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Sex differences in behavioral outcome following neonatal hypoxia ischemia: Insights from a clinical meta-analysis and a rodent model of induced hypoxic ischemic brain injury

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ABSTRACT

Hypoxia ischemia (HI; reduced oxygen and/or blood flow to the brain) is one of the most common injuries among preterm infants and term infants with birth complications. Both populations show cognitive/behavioral deficits, including impairments in sensory, learning/memory, and attention domains. Clinical data suggests a sex difference in HI outcomes, with males exhibiting more severe cognitive/behavioral deficits relative to matched females. Our laboratory has also reported more severe behavioral deficits among male rats with induced HI relative to females with comparable injury (Hill et al., 2011a,b). The current study initially examined published clinical studies from the past 20 years where long-term IQ outcome scores for matched groups of male and female premature infants were reported separately (IQ being the most common outcome measure). A meta-analysis revealed a female "advantage," as indicated by significantly better scores on performance and full scale IQ (but not verbal IQ) for premature females. We then utilized a rodent model of neonatal HI injury to assess sham and postnatal day 7 (P7) HI male and female rats on a battery of behavioral tasks. Results showed expected deficits in HI male rats, but also showed task-dependent sex differences, with HI males having significantly larger deficits than HI females on some tasks but equivalent deficits on other tasks. In contrast to behavioral results, post mortem neuropathology associated with HI was comparable across sex. These findings suggest: 1) neonatal female "protection" in some behavioral domains, as indexed by superior outcome following early injury relative to males; and 2) female protection may entail sex-specific plasticity or compensation, rather than a reduction in gross neuropathology. Further exploration of the mechanisms underlying this sex effect could aid in neuroprotection efforts for at-risk neonates in general, and males in particular. Moreover, our current report of comparable anatomical damage coupled with differences in cognitive outcomes (by sex) provides a framework for future studies to examine neural mechanisms underlying sex differences in cognition and behavior in general.

Introduction

Approximately 12–14% of US births are premature (<37 weeks gestational age (GA)) or very low birth-weight (VLBW, <1500 g; Kochanek et al., 2012). Within this infant population, hypoxia-ischemia (HI; decreased blood and/or oxygen to the brain) is a major cause of brain damage and associated adverse outcomes (Barrett et al., 2007; Fatemi et al., 2009). The fragility/immaturity of the premature neurovascular system can lead to HI, for example blood pressure fluctuations that rupture capillaries and cause intraventricular (IVH) or periventricular (PVH) hemorrhage (du Plessis and Volpe 2002; Volpe, 2001). These bleeds typically occur in the subependymal germinal matrix, with necrosis and prolonged apoptosis of ventricular zone cells (e.g., glial precursors; du Plessis and Volpe 2002; Volpe, 2001). Reperfusion failure (i.e., capillary collapse) is another common preterm injury, with resulting ischemia often leading to periventricular leukomalacia (PVL; white matter tissue loss around the ventricles; Back et al., 2012; Perlman, 1998). Preterm HI can also follow chronic lung dysfunction (Barrett et al., 2007; Kragelohmann et al., 1999; Peterson, 2003).

Though less common, HI can also occur in term infants (2–4/1000 full-term births (.2–.4%)), with hypoxic/anoxic injury caused by labor complications, cord compression, placental abnormalities, or prolonged labor (Barrett et al., 2007; Fatemi et al., 2009; Vannucci and Hagberg, 2004; Vannucci and Vannucci, 2005). Neural injury following term HI typically includes diffuse tissue loss (particularly in gray matter; Fatemi et al., 2009; McLean and Ferriero, 2004), as well as altered

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neurochemical metabolite ratios that can predict long-term neurodevelopmental outcomes (Izbudak and Grant, 2011; Thayyil et al., 2010). Anatomically, term HI injury manifests as tissue damage/loss in cortex, basal ganglia and/or hippocampus (Fatemi et al., 2009; Huang and Castillo, 2008; Jyoti et al., 2006; Martinez-Biarge et al., 2011; Vannucci, 2000; Volpe, 2001), reflecting particular susceptibility of gray matter to glutamatergic excitotoxicity at this age (Alvarez-Diaz et al., 2007; Fields, 2010; Huang and Castillo, 2008; Sie et al., 2000a). Term HI generally leads to a global form of brain injury called hypoxic ischemic encephalopathy (HIE; de Vries and Cowan, 2009; Fatemi et al., 2009; Izbudak and Grant, 2011; Volpe, 2001).

