CNS safety evaluation in drug development: Signals from animal studies that suggest the need for further investigations. Case study: Anesthetic-induced neurodegeneration in the developing brain

FDA Public Workshop
Advancing the Development of Pediatric Therapeutics
Evaluation of Long-term Neurocognitive Development in Pediatrics

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Disclaimer
The content of this talk does not necessarily reflect the views of the FDA and is entirely based on my own observations and viewpoints.

Disclosures
I have no financial interests to disclose.
Nonclinical CNS Safety Evaluation in Drug Development

Prior to First-in-Human

- **Basic Pharmacology/ADME Studies**
  - Receptor Binding Profile, Functional Studies, Tissue Distribution?
- **CNS Safety Pharmacology Studies**
  - Functional Observation Battery (FOB)
- **General Toxicology Studies**
  - General Clinical Signs
  - Clinical Examinations
  - Gross Macroscopic Findings and Brain Weight
  - Histopathology

**Generally After Phase 3**

- **Reproductive and Developmental Toxicology Studies**
  - Pre- and Post-Natal Development (PPD) Study (with NDA)
    - Learning and Memory Functional Assessments
- **Juvenile Animal Study (case-by-case)**

“Tier 1 Testing”
Pre- and Postnatal Development Study (PPD or Segment 3)

- Pregnant animals exposed to drug from implantation through weaning
- Pup exposure in utero and possibly via milk
- Generally done in the rat model (could be primate for biologic)

Pup Endpoints:
- Pre- and post-weaning survival and growth/body weight
- Physical development
  - Landmarks: pinna unfolding, coat growth, incisor eruption, etc.
- Sensory functions and reflexes
  - Surface righting, auditory startle, air righting, response to light, etc.
- Behavior
  - Learning and memory (no specific test recommended)
Juvenile Animal Studies
Bridging the Gap

Direct Dosing
Juvenile animal studies

Rats ~6-8 weeks old
(young adults)

Repeat dose studies

Repro Seg III

Rats = PND 21

birth
weaning

Indirect exposure
Nonclinical Cognitive Testing
Mazes (Puzzles or Navigating Kindergarten? )

- **Morris water maze**
- **Biel water maze**
- **Cincinnati water maze**
- **T-maze** (dry, appetitive)
- **Radial arm** (dry, appetitive)

Image Source: [www.youtube.com](http://www.youtube.com)
Reader Rabbit Kindergarten
Part 7: Number Lumber (Navigating your Hike)

- All mazes include three aspects that are essential to learning and memory: learning, consolidation, and retrieval
**Morris Water Maze**

**Allocentric Navigation** (external cues)

a.k.a. Turn left after the big tree

Hippocampus, entorhinal cortex, surrounding structures

Humans: memory for people, places, things, and events

Image Source:  http://www.nxdomain.nl/~anja/brains/watermaze.html
Cincinnati Water Maze

Egocentric Navigation
(internal cues)
a.k.a.: Finding the way to the bathroom in the dark

Dorsal striatum and connecting structures

Humans: encoded or Implicit or procedural memory

Vorhees and Williams (2014) Neurotoxicology and Teratology 45:75-90
Case Study: Anesthetic-induced Neurodegeneration in the Developing Brain
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 (PND 7)
- MK-801 (0.5 mg/kg, IP)

Science 283: 70-74
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.

Science 283: 70-74
Ikonomidou et al. (2000)

Fig. 3. Age Dependency of ethanol-induced apoptosis in the brains of developing rats

Early: VMH, DM & V Thalamus
Mid: subiculum, hippocampus, caudate, LD & AV thalamus
Late: Frontal, parietal, temporal, cingulate, retrosplenial cortices

Science 287: 1056-1060
Comparative Brain Growth Spurt

Fig. 1. The brain growth spurs of 7 mammalian species expressed as first-order velocity curves of the increase in weight with age. The units of time for each species are as follows: guinea pig [3]: days; rhesus monkey [1]: 4 days; sheep [9]: 5 days; pig [2]: weeks; man [5]: months; rabbit [8]: 2 days; rat [4]: days. Rates are expressed as weight gain as a percentage of adult weight for each unit of time.


