

Adult attachment insecurity and hippocampal cell density

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Recent findings associate attachment insecurity (assessed as levels of attachment anxiety and avoidance) with poor emotion regulation. In turn, emotion regulation has been shown to be associated with hippocampus (HC) functioning and structure. Clinical disorders such as depression and PTSD, which have been previously associated with attachment insecurity, are also known to be linked with reduced hippocampal cell density. This suggests that attachment insecurity may also be associated with reduced hippocampal cell density. We examined this hypothesis using T1 images of 22 healthy young adults. In line with our hypothesis, attachment avoidance was associated with bilateral HC reduction, whereas attachment anxiety was significantly related to reduced cell concentration in the left HC. The findings are compatible with a neurotoxic model of stress-induced cell reduction in the HC, providing further information on emotion regulation abilities among insecurely attached individuals.

Keywords: adult attachment styles; brain; hippocampus cell density; stress; emotion regulation

Structural and functional hippocampal abnormalities have been previously associated with various disorders such as PTSD and depression as well as with psychopathic and aggressive behavior (e.g. Bremner, 1999; Sheline, 2003; Soares and Mann, 1997). As suggested by Chrousos and Gold (1992), the link between mood or emotion-related disorders and hippocampal structure and function might be mediated by the hypothalamic-pituitary-adrenocortical (HPA) axis, the major hormonal stress axis of the organism. Specifically, chronic stress is often associated with a dysregulation of the HPA axis, which might manifest with an increased or decreased cortisol secretion pending various other moderators (see Sbarra and Hazan, 2008 for a comprehensive review). Out of these moderators, a meta-analysis by Dickerson and Kemeny (2004) revealed that negative social evaluation and perceived uncontrollability of a situation are the two factors that have the strongest impact on HPA axis activity.

Increased levels of cortisol were found to have a global impact on cerebral volume in both animals and humans (e.g. McEwen, 2007; Sheline, 2003). For instance, patients suffering from Cushing's Disease, which is characterized by

hypercortisolemia (higher levels of cortisol in the blood), were found to have premature brain atrophy (Simmons, Do, Lipper, and Laws, 2000). Similarly, people who experienced high levels of stress, negative affect, or trauma like PTSD patients also manifest brain atrophy (see Bremner, 1999; Gross, 2007). This raises the question whether individual conditions associated with increased stress reactivity, impaired emotion regulation and HPA dysregulation, such as high attachment insecurity, are also associated with brain cell density reductions. In the current study, we investigated whether attachment insecurity, which has been shown to be associated with poor emotion regulation (for a review see Shaver and Mikulincer, 2007) and cortisol dysregulation (Adam and Gunnar, 2001; Quirin, Pruessner, and Kuhl, 2008), is associated with reduced HC cell density.

According to Bowlby's (1969, 1973, 1980) and Ainsworth's (Ainsworth, Blehar, Waters, and Wall, 1978) attachment theory, a person's approach to close relationships or his or her *attachment style* is shaped by his or her interactions with primary care-givers. Sensitive supportive care-giving environment is likely to result in a secure attachment style, whereas an inconsistent, insensitive, intrusive, or rejecting care-giving is likely to result in an insecure attachment style. These individual differences in attachment style are commonly conceptualized along two dimensions: attachment anxiety, and avoidant attachment (e.g. Brennan, Clark, and Shaver, 1998). Attachment anxiety is associated with continuous worries about rejection and abandonment, whereas attachment avoidance refers to the reluctance to get close or intimate with a relationship partner and with suppression of rather than efficient coping with emotions (for review see Shaver and Mikulincer, 2007).

