Anesthetic-Induced Neurodegeneration: Update of Nonclinical Data Since 2007

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Ikonomidou et al. (1999)

Blockade of NMDA Receptors and Apoptotic Neurodegeneration in the Developing Brain

Chrysanthy Ikonomidou,* Friederike Bosch, Michael Miksa, Petra Bittigau, Jessica Vöckler, Krikor Dikranian, Tanya I. Tenkova, Vanya Stefovska, Lechoslaw Turski, John W. Olney

- Model: 7-day old rat (PND7)
- MK-801 (0.5 mg/kg, i.p.)
Stained with TUNEL Method (Apoptosis)
Brain slices from 8-Day old rats treated with (A) Vehicle or (B) MK-801 24 hours previously.
IP Injection 0.5 mg/kg single dose.
NOTED: Ketamine (20 mg/kg, sc), injected every 90 minutes, 7 injections produced similar results.
Approximate Exposure Margin for Ketamine-induced Neuroapoptosis RAT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Evidence of Neuro-apoptosis</th>
<th>Exposure Margin&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine 10 mg/kg x 7</td>
<td>No ✗</td>
<td>~1</td>
</tr>
<tr>
<td>Ketamine 20 mg/kg x 1</td>
<td>No ✗</td>
<td>~2.7</td>
</tr>
<tr>
<td>Ketamine 20 mg/kg x 7</td>
<td>Yes ✓</td>
<td>~7</td>
</tr>
</tbody>
</table>

<sup>1</sup> Based on rat PK data from Scallet et al. (2004) compared to reported concentrations in humans that are adequate for major surgery (2 ug/mL = “worst case scenario”).

March 10, 2011
R. Daniel Mellon, Ph.D.
Ikonomidou et al. (2000)

Ethanol-Induced Apoptotic Neurodegeneration and Fetal Alcohol Syndrome

Chrysanthy Ikonomidou, Petra Bittigau, Masahiko J. Ishimaru, David F. Wozniak, Christian Koch, Kerstin Genz, Madelon T. Price, Vanya Stefovska, Friederike Hörster, Tanya Tenkova, Krikor Dikranian, John W. Olney

- Model: 7-day old rat
- 20% solution ethanol 2.5 g/kg SC twice 2 hours apart
- MK-801 (0.5 mg/kg x 3, IP)
- Phenobarbital (50-70 mg/kg, IP)
- Diazepam (30 mg/kg x 1, IP)

Science 287: 1056-1060
Stained with silver stain (degenerating neurons)
Brain slices from 8-Day old rats treated with (A) Vehicle or (B) MK-801, (C) Phenobarbital, or (D) ethanol treated rats, 24 hours previously.

Ikonomidou et al. (2000)
Fig. 3. Age Dependency of ethanol-induced apoptosis in the brains of developing rats

Ikonomidou et al. (2000)

Early: VMH, DM & V Thalamus
Mid: subiculum, hippocampus, caudate, LD & AV thalamus
Late: Frontal, parietal, temporal, cingulate, retrosplenial cortices
Jevtovic-Todorovic et al. (2003)

Early Exposure to Common Anesthetic Agents Causes Widespread Neurodegeneration in the Developing Rat Brain and Persistent Learning Deficits

Vesna Jevtovic-Todorovic,1 Richard E. Hartman,2 Yukitoshi Izumi,3 Nicholas D. Benshoff,3 Krikor Dikranian,3 Charles F. Zorumski,3 John W. Olney,3 and David F. Wozniak3
1Department of Anesthesiology, University of Virginia Health System, Charlottesville, Virginia 22908, and Departments of 2Neurology and 3Psychiatry, Washington University School of Medicine, St. Louis, Missouri 63110

- **Model:** Neonatal Rat (PND 7), 6 hours of anesthesia.
- **Anesthetic Regimen:** cocktail of nitrous oxide, oxygen, isoflurane and midazolam.
- **Endpoints:** Histopathology, behavioral testing over 160 days, and electrophysiology testing in hippocampal slices (P29-p33)
MORRIS WATER MAZE

(a. PLACE TRIALS (Age P32)  

(b. PLACE TRIALS (Age P131)
Time Windows of Vulnerability to the Neurotoxic Effects of NMDA Receptor Antagonists for Rat (Postulated for Monkey and Human)

- **Rats**
  - Conception
  - Birth
  - Apoptotic Neurodegeneration: 14 d
  - No Neurodegeneration: 1.5 mo

- **Rhesus Monkeys**
  - Conception
  - Birth
  - Apoptotic Neurodegeneration: 2 mo
  - No Neurodegeneration: 3 yr

- **Humans**
  - Conception
  - Birth
  - Apoptotic Neurodegeneration: 3 yr
  - No Neurodegeneration: 11 yr

Slikker et al. (2007)

Ketamine-Induced Neuronal Cell Death in the Perinatal Rhesus Monkey


Model: Rhesus monkey (Gestational day 122 and postnatal day 5 and 35)

– Ketamine IV 24 hours, 6 hour withdrawal period.
– Ketamine IV 3 hours in postnatal day 5 animals.
– Ketamine (20-50 mg/kg/h) with PND35 requiring more to maintain anesthetic plane
FIG. 3. Quantitative analyses of ketamine-induced neurodegeneration assessed using caspase 3 immunostaining (A), silver staining (B) and Fluoro-Jade C staining (C). For each condition, three animals were randomly assigned to treatment and control groups ($N = 3$/group). Data are presented as means ± SD. * A probability of $p < 0.05$ was considered significant (two-way ANOVA).
### Approximate Exposure Margin for Ketamine-induced Neuroapoptosis MONKEY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Evidence of Neuro-apoptosis</th>
<th>Mean Maximum Plasma Concentration</th>
<th>PK Exposure Margin(^1)</th>
<th>PD Exposure Margin</th>
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<tr>
<td>GD122 (24 hrs)</td>
<td>Yes ✓</td>
<td>~10-15 ug/mL</td>
<td>~5-7</td>
<td>~1</td>
</tr>
<tr>
<td>PND5 (24 hrs)</td>
<td>Yes ✓</td>
<td>~10-15 ug/mL</td>
<td>~5-7</td>
<td>~1</td>
</tr>
<tr>
<td>PND35 (24 hrs)</td>
<td>No ×</td>
<td>~25 ug/mL</td>
<td>~12</td>
<td>~1</td>
</tr>
<tr>
<td>PND5 (3 hrs)</td>
<td>No ×</td>
<td>~12 ug/mL</td>
<td>~6</td>
<td>~1</td>
</tr>
</tbody>
</table>

