JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2009, 60, Suppl 4, 95-104
www.jpp.krakow.pl

J.A. MADRI

MODELING THE NEUROVASCULAR NICHE: IMPLICATIONS FOR RECOVERY FROM CNS INJURY

Department of Pathology, Yale University School of Medicine, New Haven, CT, USA

While survival from stroke, traumatic brain and spinal cord injuries, neurodegenerative diseases and hypoxia has improved over the past several years, treatments are limited and impacts of these injuries and diseases to patients, families and society can be devastating. Recovery from these injuries is variable and involves in part an orchestrated angiogenesis and neurogenesis in the neurogenic zones (neurovascular niches) of the CNS. In this focused review the roles of HIF-1α mediated responses to hypoxia in CNS neurovascular niches is discussed. Using *in vivo* and *in vitro* murine models of sublethal hypoxia we mimicked the variable responses observed in the human population and correlated differences in baseline and hypoxia-induced induction of HIF-1α and several downstream signaling components including BDNF, VEGF, SDF-1, TrkB, Nrp-1, CXCR4 and NO with differences in survival as well as endothelial cell and neural stem cell survival and proliferation, providing insight into this important and timely problem and suggesting that optimization of expression levels of some or all of these signaling components may have the potential of maximizing recovery following CNS injury.

Key words: *neurovascular niche, neural stem cell, hypoxia, endothelial cell, hydrogel, co-culture, recovery*

INTRODUCTION

In the course of one year in the United States approximately 80000 individuals will suffer strokes, over 10000 will have spinal cord injuries, over 200000 will experience traumatic brain injury and of the 60000 very low birth weight preterm infants born, 5000 will test in the mentally retarded range. The impacts of these injuries to the patients, their families, the health care system and society are devastating. While survival from these injuries has improved over the years, treatments affecting recovery are still limited.

Following all these injuries the repair/recovery process is thought to involve both angiogenesis and neurogenesis. During development and throughout our lifetime neuronal death, maintenance and neurogenesis occur in a tightly controlled fashion (1-4). The neurogenic zones, replete with neural stem cells (NSC) are found in the subventricular zones, located along the lateral aspects of the lateral ventricles and the subgranular zones (SGZ), located in the dentate gyrus of the hippocampus. These neurogenic zones are intimately associated with their local microvasculature beds (EC). Further, the behaviors of the neuronal stem cells and microvasculature in these zones are thought to be reciprocally co-regulated, in part, *via* oxygen tension, local NSC and EC soluble and solid phase factors including neurotrophins, vascular growth factors, cytokines and nitric oxide (5-9). Following injury, NSC, supported by their local vasculature, are thought to proliferate, migrate to and differentiate at injury sites, affecting variable degrees of structural and functional recovery (10). Unfortunately, in many instances recovery from injury is incomplete.

THE ROLE OF THE SVZ NEUROVASCULAR NICHE IN RECOVERY FROM NEURODEVELOPMENTAL HANDICAPS ASSOCIATED WITH PRETERM BIRTH

While preterm birth results in significant cognitive and motor disabilities, recent evidence suggests that there can be some recovery over time (11-14). Analysis of recent data indicates that over 1% of the offspring of all live births in the USA weigh under 1000 grams at birth and the survival rate for these often critically ill individuals ranges from approximately 60 to 85% (15). While survival rates of these infants has improved, there has been an increase in neonatal illness, mortality and/or severe intraventricular hemorrhage (IVH) over time, representing a shift of severely compromised patients that now survive the fetal time period and are presented for care in the neonatal unit (16, 17). Over two-thirds of very low birth weight preterm infants suffer from both apnea and respiratory distress syndromes that result in cerebral hypoxemia. Of this cohort, almost one quarter are functioning in the mentally retarded or borderline ranges at school; approximately 10% have cerebral palsy; and at eight years of age approximately one half of these neonates require special assistance in school, resulting in estimated annual life time care costs in excess of four billion dollars. The deleterious effects of low O_2 in the perinatal period are thought to be the consequences of altered neuronal differentiation, synaptogenesis and loss of neurons, glia, and their progenitor cells due to excessive apoptosis (18). Recently, several studies have reported significant improvement in academic functioning over time in this population, which correlated with increases in brain volumes (11). Although

encouraging, the cognitive improvement is variable and the repair/recovery mechanisms involved are undefined.

One possibility that may explain this variable recovery would entail variable neurogenic responses in the SVZ (a known area of neurogenesis) following the period of chronic sublethal hypoxia experienced by these neonates. Indeed, in a murine model mimicking the chronic hypoxia associated with premature birth demonstrated twice as many BrdU-labeled cells expressed neuronal markers in the neocortex in mice recovering from hypoxia as compared to controls (10). In both hypoxic-reared infant/juvenile mice, putative neuroblasts could be seen detaching from the forebrain subventricular zone, migrating through the subcortical white matter and entering the lower cortical layers 5 to 11 days after their last mitotic division (19). These data suggest that cortical neurogenesis may play a significant role in repairing neuronal losses after hypoxic neonatal injury. These findings are consistent with observations made on adult mice that demonstrated cortical, striatal and hippocampal neurogenesis following a variety of injuries and responses to several treatment modalities (20). Thus, the induction of neurogenesis following the insult of chronic sublethal hypoxia in the premature newborn may explain the cognitive improvement observed over time (19). However, the variability of this improvement warrants a more complete understanding of the mechanisms involved in modulating neurogenesis occurring in the subventricular zone (SVZ) so treatment modalities can be investigated to elicit a greater and more complete recovery.

The SVZ has been characterized as a “neurovascular niche” - an area bordered by ependymal cells and comprised of neuroblasts, astrocytes and neural stem cells/neuronal precursor cells in close apposition to a rich microvascular network (21-23). Distinct neurogenic regions including the SVZ and the subgranular zone (SGZ) have been shown to respond differentially to injuries and stimuli. The vasculature is an integral component of these neurogenic areas and angio- and neuro-genesis in these areas appears to be co-regulated and reciprocally signaled (7). Signaling among the cell types present is thought occur *via* soluble and solid phase factors and their cognate receptors (5-8, 24).

Endothelial cells are known to modulate neurogenesis, in part, through the secretion of soluble factors including NGF, BDNF and VEGF (7-9), several of which are also known to be synthesized by neuronal cells and modulate endothelial cells, suggesting a complex, dynamic cross-talk between endothelial cells and neuronal cells including neural stem cells (2-4, 7, 22, 25, 26).

This sophisticated cross-talk among the cellular elements comprising the neurovascular niche is illustrated in *Fig. 1*.

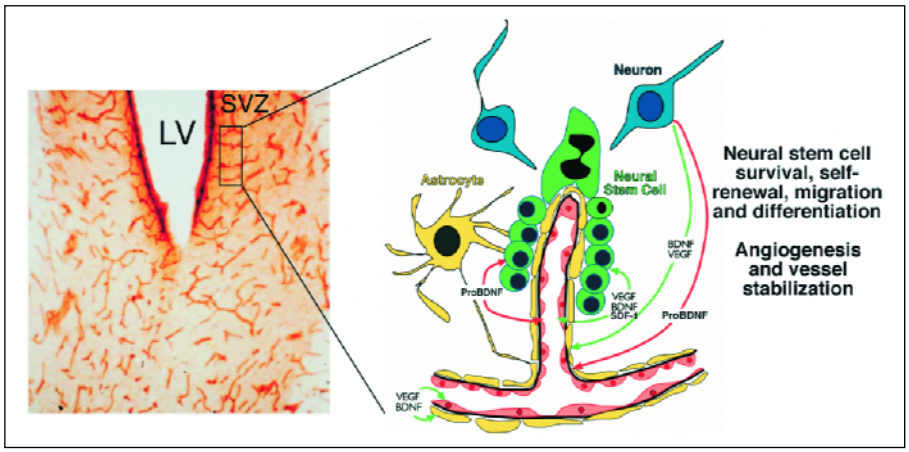

Fig. 1 .Schematic of the neurovascular niche. Distinct neurogenic regions including the SVZ (illustrated here) and the subgranular zone (SGZ) that have been shown to respond differentially to injuries and stimuli. The vasculature is an integral component of these neurogenic areas and angio- and neuro-genesis in these areas appears to be co-regulated and reciprocally signaled (7). Signaling among the cell types present is thought occur *via* soluble and solid phase factors and their cognate receptors).

