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Inhaled Gases for Neuroprotection in Neonates with HIE

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Disclosures

- iNO for this study has been provided by Mallinckrodt Pharmaceuticals

Learning objectives

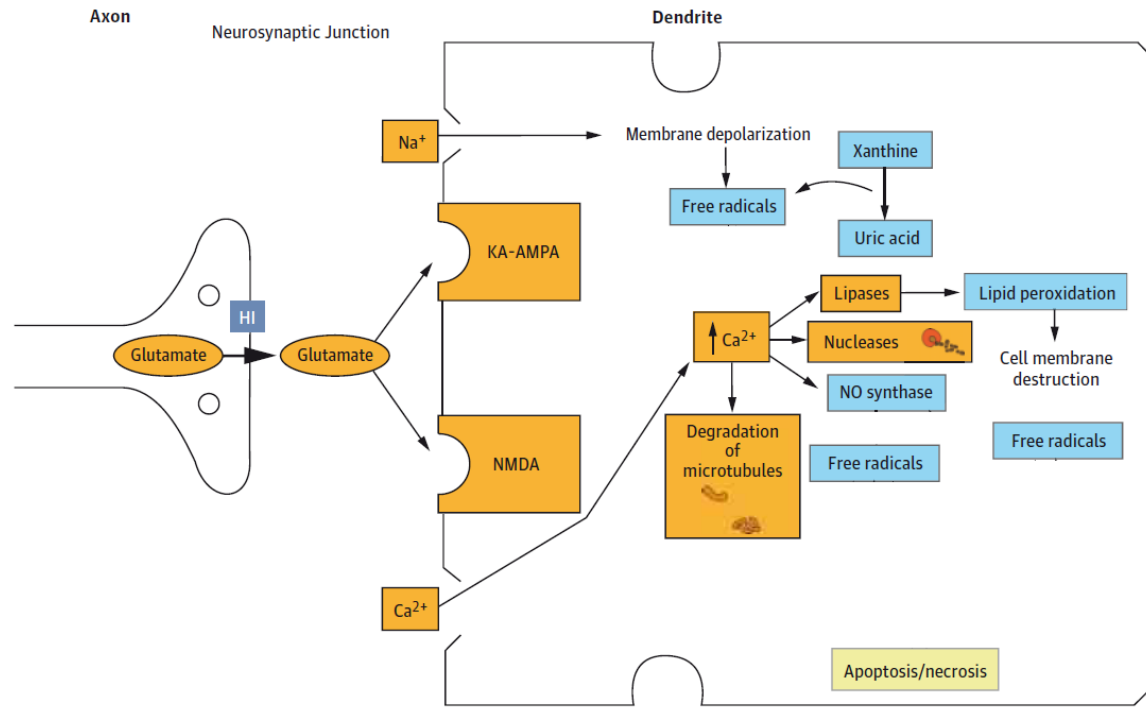
1. Identify new therapeutic inhaled gases for neuroprotection in neonates with HIE
2. Comprehend the mechanism of action of the inhaled gases as it relates to pathophysiology of HIE
3. Discuss ongoing clinical trials for inhaled gases as neuroprotection for HIE

HIE Background

- Incidence: 20 per 1000 live births
- Whole body hypothermia is standard of care for HIE
- 1/8 will will have normal neurologic function
- There is room for improvement in survival without significant neurologic impairment

HIE Pathophysiology

Figure 2. Complex Cascade of Events Producing Cellular Damage and Destruction After Hypoxia-Ischemia (HI)



Xenon

Xenon

- Noble gas
- One of the most studied inhaled gases
- Expensive to manufacture
- Lacks fetotoxicity
- Antagonizes NMDA, AMPA and CaMKII

Natale G, Cattano D, Abramo A, et al. Morphological evidence that xenon neuroprotects against N-methyl-DL-aspartic acid-induced damage in the rat arcuate nucleus: A time-dependent study. *Ann N Y Acad Sci.* 2006

Dinse A, Föhr KJ, Georgieff M, Beyer C, Bulling A, Weigt HU. Xenon reduces glutamate-, AMPA-, and kainate-induced membrane currents in cortical neurones. *Br J Anaesth.* 2005

Petzelt C. PARTICIPATION OF THE Ca²⁺-CALMODULIN-ACTIVATED KINASE II IN THE CONTROL OF METAPHASE-ANAPHASE TRANSITION IN HUMAN CELLS. *Cell Biol Int.* 2001

[Lancet Neurol](#). 2016 Feb; 15(2): 145–153.
doi: [10.1016/S1474-4422\(15\)00347-6](#)

PMCID: PMC4710577
PMID: [26708675](#)

Moderate hypothermia within 6 h of birth plus inhaled xenon versus moderate hypothermia alone after birth asphyxia (TOBY-Xe): a proof-of-concept, open-label, randomised controlled trial

