History of Preclinical Data: Anesthetic-induced Neuroapoptosis

Anesthetics and Life Support Drugs Advisory Committee Meeting
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Objective

- Summarize published in vivo preclinical data characterizing the effects of anesthetic drugs on the developing brain.
- Outline the steps taken by the Agency to further characterize the potential clinical significance of these findings.

Ikonomidou et al. (1999)

Blockade of NMDA Receptors and Apoptotic Neurodegeneration in the Developing Brain

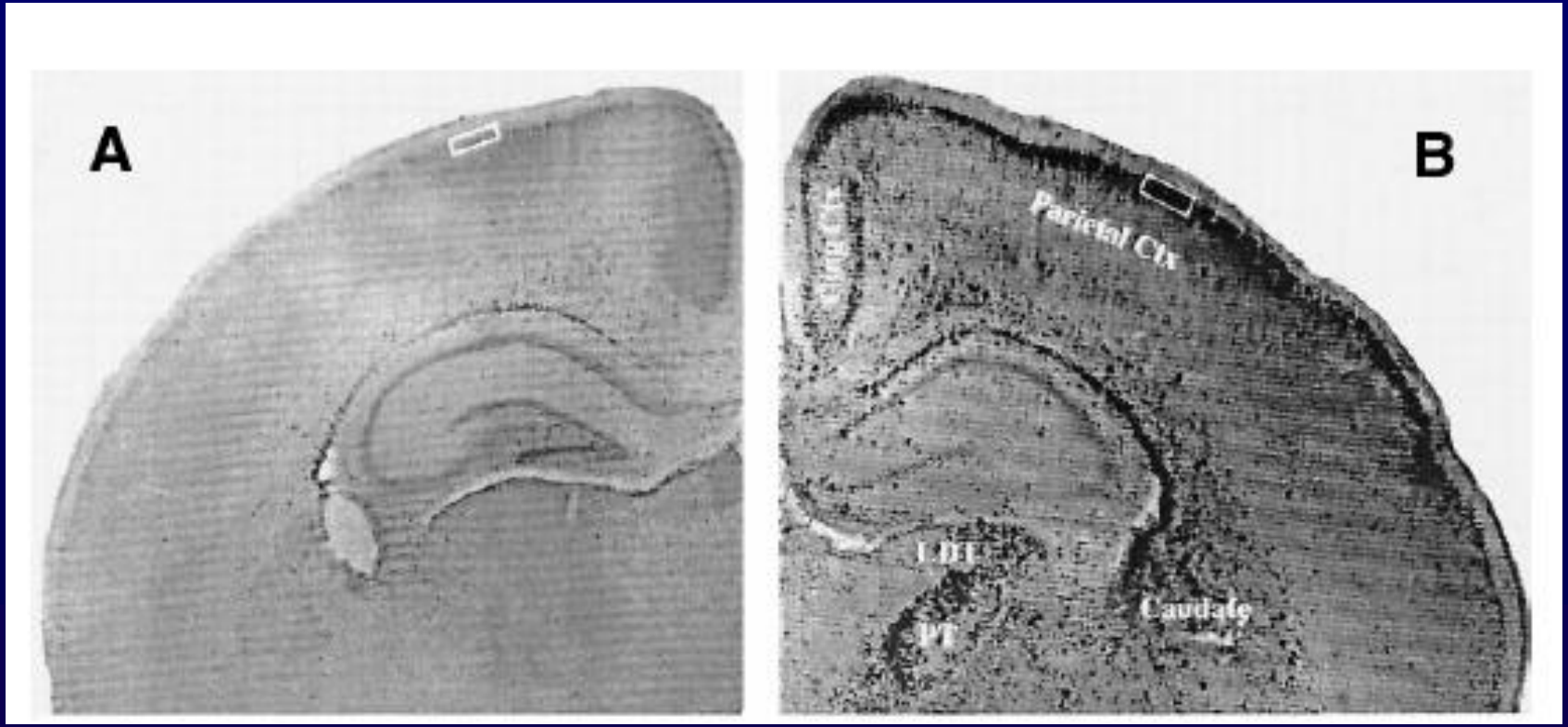
Chrysanthy Ikonomidou,* Friederike Bosch, Michael Miksa, Petra Bittigau, Jessica Vöckler, Krikor Dikranian, Tanya I. Tenkova, Vanya Stefovska, Lechoslaw Turski, John W. Olney

Model: 7-day old rat
MK-801 (0.5 mg/kg, i.p.)
Reported findings with Ketamine (20 mg/kg, s.c. x 7 over 9 h)

Science 283: 70-74

Ikonomidou et al. (1999)

Saline Treatment **MK-801 Treatment**



Stained with TUNEL Method (Apoptosis)
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.