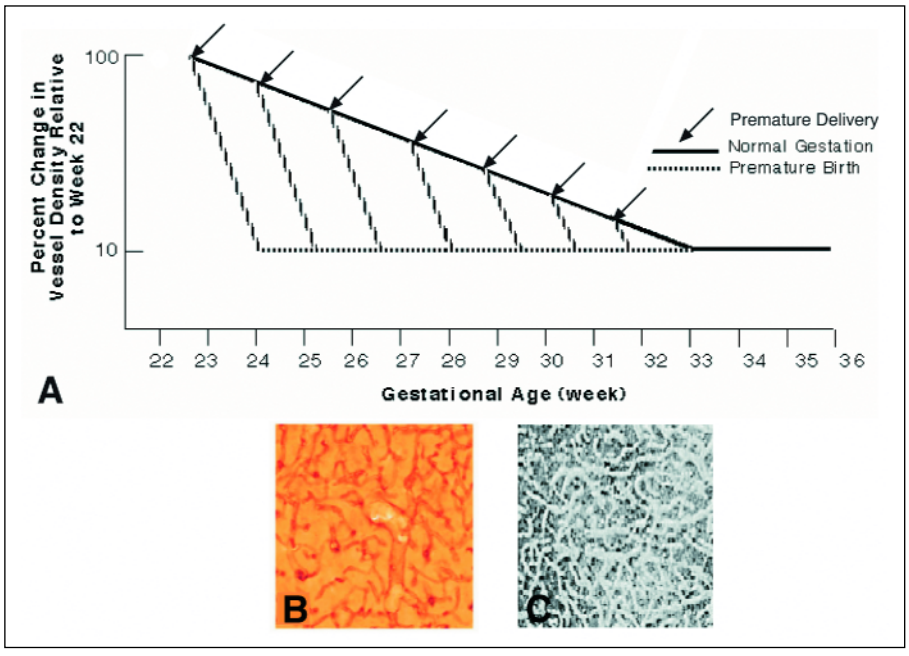

Fig. 2. Premature delivery causes an uncoupling of the involution/ stabilization of the germinal matrix (GM) subventricular zone (SVZ) vasculature. During normal in utero development the fetal brain undergoes a coupled involution/stabilization of the vasculature of the germinal matrix (SVZ) over several weeks. Premature delivery (arrows) elicits rapid, accelerated involution of the SVZ vasculature, resulting in GM intraparenchymal and interventricular hemorrhage (not shown) and chronic sublethal hypoxia eliciting ectasia (B) and increased permeability (C) of the microvasculature, resulting in dysfunction of neurovascular niche-mediated repair/recovery.

Over the past several years our knowledge and appreciation of stem cells and their potential as therapeutic agents in a wide variety of disease and repair conditions has blossomed. One particular area, that of neural stem cells and their biology, has become of great interest to neuroscientists and clinicians alike). Similar to stem cells in other tissues and organs, NSC appear to occupy specific niches in the CNS (the subventricular and subgranular zones as described above) and have been found to be closely associated with microvessels (1, 3, 22, 23, 25, 27). Several recent publications have described roles of endothelial cells in modulating the self-renewal and neurogenesis of neural stem cells (3) dorsal root ganglion (28) and differentiation of neural stem cells to the endothelial lineage (21, 29, 30). These studies are consistent with a complex, dynamic coupling of neurogenesis and vasculogenesis (21). Consistent with these observations and concepts is the evidence of sophisticated cross-talk between neurons and endothelial cells *via* VEGF, neurotrophins and their cognate receptors on both neurons and endothelial cells (5, 9, 24, 31). These data, coupled with the recent findings that demonstrated CNS niche-specific neurogenesis following sublethal chronic hypoxia in a murine animal model mimicking the chronic sublethal hypoxia of the premature newborn (10), suggest a dynamic cross-talk among cells comprising the neurovascular niche.

MODELING THE NEUROVASCULAR NICHE

Using animal models (canine and rodent), we determined that there was an uncoupling of the tightly controlled involution/stabilization of the SVZ vasculature following premature delivery resulting in involution of the SVZ vasculature without stabilization of the surviving vessels, leading to increased intraparenchymal and interventricular hemorrhage. The sublethal hypoxia associated with premature delivery also elicited brain microvascular ectasia and persistent increased permeability (32-34) (*Fig. 2*).

We then went on to demonstrate that perinatal indomethicin treatment (prescribed for closure of the ductus arteriosis) reduces this hemorrhage by inducing earlier secretion, deposition and organization of the SVZ endothelial basement membrane, endothelial tight junction formaion and astrocytic end-foot apposition, stabilizing the SVZ vascular bed (32-34) (*Fig. 3*). Interestingly, indomethicin treatment has been shown to elicit increases in VEGF, FGF-2, TGFβ receptor III and PDGF receptor α and β in rat renal cortex, all of which are known to modulate vascular behavior (35). It therefore would be of interest to assess brain and serum levels of these entities in our transient sublethal hypoxia model and to correlate these findings with cognitive and motor development.

In additional studies (not shown) we determined that chronic sublethal hypoxia, in addition to altering the permeability characteristics of the cerebral vasculature, elicited persistent angiogenesis, induced, in part, by increased VEGF and BDNF secretion by endothelial cells and astrocytes (5, 6, 9, 24, 36, 37).

Using co-cultures of astrocytes and endothelial cells in two- and three-dimensional culture we determined that the presence of astrocytes mimicked endothelial cell-astrocyte interactions *in vivo* (astrocytic end-foot process investiture of endothelial cell tube-like structures), modulated endothelial cell metabolism and blunted hypoxic insult to endothelial cells by providing increased secretion of VEGF (24, 36, 37).

In more recent studies we demonstrated a complex cross-talk between cerebral endothelial cells and neurons *via* VEGF and BDNF, resulting in activation of distinct signaling pathways modulating cell survival, proliferation and differentiation (5, 9). To better mimic the architecture and organization of the SVZ we utilized porous biocompatible hydrogels to co-culture brain-derived endothelial cells and neural stem cells in three-dimensional culture. These culture systems permit organization of endothelial cells into stable tube-like structures with neural stem cells and/or astrocytes in intimate contact with the abluminal aspects of the tubes. When implanted subcutaneously in syngenic mice and rats, these implants exhibited inosculation of their vessels with host vessels and resulted in blood circulation through the implants providing a model system to investigate the role of blood flow in an ectopic model of the SVZ (38, 39) (*Fig. 4*).

These *in vitro* studies to date have illustrated the importance of VEGF and BDNF in mediating the complex cross-talk between these cell types and confirm our recent *in vivo* studies, indicating the importance of these two factors in the SVZ responses and recovery following hypoxia (7, 8, 24) (*Fig. 5*).

In other studies, utilizing DNA microarray methods combined with immunochemical and protein assays, we investigated the changes in protein expression and brain structure following chronic sublethal hypoxia in our murine model and observed that sublethal hypoxia accentuates genes subserving presynaptic function and vasculogenesis/angiogenesis, suppresses genes involved with synaptic maturation, postsynaptic function, and neurotransmission as well as those involved with glial maturation, and components of the cortical and microtubular

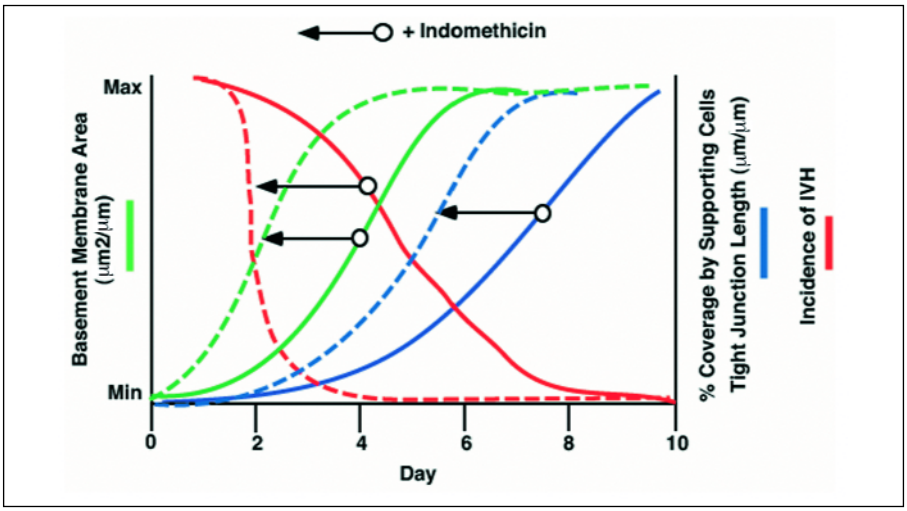

Fig. 3. Indomethicin treatment (dashed lines) reduces intraparenchymal and interventricular hemorrhages (solid lines = no indomethicin), eliciting earlier basement membrane deposition (green lines), tight junction formation and astrocytic endfoot apposition (blue lines) of the SVZ microvasculature. In additional studies (not shown) we determined that chronic sublethal hypoxia altered the permeability characteristics of the cerebral vasculature and elicited persistent angiogenesis, induced, in part, by increased VEGF and BDNF secretion by endothelial cells and astrocytes (5, 6, 9, 24, 35, 36).

