The Signature of Early-life Stress on the Vulnerable Developing Brain

4th Biennial Healthy Mothers and Healthy Babies Conference - Innovation and Equity: The Foundation to Quality Perinatal Care in 2020
February 21–22, 2020

Manon Ranger, PhD RN
Assistant Prof, School of Nursing
University of British Columbia

Investigator,
BC Children’s Hospital Research Institute
Vancouver, Canada
Jack was born 4 months early at 25wks GA
Weighed ~700g
Spent 118 days in the NICU

Picture courtesy of Dr. Marsha Campbell-Yeo; MOM-LINC Lab, IWK. Halifax, NS
118 days in the NICU (~17 wks)

1152 procedures

528 hours of fentanyl exposure

852 exposures to 24% sucrose

2 general anesthesia

1960 hours of extreme lighting

2296 hours of excessive noise

2346 hours of maternal separation

~100/118 = 83% time
Multi-System & Multi-Factorial

- Biomarkers
  - Neuroinflammatory
  - Immune
  - Neurotransmitters

- Gene - Environment Interactions

- Infection

- Early Repetitive
  - Pain/Stress

- Treatments
  - Opioids, Sedatives, Sucrose,
  - Antibiotics

- Effects of Maternal
  - Behaviour & Care

- Melatonin, Oxytocin

- Probiotics

- Brain-Gut-Microbiome Axis
Critical Period during Very Rapid Brain Development

Brain at 24 weeks

Brain at 40 weeks (term)
Clinical Evidence Adverse Effect of Early Pain/Stress

• Altered brain development (Brummelte et al., 2012; Chau et al., 2019; Ranger et al., 2013/2015; Vinall et al. 2014; Zwicker et al. 2013) and function (Doesburg et al., 2013)

• Poorer cognition, motor function (Grunau et al., 2009; Vinall et al., 2014; Zwicker et al., 2013), and more internalizing behaviours (Ranger et al. 2014; Vinall et al. 2013)

• Altered hypothalamic-pituitary-adrenal (HPA) axis (cortisol) levels (Grunau et al., 2004/2009/2013; Brummelte et al., 2015), related to attention and internalizing behaviours (Brummelte et al., 2011)
Greater PAIN associated with THINNER CORTEX in 21 brain regions

Pain exposure was the most robust predictor of cortical thickness
In very preterm children, greater exposure to procedural pain & infection in NICU associated with:

• Smaller cerebellar volumes in specific subregions
• Poorer outcomes (cognition & visual-motor integration)
Higher number of procedural pain and concurrent parenting stress associated with greater internalizing (anxiety/depressive) symptoms in very preterm children (non-ventilated sub-group)

Greater morphine exposure while receiving mechanical ventilation in the NICU was related to higher internalizing behaviours at school age
Procedural Pain Treatment NICU

• Sucrose for procedural pain management is used worldwide in NICU

• Sugar consumption activates common reward pathways and repeated sugar exposure causes excessive dopamine & acetylcholine release, and opioid stimulation (Holsti & Grunau, 2010)

• American Academy of Pediatrics: sucrose should be viewed as a prescribed medication that must be tracked; more research is needed (Pediatrics, 2016)

Recent recommendation: 0.1ml minimal dose of 24% sucrose to treat procedural pain (e.g. heel lance) (Stevens et al., 2018)
Translational Mouse Model: Bedside to Bench

Human 24-32 weeks GA

Mice 1st week life (P0-P7)

Postnatal day 7 = 32 weeks GA

P10-12 = 38-40 weeks GA = Term

Biran, Verney & Ferriero, 2012
Aim & How

Examine effects of repeated neonatal sucrose treatment before an intervention on long-term behaviour & brain development in mice

P1 - P6
Oral Water vs Sucrose

P21
Needle-prick

P60-85
Touch

P85 – P95
Brain collection & 7T MRI (N=109)

P85 – P95

Behaviour Testing (N=160)

Tremblay, Ranger, Chau, Ellegood, Lerch, Holsti, Goldowitz, Grunau. PAIN, 2017
Ranger, Tremblay, Chau, Holsti, Grunau, Goldowitz. Frontiers in Psychology, 2019
Repeated Neonatal Exposure to Sucrose Adversely Affects Brain Development in Mice

Mice exposed to sucrose compared to water during the first week of life, irrespective of having a painful procedure or not had significantly smaller volumes in ($p<0.0001$; FDR<5%):

- Corpus callosum
- Stria terminalis
- Fimbria
- Cerebellum
- Hippocampus

Important structures for stress regulation, anxiety/fear network, and memory formation

Tremblay et al., 2017
Main Behavioural Findings

• Memory in adulthood was poorer for mice exposed to pain, regardless of sucrose treatment, suggesting that sucrose is not protective for memory performance when administered for pain.

