

**Repeated exposure to low levels of
the chemical warfare agent VX
activates cell survival related gene in
mouse brain**

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WRAIR**

Report Documentation Page

*Form Approved
OMB No. 0704-0188*

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1. REPORT DATE 01 OCT 2005	2. REPORT TYPE N/A	3. DATES COVERED -	
4. TITLE AND SUBTITLE Repeated exposure to low levels of the chemical warfare agent VX activates cell survival related gene in mouse brain mouse brain		5a. CONTRACT NUMBER	
		5b. GRANT NUMBER	
		5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)		5d. PROJECT NUMBER	
		5e. TASK NUMBER	
		5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Division of Neuroscience Walter Reed Army Institute of Research		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)	
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited			
13. SUPPLEMENTARY NOTES See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003. , The original document contains color images.			
14. ABSTRACT			
15. SUBJECT TERMS			
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified	UU
			18. NUMBER OF PAGES 19
			19a. NAME OF RESPONSIBLE PERSON

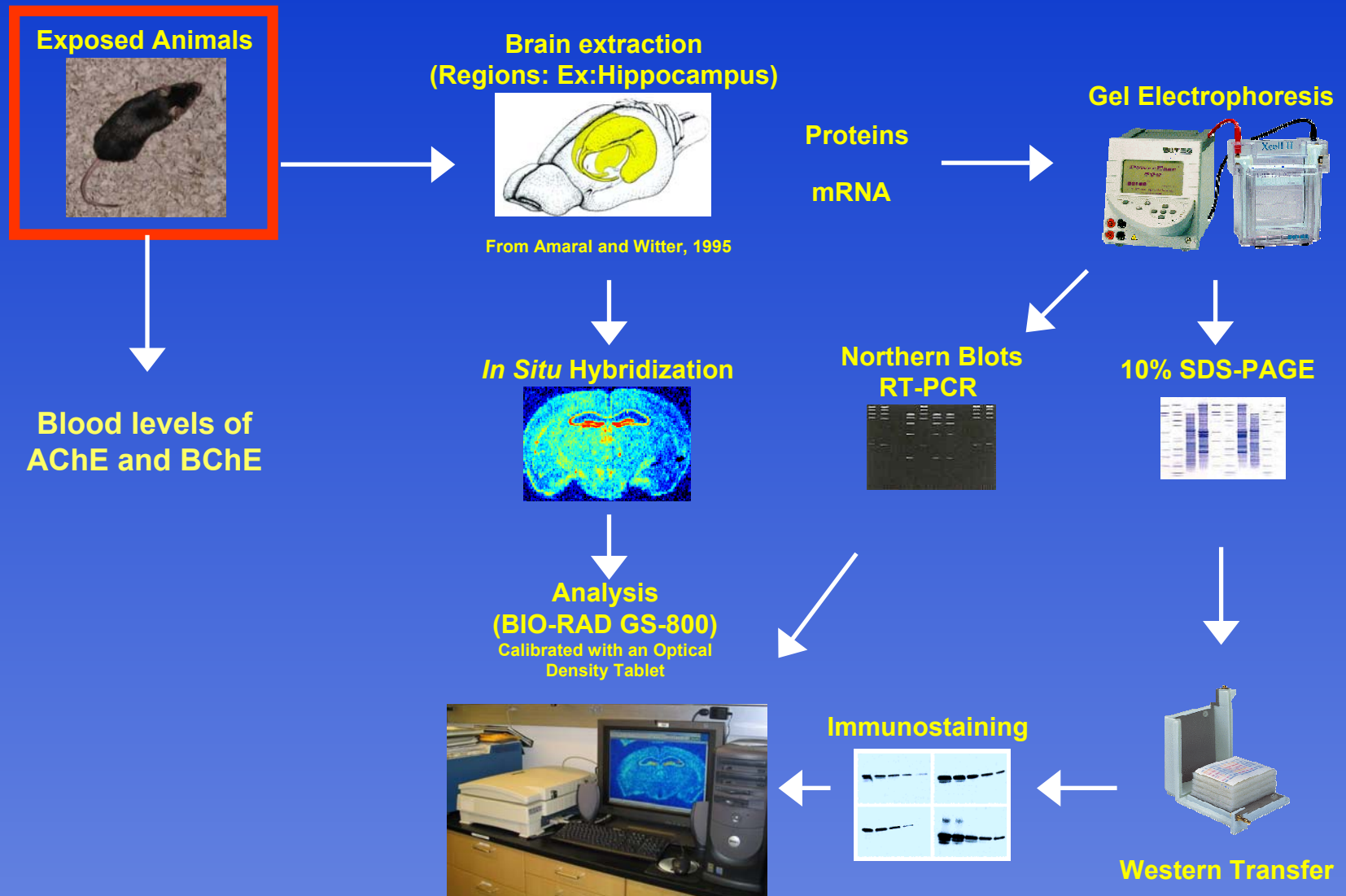
Introduction

- VX is an excitotoxic compound.
- Neurons may respond to toxicity by expressing genes associated with cell survival.
- Neurotrophins:
 - neuronal development
 - neuronal plasticity and remodeling
 - cell survival
- Brain derived neurotrophic factor (BDNF) is modulated:
 - traumatic brain injury
 - ischemia
 - toxin exposure
- BDNF may be important for neuronal plasticity, cell survival and remodeling following brain trauma.

Purpose

- Evaluate whether repeated exposure to low levels of VX affects genes associated with cell survival and neuronal plasticity.

