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Authors

Baptista, Joana
Belsky, Jay
Mesquita, Ana
et al.

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ORIGINAL CONTRIBUTION

Serotonin transporter polymorphism moderates the effects of caregiver intrusiveness on ADHD symptoms among institutionalized preschoolers

Joana Baptista[1] · Jay Belsky[2] · Ana Mesquita[1] · Isabel Soares[1]

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Abstract Research consistently chronicles a variety of mental health difficulties that plague institutionally reared children, including attention-deficit/hyperactivity disorder (ADHD), even if not all institutionalized children evince such problems. In seeking to extend work in this area, this research on gene × environment (GXE) interplay investigated whether the effect of the quality of institutional care—most notably, caregiver intrusiveness—on ADHD symptoms is moderated by the serotonin transporter (*5-HTTLPR*) polymorphism. One hundred and twenty-seven institutionalized preschoolers were evaluated using the Child Behavior Checklist. Caregiver-rated attention problems and hyperactivity were unrelated to both *5-HTTLPR* polymorphism and caregiver intrusiveness. A significant GXE effect, independent of age at placement or duration of institutionalization, emerged, however, consistent with the differential-susceptibility hypothesis: s/s homozygotes manifest the most and least ADHD symptoms when they experienced, respectively, more and less intrusive caregiving. These results provide new insight into the reasons why some institutionalized children, but not others, exhibit ADHD symptoms.

Keywords Institutionalized children · ADHD · Caregiver intrusiveness · Serotonin transporter polymorphism · Differential susceptibility

✉ Isabel Soares
isoares@psi.uminho.pt

[1] Psychology Research Center-CIPsi, School of Psychology, University of Minho, Campus de Gualtar, 4710-050 Braga, Portugal

[2] University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

Introduction

For many countries worldwide, institutional care remains a widely—and over—used form of alternative care for young children who, for various reasons, are living without their parents [1]. In Portugal, where the research reported herein was conducted, around 8500 children under the age of 18 were living in residential institutions in 2013, with the majority spending more than 1 year in such a placement. Of those, almost 13 % were younger than age 5 [2]. Despite the best intentions of such institutions, conditions within these facilities are often marked by limited quality of care, as they are usually characterized by unfavorable caregiver-to-child ratios, limited physical conditions, regimented daily schedules, rotating caregiving shifts, and unresponsive caregiving practices [3].

Not surprisingly, children with a history of institutional rearing are at heightened risk for a variety of mental health problems, including attention-deficit/hyperactivity disorder (ADHD), whose symptoms are the focus of this report. In fact, recent work indicates that institution-reared children, relative to their family-raised peers, have a higher incidence of ADHD symptoms of inattention and hyperactivity [4–6], with problems persisting years after they have departed the institution, often due to adoption [7–10]. Such high prevalence and resistance to intervention led some to argue that these difficulties represent a persistent impairment and constitute a specific deprivation syndrome associated with institutional rearing [11, 12].

Studies of post-institutionalized international adoptees indicate that the duration of institutional deprivation influences the risk of developing attention problems and hyperactivity. Particularly relevant are the findings from the English and Romanian Adoptees [ERA] project, a prospective longitudinal study investigating children from Romanian

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institutions of the Ceauşescu regime adopted by UK families. It found that children aged 6 and 11 years who had spent more than 6 months in an institution before being adopted exhibited more ADHD symptoms than those who had experienced fewer months of institutional deprivation [11].

Despite such disconcerting evidence documenting the link between duration of exposure to institutional rearing and ADHD symptoms, the fact remains that there is considerable heterogeneity in response to early deprivation. Thus, not all children exposed to institutional care, even for 6 months or more, evince signs of attention problems and hyperactivity [8, 9]. This certainly calls attention to the need to consider non-institutional factors as contributors to the development of attention problems and hyperactivity. It may well be the case that child-specific factors, including genetic ones, could account for why some institutionalized children, but not others, are more likely to develop ADHD. Indeed, this may be especially so when genetic variation across children is considered in the context of their institutional experiences. This is the issue empirically addressed herein.

