



Kent Academic Repository

Moutsiana, Christina, Johnstone, Tom, Murray, Lynne, Fearon, Pasco, Cooper, Peter J., Pliatsikas, Christos, Goodyer, Ian and Halligan, Sarah L. (2014) *Insecure attachment during infancy predicts greater amygdala volumes in early adulthood*. Journal of Child Psychology and Psychiatry . ISSN 0021-9630.

Downloaded from

<https://kar.kent.ac.uk/42759/> The University of Kent's Academic Repository KAR

The version of record is available from

<https://doi.org/10.1111/jcpp.12317>

This document version

Author's Accepted Manuscript

DOI for this version

Licence for this version

CC BY (Attribution)

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in *Title of Journal* , Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our [Take Down policy](https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies) (available from <https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies>).

Insecure attachment during infancy predicts greater amygdala volumes in early adulthood

Christina Moutsiana,¹ Tom Johnstone,² Lynne Murray,^{2,3} Pasco Fearon,¹
Peter J. Cooper,^{2,3} Christos Pliatsikas,⁴ Ian Goodyer,⁵ and Sarah L. Halligan⁶

¹Division of Psychology and Language Sciences, University College London, London; ²School of Psychology and CLS, University of Reading, Reading; ³Department of Psychology, Stellenbosch University, Stellenbosch; ⁴School of Psychology, University of Kent, Canterbury; ⁵Department of Psychiatry, University of Cambridge, Cambridge; ⁶Department of Psychology, University of Bath, Bath, UK

Background: The quality of the early environment is hypothesized to be an influence on morphological development in key neural areas related to affective responding, but direct evidence to support this possibility is limited. In a 22-year longitudinal study, we examined hippocampal and amygdala volumes in adulthood in relation to early infant attachment status, an important indicator of the quality of the early caregiving environment. **Methods:** Participants ($N = 59$) were derived from a prospective longitudinal study of the impact of maternal postnatal depression on child development. Infant attachment status (24 Secure; 35 Insecure) was observed at 18 months of age, and MRI assessments were completed at 22 years. **Results:** In line with hypotheses, insecure versus secure infant attachment status was associated with larger amygdala volumes in young adults, an effect that was not accounted for by maternal depression history. We did not find early infant attachment status to predict hippocampal volumes. **Conclusions:** Common variations in the quality of early environment are associated with gross alterations in amygdala morphology in the adult brain. Further research is required to establish the neural changes that underpin the volumetric differences reported here, and any functional implications. **Keywords:** Attachment, brain development, amygdala, longitudinal, maternal depression.

Introduction

Animal research has established that early stress exposure is a key influence on neural development in regions that are central to affective responding and stress reactivity (Huang & Lin, 2006; Meaney, 2001; Ono et al., 2008; Rainekei, Cortes, Belnoue, & Sullivan, 2012). In particular, rodent studies have demonstrated that maternal separation or poor care may result in decreased neurogenesis and glucocorticoid receptor density in the hippocampus (Huot, Plotsky, Lenox, & McNamara, 2002; Ladd, Huot, & Plotsky, 2000), with associated hippocampal volumetric reductions (Meaney, 2001). Early stress exposure has also been linked to altered amygdala morphology and function in animal studies (Caldji et al., 1998); disrupted maternal care in rodents has been associated with precocious myelination (Ono et al., 2008) and enhanced amygdala activation in response to stress (Huang & Lin, 2006; Rainekei et al., 2012). These observations are of considerable interest, given the central role that these regions play in emotional processing and responding. The amygdala is a major component of the neural circuitry of emotion, contributing to detection and evaluation of emotionally salient stimuli, as well as the expression of emotional responses such as fear (Whalen & Phelps, 2010). By contrast, the hippocampus exerts strong regulatory control over the hypothalamic–pituitary–adrenal (HPA) axis stress response system,

ultimately serving to modulate cortisol output via hippocampal glucocorticoid receptors (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). Although the findings from nonhuman primate studies concerning longer term effects of maternal separation on brain development are more complicated, such observations from the animal literature have stimulated investigation of whether human neural development may be similarly sensitive to the quality of care received in early life (Gunnar & Quevedo, 2007).

Early experience has been examined in relation to human hippocampal and amygdala morphology across several contexts, with some consistent observations emerging. Adults with PTSD who experienced maltreatment in childhood show smaller hippocampi than their nonmaltreated counterparts, although an equivalent effect has not been found in children (McCrary, De Brito, & Viding, 2010). In addition, in a study of normative variations in maternal care, higher levels of maternal support in early childhood were found to be strongly predictive of larger hippocampal volumes in school-age children (Luby et al., 2012). With regard to the amygdala, overt childhood maltreatment has not been linked to morphology, at least as evidenced by the gross alterations indexed by volumetric measurement (McCrary et al., 2010). However, enlarged amygdala volumes have been observed in two samples of orphanage-reared children, where environments are characterized by neglect (Mehta et al., 2009; Tottenham et al., 2010). In addition, Lupien and colleagues found amygdala

Conflict of interest statement: No conflicts declared.

volumes to be increased in the context of chronic maternal depressive symptoms, an association that was hypothesized to be a consequence of the withdrawn parenting that typically occurs in this context (Lupien et al., 2011). Direct observation of aspects of the parent–child relationship is required to confirm that variations in the early nurturing environment are linked to gross alternations in amygdala morphology.