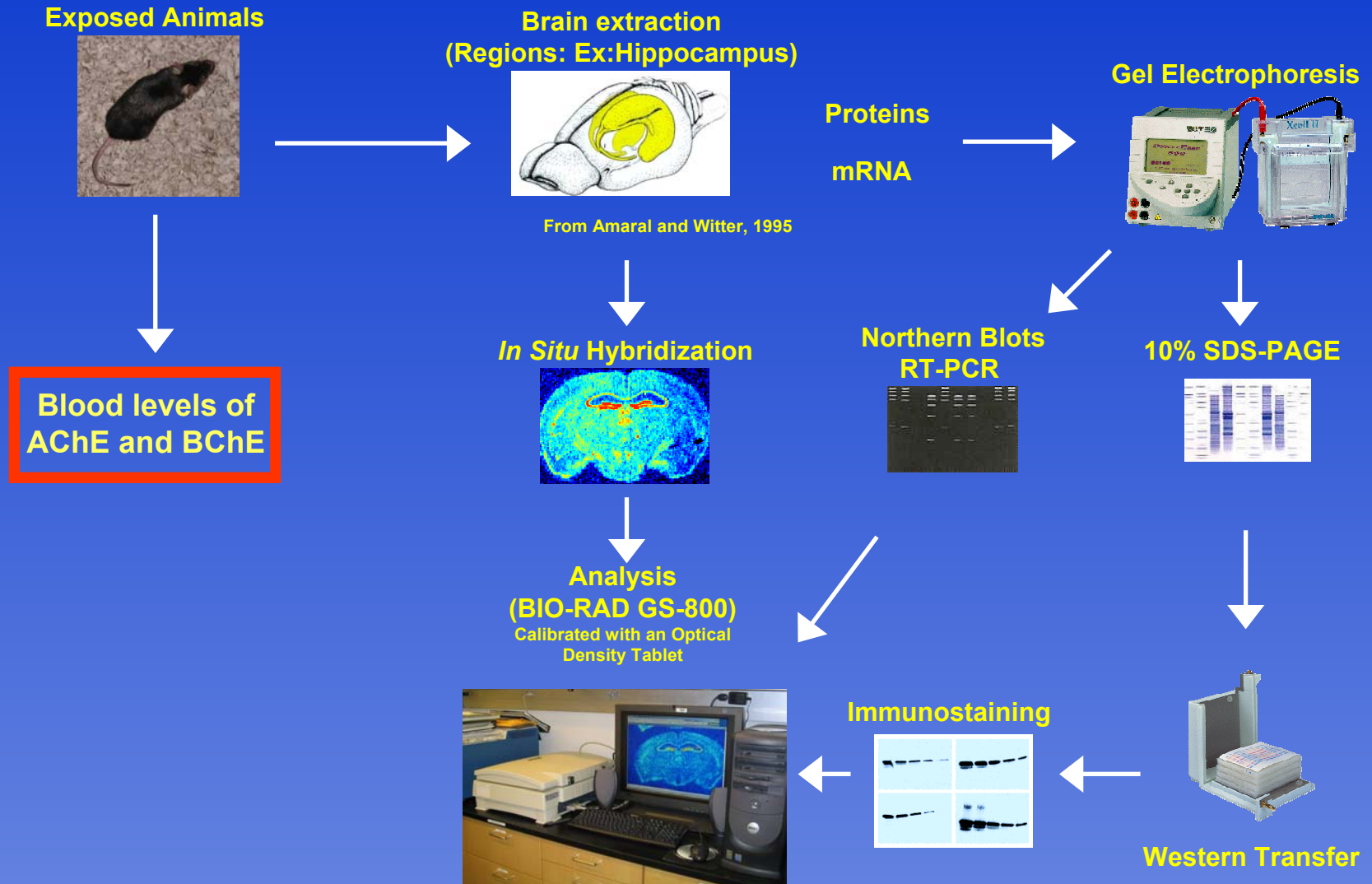
Experimental Design



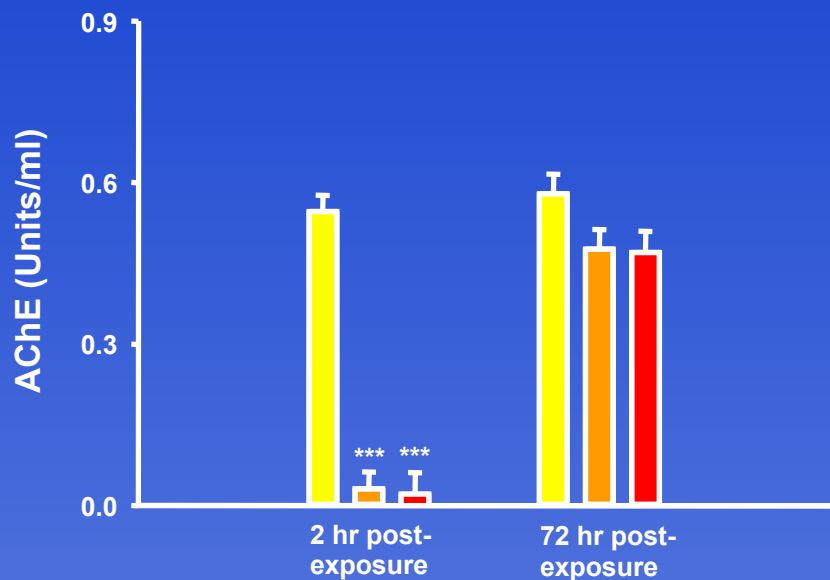
Animal Exposure

- Repeated low-level exposure
 - Three groups:
 - Saline controls
 - 0.2 LD₅₀
 - 0.4 LD₅₀
 - 1/day for 5 days/wk for 2 wk