\(^1\) Based on reported concentrations in humans that are adequate for major surgery (2 ug/mL = “worst case scenario”). Plasma concentrations for procedural sedation in newborns reported to range as high as 437 ng/mL 15 minutes post infusion of 2 mg/kg dose (Saarenmaa 2005). Continuous infusion of 2 mg/kg/h results in about 2 ug/mL in human neonates (Hartvig 1993).
“The lack of information to date precludes the ability to designate any one anesthetic agent or regimen as safer than any other... The FDA anticipates working with the anesthesia community and pharmaceutical industry to develop strategies for further assessing the safety of anesthetics in neonates and young children, and for providing data to guide clinicians in making the most informed decisions possible when choosing anesthetic regimens for their pediatric patients.”
FDA ALSDAC Advisory Committee
(March 29, 2007)

• Discussion Item 1: Are there sufficient data to apply the findings in animals to humans?
  – 14 of 15 responded No
  – 1 of 15 neither yes or no

• Synopsis: All agreed that the data are worrisome, that more studies are needed, and that this is a high priority issue.
Overview of Significant New Nonclinical Findings Reported in the Literature
(in no particular order)
Finding #1: Primate Functional Data

NCTR’s primate behavioral data with 24-hr ketamine demonstrating long-term cognitive deficits (Paule et al., 2011)

- To be presented by Dr. Merle Paule, NCTR
- Compare these data to the rodent data reported to date
- Compare these data to the clinical study results reported to date with multiple anesthetic exposures (Dr. Simone’s presentation)
- Which endpoint is more sensitive, histopathology or functional changes?
Finding #2: Oligoapoptosis

Dr. Olney’s group has reported that immature oligodendrocytes are also susceptible to anesthesia-induced apoptosis

– To be presented by Dr. Olney.
– Do oligodendrocytes show the same window of vulnerability as neurons?
– What are the long term clinical implications, if any?
– What clinical endpoints should we look for and when would we expect to see them?
Finding #3: Synaptic Architecture

Anesthesia-induced alterations in synaptic architecture (De Roo et al., 2009; Briner et al., 2010)

- PND 15 anesthesia exposure INCREASED dendritic spine density, effects occur after the peak in synaptogenesis
- Significance unclear, but may broaden the potential window of vulnerability
- Are these findings adverse or adaptive?
Objective: Examine impact of anesthesia on synaptic growth and dynamics – the development of synaptic networks

Model: Mice expressing YFP in layer 5B cortical neurons (PND 15, 20 and 30)

- IP injection of midazolam (25 mg/kg), propofol (50 mg/kg), ketamine (30 mg/kg) + subsequent doses to produce deep sedation for approximately 5 hours (vs. saline)
- Examined neuronal cytoarchitecture and cell death either immediately after the 5 hour treatment or at later time points.
Figure 2. Effects of propofol and ketamine anesthesia on dendritic protrusions in the somatosensory cortex in vivo. (a) 3D volume rendering of confocal microscopy images of apical dendrites in control (left), propofol-treated (middle) and ketamine-treated (right) PND 15 mice. Scale bars: 1 µm. (b) Protrusion density of pyramidal neurons of the SCC of mice sacrificed at PND 15; 20 and 30 just after a 5 h-anesthesia with propofol (Propo) or ketamine (Keta) compared to control conditions (ctrl). (c) spine head width at PND 15 after 5 h-propofol (Propo) or Ketamine (Keta) anesthesia compared to control group. (b, c): n = 4 animals per group, 6945 spines; ***: P<0.001, **: P<0.01, *: P<0.05, two-way ANOVA with Bonferroni post tests.
Finding #4: Neurogenesis

Anesthesia exposure during early brain development results in decreased neurogenesis in the rat model (Stefovska et al., 2008; Stratmann et al., 2009, Zhu et al., 2010)

- Impact on window of vulnerability?
- Impact on neuronal adaptability or reserve capacity?
- Impact of repeated exposures?

Image Source: Mindsparkle.com
Zhu et al. (2010)

Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents

Changlian Zhu¹,², Jianfeng Gao¹,², Niklas Karlsson¹, Qian Li¹,², Yu Zhang¹,², Zhiheng Huang¹,², Hongfu Li¹,², H Georg Kuhn¹ and Klas Blomgren¹,³

¹Center for Brain Repair and Rehabilitation, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; ²Department of Pediatrics, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou, China; ³Department of Pediatric Oncology, The Queen Silvia Children’s Hospital, Gothenburg, Sweden

- **Model**: PND14 rats and mice and PND60 rats anesthetized with isoflurane (1.7%) for 35 minutes daily for 4 days.
- **Endpoints**: histopathology, cytogenesis, cognitive endpoints (object recognition, place examined.)
Zhu et al. (2010)

March 10, 2011

R. Daniel Mellon, Ph.D.
J Cerebral Blood Flow Met 30:1017-30
Figure 8 Isoflurane reduced the neural stem cell pool in the immature rat GCL. (A) A confocal image showing a representative SOX-2 (green) and GFAP (red) double labeling in the GCL. Scale bar = 20 μm. (B) The numbers of undifferentiated neural stem cells (SOX-2/GFAP double-positive cells) were counted in the entire GCL and the bar graph shows a significant decrease in the number of stem cells in the immature rat GCL after isoflurane exposure (n = 6/group), but not in the adult rat GCL (n = 7/group). ***P < 0.001.
Finding #5: Spinal neurons

Anesthesia-induced neurodegeneration in the spinal column

• Combination of Isoflurane and nitrous oxide increased neuroapoptosis in the dorsal horn of the spinal column (Sanders et al., 2008)

• Spinal ketamine-induced neurotoxicity, but not spinal morphine (Walker et al., 2010; Westin et al., 2010)

• Are there functional consequences?