[Denis Azzopardi](#), Prof, FMedSci,^{a,*} [Nicola J Robertson](#), Prof, PhD,^b [Alan Bainbridge](#), PhD,^d [Ernest Cady](#), FlinstP,^c [Geoffrey Charles-Edwards](#), PhD,^e [Aniko Deierl](#), PhD,^f [Gianlorenzo Fagiolo](#), PhD,^a [Nicholas P Franks](#), Prof, FRS,^g [James Griffiths](#), MSc,^h [Joseph Hajnal](#), Prof, PhD,^a [Edmund Juszczak](#), Prof, MSc,^h [Basil Kapetanakis](#), MD,^{a,†} [Louise Linsell](#), MSc,^h [Mervyn Maze](#), Prof, FRCP,ⁱ [Omar Omar](#), BSc,^h [Brenda Strohm](#), RN,^h [Nora Tusor](#), PhD,^a and [A David Edwards](#), Prof, FMedSci^a

Short research report

Anticonvulsant effect of xenon on neonatal asphyxial seizures

[Denis Azzopardi](#)^{1, 2}, [Nicola J Robertson](#)³, [Andrew Kapetanakis](#)¹, [James Griffiths](#)⁴, [Janet M Rennie](#)⁵, [Sean R Mathieson](#)³, [A David Edwards](#)¹

Xenon

- TOBY-Xe study (NCT 00934700)
 - Randomized, open label, parallel group trial in UK. Completed in 2014
 - Moderate to severe HIE
 - Compared 72h of hypothermia alone to 72h hypothermia + 30% xenon for 24h
 - Primary outcome: reduction in Lac/NAA ratio on magnetic resonance spectroscopy or preserved fractional anisotropy measured on diffusion weighted magnetic resonance imaging

Azzopardi D, Robertson NJ, Bainbridge A, et al. Moderate hypothermia within 6 h of birth plus inhaled xenon versus moderate hypothermia alone after birth asphyxia (TOBY-Xe): A proof-of-concept, open-label, randomised controlled trial. *Lancet Neurol.* 2016

Azzopardi D, Robertson NJ, Kapetanakis A, et al. Anticonvulsant effect of xenon on neonatal asphyxial seizures. *Arch Dis Child Fetal Neonatal Ed.* 2013

Xenon

- CoolXenon3 (NCT02071394)
 - Randomized open label study. Phase II is currently recruiting
 - Estimated completion in 2019
 - Comparing 72h of hypothermia alone to 72h hypothermia + 50% xenon for 18h
 - Primary outcome: Death or severe disability (Baily III)
 - Secondary outcome: Brain MRI and aEEG grading

Argon

Argon

- Noble gas
- More abundant and less expensive than xenon
- GABA receptor antagonist
- Upregulates HO-1 and bcl-2
- Decreases NF-kB activation
- No clinical studies of argon

Helium

Helium

- Noble gas
- Heliox is used for deep diving
- Helium is used to cool MRI machines
- Promotes endogenous NO production and activates NrF2 pathway
- There are no current clinical studies looking at helium as a neuroprotective agent

Hydrogen

Hydrogen

- The most abundant element in the universe
- Binds free radicals formed by oxidative stress
- Reduces of caspase-3 and caspase-13 activity
- There are currently no ongoing or completed clinical studies using hydrogen for neuroprotection

Fukuda K ichi, Asoh S, Ishikawa M, Yamamoto Y, Ohsawa I, Ohta S. Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress. *Biochem Biophys Res Commun*. 2007

Ohsawa I, Ishikawa M, Takahashi K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med*. 2007

Cai J, Kang Z, Liu WW, et al. Hydrogen therapy reduces apoptosis in neonatal hypoxia-ischemia rat model. *Neurosci Lett*. 2008

Carbon Dioxide

Carbon Dioxide

- Fourth most common gas in atmospheric air
- Regulates minute ventilation and cerebral blood flow
- hypocapnia increases risk of PVL and IVH
- Permissive hypercapnia shown to decrease incidence of BPD
- In animal studies mild hypercapnia and normocapnia at time of hypoxia-ischemia preserves cerebral blood flow in contrast to hypocapnia and severe hypercapnia

Vannucci RC, Brucklacher RM, Vannucci SJ. Effect of carbon dioxide on cerebral metabolism during hypoxia-ischemia in the immature rat. *Pediatr Res*. 1997

Vannucci RC, Towfighi J, Brucklacher RM, Vannucci SJ. Effect of extreme hypercapnia on hypoxic-ischemic brain damage in the immature rat. *Pediatr Res*. 2001

Carbon Dioxide – Clinical trials

- Hypoxic-Ischemic Encephalopathy Therapy Optimization in Neonates for Better Neuroprotection with Inhalative CO₂ (HENRIC) - NCT02700854
 - Hungarian single center, open label study, actively recruiting
 - Estimated completion in 2020
 - Using low concentration CO₂ gas mixture (5% CO₂ + 95% air) in mechanically ventilated term infants

