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Institutionalization and indiscriminate social behavior: Differential-susceptibility versus diathesis-stress models for the *5-HTTLPR* and *BDNF* genotypes

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HIGHLIGHTS

- We use a GXE approach to understand indiscriminate attachment disordered.
- We focus on *5-HTTLPR* and *BDNF* in children reared in distinct relational contexts.
- We employed a confirmatory model-fitting strategy.
- A vulnerability-model for the s/s genotype emerged for institutionalized children.

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ABSTRACT

Institutionalization adversely impacts children's emotional functioning, proving related to attachment disorders, perhaps most notably that involving indiscriminate behavior, the subject of this report. In seeking to extend work in this area, this research on gene X environment (GXE) interplay investigated whether the serotonin transporter (*5-HTTLPR*) and val66met Brain-Derived Neurotrophic Factor (*BDNF*) polymorphisms moderated the effect of institutional care on indiscriminate behavior in preschoolers. Eighty-five institutionalized and 135 home-reared Portuguese children were assessed using Disturbances of Attachment Interview (DAI). GXE results indicated that s/s homozygotes of the *5-HTTLPR* gene displayed significantly higher levels of indiscriminate behavior than all other children if institutionalized, something not true of such children when family reared. These findings proved consistent with the diathesis-stress rather than differential-susceptibility model of person × environment interaction. *BDNF* proved unrelated to indiscriminate behavior. Results are discussed in relation to previous work on this subject of indiscriminate behavior, institutionalization and GXE interaction.

1. Introduction

Institutionalization of children at risk, whose parents, for various reasons, cannot guarantee sufficiently supportive care, remains a major intervention in many countries. In 2012, more than 8500 children younger than age 20 were living in residential institutions in Portugal, where the research reported herein was conducted. Of these, 13.9% were younger than age 5, with the majority (55.6%) spending more than one year in the institution [1].

Such early and extensive use of residential institutions occurs despite six decades of research compellingly documenting adverse impacts of such relational experience on children's development, particularly social-emotional functioning, including emotional/behavioral and attachment problems. Indeed, clinicians and researchers have repeatedly chronicled associations between institutional rearing and strikingly atypical attachment behavior that departs markedly from what is routinely observed in family-reared children (for review, see [2,3]). The absence of sensitive and responsive care, especially provided by consistent and reliable caregivers, is characteristic of these institutional settings and contributes to the emergence of attachment-

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disordered behavior (ADB), which is known to be associated with maladaptive developmental trajectories (for review see [4]).

Disinhibited social engagement disorder (DSED) is one frequently observed ADB among institutionalized children and is the focus of this report. It is characterized by a pattern of diffuse attachment, indiscriminate sociability, and by overfriendly attention, comfort seeking and affectionate behavior directed toward unfamiliar people [5] (for review see [2]). Even after several years of placement in adoptive families, a significant number of children who spent their early years in depriving orphanages continue to show mild to high levels of indiscriminate behavior [6].

Although strong empirical evidence showing that adverse contextual conditions – like institutionalization – promote attachment disorders, not all institutionalized children develop DSED. This calls attention to the need to consider non-institutional factors as contributors to the development of DSED. This is exactly the issue Drury and her colleagues [7] pursued upon raising the possibility that the child's genetic make-up, in interaction with institutionalization, might account for why some institutionalized children but not others develop DSED [7]. Indeed, their randomized control trial (RCT) revealed that children with two short alleles (s/s) of the serotonin transporter gene, *5-HTTLPR*, and, separately, with a met allele of the *BDNF* polymorphism, manifest the *most* indiscriminate friendliness of all children if they were institution reared, but the *least* if they were randomly assigned to high-quality foster care; indeed, effects of rearing condition in this investigation of Romanian children did not emerge for those with other genotypes. Such results were in line with the differential-susceptibility hypothesis, which stipulates that some children are more susceptible to environmental influences than others, "for better and for worse"; that is, while they are more likely than others to develop poorly under adverse conditions, they are also more likely to benefit developmentally from supportive ones [8–11].