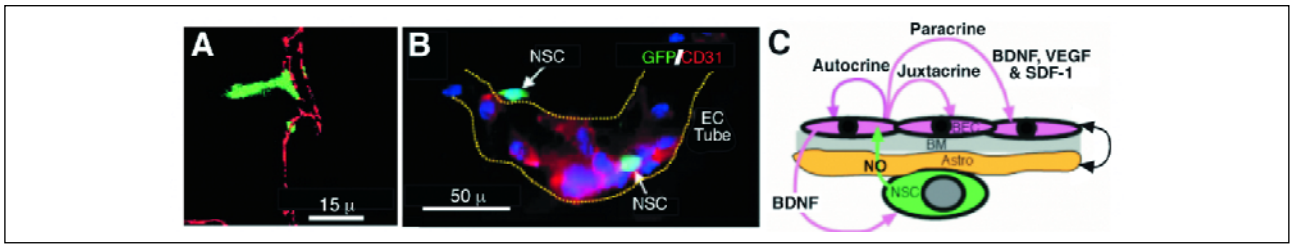

Fig. 4. Examples of three-dimensional endothelial cell-astrocyte (A) (reproduced from (24)) and endothelial cell-neural stem cell (B) (reproduced from (37)) co-cultures illustrating the close, intimate contact between these cell types that approximates their interactions *in vivo*. In both instances there is endothelial cell tube formation noted by organized rhodamine-labeled CD31 positive endothelial cells. Astrocytic end-feet are GFAP fluorescein-labelled in panel (A) and NSC are GFP-labeled in panel (B). Panel (C) illustrates some of the autocrine, paracrine and juxtacrine signaling occurring between and among these cell types comprising the SVZ. We are currently developing a tri-culture three-dimensional model consisting of astrocytes, endothelial cells and neural stem cells. BDNF = Brain Derived Neurotrophic Factor; VEGF = Vascular Endothelial Growth Factor; SDF-1 = Stromal Cell-Derived Factor-1; NO = Nitric Oxide; BEC = Endotheial Cell; Astro = Astrocyte; NSC = Neural Stem Cell; BM = Basement Membrane; GFP = Green Fluorescent Protein; CD31 = PECAM-1; GFAP = glial fibrillary acidic protein.

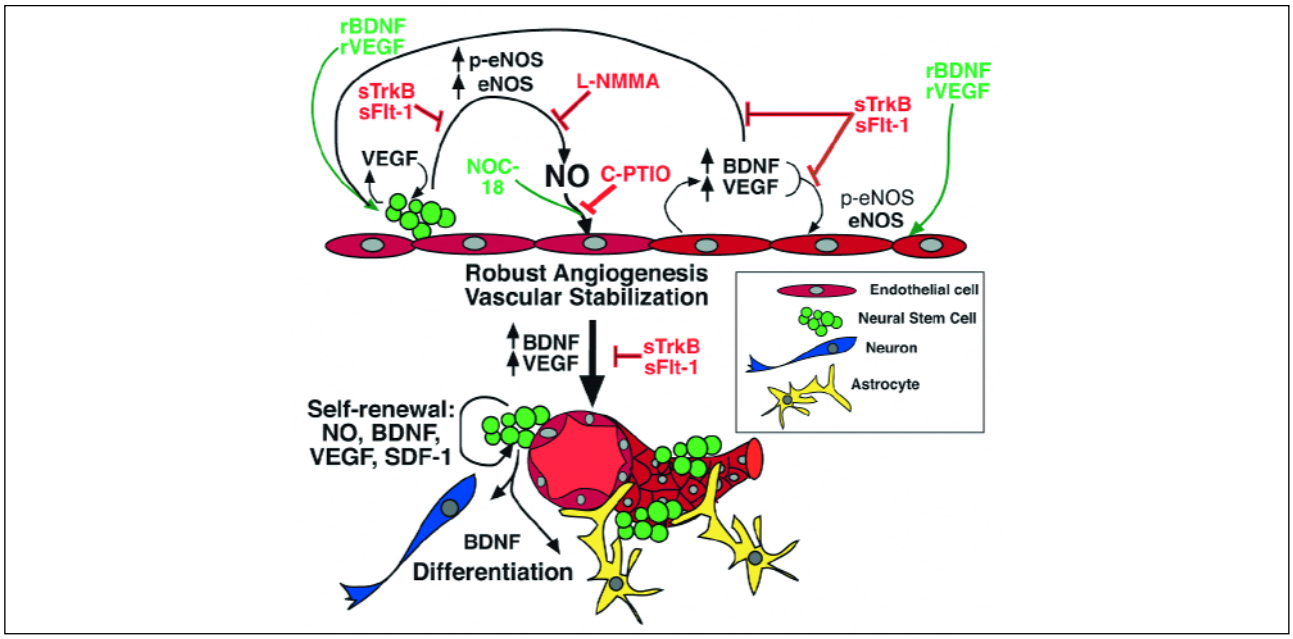

Fig. 5. Our working model of the dynamic interactions between NSC (Green Cells), BEC (Red Cells), Astrocytes (Yellow Cells) and Neurons (Blue Cells). NSC-derived NO induces the BECs to increase their expression of VEGF and BDNF. The BDNF and VEGF then engage and activate BEC VEGFR2 and TrkB receptors and induce angiogenesis in the BEC *via* autocrine and paracrine pathways. The VEGF and BDNF induced by NSC NO also stimulate NSC activation (phosphorylation) of eNOS that results in increased NSC NO generation and NSC proliferation. The persistent NO induction elicits a persistence of BEC VEGF and BDNF expression, which in turn stabilizes the formed tube-like structures and elicits NSC self-renewal. Addition of rVEGF and rBDNF (green color) to NSC cultures results in increased expression of eNOS, increased eNOS activation (phosphorylation) and increased NO generation. This induction is abrogated by addition of L-NMMA and sTrkB and sFlt-1 (red color). Addition of NO donor (NOC-18) (green color) to BEC cultures induces increased expression of BDNF and VEGF. Addition of an NO scavenger (C-PTIO) (red color) abrogates this induction. Addition of rBDNF and rVEGF (green color) to BEC cultures induces robust angiogenesis. Addition of sTRkB or sFlt-1 (red color) abrogates this induction.

cytoskeleton (18). Additionally, we observed significant ventriculomegaly of the ventricles of the hypoxic pups (*Fig. 6*).

The distribution of synapsin 1 in the hypoxic postnatal brain is consistent with alterations in synapse development (*Fig. 7*). By P11 of normoxic development, cortical synapses are abundant. This pattern was evident in the normoxic animals examined, (measured by the diffuse appearance of fine punctate staining of the cortical neuropil with synapsin 1, with modest residual staining of soma, axons, and white matter). Under normoxic conditions, cortical structures develop a mature synapsin 1 pattern prior to limbic structures, recapitulating the pattern of fetal neurogenesis. Synapses in the cingulate gyrus are less mature, as illustrated by a coarser pattern of synapsin 1 staining and a preponderance of synapsin 1 concentrated in the soma and axons. In the hippocampus, coarse staining is evident in the dentate gyrus and CA3 and CA4 regions at P11. In contrast, hypoxic brains display a developmentally inappropriate pattern of synaptic maturation in all regions. Specifically, the coarse synapsin 1 staining of the cortical plate, observed in early postpartum animals is absent, significant residual synapsin was noted in the white matter, as well as in the soma and axonal processes of the cortical neurons. The density of mature synapses, (measured by the abundance of punctate synapsin 1-positive boutons), is significantly reduced by hypoxia (*Fig. 7*).