HENRIC Trial

- Primary Outcome Measures :
 - Percentage of time spent in the desired pCO₂ range of 40-60 mmHg (temp. corrected) during CO₂ inhalation
- Secondary Outcome Measures : Number of seizures, either detected clinically or by amplitude integrated EEG monitoring
 - Time until the end point of metabolic acidosis (BE > -5 mmol/L)
 - Time until the end point of acidosis (pH > 7.25)
 - Severe hypotension (mean arterial pressure less than 25 mmHg), despite full inotrope support and volume replacement.
 - Intracranial hemorrhage detected by MRI
 - Reduction in Lac/NAA ratio on magnetic resonance spectroscopy
 - Preserved fractional anisotropy measured on diffusion weighted MRI
 - Death

Carbon Monoxide

Carbon monoxide

- Neuroprotective at low concentrations
- Upregulates Bcl-2
- Reduces caspase-3 cytochrome c release
- Promotes Nrf2 dissociation and binding to ARE
- There are currently no ongoing or completed clinical studies utilizing carbon monoxide for neuroprotection

Queiroga CSF, Almeida AS, Martel C, Brenner C, Alves PM, Vieira HLA. Glutathionylation of adenine nucleotide translocase induced by carbon monoxide prevents mitochondrial membrane permeabilization and apoptosis. *J Biol Chem*. 2010

Chora AA, Fontoura P, Cunha A, et al. Heme oxygenase-1 and carbon monoxide suppress autoimmune neuroinflammation. *J Clin Invest*. 2007

Wang B, Cao W, Biswal S, Doré S. Carbon monoxide-activated Nrf2 pathway leads to protection against permanent focal cerebral ischemia. *Stroke*. 2011

Oxygen

Oxygen

- Reactive oxygen species are exaggerated by cellular injury like HIE
- Hyperbaric oxygen is controversial
- Resuscitating with 100% Oxygen is also controversial

Resuscitation With Room Air Instead of 100% Oxygen Prevents Oxidative Stress in Moderately Asphyxiated Term Neonates

Máximo Vento, Miguel Asensi, Juan Sastre, Fernando García-Sala, Federico V. Pallardó, José Viña

THE LANCET

Volume 364, Issue 9442, 9–15 October 2004, Pages 1329–1333



Articles

Resuscitation of newborn infants with 100% oxygen or air: a systematic review and meta-analysis

Dr Peter G Davis MD ^{a, b, c, d}, Anton Tan MRCPCH ^e, Colm PF O'Donnell MRCPCH ^{a, b}, Prof Andreas Schulze MD

THE JOURNAL OF PEDIATRICS • www.jpeds.com



ORIGINAL
ARTICLES

Impact of the Neonatal Resuscitation Program—Recommended Low Oxygen Strategy on Outcomes of Infants Born Preterm

Vishal S. Kapadia, MD¹, Charitharth V. Lal, MD², Venkat Kakkilaya, MD¹, Roy Heyne, MD¹, Rashmin C. Savani, MBChB¹, and Myra H. Wyckoff, MD¹

Oxygen – Clinical trials

- Hyperbaric Oxygen Therapy Improves Outcome of Hypoxic-Ischemic Encephalopathy (NCT02894866)
 - Ongoing Chinese multicenter, randomized trial
 - The aim of study is to evaluate the safety and efficacy of hyperbaric oxygen in term infants with HIE
 - The hyperbaric oxygen treatment will be administered for 60 min in a baby hyperbaric oxygen chamber pressured with 100% oxygen.
 - The treatment will be administered once a day within 7 days after birth, 7 days is a course of treatment, at least for 4 courses.

Oxygen – Clinical trials

- **Primary Outcome Measures :**
 - Number of Death or Moderate to Severe Disability Which is Graded According to Gross Motor Function Classification System (GMFCS)
- **Secondary Outcome Measures :**
 - Number of Infants with abnormal ambulatory electroencephalography monitoring Abnormalities
 - Number of Infants with Brain Magnetic Resonance Imaging abnormalities
 - Number of Infants with Brain-stem Auditory Evoked Potentials Abnormalities at 3 Months of Life
 - Neonatal behavioral neurological assessment
 - Bayley Scales of Infant Development
 - Gross Motor Function Classification System
 - Adverse Events That Are Related to Treatment