Genetics and ADHD

There is considerable evidence that genetic factors are important in the etiology of ADHD [13, 14]. Like most complex disorders, ADHD is presumed to have a polygenic etiology. Indeed, genes associated with monoamine neurotransmission have been implicated in its pathogenesis. Although significant research has focused on associations between inattention and hyperactivity symptom phenotypes and polymorphisms in genes such as dopamine receptors [15] or dopamine transporter [16], recent interest has been directed at the potential role of the serotonin transporter length polymorphism present in the promoter region of the gene that codes for the serotonin transporter (*5-HTTLPR*), at least in the case of home-reared children. This is one of two reasons why we focus on this polymorphism in the current inquiry.

The *5-HTTLPR* is a degenerate repeat polymorphic region in the SLC6A4, the gene that codes for the serotonin transporter, which comprises a short (s) and a long (l) allele. The short allelic variant has been linked to a lower transcriptional rate of the gene and diminished functional capacity of serotonin transporter protein, resulting in reduced serotonin reuptake—and consequently higher serotonin levels—in the synaptic cleft [17]. Considering the functional relevance of this polymorphism, it has been studied as a risk marker across different psychiatric disorders, including major depression and obsessive–compulsive disorder, and thus may be particularly relevant for the present inquiry focused on ADHD (for a review, see [18]). In fact, a number of studies document associations between the *5-HTTLPR* genotype and symptoms of inattention, with evidence indicating that children with at least one s-allele, and particularly those homozygous for this allele, evince elevated levels of ADHD symptoms [19–21]. Having said that, it remains true that not all relevant genotype–phenotype association studies chronicle such links [22].

5-HTTLPR and sensitivity to the environment

It is also of interest that mounting evidence indicates that variation in *5-HTTLPR* is associated with sensitivity and responsiveness to environmental stress exposure, the second reason for focusing on this polymorphism in this gene-by-environment (GXE) inquiry [23, 24]. For instance, in a study carried out with 184 delinquents, Retz and colleagues [25], using a retrospective assessment of childhood ADHD, as well as of early adverse family environment, detected a significant GXE interaction: carriers of the *5-HTTLPR* s-allele evinced more and less persistent ADHD than non-carriers, depending on whether they were exposed to, respectively, an adverse family environment or not.

Even more directly pertinent to the research reported herein are results of other GXE studies showing that the *5-HTTLPR* polymorphism moderates the effects of early institutional deprivation on a variety of mental health outcomes. For example, Kumsta and colleagues [26] observed that *s* homozygotes who spent between 6 and 42 months after birth in a Romanian orphanage before being adopted into UK families, and who had experienced many stressful life events between ages 11 and 15, evinced the greatest increases in emotional problems over this 4-year period. Relatedly, findings from the Bucharest Early Intervention Project (BEIP) indicate that the *5-HTTLPR* s/s homozygotes manifest the most indiscriminate social behavior when they remained institutionalized, whereas their genetic counterparts randomly assigned to high-quality foster care manifest the least such behavior relative to all other children [27]. Such findings are consistent with the differential-susceptibility hypothesis, stipulating that certain individuals, for genetic or other organismic reasons, are more susceptible to environmental influences for better or worse [28–31]. Similarly, Brett and colleagues [32], also analyzing BEIP longitudinal data, reported that at 54 months of age, children with the s/s genotype of the *5-HTTLPR* living in Romanian institutions had the highest levels of externalizing behavior, whereas s/s children assigned to foster care showed the lowest levels.

Current study

Here, we seek to extend such GXE interaction research involving *5-HTTLPR* and institutional care—in several

ways. First, and for reasons already outlined in discussing *5-HTTLPR*, our target of prediction is ADHD symptoms. Second, rather than focusing on the length of institutionalization or random assignment to high-quality foster care, we consider variation in the quality of care experienced by children within the institution, focusing on dynamic, interpersonal characteristics of the caregiving environment. This is because prior research on institutional care highlights the significance of the quality of caregivers' interactive behavior with the child. Consider in this regard Smyke and collaborators' [33] evidence that poorer-quality caregiving was related to more negative behavior among 5- to 31-month olds residing in institutions, even after taking into account child gender and length of institutionalization. Consider, too, Oliveira and colleagues' [34] work showing that institutionalized preschoolers who experience more sensitive caregiving evinced less indiscriminate social behavior than their counterparts who experienced poorer-quality care.

