a final extension step of 3 min at 72°C. To determine the A/G polymorphism, PCR fragments were sequenced using the forward primer and dye terminator chemistry (BigDye v3.1, Applied Biosystems). The genotype distribution ($n = 10$ AA, $n = 38$ AG, $n = 32$ GG) was in Hardy–Weinberg equilibrium, $\chi^2$ (2, $n = 80$) $= 0.07$, $P = 0.96$. Because of the skewed distribution, AA and AG genotypes were combined in the analyses.

## Analyses

### Data processing

For six participants, physiological measures were lost due to equipment failure or incorrect markers. For four

participants, physiological measures were missing during baseline. Missing data imputation was done for these missing values using regression analyses with non-missing physiological measures and age as predictors. Four participants did not complete the CES-D. These missing values were imputed using regression analyses with age and STAI trait as predictors. Depressive symptoms and anxiety are highly correlated ($r = 0.69$ in this sample) and they often occur comorbidly (Pollack, 2005).

### *Statistical analyses*

Due to dependency of the data, statistical analyses were done separately for a random half of the sample including one randomly selected sibling from a twin pair. *T*-tests were performed to examine differences in age, education, depressive symptoms, anxiety, and HR between genotypes GG and AA/AG. To examine the influence of OXTR genotype and depressive symptoms on the development of HR reactivity across the cry paradigm, a repeated measures analysis was performed with HR as the outcome measure, episode (baseline and three episodes) as within-subjects factor, OXTR genotype (GG, AA/AG) as between-subjects factor, and depressive symptoms (mean CES-D) as covariate. State anxiety (mean STAI state) was included as a covariate in order to control for temporary mood during the lab session. Degrees of freedom were corrected using Greenhouse–Geisser and Huynh–Feldt estimates of sphericity if Mauchly's test indicated that the assumption of sphericity was violated; main effects were based on pooled error. All analyses were repeated for the second half of the sample.

## RESULTS

Table 1 presents the descriptive statistics of education, depressive symptoms, trait and state anxiety and HR during baseline and the three cry episodes for each genotype. The genotype groups did not differ on any of these variables.

The repeated measures analysis did not show a significant effect of state anxiety, $F(1,31) = 0.02$, $P = 0.88$; we therefore excluded the variable from further analyses. There was a significant main effect of episode [$F(2.38,76.00) = 9.73$, $P < 0.01$, partial $\eta^2 = 0.23$, $\varepsilon = 0.79$]. Contrasts revealed that HR was significantly higher during the first cry episode [$F(1,32) = 4.27$, $P = 0.05$, partial $\eta^2 = 0.12$], the second cry episode [$F(1,32) = 19.65$, $P < 0.01$, partial $\eta^2 = 0.38$] and the third cry episode [$F(1,32) = 17.67$, $P < 0.01$, partial $\eta^2 = 0.36$] compared to baseline. There was no significant effect of OXTR genotype [$F(1,32) = 0.77$, $P = 0.39$] or depressive symptoms [$F(1,32) = 0.07$, $P = 0.79$], nor any interaction between OXTR genotype and depressive symptoms [$F(1,32) = 0.72$, $P = 0.40$] and between depressive symptoms and cry episode [$F(2.38,76.00) = 2.07$, $P = 0.12$, $\varepsilon = 0.79$].