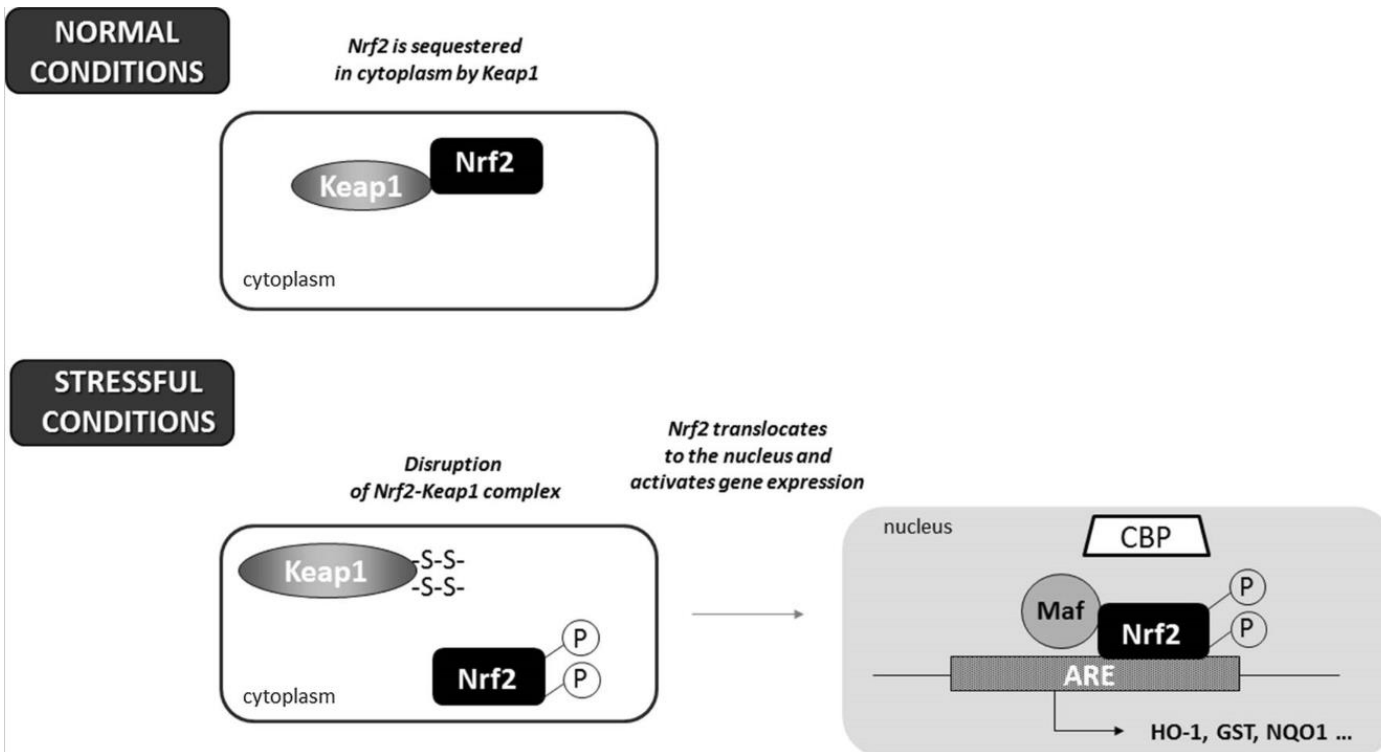
Nitric Oxide

Nitric Oxide

- Endogenous NO is a potent vasodilator, metabolized by oxyhemoglobin
- iNO is used widely to treat persistent pulmonary hypertension
- In contrary to common belief, iNO exerts effect beyond lungs due to its ability to bind to plasma proteins
- iNO activates antioxidant system through ARE-Nrf2-Keap1 pathway

Li YS, Shemmer B, Stone E, A Nardi M, Jonas S, Quartermain D. Neuroprotection by inhaled nitric oxide in a murine stroke model is concentration and duration dependent. *Brain Res.* 2013

Wang B, Cao W, Biswal S, Doré S. Carbon monoxide-activated Nrf2 pathway leads to protection against permanent focal cerebral ischemia. *Stroke.* 2011



Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cell Mol Life Sci.* 2016

Nitric Oxide

- Concurrent iNO therapy (50 PPM) and focal ischemia (Rice Vannucci model) in rats was associated with decreased infarct volumes
- iNO selectively dilates cerebral vessels in ischemic brain areas
- iNO is associated with improvement of recovery from subarachnoid hemorrhage

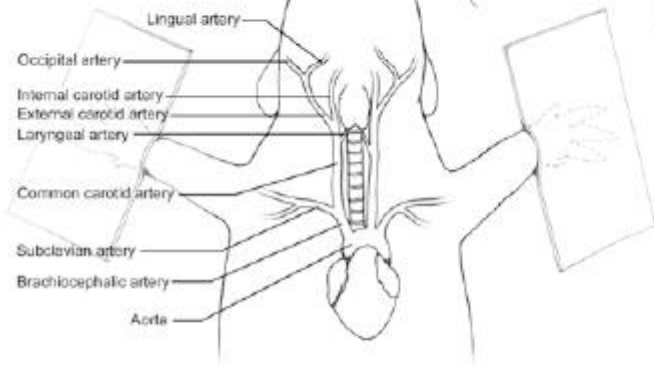


iNO as a Neuroprotective Therapy in HIE in Neonatal Mouse Model

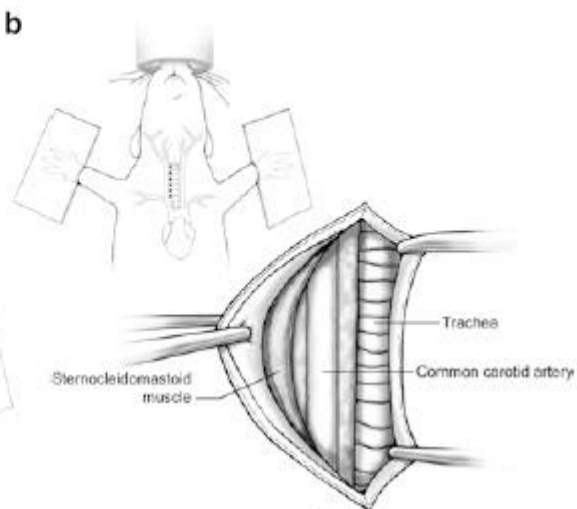
Study Hypothesis

- iNO will improve the behavioral outcomes of HI in normothermic pups by preserving cortical tissue and by preserving white matter