• Window of vulnerability?

• Are there any data examining the effects of spinal local anesthetics in an nonclinical neonatal model?
Finding #6: Effect of Pain

Impact of pain/surgery on anesthesia-induced neurotoxicity

• Analgesic dose of ketamine blocks pain-induced neuroapoptosis (Anand et al., 2007; Rovnaghi et al., 2008)
• Dr. Anand will discuss these findings.
• Would an anesthetic dose also block pain-induced neuroapoptosis?
  • Stratmann et al. 2009 model used tail-clamp during isoflurane anesthesia in the rat and still noted long-term progressive cognitive deficits.
  • Does the level of pain/discomfort associated with an procedure impact the neuronal toxicity?
Finding #7: “Better” Options?

Precedex (dexmedetomidine), morphine, fentanyl may not produce neuroapoptosis in rodent models
  • Fentanyl (Rizzi et al., 2008)
  • Morphine (Black et al., 2008)
  • Dexmedetomidine (Sanders et al., 2009 & 2010)

Published studies comparing anesthetics in rodent models suggest some drugs are better options than others
  • Is sevoflurane “better” than isoflurane?
  • Are ketamine or propofol “better” than isoflurane?

Questions:
  • Will the same findings be observed in primates?
  • Should the findings be repeated in primates?
  • What about opioid effects on neuronal development?
  • Should these medications be used instead of others when they can accomplish the clinical objective?
Isoflurane Causes Greater Neurodegeneration Than an Equivalent Exposure of Sevoflurane in the Developing Brain of Neonatal Mice

Ge Liang, M.D.,* Christopher Ward, M.D.,† Jun Peng, M.D.,‡ Yifan Zhao, M.D.,‡ Baosheng Huang, M.D.,§ Huafeng Wei, M.D., Ph.D.||

6 hours (~0.5 MAC)

Fig. 2. Isoflurane induced greater apoptosis than equipotent exposure of sevoflurane in the hippocampus CA1 region of neonatal developing brains. (4) Brain sections containing hip-
Item #9: Means to Prevent?

Published studies suggesting ways to block the anesthesia-induced neuroapoptotic effect in nonclinical models

– Can these nonclinical findings be studied in humans?

– Would animal efficacy data impact the practice of medicine?

– If yes, should these finding be reproduced in primates? How should these findings be communicated to clinicians and the public?
Prevention/Amelioration Studies

- Estradiol
  - Bittigau et al. (2002); Asimiadou et al. (2006)
- Melatonin
  - Yon et al. (2006)
- Xenon
  - Ma et al. (2007); Cattano et al. (2008)
- L-Carnitine
  - Zou et al. (2008)
- Lithium
  - Straiko et al. (2009)
- Dexmedetomidine
  - Sanders et al. (2009 & 2010)
- Hypothermia
  - Creeley and Olney (2010)
Nonclinical Conclusions

• The primate data is supporting the findings in rodents
• Research using the rodent models far outpaces the primate capabilities
• NMDA antagonists ≠ GABAergic modulators
• Studies in rodent suggest subtle changes in brain architecture and neurogenesis which may impact the perceived window of vulnerability and suggest age-dependent changes
• Collectively, these data impact the planning and interpretation of the clinical studies
Considerations for Future Nonclinical Studies

- Identify a “No Adverse Effect Level” (NOAEL) for dose and duration
- Gather and report both pharmacokinetic and pharmacodynamic endpoints
- Include comparative toxicology assessments
- Mimic the clinical setting as closely as possible
Surgical Stress
Pain
Psychological Stress etc.

Anesthesia
Thank you
Behavioral Effects in Primates
Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys

Merle G. Paule, Ph.D.
Director
Division of Neurotoxicology
FDA’s National Center for Toxicological Research
Jefferson, Arkansas 72079-9502
Merle.Paule@fda.hhs.gov
Early postnatal ketamine anesthesia and long lasting cognitive deficits in rhesus monkeys

- Sensitive period for the induction of nerve cell death in the monkey includes ~middle of third trimester (GD 120) to postnatal day 5; no effect in PND 35 offspring
- 24 hour exposure initially used as benchmark
- 3 hours does not appear to be sufficient; 9 hours is over threshold
- Cell death is both apoptotic and necrotic in the monkey; in the rat it is apoptotic
Early postnatal ketamine anesthesia and long lasting cognitive deficits in rhesus monkeys

- Given that ketamine also causes significant abnormal cell death during the brain growth spurt/synaptogenesis in the primate as it does in rodents, are there associated functional consequences as there are in rodents?
Goal: Make predictions about the effects of developmental exposures to ketamine on cognitive function in humans.

Ideally, the data needed to make such predictions are obtained from laboratory animal models in well controlled experiments under known conditions of exposure.

* The use of appropriate animal models is critical: the closer to humans, the better.