As it turns out, the gene-X-environment (GXE) interaction results of Drury and collaborators [7] differ from those of a small-sample, observational study reported by Bakermans-Kranenburg and colleagues [12] who compared children raised in Ukrainian institutions with children raised in their biological families. Although carriers of the l/l genotype of *5-HTTLPR* proved less vulnerable to the adverse institutional environment when attachment disorganization was the outcome to be explained, no such GXE interaction emerged when observed indiscriminate behavior was the focus of inquiry. Even if RCT designs afford the most compelling tests of GXE interaction, given their ability to discount gene-environment correlation (rGE) [13,14], it remains unclear given the contrasting findings just cited, whether 5-HTTLPR moderates the effect of institutional rearing on indiscriminate behavior in the case of institutionalized children. Thus, we seek to extend current research on this topic.

Toward this end, we investigate the determinants of individual differences in indiscriminate friendliness among institutionalized children, employing a non-experimental design like that of Bakermans-Kranenburg and collaborators [12], one which involves the comparison of children growing up in Portuguese institutions with home-reared children, while taking into account their genetic make-up with regard to *5-HTTLPR* and *BDNF*. Because of the specific focus here on differential susceptibility, we employ the genotypic coding and comparisons used by Drury and collaborators [7], comparing children homozygous for short alleles with all other children in the case of *5-HTTLPR* and those with and without met alleles in the case of *BDNF* [7].

Although there is considerable GXE evidence, including meta-analytic work [15], that *5-HTTLPR* moderates diverse environmental effects in a differential-susceptibility-related manner in the case of children [9,10], it is also the case that some GXE evidence is more consistent with diathesis-stress thinking which conceptualizes s alleles as "vulnerability" or "risk" genes, predisposing individuals carrying them, perhaps especially homozygotes, to problematic functioning in dangerous, risky or otherwise harmful contexts [16,17]. Met-allele carriers of the *BDNF* gene have also been found to be especially vulnerable to adversity, consistent with the diathesis-stress framework, but, in some instances, to also benefit disproportionately from supportive conditions [18], consistent with the differential-susceptibility model of person-X-environment interaction [19]. No meta-analytic work of such GXE interaction has been carried out in the case of this polymorphism, however.

In the current inquiry, a core issue is whether children carrying putative susceptibility genes will prove (a) particularly vulnerable to adversity or (b) especially susceptible to the adverse effects of institutionalization *and* the presumed beneficial effects of family rearing vis-à-vis indiscriminate social behavior. Thus, we evaluate whether children homozygous for the *5-HTTLPR* short allele and/or whether those who carry the *BDNF* met allele (i) display the highest levels of indiscriminate behavior when institutionalized, but not when home reared, or (ii) are the most likely of all children to behave in an indiscriminate friendly fashion when institutionally reared, but engage in the least such behavior when family reared. Given this comparative focus, we employ the competitive model-fitting strategy of Widaman et al. [10,20]. It affords means of testing predictions derived from alternative theoretical frameworks — differential susceptibility vs. diathesis stress. Indeed, it enables evaluation and thus comparison of "weak" and "strong" versions of each model, the difference being that in the weak model the less susceptible group still proves somewhat susceptible, just less so than the more susceptible one, whereas in the strong version of each model only the hypothesized susceptible group proves susceptible. That is, in strong models the relation between predictor (i.e., institutionalization) and outcome (i.e., indiscriminate behavior) proves not significantly different from zero in the case of the predicted less susceptible group.

2. Method

2.1. Participants

Participants included 85 institutionalized children and 135 children living with their biological parents. All children were enrolled in two larger research projects on pre-school-age Portuguese children. Criteria for exclusion of participants were the existence of moderate to severe mental or physical impairments, genetic syndromes or autism spectrum disorders. None of the children had entered elementary school when the data for this report were collected; all were Caucasian.

2.1.1. Institutionally-reared children

Eighty-five institutionalized children (50 boys, 58.8%) were recruited from 23 Portuguese institutions, along with their institutional caregivers. Children were 36 to 77 months old ($M = 54.72$, $SD = 10.52$) at the time of assessment. Their age at admission to the institution varied from 3 to 69 months ($M = 34.98$; $SD = 16.59$), with 12% admitted by 12 months of age. The reasons for children being withdrawn from their families and placed in the institution were varied, including negligence, physical and sexual abuse, parental psychopathology and substance abuse, and severely limited socioeconomic resources. Thirteen children had been previously institutionalized and one had been placed in foster care. However, at time of admission to the participating institutions all children had been living with their biological families, with the exception of two children, who were living in another institution. Length of time in institutional care ranged from 6 to 56 months ($M = 19.41$, $SD = 12.69$), with 61.2% ($n = 52$) institutionalized for more than one year.