Although there is supportive evidence showing that ADHD is among the most heritable neuropsychiatric disorders with limited environmental influences [35], relevant cross-sectional and longitudinal findings have linked intrusive caregiving with the development of attention problems and hyperactivity, at least in the case of non-institutionalized children [36–39]. For instance, Keown [40] found that more intrusive parenting behavior at age four predicted more ADHD symptoms in home-reared children; and Harold and colleagues [41], in a study with adopted children, reported that maternal negative behavior, which included intrusive parenting, was significantly linked to more ADHD symptoms at age 6. Also important to consider is GXE evidence that home-reared children homozygous for the *5-HTTLPR* s allele have more attentional deficits when exposed to more negative parenting behavior, including intrusive behavior, than other children [42, 43]. Here, we extend such research by addressing similar dynamic caregiving processes—in interaction with *5-HTTLPR*—when predicting ADHD symptoms among children being reared in Portuguese institutions.

We hypothesized that caregiver intrusiveness would be related to increased ADHD symptoms in children still residing in institutions. We further predicted that this association would be especially pronounced and perhaps even exclusively evident among children homozygous for the *5-HTTLPR s*-allele. Finally, we asked whether this anticipated GXE interaction would emerge after accounting for age at placement into the institution and length of time in institutional care, as well as whether it would take the for-better-and-for-worse form of differential susceptibility [28, 44], with *5-HTTLPR* s/s carriers being especially sensitive to both less (i.e., intrusive caregiving) and more (i.e., non-intrusive caregiving) supportive environmental conditions. Alternatively, it could prove more consistent with the traditional diathesis-stress model which stipulates only that those carrying risk alleles will be more vulnerable to adversity (i.e., intrusive caregiving), not that those carrying these (plasticity) alleles will also benefit more from supportive (i.e., non-intrusive) care.

Method

Participants

One hundred and twenty-seven children (74 boys, 58.3 %), placed in 29 Portuguese temporary care centers, participated in the present study. These institutions receive children abandoned or removed from their biological families, due to various reasons considered to endanger young children's physical and/or emotional well-being, such as abuse, neglect, or extreme economic hardship. These institutions are characterized by adequate physical resources, including medical care and nutrition [45], but simultaneously by high variability in the quality of their psychosocial care, including high child-to-caregiver ratios and frequent changes in caregivers over time [46]. By the time of assessment, children were 36–77 months old ($M = 54.67$, SD = 10.68). None of the children had entered elementary school at the time this research was conducted. The age at admission to the institution varied from 3 to 69 months ($M = 36.54$ months, SD = 15.95). Twenty-eight children had been previously institutionalized and one had been placed in foster care. However, at the time of admission to the institutions, all children had been living with their biological families, with the exception of three living in another institution. The reasons for children being withdrawn from their families and placed in the institution were varied, including negligence, abuse, parental psychopathology or intellectual disability, and extreme economic hardship. The length of time in institutional care ranged from 6 to 59 months ($M = 17.98$, SD = 11.73), with 63 % ($n = 80$) institutionalized for more than 1 year. Ninety-five institutional caregivers also participated in the study (94 female, 98.9 %), aged 21–67 years ($M = 38.58$, SD = 10.67). Twenty-two (23.2 %) of the 95 participating care providers served as primary caregiver for more than one child in the current study.

Procedure

Permission to conduct the larger investigation of which the current study is a part was provided by the 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 Portuguese

Social Services. This agency is responsible for managing the institutions and is the legal guardian of children while they remain there. The plan for the study was presented to the staff of 29 institutional care homes from the north and south of Portugal, all of which agreed to participate. Children were recruited based on their age. Exclusion criteria were the presence of severe physical or mental impairments (e.g., cerebral palsy), genetic or neurological syndromes (e.g., Down syndrome), including fetal-alcohol syndrome. Written informed consents were obtained from the biological parents and the institution director. The primary institutional caregiver of each participating child was identified based on staff interviews, thereby determining who the child showed preference for and/or who knew the child best. Caregivers also provided written informed consent.