- The LD₅₀ value for VX given subcutaneously (s.c.) in mice was 21 µg/kg (Boskovic, 1979).
- The nerve agent VX was dissolved in saline and injected s.c. in volume of 1 ml/kg.

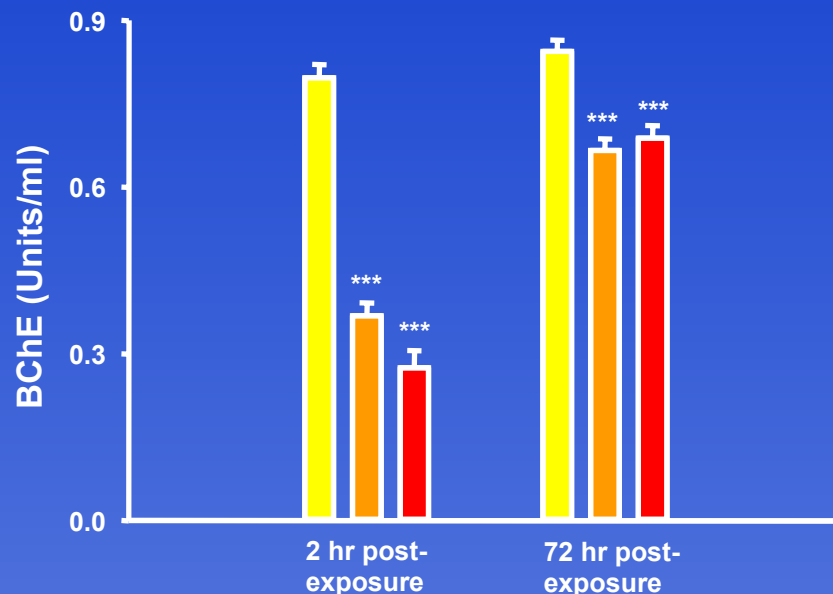
Experimental Design



Blood levels of acetylcholinesterase (AChE)

