Hormones and Behavior 94 (2017) 135–144

Contents lists available at ScienceDirect

Hormones and Behavior

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

Evening salivary cortisol and alpha-amylase at 14 months and neurodevelopment at 4 years: Sex differences

Ainara Andiarena[a,b,*], Nekane Balluerka[a,b], Mario Murcia[c,d], Jesús Ibarluzea[a,b,c,e], Vivette Glover[f], Oscar Vegas[a,b]

[a] University of the Basque Country (UPV/EHU), San Sebastian, Spain
[b] Biodonostia Health Research Institute, San Sebastian, Spain
[c] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain
[d] FISABIO, Universitat Jaume I, Universitat de València Epidemiology and Environmental Health Joint Research Unit, Valencia, Spain
[e] Departamento de Sanidad Gobierno Vasco, Subdirección de Salud Pública de Gipuzkoa, San Sebastián, Spain
[f] Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, United Kingdom

ARTICLE INFO

Keywords:
Early-life stress
Saliva
Cortisol
Alpha-amylase
Neurodevelopment
Sex differences

ABSTRACT

Stress system activity in early life can have long-term effects on neurodevelopment. The main aim of this study was to assess the association of child evening salivary cortisol and alpha-amylase basal levels at 14 months of age with longer-term neuropsychological development at 4 years in a low-risk population-based birth cohort derived from the INMA (Environment and Childhood) project in Spain. We included 186 parent-children pairs with information on both stress system activity and neurodevelopment. Both stress markers at 14 months of age showed an association with neuropsychological development at 4 years. Salivary cortisol showed a sex-specific pattern of association. In girls, cortisol levels at 14 months were negatively associated with cognitive development [long-term declarative memory ($\beta = -17.8$, $p = 0.028$; 95% CI = −33.2 to −2.5); executive function ($\beta = -9.8$, $p = 0.08$; 95% CI = −21 to 1)] and gross motor development ($\beta = -13$; $p = 0.022$; 95% CI = −24 to −2), whereas in boys cortisol levels were negatively associated with socioemotional development [autistic-like behaviours: Incidence Rate Ratio (IRR) = 1.6, $p = 0.039$; 95% CI = 1.01 to 2.41]. Salivary alpha-amylase was positively associated with socioemotional development in boys only [social competence ($\beta = 2.11$, $p = 0.013$; 95% CI = 0.47 to 3.72), autistic-like behaviours (IRR = 0.93, $p = 0.042$; 95% CI = 0.87 to 0.99) and hyperactivity symptoms (IRR = 0.81, $p = 0.021$; 95% CI = 0.69 to 0.97)]. These results suggest that stress system activity in early life is associated with longer-term neurodevelopment and that sex is an important factor in this relationship.

1. Introduction

Stress system activity in early life has been associated with long-term health and neurodevelopment (Lupien et al., 2009; Shonkoff and Garner, 2012). While regulated stress system activity is an adaptive mechanism necessary for survival and health, a dysregulated stress system (i.e. hypo- or hyper-reactive stress responses, or high/low basal activity levels) can be maladaptive for the developing nervous system and may disrupt its architecture and function, especially during the early stages of brain development (Lupien et al., 2009). The association between stress system activity and neurodevelopment has been observed both in animal studies and in adult human populations. Research involving children has often focused on the effects of prenatal maternal stress (Glover et al., 2015; Talge et al., 2007) or on children with a high risk for dysregulated stress system activity (i.e. children in orphanages) (Nelson et al., 2007). However, the association between stress system activity and neuropsychological development has been less widely studied in population-based birth cohorts than in higher risk populations.

The psychobiology of the stress system is mainly regulated by the coordination of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS) (Granger et al., 2007). Individual differences in the activity of these systems can be non-invasively measured through salivary cortisol and salivary α-amylase (sAA), respectively (Schumacher et al., 2013). The majority of studies have used cortisol levels as the biological marker of stress system activity. sAA is a more recent surrogate marker for the SNS and it is increasingly included in biobehavioural research with the aim of studying stress system

* Corresponding author.
E-mail address: ainara.andiarena@ehu.eus (A. Andiarena).

http://dx.doi.org/10.1016/j.yhbeh.2017.07.008
Received 15 September 2016; Received in revised form 19 July 2017; Accepted 20 July 2017
Available online 26 July 2017
0018-506X/

effects on neurodevelopment more comprehensively (Schumacher et al., 2013). This digestive enzyme is secreted in response to autonomic nervous system (ANS) activation (Granger et al., 2007) and is correlated with central noradrenergic activity (Bosch et al., 2011; Cubała and Landowski, 2014).

Due to their high glucocorticoid receptor (GR) concentration, the hippocampus, the amygdala and the prefrontal cortex (PFC) are brain areas highly sensitive to the effects of stress (Lupien et al., 2009). While moderate levels of glucocorticoids are beneficial for central nervous system (CNS) development (Meyer, 1983), high levels or prolonged exposure can lead to functional and structural damage in these brain areas (Lupien et al., 2009), through immediate non-genomic and long-term genomic mechanisms (Groeneweg et al., 2011). The literature concerning the biological mechanisms underlying the association between salivary alpha-amylase and neurodevelopment is still scarce. This association has been explained in terms of the relationship between central noradrenergic activity and emotional state regulation (Ramos and Arnsten, 2007), since the norepinephrine released by the locus coeruleus acts on the same brain regions as glucocorticoids (hippocampus, amygdala and PFC) (Charney and Manji, 2004). Ramos and Arnsten (2007) found that moderate levels of norepinephrine were associated with greater synaptic activity in the PFC, whereas at very high or low levels the activity was reduced. Whatever the case, the hippocampus, amygdala and PFC, in addition to the regulatory activity of the HPA axis and the SNS, are involved in cognitive and emotional functions (Lupien et al., 2009). While longitudinal studies conducted in infant populations describe negative effects of cortisol on memory (Bugental et al., 2010), executive function (EF) (Berry et al., 2012; Blair et al., 2011) and emotional/behavioural regulation (Essex et al., 2002), those studies that have included sAA propose a positive association with socioemotional development (Fortunato et al., 2008; Keller and El-Sheikh, 2009).