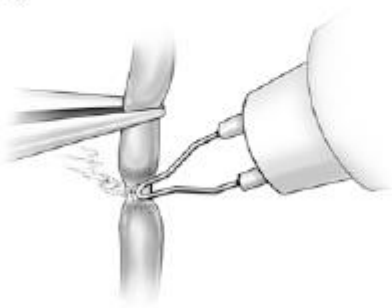
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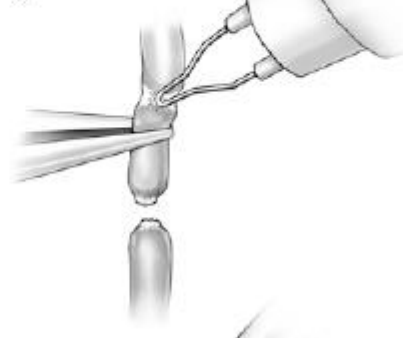
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A.



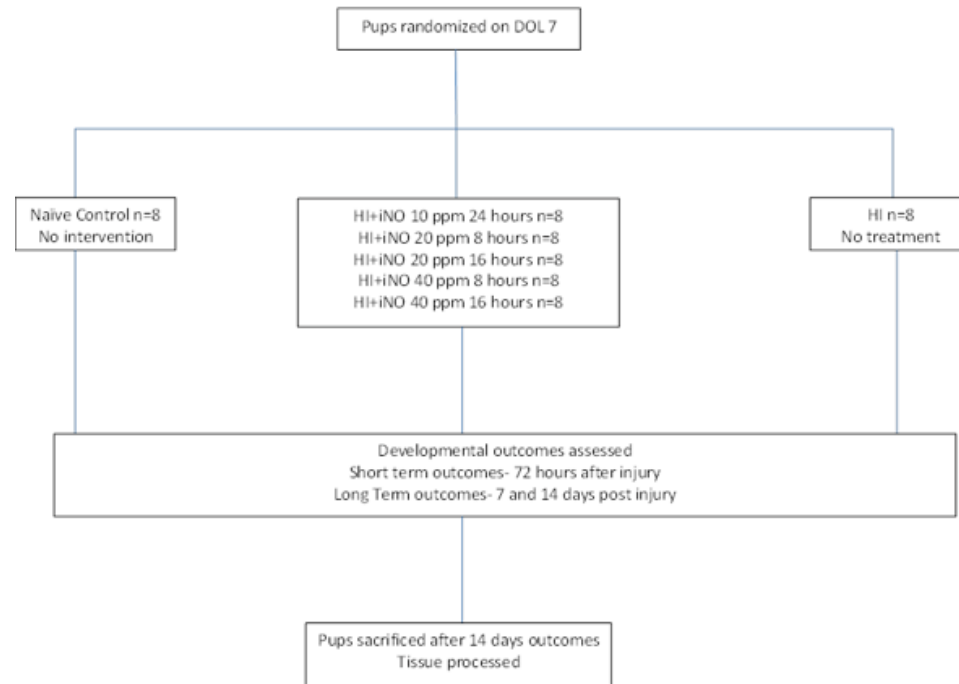
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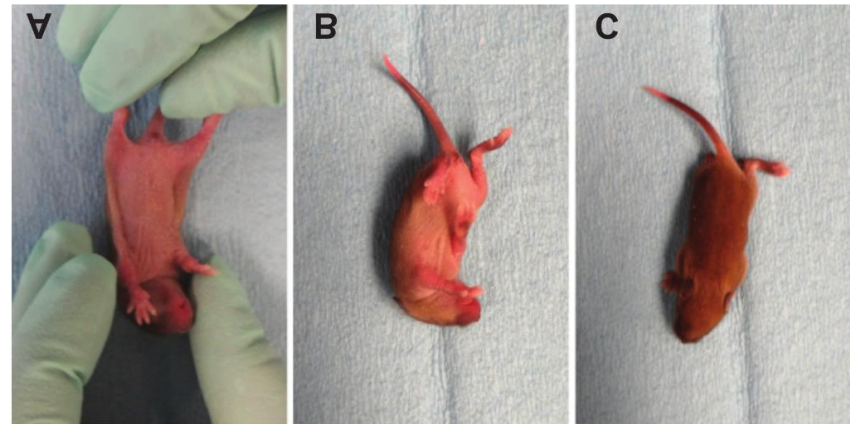
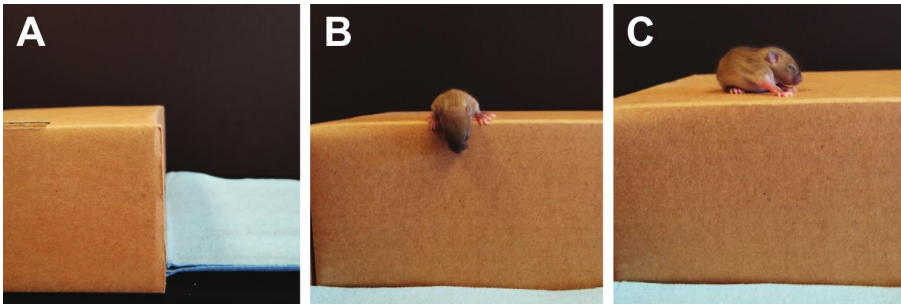
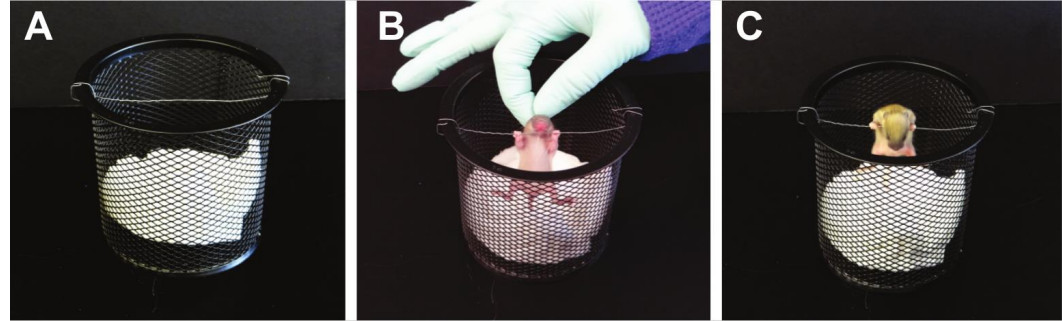
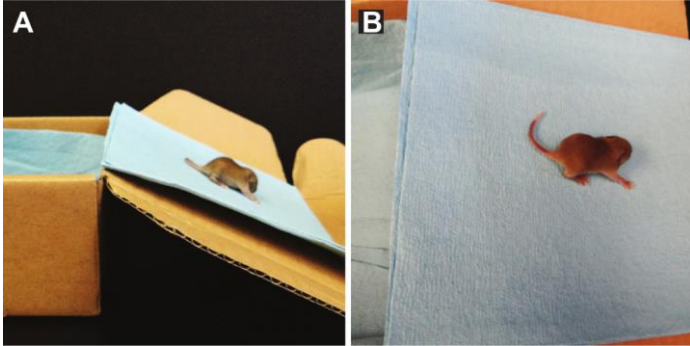
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Aim 1 Overview