Sixty-four institutional caregivers also participated in the study. Fourteen (21.9%) of the 64 participating caregivers provided care for more than one child. In this study, the maximum number of children with the same assigned caregiver was 4. All caregivers were female, ranging in age from 21 to 59 years ($M = 37.47$, $SD = 10.87$). Thirty-nine caregivers (45.3%) did not receive specific training to perform

this job, and only 17 (26.6%) had fixed, as opposed to rotating, shifts. Twenty-eight caregivers (48.3%) did not complete high school, 16 (27.6%) had obtained a high school diploma, and the remainder (n = 14, 24.1%) had completed high school.

2.1.2. Family-reared children

One hundred and thirty-five family-reared children (65 boys, 48,1%), with no history of institutionalization, were recruited from preschools. At time of assessment, children were 40 to 76 months of age (M = 57.81, SD = 7.53). The majority came from two-parent families (n = 96, 56.3%). Mothers were also invited to participate in the research. Mothers had, on average, 33.58 years of age (SD = 5.56, range 21–48). Seventy-six (n = 56.3%) mothers had not completed high school, 24.4% (n = 33) of the mothers had obtained a high school diploma, and the remaining (n = 26, 19.3%) had graduated from college.

2.2. Procedure

Permission to conduct the larger investigations of which the current study is a part was provided by Portuguese National Commission for Data Protection, which is responsible for ensuring the ethical requirements in relation to human research carried out by Portuguese entities. The research project was also approved by the Portuguese Social Services. This agency is responsible for managing the institutions and is the legal guardian of children while they remain there. Regarding the institutionally-reared children, the plan for the study was initially presented to the staff of 23 participating institutions, all of which agreed to participate. Written informed consents were obtained from the biological parents and the institution director. The institutional caregiver of each participating child was identified based on staff interviews. Specifically, caregivers were selected by asking the staff who was the key staff member whom the child showed preference for and/or who knew the child best. Caregivers also provided written informed consent. All assessments were conducted at the institutional setting. Participants from the family-reared group were recruited from preschools. Parents were first explained the purposes of the study, as well as the detailed procedure, and written informed consents were then obtained. All assessments were carried out at the preschool in which the child was enrolled or at home, according to family availability.

2.3. Measures

2.3.1. Indiscriminate behavior

The *Disturbances of Attachment Interview* (DAI) [21], a semi-structured interview administered to the institutional caregiver (institutional-reared group) and mother (family-reared group), was used to assess the presence of signs of disordered attachment in the child. The DAI is composed by 12 items, each of which are coded 0 (*rarely or minimally*), 1 (*sometimes or somewhat*), or 2 (*clearly*), according to the amount of evidence of disturbed attachment behavior which the caregiver/mother provides. Interviews were audiotaped and subsequently scored by trained researchers. For the purpose of the present study, only the three items indicative of signs of indiscriminate social behavior were used – whether the child checked back with the caregiver/mother or tended to wander off without purpose; whether the child showed initial reticence around strangers or readily approached unfamiliar persons; and whether the child would readily go off with an unfamiliar adult – yielding a total score ranging from 0 to 6, with higher scores representing increasing signs of indiscriminate behavior. 53% (n = 116) of the interviews were rated by two independent coders, and discrepancies were resolved by conferencing, leading to a consensus for each item. Agreement was more than acceptable, yielding a kappa of .90.

2.3.2. Genotyping of 5-HTTLPR and val66met BDNF polymorphisms

Saliva samples were collected with Oragene DNA collection kits (DNA Genotek, Canada) and genomic DNA was isolated as instructed by the manufacturers, using the standard protocol from PrepIT L2P (DNA Genotek). Samples concentration was accessed using Nanodrop technology. For *BDNF* rs6265 analysis, 5 ng of DNA were used, along with the corresponding KASPar assay (LGC Genomics, UK), for a final volume of 8 μL. The thermal profile was performed as instructed be the manufacturers, in a 7500 Fast Real-Time PCR System (Applied Biosystems by Life Technology, USA). Results were validated by Sanger Sequencing of representative samples for each genotype (met/met, met/val or val/val). *5-HTTLPR* allele polymorphism analysis was performed by PCR with a final reaction volume of 20 μL (60 ng of DNA, 0.5 U Taq KAPA2G HotStart (KAPA Biosystems, USA), 1× Buffer A, 1× Enhancer 1, 0.2 mM dNTPs, 5% DMSO (Sigma, USA) and 0.4 μM of each primer: Fw 5′-TCCTCCGCTTTGGCGCCTCTTCC-3′ and Rv 5′-TGGG GGTTGCAGGGGAGATCCTG-3′ [22]. The thermal profile (Eppendorf, Germany) included an initial denaturation step of 3 min at 95 °C, followed by 25 cycles of 30 s at 95 °C, 20 s at the annealing temperature of 60.4 °C and 30 s at 72 °C. The amplification products were separated on a 3% agarose gel and visualized using Gel Doc EZ system (Bio-Rad, USA). Results were also validated using Sanger Sequencing of representative samples of each genotype (s/s, s/l and l/l).

