NeuroImage: Clinical 6 (2014) 77–85

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

# Links between white matter microstructure and cortisol reactivity to stress in early childhood: Evidence for moderation by parenting

Haroon I. Sheikh[a,*], Marc F. Joanisse[b], Sarah M. Mackrell[b], Katie R. Kryski[a], Heather J. Smith[b], Shiva M. Singh[a], Elizabeth P. Hayden[b]

[a]Molecular Genetics Unit, Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada
[b]Department of Psychology, Brain and Mind Institute, University of Western Ontario, London, ON N6A 5B7, Canada

ARTICLE INFO

*Article history:*
Received 29 April 2014
Received in revised form 13 August 2014
Accepted 18 August 2014
Available online 20 August 2014

*Keywords:*
HPA axis
Stress
Cortisol
Parenting
Diffusion tensor imaging
Connectivity

ABSTRACT

Activity of the hypothalamic–pituitary–adrenal axis (measured via cortisol reactivity) may be a biological marker of risk for depression and anxiety, possibly even early in development. However, the structural neural correlates of early cortisol reactivity are not well known, although these would potentially inform broader models of mechanisms of risk, especially if the early environment further shapes these relationships. Therefore, we examined links between white matter architecture and young girls' cortisol reactivity and whether early caregiving moderated these links. We recruited 45 6-year-old girls based on whether they had previously shown high or low cortisol reactivity to a stress task at age 3. White matter integrity was assessed by calculating fractional anisotropy (FA) of diffusion-weighted magnetic resonance imaging scans. Parenting styles were measured via a standardized parent–child interaction task. Significant associations were found between FA in white matter regions adjacent to the left thalamus, the right anterior cingulate cortex, and the right superior frontal gyrus (all $ps < .001$). Further, positive early caregiving moderated the effect of high cortisol reactivity on white matter FA (all $ps \leq .05$), with high stress reactive girls who received greater parent positive affect showing white matter structure more similar to that of low stress reactive girls. Results show associations between white matter integrity of various limbic regions of the brain and early cortisol reactivity to stress and provide preliminary support for the notion that parenting may moderate associations.



## 1. Introduction

A growing literature suggests that hypothalamic–pituitary–adrenal (HPA) axis dysfunction may be an important feature of internalizing disorders (i.e., depressive and anxiety disorders). For example, distinct HPA axis responses measured via salivary cortisol to stress have been reported in adults and adolescents with depression and anxiety (Barden, 2004; Rao et al., 2008; Risbrough and Stein, 2006; Shea et al., 2005). Research also suggests that antidepressant treatment reduces HPA axis abnormalities and that such reductions may be necessary for stable remission of depression (DeBellis et al., 1993; Ising et al., 2005; Kling et al., 1994; Nemeroff et al., 1991). However, HPA axis function may be more than a state marker of disorder. Research from our group and others has shown that considerable individual differences in cortisol reactivity to stress exist from an early age (Bosch et al., 2012; Gunnar, 1989, 1992; Jansen et al., 2010; Kryski et al., 2011); further, this individual variability in HPA axis reactivity may be a mechanism by which the associations between environmental stress and disorder are mediated (Holsboer, 2000), although longitudinal work is needed to provide evidence for such models. Unfortunately, little is known about the structural brain correlates of early stress reactivity. This is an important gap in the literature, as brain circuitry and neural organization are posited to influence stress reactions, arousal, emotional regulation, brain development, and cognitive development and may thus contribute to risk for mood and emotion problems (Hart and Rubia, 2012; McCrory et al., 2010; Twardosz and Lutzker, 2010). Thus, more comprehensive models of the biology of early emerging internalizing disorder risk require a better understanding of relations between brain structure and makers of stress reactivity, such as cortisol reactivity to stress.

Most research on the neurological mechanisms related to individual differences in HPA axis reactivity comes from clinical and preclinical studies (see detailed reviews of this literature by Hart and Rubia, 2012; Jankord and Herman, 2008; Pruessner et al., 2010). These studies show that components of the brain's limbic system act as primary regulators of the HPA axis response to stress. For example, bilateral lesions of the medial prefrontal cortex (mPFC) enhance adrenocorticotrophic

* Corresponding author.
*E-mail address:* hsheikh6@uwo.ca (H.I. Sheikh).

http://dx.doi.org/10.1016/j.nicl.2014.08.013

hormone (ACTH) and corticosterone responses (Brake et al., 2000). In adult rhesus monkeys exposed to stress, lower tissue densities were observed in the limbic system, particularly the thalamic nuclei, the anterior cingulate cortex compared to controls (Willette et al., 2012), suggesting an important role of these regions in HPA axis regulation.

However, associations between cortisol reactivity to stress and brain structure likely emerge through complex pathways that also entail environmental influences. Supporting this notion is the large literature implicating early care in children's cortisol reactivity and a small literature examining early caregiving and children's brain structure. For example, significant differences in cortisol reactivity have been widely documented in young children exposed to neglect and abuse (Hunter et al., 2011). Even more normative aspects of early care may shape cortisol reactivity; for example, positive parenting was found to moderate the effect of negative life events on children's cortisol reactivity (Barry and Kochanska, 2010; Engert et al., 2010; Hagan et al., 2011; Pendry and Adam, 2007). Regarding the literature on the neural correlates of early care, Eluvathingal et al. (2006) found decreased white matter tract density in the left uncinate fasciculus in post-institutionalized children who experienced early deprivation. Additionally, decreased white matter density (a strong correlate of lower FA) was observed in adolescents and young adults who were exposed to parental verbal abuse and physical neglect during childhood (Choi et al., 2009; Huang et al., 2012). Specifically, these studies reported white matter abnormalities in areas implicated in stress evaluation and regulation, such as the anterior temporal lobe, the cingulum bundle and the fornix. However, extant research has focused on extreme forms of early stress, such as sexual or emotional abuse and physical neglect. While these are important aspects of early stress, additional research is needed on links between more common forms of contextual risk factors, such as more normative aspects of parenting, and children's neural structure. Given the literature implicating parent positive and negative caregiving behaviors in an array of child outcomes (Lueken and Lemery, 2004; Tarullo and Gunnar, 2006), such behaviors may be relevant to associations between stress reactivity and neural structure as well.