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Attachment anxiety has been consistently found to be associated with an increased reactivity to both attachment-related and nonattachment-related stressors among adults (see Sbarra and Hazan, 2008, as well as Shaver and Mikulincer, 2007, for reviews). Specifically, attachment anxiety was found to be positively correlated with increased activity in brain regions typically involved in the generation of negative affect (e.g. temporal pole) and memories (HC). The activation was found especially when participants were retrieving negative memories, and co-occurred with reduced activity in brain regions typically involved in down regulating negative emotions such as the orbito-frontal cortex (Gillath, Bunge, Shaver, Wendelken, and Mikulincer, 2005). Moreover, attachment anxiety has been found to be related with poorer ability to down-regulate physiological arousal when needed, and with longer time getting back to baseline after experiencing stress (e.g. Fraley and Shaver, 1997; Gillath, Shaver, Mendoza, Maninger and Ferrer, 2006).

Attachment avoidance, on the other hand, has been found to be associated with a pronounced tendency to downplay or suppress one's emotions as a way to defend oneself from dealing with intimacy and emotions that are threatening for highly avoidant people (Shaver and Mikulincer, 2007). Using suppression as a defense mechanism as employed by people high on attachment avoidance, however, does not successfully attenuate physiological stress levels, as indicated by increased heart rate or skin conductance level when dealing with stressors (e.g. Carpenter and Kirkpatrick, 1996; Diamond, Hicks, and Otter-Henderson, 2006; Mikulincer, 1998; Roisman, Tsai, and Chiang, 2004; for a review see Sbarra and Hazan, 2008). For example, Diamond *et al.* (2006) found that avoidantly attached individuals show increased skin conductance levels across a diversity of stressors, namely separation-related stress, mathematical calculations, public speaking, and anger recall. Moreover, when avoidant people experience additional load, their defenses seem to collapse and they look similar to their anxiously attached counterparts (e.g. Mikulincer, Dolev, and Shaver, 2004).

Recently, this line of research was extended to further describe the association between the two attachment dimensions and HPA axis regulation among adults (Diamond, Hicks, and Otter-Henderson, 2008; Gillath *et al.*, 2006; Powers, Pietromonaco, Gunlicks, and Sayer, 2006; Quirin *et al.*, 2008). In these studies, attachment anxiety was positively related to stress-induced cortisol increase. Additionally, Quirin *et al.* (2008) found that attachment anxiety was inversely related to the cortisol awakening response, in line with an earlier study investigating the same associations (Adam and Gunnar, 2001). Previous research showed that the HC mediates negative feedback of HPA axis activity after acute stress (Jacobson and Sapolsky, 1991), whereas it mediates positive feedback of the HPA axis at awakening (Buchanan *et al.*, 2004; Pruessner *et al.*, 2005; Wolf, Witt, and Hellhammer, 2004). Based on these findings,

Quirin *et al.* (2008) suggested that the patterns found for individuals high on attachment anxiety might result from impaired HC functioning or reduced HC structure. By contrast, although attachment theory posits that avoidantly-attached individuals suffered from early childhood stress (Shaver and Mikulincer, 2007), there is less evidence for cortisol dysregulations in this population as compared to anxiously-attached adults. However, Powers *et al.* (2006) found that attachment avoidance in women (and not in men) was associated with increased cortisol levels in anticipation of and during a relationship conflict.

Although attachment anxiety and avoidance seem to differ with respect to behavioral reactions to stress – perhaps due to learning and employment of different coping strategies (Gillath, Giesbrecht, and Shaver, *in press*; Shaver and Mikulincer, 2007), they may nevertheless show a similar neuro-development pattern with respect to the HC. The poor quality of early maternal care, which presumably leads to increased levels of stress in the early years of life and, not independently, to less efficient stress regulation abilities, characterizes both anxiously and avoidantly attached people. Impaired emotion regulation abilities have previously been linked to inappropriate functional and structural properties of the HC (Davidson, Pizzagalli, Nitschke, and Kalin, 2003; Kuhl, 2000, 2001). Indirect support to this suggested association between HC integrity and attachment insecurity comes from Buss *et al.* (2007) and Pruessner, Champagne, Meaney, and Dagher (2004). Those authors found that maternal care, which has been revealed to be lower in quality among insecure individuals (De Wolff and van Ijzendoorn, 1997), was also predictive of HC size. More specifically, poorer care combined with prenatal stressors was found to be a reliable predictor of a smaller HC size.