The attachment bond between an infant and their primary caregiver is considered to be a biologically primed, fundamental aspect of the early caregiving environment (Ainsworth, Blehar, Waters, & Wall, 1978; Bowlby, 1979). Securely attached infants experience interactions with their primary caregivers that are broadly sensitive and responsive to the infant's emotional needs, whereas insecurely attached infants are more often subject to inconsistent, withdrawn or negative/rejecting caregiver responses (Ainsworth et al., 1978; de Wolff & van IJzendoorn, 1997). Correspondingly, different patterns of infant attachment behaviours are displayed. Infants with secure attachment patterns tend to seek proximity when anxious and find contacting effective in reducing their distress. Insecure attachment patterns, where infants may avoid contact, become angry and inconsolable or disorganized in their attachment behaviours, are thought to represent suboptimal behavioural strategies for responding to threat which leave insecurely attached infants more vulnerable to stress than their secure counterparts (Cassidy & Shaver, 2008; Fox & Hane, 2008). Attachment is thus a potentially important mechanism via which the quality of care may influence the developing child's experience of and responding to stress. Consistent with this, several studies have indicated that children who show insecure attachment patterns respond with heightened cortisol responses to separation and to other stressors relative to secure children (Ahnert, Gunnar, Lamb, & Barthel, 2004; Hertsgaard, Gunnar, Erickson, & Nachmias, 1995; Spangler & Grossmann, 1993).

Early experiences of stress that occur in association with attachment insecurity may, in principle, serve to influence neural development in sensitive regions including the hippocampus and amygdala (Gunnar & Quevedo, 2007; Lupien, McEwen, Gunnar, & Heim, 2009). Little direct evidence on this point is available. However, previous work by our group supports the possibility of altered neural outcomes in relation to infant attachment security; in a functional magnetic resonance imaging (fMRI) study, we found evidence of less effective prefrontal neural engagement during attempts to upregulate positive emotions in young adults who were insecurely versus securely attached infants (Moutsiana et al., 2014). Here, in the same longitudinally studied sample, we investigated whether insecure versus secure early mother–infant attachments predict hippocampal and/or amygdala volumes in adulthood, as a preliminary test of the hypothesis that key

aspects of the caregiving environment that are captured by attachment status may influence morphological neurodevelopment. Our sample, originally recruited shortly following birth, comprised individuals whose mothers were suffering from postnatal depression (PND; $n = 28$) together with control participants whose mothers did not have PND ($n = 31$). Attachment status was assessed at 18 months via direct observation using the Strange Situation, the gold standard in the field; and structural MRI scans were completed at age 22 years. We hypothesized that insecure versus secure attachments would be associated with smaller hippocampal volumes, and/or larger amygdalae.

Several covariates were considered. First, whole brain volume, hemisphere/laterality and gender were taken account of in all analyses, as each of these typically explains important variance in neural structures (Brain Development Cooperative Group, 2011; Free et al., 1995). We also considered maternal depression and participant's own history of depression and anxiety as potential covariates for analyses. Previous research has linked the presence of youth or adult depression (Caetano et al., 2007; Campbell, Marriott, Nahmias, & MacQueen, 2004; MacMaster et al., 2008; Videbech & Ravnkilde, 2004), and also family history of depression (Baare et al., 2010; Chen, Hamilton, & Gotlib, 2010; Rao, Chen, et al., 2010) with reduced hippocampal volumes, although not always consistently (Luby et al., 2012; Lupien et al., 2011; Pannekoek et al., 2014; Rosso et al., 2005). Similarly, there have been observations of enlarged amygdalae in association with familial risk of depression (Lupien et al., 2011; Romanczuk-Seiferth et al., 2014), and with individual symptoms of anxiety (Baur, Hanggi, & Jancke, 2012; MacMillan et al., 2003; Qin et al., 2014; Tottenham et al., 2010), although again there have been some mixed findings (Munn et al., 2007). Given that maternal PND was prevalent in our sample and that significant rates of anxiety and depressive disorders were also identified in our participants by adolescence (Halligan, Murray, Martins, & Cooper, 2007), these factors were considered to be important possible confounds.

Methods

Procedures were approved by the University of Reading and the National Health Service Research Ethics Committees. Participants provided written informed consent prior to taking part.

Participants

Participants were derived from a prospective longitudinal study of the development of children of postnatally depressed and well women (Murray, 1992). The sample was originally recruited at 2 months postpartum, through screening a community sample of primiparous mothers of healthy, full-term infants for PND. To be considered for inclusion in the study, mothers needed to be aged 18 years or older, married or cohabiting, and the primary caregiver for the infant. The Edinburgh Postnatal Depression Scale (EPDS; Cox, Holden, &

Sagovsky, 1987) was administered at 6 weeks postpartum, and women scoring over 12 were interviewed; 61 women who met research diagnostic criteria (Spitzer, Endicott, & Robins, 1978) for depressive disorder were identified, 58 of whom were recruited for the study. Forty-two nondepressed mothers were also recruited from the same postnatal population; once a PND group mother was recruited, the next mother to screen negative for PND was invited to take part in this control group. All control group mothers identified agreed to participate. (For full details of recruitment see Murray, 1992).

At 22 years of age, 45 (76%) offspring from the PND group and 39 (93%) from the comparison group were available for the study. Of these, 63 (i.e. 75% of the available sample) were able to complete MRI scanning (exclusions were due to diabetes, epilepsy, metal implants, and inability to attend a session at the University). Complete structural scans were not obtained for one participant, and a further three participants were excluded due to structural abnormalities ($n = 2$) or inadequate image resolution ($n = 1$). The final sample comprised 29 females and 30 males, mean age 22 years.

Measures

Infant attachment. At 18 months infant attachment was assessed using the Ainsworth's Strange Situation Procedure, the gold standard observational measure of attachment security (Ainsworth et al., 1978; Main & Solomon, 1990). Attachment patterns are categorized according to the infant's response to separation from, and particularly reunion with, a primary caregiver (in this case the mother) in an unfamiliar environment. The assessment yields four classifications: a 'secure' category, and three nonsecure categories (insecure avoidant, insecure ambivalent, and disorganized). Securely attached infants show a positive response to being reunited with their parent, and, if distressed by the separation, are easily comforted. Insecure avoidant infants show little distress at separation and limited proximity seeking and/or active avoidance of the parent upon reunion. Insecure ambivalent infants show high levels of distress at separation and proximity seeking upon reunion, but cannot be readily consoled by their parent. Finally, infant disorganization/disorientation is characterized by a range of incoherent or contradictory behaviours during separations and reunions or signs of fear in relation to the parent. Attachment was classified from videotapes following the standard procedures (Ainsworth et al., 1978; Main & Solomon, 1990). Cohen's kappa coefficient for the secure, avoidant, ambivalent, and disorganized attachment classifications of the two researchers who independently scored 63 randomly selected videotapes of the Strange Situation was .94. Following the majority of previous studies, we based analyses on comparisons of secure versus insecure infants.

