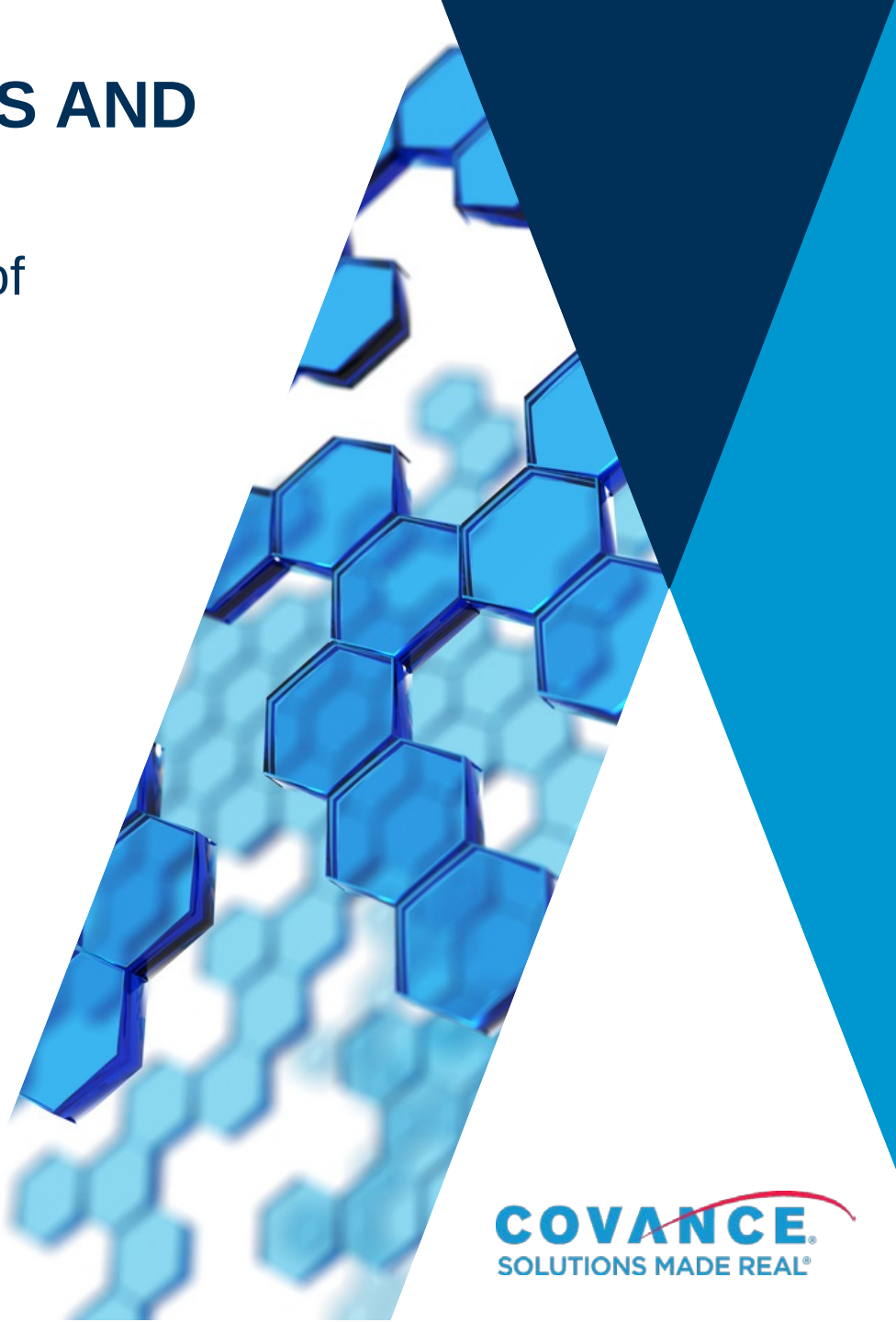
TOXIC NEURONOPATHIES AND GLIOPATHIES

Sixth Conference of the Society of Toxicologic Pathology - India
21-23 October, 2016
Pune, India

Alok Sharma, BVSc, MVSc, MS, PhD
Diplomate ACVP, DABT
Covance Laboratories, Inc.
3301 Kinsman Bvd, Madison, WI 53704
Office: 608-310-2856
Mobile: 608-216-8270
Email: alok.sharma@covance.com



Acknowledgements

- Dr. Georg J. Krinke, Pathology Evaluations, Frenkendorf, Switzerland
- Dr. Ingrid Pardo, Pfizer, Groton, CT
- Dr. William H. Jordan, Vet Path Services, Inc., Mason, OH
- Dr. D. Greg Hall, Lilly Research Laboratories, Indianapolis, IN
- Dr. Bernard S. Jortner, Virginia Tech, Blacksburg, VA
- Dr. Michael Owston Texas Biomedical Research Institute
- The Joint Pathology Center (AFIP)
- Mr. Steve Van Adestine, Covanace Lab, Inc

Goals of this Presentation

- Focus on neuronopathies & gliopathies
- Discuss mechanism of toxicity
- Familiarity with anatomic locations affected in the brain

Neuropathies

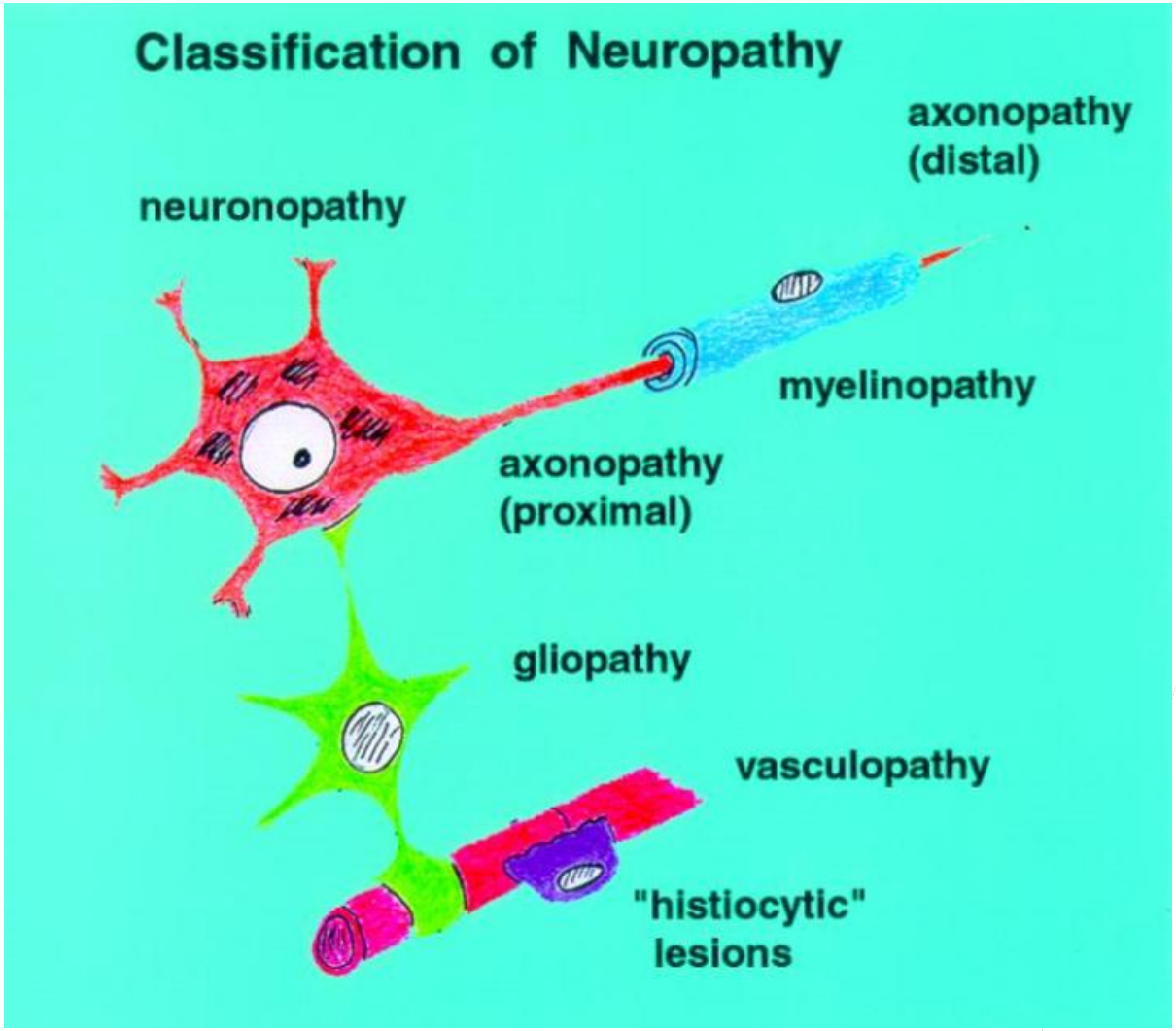


Image courtesy – G Krinke

Mechanisms of Chemically-Induced Neuronopathies

- Oxidative stress
  - Artemether
- Excessive activation of ionotropic receptors
  - Glutamate, kainic acid, domoic acid
- Excessive inhibition of receptors
  - NMDA antagonists
- False neurotransmitters
  - Hydroxylated precursors of amines

Mechanisms of Chemically Induced Neuronopathies

- Suicide transport
  - Cytotoxic lectins e.g. ricin, abrin and modeccin
- Inhibition of DNA-directed RNA synthesis
  - Doxorubicin
- Energy deprivation/cytoskeletal disruption
  - 3-acetylpyridine, pyridoxine

Artemether

- Antimalarial drug against multidrug-resistant falciparum malaria
  - Schizontocide
- Found in Chinese herb Quinghaosu [Qing hao su]

Artemether (cont.)

High parentral doses

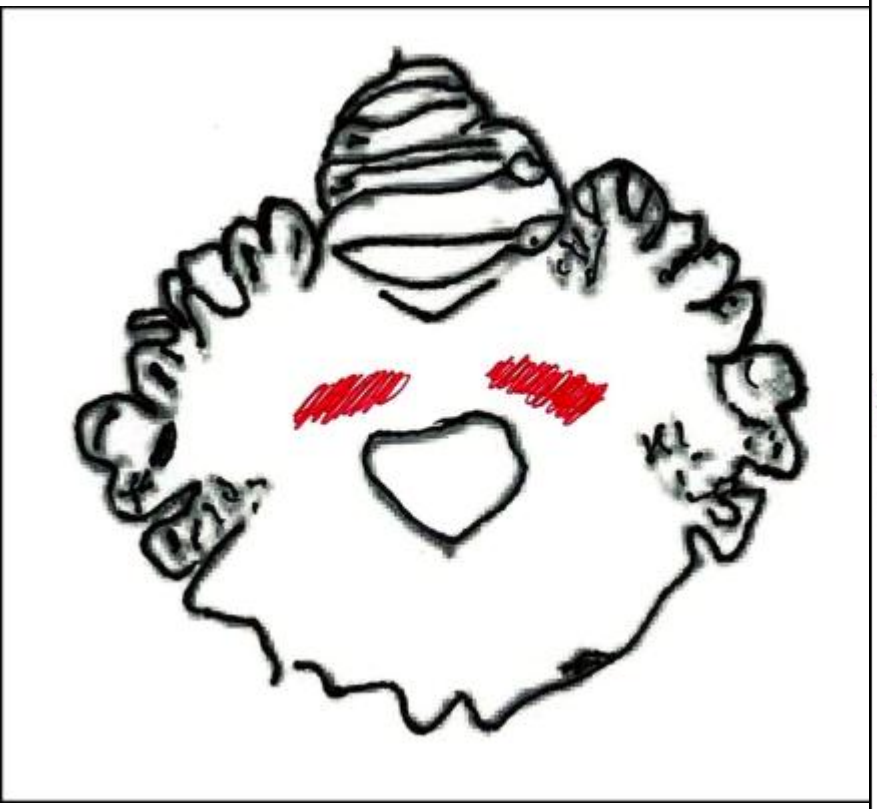
- Damage to certain brain stem nuclei in rats, mice, dogs & primates

Oral route

- Causes no brain lesions due to low systemic exposure

Artemether Lesion

Cerebellar roof nuclei (dog)