Based on this literature review, we hypothesized that insecure attachment as expressed by high levels of attachment anxiety or avoidance would be inversely related to gray matter cell concentration in the HC. Although we did not have specific hypotheses about other regions, we explored the possibility that gray matter concentration in other regions of the brain would also be correlated with levels of attachment anxiety and avoidance.

METHOD

Participants

Twenty-two students (11 females, age ranged from 20 to 35 years, $M = 24.09$, $s.d. = 3.68$) with no reported history of psychiatric or neurological illness volunteered in this study. Participants were screened for substance abuse within the past 6 months, and were not on any psychiatric medication. All participants were right-handed and Caucasian.

Psychological assessment

Participants completed the Experiences in Close Relationships scale (ECR; Brennan *et al.*, 1998; German

Version by Neumann, Rohmann, and Bierhoff, 2007). Eighteen items on the scale assess attachment anxiety (e.g. "I worry about being abandoned") and 18 items assess avoidance (e.g. "I prefer not to show a partner how I feel deep down"). Participants rated the extent to which each item was descriptive of their experiences in close relationships on a 7-point scale ranging from 1 (*not at all*) to 7 (*very much*). The reliability and validity of the scales have been repeatedly demonstrated in numerous studies, and the scales scores were found to correlate with attitudes, emotions, behaviors, genetic polymorphisms, and brain activation (for reviews, see Cassidy and Shaver, 2008, Shaver and Mikulincer, 2007). In the present study, attachment anxiety and avoidance were positively correlated but not significantly, $r=0.55$, $P<0.10$.

For control purposes, we assessed neuroticism using the NEO-FFI (Costa and McCrae, 1992). Although attachment anxiety and neuroticism are typically correlated (e.g. Nofle and Shaver, 2006), they are known to represent separate constructs, which were shown to relate with different brain activation patterns (Gillath *et al.*, 2005). In the present study, neuroticism was not significantly correlated with attachment avoidance, $r=0.38$, ns, but was positively correlated with attachment anxiety, $r=0.58$, $P<0.05$. Additionally, we assessed self-reported chronic stress with the Perceived Stress Scale (PSS; Cohen, Kamarck, and Mermelstein, 1983) since reduced hippocampal volume has been associated with stress-related disorders such as depression or PTSD (e.g. Bremner, 1999), or cortisol dysregulation (Lupien *et al.*, 1998; Pruessner *et al.*, 2005). In the present sample perceived stress was negatively correlated with both attachment anxiety, $r=-0.50$, $P<0.05$, and (nonsignificantly) with attachment avoidance, $r=-0.42$, $P<0.10$.

MRI acquisition

High-resolution morphological brain images were acquired at 3T on a Siemens head-scanner (Allegra, Siemens, Erlangen, Germany) with a circular polarized birdcage head coil using a T1-weighted 3D-MPRAGE (Magnetization Prepared Rapid Gradient Echo) sequence with the following parameters: TE = 4.83 ms, TR = 2.3 s, TI = 900 ms, flip angle = 8°, band width = 130 Hz/pixel, matrix size = 256 × 256 × 160, spatial resolution = 1 × 1 × 1 mm.

Voxel-Based Morphometry. We drew on Voxel-Based Morphometry to quantify localized gray matter concentration in the whole brain (cf. Ashburner and Friston, 2000). All structural images were converted into MINC-file format (Neelin, 1992) using NeuroLens 1.4.8 (www.neurolens.org) and visually inspected to ensure image quality and to check for abnormalities. Images were corrected for non-uniformity and normalized for intensity (Sled, Zijdenbos, and Evans, 1998). In the next step images were linearly registered (Collins, Neelin, Peters, and Evans, 1994) to the Montreal Neurological Institute (MNI) 305 average brain.

