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Early life stress alters pituitary growth during adolescence - A longitudinal study

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Abstract

The pituitary gland is integral in mediating the stress-response via its role in hypothalamic-pituitary-adrenal (HPA) axis function. Pituitary gland volume (PGV) has been found to be altered in stress-related psychopathology, and one study to date has shown stress to be associated with age-related change during adolescence. The current study investigated the effects of a number of different types of early life (i.e., childhood and adolescent) stress (including childhood maltreatment, stressful life events, and maternal affective behavior) on PGV development from mid- to late adolescence using a longitudinal design. Ninety one (49 male) adolescents took part in mother-child dyadic interaction tasks when they were approximately 12 years old, reported on childhood maltreatment and stressful life events when they were approximately 15 years old, and underwent structural magnetic resonance imaging (MRI) scans when they were approximately 16 and 19 years old. Results revealed that childhood maltreatment predicted accelerated PGV development in females, and maternal dysphoric behavior predicted accelerated PGV development in the whole sample. PGV development was not associated with depressive or anxiety symptoms. These results suggest an effect of early life stress on altered HPA axis function across mid- to late adolescence. Further research is required to assess whether these changes might be associated with risk for subsequent psychopathology.

Highlights:

Keywords: Pituitary gland, development, stress, adolescence, magnetic resonance imaging, maternal care

1. Introduction

The pituitary gland is integral in mediating the stress-response via its role in hypothalamic-pituitary-adrenal (HPA) axis function. The pituitary is under the influence of hypothalamic corticotropin releasing factor (CRF) and secretes adrenocorticotropin hormone (ACTH) which works in a feedback loop to propagate the stress response, and therefore may be a region which is critically affected by stress dysregulation. The HPA system is not fully mature at birth and there are changes that occur throughout childhood and adolescence in both basal HPA activity and cortisol reactivity (Gunnar & Donzella, 2002). During this extended period of development, experiences play a role in shaping the basal rhythms and reactivity of the HPA system. Consequently, the developing HPA axis is particularly sensitive to early life (i.e., childhood and adolescent) experience.

It has been well established in both preclinical and clinical studies that early life stress is associated with changes in neurobiological responses to stress, which may underlie the increased susceptibility to psychopathology, see (Heim & Nemeroff, 2001) for review. In rodents, prolonged early life stress caused by chronic maternal separation is associated with protracted increases in CRF, ACTH and corticosterone in response to restraint stress in adulthood (Dent, Okimoto, Smith, & Levine, 2000; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Mirescu, Peters, & Gould, 2004; Plotsky & Meaney, 1993). Complementary findings reveal that increased maternal caregiving behaviour (licking, grooming and arched-back nursing) is highly correlated with reduced ACTH and CORT and CRF mRNA in response to stress (Caldji et al., 1998; Liu et al., 1997). Taken together, these studies suggest that early life stress and the level of maternal care can induce long-lived hyper(re)activity of the CRF system as well as altering other neurotransmitter systems, resulting in increased stress responsiveness (Nemeroff, 1999).

In humans, there is evidence that stressors such as childhood maltreatment and other adverse early life experiences may have long-term influences on HPA function (Tarullo & Gunnar, 2006). Magnetic resonance imaging (MRI) studies suggest morphological and functional changes in brain structures involved in the control of the stress response, including the hippocampus, in adult patients with depression or post-traumatic stress disorder (PTSD) who have undergone early life stress (Bremner et al., 2000; Bremner et al., 1997). Other neuroimaging studies have documented alterations in the structure of the prefrontal cortex and amygdala (Sheline, Gado, & Price, 1998; Whittle et al., 2008) (Whittle et al., 2011).

Despite the central role of the pituitary gland in the HPA axis stress response, few studies have investigated the relationship between the effects of stress on pituitary gland morphology. There is general consensus that the size of the pituitary gland may be used as an index of HPA function. The

chronic effects of HPA dysfunction causing long-term changes in morphology and subsequent pituitary gland volume are considered more stable and less prone to state effects or temporal changes, which is an issue when measuring hormone markers of stress such as cortisol or CRF that are sensitive to time of day or present state, respectively (Kunugi et al., 2005; Weitzman et al., 1971). Also, given that the HPA system is self-regulating, absolute basal levels of hormones may not be conclusive. Pituitary gland volume has been hypothesized to represent an increased number and/or size of CRF cells in the area (Gertz et al., 1987; Kurosumi & Kobayashi, 1966). Such assertions are supported by evidence that enlarged pituitary gland volume reflects an increase in the size and number of the corticotrophic cells that produce and secrete ACTH, and it is hypothesized to be a consequence of either HPA axis hyperactivity or of a specific dysfunction of a subgroup of CRF neurons that provokes HPA axis hyperactivity (Axelson et al., 1992; Garner et al., 2005; Krishnan et al., 1991; Pariante & Miller, 2001; Pariante et al., 2004; Swaab, Bao, & Lucassen, 2005).

