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Implicit Association to Infant Faces: How Genetics, Early Care Experiences, and Cultural Factors Influence Caregiving Propensities. *Behavioural Brain Research*.

Implicit association to infant faces: Genetics, early care experiences, and cultural factors influence caregiving propensities

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Abstract

Genetics, early experience, and culture shape caregiving, but it is still not clear how genetics, early experiences, and cultural factors might interact to influence specific caregiving propensities, such as adult responsiveness to infant cues. To address this gap, 80 Italian adults (50% M; 18–25 years) were (1) genotyped for two oxytocin receptor gene polymorphisms (rs53576 and rs2254298) and the serotonin transporter gene polymorphism (5-HTTLPR), which are implicated in parenting behaviour, (2) completed the Adult Parental Acceptance/Rejection Questionnaire to evaluate their recollections of parental behaviours toward them in childhood, and (3) were administered a Single Category Implicit Association Test to evaluate their implicit responses to faces of Italian infants, Japanese infants, and Italian adults. Analysis of implicit associations revealed that Italian infant faces were evaluated as most positive; participants in the rs53576 GG group had the most positive implicit associations to Italian infant faces; the serotonin polymorphism moderated the

effect of early care experiences on adults' implicit association to both Italian infant and adult female faces. Finally, 5-HTTLPR S carriers showed less positive implicit responses to Japanese infant faces. We conclude that adult in-group preference extends to in-group infant faces and that implicit responses to social cues are influenced by interactions of genetics, early care experiences, and cultural factors. These findings have implications for understanding processes that regulate adult caregiving.

1.Introduction

Modern societies are becoming more and more multicultural; therefore, the investigation of psychological factors that affect the way individuals of different ethnic groups interact is becoming essential [1]. The quality of the multi-ethnic interaction is particularly significant when adults (e.g., children's caregivers, nannies, teachers, et al.) interact with an infant [2] as the nature and valence of adults' interactions can affect the child development [3].

1.1 Adults' responsiveness to in- and out-group infant faces

Numerous empirical investigations show that people perceive and evaluate in-group individuals compared to out-group individuals in specific ways. The Other-Race-Effect (ORE; or Other-Race bias), the tendency to more readily recognize members of one's own ethnic group, is a robust finding in adult face perception and recognition [4]. ORE is considered a universal phenomenon because it emerges very early in life (about 3–9 months of age) and is independent of the ethnic group [5,6]. As regards evaluations or valence, people also manifest a tendency to favour the social group to which they belong, an effect known as in-group bias [7–9]. In-group bias is also robust and universal and has been observed in adults and children in many social groups [8,10].

Infant faces represent biologically relevant stimuli that immediately and automatically capture attention and are implicitly associated with positive evaluations [11–13]. Infant cues are processed both at conscious levels (controlled) and at relatively automatic levels (without deliberate thought or awareness [14];see also [15,16]). Therefore, it is crucial and revealing to evaluate and compare adults' response to infants at both explicit and implicit levels.

Esposito et al. [17] investigated in-group preferences for infant faces by comparing responses to infant and adult faces of own and other groups in Japanese and Italian females. These authors considered implicit and explicit measures and found that ingroup preference was moderated by the measure and by the facial stimuli. At an explicit level (willingness to interact), adults showed an ingroup preference for adult faces, not infant ones, whereas at an

implicit level (autonomic reaction), adults showed a general preference toward babies that was independent of ethnic group, and no ingroup preference was observed. Hodsoll et al. [18] investigated if the baby schema influenced preferential allocation of attention independently of infant race by means of a dotprobet ask. These investigators compared responses of South Asian and UK-born White females to Asian and Caucasian infant faces and found that infant faces captured attention only when they matched the ethnicity of the participant, suggesting that, in absence of specific condition (e.g., motivation, expertise or training), sensitivity to other-group infant cues is reduced. Despite their several merits, in both studies the facial stimuli were not matched for attractiveness(see for example [19,20]), only females were considered (limiting the generalizability of the results), and more germane here the implicit responses considered could not assess response valence(see [13]).

The importance of adults' reactions to infant cues has been con-firmed in studies showing that adults' reactions to infant stimuli predict child outcomes [21–23]; for example, adults' emotional reactions to baby cues predict later attachment security [22].Senese et al. [13] adapted the Single Category Implicit AssociationTest (SC-IAT) to evaluate the valence of adults' implicit associations to infant cues. These authors found that human infant faces are implicitly and uniquely associated with a greater positive valence (apart from social desirability bias) and that individual differences in adults' implicit reactions correlated with their expressed desire to interact socially with infants. However, no study has compared the valence of adults' implicit reactions to infant faces of different ethnicities. Such a test would contribute to understanding the extent adults' responses to infant cues generalize or whether specificity of physiognomic characteristics of the face triggers sensitivity toward infants.

1.2. Genes and adults' responsiveness to infant cues

Even though molecular determinants of parenting have been studied to a far lesser extent, several studies have suggested that certain genotypes are implicated in caregiving (e.g., [24–27]; for a review see [24]) and adults' reactions to infants [26,28–30].Two prominent ones are oxytocin receptor gene polymorphisms,rs53576 (genotype: AA, AG, GG) and rs2254298 (genotype: GG, GT,TT); a third is the promoter region of the serotonin transporter gene5-HTTLPR (genotype: SS, SL, LL).

Oxytocin (OXT) is a hormone involved in moderating “social and non social functions in species ranging from nematodes to humans” [24] (p. 174). OXT receptor gene polymorphisms regulate attachment-related behaviours and social cognitions in humans[31,32]. Bakermans-Kranenburg and van I J zendoorn [26] showed that mothers carrying the GG genotype of the rs53576 (OXT) poly-morphism engaged in more sensitive interactions with their infants than mothers with the A-allele. A similar result was described by Klahr et al. [33], showing that, independent of child age and gender and participant ethnicity, mothers with GG genotype exhibited greater warmth during parent-

child interactions than A-allele carriers (no association between the genotype and parental behaviour was observed for fathers). However, Michalska et al. [30] found that A-carriers exhibited higher levels of positive parenting. GG carriers of the rs2254298 (OXT) polymorphisms showed less parental touch during parent-infant interactions than A-allele carriers [29]. Recently, Esposito et al. [34] showed an OXT gene \times environment (G \times E) interaction on adults' reactions to infant cues. The rs2254298 polymorphism moderated the effect of early care experiences (remembrances of parental bonding) on physiological responses (heart rate and facial skin temperature).