This procedure consists of creating the transformation matrix of the alignment of images and co-planar, stereotaxic brain atlas and, after recovering the transformation matrix, re-sampling of the images along new spatial dimensions with new voxel positions to the ICBM 1mm-template. It could be shown that VBM is more sensitive when the MNI template is used for normalization instead of study specific templates, although Type I error may occur (Shen, Sterr, and Szameitat, 2005). Subsequently, tissue classification was conducted using the INSECT algorithm (Collins and Evans, 1999; Evans, Collins, and Holmes, 1996; Zijdenbos, Forghani, and Evans, 1998). Briefly, INSECT relies on an artificial neural network classifier, which labels each voxel based on the according MRI signal and therefore results in the automatic separation of tissue into its three types: white matter, gray matter, and cerebrospinal fluid (CSF).

After classification, the image was re-sampled to only contain gray-matter tissue type. A cortical surface was then created for each volume. Using the cortical surface, the skull and the dura were masked from the brain so that they did not interfere with the data. After the corresponding binary mask of voxels of interest was extracted from the classified image, this mask was smoothed using a Gaussian kernel of 6 mm full-width half-maximum (fwhm) in each image (e.g. Watkins *et al.*, 2001). The average of the original corrected scans as well as the average of the classified gray matter to be used as a mask was computed using the MNI minctools (www.bic.mni.mcgill.ca/software/). The minctools were also used to choose voxels in the mask, which did not have intensity values below 0.1, i.e. anything above this value was left in the mask. Peaks and clusters as well as corresponding thresholds were computed using *frmstat* (www.math.mcgill.ca/keith/fmristat/).

Statistical analysis

VBM analyses were performed with *Glim Image*, a program developed at the MNI (Paus, Collins, Evans, Leonard, Pike, and Zijdenbos, 2001; Pruessner, Collins, Pruessner, and Evans, 2001; Watkins *et al.*, 2001; Golestani, Paus, and Zatorre, 2002; Tisserand, van Boxtel, Pruessner, Hofman, Evans, and Jolles, 2004). *Glim image* is designed for performing statistical analyses based on the general linear model with minc-image based files. One example for such an operation is Voxel Based Morphometry. The smoothed concentration maps were used to localize differences in tissue concentration, i.e. the gray matter maps were correlated with attachment styles (ECR scores) on a voxel-by-voxel basis using a linear regression model, yielding statistical maps that demarcated regions in which there was a significant difference in the concentration of gray matter. Thus, participants' attachment scores acted as independent variables, and the MR image signal intensity of each voxel acted as a dependent variable in the regression (Ashburner and Friston, 2000).

We chose a threshold of $t=3.53$ ($P<0.001$, uncorrected), and looked for clusters that consisted of voxels reaching this threshold. To correct for multiple comparisons, the P -values at cluster level were determined using Random Field Theory (Worsley et al., 1996). Because we were interested in the HC as a specific region of interest (ROI), we did not search in all peaks and clusters across the brain but chose a reduced extent threshold (cluster size) to determine the cluster P -level (Friston, 1997).

RESULTS

Preliminary analyses revealed two clusters in the left HC in which cell concentration differed for gender (cluster 1: peak x, y, z : -21, -44, 3; $t=-3.67$; 5 voxels; cluster 2: peak x, y, z : -22, -40, 7; $t=-3.69$; 24 voxels). No differences in cell concentration were found for neuroticism, perceived stress, or age. In the following analyses, we therefore controlled for the effects of gender by entering this variable as a covariate into the regression.

As hypothesized, attachment anxiety was negatively correlated with gray matter concentration in the left HC (peak x, y, z : -33, -18, -14; $t=-4.34$; 188 voxels; $P<0.01$ at cluster level; cf. Figure 1 and 4). Attachment avoidance was negatively associated with gray matter concentration in the left HC (peak x, y, z : -23, -9, -25; $t=-4.66$; 94 voxels, $P<0.05$ at cluster level) and showed a trend to be negatively associated with gray matter concentration in the right HC (peak x, y, z : 22, -8, -26; $t=-4.45$; 44 voxels, $P=0.11$ at cluster level) (Figures 2–4).¹

DISCUSSION

This is the first study to investigate associations between attachment dispositions and the gray matter density in the

hippocampal region. As expected, we found that attachment anxiety and avoidance were related to a reduced gray matter density in the HC as shown with voxel-based morphometry. Thus, the present study adds to the growing literature on physiological and neural correlates of attachment dispositions. Moreover, this work expands research on the association between HC structure, psychopathology, and chronic stress, contributing to the field of clinically relevant personality dispositions.