Variations in pituitary gland volume (smaller or larger PGV) have commonly been associated with psychiatric illnesses where stress is a known precipitating factor. For example, larger PGV has been identified in both adult and adolescent patients with major depression (Krishnan et al., 1991; MacMaster & Kusumakar, 2004; MacMaster, Russell, Mirza, Keshavan, Taormina, et al., 2006) and psychosis (Pariante et al., 2005; Pariante et al., 2004; Takahashi et al., 2009), while smaller PGV has been associated with eating disorders (Doraiswamy et al., 1991; Doraiswamy et al., 1990). Regarding anxiety disorders, smaller pituitary volumes have been found in both adult and pediatric samples with obsessive compulsive disorder (OCD) (Atmaca et al., 2009; MacMaster, Russell, Mirza, Keshavan, Banerjee, et al., 2006; Thomas & De Bellis, 2004). Thomas and De Bellis assessed the effect of paediatric maltreatment-related PTSD on PGV and found that during pre-puberty, there was no difference in PGV between PTSD and controls. However there was a significant age-by-group effect, whereby PTSD subjects had a larger PGV during the pubertal and post-pubertal period. This study highlights how adverse early life experience may be associated with pituitary abnormalities in an age-dependent manner. Also, it appears that the developmental period must be taken into account when assessing PGV, particularly during adolescence when there is a well-established pituitary hypertrophy seen in both sexes (Elster, Chen, Williams 3rd, & Key, 1990).

Given the sensitivity of the HPA axis during development to stress, and the identification that aberrant pituitary development is associated with some forms of psychiatric illness, the current study aimed to assess the effect of adverse early life experiences on pituitary development and psychiatric symptomatology. We performed a longitudinal volumetric MRI assessment of pituitary gland volume to investigate the effects of different forms of stress on pituitary development during adolescence, with a comprehensive approach to assessing the effects of stress early in life. That is, we examined a

range of stressors that might be described as extreme (i.e., childhood maltreatment), acute (i.e., stressful life events), as well as more normative and chronic (i.e., maternal parenting). We hypothesized that adverse life experiences would be associated with pituitary volume enlargement during adolescence, and that these changes would then relate to depressive and anxiety symptoms.

2. Methods

2.1 Participants. The sample consisted of 91 adolescents (54% males*)* recruited from schools across metropolitan Melbourne, Australia. Participants were recruited as part of a broader longitudinal adolescent development study (as described in (Yap, Allen, & Ladouceur, 2008)). Adolescents participated in four phases of data collection between the ages of 11 and 19 (Time 1 (T1): participants aged between 11 and 13 years, *M* = 12.63, *SD* = 0.45; Time 2 (T2): between 13 and 15 years, M = 15.02, SD = 0.43; Time 3 (T3): between 15 and 17 years, *M* = 16.45, *SD* = 0.51; Time 4 (T4): between 17 and 20 years, *M* =18.80, *SD* = 0.44). T1, T3 and T4 included a combination of data collected across the domains of structural and functional neuroimaging, cognitive functioning, diagnostic interviews of psychopathology, and questionnaire batteries. T2 included only diagnostic interviews of psychopathology, and questionnaire batteries. Measured relevant to the current paper include T1 mother-adolescent interactions, a diagnostic interview, and adolescent- and parent-rated questionnaires, T2 diagnostic interview and questionnaire measures of childhood maltreatment and stressful life events, and T3 and T4 diagnostic interviews and questionnaires in addition to Magnetic Resonance Imaging scans. The 91 adolescents included in this paper represented those who consented to brain imaging assessments at T3 *and* T4. These participants did not differ from those who participated in T1 assessments (n = 245) in SES, anxiety symptoms, or gender, or in our childhood adversity predictors measured at T1 or T2 [i.e., childhood maltreatment, stressful life events, maternal behaviour] (*p*'s > 0.05). At each wave, participants were assessed for current and past Axis I disorders by trained research assistants using the Schedule for Affective Disorder and Schizophrenia for School-Aged Children: Epidemiological Version (Orvaschel & Puig-Antich, 1987). Fifty percent of females and 49% of males had a lifetime incidence of Axis I disorder. Informed consent was obtained for all participants (and their parent or guardian) before their inclusion in the study, in accordance with the guidelines of the Human Research Ethics Committee of The University of Melbourne, Australia.