Measures

ADHD symptoms

Caregivers completed the Portuguese version of the Child Behavior Checklist for children 1.5–5 years of age (CBCL/1.5–5) [47, 48]. The CBCL/1.5–5 is composed of 100 items, each of which is coded 0 ("not true"), 1 ("sometimes or somewhat true"), or 2 ("very/frequently true"), designed to record emotional and behavior problems of young children. The CBCL/1.5–5 has strong psychometric properties and has been extensively used to assess child mental health (for instance, [44]). For the purposes of the present study, the DSM-Oriented Attention-Deficit/Hyperactivity Problems (DSM-ADH) scale was used (six items; e.g., Item 5, "Can't concentrate, can't pay attention for long"), as it has been found to be more sensitive in the identification of ADHD than the original and empirically defined CBCL Attention Problem scale [49]. In the present study, the internal consistency of the DSM-ADH scale proved to be more than adequate with a coefficient alpha of .78, a result consistent with other investigations [49, 50]. Higher scores reflect the presence of more ADHD symptoms.

Genotyping of 5-HTTLPR polymorphism

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). Sample concentration was accessed using Nanodrop technology. *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′-TGGGGGTTGCAGGGGAGATCCTG-3′ [51]. 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 validated using Sanger sequencing of representative samples of each genotype (i.e., s/s, s/l, l/l). The following genotype frequencies were found for the overall sample: s/s—19.7 % ($n = 25$), s/l—40.9 % ($n = 52$), and l/l—39.4 % ($n = 50$). The distribution is in Hardy–Weinberg-equilibrium, $\chi^2_{(1)} = 2.92$, $p = .09$. Allelic frequency is consistent with published literature and NBCI database for these genes. For further analysis, children were grouped according to the absence or presence of at least one l-allele. The *5-HTTLPR* genotype proved not to be significantly associated with child ethnicity (72.4 % Caucasian vs. 27.6 % others; see Table 1) $\chi^2_{(1)} = 2.08$, $p = .27$.

Caregiver intrusiveness

The Cooperation-Intrusiveness subscale of the Maternal Care Scales [52], adapted to the preschool years, was used by highly trained raters to assess caregiver's intrusive behavior in interaction with the child, during a 15-min videotaped task, divided in three episodes: (1) child plays with a challenging toy with the caregiver's guidance (5 min); (2) researcher provides child with uninteresting toy while placing more interesting ones out of reach, but in view, with caregiver directed to complete a (sham) questionnaire while preventing him/her from contacting the interesting toys (5 min); (3) child–caregiver play with previous out-of-reach toys (2.5 min), followed by a cleanup task for the child (2.5 min). The Cooperation-Intrusiveness subscale is a 9-point scale and aims to assess the extent to which the caregiver's interventions break into or interrupt the children's ongoing activity rather than being geared in time and quality to children's interests and mood. The degree of intrusiveness is measured with respect to the extent of physical interference with the child's activity and frequency of interruptions. A higher score reflects a more cooperative caregiver. The scale was rated by independent coders who did not know the dyads and were not aware of other data included in this inquiry; disagreements were discussed to obtain a consensus. Intraclass correlation for intercoder reliability was .92, calculated for 39 (31 %) caregiver–child interactions.

Potential covariates

Institutional placement and duration

The date of birth and date of admission to the institution were obtained from the child's case file, affording

Table 1 Demographic characteristics of the sample

	M	SD	Min–max
Gestational weeks	38.92	1.72	32–43
Age at assessment (months)	54.67	10.68	36–77
Age at admission to the institution (months)	36.54	15.95	3–69
Length of institutional care (months)	17.98	11.73	6–59
Developmental quotient	97.58	11.60	65–129
Caregiver intrusiveness	5.03	1.65	1–9
ADHD symptoms	4.26	2.70	0–12
	N		**(%)**
Gender (male)	74		58.3
Ethnicity			
Caucasian	92		72.4
Romani	2		1.6
African-Portuguese	22		17.3
African-other	11		8.7
Preterm birth (<37 gestational weeks)[a]	12		9.4
5-HTTLPR			
sl/ll	102		80.3
s/s	25		19.7

[a] $N = 102$

calculation of the child's age at placement and the length of time in the institution.