* Relevant endpoints decrease the uncertainty associated with the process; utilization of identical endpoints is best.
Early postnatal ketamine anesthesia and long lasting cognitive deficits in rhesus monkeys

- 24-hr iv anesthesia on PND 5
- Wean at 6 months of age
- Begin cognitive function assessments at 7 months of age: daily 50 min sessions (M-F)
- Monitor for at least two years (currently at >760 daily sessions, > 150 weeks/>3 years of testing; animals now ~4 years old
National Center for Toxicological Research (NCTR) Operant Test Battery (OTB) Assessments

- Motivation
- Color and Position Discrimination
- Learning
- Short-term Memory
FOR THE MOTIVATION TASK

- Only the far right retractable lever is used
- Subjects must increase the number of lever presses for each subsequent reinforcer obtained: initially, 2 presses=reinforcer, then 4 presses=reinforcer, then 6=reinforcer, and so on
FOR THE COLOR AND POSITION DISCRIMINATION TASK

- All three press-plates are used
- Initially, either a red, yellow, blue or green color is presented at the center position
- Observation of this color is indicated by a subject's response to it (color is extinguished)
- Side plates are immediately illuminated white
- If center had been either red or yellow, left is correct, if blue or green, right is correct
FOR THE SHORT-TERM MEMORY AND ATTENTION TASK

- All three press-plates are used
- Initially, one of several symbols is presented as a 'sample' at the center position
- Observation of the sample symbol is indicated by a subject's response to it (sample is extinguished)
- After one of several time delays (e.g., 1-32 seconds), three choice symbols are presented (one 'matching' the sample)
- Responding to the 'match' is correct
FOR THE LEARNING TASK

- All four retractable levers are used
- Serial position and correct and incorrect indicator lights are used
- Subjects must learn a different sequence of lever presses each test session
- Sequences of 1 to 6 levers acquired in a single session
Color and Position Discrimination Task

![Graph showing the relationship between accuracy of response and Full Scale IQ.](image)
Learning Task

![Graph showing the relationship between Full Scale IQ and Accuracy of Response. The graph displays a positive correlation, with points scattered along a trend line that slopes upward from left to right.]
### Cases of comparable behavioral effects of drugs in both humans and rhesus monkeys

#### References

<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary Acute Effect</th>
<th>Monkey</th>
<th>Human</th>
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<tr>
<td>THC</td>
<td>over-estimate time passage</td>
<td>Schulze et al. 1988</td>
<td>Hicks et al. 1984</td>
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<td>marijuana smoke</td>
<td>short-term memory impairment</td>
<td>Schulze et al. 1989</td>
<td>Darley et al. 1974</td>
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<td>chlorpromazine</td>
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<td>Ferguson et al. 1992</td>
<td>Tecce et al. 1975</td>
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<td>diazepam</td>
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<td>Schulze et al. 1989</td>
<td>Ghoneim et al. 1984</td>
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<td>morphine</td>
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<td>Goldberg et al. 1982</td>
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<td>atropine</td>
<td>learning disruption</td>
<td>Schulze et al. 1992</td>
<td>Higgins et al. 1989</td>
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<td>pentobarbital</td>
<td>overestimate time passage</td>
<td>Ferguson et al. 1993</td>
<td>Goldstone et al. 1958</td>
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**Primary Chronic Effect**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary Chronic Effect</th>
<th>Monkey</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>marijuana smoke</td>
<td>amotivational syndrome</td>
<td>Paule et al. 1992</td>
<td>Lantner 1982</td>
</tr>
</tbody>
</table>

*THC = delta-9-tetrahydrocannabinol*
IRA Percent Task Completed

Control
Ketamine
IRA Response Rate

- Control
- Ketamine

Weeks of Testing

RR

0 0.1 0.2 0.3 0.4 0.5

0 8 16 24 32 40 48 56 64 72 80 88 96 104 112 120 128 136 144 152 160

Weeks of Testing
CPR Response Rate

Weeks of Testing

RR

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1

Control
Ketamine

Weeks of Testing

U.S. Food and Drug Administration
Protecting and Promoting Public Health
www.fda.gov
CPR Accuracy

Weeks of Testing

(Mean ± SEM)

Control

Ketamine
DMTS Delay Levels

Control
Ketamine

Delay
Weeks
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80
Questions that remain

• What is the threshold duration of exposure to cause these functional deficits?
• Are they the same as that for causing abnormal cell death?
• How long will functional deficits manifest? Follow-on assessments are under way.
• What is the exact period of sensitivity to these effects? How much earlier than GD 120 and later than PND 5?
• How does this relate to human development?
Questions that remain

• Can markers of these effects be monitored *in vivo* using newer imaging techniques (microPET, etc.)?
• What might be some strategies for preventing or ameliorating the adverse effects associated with the use of anesthetic agents?
• Which drug combinations or clinical manipulations make the situation worse?
• How will these findings relate to the use of anesthetics in actual surgical or other painful/stressful circumstances?
Reference

Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys


Neurotoxicology and Teratology (2011)
doi:10.1016/jntt.2011.01.001

Funding: NICHD; CDER/FDA and NCTR/FDA
Clinical Investigations of the Potential Effects of Anesthetics on Pediatric Neurological Development

ASLDAC Meeting Presentation
March 10, 2011

Arthur Simone, MD, PhD
Medical Officer
Division of Anesthesia and Analgesia Products
Center for Drug Evaluation and Research, FDA
Disclaimer

• This presentation is limited to information that is currently in the public domain.
• FDA has not reviewed either the protocols for or the data generated by these studies.
• This presentation is to help focus the clinical discussion, not to critique the work done to date.
Clinical Investigations

• Retrospective Epidemiological Studies
  – Denmark
  – Netherlands
  – Mayo Clinic
  – Columbia University

• Prospective Epidemiological Study
  – PANDA Study

• Prospective Randomized Controlled Study
  – General Anesthesia/Spinal (GAS) Study
Denmark

- Hansen TG and Flick RP

- Anesthetic Effects on the Developing Brain: Insights from Epidemiology

- Anesthesiology 2009; 110: 1–3
Denmark

• Compares academic achievement (not IQ) of children anesthetized before the age of 1 yr versus the background Danish population
• 1977–1990, (n > 45,000)
• Utilizes links between a series registries
  – Danish Demographic Database
  – National Hospital Discharge Register
  – Register of Compulsory School Completion Assessments and Test Scores
• Awaiting publication of results

Hansen (2009)
Netherlands Study

• Bartels M, Althoff RR and Boomsma DI

• Anesthesia and Cognitive Performance in Children: No Evidence for a Causal Relationship

• Twin Research and Human Genetics 2009; 12(3): 246-253
Hypothesis

• Anesthesia administration in the first three years of life causes later learning problems.