The serotonin transporter gene (5-HTTLPR) genotype regulates serotonin level. The S-allele type is less transcriptionally efficient because it is associated with lower reuptake of serotonin in respect to the L-allele type [35]. S-allele carriers show greater emotion-related brain reaction [36], and 5-HTTLPR moderates (G \times E) experience stressors on later emotional and social behaviour [35]. Canli et al. [37] showed that amygdala and hippocampus activation to face stimuli correlated negatively with life stress in S-allele carriers but positively in L-allele carriers, indicating that 5-HTTLPR polymorphism moderates the effect of life stress on brain responses but in an opposite manner. Bakermans-Kranenburg and van IJzendoorn [26] found that mothers with S-alleles were less sensitive than mothers with L-alleles, whereas Mileva-Seitz et al. [38] and Cents et al. [27] observed that mother scarrying the S-allele exhibited higher levels of sensitive parenting than those with L-alleles. Moreover, Mileva-Seitz et al. [38] reported that the 5-HTTLPR genotype moderates the impact of the early care environment on mothers' reactions to infants (G \times E). When mothers' gaze orientation (an implicit measure) was considered, mothers with L-alleles who reported more negative early cares showed a more negative response to their babies (oriented the gaze away from the infant), whereas when self-reported maternal feelings and attitudes (an explicit measure) were considered, mothers with the S-allele who reported more positive early cares showed a more positive responses (perceived attachment to their infant).

To our knowledge, no studies have investigated how adults' implicit responses to infant cues are influenced by adults' genetic characteristics or earlier experiences in their family of origin, and infant ethnicity. This question is relevant from both practical and theoretical perspectives. A better comprehension of the psychological and biological mechanisms that influence or moderate adult responsiveness to infants could help to prevent negative adult-infant interactions and validate parental models proposed into the literature (see [39,40]) by showing how genetics, early care experiences, and cultural factors interact to regulate adults' responses to infants.

1.3. Aims of the study

Guided by the foregoing considerations, the aim of the present work was to investigate how genetics, early care experiences, and cultural factors are

related to adults' implicit responsiveness to infants. We used a paradigm that is less biased than clinical assessments or self-reports, because it reduces the impact of social desirability bias [13,41]: the Single Category Implicit Association Test (SC-IAT). We evaluated (i) implicit preferences toward ingroup infant faces compared to out-group infant faces and in-group adult female faces; (ii) whether implicit associations to infant faces are related to genetic polymorphisms, early care experiences (parenting), or the interaction between genetic and early environmental experiences (genetic sensitivity); and (iii) if the latter interaction effect is influenced by infant ethnicity.

To these aims, we administered to Italian adults three versions of the SC-IAT paradigm, adapted to evaluate the valence of their implicit associations to Italian infant and adult (female) faces and Japanese infant faces, and the Adult Parental Acceptance Rejection Questionnaire, to evaluate participants' remembrance of parental rejection in childhood. Because attractiveness is a critical factor in the regulation of infant and adult face perception [19,20], we gave particular care to matching the stimuli for attractiveness. We also collected DNA samples to classify participants as a function of three target single nucleotide polymorphisms that have been associated to adult responsiveness to infants: rs53576 marker (OXT), rs2254298 marker (OXT), and 5-HTTLPR marker (serotonin transporter gene polymorphism). For this study, we recruited non-parent adults because we wanted to investigate implicit responses independent of parental status and experience. If infant faces are universally attractive and of high biological relevance, and trigger adults' positive responsiveness, their in-group preference should be attenuated in comparisons with their responses to in-group and out-group infants. If genetic factors influence processing of social stimuli, an association with adult implicit responsiveness toward infant and adult cues should emerge. Moreover, if genetic factors moderate the association between psychological stressors and social behaviours, a significant interaction between the genetic polymorphism and participants' early care environment ($G \times E$) on implicit associations should also emerge. Toward this end, data were analysed in an exploratory way to determine if they provide evidence for diathesis-stress [42] or differential susceptibility [43]. The diathesis-stress model hypothesizes that some genetic polymorphisms could be considered as risk factors only, that if present and associated to a negative environment they amplify the risk of a negative outcome (e.g., negative implicit association). The differential susceptibility model hypothesizes that genetic polymorphisms could be considered environmental sensitivity factors that, as a function of the environment, amplify both the risk of a negative outcome if associated to a negative environment and enhancement of successful adaptation (e.g., positive implicit association) if associated to a positive environment. To our knowledge, no study has directly investigated if gene \times environment ($G \times E$) interactions on adults' reactions to infant cues are expressions of the diathesis stress or the differential susceptibility interpretations. Finally, given that in-group individuals are perceived in a preferential way [4,7–9], we expected to find a stronger association between

the three considered genetic polymorphisms and implicit reactions to in-group faces compared to out-group faces.

2. Method

2.1. Participants

A total of 80 young adults (M age = 22.5 years, SD = 2.2, range:18–25 years), including 40 males and 40 females, matched for age, $F < 1$, participated; none was a parent or worked with infants. The sample size was determined by a preliminary power analysis to ensure adequate statistical validity (minimum power of 0.80 for each test; [44]). The study was approved by the local University Ethics Committee and conducted in conformity to the Helsinki Declaration. Participants were approached individually and asked to participate in a study on genetic, individual, and cultural factors in reactions to infant cues. A written informed consent was signed by each participant before starting the experiment, and participants were then conducted into the laboratory and assessed through behavioural tasks and questionnaires. The experimental session lasted about 30 min.