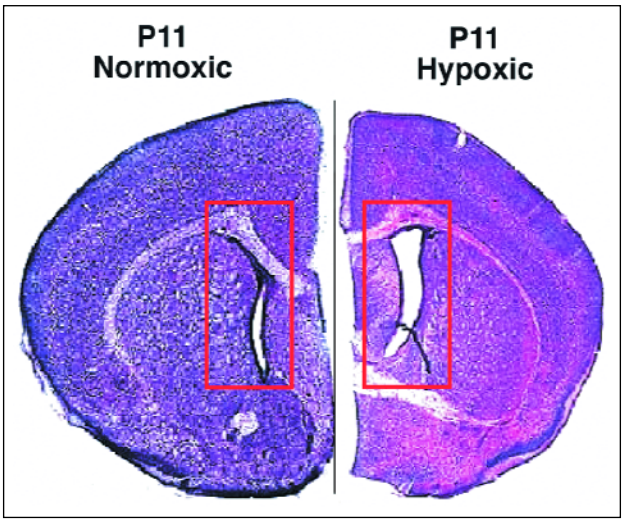

Fig. 6. Representative sections through the lateral ventricles of Normoxic P11 (left panel) and hypoxic P11 (right panel) mouse pup brains illustrating significant ventriculomegaly (boxed areas) of hypoxic reared pups.

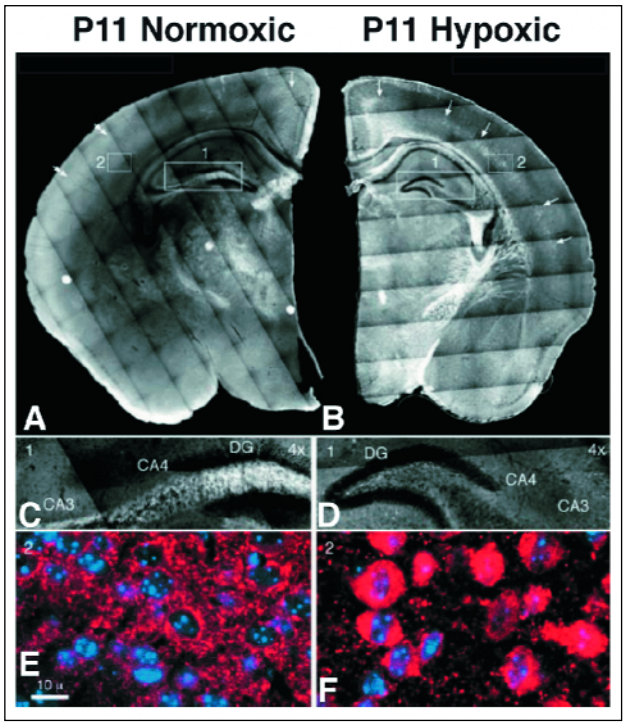

Fig. 7. Representative micrographs of P11 synapsin 1 staining of normoxic and hypoxic hippocampus and cortex. Hypoxia elicits disruption of synapse formation in the hippocampus and cortex. Under hypoxic conditions, synapsin 1 is unapparent at P11 in the hippocampus (B & D, box #1) but increased in the optic tract and internal capsule while robust staining is noted in the normoxic condition (A&C, box #1) (DG, dentate gyrus). High-resolution images of synapsin 1 (red fluorescence, panels E&F, box #2) reveal a finely punctate pattern of synaptic boutons in the Normoxic cortex (E), whereas, in the same region of the Hypoxic cortex (F), synapsin 1 is confined to the soma and coarse neurites, a pattern characteristic of immature neurons. Nuclei are stained blue with 4,6-diamidino- 2-phenylindole (DAPI). Reproduced from (18).

These changes suggest that hypoxia causes a loss of synchronization in the rate of synapse formation in different brain regions, consistent with the observed alterations in gene expression noted in our array data (18).

Other genes affecting synapse development and neural transmission concern central myelination. The oligodendrocyte genes encoding proteolipid protein, cyclic nucleotide phosphodiesterase, platelet-derived growth factor receptor-α (PDGFRα) (not shown), and myelin-associated protein (MAG) (*Fig. 8*) are down-regulated, illustrating the susceptability of glia to hypoxic stress. MAG is a marker of mature oligodendroglia, and its loss is a surrogate marker of the loss of mature glia under hypoxia. As measured by immunofluorescence, MAG appears to be reduced throughout the developing corpus callosum and fiber pathways of the P11 hypoxic brain (*Fig. 8*).

DIFFERENT MURINE STRAINS MIMIC THE RANGE OF RESPONSIVENESS TO HYPOXIA OBSERVED IN THE PREMATURE INFANT POPULATION

Recently we have observed marked differences in the responsiveness of selected mouse strains to chronic hypoxia. Specifically, CD-1 strain mice routinely survive a thirty day exposure to 9.5% O2, while C57BL6 mice succumb after thirteen days of this level of hypoxia (8, 40). This observation may shed considerable light on the variable cognitive outcomes observed in the human premature population. Using data accrued from our previous cell biological and microarray studies (5, 7, 9, 18) we have begun to develop a battery of proteins that were found to be modulated in response to the hypoxic insult and have the potential of being involved in mediating the variable survivability between the CD-1 and C57BL6 mouse strains and the variable responses observed in the human population. Given the importance of HIF-1α and several downstream signaling molecules illustrated in *Fig. 13*, we have begun to assess potential differences in these two murine strains.

In recent studies we and others discovered that different mouse strains respond differently to chronic hypoxia, mimicking the variable response/recovery observed in the human premature infant population (8, 41, 42). In these studies we determined that the differences in survival, apoptosis, proliferation and differentiation of SVZ endothelial cells and neural stem cells were due to differences in HIF-1α responsiveness to the hypoxic insult by endothelial cells and neural stem cells in the SVZ and HIF-1α's differential induction of several downstream pathways involving VEGF, BDNF, SDF-1 and NO (7, 8).

In these studies we have found that CD-1 and C57BL/6 mice exhibit differential subventricular zone (SVZ) neural progenitor cell (NPC) proliferation (*Fig. 9A*), apoptosis (*Fig. 9B*) and microvascular densities (*Fig. 9C*). P11 pups, either reared under normoxic conditions from P0 to P11 or hypoxic conditions from P3 to P11 were injected with BrdU and perfusion fixed. Immunohistochemical analysis at P11 revealed that CD-1 pups exhibited increased SVZ proliferation of nestin positive cells (30.2% *vs*. 20.6% respectively); decreased nestin positive, cleaved caspase 3 (CC3) positive cells (2.8% *vs*. 6.7% respectively) and higher microvascular densities (40.6% increase) in their SVZ areas compared to C57BL/6 P11 pups. CD-1 pups also exhibited substantially increased proliferation (21.2% *vs*. 8.2% respectively), reduced apoptosis (14.7% *vs*. 30.0% respectively) and increased vascular density (53% relative increase) compared to C57BL/6 P11 pups under hypoxic conditions.

These findings were confirmed by Western blotting and ELISA of brain lysates of similarly treated mice (*Fig. 10*) (8).

Specifically CD-1 lysates normalized for protein load and β-actin levels exhibited increased hypoxic induction levels of HIF-1α and several proteins known to be downstream of HIF-1α including BDNF, phospho-TrkB, neuropilin-1 CXCR4 and SDF-1 compared to C57BL/6 pups (8) (*Fig. 9*). These data represent global changes in whole brain lysates. Examination of brain regions (cortex, cerebellum, brain stem, periventricular areas) and specific cell types (endothelial, astrocytic, neuronal and stem cell) will likely exhibit more dramatic differences as will immunohistochemical analyses and in situ hybridizations as we have demonstrated previously (5, 6, 9, 18).

Analyses of NSC isolated from C57BL/6 and CD-1 P0 brains confirmed the tissue NSC proliferation and apoptosis findings as well as the differential hypoxic induction of HIF-1α and several known downstream proteins including VEGF, neuropilin-1, BDNF, CXCR4 and SDF-1 observed in the brain lysates (8) (*Fig. 11*).

Further, low nanogram additions of rVEGF, rSDF-1 and rBDNF enhanced C57BL/6 NSC proliferation to CD-1 levels and sequestration of these factors blunted CD-1 proliferative rates to that of C57BL/6 NSCs (8), confirming functional roles for these factors and their differential expression levels (data not shown).