It is not clear whether reductions of gray matter concentration associated with attachment insecurity have any

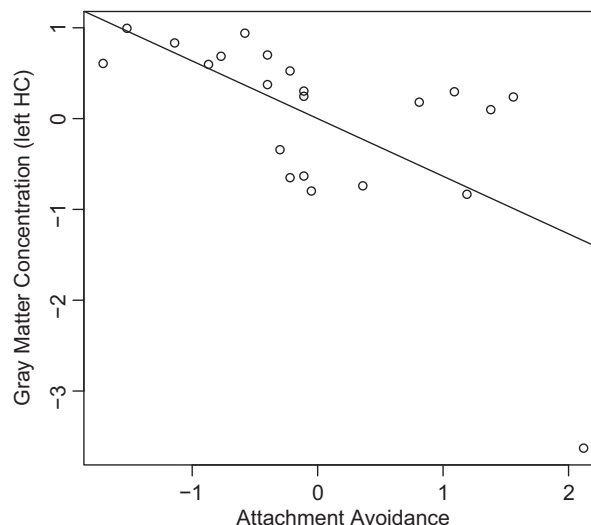


Fig. 2 Scatter plot for the correlation between attachment avoidance and gray matter concentration in the left hippocampus corrected for the influence of gender. $r=-0.63$, $P<0.01$; $r=-0.49$, $P<0.05$, after elimination of an outlier greater than $M \pm 3SD$.

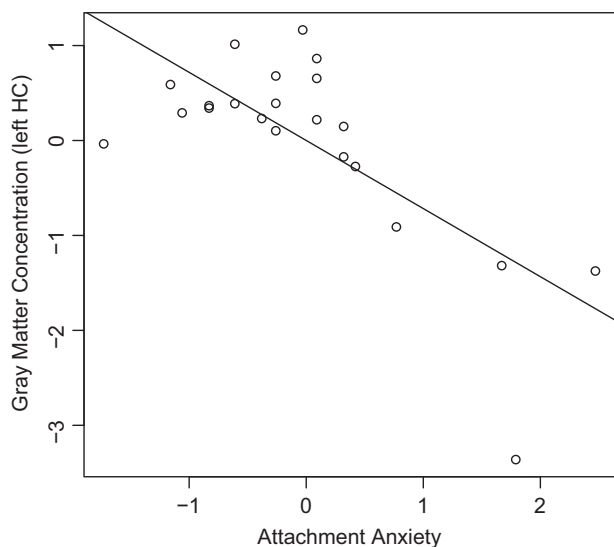


Fig. 1 Scatter plot for the correlation between attachment anxiety and gray matter concentration in the left hippocampus corrected for the influence of gender. $r=-0.72$, $P<0.001$; $r=-0.69$, $P<0.001$, after elimination of an outlier greater than $M \pm 3SD$.

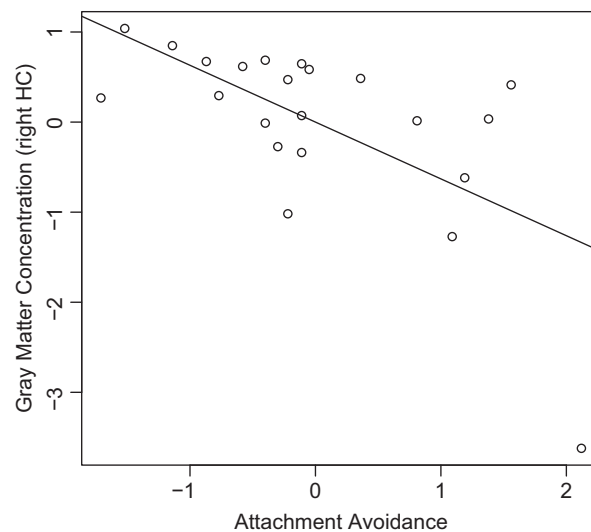


Fig. 3 Scatter plot for the correlation between attachment avoidance and gray matter concentration in the right hippocampus corrected for the influence of gender. $r=-0.63$, $P<0.01$; $r=-0.48$, $P<0.05$, after elimination of an outlier greater than $M \pm 3SD$.