2.2. Measures

2.2.1. Self-report scales

A brief sociodemographic questionnaire was administered to participants to collect basic information on age, gender, and level of education. Participants also completed the mother and father short forms of the Adult PARQ [45,46] to obtain a measure of their remembrance of parental acceptance and rejection in childhood. The two forms consist of 24 items keyed as appropriate for mothers or fathers that measure the same four scales: (1) warmth/affection (e.g., 'My [mother/father] makes me feel wanted and needed.');

(2) hostility/aggression (e.g., 'My [mother/father] treated me harshly.');

(3) indifference/neglect (e.g., 'My [mother/father] paid no attention to me as long as I did nothing to bother [her/him].');

and (4) undifferentiated rejection (e.g., 'My [mother/father] saw me as a big nuisance.'). For each item participants indicated how well each statement described their remembrance of their parents' behaviour in childhood using a 4-point Likert scale (4 = almost always true to 1 = almost never true). All scales had an adequate reliability (α s > 0.75). Total scores of the two parents were summed to obtain a single measure of parental rejection.

2.2.2. Implicit reactions

Following Karpinski and Steinman [47] and Senese et al. [13], the Single Category Implicit Association Test (SC-IAT) was adapted to evaluate participants' implicit associations to three target stimuli: Italian infant and adult (female) faces and Japanese infant faces. Because we were interested in

implicit evaluation, to reduce any bias in the selection of the stimuli, infant faces were preliminarily matched for attractiveness [19,20], and confirmed in two ancillary studies, $F < 1$. Italian adult (female) faces were included as controls; Japanese adult female faces were not included because pilot studies showed that Italian and Japanese adult faces could not be matched on explicit evaluations of attractiveness.

The SC-IAT is a two-stage measure; in each stage, target word and faces were presented on a screen in random order. In each SC-IAT one target category was presented at a time. Participants were asked to classify words or faces into the correct category as quickly as possible. The same two response keys on the keyboard were used for both classifications: the “e” and the “i” keys, respectively, for left and right responses. To force participants to be fast and accurate, a response window of 1500 ms was set for each item. Words were distinguished as “positive” and “negative” and had to be classified into a positive or negative category, respectively. Words were chosen in a preliminary study because they were associated with infants or with adults. Faces were examples of each target category. In each SC-IAT we used ten positive words (e.g., love, beautiful, luck, joy, etc.), ten negative words (e.g., bad, pain, negative, unpleasant, etc.), and four pictures of each target category. For each target category, the classification task was repeated twice. In the first condition, positive words and target faces were categorized with the same response key, and negative words with a different key (positive condition). In the second condition, negative words and target faces were categorized with the same response key, and positive words with a different key (negative condition). In each SC-IAT, one target face (Italian infant faces, Italian adult female faces, or Japanese infant faces) was presented. The implicit attitude, also referred to as the IAT effect, is measured as the difference in the latencies of responses between the two conditions. The object-attribute pair with the faster responses is interpreted as more strongly associated in memory than the pair with slower responses. If participants are faster in categorizing stimuli in the positive condition compared to the negative condition, their score is positive, and they are considered to have positive implicit attitudes to the target stimuli. If participants are faster in categorizing stimuli in the negative condition compared to the positive condition, their score is negative, and they are considered to have negative implicit attitudes to the target stimuli. The IAT score is derived by dividing the difference between the latencies of responses in the negative condition and the positive condition by the standard deviation of latencies in the two conditions (D-score; [48]). Because responses are scored using the D-score algorithm [48], values around 0 indicate the absence of a specific implicit attitude to the target. Absolute values from 0.2 to 0.3 indicate a “slight” effect, values around 0.5 a “medium” effect, and values of about 0.8 to infinity a “large” effect. All the SC-IAT scores showed adequate reliability ($\alpha > 0.73$).

2.2.3. DNA preparation and genotyping

We collected DNA samples from all (N = 80) participants using buccal swab brushes. Genomic DNA was extracted from buccal epithelial cells using DNA purification kit QIA amp DNA Mini Kit (Qiagen Inc., Tokyo, Japan). The SNP markers rs2254298 (OXT) and rs53576 (OXT) were genotyped using Light-Cycler 480 Real-Time PCR Instrument (Roche Ltd). Polymerase chain reactions were performed in 10- μ L reaction volumes in 48-well plates and contained 10 ng of DNA. Thermal cycler conditions were 95°C for 10 min and then 40 cycles of 95°C for 15 s and 60°C for 1 min. For quality control, a random subset of the sample (about 10%) was reanalysed, and genotypes were concordant with initial results. Genotyping for the serotonin transporter polymorphism (5-HTTLPR) was amplified from 10 ng genomic DNA using the following primers: forward 5'-GGCGTTGCCGCTCTGAATGC-3' and reverse 5'-GAGGGACTGAGCTGGACAACCAC-3'. PCR was performed using KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan). Amplifications were performed on Gene Amp PCR System 9700 (Applied Biosystems, CA, USA) with 1 cycle at 94°C for 2 min followed by 35 cycles of 94°C for 10 s, 63°C for 30 s, 68°C for 30 s. PCR products were separated using electrophoresis in 3% agarose gel, allowing the differentiation of the short (S) allele (484 bp) and long (L) allele (528 bp). Genotype frequencies were as follows: AG = 23 (28.8%) and GG = 57 (71.2%) for the rs2254298 marker; AA = 7 (8.8%), AG = 39 (48.8%), and GG = 34 (42.4%) for the rs53576 marker; and SS = 20 (25%), SL = 36 (45%), LL = 24 (30%) for the 5-HTTLPR. As regards the rs53576 marker the G variant was treated as dominant (see [49]) creating two groups: AG/AA = 46 (57.5%) and GG = 34 (42.5%); as regards the 5-HTTLPR two groups were considered: SS/SL = 56 (70%) and LL = 24 (30%).

2.3. Data analysis

Preliminary descriptive analyses were executed to investigate missing values and variable distributions. Univariate distributions of observed variables were examined for normality [44].

To investigate if the valence of the implicit association was affected by the stimulus features, and to investigate if responses were moderated by the gender of participants, we executed a mixed factorial 3 \times 2 ANOVA that treated stimulus as a 3-level within-subjects factor (Italian infants, Italian adult females, and Japanese infants) and gender of participants as a 2-level between-subjects factor (males vs. females). Bonferroni correction was applied to analyse post hoc effects, and the magnitude of significant effects was indicated by partial eta squared (η^2_p).

