Moderate Intrauterine Asphyxia Impairs Surface Righting in Neonatal Rats

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ABSTRACT

A lack of vestibular sensory experience during early life, including that normally produced by the Earth’s gravity, is associated with deficient neonatal responses to gravitational cues. Linking paradigms for studying the form and function of the developing vestibular system after microgravity exposure with biomedical models of inner ear dysfunction or gravistatic receptor deprivation on Earth can provide an important bridge in our understanding of the role of gravity in vestibular development. Intrauterine asphyxia is a major obstetric complication that confers risk for developing neonatal hearing impairment, possibly due to neuropathological damage to the developing hair cells of the inner ear. Vestibular function in asphyxiated infants has not been well examined but a handful of studies suggest potential vestibular deficits. Using a rat model of moderate intrauterine asphyxia, we provide evidence for impaired vestibular mediated adjustments of body position against the gravity vector.

INTRODUCTION

Pregnant rat dams flown on the shuttle during the early developmental period of their fetuses, vestibular apparatus and onset of vestibular function gave birth (after Recovery) to neonates with deficient behavioral adjustments to Earth gravity (Ronca and Alberts, 2000; Ronca et al., 2008). In the present study, we analyzed contact righting responses using a potential model of inner ear dysfunction in neonatal rats associated with a common obstetric complication. Our overall goal is to establish connections between spaceflight findings and with biomedical outcomes on Earth, thereby augmenting both types of investigation.

Intrauterine asphyxia is a condition of impaired fetal blood-gas exchange observed in 1.6 out of every 1,000 live full term births (Pierrat et al., 2005), and nearly 60% of preterm births (Vannucci et al., 1999). Intrauterine asphyxia is a component of several major obstetric complications, including infection (Eitzschig and Carmeliet, 2011), preeclampsia (Myatt and Webster, 2009), maternal diabetes (Van Lieshout and Voruganti, 2008), and intrauterine growth restriction (Marsal, 2009).

Intrauterine asphyxia has been associated with brain damage and subsequent motor, cognitive and behavioral impairment (Marlow and Budge, 2005). Even moderate asphyxia at levels often viewed in the delivery room as unremarkable, can
lead to long-term cognitive and behavioral disabilities (van Handel et al., 2007). However, these outcomes are unpredictable (Bjelke et al., 1991) and the precise causative factors unknown.

Notably, intrauterine asphyxia has been identified as a high risk factor for developing neonatal hearing loss (Borg, 1997; D’Souza et al., 1981; Eavey et al., 1995; Sanders et al., 1985). Pathological alterations of the inner ear and apoptosis in hair cells as well as impaired brainstem auditory evoked responses (Jiang et al., 2008) have been reported in infants exposed to intrauterine asphyxia (Koyama et al., 2005; Schmutzhard et al., 2009). While few studies have addressed vestibular dysfunction following intrauterine asphyxia, there appears to be a relationship between low Apgar score, sensorineural hearing loss, and reduced responses to caloric stimulation (Zagólski and Jurkiewicz, 2006).

In the present study, we tested the hypothesis that moderate intrauterine asphyxia impairs vestibular-mediated behavioral responses in rats. We utilized a translational preclinical rat model of global asphyxia established in our laboratory that provides normoxic, within-litter controls and with high clinical relevance to the third trimester of pregnancy in humans. On the first postnatal day, we analyzed surface righting, a species typical behavioral response to gravity in which rats placed in the supine position will rotate their bodies to prone.

METHODS

Subjects

Neonatal offspring derived from time-mated female Sprague-Dawley (SD) rats were used. During timed matings, vaginal cytology was examined daily until pregnancy was confirmed by presence of sperm (Gestational Day [G] 0). All experimental procedures adhered to the NRC Guide for the Care and Use of Laboratory Animals and were approved by the Wake Forest School of Medicine Animal Care and Use Committee.

