Neuroscience Letters 604 (2015) 12–17

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Contribution of maternal oxygenic state to the effects of chronic postnatal hypoxia on mouse body and brain development

Natalina Salmaso[a,1], Moises Dominguez[a], Jacob Kravitz[b], Mila Komitova[a], Flora M. Vaccarino[a,*], Michael L. Schwartz[b,*]

[a] Child Study Center, and Department of Neurobiology, Yale University, 230 South Frontage Rd., New Haven, CT 06520, USA
[b] Department of Neurobiology, Yale University, 333 Cedar St., New Haven, CT 06520, USA

HIGHLIGHTS

- Perinatal hypoxia models the effects of bioenergetics deficiency on brain development.
- Rotating dams between normoxia and hypoxia further decreased pups' body weight.
- Dam's rotation did not significantly impact the brain weight loss due to hypoxia.
- Dam's rotation caused a paradoxical mild increase in cortical volume.

ARTICLE INFO

Article history:
Received 4 January 2015
Received in revised form 22 May 2015
Accepted 24 July 2015
Available online 26 July 2015

Keywords:
Hypoxia
Brain development
Mouse
Maternal care

ABSTRACT

1–2% of live births are to very low birth weight, premature infants that often show a developmental trajectory plagued with neurological sequelae including ventriculomegaly and significant decreases in cortical volume. We are able to recapitulate these sequelae using a mouse model of hypoxia where early postnatal pups are exposed to chronic hypoxia for one week. However, because the timing of hypoxic exposure occurs so early in development, dams and pups are housed together in the hypoxic chamber, and therefore, dams are also subjected to the same hypoxic conditions as the pups. To understand the relative contribution of hypoxia directly on the pups as opposed to the indirect contribution mediated by the effects of hypoxia and potential alterations in the dam's care of the pups, we examined whether reducing the dams exposure to hypoxia may significantly increase pup outcomes on measures that we have found consistently changed immediately following chronic hypoxia exposure. To achieve this, we rotated dams between normoxic and hypoxic conditions, leaving the litters untouched in their respective conditions and compared gross anatomical measures of normoxic and hypoxic pups with non-rotating or rotating mothers. As we expected, hypoxic-rearing decreased pup body weight, brain weight and cortical volume. Reducing the dam's exposure to hypoxic conditions actually amplified the effects of hypoxia on body weight, such that hypoxic pups with rotating mothers showed significantly less growth. Interestingly, rotation of hypoxic mothers did not have the same deleterious effect on brain weight, suggesting the presence of compensatory mechanisms conserving brain weight and development even under extremely low body weight conditions. The factors that potentially contribute to these compensatory changes remain to be determined, however, nutrition, pup feeding/metabolism, or changes in maternal care are important candidates, acting either together or independently to change pup body and brain development.

1. Introduction

1–2% of all live births are to very low birth weight (VLBW), premature infants, typically less than 32 weeks gestational age and weighing under 1 kg. VLBW infants face a difficult developmental trajectory, plagued with psychological and neurological sequelae, including decreased cortical volume, ventriculomegaly, significant developmental delays and an increased incidence of psychiatric

* Corresponding authors.
E-mail addresses: natalina.salmaso@yale.edu (N. Salmaso), moises.dominguez@yale.edu (M. Dominguez), mila.komitova@yale.edu (M. Komitova), flora.vaccarino@yale.edu (F.M. Vaccarino), Michael.schwartz@yale.edu (M.L. Schwartz).
[1] Current address: Department of Neuroscience, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada.

http://dx.doi.org/10.1016/j.neulet.2015.07.033
0304-3940/

disorders such as schizophrenia, autism and anxiety disorders [1,3,17,18,21,24–27,38–40]. It is thought that the pathophysiology of these sequelae is, at least in part, due to chronic hypoxia experienced in VLBW infants during the early neonatal period as a consequence of poor oxygen exchange due to immature lung development [41]. There is also a documented deleterious effect of chronic hypoxia or simulated high altitude conditions on fetal and perinatal growth [10,34,35]. This strengthens the importance of oxygenation in achieving optimal fetal and perinatal growth and highlights the importance of chronic metabolic adaptations. Several rodent models have been developed that induce postnatal hypoxia to mimic both the neurological and psychological findings of children exposed to sub-optimal oxygenation conditions, documenting effects on the brain that include decreased brain weight, cortical volume and ventriculomegaly [33]. Using a model in which mice are reared in 10% oxygen during the early postnatal period, we have examined recovery from chronic hypoxic injury and the factors that may mediate recovery, including environmental enrichment and growth factor signaling [7,12–14,16,19,30]. We have demonstrated that, similar to the findings in VLBW children, there is heterogeneity in recovery among brain regions and cell types. In this model, cortical volume and excitatory neuron number recover by adulthood, whereas cortical interneurons fail to achieve complete maturation of their protein markers by adulthood. Indeed, we believe there is a delay in cortical maturation following chronic postnatal hypoxia, perhaps linked to an extended period of plasticity [29,31]. While delayed maturation may increase the potential for recovery, it may also increase the likelihood of missing critical developmental windows.