To investigate if implicit reactions to infant faces were affected by parental experience, the genetic polymorphism, and the ir interaction, nine hierarchical multiple regression analyses were executed on the implicit responses to faces as a function of the stimulus (Italian infants, Italian adult females, and Japanese infants) and the genetic polymorphism (rs2254298, rs53576, and 5-HTTLPR). In all hierarchical regression models, we entered in the first block the genetic polymorphism (coded: AG/AA = 0 and GG = 1, GG = 0 and AG/AA

= 1, and SS/SL = 0 and LL = 1, for the rs53576, the rs2254298, and the 5-HTTLPR markers, respectively), in the second block remembrance of the early care experiences (PARQz-score), and in the third block the PARQ × Genetic polymorphism interaction. Because of the multiple hypothesis testing, to control the increasing of type I error, for each regression step, we applied the conservative FDR correction to the p-values of the regression coefficients [50]. When the moderation effect was significant, additional analyses were carried out, according to Roisman et al. [51] recommendations, to investigate if the effects supported a diathesis-stress [42] or a differential susceptibility [43] interpretation. The analyses were carried out by means of the online application available at <http://www.yourpersonality.net/interaction/>. Specifically, we computed (1) the regions of significance (RoS) with respect to the genetic polymorphism (moderator) and the remembrances of early care experiences (predictor) to represent the upper and lower bounds of values for moderator or predictor at which the regression of outcome on predictor or moderator was statistically significant; (2) the proportion of interaction index (PoI) to measure the proportion of the area represented in the plotted interaction graph attributable to the differential susceptibility interpretation only; (3) the proportion affected index (PA), that is an alternative to the PoI, to estimate the proportion of the population that is differentially affected by the moderator; and (4) non-linear effects to ascertain whether the differential susceptibility effect were an artefact of the imposed linear model. According to Roisman et al. [51], there is evidence of differential susceptibility (1) if the RoS on the predictor shows that “the association between the moderator and the outcome is significant at both the low and the high ends of the distribution of the independent variable within the normative range (i.e., ± 2 SD)” (p. 393); (2) if the PoI is between 0.40 and 0.60, with values approaching 1 suggesting strong evidence for differential susceptibility, whereas values approaching 0 suggest strong evidence for diathesis-stress; (3) if the PA is greater than 0.16, with values around 0.50 indicating strong evidence for differential susceptibility, whereas values below 0.02 provide strong evidence for diathesis-stress; and (4) if the multiple regression with the quadratic components showed that neither X^2 (predictor), ZX^2 (interaction between the predictor and the moderator), nor the combination of the two were statistically significant; or when any of these latter effects is significant, that ‘the XZ interaction term remains statistically significant even after controlling for the nonlinear terms’ (p. 398).

3. Results

3.1. Gender and cultural effect on implicit responses

The ANOVA executed on implicit associations revealed a significant main effect of stimulus (Italian infants, Italian adult females, and Japanese infants), $F(2, 156) = 7.72$, $p < 0.001$, $\eta^2_p = 0.090$. Gender of participants and Stimulus

× Gender of participants interaction effects were not significant, $F_s < 1$. Mean comparisons showed that Italian infants were evaluated as more positive, $M = 0.30$, than both Japanese infants and Italian adult females, $M = 0.13$ and $M = 0.10$, respectively (see Fig. 1).

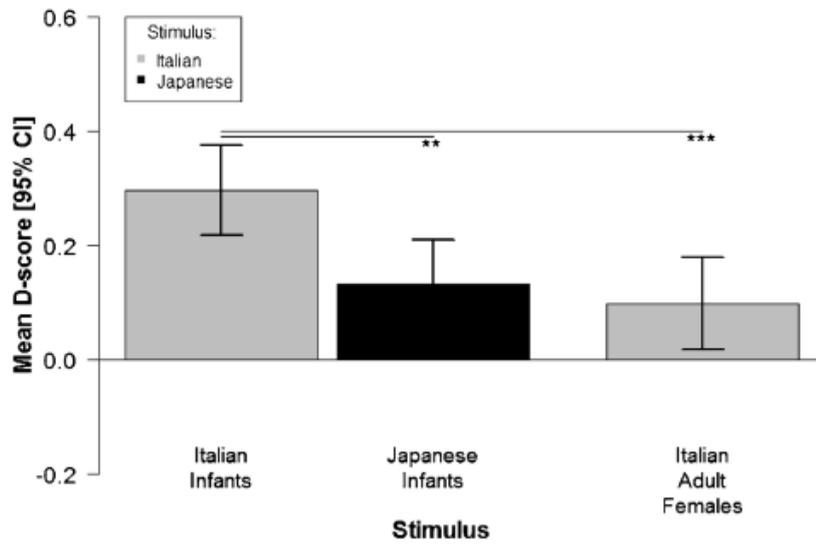


Fig. 1. Implicit associations (mean D-score) for each type of stimulus.

Note: ** $p < 0.01$; *** $p < 0.001$.