Mental development

The Griffith's Mental Development Scales [53] assesses various areas of development by means of six subscales and can be administered to children up to 8 years of age. A total score reflects general developmental level and separate subscales pertain to quotients for each area of development: locomotor (gross motor skills), personal-social (proficiency in the activities of daily living, level of independence and interaction with peers), language (both receptive and expressive), eye-and-hand co-ordination (fine motor skills and visual monitoring skills), performance (visuospatial skills including speed of working and precision), and practical reasoning (ability to solve practical problems, understanding of basic mathematical concepts and understanding of moral issues). A global quotient was calculated averaging the various sub-quotients.

Prematurity and child sex

Also serving as a potential covariate were child preterm birth, obtained from children's medical records, and child sex, as all of these factors have been linked to ADHD symptoms (for instance, [54, 55]).

Results

Descriptive statistics and bivariate associations

Descriptive statistics and bivariate associations between study variables are displayed in Tables 1 and 2. Preliminary analyses revealed no significant associations between ADHD symptoms and age at assessment, age at placement and length of time in institutional care. Inspection of Table 2 shows that children showing more ADHD symptoms had lower developmental quotients, $r = -.22$, $p = .014$; the latter was thus included as a control variable in the analyses to be reported. No other significant associations were observed between ADHD symptoms and the primary study variables, including the *5-HTTLPR* polymorphism or caregiver intrusiveness. Moreover, there were no sex differences in ADHD symptoms, $t\ (125) = .61$, $p = .54$. Likewise, no significant differences in ADHD scores emerged between children born preterm and full term, $t\ (100) = .38$, $p = .70$.

Multiple regression analysis predicting ADHD-related symptoms

Multiple regression analyses were conducted using child development quotient as covariate, entered in the first step of the model. The next step included *5-HTTLPR* genotypes (0 for s/l and l/l, and 1 for s/s) and caregiver intrusive behavior. The third and final step included the two-way

Table 2 Bivariate associations between variables

	1	2	3	4	5	6
1. Development quotient	–					
2. Age at assessment (in months)	−.07	–				
3. Age at placement (in months)	−.04	.72**	–			
4. Length of institutional care	.003	−.13	−.73***	–		
5. *5-HTTLPR* (0 = s/l and l/l, 1 = s/s)[a]	−.12	.14	.05	.06	–	
6. Caregiver intrusiveness	.21*	.15	.10	.01	.06	–
7. ADHD-related symptoms	−.22*	.02	−.02	.07	−.03	.11

* $p < .05$, ** $p < .01$, *** $p < .001$

[a] Point biserial coefficient correlation, remaining are all Pearson coefficient correlation

Table 3 Regression analysis predicting ADHD symptoms in institutional-reared preschoolers

	ADHD symptoms			
	B	SE	*β*	*T*
Step 1	[$F = 6.17$*, $R^2 = .05$]			
Developmental quotient	−.05	.02	−.22	**−2.45***
Step 2	[$F = 2.34^{+}$, $R^2 = .06$, $\Delta R^2 = .03$]			
5-HTTLPR (0 = s/l and l/l, 1 = s/s)	−.42	.60	−.06	−.70
Caregiver intrusiveness	−.06	.15	.04	.41
Step 3	[$F = 3.21$*, $R^2 = .10$, $\Delta R^2 = .07$]			
5-HTTLPR × caregiver intrusiveness	.96	.40	.23	**2.42***