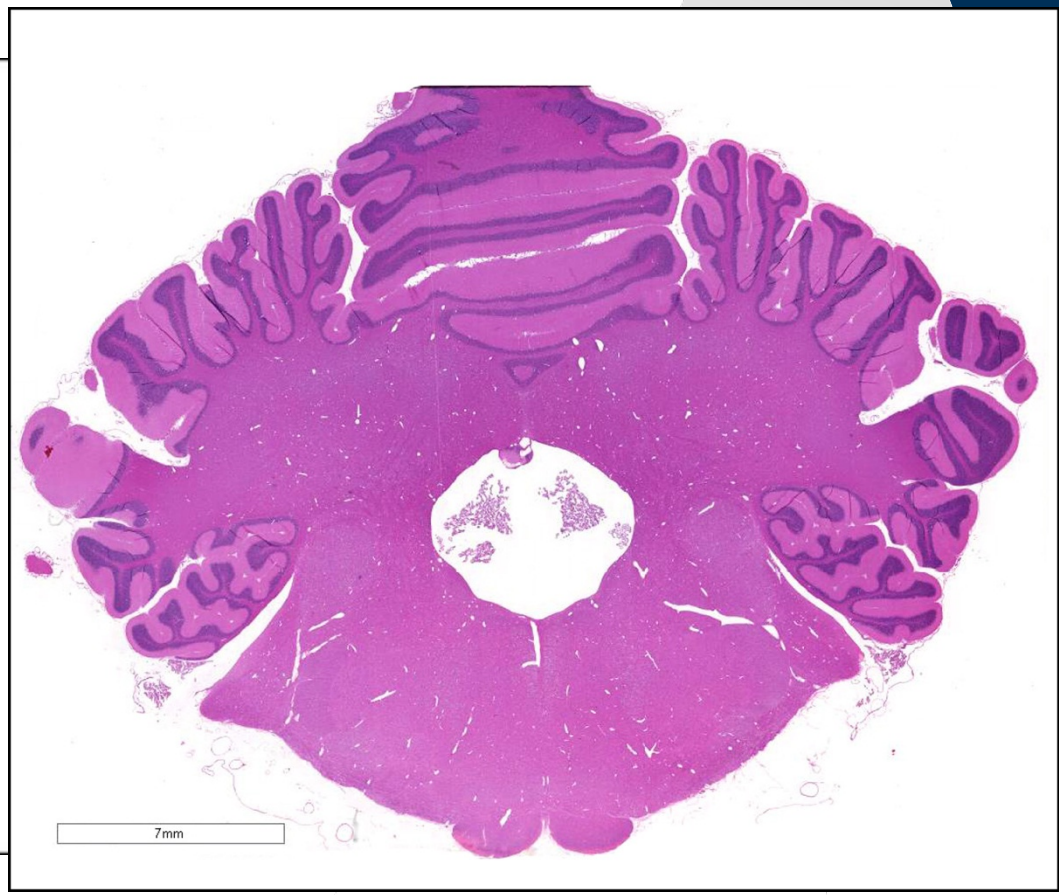


Image courtesy – G Krinke

Artemether Lesion

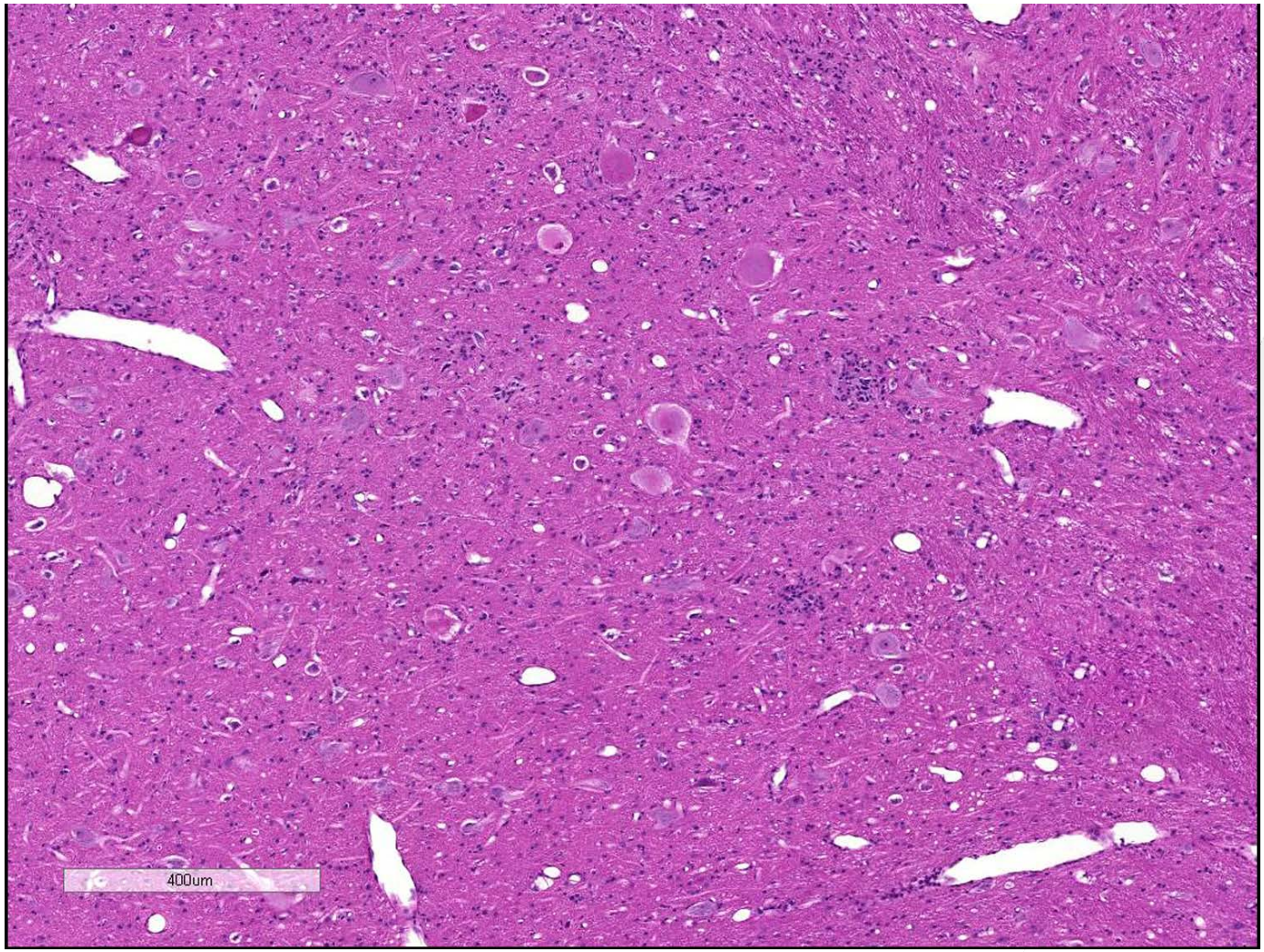


Image courtesy – G Krinke

Artemether Lesion

Neuronal necrosis, neuronophagia, gliosis, cerebellar roof nuclei, dog

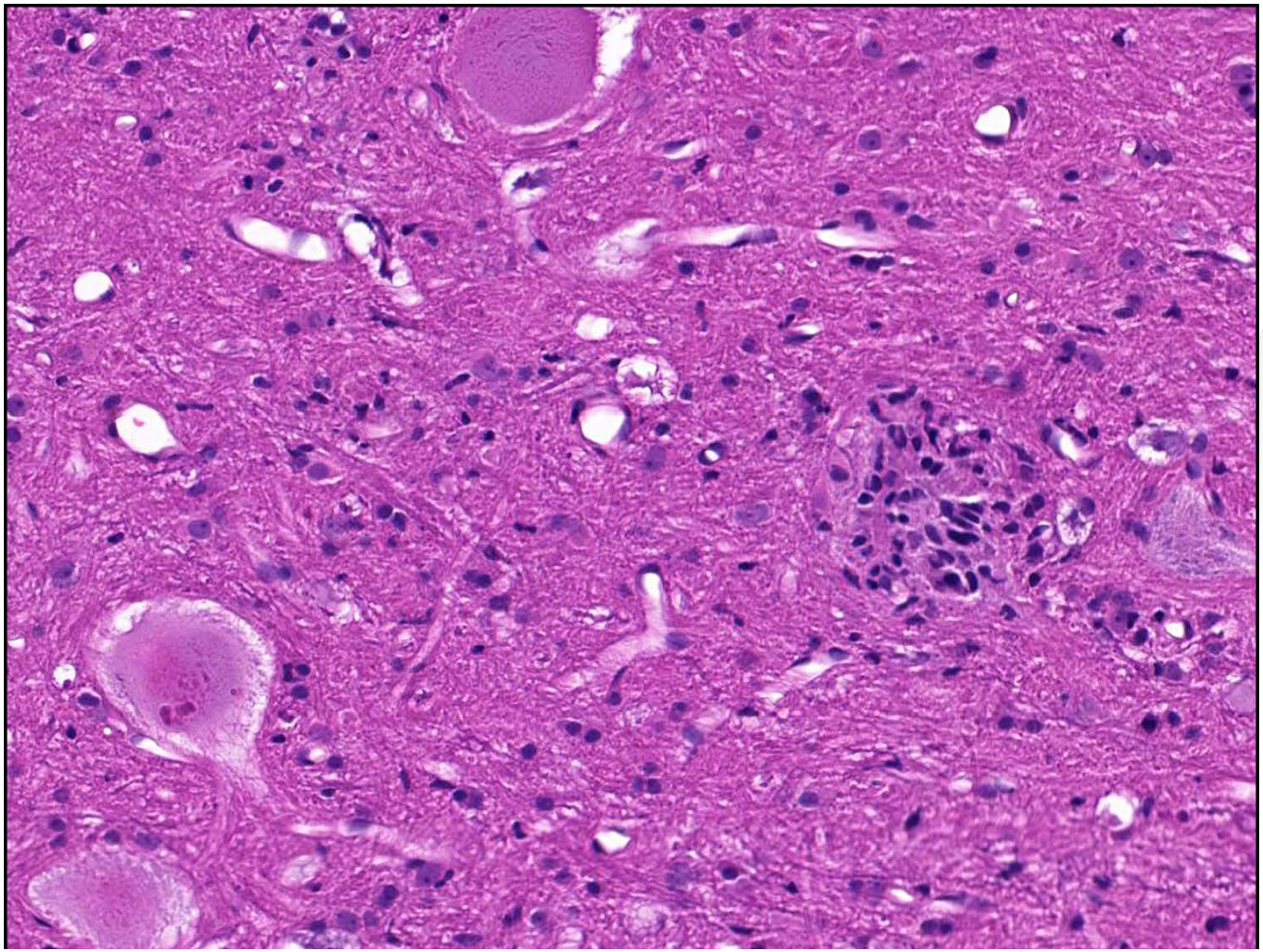
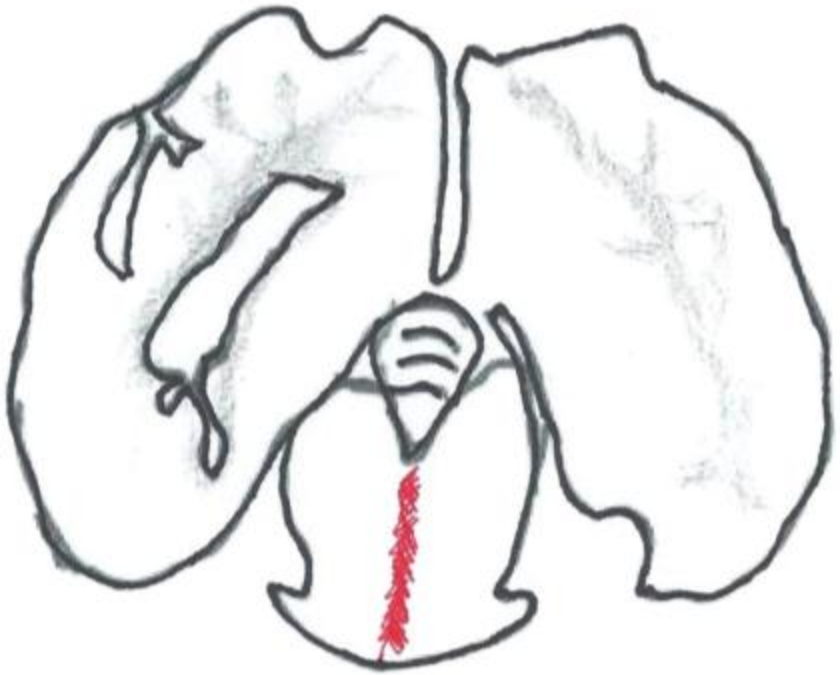


Image courtesy – G Krinke

Artemether Lesion

Dog Raphe nuclei, midbrain



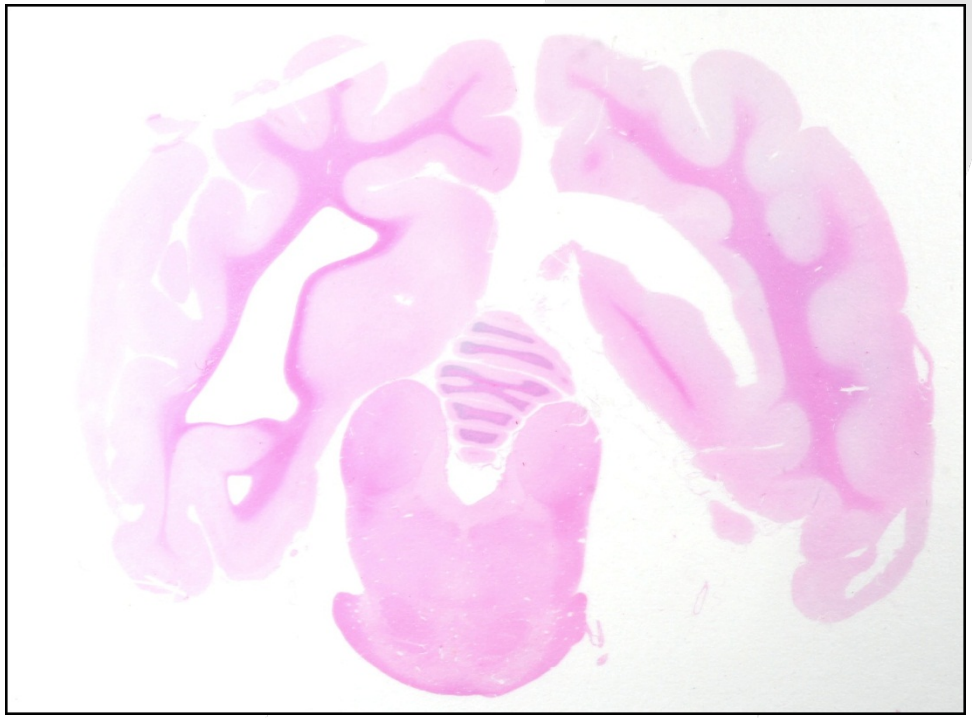


Image courtesy – G Krinke

Artemether Lesion

Artemether - Neuronal necrosis, gliosis, raphe nuclei, dog

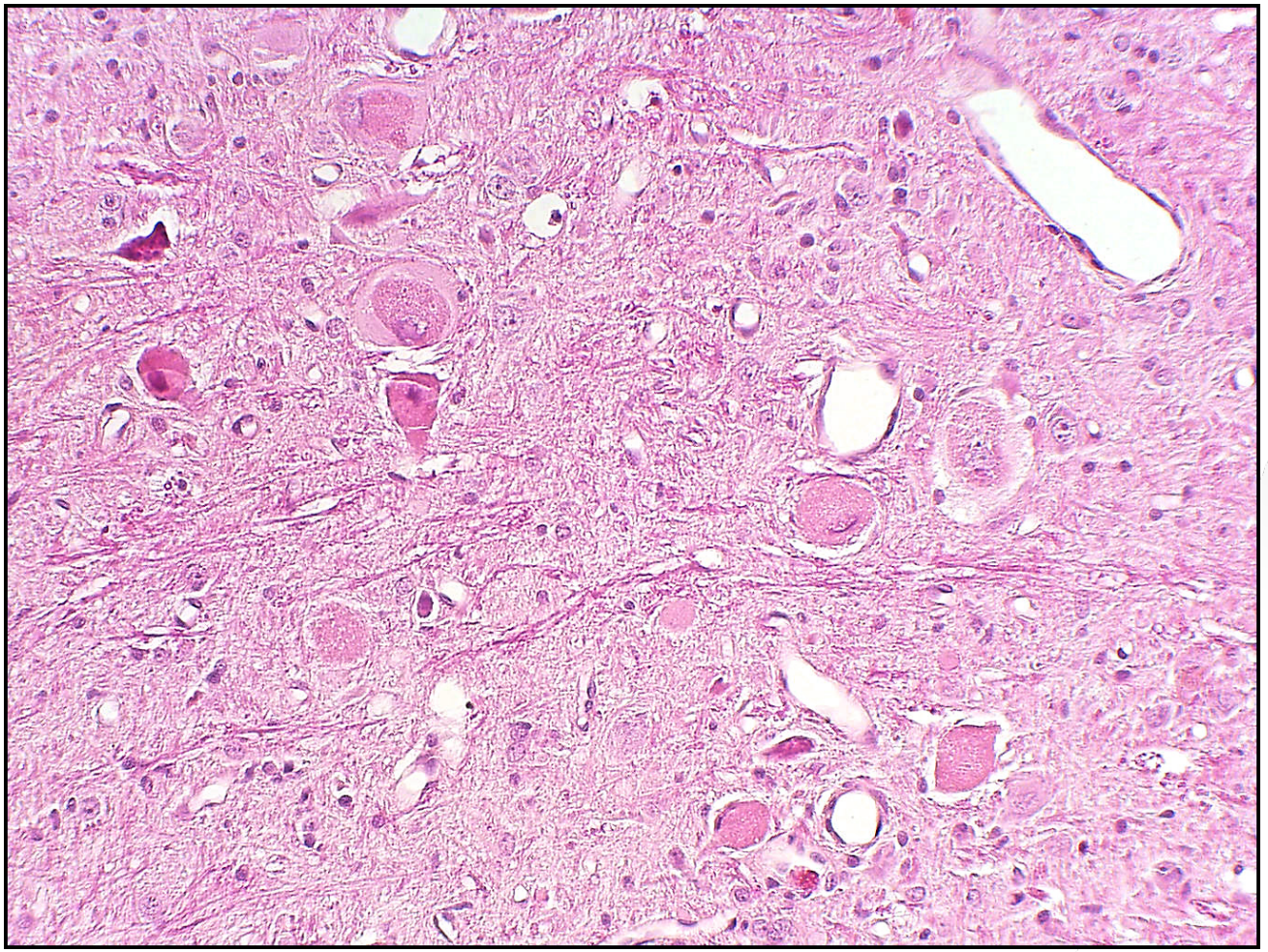


Image courtesy – G Krinke

Artemether

- Brain lesions are similar to motor neuron disease
  - I/M injection in dogs causes neuronal damage & secondary axonal lesions in cerebellar roof, pontine and vestibular nuclei and in the raphe region in the midbrain
- Doses used in patients are much lower than those that produce toxicity in animal studies