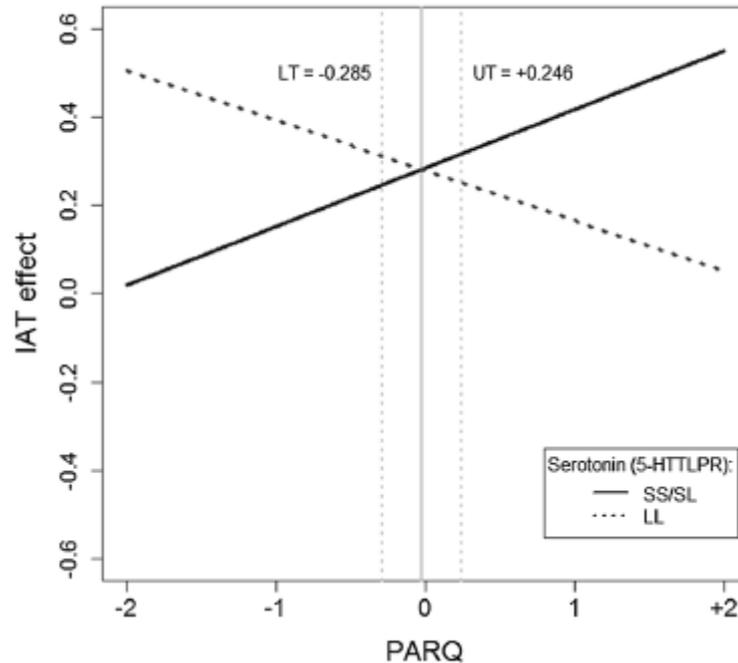


Fig. 2. Relations between early care quality (based on remembrance of maternal and paternal acceptance/rejection) and implicit reaction to Italian infant faces (IAT effect) as a function of the Serotonin polymorphism (5-HTTLPR: SS/SL, n= 56, and LL, n= 24).

Note: Dashed grey vertical bars indicate the Regions of Significance with respect to PARQ, RoS: lower threshold (LT) = -0.285, and upper threshold (UT) = 0.246 respectively; the solid grey line represents the crossover point on PARQ = -0.024; Proportion of the Interaction index (Pol) = 0.51; Proportion Affected (PA) index = 0.40.

3.2. Genetics, early care experiences, and cultural factors effect on implicit responses

3.2.1. Italian infant faces

Results from hierarchical regression analysis of implicit associations to Italian infant faces are reported in Table 1.

The rs53576 genetic polymorphism influenced the implicit association to infant faces, $R^2 = 0.070$, $p < 0.05$; this effect was also observed when controlling for the remembrance of parental rejection (PARQ score). The effects of remembered early care experiences (PARQ) and the PARQ \times Genetic polymorphism interaction were not significant. Parameter analysis of the genetic polymorphism main effect revealed that the participants with GG polymorphism had a more positive implicit association to Italian infant faces than participants with AG/AA polymorphism. The rs2254298 polymorphism analysis showed that, over and above genetic differences, remembrance of early care parental rejection (PARQ) positively influenced implicit associations to infant faces, $R^2_{diff} = 0.052$, $p < 0.05$. Therefore, negative early care experience was associated with an adult's implicit positive association to

infant cues in the own-group. The effects of the genetic polymorphism and the PARQ \times Genetic polymorphism interaction were not significant.

The 5-HTTLPR polymorphism results showed that, over and above genetic differences, remembrance of parental rejection positively influenced the implicit association to infant faces, $R^2_{diff} = 0.049$, $p < 0.05$, and this genetic polymorphism moderated the effect of parental rejection on the implicit association to infant faces, $R^2_{diff} = 0.077$, $p < 0.05$. Parameter analysis of the final model revealed that in the SS/SL group the remembrance of parental rejection was positively associated with implicit associations to Italian infant faces, whereas in the LL group the PARQ scores and IAT scores were negatively related. Therefore, in the S-carrier group a negative early care experience is associated with an adult's implicit positive association to infant cues of their own-group, whereas in the LL group a positive early care experience is associated with an implicit positive reaction to infant cues. In all cases, the expected valence of the implicit association falls on the positive side of the continuum (see Fig. 2). The interaction analysis showed that results support the differential susceptibility interpretation; both the upper and the lower regions of significance on PARQ (RoS on X) fell inside the recommended region $[-0.285, 0.246]$, both the Pol and the PA diagnostics were greater than 0.40, and neither X^2 , ZX^2 , nor the combination of both, was statistically significant.

3.2.2. Japanese infant faces

Results from hierarchical regression analysis of implicit associations to Japanese infant faces are reported in Table 2.

The rs53576 and rs2254298 polymorphisms analysis showed that implicit associations to Japanese infant faces did not relate to the genetic polymorphism, to parental experiences, or to their interaction.

Table 1

Hierarchical multiple regression analyses predicting implicit association to Italian infant faces from target genetic polymorphisms (rs53576, rs2254298, and 5-HTTLPR) and remembrance of parental rejection (N = 80).

| Predictor | Target polymorphism | | | | | |
|----------------------|----------------------|--------------------|------------------------|---------------------|-----------------------|----------------------|
| | rs53576 ^a | | rs2254298 ^b | | 5-HTTLPR ^c | |
| | ΔR^2 | Beta | ΔR^2 | Beta | ΔR^2 | Beta |
| Step 1 | 0.070 [*] | | 0.003 | | 0.000 | |
| GPoly | | 0.264 [*] | | 0.051 | | 0.005 |
| Step 2 | 0.032 | | 0.052 [*] | | 0.049 [*] | |
| GPoly | | 0.235 [*] | | 0.078 | | 0.030 |
| PARQ | | 0.128 | | 0.230 [*] | | 0.224 [*] |
| Step 3 | 0.000 | | 0.025 | | 0.077 [*] | |
| GPoly | | 0.234 [*] | | 0.052 | | -0.008 |
| PARQ | | 0.211 | | 0.308 ^{**} | | 0.368 ^{**} |
| PARQ × GPoly | | -0.036 | | -0.181 | | -0.318 ^{**} |
| Total R ² | 0.103 [*] | | 0.080 | | 0.127 [*] | |

Note: GPoly – genetic polymorphism; PARQ: composite index for the remembrance of maternal and paternal rejection.

^a AG/AA group was used as reference in the dummy coding.

^b GG group was used as reference in the dummy coding.

^c SS/SL group was used as reference in the dummy coding.

* $p < 0.05$.

** $p < 0.01$.

^{*} FDR corrected p -value < 0.05 .