$^{+}$ $p < .10$, * $p < .05$, ** $p < .01$, *** $p < .001$

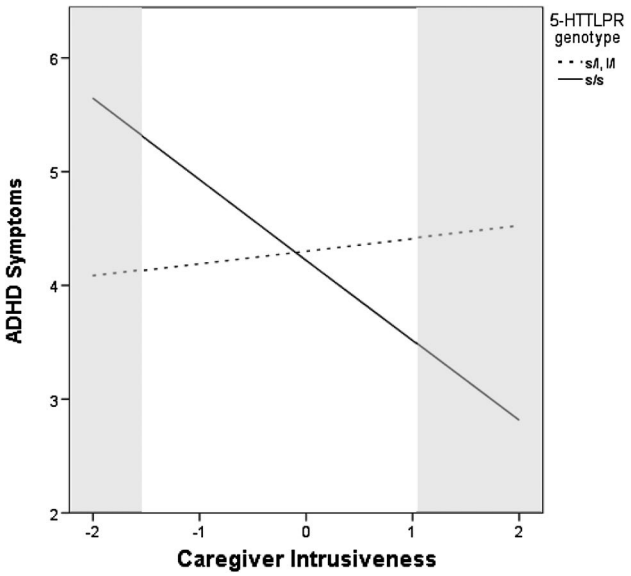

Fig. 1 The *5-HTTLPR* genotype (s/l, l/l vs. s/s) moderates the effects of caregiver intrusiveness on ADHD scores. The *shaded areas* represent the regions of significance

interaction between *5-HTTLPR* genotype and intrusiveness (Table 3). Beyond already cited evidence that children with lower developmental quotients scored higher on ADHD symptoms, $\beta = -.22$, $p = .014$, regression results revealed no significant main effects of *5-HTTLPR* or caregiver intrusive behavior. Notably, however, the GXE interaction involving *5-HTTLPR* and caregiver intrusiveness proved significant, $\beta = .23$, $p = .017$.

To illuminate the nature of this significant interaction, we plotted regression slopes of caregiver intrusiveness on ADHD symptoms separately for carriers of the s/s genotype and of at least one l-allele. Follow-up analysis [56] indicated that the effect of caregiver intrusiveness on symptoms of inattention and hyperactivity was significant for children with the s/s genotype, $\beta = -.44$, $p = .027$, but not for children with the s/l and l/l genotypes, $\beta = .08$, $p = .40$.

Following Kochanska et al. [57], we next conducted a region of significance test [58] to determine whether the GXE interaction proved more consistent with a diathesis-stress or differential-susceptibility model of environmental action. This technique defines the specific values of caregiver intrusiveness below which and above the regression lines of children with two different *5-HTTLPR* genotypes (i.e., s/l and l/l vs. s/s) differ significantly with regard to ADHD symptoms. As illustrated in Fig. 1, the slopes between *5-HTTLPR* genotypes and inattention and hyperactivity proved significant when caregiver intrusiveness scores were below −1.59 and above 2.57 and thus in a manner consistent with differential susceptibility rather than diathesis stress. More specifically, when exposed to higher levels of intrusive caregiving (i.e., <−1.59, 1.5 SD below the mean), s/s carriers scored significantly higher on ADHD symptoms than did l-allele carriers, but when exposed to lower levels of intrusiveness (i.e., >2.57, 1 SD above the mean), s/s children scored significantly lower on ADHD symptoms than l-allele carriers. This same pattern of results emerged even when controlling for age at placement into institutional care or time spent in the institution, both of which proved unrelated to ADHD symptoms.

Discussion

Children raised in institutions are at elevated risk for a variety of psychiatric problems, including ADHD symptomatology [4–6]. The current study extends research on the effects of institutionalization, most notably by exploring the interactive effect on ADHD symptoms of *5-HTTLPR* genotype and the quality of institutional care experienced by ADHD symptoms preschoolers living in institutions. Although it was surprising that no main effects of the measured environmental conditions emerged, results indicated that genetic variation moderated the effect of caregiver intrusiveness on ADHD symptoms among Portuguese institutionally reared preschoolers. Recall that *5-HTTLPR* s/s homozygotes displayed (1) the most attention problems and hyperactivity when exposed to high levels of intrusive care, but (2) the least ADHD symptoms, with a mean score similar to the general population [59], when exposed to low levels of such care; and (3) there was no detectable effect of caregiving intrusiveness in the case of l-allele carriers.