• In the absence of pain, early sucrose exposure induced poorer short-term memory, stressing importance of accurate pain assessment.

• During a sugar preference test, adult mice exposed to sucrose before an intervention in infancy consumed less sugar water.

Ranger et al., 2019
In Mice Model Paralleling NICU Care

- Early exposure to sucrose had significant deleterious effects on brain volume of important structures and on adult memory
- More research needed in both animal models & clinical longitudinal studies
- Continued recommendation is cautious use of sucrose; use minimum effective dose, use touch base approaches (skin-to-skin, facilitated tucking)
Paw Redness
“Nanny Effect”

• Evidence that *supportive* touch & maternal care can blunt the detrimental effects of early adversity *(De Medeiros et al., 2009; Maitre et al., 2017; Walker et al., 2008)*

• Additional maternal care from increased licking/grooming by mouse “nanny” could moderate adverse effects of pain
Examining Effects of Early Maternal Separation on Brain Activity in Rat Pups

• Early-life stress, such as maternal separation, has been shown to impair neurodevelopment in human and animals  
  (Curley & Champagne, 2016; Lupien et al., 2009)

• Rat pups vocalize in the ultrasonic frequency range in response to isolation; pups’ vocalizations to their mothers may be a marker of connection  (Shair, 2014)
Aims

• Determine the effects of maternal separation on:
  • Prefrontal EEG activity in developing rat pups
  • Acoustic characteristics of pups ultrasonic vocalizations (USVs)
• Examine possible modulating effects of maternal behaviour on EEG activity & USVs
Experimental group – Maternal Separation (MS)

Postnatal Day (P) 0
Birth of pups

P2 – P10
Daily 3h Separation

P10
EEG implants
n=2 pups/litter

P10-14
Home Cage EEG recording

P16
End of Study

Daily 10am-1pm, pups separated from mom + individually isolated in novel cage in incubator (T32-34C); Mom in novel cage in separate room

After last 3h separation, pups and mom reunited for minimum 1hour pre-surgery to allow feeding

Control group

Postnatal Day (P) 0
Birth of pups

P2 – P10
Daily weights only

P10
EEG implants
n=2 pups/litter

P11-14
Home Cage EEG recording

P16
End of Study

At P10 electrophysiology cortex ≈ term infant; P12 ≈ 6 week infant
Wireless telemetric device connected to an EEG electrode placed directly on prefrontal dura
Findings: Early Maternal Separation Alters Cortical Brain Activity and Ultrasonic Vocalizations in Rat Pups

- Maternally separated pups lower prefrontal brain activity during sleep especially when in contact with dam and suckling vs huddled together without contact with mom
- These findings, as do clinical findings in the NICU (Welch et al., 2017), support hypothesis that frontal brain power is impaired in absence of normal interactions with mother – possible biomarker
- Maternal care during development did not buffer damaging effects on brain
- Maternal separation alters the characteristics and patterns of USVs in response to isolation

Ranger, Myers, et al., In Progress
Kaidbey, Ranger et al., 2019 Scientific Reports
Silver Lining

• **Skin-to-skin** effective at reducing pain response & recovery from painful procedures in neonates (*Johnston et al.*, *Cochrane Database of Systematic Reviews* 2017)

• **Calmer**: Better than no treatment; equal to facilitated tucking; well accepted by mothers & staff (*Holsti et al.*, 2019); effective at stabilizing the brain’s hemodynamic response during blood collection in preterm infants (*Ranger, Albert, MacLean & Holsti, In Review* 2019)

• **Strokes** pre-experimental noxious stimulus or heel lance attenuated noxious-evoked brain activity in term infants (*Gursul et al.*, 2018)

• **Music** Intervention – Brain Network fMRI: very preterm babies exposed to tailored-made music had brain networks organisation more similar to full-term infants (*Lordier et al.*, 2019)
**Grunau Lab:** Dr. Ruth E. Grunau (PI), Cecil MY Chau