Animal and adult human studies have also reported sex differences in how stress system activity in early life affects the CNS (Bale, 2009; Goldstein et al., 2014; McEwen and Morrison, 2013), although it is unclear whether these sex differences are always present in the infant population (Panagiotakopoulos and Neigh, 2014). These sex differences could be due to the fact that HPA axis and SNS activity is — like brain anatomy, chemistry and function — sex specific. Furthermore, the stress system and the CNS interact in a sex-specific manner (Panagiotakopoulos and Neigh, 2014). However, despite evidence indicating that stress-related effects are sexually dimorphic, and despite the acknowledged importance of considering sex when studying the impact of stress-system activity on neurodevelopment (Lupien et al., 2009), the majority of studies do not perform sex-specific analyses.

The aim of the present study was to address two questions regarding associations between early markers of children's stress system activity and their longer-term neuropsychological development in a low-risk population: 1) Is there a relationship between salivary cortisol and sAA basal levels at 14 months of age and neuropsychological development at 4 years in a population-based birth cohort? 2) Are there sex differences in these associations?

Based on prior evidence of associations between measures of stress system activity and neuropsychological development in children we expected to find a negative relationship between salivary cortisol levels and neuropsychological development, especially regarding memory, EF and socioemotional development (Bugental et al., 2010; Berry et al., 2012; Cicchetti et al., 2010; Buss et al., 2011b). As few previous studies have tested the relationship between sAA and long-term neuropsychological development, we made only tentative hypotheses with respect to this association. Based on the scant evidence (Berry et al., 2012; Fortunato et al., 2008; Keller and El-Sheikh, 2009) we hypothesized that there would be a positive association between sAA and long-term neuropsychological development, especially regarding socioemotional development. Finally, based on animal studies (Mychasiuk et al., 2012; Weinstock, 2007) and the adult human literature (Goldstein et al., 2014) we hypothesized that there would be sex differences in the association between stress system activity at 14 months of age and longer-term neuropsychological development.

2. Material and methods

2.1. Study design and participants

The study participants were parent-child pairs who formed part of a population-based birth cohort recruited for the INMA (Environment and Childhood) Project in Gipuzkoa, Spain (http://www.proyectoinma.org). A total of 638 eligible pregnant women (≥ 16 years of age, intending to deliver at the reference hospital, able to communicate in Spanish or Basque, singleton pregnancy and non-assisted conception) were recruited during antenatal care visits in the first trimester of pregnancy at the Regional Hospital of Zumarraga (Gipuzkoa, Spain), which is attended by 90% of women from the study area during their pregnancy. Women were followed up during pregnancy and their children were subsequently followed up from birth through to their fourth year of life. The study protocol has been reported elsewhere (Guxens et al., 2012). The cumulative loss of enrolment from recruitment to the 4 years of age follow-up stage was 29% (10 abortions, 6 foetal/child deaths, 121 withdrawals, 25 changes of address and 26 losses of contact) (Fig. 1). Of the 450 families who remained in the study at the 4-year follow-up stage, 386 underwent a valid neuropsychological assessment, and of these, 186 had information on salivary stress biomarkers (salivary cortisol $n = 186$; salivary alpha-amylase $n = 178$). These 186 families with complete information for both the neuropsychological assessment and stress system activity formed the sample for the present study. There were no statistically significant differences between participating and non-participating families in either the main sociodemographic variables (parental occupation and education, parental age and child's sex; minimum p-value = 0.09 for father's age) or the other covariates (minimum p-value = 0.10 for breastfeeding duration). The study was approved by the hospital Ethics Committee and all participating families provided informed consent.

2.2. Assessment of cognitive and psychomotor development

A standardized version of the McCarthy Scales of Children's Abilities (MSCA) was used to assess children's cognitive and psychomotor development at 4 years of age (McCarthy, 2009). The Basque version of the instrument (MSCA-E) was applied to those children who had Basque as their first language (Andiarena et al., in press). The MSCA comprises 18 subtests that yield standardized test scores for six domains. The *Verbal* scale refers to cognitive tasks related to the processing of verbal information; the *Perceptual-Performance* scale refers to cognitive abilities related to perceptual information processing, including manual performance; the *Quantitative* scale refers to numerical abilities; the *Memory* scale considers short-term retention of information (verbal, visual or numerical); and the *Motor* scale refers to fine (e.g. drawing) and gross (e.g. balance or accuracy) abilities. The sum of the first three scales provides a *General Cognitive Index* (Supplementary Fig. 1). In addition to these scales, we also included a previously validated *Executive Function* scale, covering verbal and visual span, working memory, planning, verbal fluency and abstract thinking (Julvez et al., 2011), and a *Long-term Declarative Memory* scale, referring to long-term retention of verbal and visual information (Andiarena et al., in press). MSCA raw scores were centred on a mean of 100 and a standard deviation (SD) of 15 to homogenize the scales. All testing was performed by a trained neuropsychologist under adequate assessment conditions. The neuropsychologist was blinded to any information concerning the child, including salivary cortisol or sAA levels. A total of 4.7% ($n = 19$) of the test results were excluded from the final analysis due to the poor quality of the child's assessment or to neurodevelopmental disorders.