2.2 Neuroimaging

2.2.1. Image acquisition. Magnetic Resonance Imaging was conducted at T3 and T4 at the Royal Children's Hospital, Melbourne, Australia, on a 3-Tesla Siemens scanner. High resolution structural

images were acquired with the following sequence parameters; repetition time = 1900ms; echo time = 2.24ms; flip angle = 9°, field of view = 23cm^2, producing 176 T1-weighted contiguous 0.9mm thick slices (voxel dimensions = 0.9mm^3).

2.2.2. Image pre-processing. Non-brain tissue was removed from all T1-weighted images using FSL's Brain Extraction Tool (Smith, 2002). An automated rigid-body registration of the whole brain incorporating automatic scaling correction (FLIRT (Jenkinson & Smith, 2001) with 9 degrees of freedom) was used to align T4 images to their respective T3 images (Whitwell, Anderson, Scahill, Rossor, & Fox, 2004).

2.2.3. Image Analysis - Pituitary Volume. Each pituitary was traced by the same investigator (DG) who was blinded to participant characteristics. Images were traced using the software ANALYZE 11.0 (Mayo Clinic, Rochester, USA; http:// mayo.edu/research/labs/biomedical-imaging/software/analyze-software-system). The pituitary was defined and quantified based on a previously published technique (Pariante et al., 2004). Specifically, images were traced in the coronal plane, where the pituitary is best visualised (Garner et al., 2005; Lorenzetti et al., 2009; Whittle et al., 2012). We excluded the infindibular stalk from the tracing. The borders of the pituitary were clearly defined by the diaphragm sellae, superiorly; the sphenoid sinus, inferiorly, and the cavernous sinuses bilaterally (Lorenzetti et al., 2009; Whittle et al., 2012). The hyper-intense region of the posterior pituitary was included as it is thought to represent high concentration of vasopressin (Sassi et al., 2001). PGV estimates were calculated by summing all voxels within the traced region on each slice on consecutive 2D coronal slices. Intra- and Inter-rater reliability was assessed by tracing a sample of 10 previously traced pituitary glands which was duplicated and was 0.94 and 0.91 respectively.

2.3 Measures.

2.3.1 Socioeconomic Status

Socioeconomic status (SES) was estimated using the Australian National University Four (ANU4) scale (Jones & McMillan, 2001).

2.3.2. Stressful Life Events

At T2, participants completed the Stressful Life Events (SLE) Questionnaire. This questionnaire was adapted from one utilized by Lewinsohn and colleagues (Lewinsohn, Rohde, & Gau, 2003). The SLE Questionnaire is a 30 item self-report checklist measuring the occurrence of both normative (e.g. starting at new school) and non-normative (e.g. death of a family member) experiences representative of the types of events empirically identified to be stress-inducing for most young people (Holmes &

Masuda, 1973; Lewinsohn et al., 2003). While not all events on the scale would necessarily be considered negative or aversive, they are all usually associated with some form of coping behaviour due to a significant change in life circumstances on the part of the affected individual or significant others (e.g. parent, sibling, other close relative or close friend). Many researchers in the measurement life stress have emphasised that the requirement for adaptation in the face of life change is the core defining feature of a "stressful life event", regardless of whether it is positively or negatively valanced (Holmes & Masuda, 1973). The total number of items endorsed by participants was used as a measure of frequency of stressful life events.

2.3.3. Childhood Trauma

At T2 the Child Trauma Questionnaire (CTQ) was completed by participants. The CTQ (Bernstein, Ahluvalia, Pogge, & Handelsman, 1997; Bernstein & Fink, 1998) is a 28-item retrospective scale that measures the frequency and severity of different types of abuse and neglect. Participants were asked to rate the frequency (0 = never true, 5 = very often true) with which various events took place when they "were growing up". It is designed to assess five dimensions of childhood maltreatment: (a) physical abuse; (b) emotional abuse; (c) sexual abuse; (d) physical neglect; and (e) emotional neglect. The reliability and validity of the CTQ has been evaluated and results have been found to converge with therapist assessments of childhood trauma (Bernstein et al., 1997). Due to the comorbid nature of different types of maltreatment, a total maltreatment score was used in analyses.