The pattern of GXE interaction detected in this inquiry is consistent with a differential-susceptibility framework of person × environment interaction, rather than with the diathesis-stress model. The differential susceptibility framework stipulates that some individuals are affected more than others by both adverse environmental conditions (e.g., more intrusive caregiving/most ADHD symptoms) and relatively supportive ones (e.g., less intrusive caregiving/fewest ADHD symptoms) as a result of some characteristic of individuality, which in the current case involved the *5-HTTLPR* polymorphism. Recall that the diathesis-stress framework stipulates only that some individuals will be more vulnerable to adversity and others more resilient in the face of such conditions, with both vulnerable and resilient individuals faring equally well under supportive conditions [28–31].

It seems especially notable that the differential-susceptibility-related GXE results emerging from this inquiry are consistent with those of some related studies—and this despite important and dramatic differences in research design. Here we are referring to the fact that whereas in the current observational research all children were still residing in institutions when ADHD symptoms were measured, in other experimental work also documenting differential-susceptibility-related GXE results some children were being cared for in foster care after leaving the institution at the time of behavioral assessment [26, 27]. Considered together, such cross-study consistency suggests not only that the quality of care matters with regard to the emergence of ADHD symptoms, but that some children appear more susceptible to such quality-of-care influences for better and for worse as a result of their genetic make-up irrespective of the rearing context in which care is measured.

The research reported herein extends prior work in showing that the significant GXE effect just described and discussed emerged even after accounting for age at institutional placement and duration of institutionalization. It proved surprising, however, that these institutional features did not predict ADHD symptoms, especially given results of related investigations [8, 9]. We are not the first to fail to document such seemingly anticipated associations, however, thereby calling attention to methodological differences across inquiries that could account for variation in results. Recall that in our own and in Zeanah and colleagues' [6] work, the focus was on children still institutionalized, whereas other research focused on previously institutionalized children, living with their adoptive families [8, 9]. Another factor to consider in entertaining reasons for divergent results across studies is that the absence of an effect of duration of deprivation on ADHD may be attributable to the fact that all children from the current study were institutionalized for no less than 6 months and this was by no means the case in other work. Important to emphasize as well are risks associated with embracing null findings, such as those emerging in the current inquiry; after all, absence of evidence should not be regarded as evidence of absence.

Despite the intriguing GXE results chronicled in this report, the biological mechanisms responsible for the findings remain unclear. Of interest, nevertheless, is that some research documents an association between the *s* genotype, which shows a lower transcriptional activity of the serotonin transporter gene, with brain activity. fMRI studies chronicle increased amygdala activity among *s* carriers in response to relevant environmental stimuli, particular to unpleasant or fearful ones indicating increased stress vulnerability. Interestingly, this pattern of brain activation has been shown to be present in ADHD patients, being considered an endophenotype of this disorder [60–65]. Although this was not the focus of the present inquiry and needs further investigation in future studies, it is plausible that those alterations in amygdala response could mediate the effects of the genotype on ADHD behavior.

In the present study, development quotient was found to predict ADHD symptoms. This result is in line with previous studies, showing that ADHD is more likely to be present in the context of lower cognitive ability [66]. It is also consistent with the literature on institutionally reared children; consider in this regard Doom, Georgieff and Gunnar's [67] data showing that increased ADHD symptomatology was related to lower IQ among post-institutionalized internationally adopted children. Interestingly, our results also indicated that a lower developmental quotient was linked to more intrusive caregiving. It is important to note, however, that the cross-sectional and observational nature of the current study does not afford insight into causal directionality.

As supported by mounting evidence, it may well be the case that variations in the quality of caregiving are at the root of developmental risks among institutionalized children (see, for instance, [33]). Nevertheless, the possibility should not be ruled out that caregivers' more intrusive style may reflect their lack of preparation to deal with children who putatively are less able to signal their needs and interests, in a very stressful environment which characterizes most institutions.