Since NO is a known autocrine and paracrine modulator of neuronal behaviors and has been shown to stabilize HIF-1α *via* direct S-nitrosylation and indirectly by inhibiting prolyl hydroxylases (43-45), we examined the roles of eNOS and NO in NSC HIF-1α and prolyl hydroxylase domain 2 (PHD2) expression and NSC proliferation. In both 20% and 10% O_2 environments C57BL/6 NSCs exhibit minimal NO expression (*Fig. 12A* and *B*), while CD-1 NSCs exhibit a robust NO expression, which is not appreciably different in either the normoxic or hypoxic environments studied (*Fig. 12C* and *D*). Quantitation of eNOS protein levels revealed that both C57BL/6 and CD-1 eNOS levels were not induced under hypoxic conditions, however CD-1 NSCs expressed 2-fold more eNOS compared to C57BL/6 NPCs in both conditions (*Fig. 12E*) (2.19 fold increase, $p<0.02$). Determination of NPC proliferation in C57BL/6 NSC neurospheres using PCNA expression levels in the absence and presence of NOC-18, a slow release NO donor, revealed a two-fold increase in proliferation; while treatment of CD-1 NPC neurospheres with C-PTIO, an NO scavenger, revealed a 2.5 fold decrease in proliferation (*Fig. 12F*). Examination of HIF-1α levels in C57BL/6 NSC neurospheres in the absence and presence of NOC-18 revealed a three-fold increase in HIF-1α in the presence of NOC-18, bringing the HIF-1α level up to that observed in CD-1 NPC neurosphere cultures; while treatment of CD-1 NSC neurospheres with C-PTIO reduced HIF-1α levels to that of C57BL/6 NSCs (*Fig. 11G*). Additionally, C-PTIO treatment of CD-1 NPCs resulted in a 2.3-fold increase in prolyl hydroxylase domain 2 (*Fig. 11H*). These findings are consistent with our findings of increased HIF-1α expression and a trend toward lower PHD2 levels in CD-1 brain lysates and NSCs and are consistent with findings recently published by Berchner-Pfannschmidt *et al.*, (46) who have demonstrated NO modulation of a HIF-1α/PHD2 autoregulatory loop (8).

Very recent studies have shown that the neural stem cells (NSCs) derived from these two murine strains exhibit distinct matrix metalloproteinase-9 activities, consistent with their distinct NSC migration rates and levels of recovery following perinatal hypoxic insult (47). These data are consistent with recent findings of modulation of MMP-2 and MMP-9 activities following hypoxic-ischemic brain damage in the immature rat (48).

Our current working model of the signaling pathway components that are differentially regulated in C57BL/6 and CD-1 pup brain tissues and cultured NSCs under normoxic and hypoxic conditions is illustrated in *Fig. 13*.

In addition to our murine data, there have been several reports suggesting that plasma levels of VEGF and BDNF may be potentially useful biomarkers in the assessment of several CNS diseases (49-52).

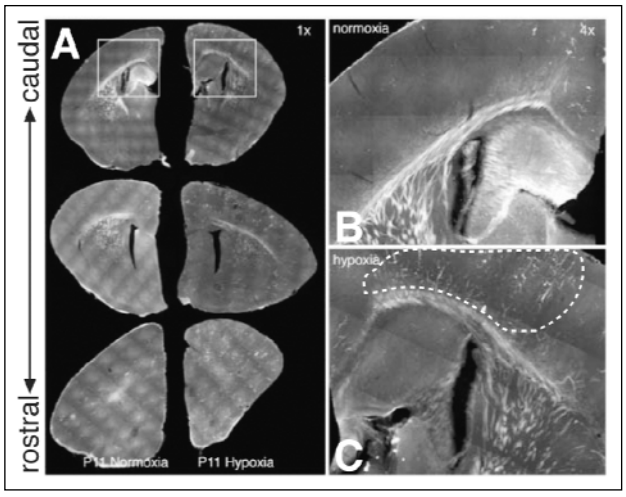

Fig. 8. Representative micrographs of P11 myelin-associated protein staining of normoxic and hypoxic brains. Hypoxia induced a loss of myelin-associated proteins (MAG). In hypoxic P11 brains (A, right panel) there was a down-regulation of MAG transcription and other myelin associated genes compared to normoxic brains (A, left panel). In hypoxic P11 brains MAG is lost from the developing corpus callosum and myelinated fiber pathways (C) compared to normoxic P11 brains (B). Hypoxia also induces an unusual MAG investiture of discrete clusters of cortical microvessels (C, area outlined by dashed line). Reproduced from (18).

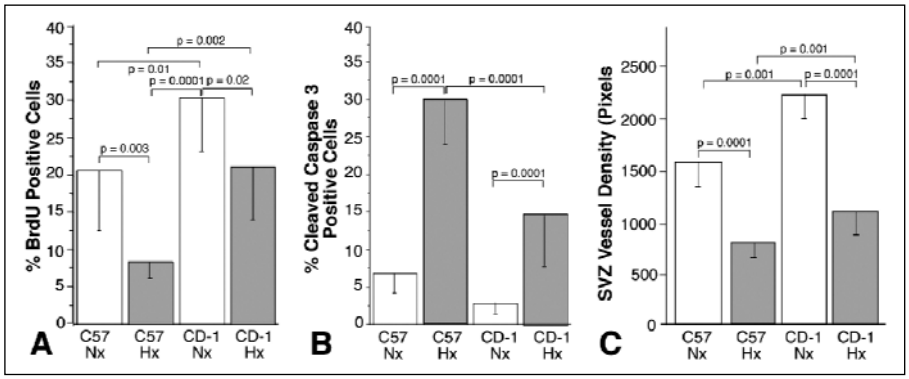

Fig. 9. Quantitation of immunohistochemical staining of normoxic and hypoxic P11 C57BL/6 and CD-1 SVZs (as illustrated in *Fig. 6*) for proliferation (A) (nestin$^+$ & BrdU$^+$ cells), apoptosis (B) (nestin$^+$ & cleaved caspase 3$^+$ cells) and vessel densities (C) (PECAM-1$^+$ structures) in the two mouse strains (n = 6).