2.3.4. Family interaction assessment and measures

Adolescents and mothers completed the lab-based interaction assessment at T1. Mother-adolescent dyads completed two 20-min interaction tasks that were video recorded for subsequent coding. An event planning interaction (EPI) was completed first, followed by a problem-solving interaction (PSI). The EPI and PSI tasks were intended to elicit positive and negative behavior, respectively. The ordering of tasks was fixed because of concern that negative affective states elicited by the PSI had the potential to persist into the positive EPI if conducted second (Gilboa & Revelle, 1994). For the EPI, mothers and adolescents were instructed to plan one or more pleasant activities to do together, with up to five activities chosen on the basis of items that both the mother and adolescent rated as being "very pleasant" on the Pleasant Events Schedule (MacPhillamy & Lewinsohn, 1976). For the PSI, mother-adolescent dyads were instructed to try to resolve one or more issues of disagreement, with up to five issues selected that the mother and adolescent endorsed as occurring the most frequently and generating the highest intensity of anger on the Issues Checklist (Prinz, Foster, Kent, & O'Leary, 1979).

2.3.5. Living in Family Environments (LIFE) coding system

The LIFE (H Hops, Biglan, Tolman, Arthur, & Longoria, 1995) is an observational, microsocial coding system that allows for a detailed analysis of individual family members' behaviors and interactive family behaviors. The LIFE system consists of 10 nonverbal affect codes (e.g., anger, dysphoria, happy) and 27 verbal content codes (e.g., validation, complaint, provoke). To code the video-recorded interactions, we used an event based protocol in which new codes were entered each time the affect or content of one of the interactants changed. The affect and content codes were used to develop composite behavior constructs. In this study, the constructs were aggressive, dysphoric, and positive behaviors. The aggressive construct included all behaviors with contemptuous, angry, or belligerent affect, as well as cruel, provocative, annoying/disruptive, or argumentative verbal statements made with neutral affect. The dysphoric construct consisted of all behaviors with dysphoric, anxious, or whining affect, as well as complaints and self-derogatory verbal comments made with neutral affect. The positive construct included all behaviors with happy or caring affect as well as approving, validating, affectionate, or humorous comments made with neutral affect. We used the LIFE data to construct a frequency variable to measure maternal expression of emotion. These variables indicate the average number of times a mother expressed each behaviour type (i.e., aggressive, dysphoric, and positive) per minute and were calculated separately for the EPI and PSI. Given that negative behaviors during the EPI and positive behaviors during the PSI have been shown to be particularly predictive of poor psychological outcomes in previous research (Schwartz et al., 2014), frequency of aggressive and dysphoric behavior during the EPI, and of positive behavior during the PSI, were used in analyses. Coders were extensively trained and blind to the clinical and demographic characteristics of the participants. Approximately 20% of the interactions were coded by a second observer to provide an estimate of observer agreement. Random pairs of observers were assigned to the interactions to minimize "drift" between any two observers. The reliability estimate used to determine inter-rater reliability (Kappa) is a stringent test based on point-by-point agreement, and has been strongly recommended for the assessment of inter-observer agreement in behavioural research (Watkins & Pacheco, 2000). Kappa coefficients for the Aggressive, Dysphoric, and Positive constructs were 0.70, 0.60, and 0.86, respectively.

2.3.6. Psychiatric symptoms

At T3 and T4, participants completed the Center for Epidemiological Studies – Depression Scale (CES-D; (Radloff, 1977)) and the Beck anxiety inventory (BAI) (Beck, Epstein, Brown, & Steer, 1988), to provide self-report measures of depressive and anxiety symptoms, respectively. Mothers completed the same instruments at T1.

2.4 *Statistical Analyses*

Our primary outcome measure was PGV development from age 16 (T3) to 19 (T4), operationalized as a residualized change score obtained from regressing T4 PGV on to T3 PGV. Five hierarchical linear regressions were performed to assess the association between each type of early life stress (or absence of positivity: maltreatment, stressful life events, frequency of maternal aggressive, dysphoric and positive behavior) and pituitary development from age 16 to 19. Age at T3 (i.e., at the baseline MRI scan) and SES were used as covariates of no interest in the first block of each regression. Lifetime diagnosis of an Axis I disorder was also included as a covariate in analyses so as to exclude the possibility that any results might be driven by psychopathology. For analyses involving maternal behaviors, maternal depressive and anxiety symptoms were also included as covariates of no interest so as to reduce the influence of any state mood effects. The measures of early life stress and sex were entered in the second block of each regression. The interaction between stress and sex was included in the final block of each regression (to assess potential sex differences in associations). All continuous variables were mean-centred prior to creating interaction terms.

A second set of analyses were performed to assess whether PGV development from 16 to 19 was associated with psychiatric symptoms at age 19. For any significant stress predictor identified in initial analyses, mediation analyses were performed to investigate whether measures of stress predict psychiatric symptoms at T4 (controlling for symptoms at T3) via their effects on PGV development.