Genotypes of both genes were in Hardy-Weinberg equilibrium. Regarding the home-reared group *5-HTTLPR* (s/s = 21; s/l = 68; l/l = 46), $p = 0.62$ and *BDNF* (met/met = 5; met/val = 56; val/val = 74), $p = 0.15$; and for the institutionalized group *5-HTTLPR* (s/s = 20; s/l = 36; l/l = 29), $p = 0.19$ and *BDNF* (met/met = 1; met/val = 32; val/val = 52), $p = 0.10$. Allelic frequency is consistent with published literature and NBCI database for these genes.

2.4. Date analysis plan

We employed SAS PROC NLIN and NLMIXED to fit all of the reparameterized models adapted from Widaman et al. [20]. The reparameterized model took the following form:

$$Y = B_0 + B_1(X_1 - C) + B_3((X_1 - C) \cdot X_2) + E. \quad (1)$$

Here Y represents the dependent variable, indiscriminate behavior, X_1 the environmental condition (i.e., family = 0, institutionalization = 1), X_2 the malleable allelic condition (i.e., *5-HTTLPR*: 1 = s/s, 0 = l-carriers; *BDNF*: 1 = met-carrier, 0 = val/val), and C the crossover point. Rewriting Eq. (1) leads to the following format with regression equations for different allelic groups separately represented:

$$Y = \begin{cases} X_2 = 0: & Y = B_0 + B_1(X_1 - C) + E \\ X_2 = 1: & Y = B_0 + B_2(X_1 - C) + E \end{cases} \quad (2)$$

where $B_2 = B_1 + B_2$ from Eq. (1). Essentially, Eqs. (1) and (2) are the same whereas in Eq. (2), B_0 represents the intercept and B_1 and B_2 the slopes for each allelic group.

The model presented in Eqs. (1) and (2) is consistent with what Widaman et al. [10,20] refers to as the "weak differential susceptibility" model (Model2 1w) where (a) the crossover point falls *within* the range of environmental measurement and (b) all allelic groups prove susceptible to environmental influence to some extent, (i.e., estimates of slopes all different from zero), though one is more so than the other. In contrast, by constraining $B_1 = 0$ in Eq. (2), the "strong differential susceptibility" model (Model 1s) posits that the association between environmental conditions and behavioral outcome is non-significant for the non-malleable allelic group, with the reverse being true for the malleable allelic group. "Weak (Model 2w) and strong (Model 2s) diathesis-stress models" differ in a similar way from each other, although the crossover point is fixed at the positive end of the

environment (i.e., family-reared condition). Based on the findings of Drury et al. [7], the strong differential-susceptibility model (Model 1s) reflects our a priori expectation and should hold for both *5-HTTLPR* and *BDNF*, with *5-HTTLPR* s/s individuals and/or *BDNF* met-allele carriers proving environmentally susceptible for better and for worse and non-s/s *5-HTTLPR* individuals and non-met carriers of *BDNF* proving not responsive to the environment at all, at least with regard to indiscriminate behavior.

To underscore the confirmatory nature of the current inquiry, all four models (i.e., Models 1w, 1s, 2w and 2s) were fitted and tested against each other by comparing representation of the data on the basis of variance accounted for (i.e., R^2) and Akaike and Bayesian information criteria (i.e., AIC and BIC, respectively). Models explaining more variance, hence better representing the data (i.e., higher R^2) are favored. Furthermore, smaller values of AIC and BIC indicate better model fit and are particularly useful when comparing non-nested models (e.g., Model 1s and Model 2w). Both AIC and BIC penalize for model complexity; therefore, adding unnecessary parameters result in increased AIC and BIC values. Additional statistical details can be found in [20,10]. Finally, we evaluated the effect sizes of the current GXE interactions by magnitude of differences using, Cohen's *d* [23] (http://www.campbellcollaboration.org/resources/effect_size_input.php).