Table 2

Hierarchical multiple regression analyses predicting implicit association to Japanese infant faces from target genetic polymorphisms (rs53576, rs2254298, and 5-HTTLPR) and remembrance of parental rejection (N = 80).

| Predictor | Target polymorphism | | | | | |
|----------------------|----------------------|--------|------------------------|-------|-----------------------|----------------------|
| | rs53576 ^a | | rs2254298 ^b | | 5-HTTLPR ^c | |
| | ΔR^2 | Beta | ΔR^2 | Beta | ΔR^2 | Beta |
| Step 1 | 0.018 | | 0.021 | | 0.124 ^{***} | |
| GPoly | | 0.136 | | 0.144 | | 0.352 ^{***} |
| Step 2 | 0.000 | | 0.003 | | 0.005 | |
| GPoly | | 0.134 | | 0.150 | | 0.360 ^{***} |
| PARQ | | 0.010 | | 0.050 | | 0.073 |
| Step 3 | 0.033 | | 0.007 | | 0.001 | |
| GPoly | | 0.130 | | 0.164 | | 0.363 ^{***} |
| PARQ | | 0.253 | | 0.008 | | 0.065 |
| PARQ × GPoly | | -0.303 | | 0.098 | | 0.018 |
| Total R ² | 0.051 | | 0.031 | | 0.130 [*] | |

Note: GPoly – genetic polymorphism; PARQ: composite index for the remembrance of maternal and paternal rejection.

^a AG/AA group was used as reference in the dummy coding.

^b GG group was used as reference in the dummy coding.

^c SS/SL group was used as reference in the dummy coding.

* $p < 0.05$.

*** $p < 0.001$.

^{*} FDR corrected p -value < 0.05 .

The results of the 5-HTTLPR polymorphism analysis showed that this genetic polymorphism influenced the implicit association to Japanese infant faces, $R^2 = 0.124$, $p < 0.001$, and this effect was also observed when controlling for remembrance of parental rejection. Participants with the LL polymorphism had a more positive implicit association to Japanese infant faces than participants with the SS/SL polymorphism. The PARQ × Genetic polymorphism interaction was not significant.

3.2.3. Italian adult female faces

Results from hierarchical regression analysis of implicit association to Italian adult female faces are reported in Table 3.

The rs53576 polymorphism analysis showed that, over and above genetic differences, remembrance of parental rejection negatively influenced the implicit association to in-group adult female faces and this latter effect was moderated by the genetic poly-morphism, $R^2_{diff} = 0.049$, $p < 0.05$ (see Table 3). Parameter analysis of the final model revealed that in the AG/AA group the

remembrance of parental rejection was negatively associated with implicit associations to Italian adult female faces, whereas in the GG group PARQ scores and IAT scores were not related. A negative early care experience correlated with an adult's implicit negative association to adult female cues of the own-group only in the A-carrier group, whereas in the GG group the implicit association to adult female cues was independent of early care experience. The interaction analysis seems to support the diathesis-stress interpretation, although interpretation is not unequivocal. Although both the upper and the lower regions of significance on PARQ (RoS on X) fell inside the recommended region [-1.578, -0.518], the Pol and the PA diagnostics were 0.12, and 0.15 respectively, the ZX2 interaction was statistically significant, and, when controlling for the non linear effect, the linear interaction was not statistically significant (see Fig. 3).

The rs2254298 polymorphism analysis showed that an implicit association to Italian adult female faces did not relate to the genetic polymorphism, to parental experiences, or to their interaction. The results of the 5-HTTLPR polymorphism analysis showed that, over and above genetic differences and remembrance of parental rejection, the genetic polymorphism moderated the effect of parental rejection on the implicit association to adult female faces, $R^2_{diff} = 0.077$, $p < 0.05$. Parameter analysis of the final model revealed that, in the SS/SL group, remembrance of parental rejection was not associated with IAT scores, whereas in the LL group PARQ scores were negatively related to implicit associations to Italian adult faces (see Fig. 4). A negative early care experience correlated with an adult's implicit negative association to adult female cues of the own-group only in the LL group, whereas in the S-carrier group the implicit association to adult female cues was independent of early care experience. The interaction analysis showed that results support the differential susceptibility interpretation, both the upper and the lower regions of significance on PARQ (RoS on X) fell inside the recommended region [-0.597, -0.062], both the Pol Fig and the PA diagnostics were greater than 0.35, and that neither X2, ZX2, nor their combination were statistically significant.

Table 3

Hierarchical multiple regression analyses predicting implicit association to Italian adult female faces from target genetic polymorphisms (rs53576, rs2254298, and 5HTTLPR) and remembrance of parental rejection (N = 80).

| Predictor | Target polymorphism | | | | | |
|----------------------|----------------------|----------------------|------------------------|--------|-----------------------|----------------------|
| | rs53576 ^a | | rs2254298 ^b | | 5-HTTLPR ^c | |
| | ΔR^2 | Beta | ΔR^2 | Beta | ΔR^2 | Beta |
| Step 1 | 0.034 | | 0.002 | | 0.002 | |
| GPoly | | 0.186 | | -0.038 | | -0.049 |
| Step 2 | 0.017 | | 0.010 | | 0.011 | |
| GPoly | | 0.207 | | -0.050 | | -0.061 |
| PARQ | | -0.130 | | -0.103 | | -0.104 |
| Step 3 | 0.049 [*] | | 0.003 | | 0.076 [*] | |
| GPoly | | 0.212 | | -0.059 | | -0.099 |
| PARQ | | -0.427 ^{*†} | | -0.076 | | 0.039 |
| PARQ × GPoly | | 0.369 [*] | | -0.062 | | -0.314 ^{*†} |
| Total R ² | 0.100 [*] | | 0.015 | | 0.089 | |

Note: GPoly = genetic polymorphism; PARQ: composite index for the remembrance of maternal and paternal rejection.

^a AG/AA group was used as reference in the dummy coding.

^b GG group was used as reference in the dummy coding.

^c SS/SL group was used as reference in the dummy coding.

^{*} $p < 0.05$.

[†] FDR corrected p -value < 0.05 .