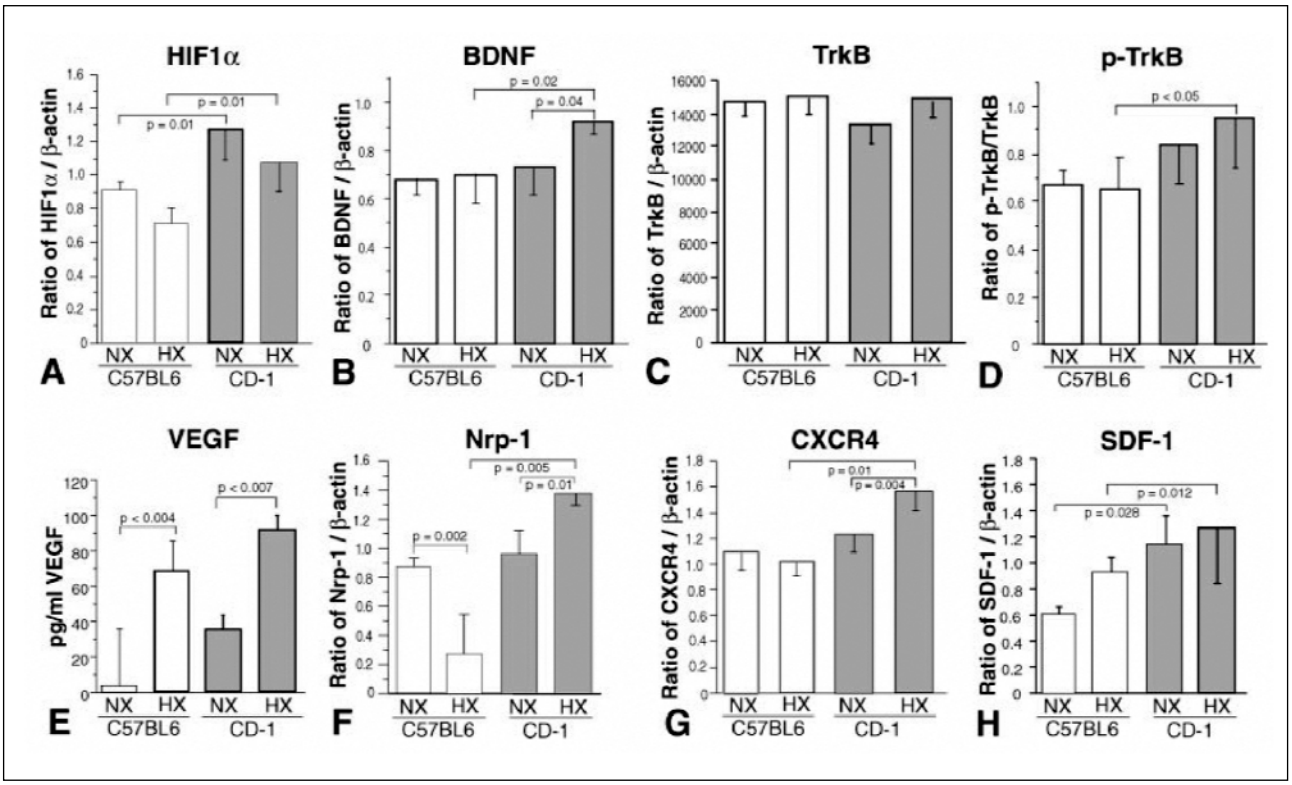

Fig. 10. P11 C57BL/6 brain homogenates exhibit distinct normoxic and hypoxic selected transcription factor, growth factor and receptor protein expression levels compared to CD-1 pups. Western blot (A,B,C,E,F,G &H) and ELISA (D) analyses of expression levels of HIF1α (A), BDNF (B), TrkB (C), p-TrkB (D), VEGF (E), Nrp-1 (F), CXCR4 (G) and SDF-1 (H) in C57BL/6 (open boxes) and CD-1 (shaded boxes) brain homogenates under 21% O_2 normoxic (NX) and a 10% O_2 hypoxic (HX) conditions (vertical bars represent standard deviations; n = 3). Reproduced from (8).

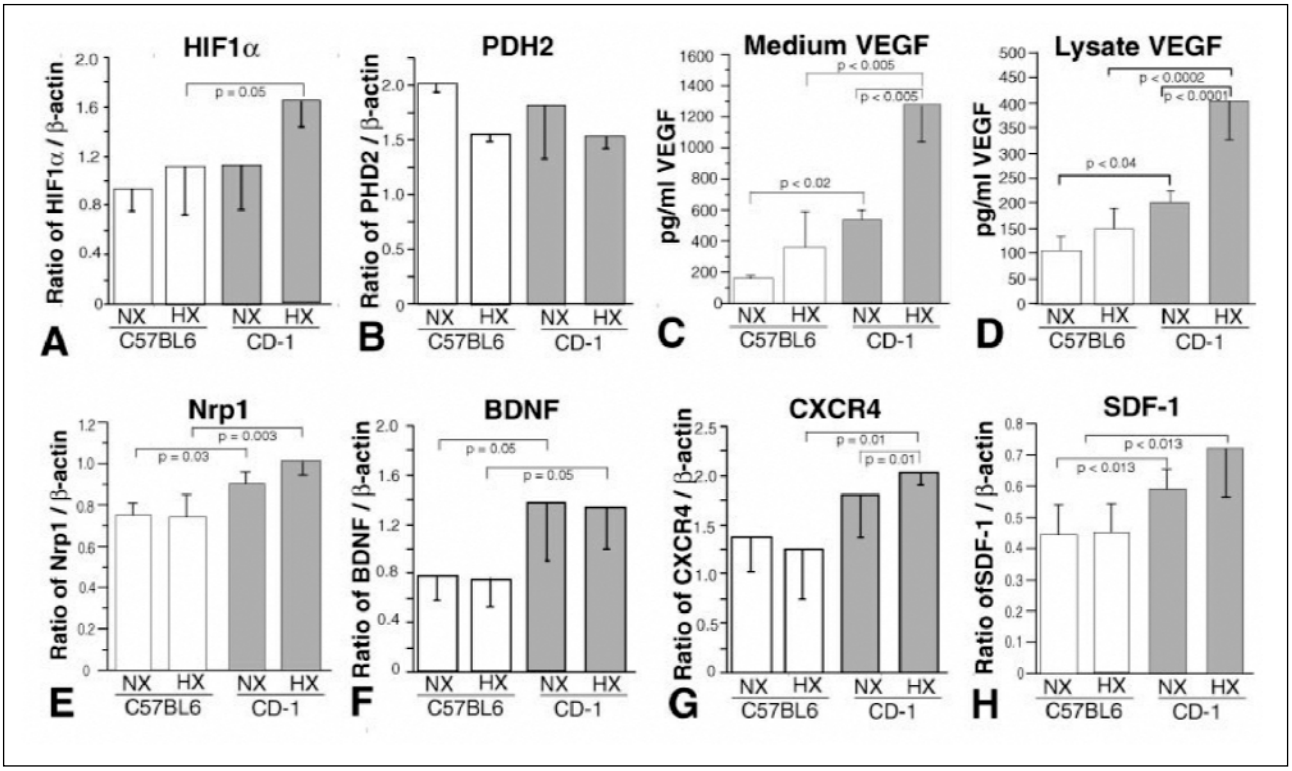

Fig. 11. C57BL/6 NSC culture homogenates exhibit distinct normoxic and hypoxic selected transcription factor, growth factor and receptor protein expression levels compared to CD-1 NSC. Western blot (A,B,E,F,G & H) and ELISA (C & D) analyses of expression levels of HIF1α (A), PHD2 (B), VEGF (C & D), (E), Nrp-1, (F) BDNF, (G) CXCR4, (H) SDF-1 in C57BL/6 (open boxes) and CD-1 (shaded boxes) NSC homogenates in a 20% O_2 culture environment (NX) and a 10% O_2 culture environment (HX) - which induces a HIF-1α response (vertical bars represent standard deviations; n = 3) Reproduced from (8).

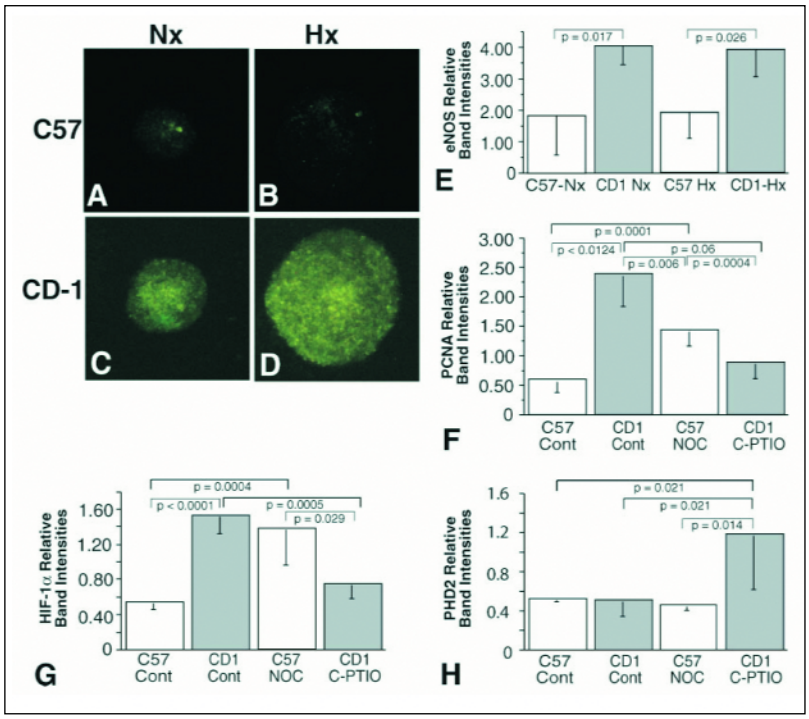

Fig. 12. Primary cultured C57BL/6 neurospheres exhibit reduced NO expression compared to similarly cultured CD-1 neurospheres. Representative fluorescence micrographs of DAF-FM labeled normoxic (A) and hypoxic (B) C57BL/6 neurospheres and normoxic (C) and hypoxic (D) CD-1 neurospheres that had been cultured for six days illustrating relative NO expression levels. (E) Western blot analysis of expression levels of eNOS in C57 and CD-1 NPC neurosphere cultures under normoxic and hypoxic conditions. Western blot analysis of expression levels of PCNA (F) and HIF-1α (G) in C57 NSC neurospheres in the absence (Cont) and presence of the NO donor NOC-18 (NOC) and CD-1 NSC neurosphere in the absence (Cont) and presence of the NO scavenger C-PTIO. (H) Western blot analysis of expression levels of PHD2 in C57 NSC neurospheres in the absence (Cont) and presence of NOC-18 (NOC) and CD-1 NSC neurosphere in the absence (Cont) and presence of C-PTIO. (vertical bars represent standard deviations; n = 3) Reproduced from (8).