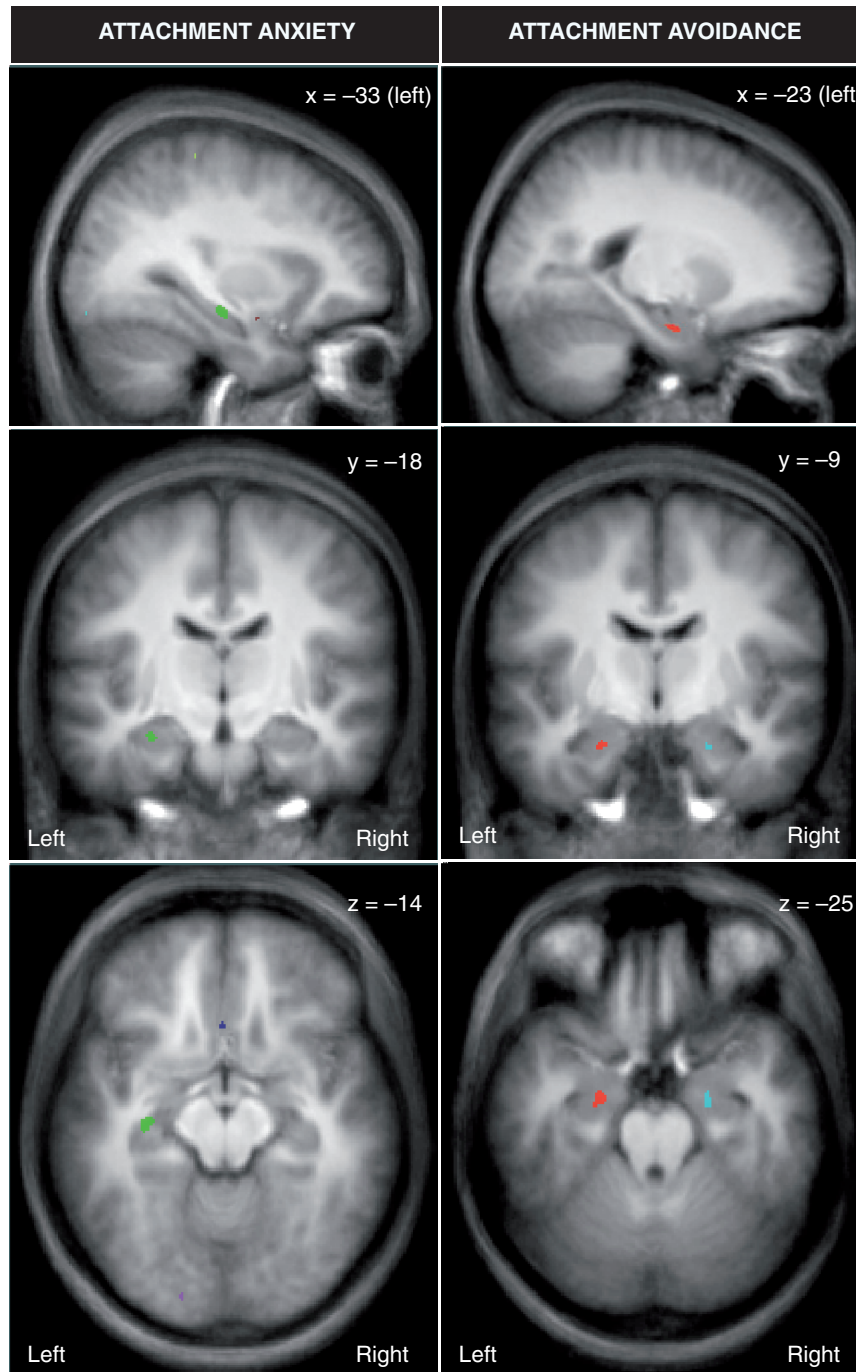


Fig. 4 Clusters of gray matter concentration in the hippocampus correlated with attachment styles. Lower gray matter concentrations in the left hippocampus were found for participants with high attachment anxiety scores (left). Lower gray matter concentration in the left as well as in the right hippocampus were found for participants with higher attachment avoidance scores (right). Statistical cluster maps are overlaid on the average brain of the sample; *t*-maps show differences in gray matter concentration at each gray matter voxel of the brain; all clusters (defining threshold of $P < 0.001$) are shown in the sagittal, coronal and horizontal view.

significance with respect to the functional properties of the HC – they may simply provide an additional marker of stress reactivity (e.g. Erickson *et al.*, 2006, for a debate about the association between functional and structural properties of brain regions). However, with respect to the role of the HC in glucocorticoid stress regulation (Herman, Ostrander, Mueller, and Figueiredo, 2005), the present

findings are compatible with broad evidence of reduced stress regulation capabilities in insecurely attached individuals (e.g. Shaver and Mikulincer, 2007). It may thus be speculated that reduced hippocampal cell density constitutes a neural underpinning of reduced stress regulation ability among insecurely attached individuals. This idea is in accordance with previous claims about the ways by which

the HC may support affect regulation apart from glucocorticoid regulation (e.g. Kuhl, 2000). For example, the HC was found to be involved in the modulation of unconditioned and conditioned associations between emotional and motor reactions to complex stimuli (e.g. Schmajuk and DiCarlo, 1992). As the operability of the HC increases within the first years of life, children's ability to inhibit emotional reactions towards threatening stimuli when they occur along with a discriminating stimulus increases. For example, a lion in the zoo is perceived as less threatening when it shows up with bars in front of it. This process, in turn, leads to an extinction of the emotional reaction (Kuhl, 2001). By contrast, the development of such HC-dependent affect regulatory abilities may be impaired in the case of early glucocorticoid intoxication. Insecurely attached individuals might experience such a condition, which in turn might lead to impaired regulatory abilities. These underdeveloped or impaired abilities are associated with HC functions such as negative patterning, which may persist throughout life (see Kuhl, 2001, for a review of hippocampal functions potentially relevant for affect regulation).