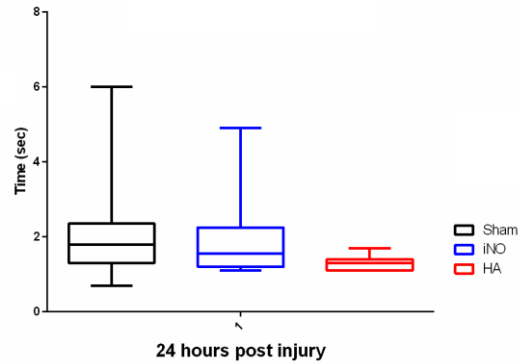


Short Term Outcomes

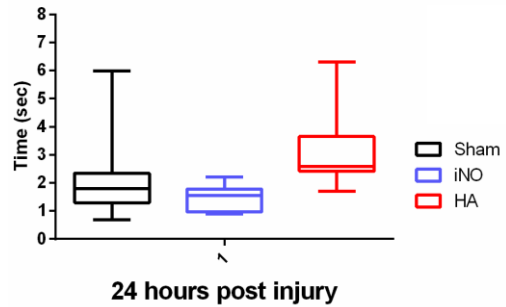
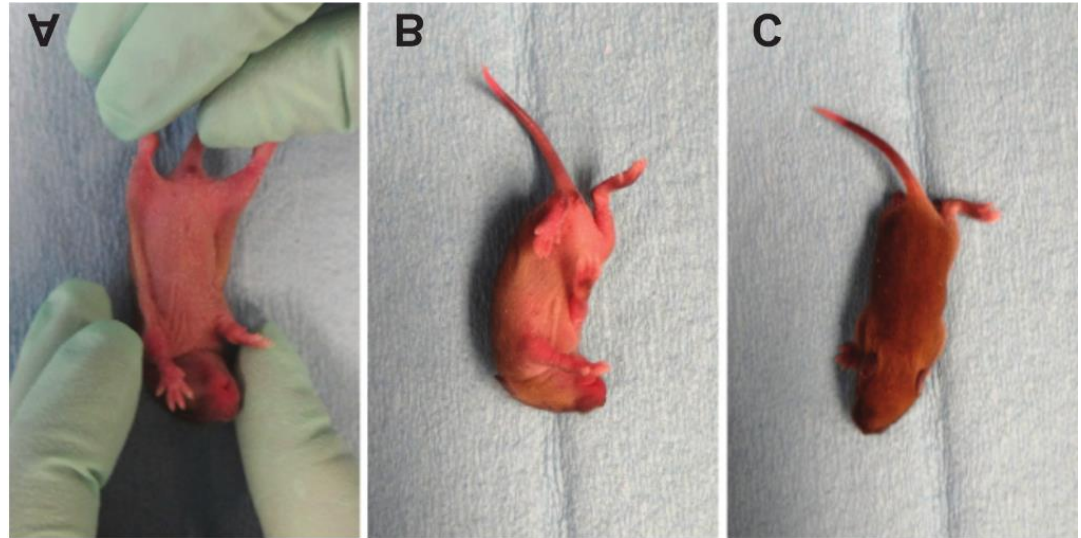