• Children who need surgery early in life have medical problems that are associated with a vulnerability to learning disabilities.

Bartels (2009)
Methods

• Data source: Young Netherlands Twin Registry
• 1,143 monozygotic twin pairs (56% female)
  – Born 1986-1995
  – Gestational age ≥ 32 weeks; birth weight ≥ 2000 grams
• Anesthesia use
  – before age 3
  – between ages 3 and 12 years
• Learning disability assessed at ~12 years
  – Educational achievement (EA) (Dutch CITO-elementary test)
  – Cognitive problems (CP) (Cognitive Problems/Inattention subscale of the Conners’ Teacher Rating Scale – Revised)
• Twins were categorized:
  – Concordant exposed; concordant non-exposed; discordant
  – Before age 3 years and ever exposed before age 12 years

Bartels (2009)
Results

• Twins exposed before age 3 had lower educational achievement scores and significantly more cognitive problems than twins not exposed to anesthesia.
• The unexposed co-twin from discordant pairs did not differ from their exposed co-twin EA and CP.
• The results are the same when the analyses are for exposure restricted to before age 3 years or ever before age 12.
• For males exposed at any time point, their EA score was lower (538) \( (p=.004) \) than the EA score in the CON-NE group (540).
• For females a non-significant difference in the same direction was found.
• CP was not significantly different between never or ever exposed individuals.

Bartels (2009)
Conclusions/Recommendations

• Monozygotic twins discordant for having received anesthesia have equivalent levels of learning-related outcomes.
• The data provide evidence against a causal effect of anesthesia on cognitive functioning.
• Using a genetically-informed design suggests a different conclusion than the work of Wilder et al., (2009).
• The vulnerability for learning-related problems may already be present at the time the decision for surgery is made; screening for learning problems should be considered then.
Caveats and Limitations

- Did not directly assess for learning disabilities instead evaluated learning-related outcomes.
- Did not assess for the specific types of anesthesia.
Mayo Clinic – Study 1


• Neuraxial Labor Analgesia for Vaginal Delivery and Its Effects on Childhood Learning Disabilities.

• Anesthesia Analgesia 2010 Aug 24 [Epub ahead of print]
Hypothesis/Methods

• Hypothesis:
  Reducing stress responses to delivery with neuraxial anesthesia, might affect subsequent neurodevelopmental outcomes.

• Methods:
  – Population-based birth cohort
    • delivered vaginally between 1976 and 1982
    • remained in Olmstead County at age 5 years
    • Followed to age 19 years
  – Determined whether neuraxial labor analgesia was used.
  – Identified those with learning disabilities.
  – Comparison utilized the Cox proportional hazards regression

Flick (2010)
Results/Conclusion

• Results:
  – 4684 vaginal deliveries
  – 1495 with neuraxial labor analgesia.
  – adjusted hazard ratio (HR) = 1.05
  – 95% Confidence Interval (CI): 0.85-1.31

• Conclusion:
  The use of neuraxial analgesia during labor and vaginal delivery was not independently associated with learning disabilities diagnosed before age 19 years.

Flick (2010)
Mayo Clinic - Study 2


- Anesthesia for Cesarean Delivery and Learning Disabilities in a Population-based Birth Cohort

- Anesthesiology 2009; 111: 302-10
Methods

• Database: same as previous study

• Cox proportional hazards regression was used to compare rates of LD between children delivered vaginally and via CD (with general or regional anesthesia).

Sprung (2009)
Results

• 5,320 children in the cohort
  – 497 were delivered via CD
    • General anesthesia n=193
    • Regional anesthesia n=304

• Children delivered via CD under general anesthesia had:
  – Lower mean birth weight, lower gestational age, and lower APGAR scores at 1 and 5 min
  – Mothers were also more likely to experience complications of pregnancy and delivery such as hemorrhage and eclampsia/preeclampsia
  – An emergency indication (68% vs. 31% for CD under regional anesthesia)

Sprung (2009)
## Results

<table>
<thead>
<tr>
<th>Type of Delivery</th>
<th>Hazard Ratio*</th>
<th>95% Confidence Interval</th>
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<tbody>
<tr>
<td>Vaginal</td>
<td>1.00</td>
<td></td>
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<tr>
<td>CD - general anesthesia</td>
<td>0.88</td>
<td>0.59-1.31</td>
</tr>
<tr>
<td>CD - regional anesthesia</td>
<td>0.64</td>
<td>0.44-0.92</td>
</tr>
</tbody>
</table>

* adjusted for gender, birth weight, gestational age, exposure to anesthesia before age 4 yr, and maternal education

Sprung (2009)
Results (continued)

Sprung (2009)
Conclusions

• Children exposed to anesthesia during CD are not more likely to develop LD compared to children delivered vaginally

• Data suggest that a brief perinatal exposure to anesthetic drugs does not adversely affect long-term neurodevelopmental outcomes.

• The risk of LD may be lower in children delivered by CD whose mothers received regional anesthesia.

Sprung (2009)
Caveats/Limitations

• Population is primarily Caucasian.
• Incidence of CD in this series was considerably less than in contemporary practice.
• It is not known how changes in the indications for CD may affect the results.
• Halothane and methoxyflurane were the potent inhalational agents used in many of the mothers.