Limitations of the study and future directions

While there are a number of strengths to the present study, including the assessment of the quality of the proximal caregiving environment, there are limitations to this research that merit attention. The sample is small, and, thus, this study is limited in its statistical power [68], which might have contributed to some (or all) of the null results reported and discussed, and to the small amount of variance accounted by the two-way interaction involving *5-HTTLPR* and intrusiveness. In consequence, interpretation and generalization of the results must be made carefully, and replication in larger samples of institutionally reared children is warranted. In addition, information regarding the main study variables was available at only a single point in time. Thus, the correlational design of this cross-sectional study limits the interpretation of results. It is important to note that in the present investigation, other indices of the quality of institutional care beyond intrusiveness were not measured. Expanding the scope of caregiving assessment might further illuminate additional environmental and GXE interaction influences on ADHD symptoms among institutionalized children. Such expanded assessment might focus on indicators of caregiving stability and consistency (e.g., daily child-to-caregiver ratio, the format and predictability of the caregivers' working shifts), as suggested by results of other investigations [46]. Moreover, the primary caregiver provided information about ADHD symptoms, making it impossible to rule out informant bias. Incorporating diagnostic interviews with caregivers and even observational measures could provide a more comprehensive view of institutionalized preschoolers' attention problems and hyperactivity in future studies.

Especially in need of consideration is that the moderating role of only a single genetic variant, the *5-HTTLPR* genotype, was examined. In view of the fact that virtually all phenotypes are shaped by multiple genes and that multiple genes have also been found to moderate environmental susceptibility, it is clear that future GXE work dealing with institutionalized children and focusing on ADHD symptoms should expand the focus to other candidate genes and even consider utilization of polygenic indices.

A final consideration regarding the strengths and limits of this work is that it focused on children placed in institutions in Western Europe. Most other investigations have focused on children placed in Eastern European institutions. These latter institutions not only have severe deficiencies in psychosocial care, but also in physical resources (see, for instance, [69]). In contrast, Portuguese institutions meet children's nutritional and health needs, even if failing to do so with regard to social and emotional support, and cognitive stimulation [45, 46]. Thus, because Portuguese institutional care offers higher quality than typically found in Eastern Europe, this needs to be kept in mind when considering results reported here and elsewhere.

Clinical implications

In highlighting the interactive influence of genes and proximate caregiving processes in accounting for variation in ADHD symptomatology among children growing up in institutions, this study carries important implications for practice. Most notably, perhaps, the GXE results underscore the centrality of the proximal caregiving environment—especially its intrusiveness—even in the unfavorable environment of an institution, by clearly suggesting that it can undermine behavioral development when the quality of care is poor (i.e., more ADHD symptoms), but contribute to the emergence of fewer mental health problems (i.e., fewer ADHD symptoms) when the quality of care is better. Although the replacement of institutional care for more family-like forms of caregiving is urgent, given that there are currently around 8500 institutionalized children in Portugal [2], efforts should be made to rapidly improve the quality of institutional caregiving. Perhaps, in fact, great strides could be made in reducing the development of ADHD simply by educating caregivers about the nature of intrusive caregiving, while affording these stressed individuals alternative ways of relating to their charges. Important to mention in this regard is McCall and colleagues' (see [70]) intervention work designed—and found—to improve the quality of caregiving in institutions in Russia—and thereby enhance children's development.

Conclusion

The current results document the moderating role of *5-HTTLPR* on the relation between caregiver intrusiveness and ADHD symptoms among institutionalized preschoolers. Most notably, s/s homozygotes of the *5-HTTLPR* displayed the most and the least ADHD symptoms when exposed to more and less intrusive caregiving, respectively.

These findings proved more consistent with the differential susceptibility rather than with the diathesis-stress model of person × environment interaction. No relations emerged between caregiver intrusiveness and attention problems and hyperactivity in the case of l-allele carriers. Our findings not only suggest that the quality of caregiving matters with regard to the emergence of ADHD symptomatology, but also that some institutionalized children are more susceptible to such quality-of-care influences—for better and for worse—than others as a result of their individual characteristics, in this case their genetic makeup.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical standards The ethical requirements followed the guidelines present in the 1964 Declaration of Helsinki and its later amendments.

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