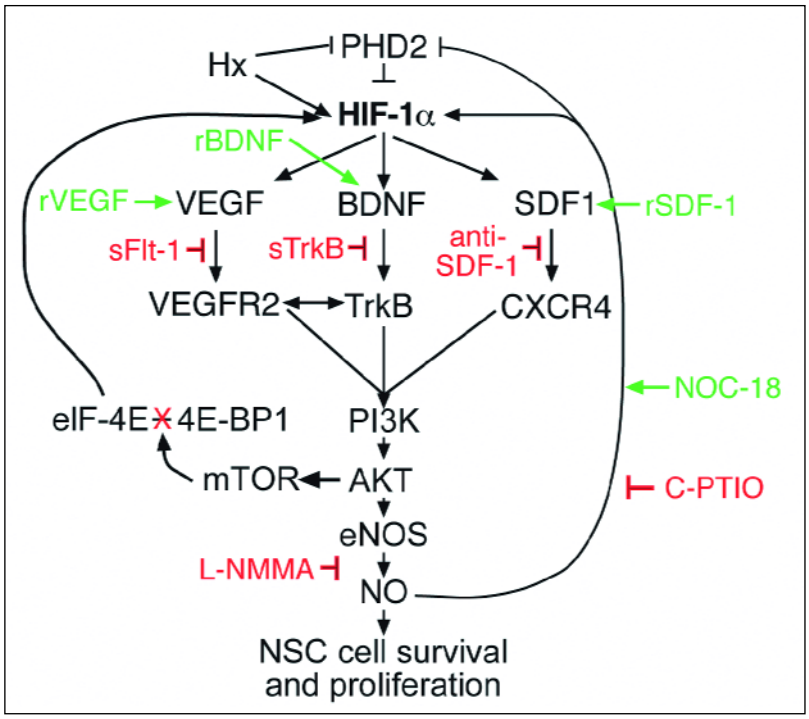

Fig. 13. Working model of the HIF-1α mediated signaling cascades differentially activated in C57BL/6 and CD-1 pups in response to hypoxic insult affecting survival, hematopoietic stem cell and neural stem cell proliferative and survival behaviors and SVZ vessel densities in these two mouse strains. These differences may, in part, be explained by differences in HIF-1α modulated signaling pathways mediated by VEGF, BDNF and SDF-1, all of which are known to exhibit modulation by HIF-1α, whose expression level is regulated by an NO-mediated HIF-1α/PHD2 autoregulatory loop (7, 8). We have also recently determined that HIF-1α translation mediated in part by mTOR is more robust in CD-1 NSCs. Green colored agents = stimulators of this loop; Red colored agents = inhibitors of this loop.

CONCLUSIONS

With the continued advent of new techniques and equipment enabling the medical community to rescue and maintain premature infants of increasingly low birth weight, the pediatric neurology community continues to see increasing numbers of infants, toddlers and school age children suffering from a variety and range of neurodevelopmental abnormalities (11, 16). A major risk factor associated with increased neurodevelopmental abnormalities continues to be chronic sublethal hypoxia. This common complication (due to immaturity of the lungs at time of birth) results in changes in the neuropil and the neurovasculature, which, in turn, is thought to be a cause of the subsequent observed motor and cognitive handicaps. Although recent studies have documented improvement of cognitive function over time in this population, the improvement is variable and mechanisms of recovery are still incompletely understood (14).

The emerging role of the neurovascular niche areas (SVZ and SGZ) in recovery from this and other CNS injuries warrant continued investigation into the control of neuro- and vasculogenesis in these areas and the factors that mediate these processes. Thus, a better understanding of the consequences of chronic sublethal hypoxia to CNS tissues and cells (in particular the neurovascular niches) and the perturbations of endothelial cells, neural stem cells, astrocytes and neuronal cells

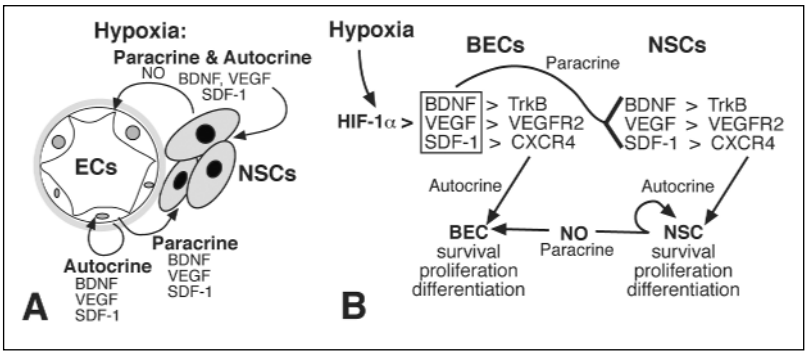

Fig. 14. Working model of the effects of hypoxia on the endothelial cells and neural stem cells comprising the SVZ neurovasular niche. (A) illustrates the dynamic autocrine and paracrine signaling loops thought to be operational in these two cell types following hypoxic insult. (B) illustrates the HIF-1α mediated induction of BDNF, VEGF and SDF-1 and their subsequent autocrine and paracrine signaling, mediating BEC and NSC survival proliferation and differentiation driven in part by NO.

comprising these niches and their dynamic interactions are warranted (*Fig. 14*). The information gained from such studies will aid in the rational design and effective use of novel preventive therapies (directed at specific receptors and signaling pathway components) in the very low weight premature infant population.

Acknowledgements: The author wishes to acknowledge the contributions of the past and current members of the Madri laboratory as well as collaborations with Drs. Laura Ment, Michael Schwartz, William Stewart and Erin Lavik. The work presented here was supported in part by USPHS grants R37-HL28373, RO1- HL51018, PO1-NS35476 and The Reed Foundation (to JAM).

Conflict of interests: None declared.

