

Oxytocin receptor (*OXTR*) and serotonin transporter (*5-HTT*) genes associated with observed parenting

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Both oxytocin and serotonin modulate affiliative responses to partners and offspring. Animal studies suggest a crucial role of oxytocin in mammalian parturition and lactation but also in parenting and social interactions with offspring. The serotonergic system may also be important through its influence on mood and the release of oxytocin. We examined the role of serotonin transporter (*5-HTT*) and oxytocin receptor (*OXTR*) genes in explaining differences in sensitive parenting in a community sample of 159 Caucasian, middle-class mothers with their 2-year-old toddlers at risk for externalizing behavior problems, taking into account maternal educational level, maternal depression and the quality of the marital relationship. Independent genetic effects of *5-HTTLPR SCL6A4* and *OXTR rs53576* on observed maternal sensitivity were found. Controlling for differences in maternal education, depression and marital discord, parents with the possibly less efficient variants of the serotonergic (*5-HTT ss*) and oxytonergic (*AA/AG*) system genes showed lower levels of sensitive responsiveness to their toddlers. Two-way and three-way interactions with marital discord or depression were not significant. This first study on the role of both *OXTR* and *5-HTT* genes in human parenting points to molecular genetic differences that may be implicated in the production of oxytocin explaining differences in sensitive parenting.

Keywords: parenting; sensitivity; oxytocin receptor (*OXTR*) gene; serotonin transporter (*5-HTT*) gene; marital discord; maternal depression

Although the development of offspring is shaped by their genetics, parenting also plays a central role in determining the individual child's adaptation to the social environment in various non-human species (Suomi, 1999; Meaney, 2001; Meaney and Szyf, 2005), as well as in humans (Cummings and Davies, 2002; Repetti *et al.*, 2002; Sroufe *et al.*, 2005). In particular parental sensitivity, defined as the ability to accurately perceive children's signals and to respond in an adequate and prompt way (Ainsworth *et al.*, 1978) has been documented to be crucial in the development of the offspring's capacity to establish attachments to protective adults and to regulate stress (Bowlby, 1969/1982; Cassidy and Shaver, 1999; Bakermans-Kranenburg *et al.*, 2003).

Almost no molecular genetic studies of parenting in humans have been conducted (Swain *et al.*, 2007). In a

previous report on the current sample we focused on two dopamine-related genes (Dopamine D4 Receptor, *DRD4* and Catechol-O-Methyltransferase, *COMT*), showing that daily hassles were strongly associated with less sensitive parenting in parents with the combination of genes leading to less efficient dopaminergic system functioning, whereas the most efficient combination (*COMT* met/met, no *DRD4-7 Repeat*) appeared to buffer the negative effect of daily hassles on maternal sensitivity (Van IJzendoorn *et al.*, 2007). In the current report we examine the role of oxytocin receptor (*OXTR*) and serotonin transporter (*5-HTT*) genes in explaining differences in sensitive parenting, taking into account maternal depression and the quality of the marital relationship. Marital relationships belong to the most crucial affiliative social systems with great import for the way in which mothers care for their children (Belsky, 1984; Cummings and Davies, 2002; Repetti *et al.*, 2002).

Carter (1998) in her seminal 'Neuroendocrine perspectives on social attachments and love' proposed a crucial role of oxytocin not only in mammalian parturition and lactation but also in parenting, by reducing neophobia and feelings of stress and enhancing the reward value of social interactions with the offspring. The important role of oxytocin in parental behavior of rodents (Lim and Young, 2006; Olazábal and Young, 2006) and sheep (Keverne and Kendrick, 1992) has been documented extensively. Similar data for non-human primates and humans are almost absent

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(Maestriperi, 1999; Numan and Insel, 2003), although recently oxytocin levels in human mothers were found to be related to parenting in the first month after birth (Feldman et al., 2007).