3. Results

Frequencies of the individual genotypes for each rearing group and for the males and females are presented in Table 1. Tests revealed no significant differences in genotype frequency as a function of gender or rearing group (see Table 1).

Recall that we first fit the re-parameterized models stipulated in Eqs. (1) and (2) (see Table 2, Model 1w). Next we evaluated alternative models (upon setting different constraints) to determine whether the predicted interaction pattern – strong differential susceptibility (Model 1s) – best fit the data shown in Fig. 1. A summary of the results, including parameter estimates and tests of model fit (i.e., R^2, AIC and BIC), are presented in Table 2.

Regarding *5-HTTLPR*, (a) all four models explained significant variance in children's indiscriminate behavior, suggesting that all four models represent the data relatively well. (b) Compared to the weak differential susceptibility model (i.e., 1w), none of the other three models (i.e., 2w, 1s, 2s) accounted for significantly less variance. (c) Based on the parameter estimates of Model 1w in which all parameters are freely estimated, the slope reflecting the association between rearing condition and indiscriminate behavior proved significant for *5-HTTLPR* s/s homozygotes but was not different from zero in the case of other children (i.e., long allele carriers). (d) Although point estimates of the crossover point (i.e., C) in Model 1w falls in the range of the environmental measurement ($C = 0.30$, $SE = 0.19$), seemingly consistent with the differential-susceptibility pattern, the 95% confidence interval falls outside the possible range of the contextual measurement (i.e., lower bond $= -0.09$), thereby precluding differential susceptibility while supporting diathesis-stress. (e) AIC and BIC favored the strong diathesis stress model (i.e., Model 2s). Consequently, we conclude that the strong diathesis-stress model (i.e., Model 2s) fit the data best (i.e., based on the five points made above regarding explained variance, parameter estimates and AIC and BIC). Thus, children homozygous for the short allele displayed the most indiscriminate behavior when institutionally reared — but not the least when family reared; and no relation emerged between rearing experience and indiscriminate behavior in the case of long-allele carriers.

With respect to *BDNF*, none of the fitted models explained a significant amount of variance, indicating that none of the models represented the data well, although both parameter estimates, AIC and BIC, favored the weak diathesis-stress model (i.e., Model 2w).

Regarding the effect sizes, home- and institutionally reared children had a small but non-significant difference (Cohen's $d = 0.196$, 95% CI: [−0.08, 0.47]), with the latter manifesting slightly more indiscriminate behavior. Comparisons between individual genetic and environmental groups are presented in Table 3. Notably, among 5-HTTLPR s/s homozygotes, the difference between children exposed to different environmental conditions (i.e., institutionalized vs. home-reared) was large and significant ($d = 0.807$, 95% CI = [0.17, 1.44]), with the institutionalized (s/s) individuals exhibiting more indiscriminate behavior than (s/s) home-reared children. Importantly, this effect size was much greater than that between 5-HTTLPR l-carriers who experienced different rearing conditions. Furthermore, institutionalized children homozygous for the s allele proved more indiscriminately friendly than those carrying the l-allele, resulting in a large effect size ($d = 0.574$, 95% CI = [0.07, 1.08]).

4. Discussion

Much attention has been paid in recent years to the importance of replication in science in general [24,25] and in studies of gene-behavior relations in particular [26], perhaps most notably with regard to putative evidence of GXE interaction [27]. In the spirit of seeking to "conceptually" replicate prior results, particularly those of Drury and collaborators [7], we conducted a GXE study pertaining to the relation between institutionalization and indiscriminate social behavior using a model-fitting approach rather than an exploratory test of an interaction term within a traditional regression framework. This allowed us to directly contrast a differential susceptibility model with a diathesis-stress one of person-X-environment interaction.

Recall that results proved consistent with the (strong) diathesis-stress model, but only in the case of *5-HTTLPR*; no significant findings emerged for the *BDNF* gene. More specifically, children homozygous for the short *5-HTTLPR* allele displayed the most indiscriminate behavior when institutionally reared — but not the least when family reared; and in the case of those carrying long alleles, rearing experience proved unrelated to the behavioral outcome that is the focus of this report. In consequence, our results failed to fully reproduce Drury et al.'s [7] differential-susceptibility-related findings in the case of the *5-HTTLPR* s/s genotype. Nevertheless, the data presented herein proved consistent with those of Drury et al.'s [7] in showing that s-homozygotes were most susceptible to adversity, manifesting the most indiscriminate friendliness of all children when reared in an institutional setting. Such a diathesis-stress-like pattern has emerged in other research focused on individuals homozygous for the s allele and living under adverse contextual conditions (e.g., many stressful life events) [16,17].