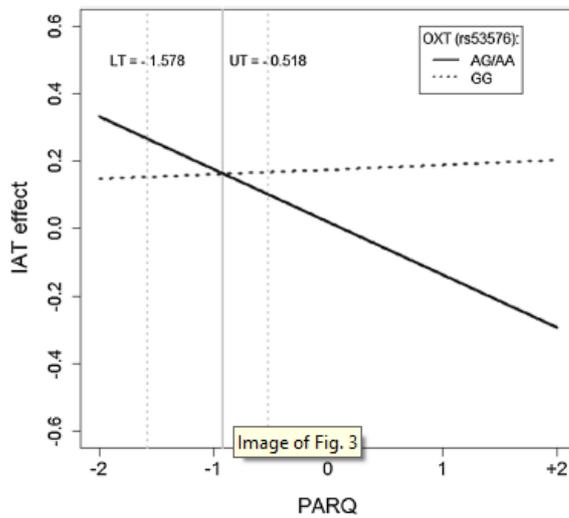


Fig. 3. Relations between early care quality (based on remembrance of maternal and paternal acceptance/rejection) and implicit reaction to Italian adult female faces (IAT effect) as a function of the Oxytocin polymorphism (rs53576; AG/AA, $n=46$, and GG, $n=34$).
 Note: Dashed grey vertical bars indicate the Regions of Significance with respect to PARQ, RoS: lower threshold (LT) -1.578 , and upper threshold (UT) -0.518 respectively; the solid grey line represents the crossover point on PARQ -0.918 ; Proportion of the Interaction index (Pol) 0.12 ; Proportion Affected (PA) index 0.15 .

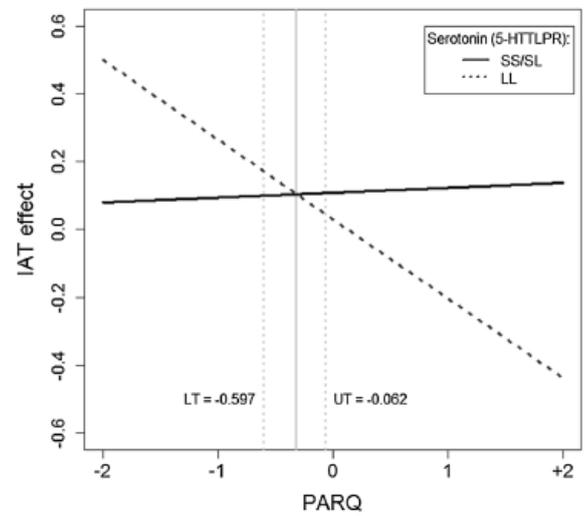


Fig. 4. Relations between early care quality (based on remembrance of maternal and paternal acceptance/rejection) and implicit reaction to Italian adult female faces (IAT effect) as a function of the Serotonin polymorphism (5-HTTLPR; SS/SL, $n=56$, and LL, $n=24$).
 Note: Dashed grey vertical bars indicate the Regions of Significance with respect to PARQ, RoS: lower threshold (LT) -0.597 , and upper threshold (UT) -0.062 respectively; the solid grey line represents the crossover point on PARQ -0.315 ; Proportion of the Interaction index (Pol) 0.35 ; Proportion Affected (PA) index 0.49 .

4. Discussion

The present work investigated the roles of genetics, early care experiences, and cultural factors on the valence of implicit preferences toward in-group and out-group faces, by means of a paradigm (SC-IAT) that reduces the social desirability bias and is sensitive to subtle cues [13]. To reduce any bias in the selection of the stimuli, own-group and other-group infant faces were also matched for explicit attractiveness [19,20]. As expected, results confirmed that infant faces were implicitly evaluated as more positive than adult faces [13]. As regards own-group bias, contrary to our expectation (see [17]), the implicit preference for own-group is also observed when comparing infant faces. That is, Italian infant faces were evaluated implicitly as more positive than Japanese infant faces. Therefore, similar to what has been observed in perception [4–6], it is plausible that different processes are triggered by the perception of infant cues of different ethnic group (see also [18]). That said, the infant face preference and the own-group preference for the Italian infant faces were not moderated by the gender of participants: Males and females showed similar reactions. This finding replicates previous studies [13,52] and further confirms that the valence of implicit associations to infant cues for males and females is similar. This finding also seems to suggest that gender differences in observed responses to infant cues relate to conscious or controlled thoughts and beliefs [13,52], such as sex-linked traits (e.g., prenatal

hormone exposure, self-reported interest in infants, individual differences, etc.; see also [53]), and not to the valence of the implicit associations.

A specific goal of the present research was to explore whether DNA polymorphic variation of Oxytocin and Serotonin coding were associated with implicit evaluation of social stimuli (faces) of one's own and other ethnic group, and if genetic factors moderated the association between early care experiences (parenting) and implicit responses to infant and adult social cues; that is, to directly test if a diathesis-stress [42] or differential susceptibility [43] effect was observed. As expected, results confirmed that Oxytocin and Serotonin are both related to adults' reactions to social stimuli, although with slight differences. Similar to Bakermans-Kranenburg and van IJzendoorn [26] and Klahr et al. [33], participants with GGrs53576 (OXT) polymorphism exhibited a more positive implicit association to Italian infant faces than A-allele carriers (but see [30] for a different pattern of results). Therefore, homozygotes for the G allele on rs53576 are expected to have more positive implicit association to an in-group child than A-allele carriers. Not significant were the association of the same target polymorphism and implicit responses to Japanese infant faces, whereas an OXT gene \times environment (G \times E) interaction effect was observed on adults' implicit association to Italian adult female faces only.

As regards the rs2254298 (OXT) polymorphisms, no main effector interaction was observed on responses to Italian infant and adult female faces or Japanese infant faces. This result did not replicate the G \times E effect observed by Esposito et al. [34]. One possible explanation could be that in that study the authors investigated adults' physiological responses to distressing social stimuli (cry), that are associated with negative implicit evaluations (see [52]), whereas here we considered neutral faces, that are associated with positive implicit evaluations (see also [13]). In other words, it is possible that the different OXT polymorphisms are related to specific social cues.

In accord with Bakermans-Kranenburg and van IJzendoorn [26], adults' implicit reaction to Japanese infant faces was influenced by the 5-HTTLPR, with S-allele carriers showing a less positive implicit reaction (but see [27], and [38], for a different result). Therefore, homozygotes for the L allele on 5-HTTLPR are expected to have more positive implicit association to out-group infants only than S-allele carriers. Not significant were the main effects of the 5-HTTLPR polymorphism on implicit responses to in-group Italian infant and adult faces, but, more germane here, a significant Gene \times Environment (G \times E) effect was observed on adults' implicit response to both in-group cues not to out-group ones.