Circumstantial evidence for the potentially important role of oxytocin in human parenting may be derived from experimental studies administering oxytocin to patients with autism, which enhanced their social cognitions and empathic feelings (Bartz and Hollander, 2006), and in studies relating autism to variations in the oxytocin receptor gene (Wu et al., 2005; Ylisaukko-oja et al., 2006; Jacob et al., 2007). In a study with non-clinical individuals, intranasal oxytocin administration increased feelings of interpersonal trust (Kosfeld et al., 2005). In a non-clinical adult female sample Tops et al. (2007) found plasma oxytocin levels to be strongly associated with attachment defined as the tendency to express and share emotions and feelings with partners or close friends. These findings appear to support Carter's (1998) suggestion that oxytocin is important for intimate attachments such as marital relationships and interactions with offspring.

The serotonergic system may be important in this regard through its association with depression (particularly in the face of stressful life events, see for a review Uher and McGuffin, 2008) but also through its potential influence on the release of oxytocin. Serotonergic fibers have preferential input to oxytonergic regions in macaques and other animals, and both oxytocin and serotonin can modulate affiliative responses to partners and offspring (Emiliano et al., 2007). Stimulation of the hypothalamus by serotonin has been shown to lead to release of oxytocin as a precursor molecule (Lee et al., 2003). Galfi et al. (2005) found that in rats oxytocin secretion was influenced directly by the serotonergic system (Jorgensen et al., 2003). Although the serotonergic system has been implicated in anxiety, affiliation and reward, little evidence for its role in parental behavior is available (Numan and Insel, 2003; D'Souza and Craik, 2006). The serotonergic system may however be important for responding sensitively to the partner as well as to the offspring, partly through its influence on the release of oxytocin.

Hypotheses

In the current investigation we examine the associations between *5-HTT* and *OXTR* genes and parenting, focusing on one of the variants of each gene (*5-HTTLPR SCL6A4* and *OXTR rs53576*). We expect to find lower levels of parental sensitive responsiveness in parents who as a result of their serotonergic and oxytonergic system genes are supposed to have less efficient oxytocin production. We test whether this association is dependent on the quality of the marital relationship and we control for differences in maternal educational level and depression. Finally we examine whether the effect sizes related to the genetic differences are comparable to those implicated in the environmental factors.

METHODS

Participants

The current article is based on data obtained in the SCRIPT study (Screening and Intervention of Problem behavior in Toddlerhood), which investigated the effectiveness of an early intervention program aimed at reducing externalizing problems in 1–3-year-old children by enhancing maternal sensitivity and adequate discipline strategies (Van Zeijl et al., 2006). It consisted of a screening phase in a general population sample and a pretest-posttest randomized case-control intervention in a subsample of children ($n=237$) with scores above the 75th percentile on the CBCL Externalizing Problems scale (Achenbach and Rescorla, 2000). Mother and child completed several tasks (e.g. free play, puzzles, see Van Zeijl et al., 2006) during a 1.5 h (pretest) laboratory session. About 3 years after this pretest session the participants were contacted to take part in the collection of DNA material. Cheek cells were collected from 176 mothers. These mothers (mean age 33 years, $s.d.=4.1$) did not significantly differ from mothers who did not participate in the genetic part of the study on age, sensitivity, marital discord, depression, child age or gender and number of children. They were however slightly better educated ($M=3.66$, $s.d.=1.06$ on a scale ranging from 1 = elementary school to 5 = at least BA degree) than non-participating mothers ($M=3.33$, $s.d.=1.08$). Fifty-seven percent of the children were boys. Since data for the current article were derived from the screening and pretest phases the intervention and control groups were combined in the analyses. Permission for the study was obtained from the Committee for Medical Ethics of Leiden University Medical Centre and the Ethics Committee of the Faculty of Social and Behavioral Sciences of Leiden University.

Measures

Sensitivity. Mothers' sensitive interaction with their toddlers was observed in the laboratory session during a series of problem-solving tasks. Dyads were given three tasks during a total time of 15 min; they were asked to solve puzzles that were too difficult for the child, and mothers were instructed to help their child in the way they usually did. Mothers' supportive presence, intrusiveness and clarity of instruction were rated on 7-point scales drawn from Egeland et al. (1990). These scales include and extend Ainsworth et al.'s (1978) original scales for 'sensitive responsiveness' developed for parent–infant interaction in the first year after birth. The Egeland et al. (1990) observational scales take the wider age range of the current sample into account and measure an age-appropriate concept of sensitivity that also pertains to the developmental domain of coping with cognitive challenges. The average intraclass correlation (single rater, absolute agreement) for intercoder reliability (for all separate pairs of seven coders) was .75 ($n=30$). For the overall sensitivity score, ratings of the separate tasks were averaged,

the intrusiveness scores were reversed and the standardized subscale scores were added.