Origin of the FDA Investigations

- In 2000, FDA raises concerns regarding proposed NIH clinical trial to study ketamine in children based on a preclinical study published in 1999 by Dr. Olney and colleagues.
- Formation of an FDA-wide Expert Working Group: FDA neurotoxicologists CDER and NCTR.
- Rapid Response Team: CDER's Office of Pharmaceutical Sciences.
- Research Subcommittee of the Pharmacology Toxicology Coordinating Committee (PTCC)

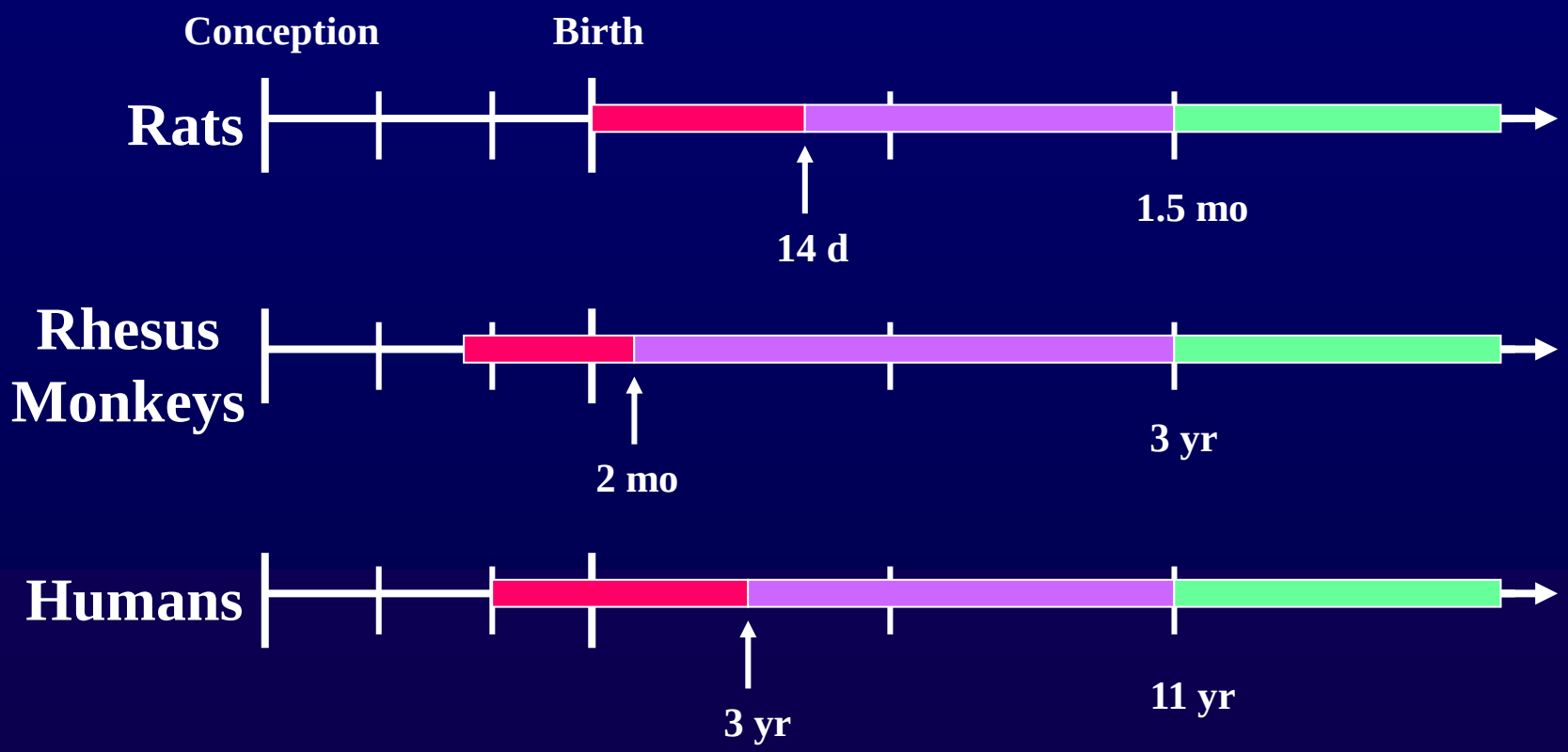
FDA Investigations (continued)

- Reviewed literature on NMDA receptor system and susceptibility to neurotoxicity (Haberny et al., 2002).
- Duplicated and extended the findings reported by Dr. Olney's group in the 7-day old rat (Scallet et al., 2004).
- Recommendations:
  - Duplication of Dr. Olney's findings in the rat brain support the need for studies in the nonhuman primate model.
  - The rat model could be used to pursue mechanistic studies.
  - CDER/NCTR would nominate ketamine to the National Toxicology Program to obtain funds to support the nonhuman primate studies.