However, there was a significant three-way interaction between cry episode, OXTR genotype and depressive symptoms [$F(2.38,76.00) = 4.11$, $P = 0.02$, partial $\eta^2 = 0.11$, $\varepsilon = 0.79$]. Participants with the GG genotype *and* low scores on CES-D had the largest increase in HR across the cry paradigm, especially during the second cry episode [$F(1,32) = 11.15$, $P < 0.01$, partial $\eta^2 = 0.26$] and the third cry episode [$F(1,32) = 7.37$, $P = 0.01$, partial $\eta^2 = 0.19$]. The three-way interaction is illustrated in Figure 1, with depressive symptoms dichotomized into low (Figure 1A, $M = 3.66$, s.d. $= 2.64$) versus high (Figure 1B, $M = 13.02$, s.d. $= 6.04$) CES-D scores using a median split (median $= 8.12$). There was a significant main effect of episode for participants with the AA/AG genotype and low CES-D scores [$F(3,36) = 6.73$, $P < 0.01$, partial $\eta^2 = 0.36$] or high CES-D scores [$F(3,24) = 10.96$, $P < 0.01$, partial $\eta^2 = 0.58$], as well as for the participants with the GG genotype and low CES-D scores [$F(3,15) = 4.69$, $P = 0.02$, partial $\eta^2 = 0.48$], but not for participants with the GG genotype and high CES-D scores [$F(3,21) = 2.75$, $P = 0.07$]. To ensure that the effects were not confounded by population stratification, analyses were repeated after exclusion of participants who were not from Caucasian origin ($n = 5$). The three-way interaction between cry episode, OXTR genotype and depressive symptoms remained significant [$F(2.40,69.58) = 5.70$, $P < 0.01$, partial $\eta^2 = 0.16$, $\varepsilon = 0.80$].

Analyses were repeated for the second half of the twin pair sample. Again state anxiety was excluded from the analyses, because it did not show a significant effect on HR [$F(1,33) = 0.06$, $P = 0.81$]. The main effect of episode was significant [$F(2.05,69.64) = 15.39$, $P < 0.01$, partial $\eta^2 = 0.31$, $\varepsilon = 0.68$]. Contrasts revealed that HR was significantly higher during the first cry episode [$F(1,34) = 13.75$, $P < 0.01$, partial $\eta^2 = 0.29$], the second cry episode [$F(1,34) = 25.40$, $P < 0.01$, partial $\eta^2 = 0.43$] and the third cry episode [$F(1,34) = 22.63$, $P < 0.01$, partial $\eta^2 = 0.40$] compared to baseline. Similar to the first half of the twin pair sample, there was no significant effect of OXTR genotype [$F(1,34) = 1.67$, $P = 0.21$] or depressive symptoms [$F(1,34) = 1.12$, $P = 0.30$], nor any interaction between OXTR genotype and depressive symptoms [$F(1,34) = 3.96$, $P = 0.06$] and between depressive symptoms and cry episode [$F(2.05,69.64) = 2.90$, $P = 0.06$, $\varepsilon = 0.68$]. As in the first sample, the three-way interaction of episode, OXTR and depressive symptoms was significant [$F(2.05,69.64) = 3.77$, $P = 0.03$, partial $\eta^2 = 0.10$, $\varepsilon = 0.68$]. Again, participants with the GG genotype and low scores on CES-D had the largest increase in HR across the cry paradigm, especially during the second cry episode [$F(1,34) = 6.06$, $P = 0.02$, partial $\eta^2 = 0.15$] and the third cry episode [$F(1,34) = 5.49$, $P = 0.03$, partial $\eta^2 = 0.14$], thus replicating the results in the first half of the sample. The three-way interaction in the second half of the twin pair sample is illustrated in Figure 2. As shown in Figure 2, participants with the GG genotype with low CES-D scores show an earlier increase in HR reactivity (during cry episode 2) compared to other participants. Mean CES-D score in the group with low depressive symptoms was 4.24 (s.d. $= 2.33$) and 14.15 (s.d. $= 5.83$) in the group with high depressive symptoms. There was a

**Table 1** Means and standard deviations for CES-D, STAI state, STAI trait, education and HR during baseline and three cry episodes for the OXTR GG and AA/AG genotypes