Our findings highlight the importance of considering individual differences in stress reactivity (as can be measured by attachment dimensions) when modeling the effects of psychological trauma on biological systems. For example, reduced HC volume has been found in PTSD (e.g. Bremner, 1999). However, there is an ongoing controversy as to whether PTSD causes HC reduction or whether individuals with a reduced HC (Gilbertson *et al.*, 2002) are inclined to develop PTSD. Because attachment insecurity is thought to develop in an early age and to stay relatively stable (e.g. Fraley and Brumbaugh, 2004), and has been found to be increased in individuals with PTSD (e.g. Fraley, Fazzari, Bonanno, and Dekel, 2006, Selcuk and Gillath, 2009; for a review see Shaver and Mikulincer, 2007), it has the potential to act as a predisposing factor to changes in regional cell densities, which later leads to the development of PTSD; obviously these links will have to be examined in further research before clear conclusions can be drawn.

The present findings referring to attachment insecurity are in line with research in the context of intellectual disability revealing significant negative correlations between general anxiety assessed through a clinical interview and gray matter concentration in the left HC (Spencer *et al.*, 2007). In the present study, however, attachment anxiety was associated with reduced gray matter concentration in the HC, after controlling for the effects of neuroticism. This way of proceeding allows us to rule out the alternative explanation that our results are due to general anxiety or negative perception of the world rather than attachment anxiety. Although attachment anxiety and neuroticism are known to be moderately associated (r s range between 0.3 and 0.4, Nofle and Shaver, 2006), they are considered to be two distinct concepts. Whereas attachment anxiety involves

feelings, beliefs, cognitions, and behaviors that arise mainly in the context of close relationships, neuroticism is conceptualized and measured as a broader trait linked with a range of negative emotions in relational and non-relational situations and a general negative perception of the world (Nofle and Shaver, 2006). As such, Gillath *et al.* (2005) found that attachment anxiety and neuroticism, despite some overlap, predicted different regions of neural activity (e.g. regions linked to cognitive information processing were predicted by neuroticism rather than attachment anxiety).