- What makes the assessment of potential risk of neurotoxicity difficult in these patients?
  - Severely ill patients often suffer with cerebral malaria and coma
- The drug has been used in more than 2 million patients with malaria

Other Drugs Causing Neuronal Damage at High Doses

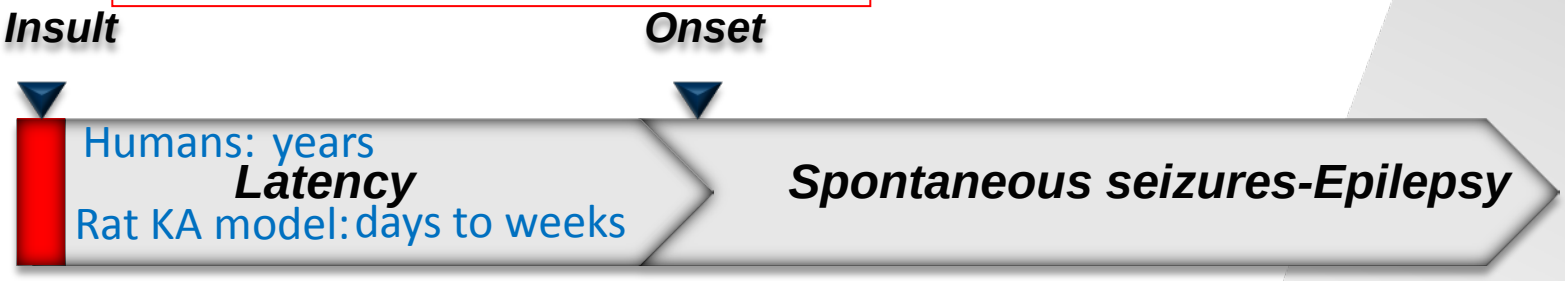
- Which other drugs produce neuronal damage at high doses in animal studies?
  - Nifurtimox for Chaga‘s disease (Trypanosoma cruzi)
    - Cerebral neuronal damage in mice & rats
    - Neurological symptoms in patients
  - Still a widely accepted drug of choice for Chaga‘s disease
  - Metronidazole for certain protozoal infestations and anaerobic bacteria
    - Neuronal damage in vestibular, cochlear & olivary nuclei (brain stem)
    - Neurological side effects reported in patients

History/ Signalment/ Clinical Signs

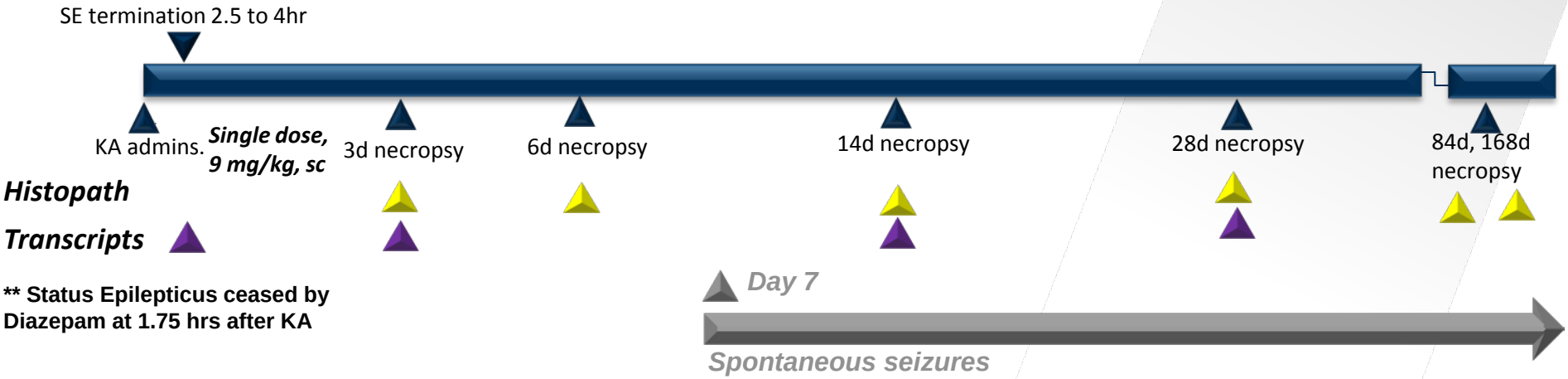
- Male Fisher 344 rats
  - 7 to 8-week-old
  - 200 to 300 g body weight
- A single subcutaneous dose of a xenobiotic
  - Changes in physical activity
  - Hypersalivation
  - Stereotypic grooming
  - Wet dog shakes
  - Varying intensities of continuous tremors & continuous clonus for 2.5 to 3 hrs.
  - Latency period from 1 to 8 weeks
  - Stage 3 to 5 spontaneous motor seizures

Rat Kainic Acid–induced MTLE Model

*Isolated from seaweed – excitatory and neurotoxic activity - acts on glutamate receptors (Kainate and AMPA)



Rat KA MTLE Model: study time course



** Status Epilepticus ceased by Diazepam at 1.75 hrs after KA

Toxicologic Pathology, 36: 932-943, 2008
Copyright © 2008 by Society of Toxicologic Pathology
ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1177/0192623308326093

Temporal Profile of Clinical Signs and Histopathologic Changes in an F-344 Rat Model of Kainic Acid–induced Mesial Temporal Lobe Epilepsy

Alok K. Sharma,[1,3] William H. Jordan,[2] Rachel Y. Reams,[3] D. Greg Hall,[2] and Paul W. Snyder[1]

[1]*Department of Comparative Pathobiology, Purdue University, West Lafayette, Indiana, USA*
[2]*Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA*
[3]*Covance Laboratories Inc., Greenfield, Indiana, USA*

Toxicologic Pathology, 000: 1-14, 2009
Copyright © 2009 by The Author(s)
ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1177/0192623309344202

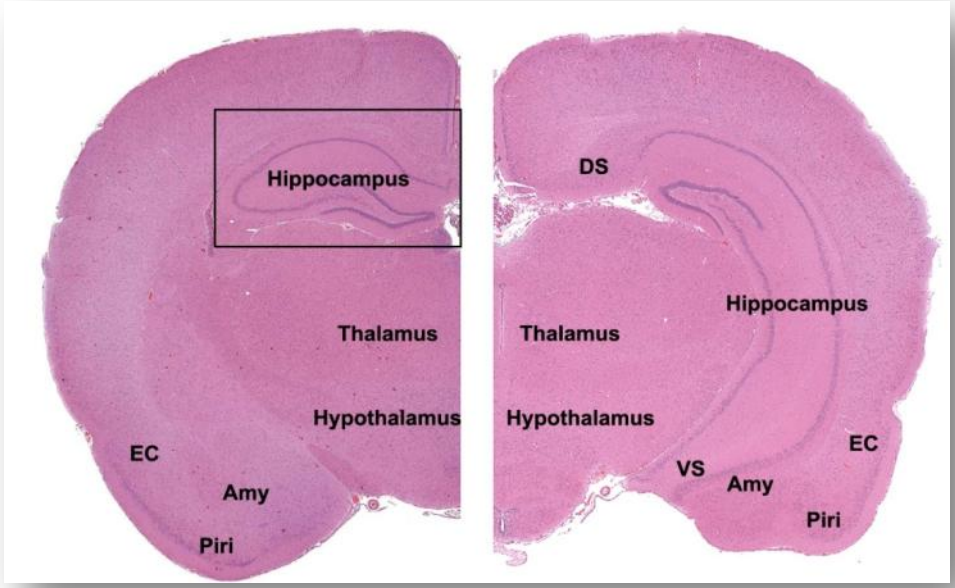
Kainic Acid-induced F-344 Rat model of Mesial Temporal Lobe Epilepsy: Gene Expression and Canonical Pathways

Alok K. Sharma,[1,4] George H. Searfoss,[3] Rachel Y. Reams,[2] William H. Jordan,[3] Paul W. Snyder,[4] Alan Y. Chiang,[3] Robert A. Jolly,[3] and Timothy P. Ryan[3]

[1]*Department of Pathology, Covance Laboratories Inc., Madison, WI 53704, USA*
[2]*Department of Pathology, Covance Laboratories Inc., Greenfield, IN 46140, USA*
[3]*Lilly Research Laboratories, Division of Eli Lilly and Co., Indianapolis, IN 46285, USA*
[4]*Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907, USA*

Getting Our Bearings for Site of Injury and Histopathological Observations

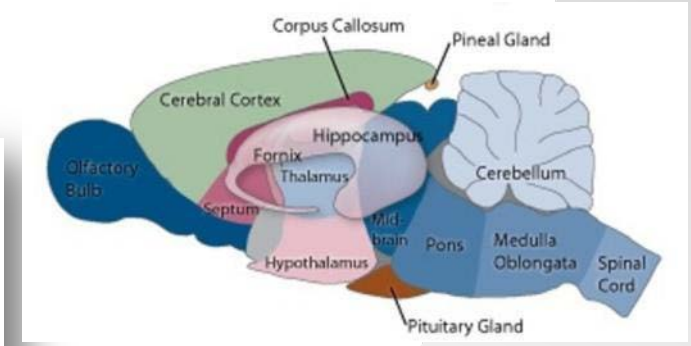
Rat brain coronal sections



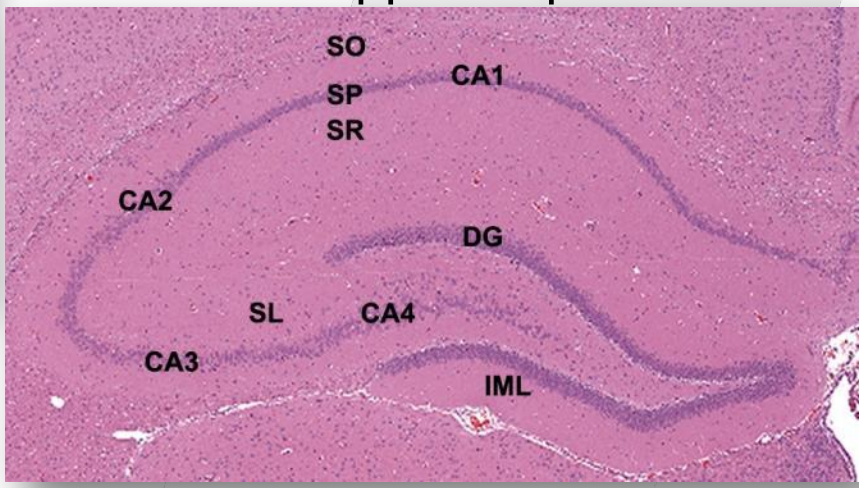
Bregma -3.12 **Bregma -5.28**

DS & VS – dorsal & ventral subiculum
EC & Piri – entorhinal & piriform cortices
Amy – amygdala