| | Sample 1 | | | | | | | | Sample 2 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Genotype GG | | | Genotype AA/AG | | | | | Genotype GG | | | Genotype AA/AG | | | | |
| Variable | *M* | s.d. | Range | *M* | s.d. | Range | *t* | *P* | *M* | s.d. | Range | *M* | s.d. | Range | *t* | *P* |
| Education[a] | 3.85 | 0.67 | 2–5 | 3.57 | 0.93 | 3–5 | −0.92 | 0.36 | 3.64 | 0.33 | 2–5 | 3.43 | 0.79 | 2–5 | −0.84 | 0.41 |
| CES-D[b] | 8.67 | 3.05 | 4–14 | 7.70 | 8.06 | 0–34 | −0.43 | 0.67 | 10.37 | 7.51 | 0–30 | 8.86 | 6.25 | 1–25 | −0.67 | 0.51 |
| STAI state[b] | 1.69 | 0.28 | 1.25–2.15 | 1.71 | 0.51 | 1.05–3.55 | 0.12 | 0.91 | 1.54 | 0.30 | 1.00–2.05 | 1.58 | 0.39 | 1.00–2.35 | 0.33 | 0.74 |
| STAI trait[b] | 1.79 | 0.39 | 1.20–2.80 | 1.82 | 0.55 | 1.15–3.55 | 0.18 | 0.38 | 1.79 | 0.38 | 1.20–2.45 | 1.68 | 0.34 | 1.25–2.50 | −0.88 | 0.38 |
| HR Baseline[b] | 67.70 | 6.74 | 58.18–80.55 | 67.08 | 9.85 | 47.50–92.54 | −0.21 | 0.84 | 65.25 | 8.90 | 49.67–84.46 | 68.67 | 9.41 | 51.58–85.47 | 1.12 | 0.27 |
| HR Episode 1[b] | 70.39 | 8.65 | 57.87–82.55 | 69.53 | 9.64 | 49.49–96.06 | −0.27 | 0.79 | 67.91 | 10.51 | 50.77–86.46 | 70.36 | 8.76 | 51.62–83.30 | 0.78 | 0.44 |
| HR Episode 2[b] | 70.85 | 7.64 | 59.32–80.94 | 70.78 | 10.61 | 51.63–97.84 | −0.02 | 0.98 | 70.09 | 10.72 | 53.97–91.03 | 71.22 | 8.28 | 53.25–85.02 | 0.37 | 0.72 |
| HR Episode 3[b] | 73.23 | 10.00 | 58.21–89.07 | 70.97 | 10.01 | 51.26–97.88 | −0.69 | 0.50 | 71.36 | 11.87 | 53.18–92.02 | 72.49 | 7.92 | 53.27–86.13 | 0.35 | 0.73 |

*Note.* Sample 1: [b]$n = 36$ ($n = 14$ GG, $n = 22$ AA/AG), [a]$n = 34$ ($n = 13$ GG, $n = 21$ AA/AG), Sample 2: [b]$n = 38$ ($n = 15$ GG, $n = 23$ AA/AG), [a]$n = 36$ ($n = 15$ GG, $n = 21$ AA/AG).

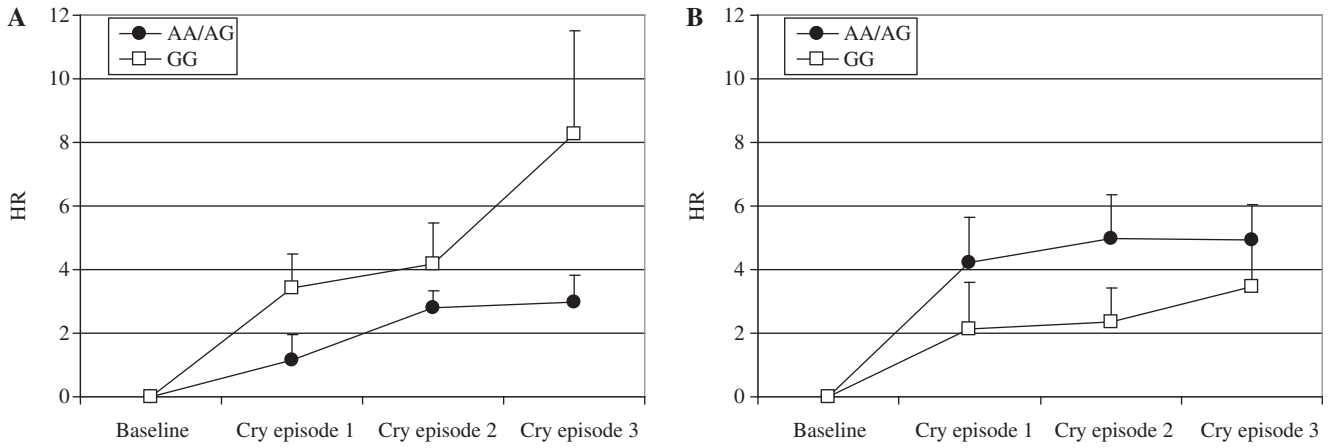


**Fig. 1** HR reactivity across the three cry episodes for participants in the first half of the twin pair sample with OXTR AA/AG or GG and low (**A**: $n = 13$ AA/AG, $n = 6$ GG) and high (**B**: $n = 9$ AA/AG, $n = 8$ GG) levels of depression.