Ketamine Nomination National Toxicology Program

- FDA's nomination proposed the following general studies:
  - Studies to characterize potential for ketamine to produce neurodegeneration in developing nonhuman primate.
  - Behavioral assessments of nonhuman primate infants exposed to ketamine during development.
- Studies unanimously approved by NTP but not funded.
- These studies are currently being completed by NCTR.

Time Windows of Vulnerability to the Neurotoxic Effects of NMDA Receptor Antagonists for Rat (Postulated for Monkey and Human)



Source: William Slikker, NCTR

Two Types of NMDA-Receptor Mediated Neurotoxicity

Neuroapoptotic Degeneration	Excitotoxic Degeneration
Developing brain	Adult brain
Cell death without necrosis	Neuronal vacuoles and eventual necrosis
Widespread in brain	Distinct brain regions
Can be physiological (example: synaptogenesis)	Always pathological (example: ischemia)

Hayashi et al. (2002)

Repeated administration of ketamine may lead to neuronal degeneration in the developing rat brain

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- Model: Neonatal Rat (PND 7), intraperitoneal injections, histology at 24 hours post last injection
  - Saline injection
  - Single dose ketamine (25, 50, 75 mg/kg, i.p.) → No neurodegeneration
  - Repeated Doses (7) once every 90 minutes, 25 mg/kg Ketamine → Neurodegeneration

Paediatric Anaesthesia 12:770-774

Hayashi et al. (2002)

- Single doses of ketamine did not produce evidence of neurodegeneration.
- Confirmed that repeated doses of ketamine can produce evidence of neurodegeneration in the rat model.
- Suggests that there are exposure conditions that do not produce neurodegeneration.

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. Wozniak[3]

[1]Department of Anesthesiology, University of Virginia Health System, Charlottesville, Virginia 22908, and Departments of [2]Neurology and [3]Psychiatry, 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)

J. Neuroscience 23(3): 876-882

Jevtovic-Todorovic et al. (2003)

- First published report to suggest that both nitrous oxide, isoflurane and midazolam can also produce neuroapoptosis in rat model.
- First study to attempt to mimic the clinical anesthetic setting.
- Exposure of neonatal rats to 6 hrs of "mock anesthesia" (nitrous oxide, oxygen, isoflurane, midazolam) caused:
  - widespread apoptotic neurodegeneration in the developing brain,
  - deficits in hippocampal synaptic function, and
  - persistent memory/learning impairments.

The Challenge of Animal Models: How to Extrapolate Risk to Humans?

- **Species Differences:**
  - Most sensitive species vs. most appropriate species
  - Metabolism Differences
  - Developmental Differences
- **Technical Study Design Challenges:**
  - How to mimic the clinical setting as closely as possible
    - Concurrent medications, blood gases, nutritional support, hemodynamic stability
  - How to extrapolate dose administered to clinical setting.
    - Body Surface Area, pharmacokinetic comparison vs.
    - Pharmacodynamic effect

Scallet et al. (2004)

Developmental Neurotoxicity of Ketamine: Morphometric Confirmation, Exposure Parameters, and Multiple Fluorescent Labeling of Apoptotic Neurons

A. C. Scallet,*[1] L. C. Schmued,* W. Slikker, Jr.,* N. Grunberg,† P. J. Faustino,‡ H. Davis,§ D. Lester,‡ P. S. Pine,‡ F. Sistare,‡ and J. P. Hanig,‡

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- Objective: Confirm and extend results of Ikonomidou 1999 Science Paper
- Model: Neonatal Rat Model (PND 7), subcutaneous injections, histology at 24 hours post last injection
  - Saline injection
  - Repeated Doses (7) once every 90 minutes, 10 mg/kg Ketamine
  - Repeated Doses (7) once every 90 minutes, 20 mg/kg Ketamine
  - Single Dose Ketamine 20 mg/kg

Toxicological Sciences 81: 364-370

Approximate Exposure Margin for Ketamine-induced Neuroapoptosis

Treatment	Evidence of Neuro-apoptosis	Exposure Margin[1]
Ketamine 10 mg/kg x 7	No ✗	~1
Ketamine 20 mg/kg x 1	No ✗	~2.7
Ketamine 20 mg/kg x 7	Yes ✓	~7

[1] Based on reported concentrations in humans that are adequate for major surgery (2 ug/mL = "worst case scenario").