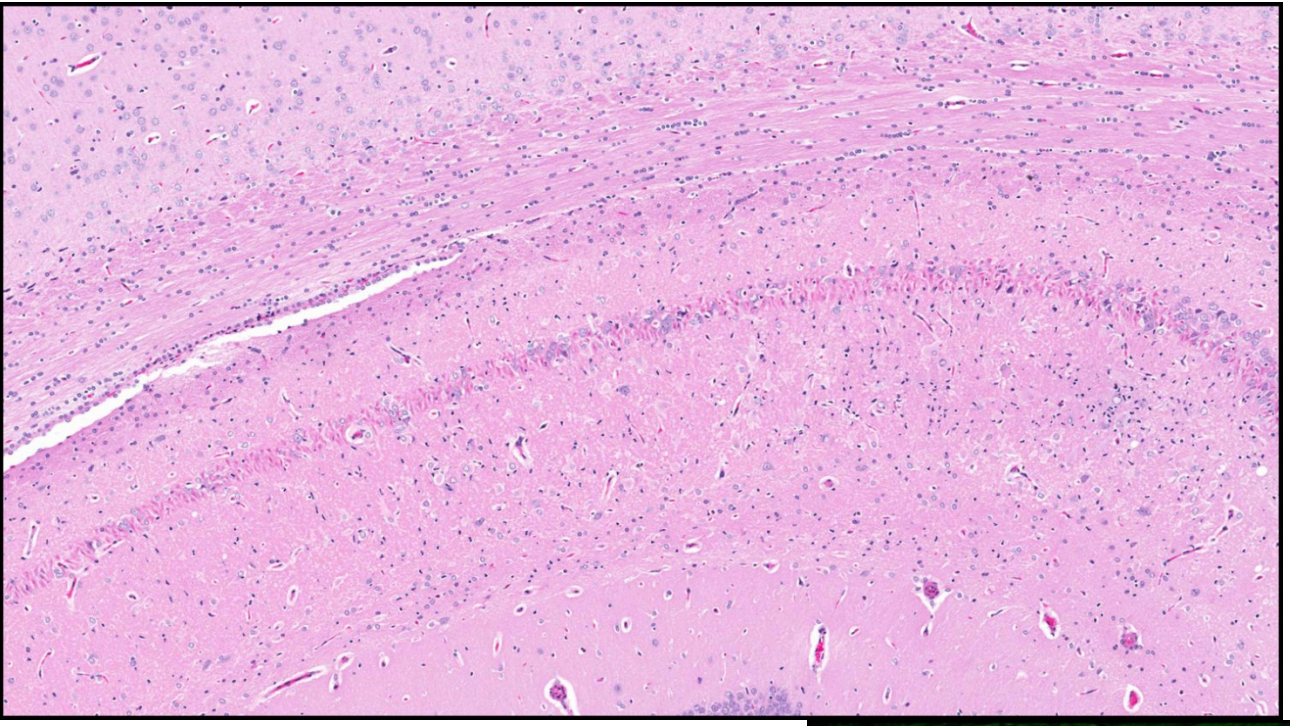
Rat brain diagram



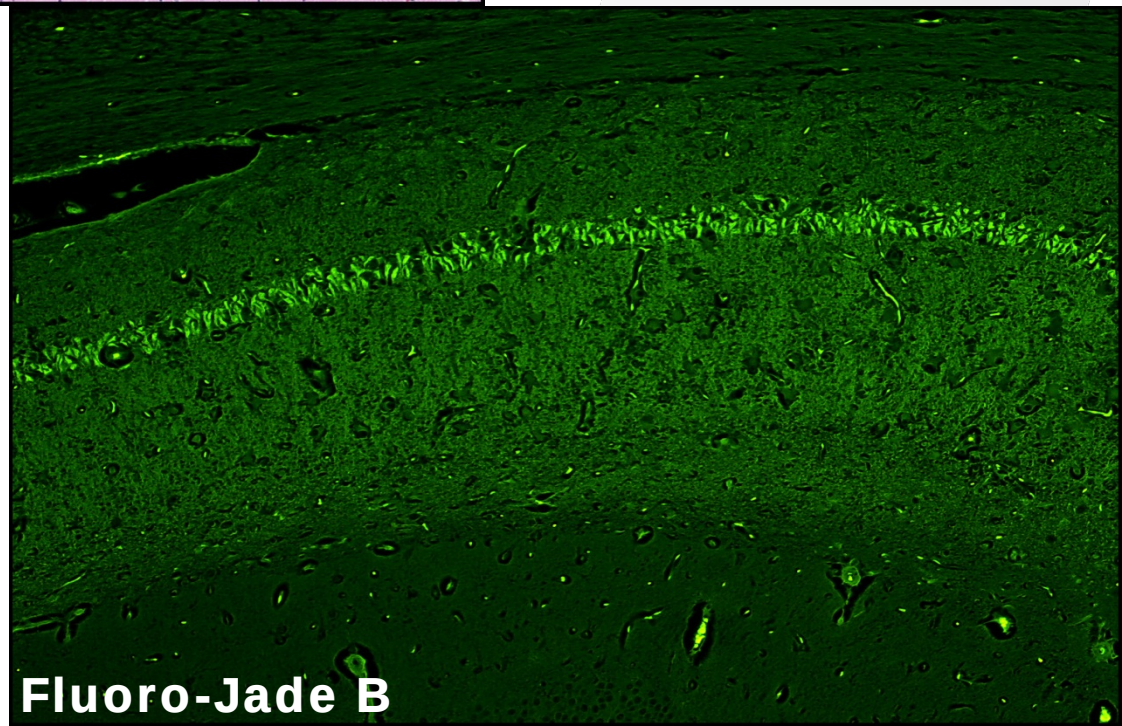
Detail of Hippocampus



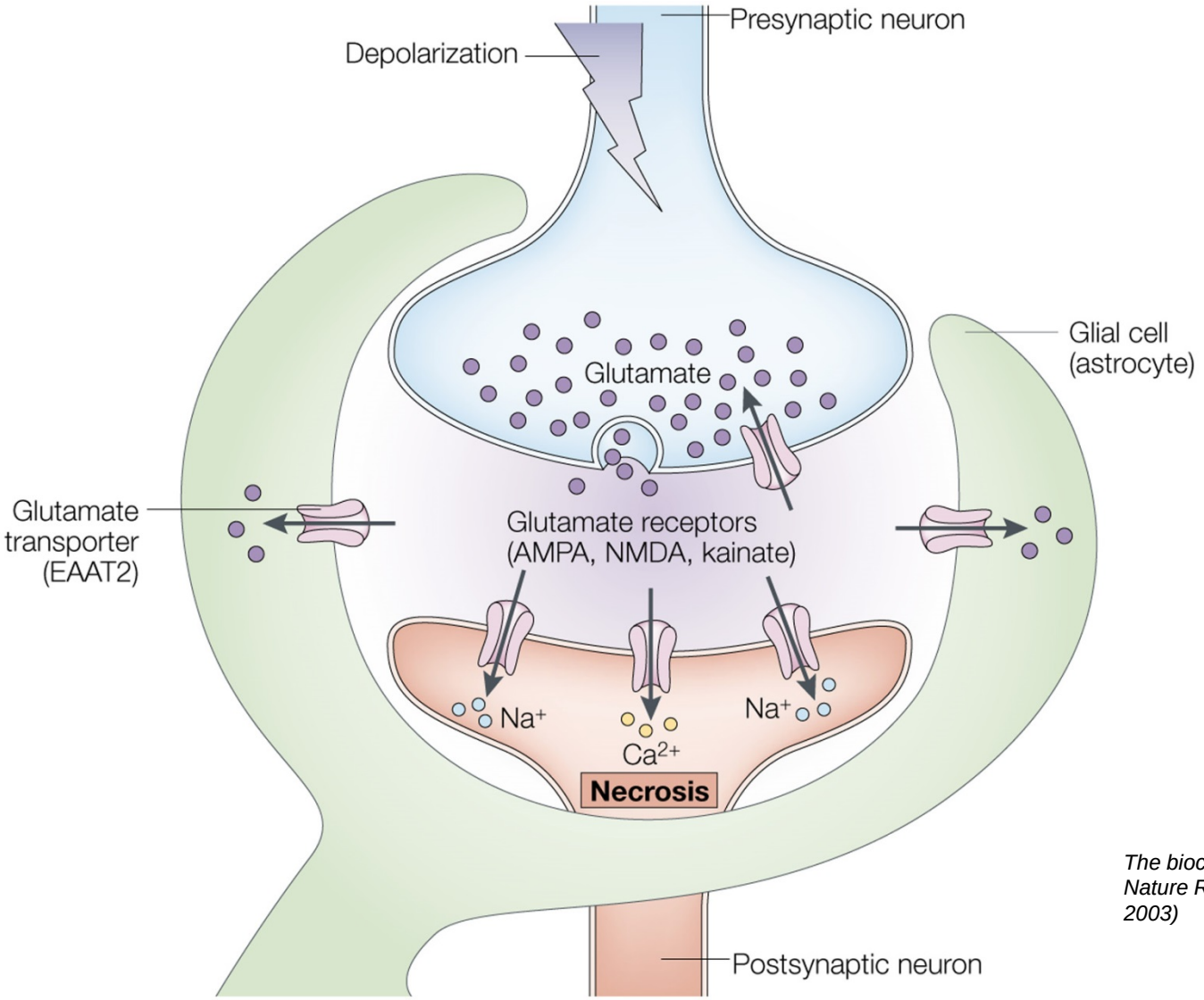
CA – Cornu Ammonis **Bregma -3.12**
SO, SP, SR & SL – stratum -oriens, -pyramidale, -radiatum & -lucidum
DG – dentate gyrus
IML – inner molecular layer



Day 6

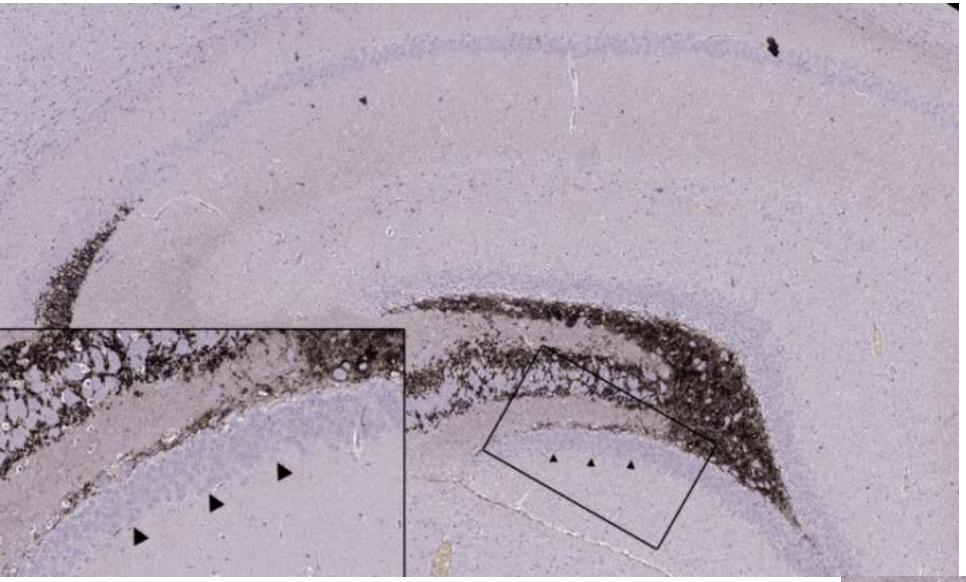


Receptors of Excitotoxicity



The biochemistry of neuronal necrosis: rogue biology? Nature Reviews Neuroscience 4, 672-684 (August 2003)

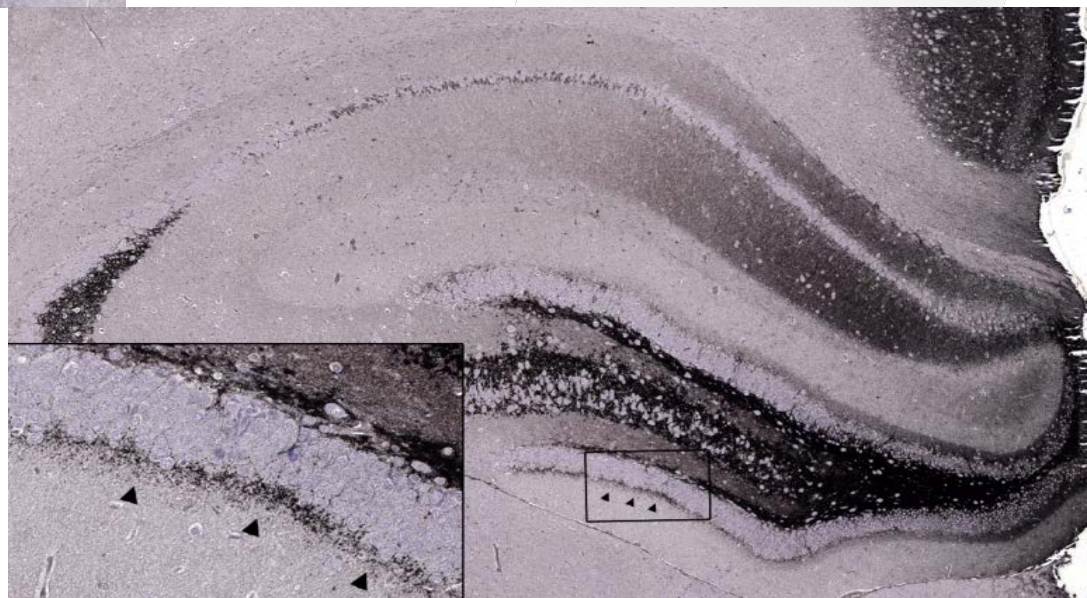
Hypothesis



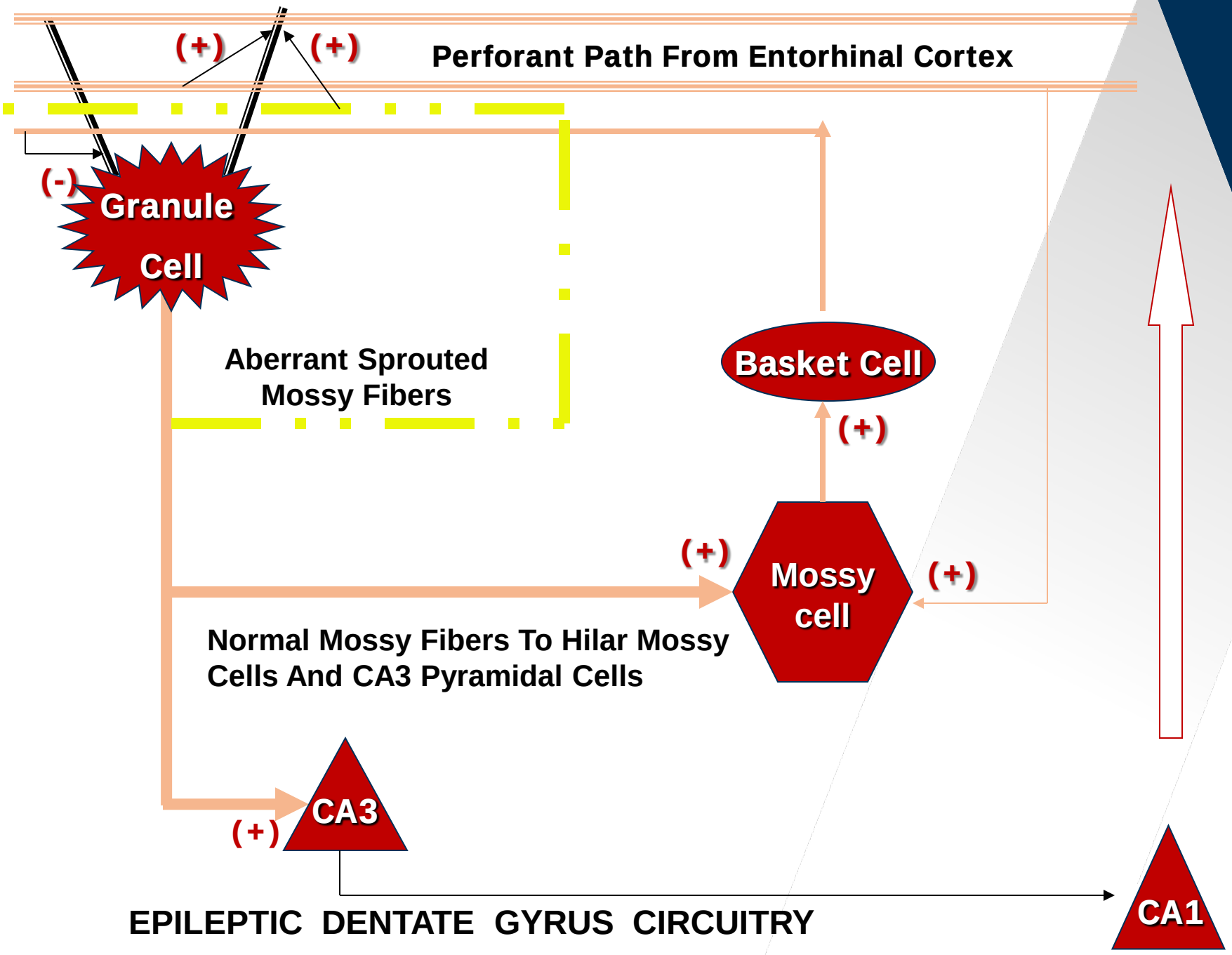
Control

Day 14 Timm's stain – for Mossy Fiber Sprouting

Kainic acid



▲ Inner Molecular Layer of the Dentate Gyrus

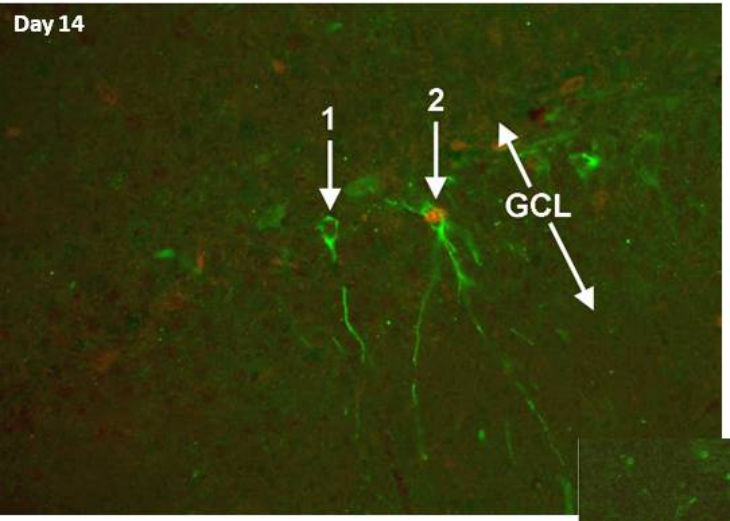


EPILEPTIC DENTATE GYRUS CIRCUITRY

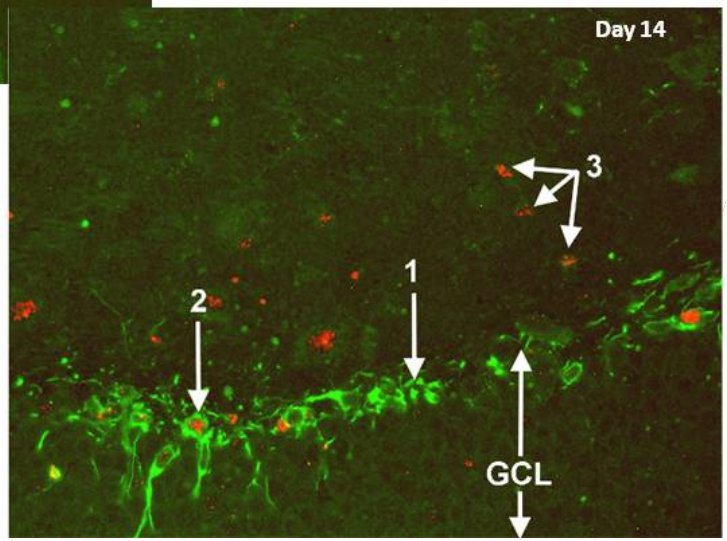
Adapted From: Epilepsy - Scientific Foundation of Clinical Practice (2004)-JM Rho, R Sankar, JE Cavazos