Marital discord. Marital discord was assessed using a subscale of the Dutch Family Problems Questionnaire (Koot, 1997). The mothers indicated on a 3-point scale whether five statements about their partner relationship and partner support were 0 *not true*, 1 *somewhat or sometimes true*, or 2 *true or often true*. Reliability and validity of this scale were demonstrated by Koot (1997); the internal consistency (Cronbach's alpha) in our sample was 0.66. A total score was computed by summing item scores.

Maternal depression. Mothers completed the short form of the Young Adult Self-Report (YASR, Achenbach, 1997). We used a short form, consisting of the 29 items that were found to discriminate best between referred and non-referred samples (Achenbach, 1997). The internal consistency (Cronbach's alpha) in our sample was 0.89. A total score was computed by summing item scores.

Genotyping. Buccal swabs from the mothers were collected in lysis buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 0.1 mg/ml 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 *5-HTTLPR* polymorphism in the promoter region of the *SLC6A4* gene was genotyped by PCR amplification followed by agarose gel electrophoresis. The forward primer was 5'-ATGCCAGCACCTAACCCCTAATGT-3' and the reverse primer was 5'-GGACCGCAAGGTGGGCGGA-3' (Gelernter *et al.*, 1997). These primers produce a short fragment of 375 bp representing the 14 repeat allele ('s') and a long fragment of 419 bp representing the 16 repeat allele ('l'). PCR fragments containing the *5-HTTLPR* polymorphism were obtained in a total reaction volume of 25 µl, containing 50 ng of genomic DNA, 0.3 mM dNTPs, 1.5 mM MgCl²⁺, 10 pmol of each primer and 0.3 U of BioThermAB polymerase (Genecraft, Munster, Germany). PCR conditions were the following: an initial denaturation step of 10 min at 94°C, 36 cycles of 30 s at 94°C, 1 min at 68°C and 1 min at 72°C, followed by a final extension step of 15 min at 72°C. The amplification products were separated on a 2% agarose gel with 0.001% ethidium bromide and visualized by ultraviolet transillumination. Difficult cases were re-genotyped two more times. Genotyping was unambiguous in 159 cases. Genotypes ($n=38$ ss, $n=65$ sl, $n=56$ ll) were in Hardy-Weinberg equilibrium.

The region of interest from the Oxytocin receptor gene (*OXTR* rs53576) was amplified by PCR using a forward primer (5'-GCCACCATGCTCTCCACATC-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% DMSO 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 ($n=17$ AA, $n=71$ AG, $n=89$ GG) was in Hardy-Weinberg equilibrium. Because of the skewed distribution AA and AG genotypes were combined in the analyses. The distributions of *5HTT SLC6A4* and *OXTR* were independent ($P=0.69$).

RESULTS

Table 1 presents the means, s.d. and bivariate correlations for the main variables. *OXTR* and *5-HTT* genotypes were not associated with age of child, maternal educational level, depression, maternal sensitivity, or marital discord (Table 1). Mothers with a higher educational level appeared significantly more sensitive to their children [$r(157)=0.38$, $P<0.01$] and less depressed [$r(157)=-0.19$, $P=0.02$] but educational level was not related to marital discord. Marital discord was related to depression [$r(157)=0.33$, $P<0.01$].