Fredriksson et al. (2004)

Research report

Neurofunctional deficits and potentiated apoptosis by neonatal NMDA antagonist administration

Anders Fredriksson [a,*], Trevor Archer [b], Henrik Alm [a], Torsten Gordh [c], Per Eriksson [d]

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[d] *Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden*

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Available online 13 April 2004

- Model: Mouse pups, Neonatal day 10
  - Ketamine (50 mg/kg, s.c.)
  - Diazepam (5 mg/kg, s.c.)
  - Ketamine (50 mg/kg, s.c.) + Diazepam (5 mg/kg, s.c.)
  - Vehicle (0.9% Saline)

Behavior. Brain Res. 153:367-376

Fredriksson et al. (2004)

- First report of neuroapoptosis in a second rodent species.
- Ketamine and diazepam alone produced neurodegeneration in the mouse model.
- Ketamine and diazepam produced different neuroanatomical patterns of neurodegeneration.
- The combination of both ketamine and diazepam produced a greater degree of neurodegeneration than either drug alone.
- Functional deficits noted at 2 months of age in motor activity and learning performance (ketamine and ketamine + diazepam groups).

Mickley et al. (2004)

BMC Pharmacology

BioMed Central

Research article

Open Access

Long-term age-dependent behavioral changes following a single episode of fetal N-methyl-D-Aspartate (NMDA) receptor blockade

G Andrew Mickley*†, Cynthia L Kenmuir†, Colleen A McMullen†, Alicia Snyder†, Anna M Yocom, Deborah Likins-Fowler, Elizabeth L Valentine, Bettina Weber and Jaclyn M Biada

- Model: Embryonic rat fetuses treated through the maternal circulation
- Conditioned taste aversion (CTA) model for learning and memory.

BMC Pharm 4:28

Mickley et al. (2004)

- E18 rat fetuses pretreated with ketamine (100 mg/kg, i.p. through maternal circulation) and taught a conditioned taste aversion (CTA) learn and remember the CTA, whereas treated of E19 fetuses with ketamine do not.
- Exposure of rat fetus to ketamine in utero results in long-term behavioral deficits in the adult animal.
- Data suggest that there are critical periods of gestational development in which the rat is susceptible to long-term behavioral neurotoxicity.

Young et al. (2005)

Potential of ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain

[1]Chainllie Young, [2]Vesna Jevtovic-Todorovic, [1]Yue-Qin Qin, [1]Tatyana Tenkova, [1]Haihui Wang, [1]Joann Labruyere & *,[1]John W. Olney

- Model: 7-day old mouse
- Ketamine 10, 20, 30, 40 mg/kg, s.c.
- Midazolam 9 mg/kg, s.c.
- Ketamine 40 mg/kg + Midazolam 9 mg/kg

Br. J Pharmacol. 146:189-197

Young et al. (2005)

- Ketamine 10 mg/kg produced a slight, nonsignificant, increase in neuroapoptosis.
- Ketamine at ≥ 20 mg/kg produced significant increase in neuroapoptosis and at doses between 30 mg/kg and 40 mg/kg sharp increase in neuroapoptosis.
- Midazolam produced a dose-dependent increase in neuroapoptosis.
- Ketamine plus midazolam produced a greater increase in neuroapoptosis than either drug alone.

Rudin et al. (2005)

SINGLE-DOSE KETAMINE ADMINISTRATION INDUCES APOPTOSIS IN NEONATAL MOUSE BRAIN

Michael Rudin,[1] Ron Ben-Abraham,[2] Vered Gazit,[3] Yevgeney Tendler,[3], Vadim Tashlykov,[3], and Yeshayahu Katz

[1]*Dept. of Anesthesiology, HaEmek Medical Center, Afula, Israel;* [2]*Dept. of Anesthesiology and Critical Care, Tel Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel;* [3]*Laboratory of Anesthesia, Pain and Neural Research, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

- **Model: 7-day old ICR mice**
- **Ketamine (1.25, 2.5, 5, 10, 20, 40 mg/kg, s.c.)**

J Basic & Clin Physiol & Pharm 16(4): 231-243

Rudin et al. (2005)

- Ketamine produced neuroapoptosis at 5 mg/kg and above.
- Neuroapoptotic neurons peaked at 72 hours after dosing but were still evident out to day 7 post treatment.
- No gross neurobehavioral effects noted at day 7.

Slikker et al. (in press)

Ketamine-induced neurodegeneration in the perinatal rhesus monkey

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

Ketamine i.v. 24 hours, 6 hour withdrawal period.

Ketamine i.v. 3 hours in postnatal day 5 animals.

Nonclinical Summary

- Multiple anesthetic drugs:
  - NMDA receptor antagonists
  - GABA-ergic drugs
- Multiple species
- Long-term behavioral changes
- Combinations of drugs
- Potential means to block these effects