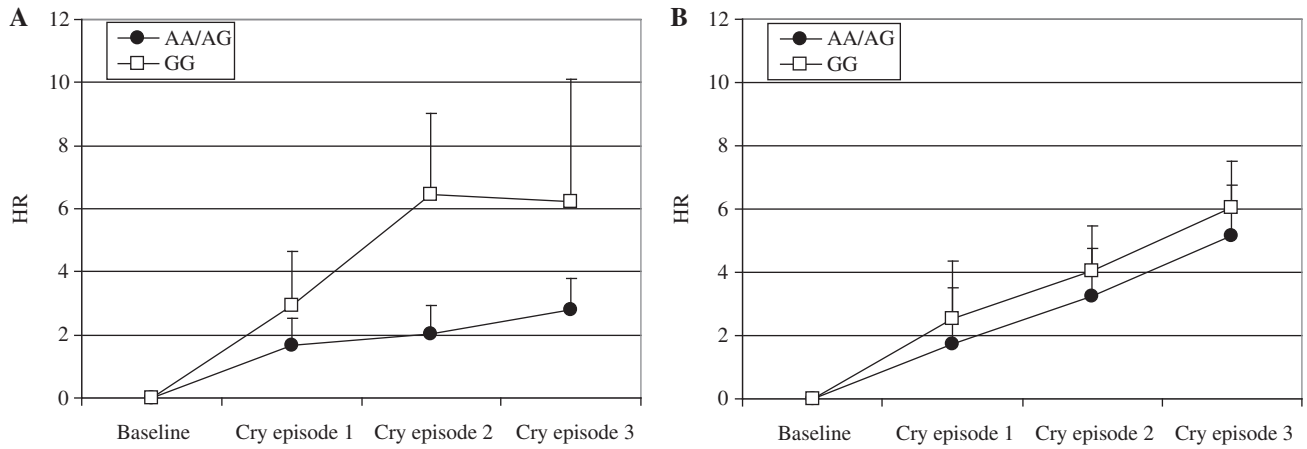


**Fig. 2** HR reactivity across the three cry episodes for participants in the second half of the twin pair sample with OXTR AA/AG or GG and low (**A**: $n = 13$ AA/AG, $n = 5$ GG) and high (**B**: $n = 10$ AA/AG, $n = 10$ GG) levels of depression.

significant main effect of episode for participants with the AA/AG genotype and low CES-D scores [$F(3,36) = 4.10$, $P = 0.01$, partial $\eta^2 = 0.26$] or high CES-D scores [$F(3,27) = 5.35$, $P < 0.01$, partial $\eta^2 = 0.37$], as well as for the participants with the GG genotype and low CES-D scores [$F(3,12) = 3.43$, $P = 0.05$, partial $\eta^2 = 0.46$] or high CES-D scores [$F(3,27) = 8.73$, $P < 0.01$, partial $\eta^2 = 0.49$]. After exclusion of the non-Caucasian participants ($n = 5$) the three-way interaction between cry episode, OXTR genotype and depressive symptoms remained significant [$F(2.08,60.42) = 5.60$, $P < 0.01$, partial $\eta^2 = 0.16$, $\varepsilon = 0.69$].

## DISCUSSION

Participants with the OXTR GG genotype displayed more pronounced physiological reactivity across the cry paradigm than participants with the AA or AG genotype. Similar to D.Out *et al.* (submitted for publication), the results point to genetic effects on increased HR to repeated infant cries, and we documented the role of the OXTR genotype in this sensitization response. However, participants with the GG genotype did *not* show more pronounced physiological reactivity when they had more symptoms of depression. Thus, adults with the GG genotype became more sensitized to repeated infant cries than participants with the potentially less effective variants of the oxytonergic system gene, unless they showed more symptoms of depression. Our findings cannot be explained by an association between depressive symptoms and OXTR genotype, since depressive symptoms and OXTR genotype were unrelated. We replicated the results in the other half of the twin sample.