Neurogenesis and Seizure Generation

Aberrant neurogenesis in the hippocampus during epileptogenesis is also implicated for seizure generation

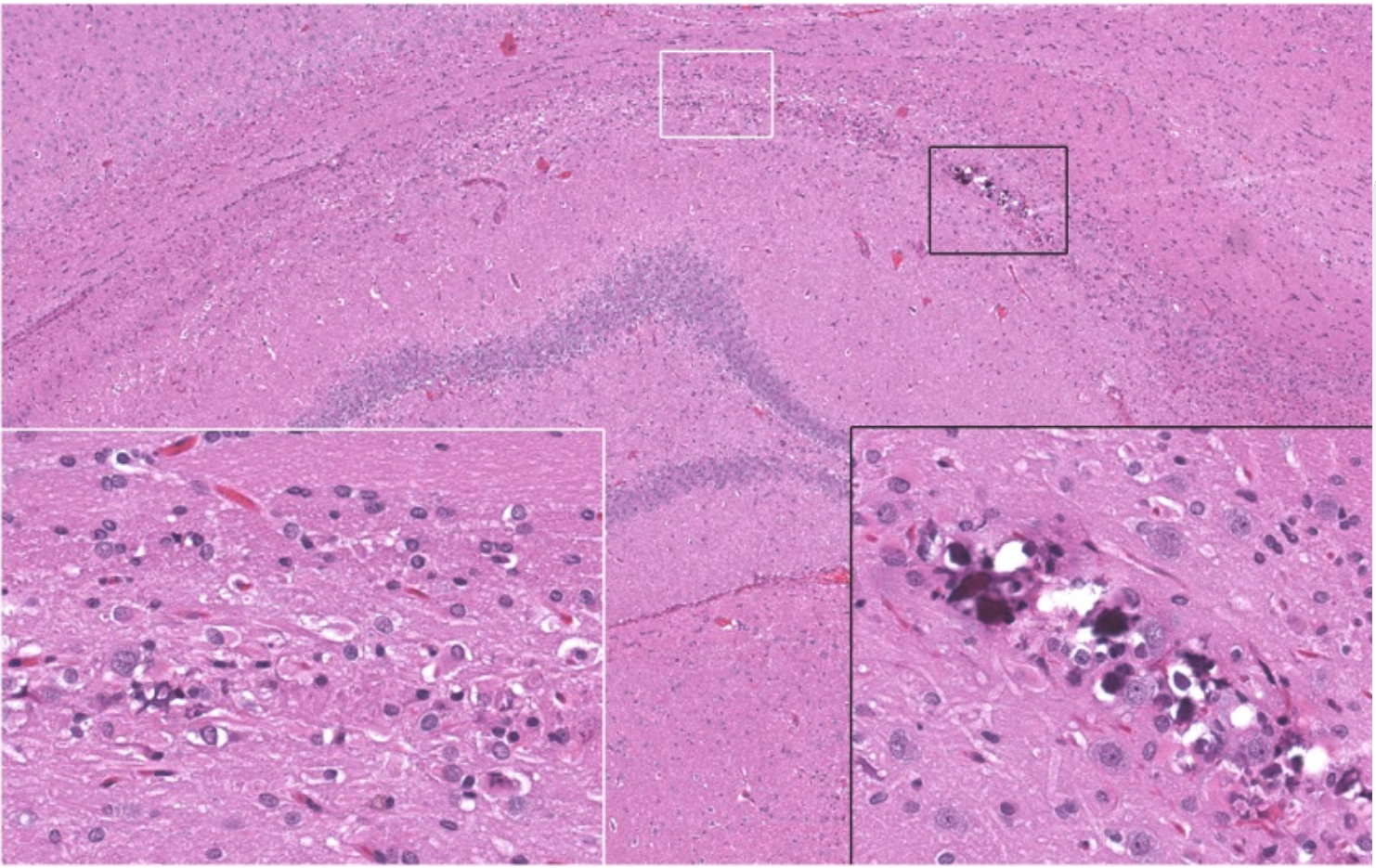


Evidence of neurogenesis (by DCX labeling cells in the GCL of the dentate gyrus) was first seen at day 6 and peaked at day 14

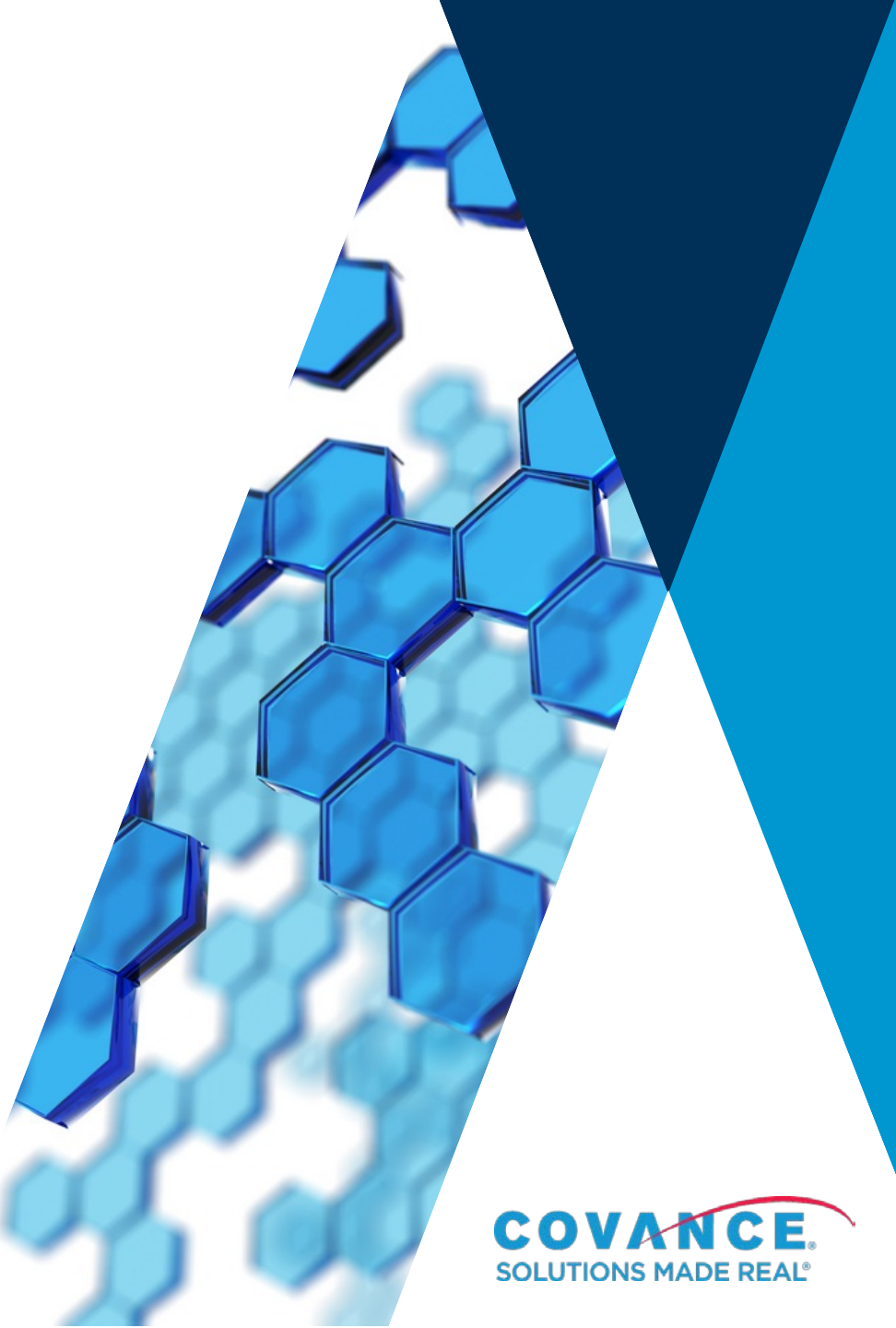


- 1 = DCX-positive post-mitotic immature neuron
- 2 = DCX- and Ki-67-positive immature mitotic neuron
- 3 = Ki-67 positive presumably dividing glial cells
- GCL = dentate gyrus granule cell layer

Day 168 Lesion



Role of Blood-Brain Barrier



COVANCE
SOLUTIONS MADE REAL®

Doxorubicin

- aka Adriamycin
- Glycosidic anthracycline antibiotic
- Antineoplastic
- Neurotoxicity (not a feature in man but seen in NHPs)
- Noncovalently binds with DNA
- Neurotoxic in rats at 10mg/kg I/V
  - Lesions in dorsal root ganglia (DRG) in few hrs to day 4
  - Lesion severity in DRG > trigeminal > autonomic ganglia

Doxorubicin

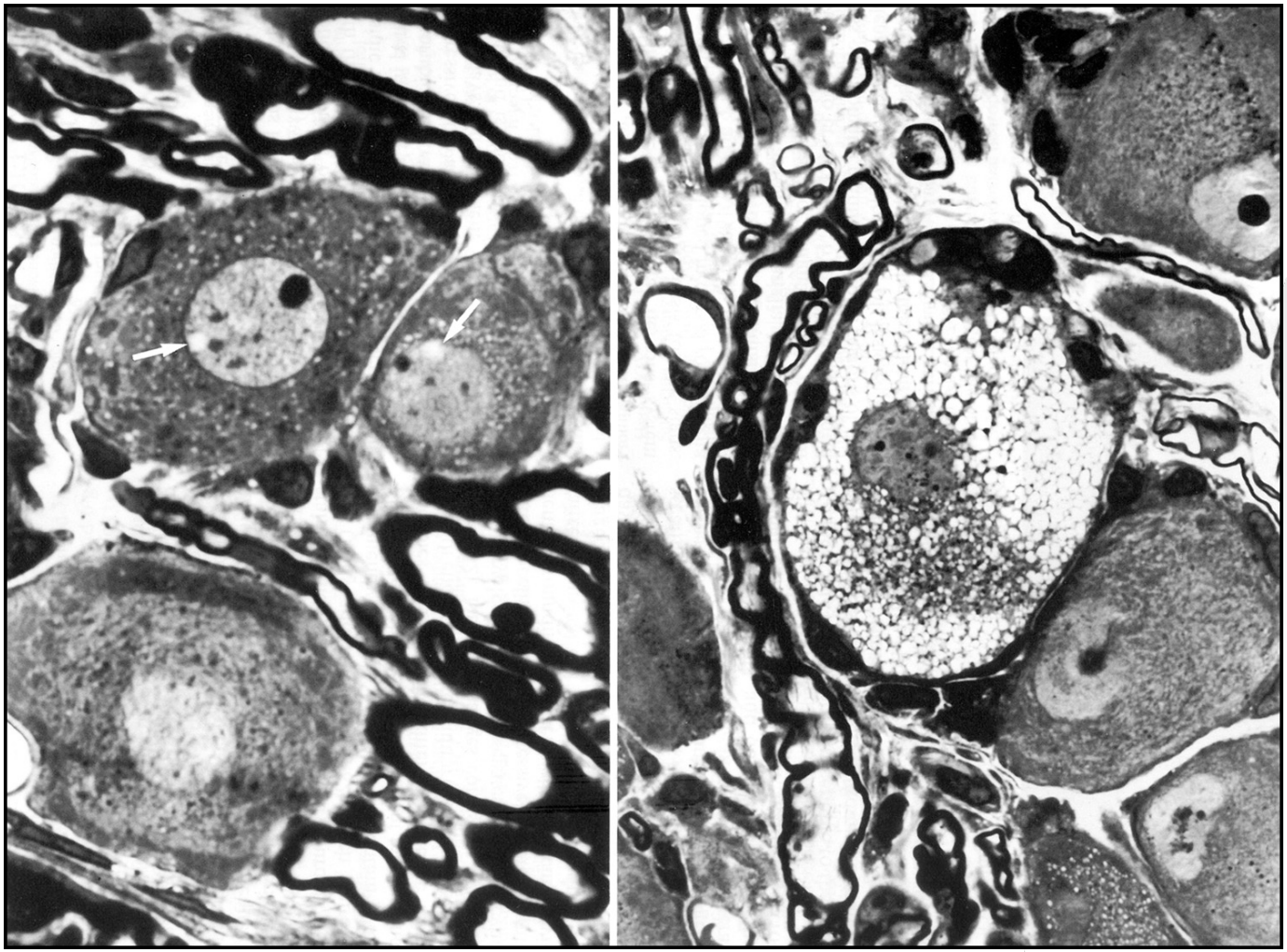


Image courtesy – B. Jortner

Doxorubicin (cont.)

- Initial lesion
  - Discrete regions of nuclear clearing
- Intermediate lesion
  - Marked neuroplasmic vacuolization
- By Day 9: changes progress to nuclear necrosis
  - Pyknosis
  - Karyolysis
- Wallerian-type degeneration
  - Peripheral nerves
  - Dorsal roots and dorsal columns of the spinal cord

Doxorubicin (cont.)

Pathogenesis

- Doxorubicin can not cross the blood brain barrier (BBB)
- Peripheral sensory and autonomic ganglia lack such blood-tissue barriers
- I/V injection
  - Parts of CVO
    - Area postrema
    - Median eminence
    - Neurohypophysis
- I/V mannitol before doxorubicin
  - Damage to brain
    - Injury of neurons in the cortex and subcortical nuclei of the brain; hemorrhagic infarcts

Role of Blood–Brain Barrier

Blood-brain barrier

- Is incompletely developed at birth
- Even less so in premature infants
- Predisposes the premature infant to brain injury by toxins
  - Unconjugated bilirubin
  - Hexachlorophene
    - Excluded from the nervous system in adults

Blood-Brain Barrier

- Brain capillary endothelial cells possess
  - ATP-dependent membrane transporters (ATP-binding cassette or ABC transporters) in their luminal membrane (Schinkel, 1999)
    - Such as the multidrug-resistance protein (MDR1), or P-glycoprotein
    - Extrude chemicals and contributes to the blood-brain barrier

Schinkel *Adv Drug Delivery Rev* 36:179–194, 1999.

Blood-Brain Barrier

- P-glycoprotein (encoded by the multi-drug resistance gene or MDR1)
  - Highly impedes the ivermectin (a neurotoxic pesticide and human antihelmintic drug) entry into the brain
  - By an ATP-driven efflux mechanism at the blood-brain barrier
  - mdr1a gene knock out mice reveal 100-fold higher brain levels of ivermectin

Schinkel Adv Drug Delivery Rev 36:179–194, 1999.