Despite the considerable evidence generated from the use of chronic postnatal hypoxia, a potential difficulty in the mechanistic interpretation of these studies is the inseparable interaction between the pups and dam during the period of low oxygen exposure (i.e., from postnatal day 3 (P3) to P11). During hypoxic exposure, dams and pups (typically in a C57/B6 genetic background) are housed together in the hypoxic chamber and therefore the dams are also subjected to the same hypoxic conditions as the pups. In order to minimize the adverse effects of maternal stress due to hypoxic exposure on the pups, we include a CD1 foster dam along with each C57 dam and her pups. These litters are culled to 8–10 pups to achieve homogeneity of total "maternal workload". Nevertheless, the question remains as to the relative contribution of hypoxia directly on the pups as opposed to the indirect contribution mediated by the effects of hypoxia and potential alterations in the dam's care of the pups. To disentangle these effects, we examined whether reducing the dams exposure to hypoxia may significantly increase pup outcomes on measures that we have found consistently changed immediately following chronic hypoxia exposure (i.e., body weight, brain weight, cortical volume and cortical neuronal number). In order to achieve this, we rotated dams between normoxic and hypoxic conditions, leaving the litters untouched in their respective conditions and compared gross anatomical measures of normoxic and hypoxic pups with non-rotating or rotating mothers.

2. Methods

2.1. Mice

Ten C57/B6 mice pregnant dams and ten CD1 pregnant fosters were ordered from Charles River and housed in single cages until birth. All litters were fostered to CD1 mothers, culling litters to a total of 8 pups for all dams on Postnatal Day 2 (P2). Dams and litters were housed in hypoxic (approx. 10% O2) or normoxic control conditions from P3–P11 as previously described [17,18,20,21]. The mean O2 level during a typical 8 day hypoxic exposure period was 10%, with a range from 10.2 to 9.9% and a standard error of 0.0. Two litters remained with their dams in normoxic conditions for the entire period (P3–P11), while two litters remained with their dams in hypoxia for this period. In addition, two cohorts of three litters (2 normoxic and 1 hypoxic litter in each cohort) had the dams rotated every 12 h among the normoxic litters and one hypoxic litter. We choose 12 h based upon pilot experiments using rotation periods of 8 and 12 h. The 12 h period allowed each dam to appropriately acclimate to each new litter ensuring that the majority of time with each litter was spent in appropriate nursing behavior and care of the pups. For example, a dam for one of the normoxic litters would be with it's normoxic litter for 12 h, then be rotated to the second normoxic litter for 12 h and then to a hypoxic litter for 12 h after which it would start again with the original litter. Each dam during this cycle was exposed to 24 h in normoxia and 12 h in hypoxia. All experimental groups were comprised of pups from at least 2 different litters in order to control for any litter effects.

Table 1
Number of mice in each experimental group that were used for statistical analyses.

	Norm-NR	Norm-rot	Hyp-NR	Hyp-rot
Body weight	$n=9$	$n=31$	$n=8$	$n=25$
Brain weight	$n=9$	$n=24$	$n=8$	$n=12$
Histology	$n=3$	$n=4$	$n=4$	$n=3$

2.2. Body and brain weights

Body weights for all pups were recorded just prior to placement of the hypoxia litters in the hypoxia chamber on P1. All pups were again weighed on P7 and on removal from the hypoxia chamber on P11. The day following removal from the chamber (P12) mice were perfused as described below. The spinal cord was removed and the brain weights were recorded.

2.3. Histology

Mice were overdosed using xylazine/ketamine and transcardially perfused with phosphate buffered saline and 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 24 h and then cryoprotected in 30% sucrose for 48 h before storing at −80. 20 μm sagittal sections were collected using a cryostat, placed on Superfrost++ slides and stored at −80 °C until staining. Sister sections (1 in 30) were pre-blocked at room temperature for one hour in 10% Normal Donkey Serum diluted in 0.3% Triton in 1XPBS (NDS-PBST) and subsequently stained for NeuN (mouse anti-Neuronal Nuclei, Chemicon) at a dilution of 1:500 in NDS-PBST overnight at room temperature. Sections were washed 3×10 min with PBS and then incubated in anti-mouse 488 (Jackson Laboratories) at 1:1000 for 1.5 h at room temperature. Slides were coverslipped with Vectashield Hard Set (Vector Laboratories) contacting DAPI counterstain and images taken using Zeiss ApoTome and cortical volume and cell number were assessed using Microbrightfield StereoInvestigator software as in our previous studies [7,19]. The number of mice in each group that were used for statistical analyses are shown in Table 1.

2.4. Statistical analysis

All dependent variables were analyzed by factorial analysis of variance, with oxygenic state, maternal status, and age (for body weights only) included as independent variables, where appropriate. Simple analysis and comparisons were conducted only when a significant interaction or main effect was observed ($p < 0.05$) and