

Review of pathology reports on Vigabatrin (VGB) exposure in the rat

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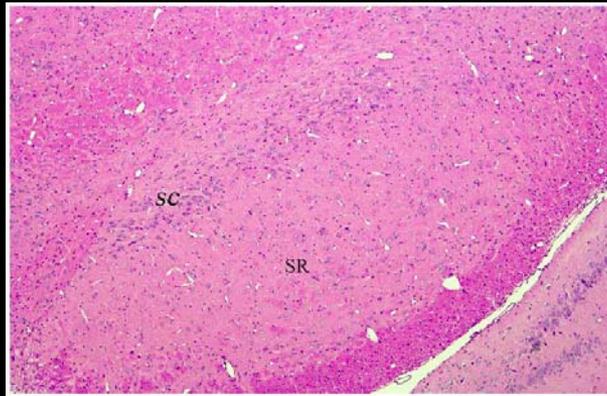
Findings of study #1007 and associated PWG report

- 1) This study reported the occurrence of vacuolization of the neuropil and gray matter within the brains of juvenile rats exposed to VGB from day 4 to 60 (PND)**
- 2) Intramyelinic edema (IME), as seen in the adult, was not reported**
- 3) Neuronal degeneration was not detected**

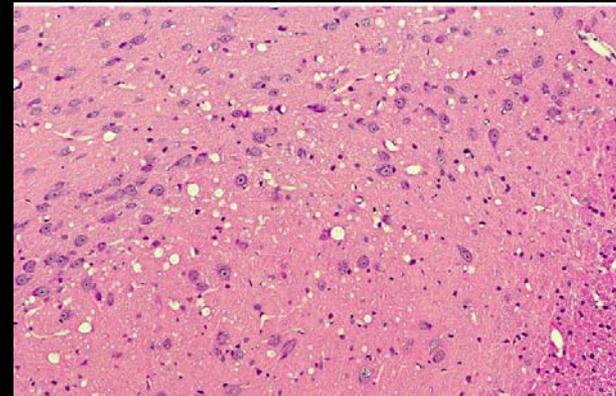
H & E photomicrographs from study # 1007

SN

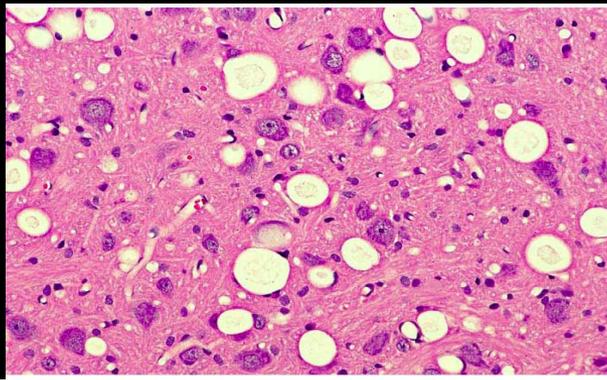
Control



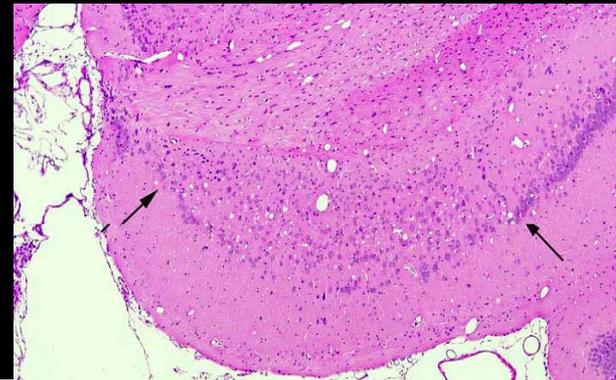
Treated



Treated



Treated



Deep CBN

HIP

Evaluation of Study # 1007

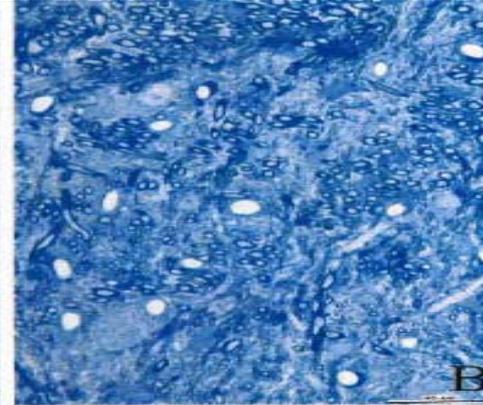
- 1) We found credible the specific loci and nature of the lesions described, within the limits of the experimental design used
- 2) The studies' inability to confirm neurodegeneration does not prove the absence of neuronal degeneration