Taken together, these lines of research suggest that white matter connectivity may be linked to both children's cortisol reactivity to stress as well as early caregiving. We therefore conducted a preliminary investigation of links between cortisol reactivity to stress and structural connectivity, indexed using diffusion tensor imaging (DTI), which yields fractional anisotropy (FA) indices of white matter. FA is a measure of directionally dependent restriction of water diffusion due to axonal myelin insulation and is widely used to evaluate white matter integrity in the brain. Myelination increases nerve conduction velocities and facilitates synchronous firing of neurons by reducing travel distance effects in distributed networks (Fields, 2008). Therefore, high anisotropy is generally interpreted as white matter with more efficient movement of water molecules by diffusion parallel to the course of the axon fiber bundles. Lower FA in white matter suggests underlying disruption of myelin insulation or axonal integrity (Fields, 2008; Song et al., 2002).

In a community sample of 6-year-old girls, we examined what is, to our knowledge, the first investigation of the neural correlates of early neuroendocrine stress reactivity. Because we were interested in identifying associations between constructs implicated in depression vulnerability (i.e., cortisol stress reactivity, early care; Azar et al., 2007; Dougherty et al., 2011, 2013), we examined girls prior to the age of risk for depression (Kendler et al., 1993, 1997; Wittchen et al., 1994) to increase the likelihood that we were examining risk markers, rather than the sequelae of current or past depression, and used a community sample to increase the representativeness of any findings to typically developing girls and families. Based on evidence for white matter disruptions in limbic regions of the brain and stress-related mood disorders (Osoba et al., 2013; Yap et al., 2008), we hypothesized that early cortisol reactivity would be associated with white matter differences in the limbic regions of the brain. Additionally, based on research linking early care and cortisol function, we investigated whether parenting moderated associations between cortisol reactivity and the brain's white matter microstructure.

## 2. Methods

### 2.1. Participants

We recruited 45 6-year-old (mean = 6.13 years, $SD = 0.73$) girls for the current imaging study from a larger community sample of 409 (208 girls) children participating in an ongoing longitudinal study. Children were aged three at the time the larger study was initiated and were screened for significant medical or psychological problems via a procedure administered by trained study personnel; children with such problems were ineligible to participate. Because this was a pilot study of a small sample, only girls were recruited to eliminate the need to use sex as a covariate in a small data set, given the well-established impact of sex differences in young children's brain development (Gong et al., 2011; Paus et al., 2008), although we discuss the significant limitations of this decision in the Discussion section. Girls were either high or low in cortisol reactivity to stress based on data collected at the baseline assessment, operationalized as described in the following section.

Demographics for the current study subsample are provided in Table 1. Girls were administered the Peabody Picture Vocabulary Test (PPVT; Dunn and Dunn, 1997) at baseline to screen for gross cognitive impairment and English proficiency; girls were of average cognitive ability ($M = 112.3$, $SD = 13.9$). We found no differences in family demographics such as family income, parent education and ethnicity and child cognitive ability between the sample recruited for imaging analyses and the original study sample (all $ps > 0.28$), which was representative of the region from which participants were recruited. The study protocols were reviewed and approved by the University of Western Ontario Health Sciences Research Ethics Board.

### 2.2. Assessment of cortisol reactivity to stress

The stress task and cortisol collection procedures used have been described in great detail previously (Kryski et al., 2011; Kryski et al., 2013). In brief, at the baseline assessment, cortisol data were collected from children during a visit to the family's home.[1] All visits began between 12:00 pm and 3:30 pm to minimize the effects of diurnal variation on cortisol samples (de Weerth et al., 2003; Donzella et al., 2008). Caregivers were asked to prevent children from eating or drinking for a half hour prior to the visit to remove the influence of food/drink on cortisol assays (Magnano et al., 1989; Schwartz et al., 1998). None of the children were taking corticosteroids. After 30 min of quiet play with the experimenter, a baseline salivary cortisol sample was collected, followed by the stress task described below.

The stress task was a downward adaptation of one developed and validated by Lewis and Ramsey (2002) for use with older children and was designed to emphasize social evaluation under motivated and uncontrollable circumstances, which has been shown to elicit large cortisol responses in both adults (Dickerson and Kemeny, 2004; Magnano et al., 1989) and preschool-aged children (Gunnar et al., 2009). Briefly, each child attempted to complete a matching task by matching colored game pieces to animal icons based on an answer key. A large toy replica of a traffic light was placed adjacent to the board, and the child was instructed that the traffic light would show how much time they had to complete the task and win a prize, with "green" indicating that they had time to work and "red" indicating that they were out of time. The experimenter surreptitiously controlled the traffic light via remote control so that no child could complete the task on time during any of the three trials conducted. The mean duration of the task for children who completed all three trials in Study 1 was 15.01 min ($SD = 1.5$), including

[1] In total, 392 (95.8%) children provided all six cortisol samples, while the remaining fourteen (3.4%) children did not provide at least one sample.