Recently, Bakermans-Kranenburg and Van IJzendoorn (2008) found that mothers with OXTR GG genotype were more sensitive to their toddlers' signals than mothers with the AA or AG genotype. More pronounced physiological reactivity might form the basis for maternal sensitivity, because high arousal has been shown to lead to quick responding to the infant (Del Vecchio *et al.*, 2009), though it should be mentioned that very strong physiological reactivity to infant cries may also be associated with harsh responses (Crowe and Zeskind, 1992). Several studies have demonstrated the important role of oxytocin in maternal behavior (e.g. Feldman *et al.*, 2007, 2010). Oxytocin prompts affiliative behavior as a response to stress, such as tending and protective responses to infants (Taylor, 2006). At the same time, depressive symptoms have been shown to dampen maternal sensitivity (Donovan *et al.*, 1998; Murray *et al.*, 1993; Murray *et al.*, 1996). To our knowledge, this is the first study to suggest effects of OXTR genotype in combination with depressive symptoms on physiological reactivity to infant crying.

The current study is limited in several ways. First, our sample size is relatively small compared to other studies that examine the association between sensitivity and depressive symptoms or genotype. This resulted in small genotype group sizes and the necessary combination of the OXTR AA and AG genotypes in the analyses (similar to Bakermans-Kranenburg and Van IJzendoorn, 2008). However, our results were replicated in the other half of the twins, consolidating our findings in the first half. Of course, the second sample was genetically and otherwise associated with the first sample which makes it easier to replicate although assessments were conducted independently. Second, the participants did not have a diagnosis of clinical depression. Our findings indicate that more depressive symptoms dampen sensitivity to infant crying even in a subclinical population. Sensitivity to infant crying might be reduced even more in adults who are diagnosed with clinical depression, as shown by Schuetze and Zeskind (2001). Furthermore, it should be mentioned that we focused on variants of the OXTR gene that have not yet been shown to be functional. The Bakermans-Kranenburg and Van IJzendoorn (2008) study was one of the first suggesting functional implications for GG *vs* AG and AA variants of OXTR. In previous studies, variations in OXTR have been related to autism (Wu *et al.*, 2005), indicating genetic vulnerability to autism in carriers of the A allele. The processes linking variants of the OXTR gene to actual oxytocin levels in humans have however not yet been clarified. Carter *et al.* (2007) detected variations of oxytocin levels in saliva samples as a function of lactation and massage. Measurement of oxytocin in saliva may be a promising future method to test the association between oxytocin and sensitivity to infant crying more directly.

Further research is needed to investigate how OXTR genotype and depressive symptoms affect behavior of typical, non-twin parents instead of twin adults who did not (yet) have children of their own. In the current study we focused on physiological measures of sensitivity to infant cries. Elsewhere, we documented that parents and non-parents did not differ in the heritability of HR while listening to infant cries (D.Out *et al.*, submitted for publication). Since physiological reactivity to infant cries is associated with responsivity to the infant (Del Vecchio *et al.*, 2009), OXTR genotype and depressive symptoms are expected to influence parent-child interaction as well. In addition, future studies may target the processes underlying the association between OXTR genotype, depressive symptoms and physiological reactivity to infant crying. OXTR genotype might affect neural networks involved in emotional processes, such as the perception of infant cries. In a future neuroimaging study we aim at clarifying how OXTR genotype and depression affect these neurological processes.

In conclusion, the current study is the first to highlight the role of OXTR genotype in combination with depressive symptoms on physiological reactivity to repeated infant crying. The findings support previous results of decreased sensitivity in depressed mothers and increased sensitivity in the OXTR GG genotype. Adults with the OXTR GG genotype showed more pronounced physiological reactivity to repeated infant cries than adults with the potentially less

efficient variant of the oxytonergic system gene, but depressive symptoms may suppress the effect of the OXTR GG genotype.

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