Offspring psychological disorder. Offspring depressive and anxiety disorders were indexed via the Structural Clinical Interview for DSM-IV at 22 years (Spitzer, Williams, & Gibbon, 1995); and at 8, 13 and 16 years via the Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Lifetime Version (Kaufman et al., 1997). At 22 years alcohol and substance abuse/dependence were also assessed. Interviews were conducted by trained researchers blind to group status. At every time point, participants reported on current and previous disorder, and the resultant information was used to establish lifetime diagnoses (present/absent).

Maternal depression. Maternal depression was assessed using the SCID-IV at 22 years by interviewers blind to group. Clinical interviews were similarly conducted at 18 month, 5 year, 8 year, 13 year and 16 year assessments,

meaning that we were able to establish total number of months of maternal depression over the offspring's lifetime.

MRI data acquisition and processing. High-resolution three-dimensional (3D) T1-weighted images were acquired on a 3-T whole-body scanner (Siemens MAGNETOM Trio) with a 12-channel Head Matrix coil. The MRI parameters of the 3D magnetization-prepared rapid gradient-echo (3D-MPRAGE sequence) were the following: FOV = 250×250 mm², TR/TE/TI/FA = 2020 ms/2.52 ms/1.1/9°. The images were acquired with an in-plane spatial resolution of 0.9765 mm and with 176 contiguous sagittal 1-mm-thick slices. Thus, nearly isotropic three-dimensional MR data sets were obtained, to allow accurate volumetric MR measurements.

Raw structural data were converted from the DICOM format into the NIfTI format through the dcm2nii software (Rorden & Brett, 2000). We first inspected all raw images for abnormalities. Images from three participants were excluded from the analysis, two of them due to structural abnormalities, and one due to very poor resolution because of head motion. All data processing was carried out using FSL version 4.1.8 (FMRIB's Software Library: www.fmrib.ox.ac.uk/fsl; Smith et al., 2004; Woolrich et al., 2009). Nonbrain tissue was removed from the high-resolution anatomical images using BET (Smith, 2002), and the remaining voxels were summed up to give an estimate of the total intracranial volume (TIV) per participant, which we used as a covariate in subsequent analysis. Volume of interest analysis was carried out with individual hippocampal and amygdala masks using the automated FSL tool, FIRST version 1.2 (FMRIB's Integrated Registration and Segmentation Tool). FIRST is widely used for volumetric analyses of subcortical brain units, with results comparable with those obtained through semiautomated manual segmentation; and yields volumetric estimates on bilateral amygdalae and hippocampal units that are highly comparable to manual tracing (Morey et al., 2009). Nonzero voxels/volumes within the masks were calculated in mm³ using Fslstats (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Fslutils>). All volumetric analyses were performed in subject-native space (i.e. not in MNI template space).

Results

Sample characteristics are presented in Table 1, by attachment status; age was highly constrained

Table 1 Sample characteristics reported by attachment status

	18-month attachment status	
	Secure ($N = 35$)	Insecure ($N = 24$)
Age in years, M (SD)	22.3 (.65)	22.5 (.64)
Proportion female, N (%)	20 (57.1)	9 (37.5)
Social class I, II and III nonmanual, N (%)	33 (71.7)	19 (61.3)
Education after 18 years, N (%)	27 (61.4)	22 (75.9)
Lifetime depressive disorder, N (%)	8 (22.9)	10 (41.7)
Lifetime anxiety disorder, N (%)	9 (25.7)	7 (29.2)
Any psychotropic medication use, N (%)	2 (6.9)	1 (3.3)
Maternal postnatal depression, N (%)	12 (34.3)	16 (66.7)**
Total months maternal depression, M (SD)	16.2 (24.8)	29.5 (29.8)*

* $p < .10$; ** $p < .001$; PND, postnatal depression.

Table 2 Results of linear regression analyses examining hippocampal and amygdala volume in relation to maternal postnatal depression, total study months of depression, participant history of depression and anxiety, education and socioeconomic status; separate analyses conducted for each variable

	Total hippocampal volume		Total amygdala volume	
	Parameter	Model ^a	Parameter	Model ^b
Maternal postnatal depression ^c	$\beta = .10, p = .39$	$R^2 = .26, p = .001$	$\beta = .01, p = .93$	$R^2 = .07, p = .25$
Maternal total months depression	$\beta = .24, p = .038$	$R^2 = .32, p < .001$	$\beta = .12, p = .38$	$R^2 = .08, p = .22$
Participant lifetime depressive disorder ^c	$\beta = .23, p = .046$	$R^2 = .30, p < .001$	$\beta = .10, p = .46$	$R^2 = .08, p = .30$
Participant lifetime anxiety disorder ^c	$\beta = .12, p = .34$	$R^2 = .26, p = .001$	$\beta = -.04, p = .77$	$R^2 = .07, p = .25$
Education ^d	$\beta = .16, p = .18$	$R^2 = .29, p = .001$	$\beta = -.02, p = .86$	$R^2 = .07, p = .29$
Socioeconomic status	$\beta = -.17, p = .15$	$R^2 = .27, p < .001$	$\beta = .05, p = .69$	$R^2 = .07, p = .24$

^aModel includes total intracranial volume and gender, $R^2 = .25, F_{2,56} = 9.27, p < .001$.

^bModel includes total intracranial volume and gender, $R^2 = .07, F_{2,56} = 2.15, p = .13$.