The results of the analysis of variance of maternal sensitivity with *OXTR* and *5-HTT SLC6A4* as factors and maternal education, depression and marital discord as covariates are presented in Table 2. Controlling for maternal education, depression and marital discord, both *OXTR* [$F(1, 152)=4.32$, $P=0.04$, partial $\eta^2=0.03$] and *5-HTT* [$F(1, 152)=4.67$, $P=0.03$, partial $\eta^2=0.03$] genes were significantly associated with maternal sensitivity. Mothers with *OXTR* AA or AG genotypes were less sensitive than mothers with the GG genotype, and mothers with *5-HTT* ss were less sensitive than mothers with *5-HTT* sl or ll (Table 1). The interaction between *OXTR* and *5-HTT* genes, and the two-way and three-way interactions of the genotypes with marital discord and depression did not contribute significantly to the prediction. *5-HTT* ll vs ss/sl did not contribute significantly to the prediction of maternal sensitivity in a separate analysis.

DISCUSSION

These results point to independent genetic effects on maternal sensitivity of *5-HTT SLC6A4* and *OXTR* rs53576 polymorphisms, taking into account differences in educational level, depression and marital discord. We found lower levels of sensitive responsiveness to their toddlers in parents with the potentially less efficient variants of the serotonergic and oxytonergic system genes. The genetic effects did not interact with depression or the quality of the marital relationship and neither depression nor the marital relationship was associated with the genotypes examined in the

Table 1 Descriptives and correlations among the variables^a

	Total		Maternal genotype								Education	Sensitivity	Discord	Depression		
			OXTR				5HTTLPR									
	M	s.d.	GG (n = 79)		AG/AA (n = 80)		t	ss (n = 38)		sl/ll (n = 121)						
			M	s.d.	M	s.d.		M	s.d.	M	s.d.					
Child age (months)	23.30	10.14	24.54	9.83	22.08	10.36	1.54	23.13	9.98	23.36	10.24	0.12	-0.11	0.10	0.14	-0.06
Maternal education	3.66	1.06	3.67	1.03	3.65	1.09	0.12	3.71	1.06	3.64	1.06	0.33		0.38**	-0.04	-0.19*
Maternal sensitivity	0.11	2.32	0.42	2.13	-0.21	2.47	1.74	-0.45	2.31	0.28	2.30	1.70			-0.09	-0.11
Marital discord	1.97	1.63	1.96	1.38	1.99	1.85	0.10	1.84	1.67	2.02	1.62	0.58				0.33**
Maternal depression	6.72	6.54	6.38	5.75	7.05	7.26	0.64	5.08	4.80	7.23	6.94	1.78				

* $P < 0.05$ ** $P < 0.01$.^a $n = 159$.**Table 2** Analysis of variance of maternal sensitivity with OXTR and 5-HTTLPR as factors and maternal education, depression and marital discord as covariates^a

Predictors	F(1,152)	P	Partial η^2
Maternal education	26.66	<0.01	0.15
Maternal depression	0.10	0.75	<0.01
Marital discord	1.15	0.29	0.01
OXTR	4.32	0.04	0.03
5-HTT	4.67	0.03	0.03
OXTR \times 5-HTT	0.82	0.37	0.01

^a $n = 159$.

current study. Thus, differences in sensitive parenting appear to be associated with molecular genetic differences that may implicate the production of oxytocin. Sensitive parenting is a well-documented crucial determinant of young children's socio-emotional development with long-lasting consequences (Cassidy and Shaver, 1999; Sroufe *et al.*, 2005; Belsky *et al.*, 2006), underscoring the relevance of our findings.

Given the important role of the oxytocin system in affiliative relationships OXTR is an excellent target in a candidate gene approach involving parental caregiving of offspring (Carter, 1998). Oxytocin not only has a critical role in birth and lactation but also in the emergence of an intimate bond with offspring, as it may lower feelings of stress and fear (Carter, 1998; Young, 1999, 2001; Numan, 2006; Emiliano *et al.*, 2007). Beyond the reduction of anxiety oxytocin is suggested to have specific rewarding or hedonic effects that may facilitate parenting (Numan and Insel, 2003). Our study is one of the first suggesting functional implications for GG *vs* AG and AA variants of OXTR, although the underlying processes linking variants of the OXTR gene to actual oxytocin levels in humans have not yet been clarified. In previous studies, variations in OXTR have been related to autism. In a study in the Chinese Han population preferential transmission of A over G was found for rs53576 (Wu *et al.*, 2005), indicating genetic vulnerability

to autism in carriers of the A allele—the same genotypic variant that was related to lower levels of sensitivity in our study. Replication in a Caucasian sample showed preferential transmission of G over A at rs2254298 for children with autism, but no significant association with rs53576 (Jacob *et al.*, 2007). These findings point to OXTR as an excellent candidate for mediating genetic vulnerability to autism, but they also indicate its potential for studies on the association with sensitive parenting, which presupposes awareness of and empathy with children's needs and subtle emotional signals.