Sprung (2009)
Mayo Clinic – Study 3


- Early Exposure to Anesthesia and Learning Disabilities in a Population-based Birth Cohort

- Anesthesiology 2009; 110: 796-804
Methods

• Used same database as previous studies.
• Identified children who underwent general anesthesia for any type of surgery or diagnostic procedure before their fourth birthday.
• Educational and medical records were reviewed to identify children with learning disabilities (LD).
  – Reading, written language, and math disabilities.
  – Diagnosed using three formulas involving IQ and achievement test scores.
• Cox proportional hazards regression was used.
  – Adjustments for gestational age at birth, sex, and birth weight.
• Individuals were followed from birth until age 19 years.

Wilder (2009)
Results

• 8,548 children born during the period

• 5,357 children in included in the cohort

• 4,764 had no anesthesia exposure

• 593 received GA before age 4 yr
  – 449 had a single anesthetic exposure
  – 100 had received two anesthetics
  – 44 had received three anesthetics

Wilder (2009)
Results (continued)

- Exposed before age 4 yr, versus unexposed children:
  - lower birth weight ($P < 0.001$)
  - lower gestational age ($P < 0.001$)
  - were more likely to be male ($P < 0.001$)
  - higher levels of maternal education ($P < 0.039$);
- Analysis was adjusted for first 3 differences
- No adjustment for 4th factor; data missing for 10% of children.
- Apgar scores and peripartum complications were not different between two groups.
Results (continued)

Wilder (2009)
## Results

<table>
<thead>
<tr>
<th>Type of Exposure</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single anesthetic</td>
<td>1.0</td>
<td>0.79 - 1.27</td>
</tr>
<tr>
<td>Two anesthetics</td>
<td>1.59</td>
<td>1.06 - 2.37</td>
</tr>
<tr>
<td>Three or more anesthetics</td>
<td>2.60</td>
<td>1.60 - 4.24</td>
</tr>
<tr>
<td>Anesthetic duration $\leq$ 30 minutes</td>
<td>0.93</td>
<td>0.56 - 1.55</td>
</tr>
<tr>
<td>Anesthetic duration $\geq$ 120 minutes</td>
<td>1.65</td>
<td>1.19 - 2.29</td>
</tr>
</tbody>
</table>

Wilder (2009)
Cumulative percentage of learning disabilities diagnosis by the age at exposure shown separately for those who have zero, one, or multiple anesthetic exposures before 4 years of age. Reproduced from Wilder et al. [6**].
Conclusions

• A single anesthetic exposure was not a risk factor for development of a LD
• Multiple exposures were a significant risk factor.
• The data do not discern whether anesthesia contributes to LD or is a marker for other factors that do.

Wilder (2009)
Caveats and Limitations

• Cannot distinguish between potential effects of anesthesia itself and other factors associated with anesthesia, e.g., stress response to surgical procedure.

• Cannot discern if children requiring surgery differ from those who do not in a way that affect risks for LD.

• Cannot exclude that requiring multiple anesthetics is a marker for conditions that increase LD risk.

Wilder (2009)
Caveats/Limitations

- Children requiring repeated procedures may have a higher burden of illness, which may increase risk for LD.
- Rochester was a predominantly white, middle-class community limits the generalization of the results.
- It is not certain whether LD is a relevant outcome measure for assessing potential neurotoxic effects of anesthesia.
Mayo Clinic – Study 4

- Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, Sprung J, Weaver AL, Schroeder DR, and Warner DO

- Learning and behavior after early anesthetic exposure: a comorbidity adjusted study in Rochester, Minnesota

- Pediatrics (Under review)
Methods

- Database used for previous studies
- Exposed to anesthesia before two years of age (n = 350)
- Matched to 2 unexposed controls (n=700)
  - Based on risk factors for learning disabilities (LD)
  - Adjusted for co-morbidities
- Risk factors included
  - Gender
  - Mother’s level of education
  - Birth weight
  - Gestational age
- Health status was quantified using:
  - ASA-PS system
  - Johns Hopkins Adjusted Clinical Groups Case Mix System

Flick (2011)
Methods (continued)

• Educational records were evaluated
  – Identify children with a LD
  – Need for an individualized education program (IEP)
  – Results of tests of cognition and achievement.

• IEPs were separated:
  – Emotional behavioral disorders (IEP-EBD)
  – Speech and language impairments (IEP-SL)
## Results

<table>
<thead>
<tr>
<th>IEP Type</th>
<th>No. of exposures</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBD</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.71</td>
<td>0.35-1.44</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>0</td>
<td>(none identified)</td>
</tr>
<tr>
<td></td>
<td>(none identified)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SL</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.30</td>
<td>0.75-2.25</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>5.08</td>
<td>2.66-9.69</td>
</tr>
</tbody>
</table>

Flick (2011)
Conclusions

• Multiple exposures to anesthesia prior to age 2 years are a risk factor for deficits in learning but not behavior, even with adjustment for health status.

• Multiple anesthetic exposures were associated with LD and the need for an IEP-SL, but not for IEP-EBD.

• Group administered tests of cognitive ability and achievement were consistent with, but appeared less sensitive than, the results of individual testing.
Caveats/Limitations

• Unknown/unmeasured confounding factors associated with the need for anesthesia and surgery may influence outcomes.