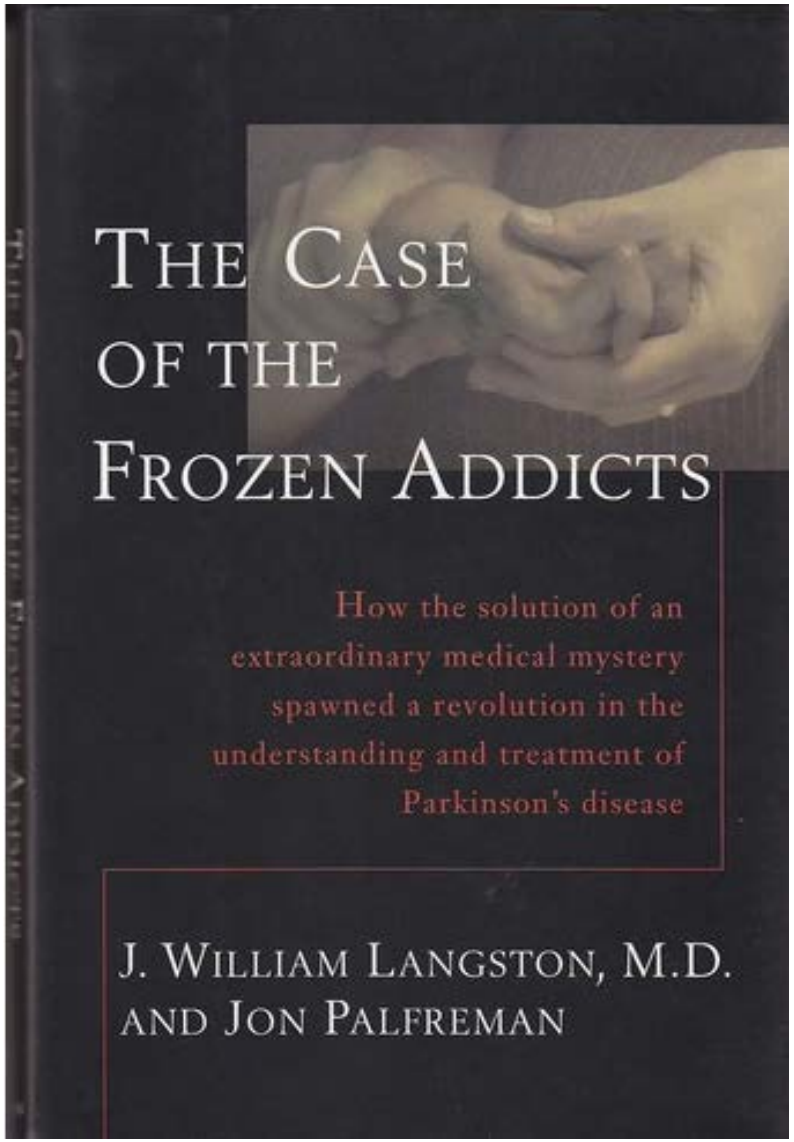
Blood-Brain Barrier

- In 1950s clinical trial were conducted to compare the efficacy of tetracycline and penicillin–sulfonamide mixture in the management of bacterial infections in premature infants (Silverman et al., 1956)
- The penicillin–sulfonamide mixture caused much higher mortality than tetracycline
  - Because sulfonamide displaced a significant amount of bilirubin from albumin
  - In newborns, blood–brain barrier is not fully developed
  - Free bilirubin diffused into the brain and caused severe brain damage termed *kernicterus.*
- In adults, bilirubin does not cross the blood-brain barrier

Silverman et al. Pediatrics 18:614–625,1956

1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine (MPTP)

- Parkinson's disease is better understood because of studies on the mechanism of toxicity of MPTP
- How was MPTP discovered?
  - A mistake on part of a designer chemist while synthesizing illicit version of meperidine (Demerol®)
    - Unsuspecting users injected themselves and developed Parkinsonism
      - Responded to levo-dopa
      - Autopsy – severe brain damage identical to Parkinson

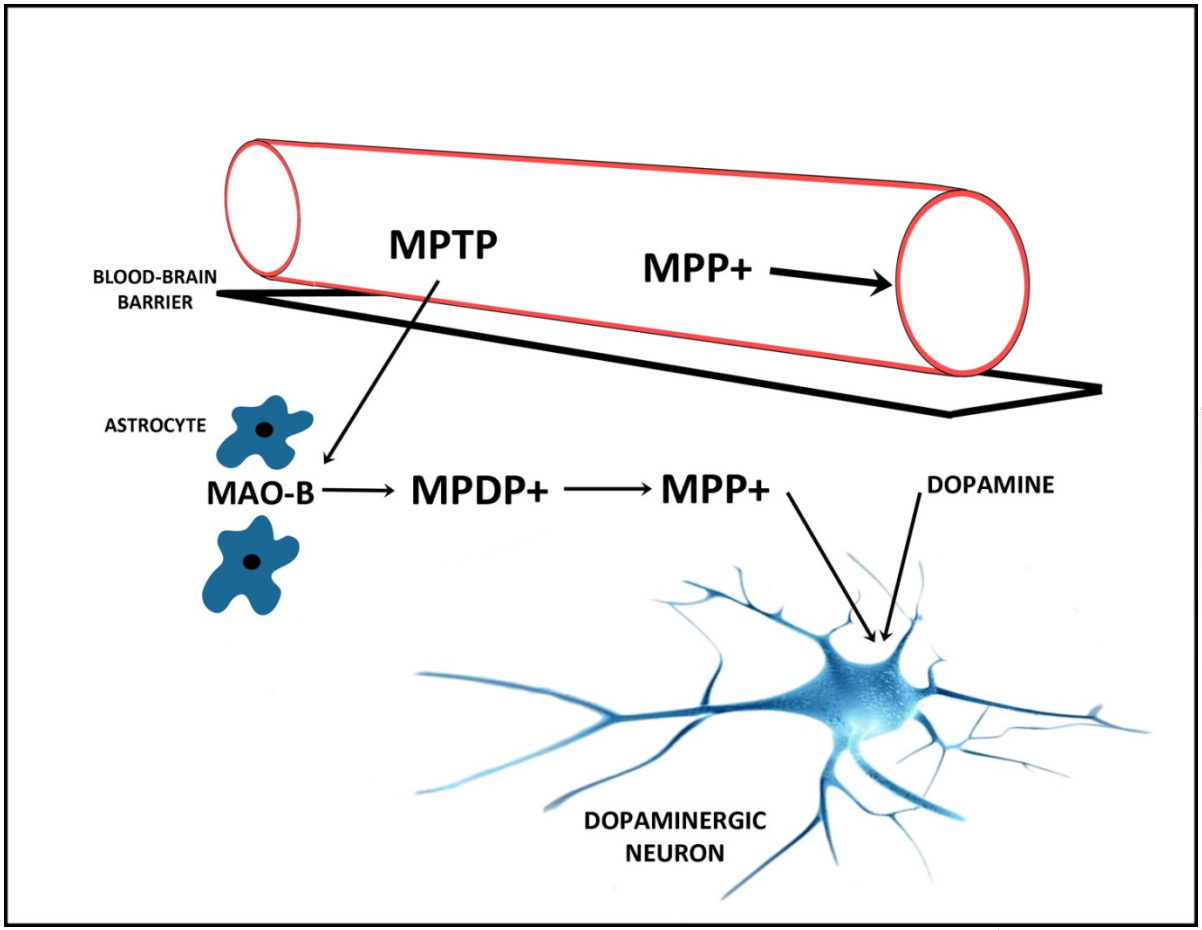


- Neurosurgeon J. William Langston was the first to deal with six patients at San Francisco area ER
- Patients were fully conscious but unable to move or speak
- Langston diagnosed them as having advanced Parkinson's disease
- History of using a synthetic analogue of heroin

1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine (MPTP), cont.

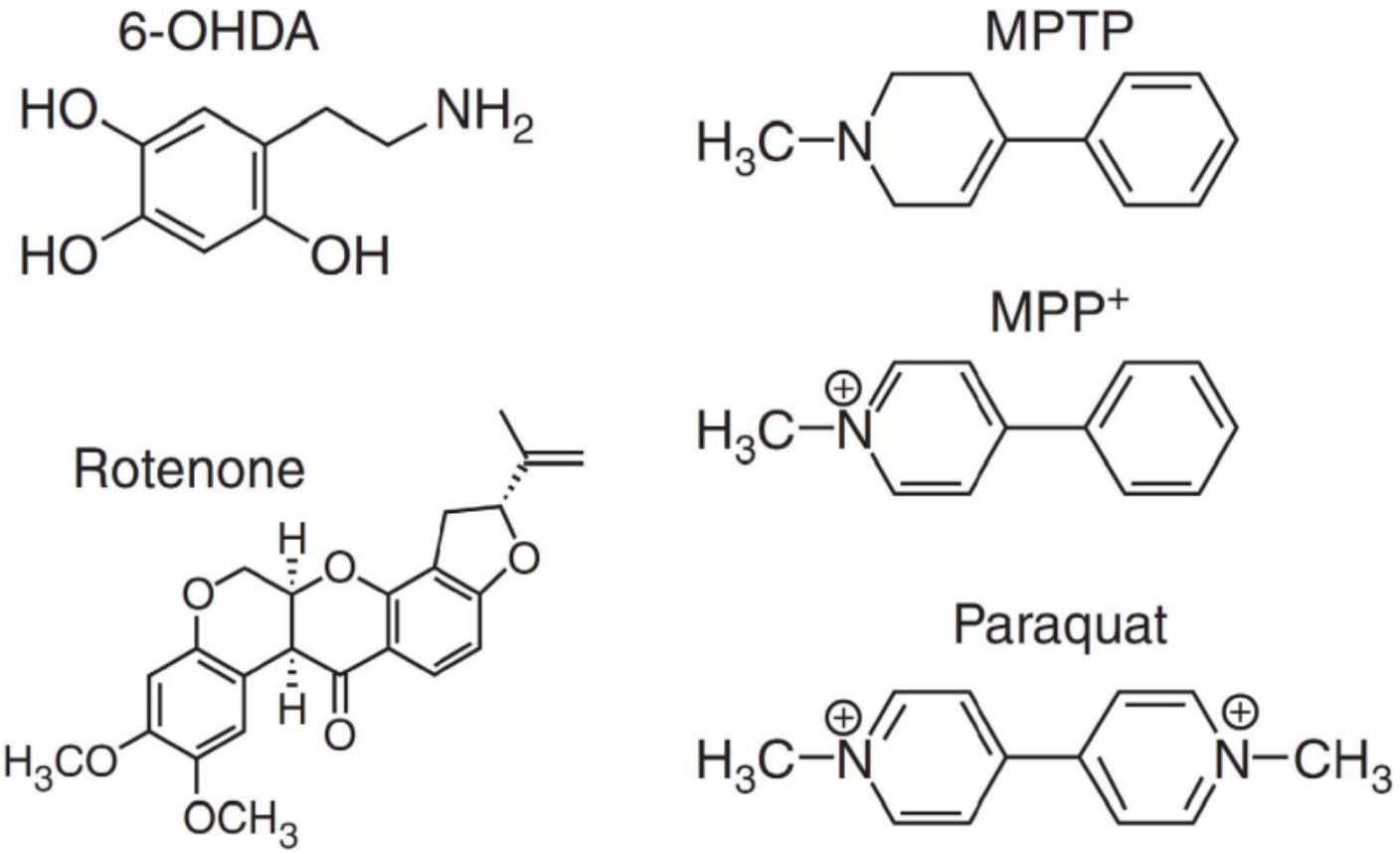
- Cationic neurotoxic metabolite of MPTP (MPP^+)
  - Electrophoretically accumulates in the mitochondria of dopaminergic neurons
    - Mitochondrial dysfunction and cell death
    - Also causes inhibition of dopamine uptake

1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine (MPTP), cont.



Monoamine oxidase B in astrocytes activates MPTP to MPP^+ (1-methyl-4-phenylpyridine)

Neurotoxic Molecules to Induce Nigrostriatal Damage in Animal Models of PD



MPP+ is structurally similar to most of these xenobiotics.