REFERENCES

1. Louissaint A, Rao S, Leventhal C, Goldman SA. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. *Neuron* 2002; 34: 945-960.
2. Tavazoie M, Van der Veken L, Silva-Vargas V, *et al.* A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* 2008; 3: 279-288.
3. Shen Q, Goderie SK, Jin L, *et al.* Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* 2004; 304: 1338-1340.
4. Shen Q, Wang Y, Kokovay E, *et al.* Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell* 2008; 3: 289-300.
5. Ogunshola OO, Antic A, Donoghue MJ, *et al.* Paracrine and autocrine functions of neuronal vascular endothelial growth factor (VEGF) in the central nervous system. *J Biol Chem* 2002; 277: 11410-1145.
6. Ogunshola OO, Stewart WB, Mihalcik V, Solli T, Madri JA, Ment LR. Neuronal VEGF expression correlates with angiogenesis in postnatal developing rat brain. *Brain Res Dev Brain Res* 2000; 119: 139-153.
7. Li Q, Ford MC, Lavik EB, Madri JA. Modeling the neurovascular niche: VEGF- and BDNF-mediated cross-talk between neural stem cells and endothelial cells: An in vitro study. *J Neurosci Res* 2006; 84: 1656-1668.
8. Li Q, Michaud M, Stewart W, Schwartz M, Madri JA. Modeling the neurovascular niche: murine strain differences mimic the range of responses to chronic hypoxia in the premature newborn. *J Neurosci Res* 2008; 86: 1227-1242.
9. Kim H, Li Q, Hempstead BL, Madri JA. Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells. *J Biol Chem* 2004; 279: 33538-33546.
10. Fagel DM, Ganat Y, Silbereis J, *et al.* Cortical neurogenesis enhanced by chronic perinatal hypoxia. *Exp Neurol* 2006; 199: 77-91.
11. Wilson-Costello D, Friedman H, Minich N, Fanaroff AA, Hack M. Improved survival rates with increased neurodevelopmental disability for extremely low birth weight infants in the 1990. *Pediatrics* 2005; 115: 997-1003.
12. Tyson JE, Saigal S. Outcomes for extremely low-birth-weight infants: disappointing news. *JAMA* 2005; 294: 371-373.
13. Saigal S, Stoskopf B, Streiner D, *et al.* Transition of extremely low-birth-weight infants from adolescence to young adulthood: comparison with normal birth-weight controls. *JAMA* 2006; 295: 667-675.
14. Hack M, Flannery DJ, Schluchter M, Cartar C, Borawski E, Klein N. Outcomes in young adulthood for very-low-birth-weight infants. *N Engl J Med* 2002; 346: 149-157.
15. Bassan H, Feldman HA, Limperopoulos C, *et al.* Periventricular hemorrhagic infarction: risk factors and neonatal outcome. *Pediatr Neurol* 2006; 35: 85-92.
16. Paul DA, Leef KH, Locke RG, Bartoshesky L, Walrath J, Stefano JL. Increasing illness severity in very low birth weight infants over a 9-year period. *BMC Pediatr* 2006; 6: 2.
17. Cooke RW. Preterm mortality and morbidity over 25 years. *Arch Dis Child Fetal Neonatal Ed* 2006; 91: F293-F294.
18. Curristin SM, Cao A, Stewart WB, *et al.* Disrupted synaptic development in the hypoxic newborn brain. *Proc Natl Acad Sci USA* 2002; 99: 15729-15734.
19. Fagel DM, Ganat Y, Silbereis J, *et al.* Cortical neurogenesis enhanced by chronic perinatal hypoxia. *Exp Neurol* 2005; 199: 77-91.
20. Warner-Schmidt JL, Duman RS. Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus* 2006; 16: 239-249.
21. Wurmser AE, Palmer TD, Gage FH. Cellular interactions in the stem cell niche. *Science* 2004; 304: 1253-1255.
22. Ward NL, Lamanna JC. The neurovascular unit and its growth factors: coordinated response in the vascular and nervous systems. *Neurol Res* 2004; 26: 870-883.
23. Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 2000; 425: 479-494.
24. Chow J, Ogunshola O, Fan SY, Li Y, Ment LR, Madri JA. Astrocyte-derived VEGF mediates survival and tube stabilization of hypoxic brain microvascular endothelial cells in vitro. *Brain Res Dev Brain Res* 2001; 130: 123-132.
25. Doetsch F. A niche for adult neural stem cells. *Curr Opin Genet Dev* 2003; 13: 543-550.
26. Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. *J Neurosci* 2006; 26: 13007-13016.
27. Capela A, Temple S. LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron* 2002; 35: 865-875.

28. Mompeo B, Engele J, Spanel-Borowski K. Endothelial cell influence on dorsal root ganglion cell formation. *J Neurocytol* 2003; 32: 123-129.
29. Oishi K, Kobayashi A, Fujii K, Kanehira D, Ito Y., Uchida MK. Angiogenesis in vitro: vascular tube formation from the differentiation of neural stem cells. *J Pharmacol Sci* 2004; 96: 208-218.
30. Wurmser AE, Nakashima K, Summers RG, *et al.* Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. *Nature* 2004; 430: 350-356.
31. Scharfman H, Goodman J, Macleod A, Phani S, Antonelli C, Croll S. Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Exp Neurol* 2005; 192: 348-356.
32. Ment LR, Stewart WB, Ardito TA, Madri JA. Beagle pup germinal matrix maturation studies. *Stroke* 1991; 22: 390-395.
33. Ment LR, Stewart WB, Ardito TA, Huang E, Madri JA. Indomethacin promotes germinal matrix microvessel maturation in the newborn beagle pup. *Stroke* 1992; 23: 1132-1137.
34. Ment LR, Stewart WB, Ardito TA, Madri JA. Germinal matrix microvascular maturation correlates inversely with the risk period for neonatal intraventricular hemorrhage. *Brain Res Dev Brain Res* 1995; 84: 142-149.
35. Mucha K, Foroncewicz B, Koziak K, Czarkowska-Paczek B, Paczek L. The effects of indomethacin on angiogenic factors mRNA expression in renal cortex of healthy rats. *J Physiol Pharmacol* 2007; 58: 165-178.
36. Ment LR, Stewart WB, Fronc R, *et al.* Vascular endothelial growth fact*or* mediates reactive angiogenesis in the postnatal developing brain. *Dev Brain Res* 1997; 100: 52-61.
37. Ment LR, Stewart WB, Scaramuzzino D, Madri JA. An in vitro three-dimensional coculture model of cerebral microvascular angiogenesis and differentiation. *In Vitro Cell Dev Biol Anim* 1997; 33: 684-691.
38. Ford MC, Bertram JP, Hynes SR, *et al.* A macroporous hydrogel for the coculture of neural progenitor and endothelial cells to form functional vascular networks in vivo. *Proc Natl Acad Sci USA* 2006; 103: 2512-2517.
39. Rauch MF, Michaud M, Xu H, Madri JA, Lavik EB. Primary neural progenitor and endothelial cells in a macroporous gel promote stable vascular networks in vivo. *J Biomater Sci Polymer Ed* 2008; 19: 1469-1485.
40. Antaya RJ, Cajaiba MM, Madri J, *et al.* Juvenile hyaline fibromatosis and infantile systemic hyalinosis overlap associated with a novel mutation in capillary morphogenesis protein-2 gene. *Am J Dermatopathol* 2007; 29: 99-103.
41. Ward NL, Moore E, Noon K, *et al.* Cerebral angiogenic factors, angiogenesis, and physiological response to chronic hypoxia differ among four commonly used mouse strains. *J Appl Physiol* 2007; 102: 1927-1935.
42. Sheldon RA, Sedik C, Ferriero DM. Strain-related brain injury in neonatal mice subjected to hypoxia-ischemia. *Brain Res* 1998; 810: 114-122.
43. Li F, Sonveaux P, Rabbani ZN, *et al.* Regulation of HIF-1alpha stability through s-nitrosylation. *Mol Cell* 2007; 26: 63-74.
44. Martinez-Ruiz A, Lamas S. S-nitrosylation: a potential new paradigm in signal transduction. *Cardiovasc Res* 2004; 62: 43-52.
45. Ozturk H, Tuncer MC, Ozturk H, Buyukbayram H. Nitric oxide regulates expression of Sonic hedgehog and hypoxia-inducible factor-1alpha in an experimental model of kidney ischemia-reperfusion. *Ren Fail* 2007; 29: 249-256.
46. Berchner-Pfannschmidt U, Yamac H, Trinidad B, Fandrey J. Nitric oxide modulates oxygen sensing by hypoxia-inducible factor 1-dependent induction of prolyl hydroxylase 2. *J Biol Chem* 2007; 282: 1788-1796.
47. Li Q, Michaud M, Schwartz ML, Madri JA. Modeling the neurovascular niche: strain differences in behavioral and cellular responses to perinatal hypoxia, and relationships to neural stem cell survival and self-renewal. *Am J Pathol* 2009; Oct 8 (epub ahead of print)
48. Dragun P, Makarewicz D, Wojcik L, Ziemka-Nalecz M, Slomka M, Zalewska T. Matrix metalloproteinases activity during the evolution of hypoxic-ischemic brain damage in the immature rat. The effect of 1-methylnicotinamide (MNA). *J Physiol Pharmacol* 2008; 59: 441-455.
49. Tsao PN, Wei SC, Chau HC, *et al.* Vascular endothelial growth factor in preterm infants with respiratory distress syndrome. P*ediatr Pulmonol* 2005; 39: 461-465.
50. Warner-Schmidt JL, Duman RS. VEGF as a potential target for therapeutic intervention in depression. *Curr Opin Pharmaco*l 2008; 8: 14-19.
51. Iga J, Ueno S, Yamauchi K, *et al.* Gene expression and association analysis of vascular endothelial growth factor in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; 31: 658-663.
52. Sharma A, Greenman J, Sharp DM, *et al.* Vascular endothelial growth factor and psychosocial factors in colorectal cancer. *Psychooncology* 2008; 17: 66-73.

Received: August 5, 2009
Accepted: September 10, 2009

Author's address: Joseph A. Madri, Ph.D, M.D., Department of Pathology, Yale University School of Medicine, 310 Cedar Street, P.O. Box 208023, New Haven, CT 06520-8023, USA; Phone: 203-785-276; Fax (Dept.): 203-785-7303; Fax (Office): 203-785-7213; E-mail: JOSEPH.MADRI@YALE.EDU