In contrast surprisingly little evidence supporting the significance of 5-HTT genes for parenting has been reported (Numan and Insel, 2003). Serotonin has been associated with negative emotions, such as anxiety or stress, although meta-analyses on linear relations between 5-HTT SLC6A4 and anxiety related traits have shown somewhat divergent results (Sen *et al.*, 2004; Munafo *et al.*, 2005). Here we suggest that 5-HTT SLC6A4 is associated with parenting through its potential influence on the oxytonergic system as we did exclude the possibility that its influence would be through its associations or interactions with maternal depression.

The influence of genetic differences in 5-HTT (3% explained variation) and OXTR (3% explained variation) genes on parenting is much smaller than the association between sensitive parenting and parental educational level (15% explained variation). As Kagan *et al.* (2007) recently argued, most psychological traits and behaviors may be better explained by a combination of basic characteristics such as gender and socio-economic status than by genetic markers alone, although including both genetic and environmental factors might predict such outcomes in specific sub-groups with most accuracy. The findings of the current study illustrate this point in showing that lower maternal educational level is more strongly associated with less sensitive parenting than genes potentially related to less efficient oxytocin production. It should be noted that mothers' sensitive interaction with their toddlers

was observed during problem-solving tasks potentially eliciting differences in sensitive instruction, which might explain its association with educational level. Moreover, the more accurate assessments of genetic factors compared with environmental influences may lead to less comparable effect sizes.

The role of genetic factors may also be dependent on the presence or absence of stressful life circumstances, with an increasing influence of hormonal effects on parenting in deprived contexts with low social support (Repetti *et al.*, 2002). Numan and Insel (2003) argue that in primates (as compared with rats) the balance between size and role of the medial pre-optical area and the neocortex has shifted in favor of the latter (Keverne, 2001). Primate parenting might therefore be under stronger cognitive than hormonal control, at least in normal circumstances (Numan and Insel, 2003; Kagan *et al.*, 2007). However, the medial preoptic area (MPOA) of the hypothalamus might still be involved by signaling the level of maternal motivation to the neocortex, which uses this input in the development of complex voluntary response strategies (Numan and Insel, 2003). Associations between serotonin and oxytocin system genes and parenting might be most pronounced but not limited to mothers in deprived settings, characterized by, for example, high degrees of stress or marital discord. The association between the *5-HTT* 's' allele and depression under conditions of environmental adversity (Caspi *et al.*, 2003) is a replicated finding (Uher and McGuffin, 2008). The absence of a significant gene–environment (GxE) interaction in our study does not support the claim that in certain (deprived) environments the role of hormones on behavior is more pronounced, but it should be noted that our sample is rather homogeneously well-educated, and major deprivation is absent. In a previous report on the same sample we found a negative impact of daily hassles on parental sensitivity (Van IJzendoorn *et al.*, 2007). Including daily hassles as covariate in the current analyses did not change the results. Better assessment of the environment as well as more intense levels of environmental stress or deprivation might however uncover GxE interactions that were not apparent in the current study.

It has been noted that the influence of oxytocin might be most important immediately after birth, for establishing the bond between mother and offspring instead of maintaining this bond at a later stage (Fahrbach *et al.*, 1985; Feldman *et al.*, 2007; Insel and Harbough, 1989). In the current study sensitive parenting was measured at 23 months. In order to assess the role of hormonal mechanisms such as the oxytonergic system some time after birth, Numan and Insel (2003) proposed to compare the sensitivity of adoptive mothers with the sensitivity of biological mothers to their offspring. Studying adoptive and non-adoptive parents from similar backgrounds, Juffer (1993) reported the absence of large differences in sensitive parenting. The influence of oxytocin caused by parturition

and lactation may, therefore, be overridden by other mechanisms of a more cognitive nature.