Scale:
Rat = days
Human = months
Monkey = 4 days
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, Richard E. Hartman, Yukitoshi Izumi, Nicholas D. Benshoff, Krikor Dikranian, Charles F. Zorumski, John W. Olney, and David F. Wozniak

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- **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 (PND 29 - PND 33)

J. Neuroscience 23(3): 876-882
MORRIS WATER MAZE

a. PLACE TRIALS (Age P32)

b. PLACE TRIALS (Age P131)

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 PND 35 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 \((N = 3/\text{group})\). Data are presented as means ± SD. * A probability of \(p < 0.05\) was considered significant (two-way ANOVA).
Behavioral Changes: Primates
NCTR Operant Test Battery

- Incremental repeated acquisition (IRA)
  - learning
- Conditioned Position Responding (CPR)
  - Color and position discrimination
- Progressive Ratio (PR)
  - motivation
- Delayed matching to sample (DMTS)
  - Short-term memory
Effects of Postnatal Ketamine on Monkey Cognition

- Investigating the effects of postnatal ketamine and its impact on cognition
- 24 h iv anesthesia on PND 5
- Wean at 6 mo
- Begin cognition function assessments at 7 mo

Mayo Clinic Research: One Example of Retrospective Clinical Study

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

Robert T. Wilder, M.D., Ph.D.,* Randall P. Flick, M.D., M.P.H.,† Juraj Sprung, M.D., Ph.D.,‡ Slavica K. Katusic, M.D.,§ William J. Barbaresi, M.D.,∥ Christopher Mickelson, M.D.,# Stephen J. Gleich, M.D.,** Darrell R. Schroeder, M.S.,†† Amy L. Weaver, M.S.,†† David O. Warner, M.D.‡

![Graph: Cumulative percentage of learning disabilities diagnosis by the age at exposure shown separately for those that have zero, one, or multiple anesthetic exposures before age 4 yr.](image)

Fig. 1. Cumulative percentage of learning disabilities diagnosis by the age at exposure shown separately for those that have zero, one, or multiple anesthetic exposures before age 4 yr.
SmartTots is a Public-Private Partnership between the International Anesthesia Research Society and the U.S. Food and Drug Administration formed in 2010 to investigate the effect of anesthetics and sedatives on the human brain, and to ensure that information and outcomes generated from this research can be used to benefit public health.
Ongoing Clinical Research

• **GAS Study** (General Anesthesia Safety) Study
  – Drs. Andrew Davidson (Aus), Mary Ellen McCann (US), Neil Morton (UK)
  – International Study, Children’s Hospital Boston
  – Spinal vs general anesthetic for inguinal hernia repair

• **PANDA Study** (Pediatric Anesthesia & Neurodevelopment Assessment) Study
  – Dr. Lena Sun and colleagues
  – Twin cohort studies

• **Mayo Clinic Research Studies**
  – **MASK Study** (MAYO Safety in Kids) Study
  – Mayo Clinic Research (Dr. Randal Flick and colleagues)
  – Collaborative effort with NCTR to test the NCTR OTB in children exposed to one or more anesthetics early in life.
Adolescent Brain Development
The Second Wave of Synaptogenesis


Image Source: www.developingchild.harvard.edu
Concluding Thoughts

• Nonclinical Assessments that May Suggest Pediatric Concern
  – Pharmacology and Distribution
  – Histopathology
  – Cognitive Testing - Limited to PPD (with NDA)
  – Strongly Consider the Need for a Juvenile Animal Study (or Two)
    • Age Dependent (Neonate vs. Adolescent Risk)
    • Histopathology and Cognitive Function
  – Currently No Social Behavioral Assessments

• Anesthetic Case Study Illustrates Utility
  – Nonclinical Data is Compelling
  – Translational Neurotoxicity (Rat → Monkey → Human?)
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SmartTots:  www.smarttots.org