^c0 = disorder absent, 1 = disorder present.

^d1 = age 16 years educational qualification equivalent, 2 = age 18 years equivalent, 3 = more than 18 years equivalent.

(range 21–23 years), and rate of psychotropic medication use was extremely low. As previously described for this sample, nonsecure attachments were almost exclusively of the avoidant subtype ($n = 21$) versus ambivalent ($n = 1$) or disorganized ($n = 2$); and the PND versus control group contained a higher proportion of participants who were insecurely attached as infants (Murray, Halligan, Adams, Patterson, & Goodyer, 2006). Similarly, there was a trend for total months of maternal depression during the course of the study to be higher for insecure versus secure participants.

Key sample characteristics, namely maternal PND status, total months of maternal depression, and participant's own history of depression or anxiety (present/absent), level of education and socioeconomic status, were screened as potential covariates by examining their relationship with volumetric outcomes. Multiple linear regression analyses were conducted, examining total hippocampal/amygdala volumes in relation to each potential confound, controlling for TIV and gender. Results are presented in Table 2. For hippocampal volume, significant positive associations were observed for maternal total months of depression and the presence of a lifetime history of depressive disorder in the participant, but there were no other significant effects. There was no evidence that amygdala volume was related to any of the covariates. Based on these observations, maternal total study months of depression and participant history of depression were controlled for in analyses of hippocampal volume. Gender and TIV were included as covariates for all analyses.

Infant attachment at 18 months

Infant attachment status was investigated as a predictor of hippocampal and amygdala volumes. First, we completed a 2×2 ANCOVA examining hippocampal volume in relation to infant attachment status (secure/insecure) and laterality (left vs. right hemisphere), with TIV, gender, participant history of

depression and maternal total months of depression included as covariates. Results are depicted in Figure 1. We found no significant effects of attachment on hippocampal volume, either as a main effect ($F_{1,52} = 0.12, p = .73, \text{partial } \eta^2 = .002$) or in interaction with hemisphere ($F_{1,52} = 0.04, p = .85, \text{partial } \eta^2 = .001$).

Second, an equivalent analysis was completed for amygdala volume. Results indicated a significant main effect of infant attachment ($F_{1,55} = 4.39, p = .041, \text{partial } \eta^2 = .074$). As can be seen in Figure 1, in line with hypotheses, greater amygdala volumes were found in adults who had been insecurely attached infants relative to their securely attached counterparts. There was no significant attachment \times laterality interaction ($F_{1,55} = 2.54, p = .12, \text{partial } \eta^2 = .044$). Given that the presence/absence of maternal PND was a key defining feature of the sample which was strongly related to attachment, we repeated analyses including PND status as a second independent variable. The attachment main effect was retained ($F_{1,53} = 4.66, p = 0.035, \text{partial } \eta^2 = .081$) and no significant effect of maternal PND emerged ($F_{1,53} = 0.41, p = 0.53, \text{partial } \eta^2 = .008$). Importantly, the maternal PND by attachment status interaction term was also not significant and the estimated effect size was extremely small ($F_{1,53} < 0.001, p = .99, \text{partial } \eta^2 < .001$). Thus, we had no reason to believe that associations between attachment status and amygdala volume were particularly present in one or other of the original recruitment groups.

Discussion

In our sample of young adults who had been studied from birth, we found that the quality of the early mother–child relationship was a significant predictor of amygdala volume in adulthood. Specifically, participants who had insecure attachments at 18 months of age had larger amygdala volumes at 22 years than those who had been securely attached. By contrast, we did not find the quality of the early