Feather-Schussler DN, Ferguson TS. A Battery of Motor Tests in a Neonatal Mouse Model of Cerebral Palsy. *J Vis Exp.* 2016

Righting Reflex

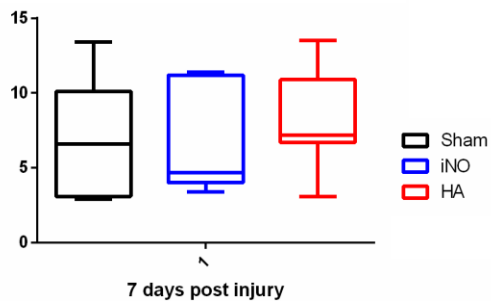
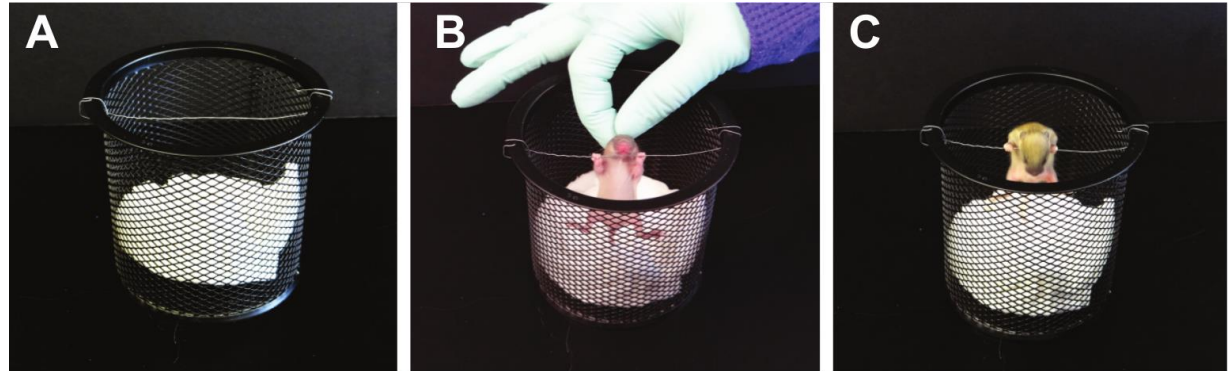
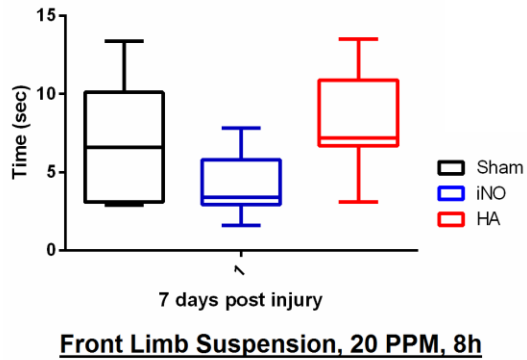