**Goldowitz Lab:** Dr. Daniel Goldowitz (PI), Dr. Sophie Tremblay (PhDc)

**Holsti Lab:** Dr. Liisa Holsti

**Mouse Imaging Centre (MICe)/UofT:** Dr. Jason P. Lerch, Dr. Jacob Ellegood, & Christine Laliberte

**Acknowledgements:** staff at the CMMT, Hannah McNeill & Zahra Ezzat-Zadeh for help during the experiments; staff BC Children’s & Women’s Hospitals

Fellowships/Funding: Canadian Institutes for Health Research (CIHR), Canadian Child Health Clinician Scientist Program, Fonds Recherche Quebec- Santé; Pain In Child Health.

**Columbia University Developmental Neuroscience & Nurture Science Program**

Dr. Martha G. Welch, Dr. Michael M. Myers, Jasmine Kaidbey, Muhammad Anwar, Dr. Philip Grieve, Dr. Judy Austin, Ali Schulz, & Robert J. Ludwig

Special thanks to: Dr. Myron A. Hofer, Dr. Harry Shair, Joseph Barone, Morgan Firestein, Dr. Raymond I. Stark, Dr. Joseph Isler, & Dr. William Fifer

Funding: Einhorn Family Charitable Trust, Fleur Fairman Family, Morgan Rutman Family, Deki Stephenson
Thank You!

manon.ranger@ubc.ca
@DrManonRanger
Cortical Response Trajectory

- Thalamocortical connections begin around 24 weeks’ gestation & can potentially encode painful stimuli but functionally immature (Fitzgerald, 2005)

- Critical phase of immaturity:
  - ↑ Neuronal activation
  - ↓ Sensory discrimination
  - ↓ Descending inhibitory input

Figure from Ranger, Beggs, Grunau. Dev. Aspects of Pain in Fetal and Neonatal Physiology (2016)
• Preterm infants show non-specific bursting activity (delta brush) in response to tactile & pain (heel lance) (Fabrizi et al., 2011)

• Shifts into modality specific response ~ 35-37 weeks
How Do Infants Feel Pain?

- Nociceptive activity (BOLD response) in newborn infant very similar to adults (18/20 brain regions)
- No activation of amygdala (i.e. emotion)
- Activation at lower sensory threshold in newborns vs adults
- More widespread nociceptive response in infants

Goksan et al., (2015) eLife; Figure 1
Procedural Pain and Brain Development in Premature Newborns

Susanne Brummelte, PhD,¹,² Ruth E. Grunau, PhD,¹,² Vann Chau, MD,¹,²
Kenneth J. Poskitt, MDCM,¹,²,³ Rollin Brant, PhD,⁴ Jillian Vinall, BA,¹,² Ayala Gover, MD,¹,²
Anne R. Synnes, MDCM,¹,² and Steven P. Miller, MDCM¹,²

ANN NEUROL 2012;71:385–396

Neonatal pain in relation to postnatal growth in infants born very preterm

Jillian Vinall a,b, Steven P. Miller b,c, Vann Chau b,c, Susanne Brummelte b,c, Anne R. Synnes b,c,
Ruth E. Grunau a,b,*

Pediatric Neurology 48 (2013) 123–129

Score for Neonatal Acute Physiology—I and Neonatal Pain Predict Corticospinal Tract Development in Premature Newborns

Jill G. Zwicker PhD, OT(C) a,b, Ruth E. Grunau PhD a,b,c, Elysa Adams MSc a, Vann Chau MD a,b,c,
Rollin Brant PhD b,d, Kenneth J. Poskitt MDCM a,b,c, Anne Synnes MHSc, MDCM a,b,e,
Steven P. Miller MDCM a,b,c,f,*
Combinations...

**Neonatal Pain + Reduced maternal care:**
Increase serum corticosterone & decreased glutamate levels in hippocampus/frontal cortex in 4 day old pups *(Mooney-Leber et al., 2018)*

Neonatal pain & maternal isolation altered long-term spatial memory at adulthood *(Mooney-Leber et al., 2019)*

**Maternal trauma:**
Greater neonatal invasive procedure exposure + mothers’ recall of these procedures were related to mothers’ post-traumatic stress symptomatology at discharge *(Vinall et al., 2018)*