Reported findings of follow up study, OVNC- 9004

- **1) Vacuolization was observed in the mid brain and brain stem**
- **2) Vacuolization was reported to be of an IME nature and confined in the white matter**

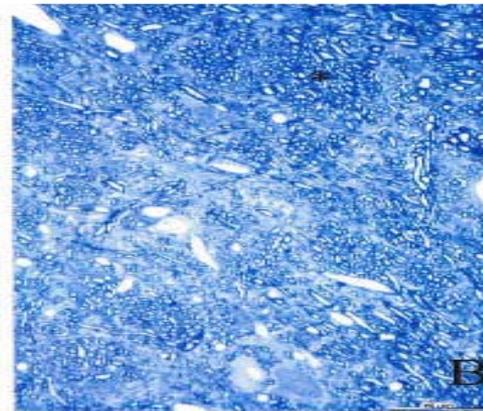
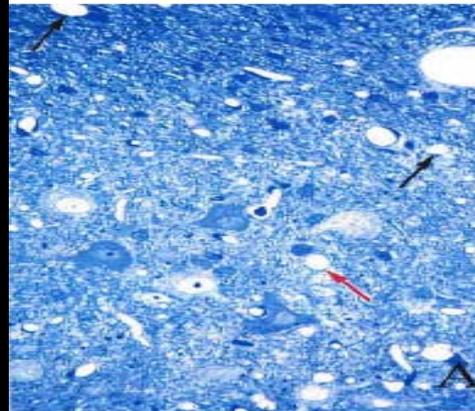
Photomicrographs of Toluidine blue stained sections (deep cerebellum) from study # OVNC- 9004

TREATED



CONTROL

TREATED



CONTROL

Evaluation of study # OVNC-9004

- 1) Areas examined typically involved grey matter structures (deep cerebellar nuclei), rather than myelinated tracts such as the adjacent cerebellar peduncles.
- 2) Many examples showed neurons in tissues of treated animals but only myelinated tracts in tissues of vehicle treated control animals.
- 3) Virtually, no forebrain areas were examined.
- 4) As in previous study, non optimal survival times and histological methods were employed.

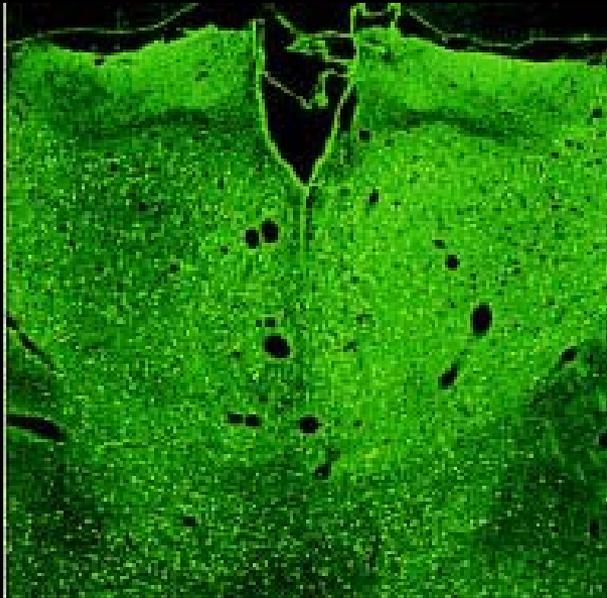
Appropriateness survival intervals used

Critical window of natural neuronal apoptosis:

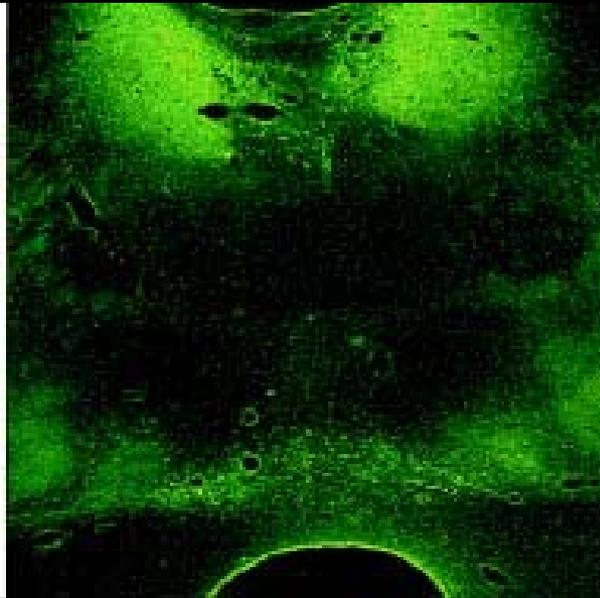
Species	Duration of neuronal apoptosis after birth
Rat	2 weeks
Monkey	1-3 months
Human	3 years

Photomicrographs of kainic acid induced neuronal degeneration at different time points as detected by Fluoro Jade dyes

2 DAYS



30 DAYS



THALAMUS

Appropriateness of histochemical techniques used

Stain

Specificity

H & E

Nuclei and cytoplasm of all cells

Toluidine Blue

Plasma membranes and nuclei

Fluoro Jade B,
Fluoro Jade C

Necrotic and apoptotic neurons

Suppressed silver

Necrotic and apoptotic neurons

Caspase 3 immunohistochemistry

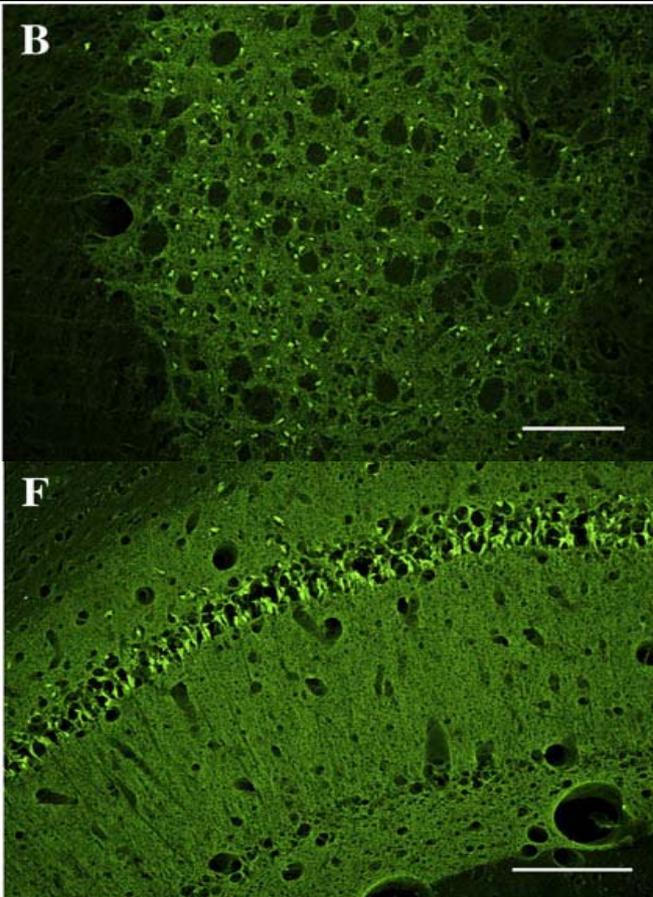
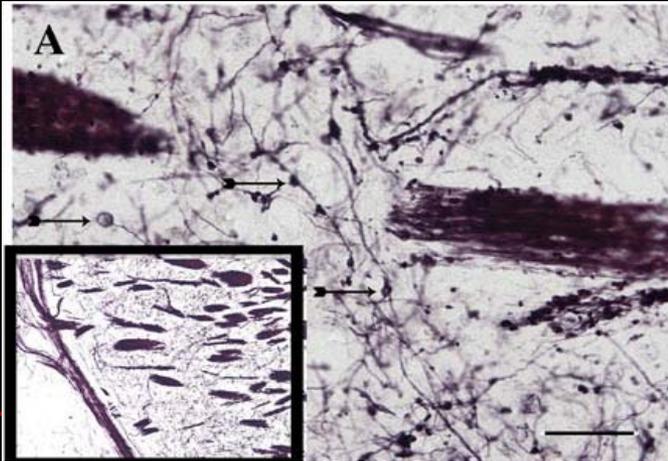
Apoptotic cells