• Despite evaluations of health status, children requiring anesthetic exposure may have an unassessed higher burden of illness potentially affecting outcomes.
Mayo Clinic – Upcoming Work

• Evaluation of an association between anesthetic exposure and autism
• Evaluation of an association between anesthetic exposure and ADHD
• Long term neurodevelopmental phenotype of children exposed to modern anesthetic agents prior to age two years
Columbia University

- DiMaggio C, Sun LS, Kakavouli A, Byrne MW, and Li G

- A Retrospective Cohort Study of the Association of Anesthesia and Hernia Repair Surgery With Behavioral and Developmental Disorders in Young Children

Design

Retrospective cohort analysis

– Children born between 1999 and 2001 and enrolled the New York State Medicaid program.
– Birth cohort of 383 children who underwent inguinal hernia repair during the first 3 years of life.
– Comparator was random sample of 5050 children.
  • Frequency-matched on age in months
  • No history of hernia-repair before age 3

DiMaggio (2009)
Methods

• Exposure = an ICD-9 procedure code related to hernia repair
  – Inpatient or outpatient
  – Principal or secondary
  – 1999 to 2002

• Outcome = the presence of a diagnostic code for
  – Unspecified delay or behavioral disorder
  – Mental retardation
  – Autism
  – Language or speech problems

• Controlled for age, sex, and complicating birth-related conditions such as low birth weight.

DiMaggio (2009)
Results

• No significant difference between groups for
  – Age
  – Loss to follow-up

• Exposed group, versus unexposed, was more likely:
  – Male (78% vs. 50%)
  – Black
  – Diagnosed with one of the following at birth
    • Any secondary diagnosis
    • Gastrointestinal disorder (including hernia)
    • CNS anomaly
    • Low birth weight (<2500 g)
    • Perinatal hypoxia

DiMaggio (2009)
## Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anesthesia exposure</td>
<td>2.3</td>
<td>1.3 - 4.1</td>
</tr>
<tr>
<td>Age</td>
<td>1.0</td>
<td>0.9 - 1.0</td>
</tr>
<tr>
<td>Gender</td>
<td>2.7</td>
<td>1.5 - 4.7</td>
</tr>
<tr>
<td>Race</td>
<td>1.1</td>
<td>1.0 - 1.1</td>
</tr>
<tr>
<td>Birth complication</td>
<td>1.6</td>
<td>1.1 - 2.5</td>
</tr>
</tbody>
</table>
Conclusions

• Hernia repair before 3 years of age is associated with an increased risk of subsequent diagnosis of behavioral/developmental disorders.

• The association cannot be explained by confounding due to:
  – Low birth weight
  – Comorbidity
  – Demographic characteristics

• Further studies are needed.

DiMaggio (2009)
Caveats/Limitations

• Database used is a blunt instrument.
  – Unable to establish the type, route and dose of anesthetic.
  – Unable to differentiate the effects of anesthesia from those of surgery.
  – Diagnoses might be susceptible to bias resulting from misclassification, underreporting and local practice patterns.

• Potential for bias from unmeasured cofounders, e.g., reasons for prematurity and resulting low birth weight.

• Potential for differences between children covered by Medicaid compared to other population groups.

• Long-term effects remain unknown.

DiMaggio (2009)
Columbia University

- DiMaggio C, Sun LS and Li G

- Early Childhood Exposure to Anesthesia and Risk of Developmental and Behavioral Disorders in a Birth Cohort of 5,824 Twin Pairs

- Anesthesiology ISS-A1 2010
Objective/Methods

Objective:
- To assess the association between exposure to anesthesia under 3 years of age and the risk of developmental and behavioral disorders in a birth cohort of twins.

Methods:
- Retrospective cohort of 5,824 twin pairs
- Born between 1999 and 2005 and enrolled in the N.Y. State Medicaid program
- Followed each study subject from birth to up to six years of age
- Exposure status based on surgical procedures before age 3 years
- Developmental and behavioral outcomes identified by screening diagnoses according to the International Classification of Diseases, Ninth Revision.
- The association of exposure to anesthesia with subsequent developmental and behavioral disorders was assessed using the various types of analyses.
Results

• 11,648 children studied
  – 668 (6%) were exposed to anesthesia at least once by age 3.
• During the follow-up, a total of 2,168 children (19%) were diagnosed with developmental and behavioral disorders,
  – 84% of the disorders - unspecified developmental delay.
• The incidence rate of developmental and behavioral disorders was:
  – 34 cases per 100 exposed children
  – 16 cases per 100 unexposed children
  – Crude relative risk 2.5; 95% CI: 2.2-2.7
  – Adjusting for birth complications and sex, RR = 2.3; 95% CI 1.9-2.7.
  – Further analysis is ongoing.

DiMaggio (2010)
Conclusions

• Children who were exposed to anesthesia under 3 years of age are more than twice as likely as their peers to be subsequently diagnosed with developmental and behavioral disorders.

• Excess risk cannot be fully explained by birth complications

• Further studies are needed.

DiMaggio (2010)
PANDA Study

• Sun L.

• Early childhood general anaesthesia exposure and neurocognitive development

• British Journal of Anaesthesia 105 (S1): i61–i68 (2010)

• http://www.kidsPANDASTudy.org
Design/Objective

Pediatric Anesthesia and NeuroDevelopment Assessment (PANDA) Study

– Large-scale, multisite (USA), ambi-directional, sibling-matched, cohort study.

– Objective: to examine the neurodevelopmental effects of exposure to general anesthesia during inguinal hernia surgery before 3 years of age.
  • Plan to enroll total 1,000 subjects
  • Sibling comparison group
  • Direct neuropsychological assessment
PANDA Participating Sites

- Children’s Hospital of New York - Columbia University
- Boston Children’s Hospital
- Children’s Hospital of Philadelphia
- Chicago Children’s Memorial Hospital
- Cincinnati Children’s Hospital
- Vanderbilt University Children’s Hospital
- Pittsburgh Children’s Hospital
- University Michigan Children’s Hospital
Inclusion Criteria

- ASA PS 1 and 2
- Gestational age > 36 weeks
- Single anesthesia exposure prior to 36 months of age during inguinal hernia surgery
- Sibling within 36 months in age who had no anesthesia before 36 months of age
- Both siblings are currently between 8 years 0 month to 15 years 0 month of age
Study Progress