3. RESULTS

Demographic information for the sample is shown in Table 1.

Table 1. Descriptive statistics of the study samples

	Entire sample	**Females**	**Males**
	Mean (s.d.), n	**Mean (s.d.), n**	**Mean (s.d.), n**
T3 pituitary volume (mm^3)[b]	506.55 (94.35), 91	542.19 (86.37), 42	476.00 (90.86), 49
T4 pituitary volume (mm^3)[a]	566.68 (94.76), 91	591.09 (101.34), 42	545.75 (84.25), 49
BAI T3	7.90 (7.38), 91	8.11 (5.51), 42	7.72 (8.72), 49
BAI T4	7.09 (7.99), 90	8.02 (8.41), 42	6.27 (7.59), 48
CESD T3	9.77 (7.36), 91	9.71 (6.95), 42	9.82 (7.76), 49
CESD T4	10.53 (9.01), 90	10.91 (9.81), 42	10.19 (8.33), 48

SES	61.48 (119.96), 90	62.52 (20.57), 41	60.61 (19.60), 49
CTQ T2[a]	52.80 (4.50), 88	53.85 (4.20), 41	51.89 (4.60), 47
SLE T2	8.66 (5.91), 88	9.30 (5.56), 40	8.13 (6.18), 48
Mother aggressive construct rpm T1	0.57 (0.41), 68	0.61 (0.43), 34	0.54 (0.39), 34
Mother dysphoric construct rpm T1	0.55 (0.34), 68	0.50 (0.32), 34	0.60 (0.37), 34
Mother positive interpersonal construct T1	1.80 (0.67), 68	1.77 (0.71), 34	1.83 (0.64), 34

s.d., standard deviation; CTQ, Childhood Trauma Questionnaire; CESD, Center for Epidemiological Studies Depression Scale; SLE, stressful life events; SES, socioeconomic status; BAI, Beck anxiety inventory; y, years; rpm, rate per minute.
[a] Significant sex difference $p < 0.05$
[b] Significant sex difference $p < 0.001$

A repeated measures ANOVA was conducted to describe the pattern of PGV growth across time by sex and lifetime psychopathology status. This analysis revealed a main effect of time (F[1,87] = 40.35, $p < 0.001$), with PGV's significantly increasing from age 16 to 19. This effect was moderated by both sex and psychopathology status (F[1,87] = 4.08, $p = 0.047$). Follow-up analyses showed that there were sex differences in PGV development only for those who had experienced lifetime psychopathology (F[1,43] = 6.10, $p = 0.018$), with males in this group showing a greater degree of PGV growth as compared to females.

Table 2 shows Pearson's bivariate correlations between all variables of interest, separately for males and females. As can be seen, for females, maternal aggressive behavior and stressful life events were both associated with depressive and anxiety symptoms at age 19, and childhood maltreatment was associated with anxiety symptoms at age 19. No significant associations were found between measures of stress and symptoms at age 19 in males.

Table 2. Bivariate correlations between all variables of interest. Female correlations above the diagonal, male correlations below the diagonal.

	SES	CESD T3	CESD T4	BAI T3	BAI T4	PGV T3	PGV T4	MAgg	MDys	MPos	CTQ T2	SLE T2
SES	1	.229	-.055	.025	-.047	-.187	-.189	.181	.036	-.384*	-.188	-.206
CESD T3		1	.441**	.405**	.301	.010	-.087	.566**	.251	-.358*	.197	.239
CESD T4	-.099	.492**	1	.189	.573**	.225	.092	.472**	.205	.012	.193	.349*
BAI T3	-.154	.562**	.374**	1	.483**	.003	-.078	.338	.189	-.325	.141	.453**
BAI T4	-0.071	.505**	.836**	.396**	1	.206	.144	.433*	.196	-.198	.316*	.383*
PGV T3	-0.004	.028	0.053	.022	-0.025	1	.504**	.035	-.067	.158	.060	.333*
PGV T4	0.039	-.039	0.021	.009	0.078	.515**	1	-.203	.067	.232	.307	.186
MAgg	-0.258	.132	0.212	-.077	0.183	-0.299	-0.039	1	.322	-.402*	-.138	.160
MDys	-0.166	-.138	0.056	-.126	0.167	-0.034	.394*	0.145	1	-.239	.214	.303
MPos	0.281	.078	-0.089	.178	-0.114	0.274	0.064	-0.292	-0.188	1	.096	-.020
CTQ T2	0.027	.357*	0.097	.278	0.198	0.127	-0.052	-0.135	-0.001	0.041	1	.417**
SLE T2	-0.106	.290*	-0.077	.209	-0.043	0.045	-0.184	-0.099	-0.06	0.067	0.153	1