Table 1
Distribution of genetic-variant subgroups as a function of rearing group and gender (n, %).

	5-HTTLPR			*BDNF*		
Genotypes	s/s	l/*	*p* (X^2)	met/*	val/val	*p* (X^2)
Group			.14 (2.19)			.35 (.86)
Family-reared children (n = 135)	21 (16%)	114 (84%)		61 (45%)	74 (55%)	
Institutionalized children (n = 85)	20 (24%)	65 (76%)		33 (39%)	52 (61%)	
Sex			.06 (3.54)			.29 (1.11)
Female (n = 105)	25 (24%)	80 (76%)		41 (39%)	64 (61%)	
Male (n = 115)	16 (14%)	99 (86%)		53 (46%)	62 (34%)	

Table 2
Results of the alternate regression models for *5-HTTLPR* and *BDNF* (N = 220).

Parameters	*5-HTTLPR*				*BDNF*			
	Differential susceptibility estimates (SE) 95% CI		Diathesis stress estimates (SE) 95% CI		Differential susceptibility estimates (SE) 95% CI		Diathesis stress estimates (SE) 95% CI	
	Model 1w	Model 1s	Model 2w	Model 2s	Model 1w	Model 1s	Model 2w	Model 2s
B_0	0.94 (0.11) [0.72, 1.17]	0.94 (0.11) [0.72, 1.17]	0.88 (0.13) [0.62, 1.14]	0.90 (0.11) [0.69, 1.11]	0.38 (1.54) [−2.65, 3.42]	1.18 (0.14) [0.91, 1.45]	0.88 (0.13) [0.62, 1.14]	1.02 (0.11) [0.80, 1.24]
B_1	−0.01 (0.24) [−0.47, 0.46]	0.00 (−) [−]	0.06 (0.23) [−0.40, 0.51]	0.00 (−) [−]	0.34 (0.28) [−0.20, 0.89]	0.00 (−) [−]	0.50 (0.25) [0.01, 1.00]	0.00 (−) [−]
C	0.30 (0.19) [−0.09, 0.68]	0.29 (0.19) [−0.09, 0.68]	0.00 (−) [−]	0.00 (−) [−]	−1.92 (5.48) [−12.72, 8.90]	3.09(5.82) [−8.38, 14.56]	0.00 (−) [−]	0.00 (−) [−]
B_2	1.43 (0.47) [0.49, 2.36]	1.43 (0.47) [0.49, 2.36]	1.07 (0.36) [0.35, 1.79]	1.05 (0.36) [0.35, 1.75]	0.16 (0.33) [−0.49, 0.81]	0.16 (0.33) [−0.49, 0.81]	−0.03 (0.30) [−0.62, 0.55]	−0.17 (0.29) [−0.75, 0.40]
R^2	0.045	0.045	0.039	0.038	0.028	0.021	0.010	0.002
F	3.38	5.09	4.37	8.72	2.06	2.31	2.20	0.35
df	3,216	2,217	2,217	1,218	3,216	2,217	2,217	1,218
p	0.02	0.007	0.01	0.004	0.11	0.10	0.11	0.55
F vs. 1w	–	0.001	1.38	0.72	–	1.54	1.77	2.91
df	–	1,216	1,216	2,216	–	1,216	1,216	2,216
p	–	0.97	0.24	0.49	–	0.22	0.19	0.06
F vs. 1s	0.001	–	–	1.45	1.54	–	–	4.27
df	1,216	–	–	1,217	1,216	–	–	1,217
p	0.97	–	–	0.23	0.22	–	–	0.04*
AIC	813.9	811.9	813.3	**811.4**	817.8	817.4	817.6	819.6
BIC	830.9	825.5	826.9	**821.5**	834.8	830.9	831.2	829.8

Note. 95% CI: 95% confidence interval. SE: standard error. Parameters fixed at reported value has no applicable SE and 95% CI, therefore were represented as "–" in the table. AIC, Akaike information criterion; BIC, Bayesian information criterion. Model 1w/1s: the weak and strong differential-susceptibility model, respectively. Model 2w/2s: the weak and strong diathesis-stress model, respectively. F vs. 1w stands for an F test of the difference in R^2 for a given model versus Model 1w. Smallest AIC and BIC values were bolded and thus indicated the model that was selected.