Image courtesy K. Tieu

MPTP-treated Monkey

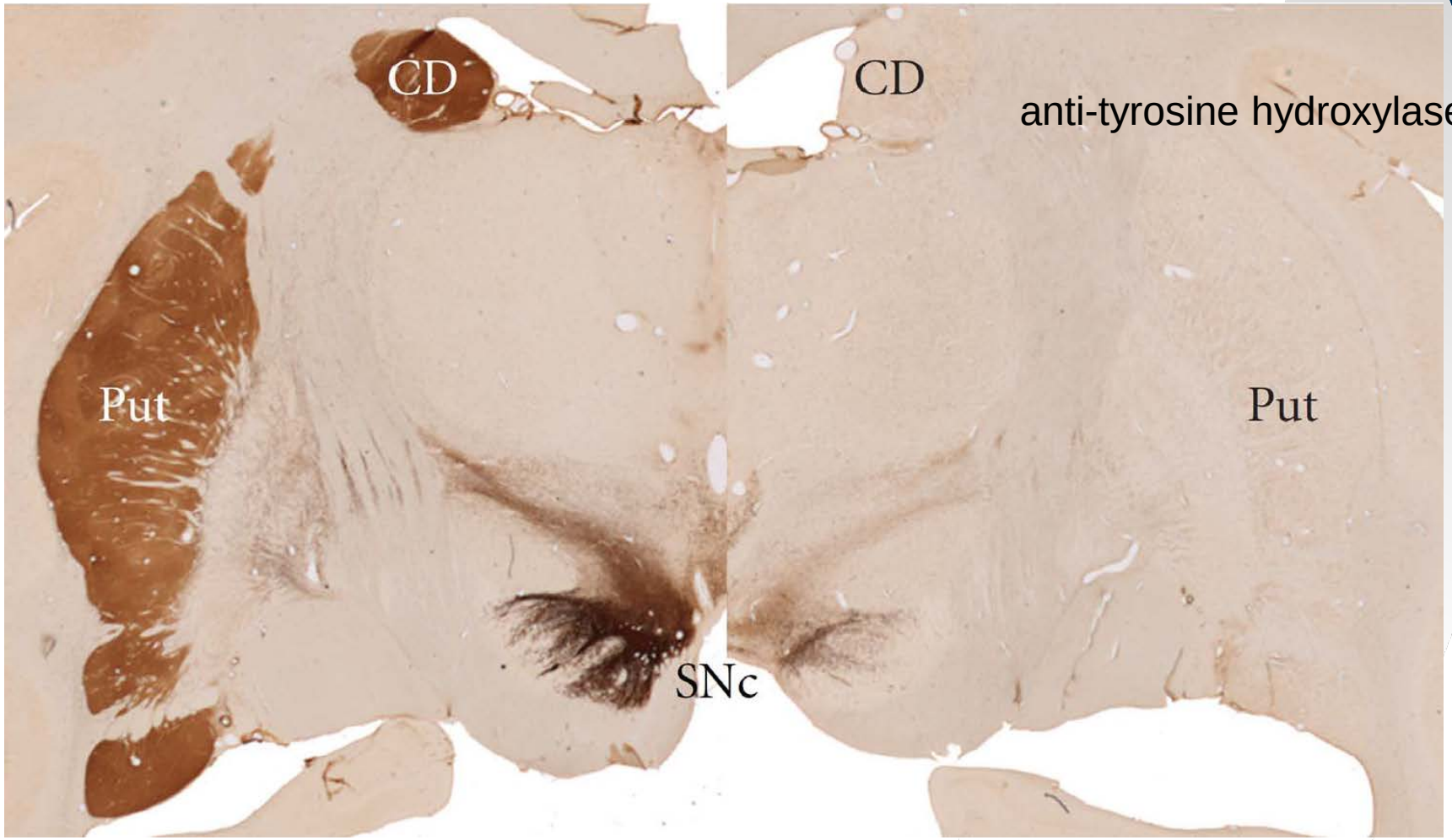


Image courtesy - J. Blesa

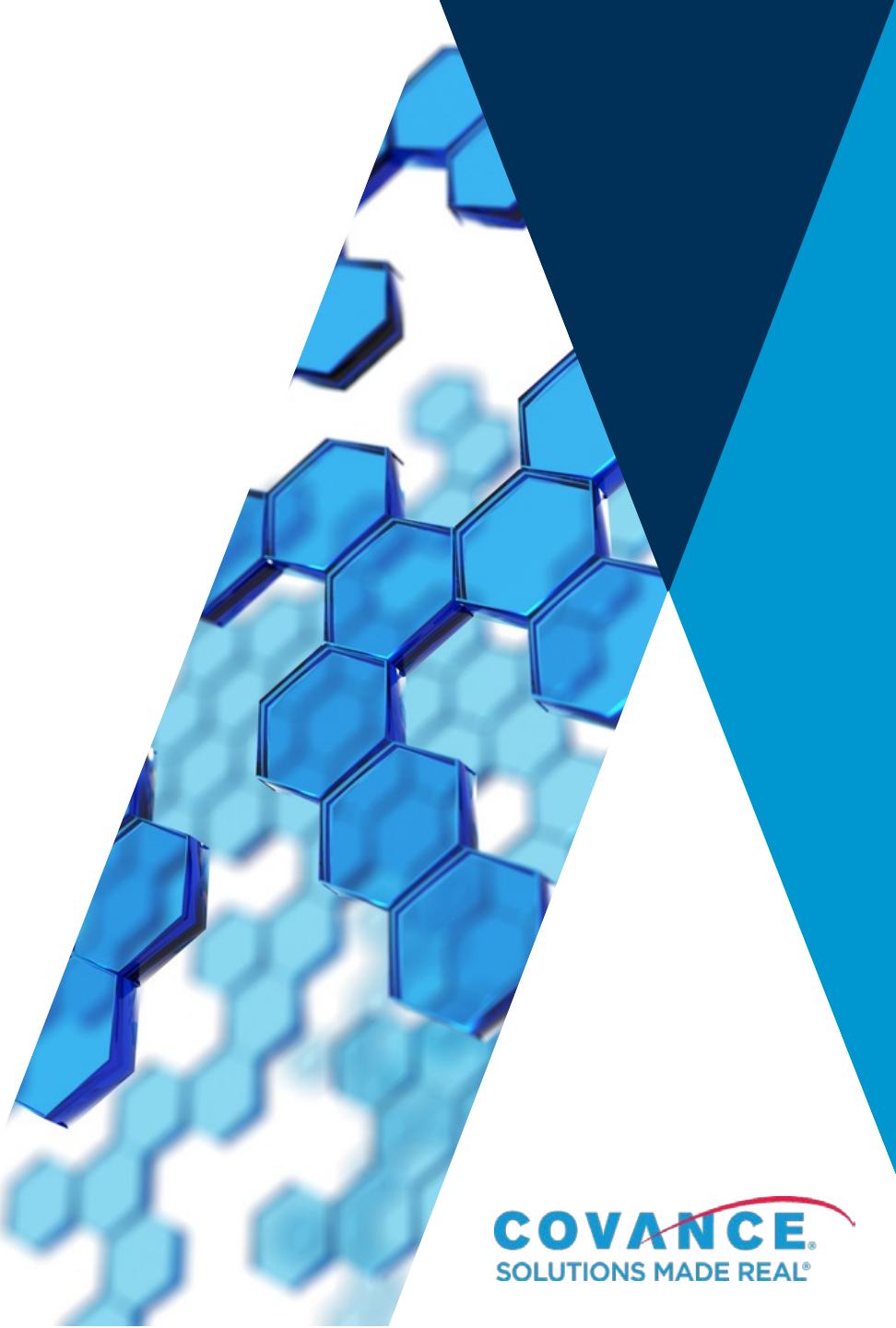
1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine (MPTP)

- Why MPTP works better in primates and not rodents?
  - Importance of melanin in the pathogenesis of xenobiotic induced Parkinsonism
    - Reversible intracellular binding
    - The release of melanin-bound MPTP contribute to injury to substantia nigra neurons

*Retinal toxicity associated with chlorpromazine and chloroquine

D'Amato, R.J., et al. Science, New Series, Vol. 231, No. 4741 (Feb. 28, 1986), pp. 987-989
Klaassen, C.D., Ed.: Casarett and Doull's Toxicology: The Basic Science of Poisons. Seventh Edition

CNS VACUOLATION



COVANCE
SOLUTIONS MADE REAL®

Sulfonylurea Derivatives

- Antidiabetic drugs for Type 2 diabetes
  - Act by increasing insulin release from the beta cells in pancreas
- Induce severe hypoglycemia
- Swelling of astrocytes in the cortex/hippocampus

Sulfonylurea Derivatives

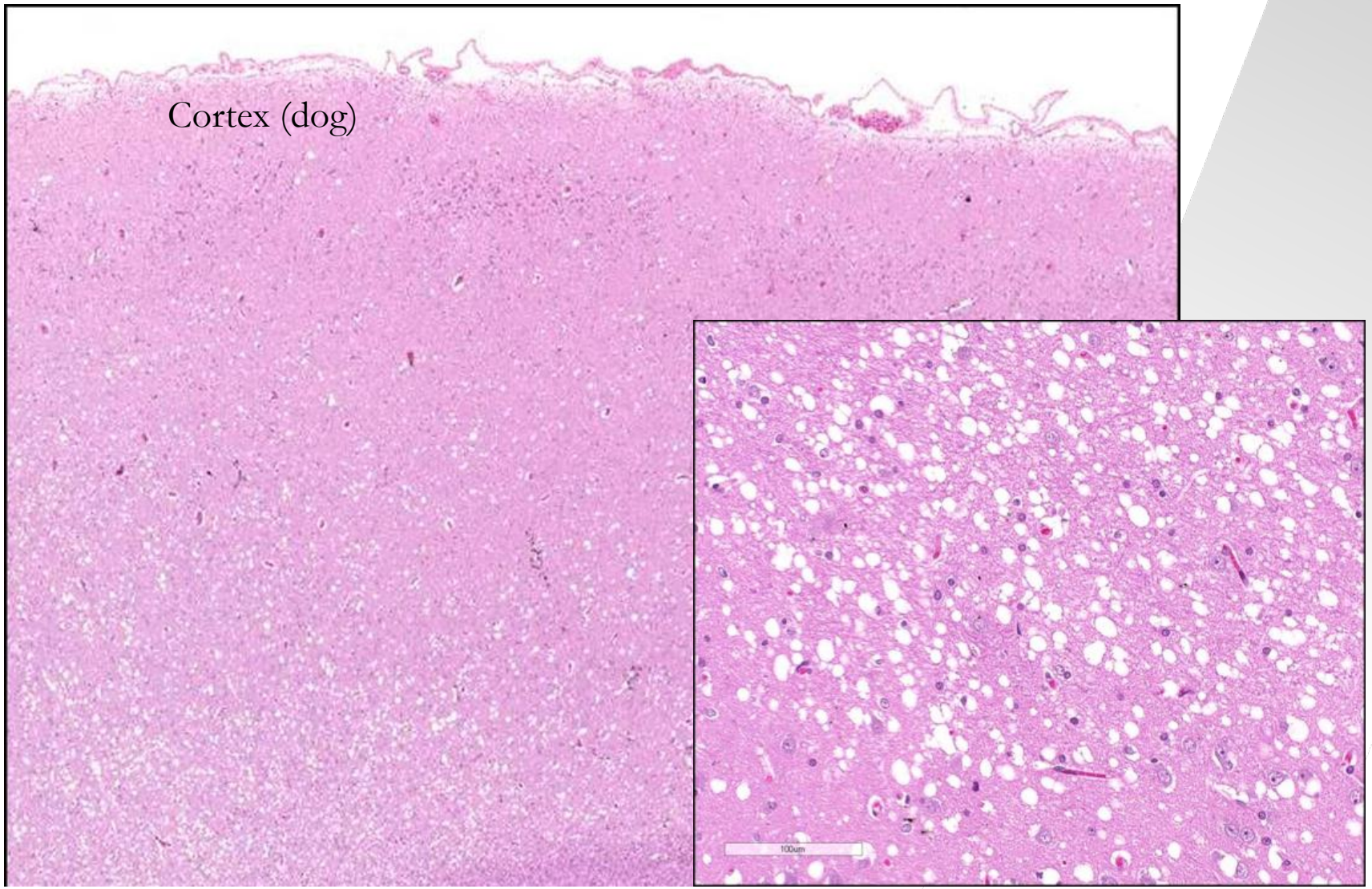


Image courtesy – G Krinke

Sulfonylurea Derivatives

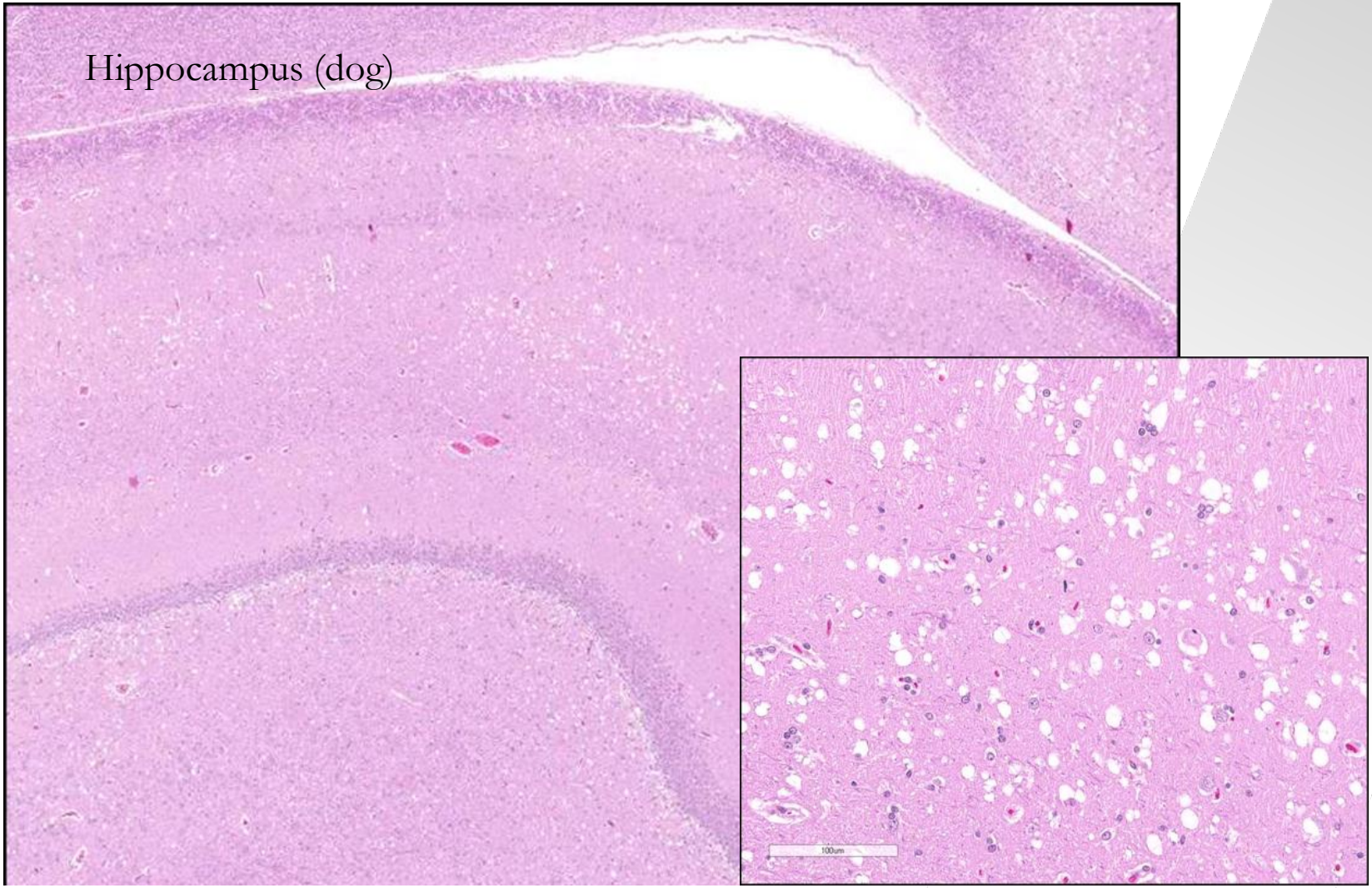


Image courtesy – G Krinke

Sulfonylurea Derivatives (cont.)

- Mechanism (?)
  - Hypoglycemia
  - Energy deprivation

6-Aminonicotinamide (6-ANA)

- Mechanism (?)
  - Inhibition of pentose phosphate pathway
    - Energy deprivation
    - Swelling of astrocytes

6-Aminonicotinamide (6-ANA)

Swelling/vacuolation of astrocytes in cerebral cortex, 6-ANA (dog)