BAI, Beck Anxiety Inventory; CESD, Center for Epidemiological Studies Depression Scale; CTQ, Childhood Treatment Questionnaire; MAgg, maternal aggressive behaviour; MDys, maternal dysphoric behaviour; MPos, maternal positive behaviour; PGV, pituitary gland volume; SLE, stressful life events.
*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

The results of all regression analyses are presented in Table 3. Note that covariates in block 1 were not significant in any analysis and are therefore not shown in the Table for simplicity. Analyses revealed that sex moderated the association between childhood maltreatment and PGV development. Follow-up analyses for each sex revealed that higher levels of childhood maltreatment predicted increased PGV development in females (β = 0.32, t = 2.04, p = 0.049), but not males (β = -0.15, t = -1.02, p = 0.321). The association for females is illustrated in Figure 1. Further analyses revealed that for females, childhood maltreatment did not predict PGV at age 16 (p > 0.9), but did predict larger PGV at a trend level at age 19 (p = 0.082).

Table 3. Regression analysis

Block	Predictor	β	T	P	Model Fit
		Childhood Maltreatment			
2	CTQ	.084	.753	.454	F(5,82) = .57, p = .726, R^2 = .033
	Sex	-.031	-.279	.781	
3	CTQ×Sex	-.378	-2.270	.026*	F(6,81) = 1.35, p = .243, R^2 = .091
		Stressful Life Events			
2	SLE	-.099	-.871	.386	F(5,84) = .57, p = .752, R^2 = .033
	Sex	-.052	-.480	.632	
3	SLE×Sex	-.117	-.710	.480	F(6,83) = .55, p = .766, R^2 = .038
		Maternal Dysphoric Behavior			
2	MDys	.291	2.239	.029*	F(7, 59) = 1.41, p = .219, R^2 = .143
	Sex	-.082	-.632	.530	
3	MDys×Sex	.202	1.069	.290	F(8,58) = 1.38, p = .225, R^2 = .160
		Maternal Aggressive Behavior			
2	MAgg	-.023	-.116	.869	F(7,59) = .64, p = .718, R^2 = .071
	Sex	-.049	-.367	.715	
3	MAgg×Sex	.260	1.517	.135	F(8,58) = .86, p = .553, R^2 = .106
		Maternal Positive Behavior			
2	MPos	.055	.406	.686	F(7,59) = .66, p = .701, R^2 = .073

	Sex	-.056	-.416	.679	
3	MPos×Sex	-.204	-1.162	.250	$F(8,58) = .75$, $p = .644$, $R^2 = .094$

CTQ = Childhood Treatment Questionnaire, MAgg = Maternal Aggressive Behavior, MDys = Maternal Dysphoric Behavior, MPos = Maternal Positive Behavior, SLE = Stressful Life Events.

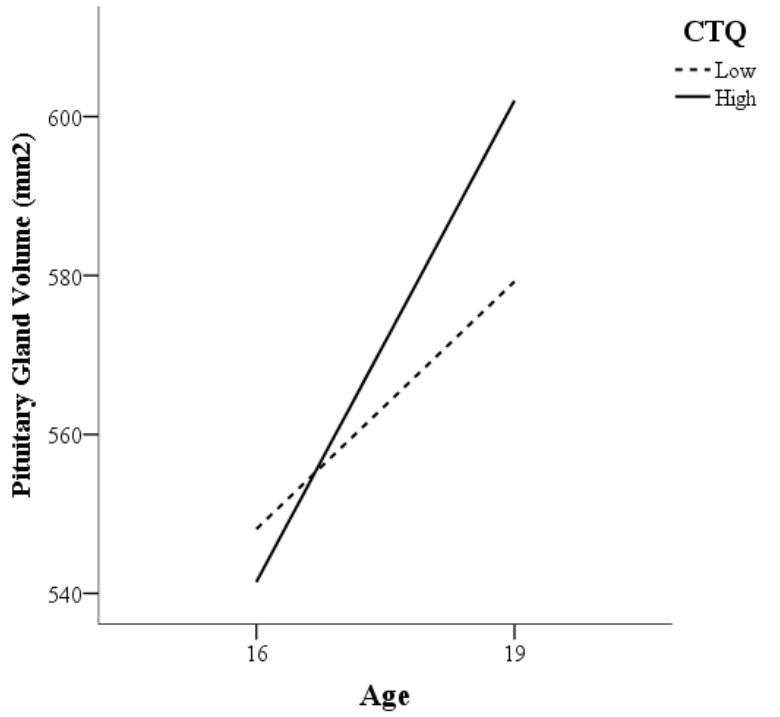


Figure 1. *Effects of childhood maltreatment on pituitary gland volume development from age 16 to 19 in females.*