Given the detrimental neural effects of hypoxia ischemia, it is not surprising that HI causes long-term behavioral consequences in preterm/term populations (Cserjesi et al., 2012; Kent et al., 2012). For example, problems are often seen with language acquisition/processing in preterm populations (as measured at later ages; Briscoe et al., 1998; Casiro et al., 1990; Jansson-Verkasalo et al., 2004a, 2004b; Marlow et al., 2005; Steinman et al., 2009). Term infants with HIE also show lower language, reading and spelling scores compared to matched control children at school-age (Badawi et al., 2001). A proposed mechanism contributing to language deficits is underlying difficulties in rapid auditory processing (RAP; the ability to discriminate differences between rapidly presented auditory cues such as /ba/ versus /da/), with early (6–12 months) RAP deficits highly predictive of later language performance scores (Benasich et al., 2006; Choudhury et al., 2007; Downie et al., 2002). In fact, early RAP scores are predictive of language outcomes in both typically developing and at-risk children (Benasich, 2002; Benasich and Tallal, 2002; Benasich et al., 2002, 2006; Fitch and Tallal, 2003; Sie et al., 2000b; Trehub and Henderson, 1996). Accordingly, deficits in rapid auditory processing (and other aspects of speech processing) are seen in VLBW children (Ortiz-Mantilla et al., 2008), as well as preterm/term populations with evidence of HI insult (Downie et al., 2002; Gallo et al., 2011; Jansson-Verkasalo et al., 2004a; Robertson and Finer, 1985).

In addition to speech/language impairments, infants with HI injury also show deficits in learning, memory and executive functioning (Edgin et al., 2008; Luu et al., 2011). For example, children that undergo an HI insult during infancy score lower than expected on memory quotient scores (Gadian et al., 2000), and display poor performance on spatial memory tasks (Baron et al., 2011; Curtis et al., 2006). Term infants with severe HI insult also display deficits on "everyday memory tasks" in adolescence as compared to matched children with mild HI insult (Marlow et al., 2005).

Finally, neonatal HI is often associated with impairments in visual attention, as well as a higher than expected diagnosis of disorders such as ADHD (Aarnoudse-Moens et al., 2009; de Kieviet et al., 2012; Getahun et al., 2013; Lindstrom et al., 2011; Lou, 1996; Perricone et al., 2013; Scott et al., 2012; Shum et al., 2008; Sun and Buys, 2012). Specifically, premature children with HI damage show deficits in visual perception, visuomotor integration, visual recognition, and visuospatial processing (Fazzi et al., 2009), and other studies report reduced visuospatial function and visual attention impairments in premature infants, as well as infants diagnosed with HIE (Baron et al., 2009; Lindstrom et al., 2011; Mercuri et al., 1997, 1999).

