Neuroscience 342 (2017) 188–199

REVIEW

THE Val66Met BRAIN-DERIVED NEUROTROPHIC FACTOR GENE VARIANT INTERACTS WITH EARLY PAIN EXPOSURE TO PREDICT CORTISOL DYSREGULATION IN 7-YEAR-OLD CHILDREN BORN VERY PRETERM: IMPLICATIONS FOR COGNITION

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Abstract—Early stress in the form of repetitive neonatal pain, in infants born very preterm, is associated with long-term dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and with poorer cognitive performance. Brain-derived neurotrophic factor (BDNF) which is important in synaptic plasticity and cognitive functions is reduced by stress. Therefore the *BDNF* Val66Met variant, which affects secretion of BDNF, may interact with early exposure to pain-related stress in children born very preterm, to differentially affect HPA regulation that in turn may be associated with altered cognitive performance. The aims of this study were to investigate whether in children born very preterm, the *BDNF* Val66Met variant modulates the association between neonatal pain-related stress and cortisol levels at age 7 years, and if cortisol levels were related to cognitive function. Furthermore, we examined whether these relationships were sex-specific. Using a longitudinal cohort design, $N = 90$ children born very preterm (24–32 weeks gestation) were followed from birth to age 7 years. Cortisol was assayed from hair as an index of cumulative stress and from saliva to measure reactivity to a cognitive challenge. *BDNF* Val66Met variant was genotyped at 7 years using real-time polymerase chain reaction (PCR). Using generalized linear modeling, in boys with the Met allele, greater neonatal pain-related stress (adjusted for clinical risk factors) predicted lower hair cortisol ($p = 0.006$) and higher reactivity salivary cortisol ($p = 0.002$). In both boys and girls with the Met allele, higher salivary cortisol reactivity was correlated with lower IQ ($r = -0.60$; $p = 0.001$) and poorer visual-motor integration ($r = -0.48$; $p = 0.008$). Our findings show associations between lower BDNF availability (presence of the Met allele) and vulnerability to neonatal pain/stress in boys, but not girls. This exploratory study suggests new directions for research into possible mechanisms underlying how neonatal pain/stress is related to cognitive performance in children born very preterm.

This article is part of a Special Issue entitled: Early Adversity.

Key words: BDNF rs6265, preterm, infant, cortisol, pain, sex.

Contents

INTRODUCTION

Early adversity is known to affect behavioral and physiological systems in the long-term. Genetic variation

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Abbreviations: ACTH, adrenocorticotropin; BDI, Beck Depression Inventory; BDNF, brain-derived neurotrophic factor; GZLM, Generalized Linear Modeling; HPA, hypothalamic–pituitary–adrenal; NICU, neonatal intensive care unit; TSST, Trier Social Stress Test; WISC, Wechsler Intelligence Scale for children.

http://dx.doi.org/10.1016/j.neuroscience.2015.08.044
0306-4522/

can offer opportunities to unveil gene-environment interactions that may explain susceptibility and resiliency to early adverse environmental effects. Brain-derived neurotrophic factor (BDNF) is an important neurotrophin which is widely expressed in the brain especially in the prefrontal and hippocampal regions, and has long-term effects on neuronal survival, development and synaptic plasticity. Human postmortem (Chen et al., 2001) and animal studies have found that stress modifies BDNF expression: decreased expression in the hippocampus and increased in amygdala (Rasmusson et al., 2002; Govindarajan et al., 2006; Lakshminarasimhan and Chattarji, 2012). BDNF is involved in cognitive functions (Hariri et al., 2003; Zhang et al., 2013, 2014), with the pathophysiology of psychiatric disorders such as schizophrenia, major depression, and bipolar disorder (see (Duman and Monteggia, 2006; Notaras et al., 2015). The *BDNF* Val66Met (rs6265) variant affects intracellular processing and secretion of BDNF (Egan et al., 2001). The Met allele encodes a precursor protein with impaired function which results in lower BDNF availability, and hence is associated with alterations of human hippocampal function and episodic memory (Egan et al., 2003). Many studies have implicated the BDNF Val66Met polymorphism in psychiatric disorders such as schizophrenia, Alzheimer's disease, and affective disorders (Ventriglia et al., 2002; Chen et al., 2006; Rybakowski, 2008; Verhagen et al., 2010). Sex differences have been found in BDNF levels (e.g. Xiu et al., 2009; Zhang et al., 2014). However, the direction of interaction differs depending on the study population and outcome measures. Availability of BDNF is also affected by early environmental physical or psychological stress, which is reported to result in decreased neurotrophic support to certain BDNF-rich regions (Rasmusson et al., 2002; Brown et al., 2003). However, the relationships between early adversity, BDNF levels, and cognition in young children are still unknown.