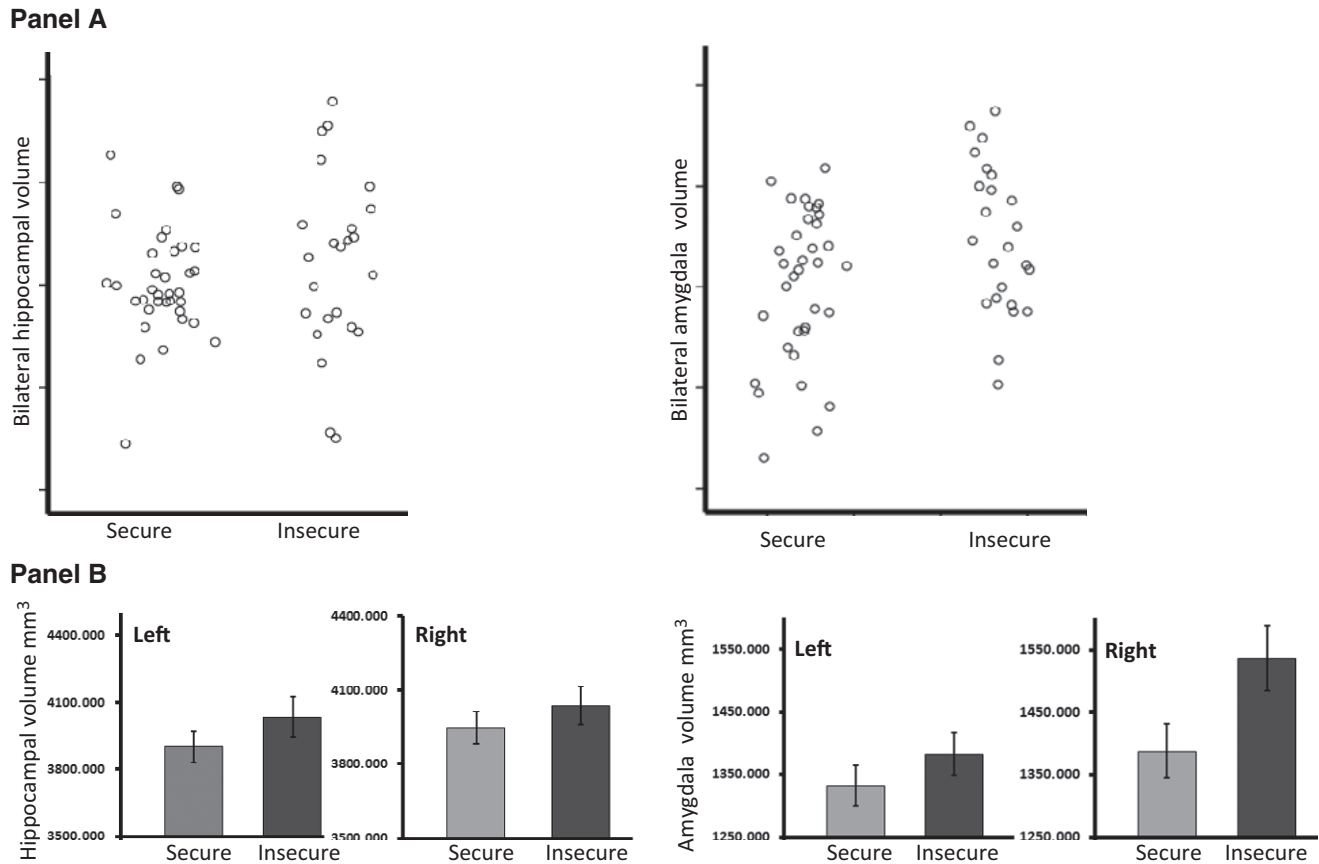


Figure 1 Hippocampal and Amygdala volumes in the secure and insecure attachment groups. Panel A: bilateral volumes are plotted with correction for total intracranial volume using regression analysis; actual values are regression residuals. Panel B: estimated marginal means (with standard errors) for left and right hippocampal and amygdala volumes, corrected for total intracranial volume. In univariate tests secure versus insecure attachment group hippocampal volumes were not significantly different for either right (insecure $M = 4,065 \text{ mm}^3$, $SD = 468$; secure $M = 3,925$, $SD = 385$; $F_{1,55} = 0.77$, $p = .39$) or left hemisphere (insecure $M = 4,059 \text{ mm}^3$, $SD = 509$; secure $M = 3,885$, $SD = 376$; $F_{1,55} = 0.77$, $p = .39$). For amygdala volumes univariate tests indicated significant group differences for right (insecure $M = 1,544 \text{ mm}^3$, $SD = 288$; secure $M = 1,362$, $SD = 266$; $F_{1,55} = 5.07$, $p = .028$), but not left hemisphere (insecure $M = 1,389 \text{ mm}^3$, $SD = 175$; secure $M = 1,327$, $SD = 204$; $F_{1,55} = 0.77$, $p = .39$)

attachment relationship to be a predictor of hippocampal volumes.

Our finding that insecure attachment in infancy predicts amygdala volume in adulthood is consistent with previous reports of enlarged amygdalae, but no changes in hippocampi, in children who experienced early institutional deprivation (Mehta et al., 2009; Tottenham et al., 2010). The majority of insecure attachments (87%) in the current sample were Type A-Avoidant, which is typical of dyads in which mothers are relatively unresponsive to their infant (Ainsworth et al., 1978). As such, our observations complement the conclusions from the postinstitutional adoption studies, by suggesting that normative variations in the extent to which the infant experiences an involved and responsive caregiver may also predict amygdala morphology. With regard to the underlying mechanisms, in principle differences in amygdala volumes may arise as a consequence of more frequent or prolonged activation of the developing child's stress response systems. Not only is attachment conceptualized as both a consequence of less optimal parenting and a behavioural mechanism for regulating resultant stress, but

several studies have also found direct evidence that insecurely attached infants show greater stress reactivity, as indicated by cortisol response to separation and other stressors (Fearon, Groh, Bakermans-Kranenburg, van IJzendoorn, & Roisman, 2014; Fox & Hane, 2008). Research from the animal literature demonstrates that early stress can significantly influence amygdala development (Lupien et al., 2009). Based on the current observations, the possibility that similar influences operate in human development warrants further investigation. Nevertheless, other mechanisms should also be considered, particularly given the correlational nature of our study. Greater amygdala volumes may be related to persistent environmental or behavioural effects occurring in association with insecure infant attachment status, may be a contributor to versus a consequence of attachment status, or may reflect underlying, shared genetic influences.

The functional implications of the current findings remain to be determined. Theoretically, attachment insecurity is held to represent a risk factor for a number of adverse outcomes, particularly in the socioemotional domain (Cassidy & Shaver, 2008).

Previous observations from the same sample studied here have found mother–infant attachment patterns to be an important predictor of offspring socioemotional adjustment and responding in childhood and adolescence (Murray et al., 2006, 2011). As already described, insecure attachments are also associated with greater physiological reactivity to stress, which is likely to be directly influenced by neural activity in the amygdala (Fox & Hane, 2008; Whalen & Phelps, 2010). Previous research has indicated that greater amygdala volumes are associated with trait negative affectivity (Holmes et al., 2012), sensitivity to negative experiences (Barros-Loscertales et al., 2006; Gerritsen et al., 2012), and with the presence of elevated levels of anxiety in both children and adults (Baur et al., 2012; MacMillan et al., 2003; Qin et al., 2014), including in the aforementioned study of postinstitutionalized children (Tottenham et al., 2010). Such findings are notable, given that meta-analysis of attachment-related outcomes has indicated that avoidant attachments, in particular, are associated with elevated rates of internalizing symptoms (Groh, Roisman, van IJzendoorn, Bakermans-Kranenburg, & Fearon, 2012), and suggest that the larger amygdalae observed in the insecure group in the current study may predispose to elevated sensitivity to stress and/or symptoms of anxiety. Nonetheless, we found no association between participant lifetime history of anxiety or depressive disorder and amygdala volumes in our sample. Moreover, in an fMRI study of the same sample studied here, we also found no differences in amygdala responding during an emotion regulation task between those who were securely versus insecurely attached during infancy (Moutsiana et al., 2014). These observations are certainly not conclusive; the fMRI task utilized in our previous report focused on effortful regulation of emotion and particularly recruited prefrontal versus limbic neural regions; and we have limited power to detect associations between amygdala volumes and the presence of psychiatric disorder. However, direct evidence to show that the volumetric differences in amygdalae we observed in association with infant attachment security are of functional importance is still needed.