Photo micrographic examples of specific stains for myelin pathology and neuronal degeneration

Black Gold-II

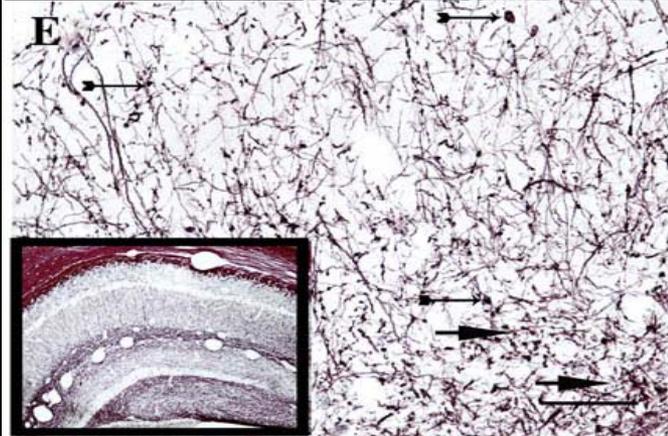
Fluoro-Jade C

ST



3-NPA

HIP



KA

Appropriateness of anatomical regions examined

- 1) The initial study (#1007) did a good job of examining a wide variety of anatomical regions including the forebrain**
- 2) It is especially important to evaluate the forebrain because it:**
 - A) Contains a high ratio of grey matter / white matter**
 - B) It employs the inhibitory neurotransmitter GABA**
 - C) It develops last**

Appropriateness of..... continued

3) In contrast, the follow up study (OVNC-9004) was limited to the brain stem, which is less appropriate because it:

A) Has a lower ratio of grey matter / white matter

B) It employs the inhibitory neurotransmitter glycine

C) It develops earliest

Other pediatric anesthetics and anticonvulsants that have resulted in neurodegeneration in juvenile animals

Drug

Mechanism of action*

- Midazolam GABA mimetic
- Ketamine NMDA receptor antagonist
- Valproic Acid GABA transaminase inhibitor

* Vigabatrin is a GABA transaminase inhibitor

SUMMARY

- Study OV-1007 and the associated PWG constituted a relatively credible study by identifying the grey matter lesions as unique and distinct from the reversible IME seen in the adults. Their inability to identify degenerating cells or the source of vacuoles may reflect suboptimal survival intervals.

Summary ...

- The follow up study, OVNC-9004 was less convincing in demonstrating lesions were of an IME nature.
- Also, the absence of any degeneration within the grey matter may have been compromised by a bias in the anatomical regions examined as well as less than optimal survival intervals and histochemical techniques

Suggested experimental design for Unequivocal resolution of VGB lesions in juvenile rats

- 1. Commence dosing of animals at 4, 7 and 14 days of age to mimic the premature, full term and 6-12 month old human infant respectively.**
- 2. Sacrifice animals at the following times after initial dose: 8 hrs, 1 day, 3 days, 10 days and 30 days.**
- 3. Histologically stain brain tissue sections using highly specific and sensitive markers. Neuronal degeneration can be detected by using Fluoro Jade dyes, caspase 3 immunohistochemistry and suppressed silver methods. Myelinopathies can be detected with Black-Gold II or myelin basic protein immunohistochemistry.**

Suggested experimental design.....

- 4. Count all examples of degenerating neurons and myelin lesions observed and compare this number with that seen in untreated animals of a comparable age.**
- 5. At the very least, all brain regions examined in the first study (#OV-1007) should be replicated, as opposed to examining only the brain stem and mid brain as was done in the second study (#OVNC-9004)**

Conclusions

- 1) In developing animals, VGB exposure can result in lesions of the neuropil and grey matter of the brain
- 2) This lesion is qualitatively different from the reversible IME seen in the adult
- 3) The possibility of irreversible neuronal degeneration or the degeneration of other cell types may be resolved by using shorter survival intervals and specialized histochemical stains.

Acknowledgement

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