Although the oxytonergic system might thus not be a necessary or sufficient condition for sensitive parenting, experimental research showing that oxytocin improves 'mind reading' suggests that oxytocin nevertheless may facilitate parental sensitivity at any stage in parents' lives and not only during the period around birth. In a double-blind placebo controlled study on 30 adult males, Domes and colleagues (2007) found that after intranasal administration of a single dose of oxytocin participants were substantially better able to infer the affective mental state of others from subtle social cues from the eye region in a standard paradigm, the Reading the Mind in the Eyes Test. The authors state that reading the mind of an interactive partner is a cornerstone of all human interactions, which would also pertain to parenting. The definition of sensitive parenting explicitly includes the reading of the child's attachment needs from subtle facial or other non-verbal signals as a first and important step to responding in a prompt and adequate manner (Ainsworth *et al.*, 1978; Egeland *et al.*, 1990; Sroufe *et al.*, 2005).

Our study is limited in several ways. First, generalization of our findings may be limited to samples similar to the rather homogeneous middle-class sample included in the current study. Moreover, the families in the current study had an externalizing toddler (75th percentile or above on the CBCL), and our findings may only apply to parents who perceive their children as difficult and non-compliant, and who already at an early stage have difficulty managing their children. It is important to note, however, that the families in our study were two-parent families from predominantly well-educated background and without psychiatric disorders. Nevertheless, replication in unselected samples is needed.

Second, although our sample size is relatively large compared with other studies including observational measures of parenting, our study may nevertheless lack power to detect gene–gene or gene–environment interaction effects. Moreover, the *OXTR* AA and AG genotypes were combined in the analyses, as were the *5-HTT* sl and ll genotypes (similar to, e.g. Kaufman *et al.*, 2004; Battaglia *et al.*, 2005; Hayden *et al.*, 2007; Young *et al.*, 2007; but see Hariri *et al.*, 2002; Caspi *et al.*, 2003 for combined ss/sl vs ll genotypes). In our study *5-HTT* ss/sl vs ll did not contribute significantly to the prediction of maternal sensitivity. It should be noted that the *5-HTT SLC6A4* gene possesses several other polymorphic loci affecting its expression and function that were not included in this study (Wendland *et al.*, 2006).

Third, because *5-HTT* and *OXTR* genes have been associated with other forms of social behavior and mental states (Uher and McGuffin, 2008), it is unclear how specific and direct their influence is on sensitive parenting. It is possible that both genes affect interpersonal sensitivity more generally, which may in turn make parents more sensitive

to their offspring. Alternatively, the genes might be associated with affective states that promote or hamper the display of sensitive parenting. Further research is needed to clarify the process underlying the association found in the current study.

Last, we did not assess oxytocin levels directly, but variants of the oxytocin receptor gene that have not yet been shown to be functional. In a promising line of research Carter and her colleagues (2007) showed that oxytocin might be extracted from saliva samples, with detectable variations of oxytocin concentrations in saliva depending on lactation in mothers and massage in male subjects. Oxytocin levels in saliva were however low and assessments labor-intensive. In the near future it may become easier to measure salivary oxytocin as a biomarker for affiliative behavior in humans, in particular in parenting, which would enable direct tests of the association between oxytocin and sensitive parenting suggested in the current report.

In conclusion, the present study is the first to suggest independent effects on maternal sensitivity of *5-HTT* and *OXTR* genes in humans. Taking into account differences in maternal educational level, depression and marital discord, we found that parents with the possibly less efficient variants of the serotonergic and oxytonergic system genes showed lower levels of sensitive responsiveness to their toddlers. The current study is among the first to examine the molecular genetic basis of human parenting. The findings support previous results of environmental effects on sensitive parenting, but additionally point to molecular genetic differences that may be implicated in the production of oxytocin as a factor explaining differences in sensitive parenting.

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