We failed to confirm previous reports of links between hippocampal volume and aspects of the caregiving environment, although we note that previous findings have been mixed. Thus, while one study found that maternal support in early childhood predicted larger hippocampal volumes in school-aged children (Luby et al., 2012), a second study found that higher levels of maternal warmth in childhood predicted smaller hippocampal volumes in adolescence (Rao, Betancourt et al., 2010). Such discrepancies are likely to reflect differences in the ages of the samples and the types of developmental

experiences studied. It is worth noting that we did find that history of offspring and maternal depression were both related to greater hippocampal volume. However, these findings were in the opposite direction to that reported in some previous research, where smaller hippocampal volumes have been linked to a familial history of depression (Baare et al., 2010; Chen et al., 2010; Rao, Chen, et al., 2010). Given that other studies have also failed to find any association between familial risk of depressive disorder and hippocampal volumes (Luby et al., 2012; Lupien et al., 2011), or have identified larger hippocampi in association with risk status (van Erp et al., 2012; Romanczuk-Seiferth et al., 2014; Ladouceur et al., 2008), the current evidence base does not allow for clear conclusions.

The current study has some key strengths, including the longitudinal design and the rigorous observational assessment of attachment status. There are also some limitations. We have applied overall volumetric measurement, which is a gross indicator of neural morphology that potentially misses fine-grained structural differences that might be detectable in larger samples, or with more sophisticated (e.g. multimodal) or higher field strength scanning protocols. Moreover, findings are essentially correlational and causal influences cannot be inferred. Finally, there are some limitations in our ability to generalize findings; insecure attachments were almost exclusively of the avoidant type and studies of insecure ambivalent or disorganized attachment groups may yield different findings; and the sample had some unique characteristics, including the low risk profile, and the inclusion of a group with maternal PND. Nonetheless, the current analyses provide a proof of principle, demonstrating that the caregiving experiences captured by early attachment status can predict amygdala structure in adulthood. It remains for future studies to evaluate the significance of our observations and to explore the environmental and/or genetic factors that may have contributed to them.

Acknowledgements

This research was funded by Medical Research Council Grant G0701514 to S.L.H. and was supported by the Tedworth Charitable Trust. We thank families in our study for their generosity in assisting with our research throughout the 22 years of the study. The authors have declared that they have no competing or potential conflicts of interest.

Correspondence

Sarah L. Halligan, Department of Psychology, University of Bath, Bath BA2 7AY, UK; Email: s.l.halligan@bath.ac.uk

Key points

- Animal research indicates that the quality of care received early in life influences morphological development in neural structures related to emotional processing.
- In humans, infant attachment status is considered a key indicator of early parenting.
- In a longitudinal study, we examined infant attachment as a predictor of hippocampal and amygdala volumes in early adulthood.
- Insecure versus secure attachment status in infancy predicted larger amygdala volume in adulthood, but was unrelated to hippocampal volume.
- We conclude that normative variations in the quality of early experience are relevant to amygdala morphological development, with possible implications for emotion processing and adjustment.