The present findings also extend previous research, suggesting that both types of attachment insecurity have similar underlying substrates. Thus, attachment avoidance and not only attachment anxiety were associated with HC cell density. These findings are in line with previous research suggesting that relationship anxiety underlies attachment avoidance working models. For example, Mikulincer, Dolev, and Shaver (2004) found that when avoidant people are hindered from using their typical defense mechanisms, they act very similar to anxiously attached individuals. Having similar HC cell density suggest one shared mechanism that may contribute to explaining the behavioral and emotional similarity.

Although the HC is strongly implicated in stress regulation (Herman *et al.*, 2005; see also Kuhl, 2001, for a link to psychological models of affect regulation), atrophy of the HC is not considered to be the sole neural underpinning of reduced emotion regulation abilities in insecurely-attached individuals (if at all, given the above debate about the causal direction). For example, the orbito-frontal cortex, which is known to be involved in emotion regulation (Beer, Shimamura, and Knight, 2004), has been found to be less activated in anxiously-attached as compared with their non-anxious counterparts during coping with separation stress (Gillath *et al.*, 2005). However, recent research on the extinction of conditioned responses suggests that the orbito-frontal/ventro-medial prefrontal cortex and the HC interact in regulating stress (Kalisch, Korenfeld, Stephan, Weiskopf, Seymour, and Dolan, 2006).

Limitations

There are some limitations to the present study. First, it should be noted that we asked participants about their history of psychiatric disorders instead of conducting a diagnostic interview. Consequently, although the study relied on young students, we cannot rule out the possibility that one or more participants may have actually suffered from a psychopathological disorder without reporting it. Likewise, we did not ask for a history of sexual, physical, or emotional abuse, which constitute early stressors that can be assumed to influence HC cell density as well (e.g. Schmahl, Vermetten, Elzinga, and Bremner (2003).

Another limitation of the study is its cross-sectional nature. Although the link between attachment styles in adulthood and childhood was already established

(Fraleigh, 2002; De Wolff and van IJzendoorn, 1997; see Simpson, Collins, Tran, and Haydon, 2007, for relationships with the strange situation procedure), other factors, in particular changes in the family environment (Waters, Hamilton, and Weinfield, 2000), may influence the development of attachment styles. Therefore, it cannot be excluded that the relationships between HC gray matter concentration and attachment styles are influenced by factors other than the quality of early rearing conditions (e.g. partnership stress). Also, it cannot be excluded that a genetic predisposition to HC gray matter concentration causally influences the development of attachment styles. Not least, HC gray matter concentration can in part vary as a function of experiences made in the adulthood (Maguire *et al.*, 2000). Therefore, it is not implausible that HC gray matter concentration may increase in response to trainings fostering emotion regulation such as meditation (Holzel *et al.*, 2008), or, speculatively, may even become reduced as a function of stress in adulthood rather than childhood. However, the latter assumption cannot be supported by the present study because perceived chronic stress did not predict HC gray matter concentration.

CONCLUSION

In summary, we found that both attachment anxiety and avoidance were positively associated with HC cell density. These findings are consistent with evidence showing that attachment insecurity is associated with factors that have in turn been found to be related to reduced hippocampal volume, such as reduced quality of parental care and dysregulated glucocorticoid regulation. Despite some limitations, the present study is relevant in several regards. First, it is pioneering neural underpinnings of attachment styles and may thus stimulate future research in this area. Second, the present findings of a link between attachment styles and HC structure gives rise to develop and test models about the role of the HC in attachment-related emotion regulation processes. Third, this evidence has strong implications for research in clinical neurobiology, which has demonstrated reduced HC cell density or volume for a diversity of clinical disorders that are likely to be associated with attachment insecurity.

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