The fact that Drury's study [7] was experimental in design, involving the random assigned of institutionalized children to different rearing conditions, whereas ours is observational, contrasting home- and institutionally reared children, could well account for our inability to reproduce her team's results. Indeed, it was this fundamental design difference that led us to consider our effort to be a "conceptual" rather than exact replication: Even though we addressed the same GXE interactions, we were doing so with a distinctly different research design. Given this, it would be mistaken to regard the results reported herein as a "failure to replicate".

What must be appreciated as well is that in Drury's RCT [7] not only were children randomly assigned to rearing condition, but the rearing condition which served as the contrast to institutionalization involved *high-quality* foster care. In the present, quasi-experimental effort, comparison children were raised by their biological parents — and thus were not selected because their parents provided high-quality care or were trained to do so, as foster parents were in the Drury work. Thus, we cannot disregard the possibility that these differences in family-rearing conditions are responsible for the differences in results, which emerged across investigations.

Also important to consider when contrasting findings from the two GXE studies of institutionalization is that the current research was conducted in Portugal, whereas the Drury et al. work was carried out in Romania. What makes this notable is that there are empirical grounds for regarding the quality of institutional care different in these two settings [28,29]. According to Gunnar's classification of quality of institutional rearing [30], Portuguese institutions perform at Level 2 because they meet children's nutrition and health needs, even if failing to do so with regard to cognitive stimulation, social-relational support and emotional care; or even as Level 3, as some of the institutions do provide sufficient support for healthy development, except with regard to the provision of a consistent and stable relationship of children with specific caregivers. Romanian institutions are classified as Level 1 [30], reflecting a lower quality of care.

Regarding the non-findings in the case of *BDNF*, it is not entirely clear why we failed to fully reproduce Drury et al.'s [7] findings, though all the

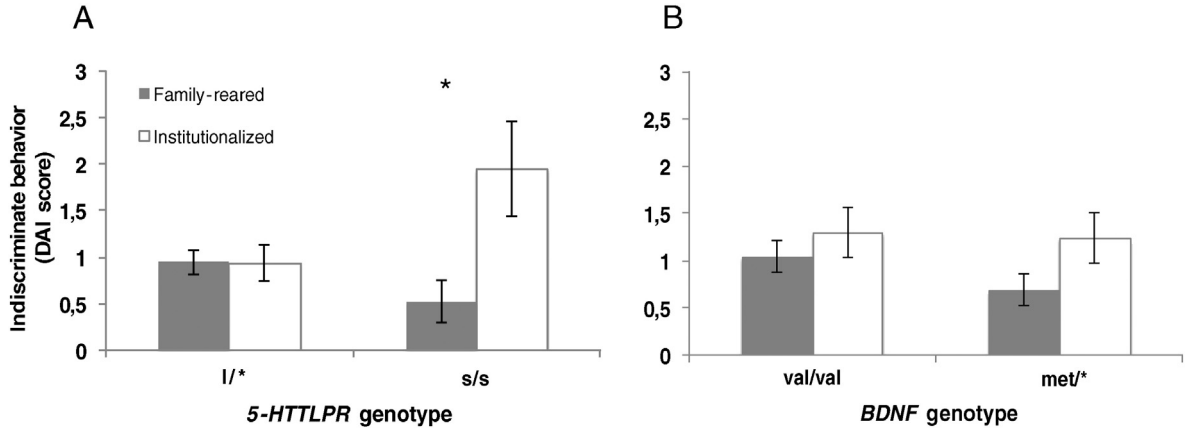

Fig. 1. Children's indiscriminate behavior by group (family-reared with biological parents and institutionalized children) and by genotype for the *5-HTTLPR* (A) and *BDNF* (B).

Table 3
Sample size, means, standard deviations and effect size (Cohen's *d*) comparisons of indiscriminate behavior by 5-HTTLPR, BDNF genotype and institutionalization exposure (N = 220).