Righting Reflex, 20 PPM, 8h

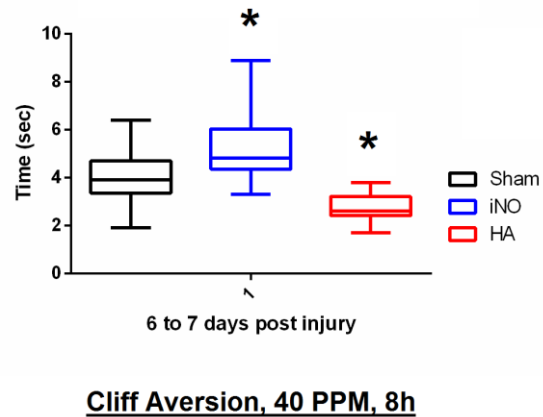
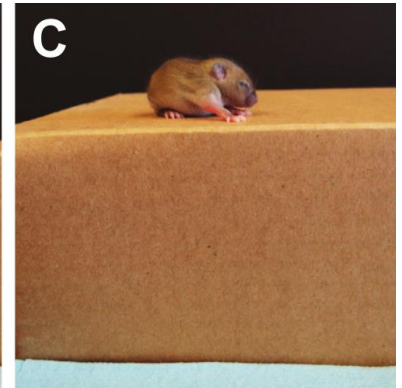
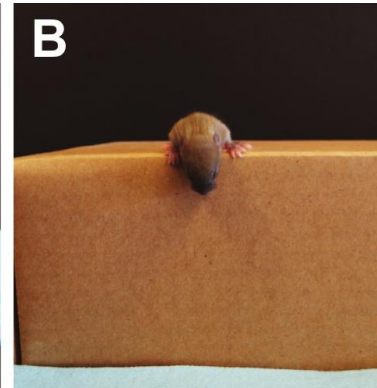
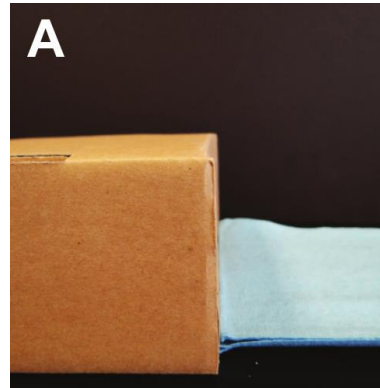
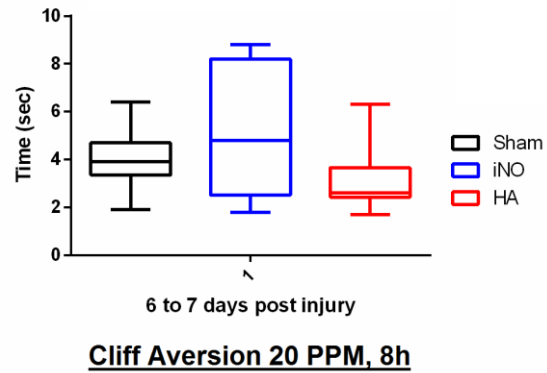


Righting Reflex, 40 PPM, 8h

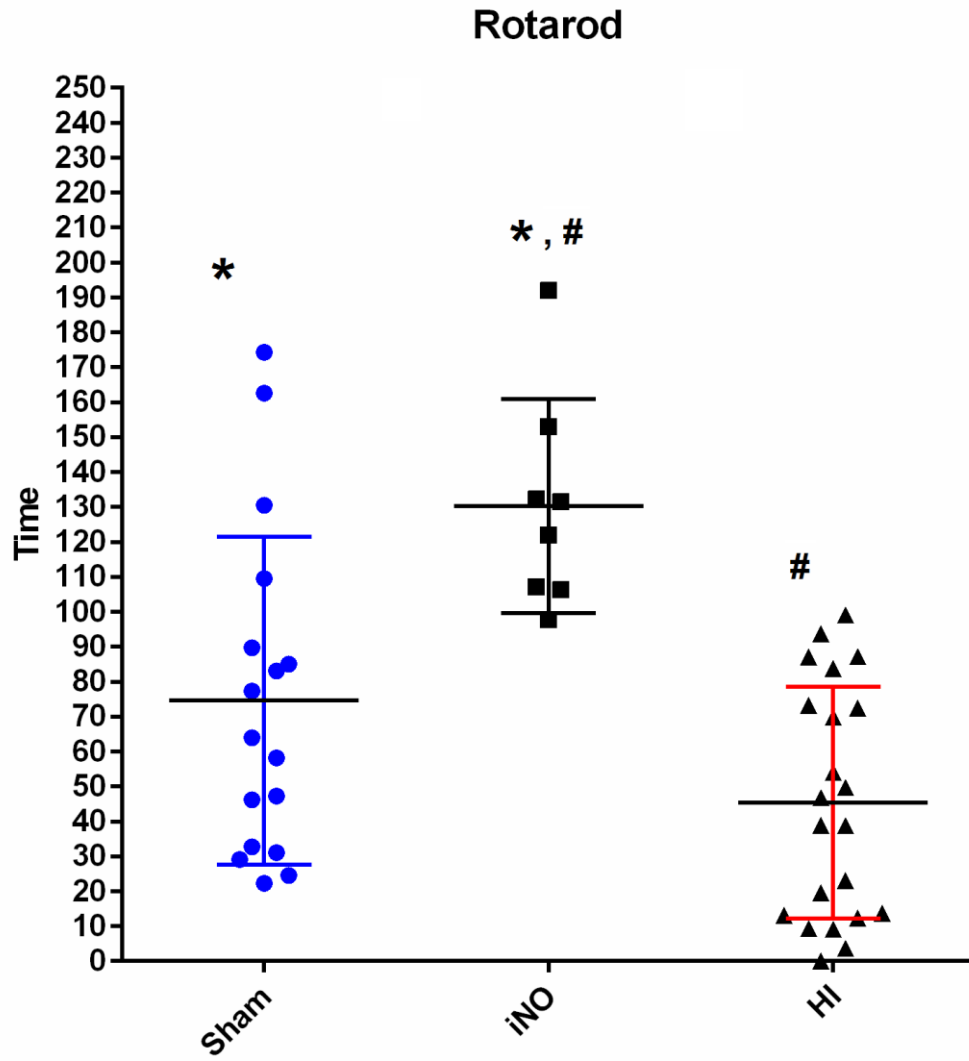
Front limb Suspension



Cliff Aversion



Long Term outcomes, Rotarod



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- Livia Sura
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Thank You!