Procedure

On G22, the expected day of parturition, each dam was administered under isoflurane anesthesia a chemical transection of the spinal cord (vertebral level L1/L2). The uterus was externalized and the blood supply feeding one of the dam’s uterine horns was ligated for 15min to produce fetal asphyxia (APX). The other uterine horn remained undisturbed (NON).

Pup Delivery

Fetuses in each uterine horn were delivered by cesarean section, stroked to stimulate respiration, and the umbilical cord occluded (Figure 1) at 36.5°C.

Figure 1. Experimental protocol for within dam comparisons of perinatally asphyxiated (left) and non-asphyxiated (right) offspring. The target fetus in each uterine horn is shown (colorized, leftmost image). Fetuses are cesarean delivered (second image from the left), stroked to stimulate respiration (third image from the left), and temperature is maintained at 36.5°C (fourth image from the left).
Comparisons were made between cesarean delivered neonatal rats derived from the APX and NON conditions. Further comparisons were made with Vaginally (VG) born pups to control for the surgical cesarean delivery. Neonates in all three conditions were placed in a heated incubator maintained at 36.5°C for 90-120min postpartum, then fostered to a non-manipulated dam.

To validate degree of asphyxia, rapid decapitation was performed in a subset of newborn pups and blood collected from the mixed venous/arterial pool using a clinical blood gas analyzer (ABL 5, Radiometer, Copenhagen, Denmark).

Whole brain lactate (WBL) was measured from snap frozen postpartum brains (Trinity Biotech, St. Louis, MO).

**RESULTS**

**Validation of Asphyxia**

We found clear evidence for asphyxia as measured by blood pH levels and whole brain lactate.

One-way Analysis of Variance revealed a main effect of condition on blood pH \[ F(d/2)=47.06; p<.0001 \] and WBL across conditions \[ F(2,12)=27.40; p<.0001 \]. Newman Keuls posthoc tests revealed the following significance APX<NON=VG, p<0.05 for blood pH; APX>NON=VG, p<0.05 for WBL. Ns=5 pups/condition.

**Surface Righting Responses**

At 24 hr postpartum, righting responses were analyzed, illustrated in Figure 2. Statistical significance determined by chi-squared analysis.

On Postnatal day (P)1, neonates were placed in the supine position on a solid surface (Figure 2, upper left). Each neonate was held gently with the experimenter’s fingers around the pelvic girdle and head and then released.

Righting from supine to prone was assessed with ‘None’ reflecting no attempt to right (Figure 2, top image) and ‘Attempt/Success’ (Figure 2, lower three images).

APX failed to show righting movements significantly more frequently and attempted to right or achieved righting success significantly less frequently as compared to NON and VG.

**Table 1.** Blood pH and whole brain lactate (WBL) in neonatal rats delivered vaginally (VAG), or by cesarean section either without asphyxia (NON) or with asphyxia (APX).

<table>
<thead>
<tr>
<th>Birth Condition</th>
<th>pH</th>
<th>WBL (ug/dl)</th>
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<tbody>
<tr>
<td>VAG</td>
<td>&gt; 7.0</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>NON</td>
<td>&gt; 7.2</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>APX</td>
<td>&lt; 6.9*</td>
<td>&gt; 55*</td>
</tr>
</tbody>
</table>
CONCLUSIONS

Past studies of sensory loss associated with perinatal asphyxia have focused on auditory impairments and identified inner ear pathology within the cochlea. The present findings provide new evidence for disrupted vestibular mediated responses in neonatal rats one day following moderate perinatal asphyxia. In this study, we were not able to rule out tactile or proprioceptive deficits associated with the placement of pups on their backs. We previously found that eliminating these sources of compensatory adjustment can be accomplished in rats that underwent gestation during spaceflight using a water immersion righting test (Ronca and Alberts, 2000). In the present study, the vestibulosensory specificity of the deficit is not yet known. By expanding the range of vestibular mediated tests and identifying inner ear damage in this model, we hope to create a bridge between findings derived from spaceflight and studies modeling biomedical concerns thereby advancing our understanding of how vestibular form and function are shaped by development on Earth and in space.

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