References

- Ahnert, L., Gunnar, M.R., Lamb, M.E., & Barthel, M. (2004). Transition to child care: associations with infant-mother attachment, infant negative emotion, and cortisol elevations. *Child Development, 75*, 639–650.
- Ainsworth, M.D.S., Blehar, M.C., Waters, E., & Wall, S. (1978). *Patterns of attachment: A psychological study of the strange situation*. Hillsdale, NJ: Erlbaum.
- Baare, W.F., Vinberg, M., Knudsen, G.M., Paulson, O.B., Langkilde, A.R., Jernigan, T.L., & Kessing, L.V. (2010). Hippocampal volume changes in healthy subjects at risk of unipolar depression. *Journal of Psychiatric Research, 44*, 655–662.
- Barros-Loscertales, A., Meseguer, V., Sanjuan, A., Belloch, V., Parcet, M.A., Torrubia, R., & Avila, C. (2006). Behavioral Inhibition System activity is associated with increased amygdala and hippocampal gray matter volume: A voxel-based morphometry study. *Neuroimage, 33*, 1011–1015.
- Baur, V., Hanggi, J., & Jancke, L. (2012). Volumetric associations between uncinate fasciculus, amygdala, and trait anxiety. *BMC Neuroscience, 13*, 4.
- Bowlby, J. (1979). *The making and breaking of affectional bonds*. London: Tavistock.
- Brain Development Cooperative Group (2011). Total and regional brain volumes in a population-based normative sample from 4 to 18 years: The NIH MRI Study of Normal Brain Development. *Cerebral Cortex, 22*, 1–12.
- Caetano, S.C., Fonseca, M., Hatch, J.P., Olvera, R.L., Nicoletti, M., Hunter, K., & Soares, J.C. (2007). Medial temporal lobe abnormalities in pediatric unipolar depression. *Neuroscience Letters, 427*, 142–147.
- Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P.M., & Meaney, M.J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 5335–5340.
- Campbell, S., Marriott, M., Nahmias, C., & MacQueen, G.M. (2004). Lower hippocampal volume in patients suffering from depression: A meta-analysis. *American Journal of Psychiatry, 161*, 598–607.
- Cassidy, J., & Shaver, P.R. (2008). *Handbook of attachment: Theory, research, and clinical applications* (2nd edn). New York: The Guilford Press.
- Chen, M.C., Hamilton, J.P., & Gotlib, I.H. (2010). Decreased hippocampal volume in healthy girls at risk of depression. *Archives of General Psychiatry, 67*, 270–276.
- Cox, J.L., Holden, J.M., & Sagovsky, R. (1987). Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *British Journal of Psychiatry, 150*, 782–786.
- Dedovic, K., Duchesne, A., Andrews, J., Engert, V., & Pruessner, J.C. (2009). The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. *Neuroimage, 47*, 864–871.
- van Erp, T.G.M., Thompson, P.M., Kieseppä, T., Bearden, C.E., Marino, A.C., Hoftman, G.D., ... & Cannon, T.D. (2012). Hippocampal morphology in lithium and non-lithium-treated bipolar I disorder patients, non-bipolar co-twins, and control twins. *Human Brain Mapping, 33*, 501–510.
- Fearon, R.M.P., Groh, A.M., Bakermans-Kranenburg, M.J., van IJzendoorn, M.H., & Roisman, G.I. (2014). Attachment and developmental psychopathology. In D. Cicchetti & D.J. Cohen (Eds.), *Developmental psychopathology, volume 1, theory and method* (2nd edn). New York, NY: Wiley.
- Fox, N.A., & Hane, A.A. (2008). Studying the biology of human attachment. In J. Cassidy & P.R. Shaver (Eds.), *Handbook of attachment: theory, research and clinical applications* (pp. 811–829). New York and London: Guilford Press.
- Free, S.L., Bergin, P.S., Fish, D.R., Cook, M.J., Shorvon, S.D., & Stevens, J.M. (1995). Methods for normalization of hippocampal volumes measured with MR. *AJNR American Journal of Neuroradiology, 16*, 637–643.
- Gerritsen, L., Rijpkema, M., van Oostrom, I., Buitelaar, J., Franke, B., Fernandez, G., & Tendolcar, I. (2012). Amygdala to hippocampal volume ratio is associated with negative memory bias in healthy subjects. *Psychological Medicine, 42*, 335–343.
- Groh, A.M., Roisman, G.I., van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., & Fearon, R.P. (2012). The significance of insecure and disorganized attachment for children's internalizing symptoms: A meta-analytic study. *Child Development, 83*, 591–610.
- Gunnar, M., & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Review of Psychology, 58*, 145–173.
- Halligan, S.L., Murray, L., Martins, C., & Cooper, P.J. (2007). Maternal depression and psychiatric outcomes in adolescent offspring: A 13-year longitudinal study. *Journal of Affective Disorders, 97*, 145–154.
- Hertsgaard, L., Gunnar, M., Erickson, M.F., & Nachmias, M. (1995). Adrenocortical responses to the strange situation in infants with disorganized/disoriented attachment relationships. *Child Development, 66*, 1100–1106.
- Holmes, A.J., Lee, P.H., Hollinshead, M.O., Bakst, L., Roffman, J.L., Smoller, J.W., & Buckner, R.L. (2012). Individual differences in amygdala-medial prefrontal anatomy link negative affect, impaired social functioning, and polygenic depression risk. *Journal of Neuroscience, 32*, 18087–18100.
- Huang, T.Y., & Lin, C.H. (2006). Role of amygdala MAPK activation on immobility behavior of forced swim rats. *Behavioural Brain Research, 173*, 104–111.