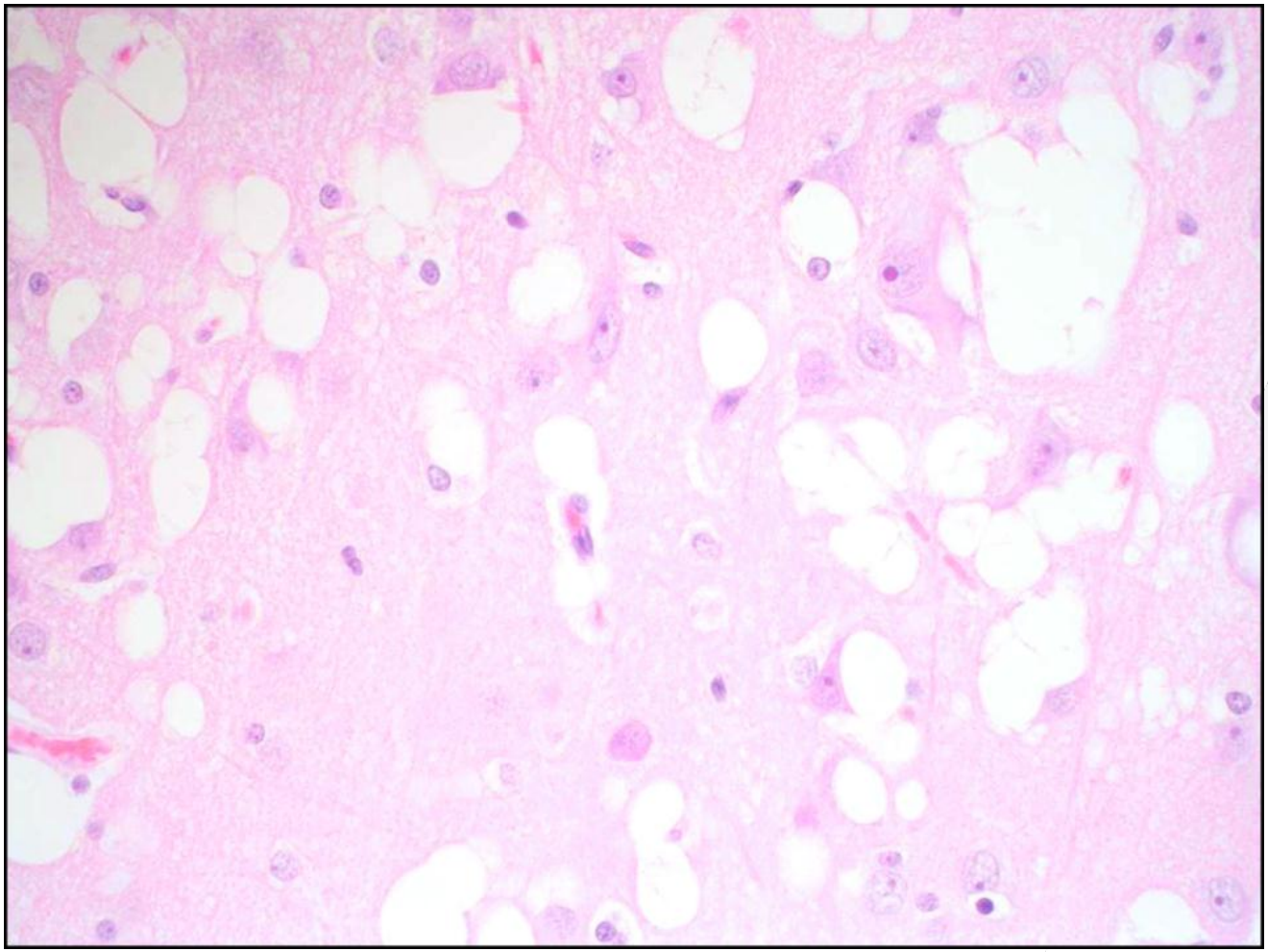
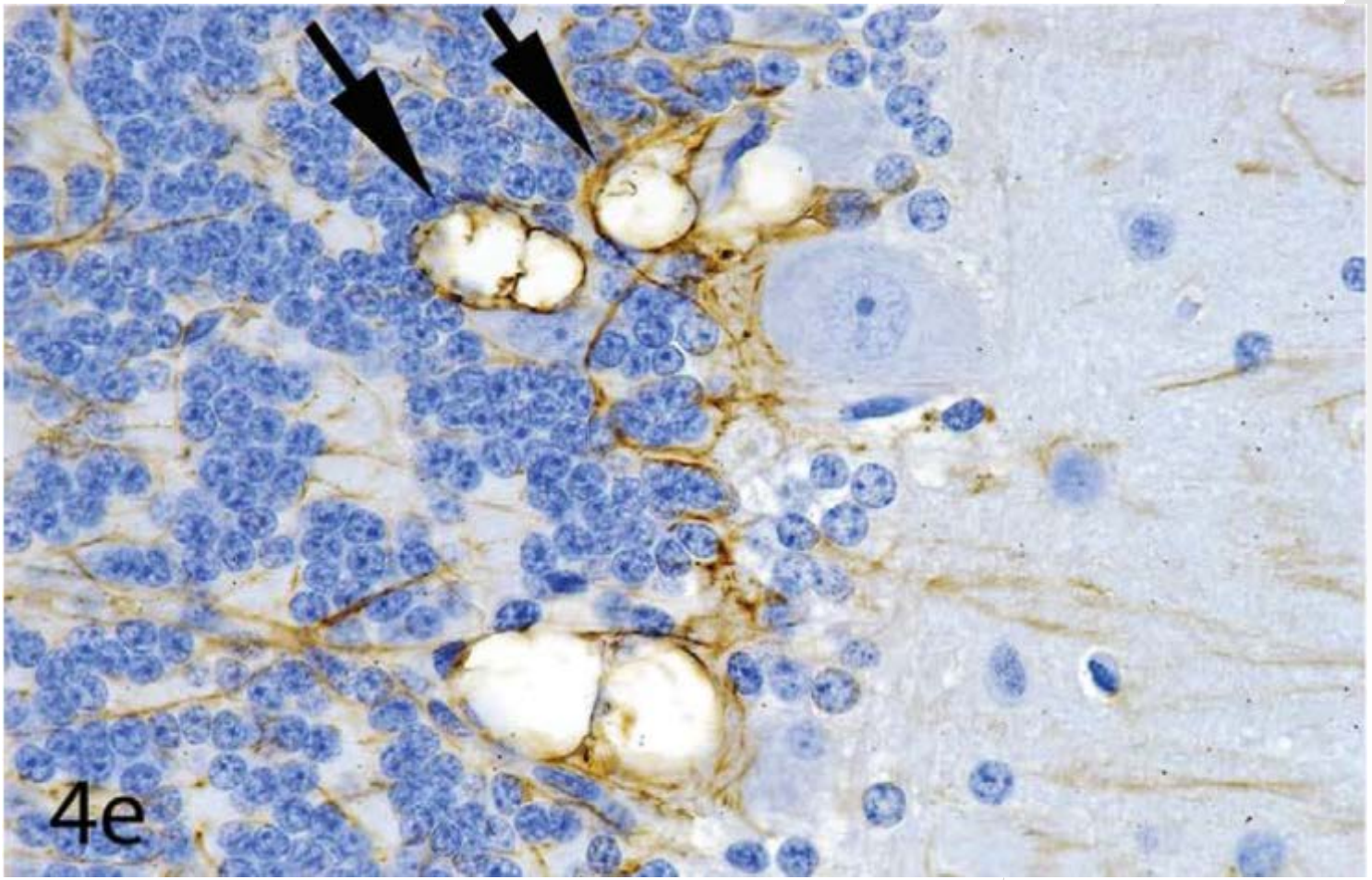


Image courtesy – G Krinke

Astrocytic Vacuoles

Macaque monkey cerebellar cortex, GFAP – Astrocytic vacuoles



Garman. Tox Path, 39: 22-35, 2011

Astrocytic Swelling & Vacuolation

- In HE stain
  - White matter is preserved
  - Neurons (perikarya) are not vacuolated
  - Neuropil in the gray matter is vacuolated = astrocytes
  - Astrocytic nuclei can be seen in some vacuoles
- GFAP stains only the margin of vacuoles, not their watery content

Primary Demyelination Intramyelinic Edema Hexachlorophene

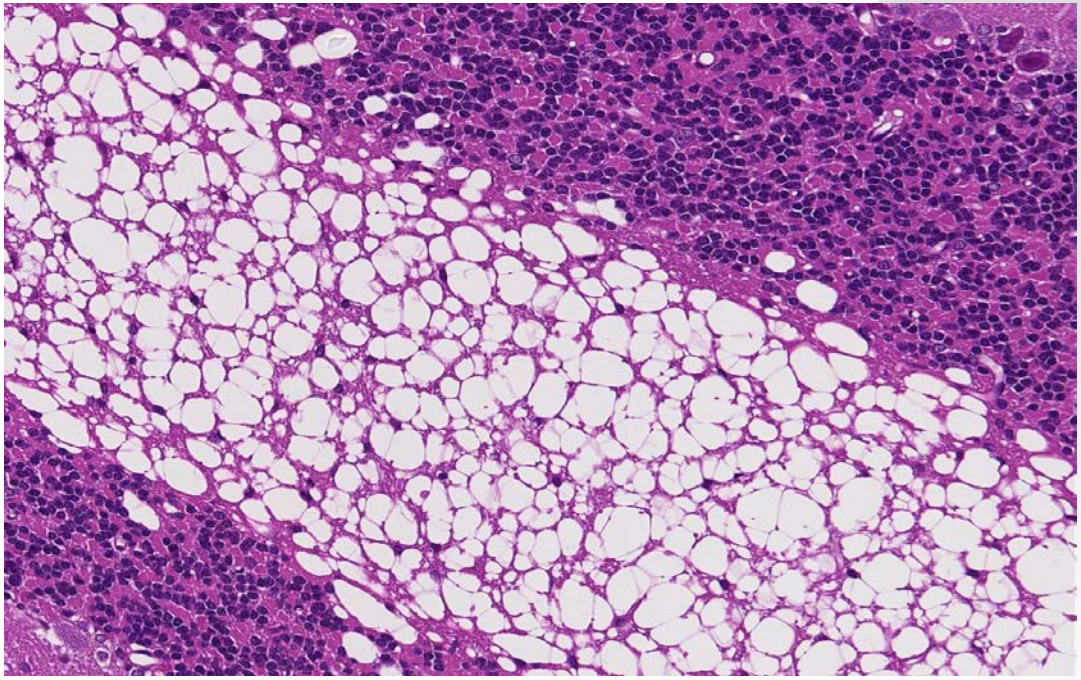


Image courtesy – G Krinke

- Mechanism
  - Direct damage to myelin
  - Similar findings can be seen with xenobiotics damaging oligodendrocytes e.g. cuprizone

Conclusion

- Showed different examples of neurotoxicities and their patterns
  - Covered neuronopathies and gliopathies
- Discussed the mechanism including the role of blood brain barrier in avoiding neurotoxicities
  - Clear in certain neurotoxicities
  - Not so much in others

Thank You! Questions?

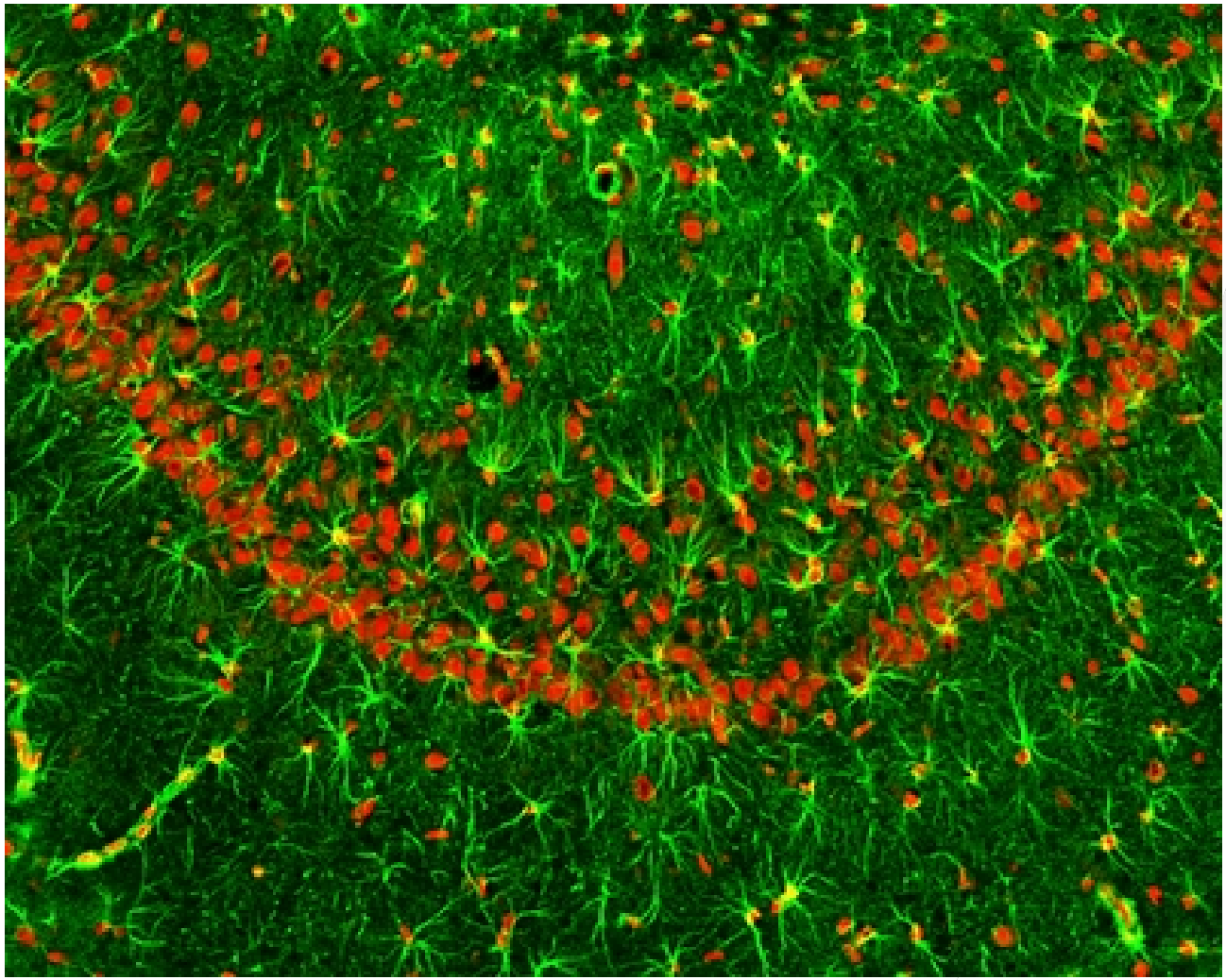
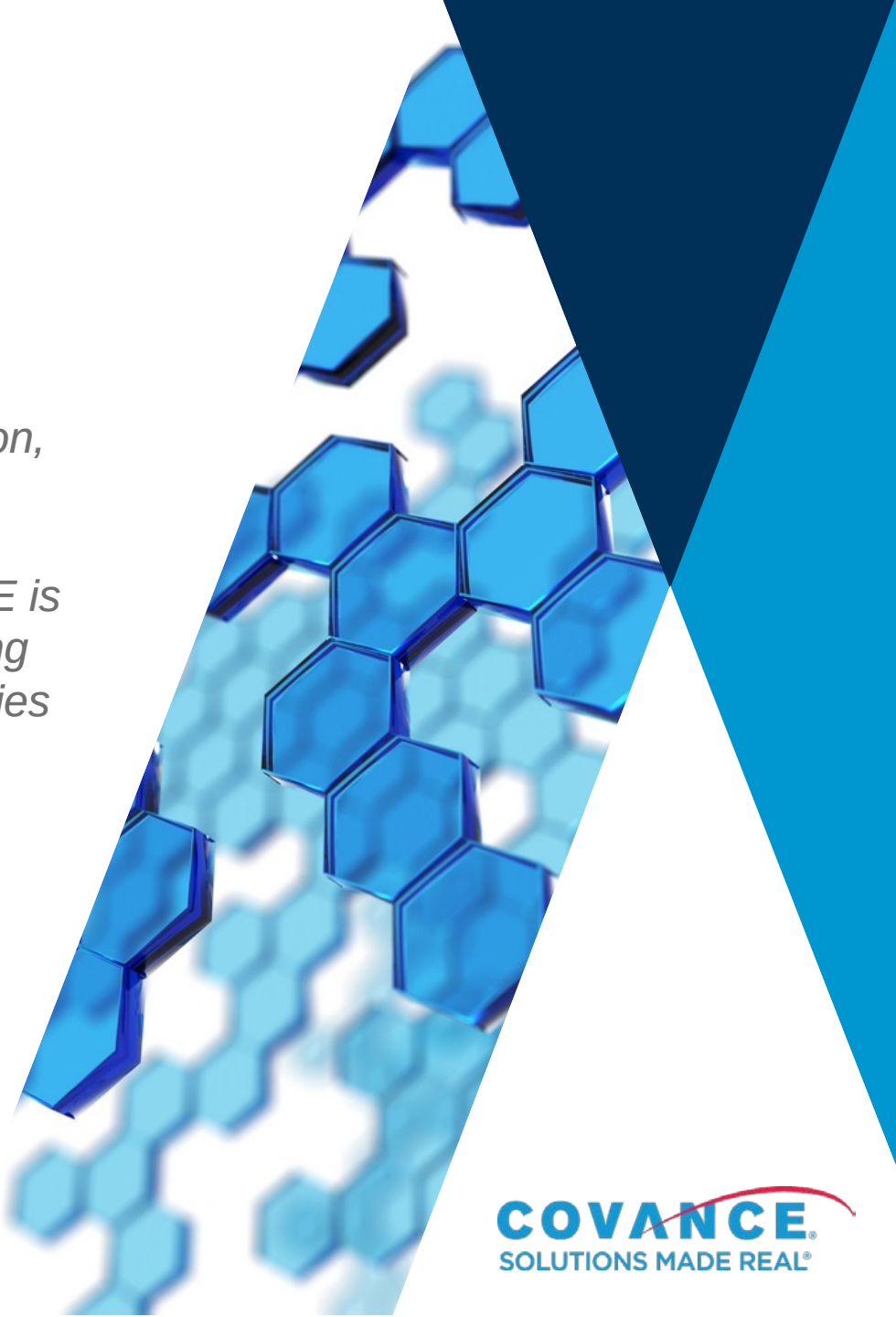


Image courtesy – Michael Davidson, Molecular Expressions

Covance Inc., headquartered in Princeton, NJ, USA, is the drug development business of Laboratory Corporation of America Holdings (LabCorp). COVANCE is a registered trademark and the marketing name for Covance Inc. and its subsidiaries around the world.



COVANCE
SOLUTIONS MADE REAL®