Infants born very preterm are exposed to considerable procedural pain-related stress during weeks to months of hospitalization in the neonatal intensive care unit (NICU) (Grunau, 2007, 2013). Since pain and stress cannot be distinguished in very preterm infants, we use the term "pain-related" stress (Grunau et al., 2013). Early-life stress can permanently alter neural, hormonal, and behavioral systems and thereby contribute to increased vulnerability to cognitive problems later in life (Heim and Nemeroff, 2002; Richards and Wadsworth, 2004). Children born very prematurely perform more poorly in tasks that require attention and inhibition (Kulseng et al., 2006; Shum et al., 2008). Executive functions in children born very preterm have been identified as an area of difficulty in this population, even when intelligence is broadly normal (Mulder et al., 2009; Aarnoudse-Moens et al., 2012). One primary mechanism underling early programing effects of stress is re-setting of the hypothalamic–pituitary–adrenal (HPA) axis, reflected by long-term changes in cortisol the primary stress hormone in humans (Grunau, 2007, 2013; Brummelte et al., 2012; Zouikr et al., 2014). Early exposure to stress has been shown to have prolonged effects on cognitive and affective functions (Heim and Nemeroff, 2002; Richards and Wadsworth, 2004; Heim et al., 2008); reviewed by Lupien et al. (2009), Pechtel and Pizzagalli (2011).

Neurodevelopmental and behavioral problems are highly prevalent among children born very preterm (Aarnoudse-Moens et al., 2009), however, little is known about the etiology of these difficulties, and particularly whether altered endogenous cortisol expression following early stress may play a role. In a longitudinal study, we found the trajectory of salivary cortisol expression was altered in very preterm infants while in the NICU (Grunau et al., 2005), and compared to infants born full-term term long after NICU discharge (Grunau et al., 2007). Importantly, cumulative neonatal stress (higher number of skin-breaking procedures from birth to term adjusted for clinical confounders related to prematurity) was associated with these altered cortisol levels, Furthermore, altered hair and salivary cortisol levels were evident at school-age in children born very preterm (Buske-Kirschbaum et al., 2007; Kajantie and Raikkonen, 2010; Grunau et al., 2013; Brummelte et al., 2015). While endogenous cortisol levels play an important role in brain function, there is a dearth of knowledge of the relationship between cognitive performance and cortisol expression at school age in children born preterm.

In rodent studies, long-term effects of pain on the HPA axis appear to depend on the type of pain induced in the neonatal period. In a model of repetitive neonatal pin prick pain, while increased anxiety was evident in adulthood there was no effect on corticosterone level (Anand et al., 1999; Walker et al., 2003). Walker et al. using the same pin-prick model, also found no effect on corticosterone, but concluded this might be due to increased maternal licking and grooming by dams of pups in the pain group, suggesting maternal behavior might buffer effects of pain thereby preventing HPA changes. On the other hand, inflammatory pain induced by carrageenan did show long-term alteration in HPA axis regulation (Victoria et al., 2013a), anxiety (Victoria et al., 2013b), and memory (Henderson et al., 2015). The conditions under which neonatal pain leads to changes in neuroendocrine function, the relationship with cognition, and underlying mechanisms are unclear.

Hair cortisol provides an integrated index of cumulative stress level during an extended period of time while salivary cortisol reflects short-term activation and reactivity of the HPA axis (Sauve et al., 2007; Steudte et al., 2011; van Holland et al., 2012). Hair and salivary cortisol serve as non-invasive complementary biomarkers of stress hormone regulation (van Holland et al., 2012).

Our previous studies have shown that cortisol levels in very preterm children at school age were associated with neonatal pain-related stress in a sex-dependent way (Grunau et al., 2013; Brummelte et al., 2015). In preterm boys with the minor allele in *NFKBIA* rs2233409 (NF-kappa-B inhibitor alpha) which regulates the NF-κB-mediated inflammatory response (Hayden and Ghosh, 2008; Lynn et al., 2008)) greater procedural neonatal pain/stress predicted lower hair cortisol at age 7 years, (Grunau et al., 2013). Moreover, greater neonatal