In accord with the literature [14,24,31,32,39], the genetic effect on responsiveness to infant and adult cues was better specified by G \times E interaction effects. Results showed a significant moderation of the genetic polymorphism on the relation between early care experiences and implicit associations to Italian infant and adult faces. As expected, the genetic

polymorphisms sensitise the impact of early care environmental experiences on implicit responses to social stimuli of one's own ethnic group in different ways.

As regards the rs53576 (OXT), the G × E interaction showed that this genetic polymorphism moderates the relation between early care experiences and adults' implicit associations to Italian adult female faces only. A-allele carriers showed that a negative early care environment is associated with an adult's implicit negative response to adult female faces, whereas no Gene × Environment(G × E) interaction was observed for GG carriers. The moderation analysis showed that this effect is more consistent with the diathesis-stress hypothesis. In other words, in the presence of negative early parental experiences, A-allele carriers (rs53576 OXT) are more vulnerable (at risk) to a negative implicit association to adult female cues; therefore, they can be expected to show more negative attitudes and/or behaviours to in-group adult females.

This result confirms that Oxytocin plays a role in social-related behaviour [24,31,32] and the interaction between genetic and environmental rearing conditions [24]. The fact that the effect is observed for own-group female faces only provides further evidence that Oxytocin is susceptible to a combination of signals from organism, environment, and social group [24].

As regards the 5-HTTLPR (serotonin transporter), the G × E interaction showed that the OXT receptor gene polymorphisms moderates the relation between early care experiences and implicit associations to salient in-group cues in both Italian infant and adult females faces, although with some specific differentiations. In both cases, results showed that implicit responses of participants with the L homozygote (5-HTTLPR) were directly associated with the quality of the early care experiences, and that both effects were more consistent with the differential susceptibility hypothesis. This result implies that the 5-HTTLPR polymorphism represents an environmental sensitivity factor that, as a function of the environment, can amplify the risk of a negative outcome (negative implicit association to in-group cues) or enhance successful environmental adaptation (positive implicit association to in-group cues). In other words, in the absence of early stressful environments, the L homozygote seems to promote an advantage for a positive in-group interpersonal or caregiving relation, over the S-allele carriers, whereas in the presence of negative early care experiences the reverse pattern is expected. For implicit responses to in-group infant faces only, independent of the genetic polymorphism, (1) the expected implicit response tended to be mainly positive or neutral not negative and (2) the data showed that S-carriers were also sensitive to the environment albeit in the opposite direction: In the presence of negative early care experiences, S-carriers showed a positive implicit association to in-group infant faces. In other words, the presence of a compensatory positive implicit reaction was seen only for in-group infant cues for S-carriers who experienced a negative early care environment (parenting).

Overall, our results show that the association between the serotonin transporter gene (5-HTTLPR) polymorphisms and implicit social responses are consistent with the results of Mileva-Seitz et al. [38] who found that LL carriers experience beneficial effects when environmental contingencies are developmentally more positive and an implicit measure is considered. Our study seems to indicate, for the first time, that this effect is specific for in-group cues. Indeed, similar to what we observed for Oxytocin, the results indicated that a genetic sensitivity effect is observed only for in-group adult and infant faces. This result further confirms that the valence of implicit reactions to social cues is related to the genetic polymorphism, early care experiences, stimulus ethnicity and their interactions; and that different processes are triggered by the perception of cues of another ethnic group [4,7–9].

4.1. Limitations

Despite of the several merits of this study, including the application of the single category implicit association paradigm to evaluate whether in-group preferences extend to infant faces, our care matching the stimuli for attractiveness, and direct investigation of interactions between genetics, early care experiences, and cultural factors in the regulation of implicit responses to in- and out-groups social cues, some limitations should be acknowledged. We studied effects of in- and out-group social cues, but participants of only one culture were included. Future studies should replicate our findings by including participants from other cultures to discern if effects are cross-culturally generalizable. We recruited non-parent adults to investigate implicit responses independent of parental status and experience, but mothers and fathers and other groups, such as parents of at-risk infants, should be considered to investigate if parental experience influences implicit reactions or genetic effects. Indeed, given the specificity of the sample, the generalizability of findings to parents should be verified (but past study has own similar SC-IAT responses in parents and nonparents [13]) as the connection between implicit associations and the actual adult-infant behaviour should be further investigated.

Finally, the sample size was relatively small, but we obtained statistically significant effects, that were substantially confirmed even when the conservative FDR correction was applied. Future studies should replicate these findings.

4.2. Conclusions

In summary, the results of this study showed that own-group implicit preferences extend to infant faces, and in line with parental models proposed into the literature [14,39], that the valence of implicit associations to infant cues is related to genetics, early care experiences, cultural factors, and their interaction. As regards the molecular level, results confirm a genetic sensitivity in adults' response to social cues (see [24] for a review) and suggest that (1) genetics moderate the relation between early care experiences and the

valence of the implicit representation of infant and adult cues, although with some specific differentiations; (2) different polymorphisms have a differential relation with early care experiences; and (3) genetic sensitivity is specifically related to in-group social stimuli. Moreover, as regards in-group infant cues only, the results seem to point to a compensatory protective effect that (in the presence of negative early care experiences) is associated with positive implicit representations of infant cues. Further studies are needed to assess the robustness of this latter effect. If confirmed, this result would support the idea that infant faces represent a protective factor that inhibits an implicit negative response in adults, and it shed light on how genetics, early care experiences, and cultural factors interact to regulate adults' responses to infants. Finally, future studies should continue to investigate how genetic programming and the environment interact to regulate adults' responsiveness to infants and adults and whether it is possible to move these social interactions in a positive direction. For example, future research could investigate if information about early care experiences and genetic polymorphisms can be used to implement tailored interventions for at-risk adult-infant dyads to facilitate the expression of more adaptive adult-infant interactions.

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