		5-HTTLPR			BDNF		
		s/s	l/*		met/*	val/val	
		Mean (SD) N		Cohen's *d* 95% CI	Mean (SD) N		Cohen's *d* 95% CI
Indiscriminate behavior	Community children (n = 135)	0.52(1.03) n = 21	0.95(1.39) n = 114	*d* = 0.320 [−0.15, 0.79]	0.69(1.26) n = 61	1.04(1.41) n = 74	*d* = 0.260 [−0.08, 0.60]
	Institutionalized children (n = 85)	1.95(2.31) n = 20	0.94(1.56) n = 65	*d* = 0.574 [0.07, 1.08]	0.85(1.58) n = 33	1.38(1.91) n = 52	*d* = 0.296 [−0.14, 0.73]
	Cohen's *d* 95% CI	*d* = 0.807 [0.17, 1.44]	*d* = 0.007 [−0.30, 0.31]		*d* = 0.116 [−0.31, 0.54]	*d* = 0.208 [−0.15, 0.56]	

Note: SD: standard deviation. 95% CI: 95% confidence interval. Cohen's $d = \frac{\overline{X}_1 - \overline{X}_2}{SD_{pooled}}$ and $SD_{pooled} = \sqrt{\frac{(n_1-1)SD_1^2+(n_2-1)SD_2^2}{n_1+n_2-2}}$. $\overline{X}_{1/2}$: mean of group 1/2. SD_{pooled}: pooled standard deviation. $SD_{1/2}$: standard deviation of group 1/2.

just delineated across-study differences merit consideration. So too, though, may be differences in the length of institutionalization across studies. Whereas in the Drury et al. inquiry, children averaged a full two years of institutional rearing, in the current work it was almost a third less, 19 months. It could be the case then, that with greater exposure to institutional care, the indiscriminate behavior of Portuguese children would reflect the same GXE interaction as discerned in the Romanian investigation. Seemingly consistent with this line of reasoning are Gunnar and colleagues' [19] recent findings documenting a GXE interaction between length of institutionalization and the presence of the met allele in youth adopted from orphanages. Notably, adopted children carrying at least one copy of the met allele had the *fewest* attention problems when adoption occurred early in life but the most when adopted later, clearly indicating that length of institutional exposure is an important consideration when investigating the moderating effect of *BDNF*.

The differences outlined here between the current and the Drury et al. investigation could also account for why we did not reproduce the GXGXE interaction that the Romanian study revealed when considering both *5-HTTLPR* and *BDNF*. So, too, could limitations in statistical power, due to limited sample size, when seeking to reproduce a three-way interaction.

Whatever the similarity and differences in findings and research design across the two studies, it is clear that the neurobiological underpinnings of indiscriminate behavior deserve further investigation. Nevertheless, it seems noteworthy that not only has being homozygous for the *5-HTTLPR s* allele been linked in both investigations with increased susceptibility to the adverse effects of institutional rearing on indiscriminate behavior, but that it also has been associated with the hyperactivity and impulsivity dimensions of Attention Deficit/Hyperactivity Disorder (ADHD) [31]. Though differences should be acknowledged regarding the phenotypic expression of both ADHD and indiscriminate friendliness, the possibility arises in light of the observations just made regarding children homozygous for the s allele that these two types of disordered functioning, which both seem to reflect limited inhibitory control, could be related [2]. And this raises the obvious question of whether the emergence of indiscriminate friendliness presages that of ADHD, something only future longitudinal research will be able to address.

Whatever the strengths of this study – most notably a clear analytic strategy for contrasting the two conceptual models of person × environment interaction while seeking to conceptually replicate Drury and colleagues [7] research – it is not without limitations. First, the indiscriminate behavior score was based on different informants for the two groups of children, caregivers for the institutionalized children and mothers for the family-reared children. Of note in this regard is that whereas caregivers may be exposed to and thus familiar with notable variation in such behavior, most parents, even if rearing more than one child, are unlikely to have similar experience. Second, the present study was *quasi*-experimental in design, so even though children in the two groups were matched on age and gender, such a design does not afford the degree of similarity across groups that is typical of an RCT. Such considerations mean that caution is called for in interpreting the results of this inquiry, especially when comparing with other studies with different designs. To repeat, then, our failure to reproduce all the findings discerned in Drury et al.'s RCT should not be regarded, at least in the strictest sense, as a "failure to replicate". In this regard, it is worth recalling the scientific adage that "the absence of evidence (in the current inquiry supporting the differential susceptibility model of person × environment interaction) is not evidence of absence".

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