- Huot, R.L., Plotsky, P.M., Lenox, R.H., & McNamara, R.K. (2002). Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. *Brain Research*, 950, 52–63.
- Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., ... & Ryan, N. (1997). Schedule for affective disorders and Schizophrenia for school-age children-present and lifetime version (K-SADS-PL): Initial reliability and validity data. *Journal of the American Academy of Child and Adolescent Psychiatry*, 36, 980–988.
- Ladd, C.O., Huot, R.L., & Plotsky, P.M. (2000). Long-term behavioral and neuroendocrine adaptations to adverse early experience. In E.A. Mayer, & C. B. Saper (Eds.), *Progress in brain research: The biological basis for mind body interactions* (pp. 81–103). Amsterdam: Elsevier.
- Ladouceur, C.D., Almeida, J.R., Birmaher, B., Axelson, D.A., Nau, S., Kalas, C., ... & Phillips, M.L. (2008). Subcortical gray matter volume abnormalities in healthy bipolar offspring: Potential neuroanatomical risk marker for bipolar disorder? *Journal of the American Academy of Child and Adolescent Psychiatry*, 47, 532–539.
- Luby, J.L., Barch, D.M., Belden, A., Gaffrey, M.S., Tillman, R., Babb, C., ... & Botteron, K.N. (2012). Maternal support in early childhood predicts larger hippocampal volumes at school age. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 2854–2859.
- Lupien, S.J., McEwen, B.S., Gunnar, M.R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, 10, 434–445.
- Lupien, S.J., Parent, S., Evans, A.C., Tremblay, R.E., Zelazo, P.D., Corbo, V., ... & Séguin, J.R. (2011). Larger amygdala but no change in hippocampal volume in 10-year-old children exposed to maternal depressive symptomatology since birth. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 14324–14329.
- MacMaster, F.P., Mirza, Y., Szeszko, P.R., Kmiecik, L.E., Easter, P.C., Taormina, S.P., ... & Rosenberg, D.R. (2008). Amygdala and hippocampal volumes in familial early onset major depressive disorder. *Biological Psychiatry*, 63, 385–390.
- MacMillan, S., Szeszko, P.R., Moore, G.J., Madden, R., Lorch, E., Ivey, J., ... & Rosenberg, D.R. (2003). Increased amygdala: Hippocampal volume ratios associated with severity of anxiety in pediatric major depression. *Journal of Child and Adolescent Psychopharmacology*, 13, 65–73.
- Main, M., & Solomon, J. (1990). Procedures for identifying infants as disorganized/disoriented during the Strange Situation. In M. Greenberg, D. Cicchetti & E.M. Cummings (Eds.), *Attachment in the preschool years: Theory, research and intervention* (pp. 121–160). Chicago: University of Chicago Press.
- McCrary, E., De Brito, S.A., & Viding, E. (2010). Research review: The neurobiology and genetics of maltreatment and adversity. *Journal of Child Psychology and Psychiatry*, 51, 1079–1095.
- Meaney, M.J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience*, 24, 1161–1192.
- Mehta, M.A., Golemboski, N.I., Nosarti, C., Colvert, E., Mota, A., Williams, S.C., ... & Sonuga-Barke, E.J. (2009). Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: The English and Romanian Adoptees study pilot. *Journal of Child Psychology and Psychiatry*, 50, 943–951.
- Morey, R.A., Petty, C.M., Xu, Y., Pannu Hayes, J., Wagner, H.R., II, Lewis, D.V., ... & McCarthy, G. (2009). A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *Neuroimage*, 45, 855–866.
- Moutsiana, C., Fearon, P., Murray, L., Cooper, P.J., Goodyer, I.M., Johnstone, T., & Halligan, S.L. (2014). Making an effort to feel positive: Insecure attachment in infancy predicts the neural underpinnings of emotion regulation in adulthood. *Journal of Child Psychology and Psychiatry*, 55, 999–1008.
- Munn, M.A., Alexopoulos, J., Nishino, T., Babb, C.M., Flake, L.A., Singer, T., ... & Botteron, K.N. (2007). Amygdala volume analysis in female twins with major depression. *Biological Psychiatry*, 62, 415–422.
- Murray, L. (1992). The impact of postnatal depression on infant development. *Journal of Child Psychology and Psychiatry*, 33, 543–561.
- Murray, L., Arteche, A., Fearon, P., Halligan, S., Goodyer, I., & Cooper, P. (2011). Maternal postnatal depression and the development of depression in offspring up to 16 years of age. *Journal of the American Academy of Child and Adolescent Psychiatry*, 50, 460–470.
- Murray, L., Halligan, S.L., Adams, G., Patterson, P., & Goodyer, I.M. (2006). Socioemotional development in adolescents at risk for depression: The role of maternal depression and attachment style. *Development and Psychopathology*, 18, 489–516.
- Ono, M., Kikusui, T., Sasaki, N., Ichikawa, M., Mori, Y., & Murakami-Murofushi, K. (2008). Early weaning induces anxiety and precocious myelination in the anterior part of the basolateral amygdala of male Balb/c mice. *Neuroscience*, 156, 1103–1110.
- Pannekoek, J.N., van der Werff, S.J., van den Bulk, B.G., van Lang, N.D., Rombouts, S.A., van Buchem, M.A., ... & van der Wee, N.J. (2014). Reduced anterior cingulate gray matter volume in treatment-naïve clinically depressed adolescents. *Neuroimage: Clinical*, 4, 336–342.
- Qin, S., Young, C.B., Duan, X., Chen, T., Supekar, K., & Menon, V. (2014). Amygdala subregional structure and intrinsic functional connectivity predicts individual differences in anxiety during early childhood. *Biological Psychiatry*, 75, 892–900.
- Raineki, C., Cortes, M.R., Belnoue, L., & Sullivan, R.M. (2012). Effects of early-life abuse differ across development: Infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *Journal of Neuroscience*, 32, 7758–7765.
- Rao, H., Betancourt, L., Giannetta, J.M., Brodsky, N.L., Korczykowski, M., Avants, B.B., ... & Farah, M.J. (2010). Early parental care is important for hippocampal maturation: Evidence from brain morphology in humans. *Neuroimage*, 49, 1144–1150.
- Rao, U., Chen, L.A., Bidesi, A.S., Shad, M.U., Thomas, M.A., & Hammén, C.L. (2010). Hippocampal changes associated with early-life adversity and vulnerability to depression. *Biological Psychiatry*, 67, 357–364.
- Romanczuk-Seiferth, N., Pöhlend, L., Mohnke, S., Garbusow, M., Erk, S., Haddad, L., ... & Heinz, A. (2014). Larger amygdala volume in first-degree relatives of patients with major depression. *NeuroImage: Clinical*, 5, 62–68.
- Rorden, C., & Brett, M. (2000). Stereotaxic display of brain lesions. *Behavioural Neurology*, 12, 191–200.
- Rosso, I.M., Cintron, C.M., Steingard, R.J., Renshaw, P.F., Young, A.D., & Yurgelun-Todd, D.A. (2005). Amygdala and hippocampus volumes in pediatric major depression. *Biological Psychiatry*, 57, 21–26.
- Smith, S.M. (2002). Fast robust automated brain extraction. *Human Brain Mapping*, 17, 143–155.
- Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H., ... & Matthews, P.M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*, 23 (Suppl 1), S208–S219.

- Spangler, G., & Grossmann, K.E. (1993). Biobehavioral organization in securely and insecurely attached infants. *Child Development, 64*, 1439–1450.
- Spitzer, R.L., Endicott, J., & Robins, E. (1978). Research diagnostic criteria: Rationale and reliability. *Archives of General Psychiatry, 35*, 773–782.
- Spitzer, R.L., Williams, J.B.W., & Gibbon, M. (1995). *Structured clinical interview for DSM-IV (SCID)*. New York: New York State Psychiatric Institute, Biometrics Research.
- Tottenham, N., Hare, T.A., Quinn, B.T., McCarry, T.W., Nurse, M., Gilhooly, T., ... & Casey, B.J. (2010). Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Developmental Science, 13*, 46–61.
- Videbech, P., & Ravnkilde, B. (2004). Hippocampal volume and depression: A meta-analysis of MRI studies. *American Journal of Psychiatry, 161*, 1957–1966.
- Whalen, P.J., & Phelps, E.A. (2010). *The human amygdala*. New York: Guilford Press.
- de Wolff, M.S., & van IJzendoorn, M.H. (1997). Sensitivity and attachment: A meta-analysis on parental antecedents of infant attachment. *Child Development, 68*, 571–591.
- Woolrich, M.W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T., ... & Smith, S.M. (2009). Bayesian analysis of neuroimaging data in FSL. *Neuroimage, 45*, S173–S186.

Accepted for publication: 22 July 2014