While stressful life events and aggressive and positive maternal behaviors had no significant effects on PGV development, we found that maternal dysphoric behavior was related to an accelerated PGV growth across time for the whole group (see Figure 2). Further analyses revealed that maternal dysphoric behavior did not predict PGV at age 16 ($p > 0.8$), but did predict larger PGV at age 19 at a trend level ($p = 0.073$).

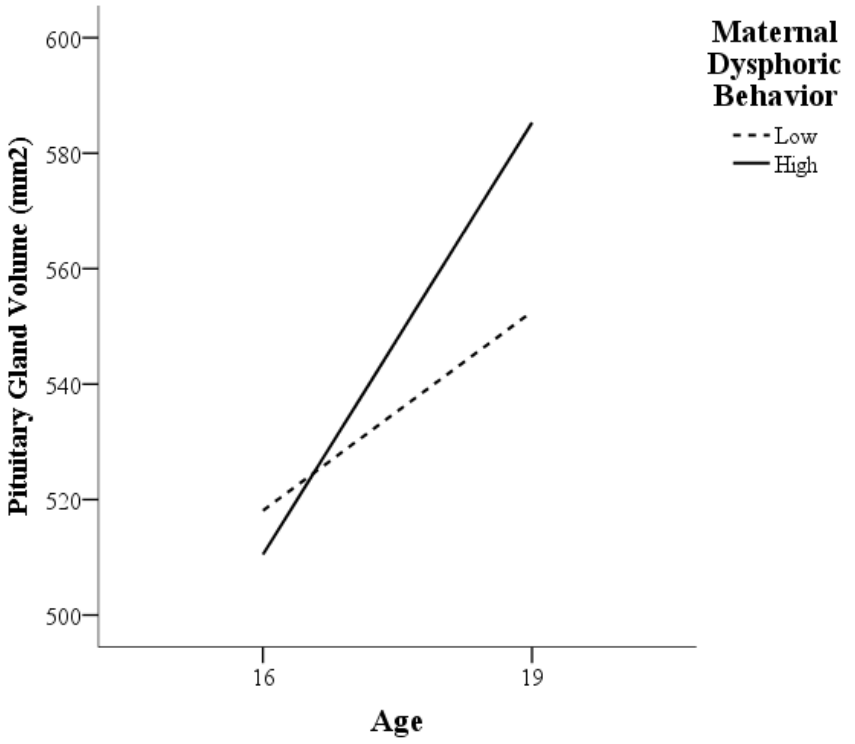


Figure 2. *Effects of Maternal dysphoric behavior on pituitary gland volume development from age 16 to 19.*

Despite the fact that childhood adverse events were associated with depressive and anxiety symptoms age 19 in females (see Table II), analyses performed to assess whether PGV development from 16 to 19 was associated with psychiatric symptoms at age 19 revealed no significant associations, either for the whole group, or for males or females separately (all p values > 0.05). Given these non-significant associations, mediation was not indicated and mediation analyses were not performed.

4. DISCUSSION

Our study has shown that early life stress can impact on the developmental trajectory of pituitary growth during adolescence. Specifically, we found that both relatively extreme adverse environments (i.e., childhood maltreatment) *and* exposure to negative environments that are (arguably) more normative (i.e., observed frequency of maternal dysphoric behavior) predicted enhanced PGV development from mid- to late adolescence. Sex differences were observed such that childhood maltreatment predicted PGV development only for females. Finally, while childhood stress was associated with later depressive anxiety symptoms, PGV development did not mediate this link.
Our finding that childhood maltreatment predicted accelerated growth of the pituitary gland during adolescence is consistent with literature showing enlarged PGVs in stress-related psychopathology