- Case Report Form for medical data has been finalized.
- Summary form to capture all neuropsychological tests results is under development.
- 16 assessment instruments have been selected to evaluate 7 cognitive domains:
  - Memory/learning
  - Motor/processing speed
  - Language
  - Behavior
  - Attention/executive function
  - Visuospatial
  - IQ
Columbia Univ. – Upcoming Works


GAS Study

• A Multi-site Randomized Controlled Trial Comparing Regional and General Anesthesia for Effects on Neurodevelopmental Outcome and Apnea in Infants (GAS)

• Collaborators:
  – Children’s Hospital of Boston (US Sponsor)
  – Royal Children's Hospital
  – Royal Hospital for Sick Children
  – Murdoch Children's Research Institute

• [www.clinicaltrials.gov](http://www.clinicaltrials.gov) Identifier: NCT00756600

McCann (2010)
GAS Study

Participating hospitals are located in:
– Australia
– Canada
– Italy
– United Kingdom
– United States
Design/Objectives

- Prospective, randomized, international, active-controlled, observer-blinded, non-inferiority study
- **Primary Objective:**
  To determine whether regional and general anesthesia administered to infants undergoing inguinal hernia repair results in equivalent neurodevelopmental outcomes
- **Secondary Objective:**
  To describe the incidence of apnea in the post-operative period after both regional and general anesthesia for inguinal hernia repair in infants

McCann (2010)
Treatments

• Regional Anesthesia: Bupivacaine up to 2.5 mg/kg total dose administered as single injection(s) by one of the following routes:
  – caudal or
  – subarachnoid or
  – both caudal and subarachnoid or
  – subarachnoid and ilioinguinal nerve blockade.

• General Anesthesia: Sevoflurane up to 8% for induction and maintenance. In addition, bupivacaine (up to 2.5 mg/kg) administered via caudal route or for ilioinguinal nerve block.
Study Population

660 infants presenting for herniorrhaphy are to be recruited

Inclusion Criteria:

• Scheduled for unilateral or bilateral inguinal hernia repair
• Gestational age is 26 weeks or more
• Post-menstrual age is up to 60 weeks
Study Population

Exclusion Criteria:

• Any contraindication to general or spinal/caudal anesthesia
• Pre-operative ventilation immediately prior to surgery
• Congenital heart disease that required ongoing pharmacotherapy
• Known chromosomal abnormality or any other known acquired or congenital abnormalities likely to affect development
• Children where follow-up would be difficult for geographic or social reasons
• Known neurological injury such as cystic periventricular leukomalacia (PVL), or grade 3 or 4 intra ventricular hemorrhage (ICH) (+/- post-hemorrhage ventricular dilation)
• Previous exposure to volatile anesthesia or benzodiazepines as a neonate or in the third trimester in utero.

McCann (2010)
Outcome Metrics

• Primary Outcome Measure:
  Wechsler Preschool and Primary Scale of Intelligence-Third Edition (WPPSI-III) full scale IQ score assessed at 5 years corrected age

• Secondary Outcome Measures:
  – Bayley neurodevelopmental scale assessed at 2 years corrected age
  – Frequency and characteristics of apnea in the post-operative period

McCann (2010)
Study Progress

• Over 400 enrolled thus far.
• Enrollment might be complete in late 2011 but more likely in 2012.
• Reporting of study results:
  – For 2-year assessment: 2014
  – For 5-year assessment: 2017

McCann (2010)
Summary

• Nonclinical findings
  – Mounting evidence of anesthetic-induced toxicity
  – Possible safety margins may exist

• Clinical findings
  – Only epidemiological studies reported to date
  – Conflicting results for single exposures
  – Causality cannot be established
  – Prospective epidemiological study and clinical trial are underway as well as additional retrospective epidemiological studies
Summary

• Issues to consider during discussions:
  – Types of additional clinical studies that will be useful
    • Design
    • Populations
    • Endpoints
  – Potential need for pre-op evaluation for neurocognitive functioning as well as post-op follow-up and interventions
What is Our Professional Responsibility? Focus on Research and Risk Communication.

Robert “Skip” Nelson, MD, PhD
Senior Pediatric Ethicist/Lead Medical Officer
Office of Pediatric Therapeutics
Office of the Commissioner, FDA
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March 10, 2011
Knowns and Unknowns

“[T]here are known knowns; there are things we know we know. We also know there are known unknowns; that is to say we know there are some things we do not know. But there are also unknown unknowns – the ones we don't know we don't know.”

– Former United States Secretary of Defense Donald Rumsfeld
Four Categories

• “Known knowns”
  – What are the facts as we know them?
• “Known unknowns”
  – What questions need to be answered?
• “Unknown knowns”
  – Is ignorance of the known facts an option?
• “Unknown unknowns”
  – How do we communicate risk?
Ethical Framework for Research

• Any research protocol enrolling children must offer a direct clinical benefit that is comparable to the non-research alternatives. Thus, any modifications of usual practice of pediatric anesthesia should be designed to mitigate the risk of neurotoxicity.

• Interventional studies to establish the anesthetic exposure necessary to induce neurotoxicity under different conditions will need to use animal models and protocols that would inform clinical practice.

• There is a role for human clinical studies of different risk reduction strategies. These trials may be of limited significance if we discover that neurotoxicity from anesthetic exposure is not linear but has a threshold below which such toxicity does not occur.

• There is no reason why a clinical trial cannot be designed to answer a safety endpoint provided that we are not doing anything to intentionally increase the risk exposure within the clinical trial.
Professional Responsibility

• We are now faced with a new challenge.
• Rather than short-term and overt adverse drug reactions such as chest wall rigidity or postoperative nausea and vomiting, we are now faced with a potential long-term and silent adverse drug reaction that could be resulting in significant neurobehavioral toxicity.
• Tackling the complicated questions that we are now facing will require partnerships, resources, and commitment.
Thank you.