Most of the studies described above report average scores for groups of "at risk" infants including both sexes, but report only combined scores. Yet evidence suggests important differences between males and females. For example, infant males are 61% more likely to suffer stroke (Golomb et al., 2009, 2010), and show a higher incidence of prematurity, anoxia, intraventricular hemorrhage, and mortality from prematurity (Lauterbach et al., 2001; Mayoral et al., 2009; Peacock et al., 2012; Raz et al., 2004, 2010). Moreover, males are more likely to be diagnosed with neurodevelopmental disorders, and are more likely to exhibit cerebral palsy compared to females with comparable injury (Donders and Hoffman, 2002; Gualtieri et al., 1985; Lauterbach et al., 2001; Rutter et al., 2003). Interestingly, the heightened susceptibility of males to severe HI insult parallels exacerbated cognitive and behavioral deficits that follow HI (Hindmarsh et al., 2000; Kent et al., 2012; Kesler et al., 2008; Peacock et al., 2012). Specifically, males with neonatal HI injuries score lower than females with comparable injury on IQ tests and other measures of cognitive/developmental outcomes (Aylward, 2002, 2005; Begega et al., 2010; Lauterbach et al., 2001; Leversen et al., 2011; Morsing et al., 2011; Raz et al., 2004; Wallace et al., 1995). Nonetheless, the mechanisms underlying a sex difference in outcomes are unknown. Some evidence suggests that testosterone may exacerbate injury (McCarthy, 2008), or that estrogen/progesterone could be protective (Carwile et al., 2009; McCullough and Hurn, 2003), and other evidence suggests sex differences in cell death pathways that may favor females (Lang and McCullough, 2008; Liu et al., 2009; Manwani and McCullough, 2011; McCullough et al., 2005).

To further explore HI effects on behavioral outcomes, rodent models have been used (Vannucci and Vannucci, 1997; Vannucci et al., 1999), with postnatal day (P)7 HI male rats showing deficits in rapid auditory processing (RAP; Alexander et al., 2013a, 2013b; Hill et al., 2011a, 2011b; McClure et al., 2005, 2006, 2007; Smith et al., 2013 in prep). Similar RAP deficits are seen in males using rodent models of neonatal brain injury (e.g., induced microgyria; Peiffer et al., 2001, 2002, 2004; Threlkeld et al., 2006, 2007) as well as prenatal teratogenic exposure (Threlkeld et al., 2009) — indicating that RAP deficits in animal models could index neural disruptions that may be associated with language impairments in human populations. Learning/memory impairments have also been reported in P7 HI male rats on Morris water maze and other spatial tasks (Arteni et al., 2003; Balduini et al., 2000; Hill et al., 2011a, 2011b; Ikeda et al., 2001; McClure et al., 2006, 2007), as well as non-spatial learning/memory tasks (Hill et al., 2011a), and choice reaction time (CRT) tasks (an assessment of attention; Arteni et al., 2010; Ikeda et al., 2001; Mishima et al., 2004). Interestingly, few of these studies used male *and* female rats with comparable injuries, although the few studies that *have* investigated behavioral outcomes in male and female rodents with comparable perinatal brain injury have repeatedly shown a female advantage (Hill et al., 2011a, 2011b; Peiffer et al., 2002, 2004).

The scarcity of research in this area forms the rationale for the current study. Initially, we performed a meta-analysis of outcome data from studies of human premature populations that provided mean and standard deviation IQ data for matched males and females separately, to confirm the sparse but consistent literature reports of sex differences in outcomes.[1] Next, we designed a rodent study to assess HI-induced sex differences on a battery of behavioral paradigms. Specifically, we employed male and female rats with HI induced on postnatal day 7 (P7)—a time point comparable to neurodevelopmental indices of human term (40 weeks gestational age).[2] We examined performance on RAP tasks, maze tasks indexing spatial and non-spatial learning/memory, and visual attention (5CSRT). We hypothesized that HI injured-males would display robust behavioral impairments on all of the behavioral tasks as compared to male shams, while female rats with HI injury might be "protected" from behavioral impairment. We also hypothesized that the pattern of HI-induced brain injury would be more severe in males as compared to females.

[1] Due to the large disparity in HI incidence for premature versus term infants (12–14% vs. 1–.2%), it is not surprising that we found no sex-specific follow-up data on HIE (term populations) for meta-analysis (noting that it would have been preferable to analyze such a dataset). For this reason we used available follow-up data (i.e., from premature infants inherently at risk for HI injury).

[2] Even though data from preterm infants were used for the meta-analysis, the P7 HI model (modeling term HI injury) was used here based on the more robust behavioral deficits seen in the P7 HI model as compared to the P3 HI model (which simulates HI during preterm birth; see Alexander et al., 2013c). Specifically, we decided that the P3 model would not be optimal for an initial examination of sex differences. Future research on sex differences using the rodent model should, however, be conducted using P3 HI.