{MacMaster, 2004 #586}, and with a previous study showing that adolescents with paediatric maltreatment-related PTSD exhibited increased pituitary volumes over time {Thomas, 2004 #571}. Our finding that childhood maltreatment predicted PGV development in females but not males is consistent with a number of studies that have previously documented gender differences in the effects of early life traumatic events on stress reactivity. For example, women with childhood trauma exposure exhibited an increased cortisol response compared to men with childhood trauma exposure, while no difference was observed in the absence of trauma (Ter Horst, Wichmann, Gerrits, Westenbroek, & Lin, 2009; Vamvakopoulos & Chrousos, 1993). This gender difference was speculated to be due to the male specific protective effect of a particular CRF receptor 1 gene allele, which only becomes effective in the presence of childhood trauma, and results in decreased CRH-dependent neuroendocrine reactivity and decreased symptoms of depression (Heim et al., 2009; Ter Horst et al., 2009; Vamvakopoulos & Chrousos, 1993). Also, abused females without current depression exhibit markedly increased plasma ACTH responses to psychosocial laboratory stress compared with control subjects and depressed females without adverse childhood experiences (Heim & Nemeroff, 2001). This may be indicative of sensitization of the neuroendocrine stress responses after early life stress, which may be related to an increased risk of psychopathology (Heim et al., 2009). Although PGV development did not predict psychiatric symptoms in our study, the gender differences we observed in PGV development associated with early childhood trauma, may suggest a female vulnerability to the development of depression or other psychopathology later in life. Interestingly, while psychopathology has been found to mediate the relation between childhood maltreatment and adolescent development of other brain regions (Whittle et al., 2011), in this study, maltreatment appeared to have an effect on PGV development independent of psychopathology, potentially suggesting a programming effect.

Maternal dysphoric, but not aggressive or positive behavior significantly predicted accelerated PGV development in both sexes. Maternal dysphoric behavior has been found to predict mental health problems in adolescents in previous work (Yap et al., 2008) (Schwartz et al., 2011). Our results regarding maternal dysphoric behavior are consistent with research showing maternal clinical depression (associated with increased dysphoric behavior), to exert an adverse impact on child functioning indirectly through disturbances in parent-child relations (Fox & Gelfand, 1994; Hops et al., 1987). There is a well-established increased risk for psychopathology and the development of emotional and behavioural problems in children of depressed parents (Downey and Coyne, 1990)(Murray and Cooper, 1997; Radke-Yarrow, 1998){LaRoche, 1989 #668}. A number of studies have associated the presence of early maternal depression with alterations in cortisol secretion in children {Essex, 2002 #667}{Ashman, 2002 #669} and adolescents {Halligan, 2004 #670}. Thus

increased levels of maternal dysphoric behavior may influence accelerated PGV development during adolescence as a result of effects on stress reactivity.

Given the role of the pituitary gland in HPA axis function, it is possible that our findings reflect an ongoing effect of early stress on HPA axis dysfunction which persists through adolescence and potentially into adulthood. Preclinical animal studies strongly suggest that the development of the HPA axis may be affected by early life stressful experience. Unfortunately, stress hormone levels were not assessed at any point in this study, so there is some speculation as to whether the proposed HPA dysfunction in these 'larger' pituitary glands has resulted in aberrant cortisol or ACTH levels. Also, given that the pituitary gland produces a number of hormones other than CRH, of particular note are the sex hormones which become up-regulated particularly throughout pubertal development; and have also been implicated in the control of HPA function (see (McCormick & Mathews, 2007) for review), we are unable to ascertain the extent to which the effects of stressors during childhood are moderated by changes in the hypothalamic-pituitary-gonadal axis, particularly during puberty.

The findings of this study must be interpreted considering some limitations. For example we did not assess stress from T2 to T4, so we can't deduce whether the changes in PGV were due specifically to stress which occurred early in life (i.e., prior to age 15), as opposed to stress which occurred closer to imaging. Also, childhood maltreatment measurements were based on self-report. As such, it is possible that some individual difference factor might lead to false or inflated recollections (Coughlin, 1990). However, is has been suggested that adolescents are more likely to deny or under-report maltreatment (Tajima, Herrenkohl, Huang, & Whitney, 2004). We did not assess outcomes in early adulthood and as such we do not know whether the developmental PGV changes observed to be associated with stress might predict psychopathology later in life. Finally, other potential moderating and mediating effects were not assessed. For example, adverse early life experience during critical periods of development may induce a vulnerability to the effects of stress later in life. It is likely that the combination of genetics, early life stress, and ongoing stress will ultimately determine the manifestation of psychopathology.

5. CONCLUSIONS

Our research findings suggest that different types of early life stress may be important in determining the developmental trajectory of the pituitary gland during adolescence. Large PGV has been implicated in various psychiatric disorders and is thought to be indicative of HPA dysfunction. Although our findings did not directly find a link between enlarged PGV and symptoms of

psychopathology, it is possible that stress-related accelerated PGV development could represent an increased vulnerability to the development of psychopathology later in life.

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Conflict of Interest

The authors have no conflict of interest to declare.

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