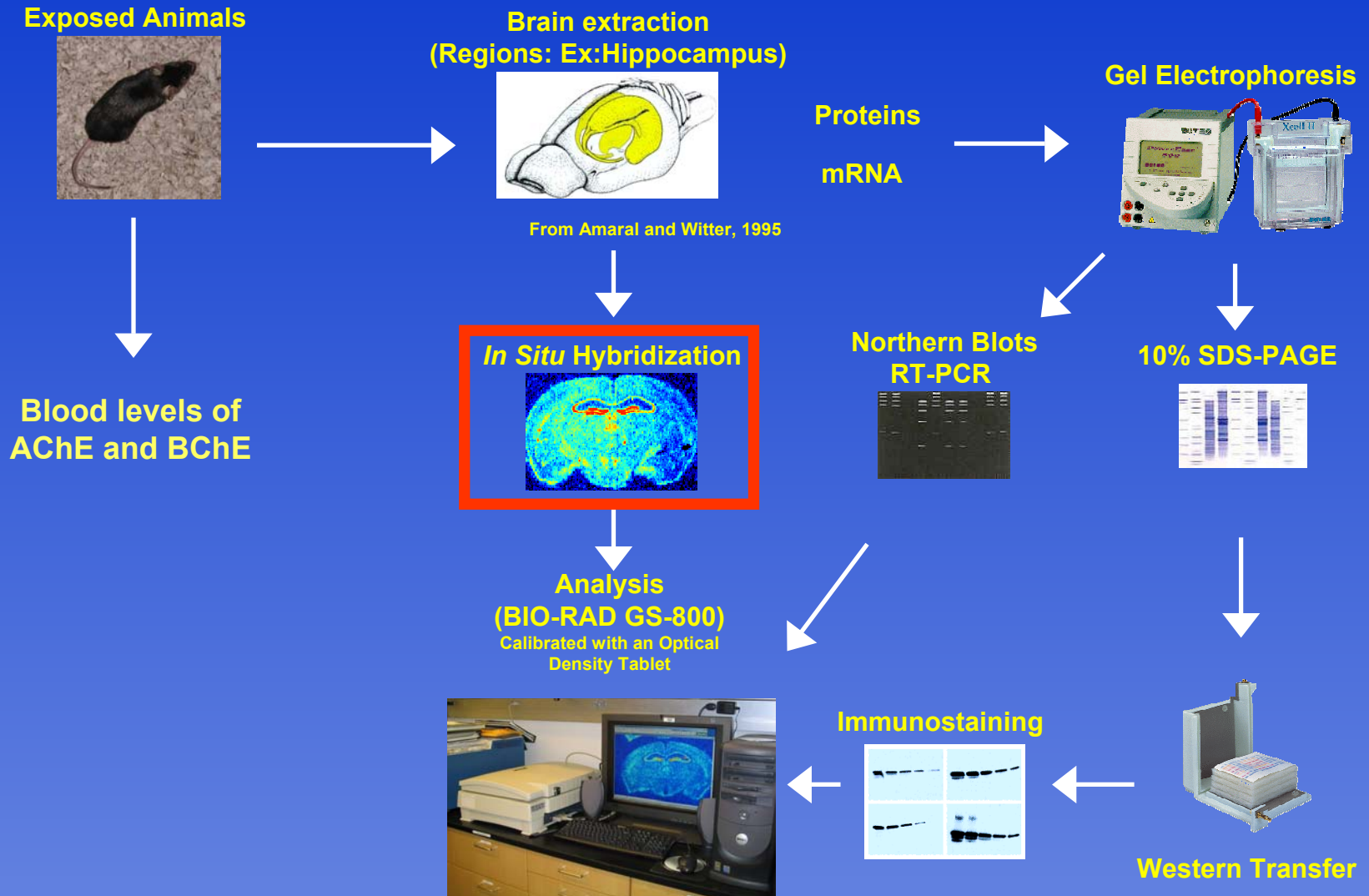


Blood levels of butyrylcholinesterase (BChE)



Saline (n = 5) 0.2 LD₅₀ VX (n = 5) 0.4 LD₅₀ VX (n = 6)

Experimental Design



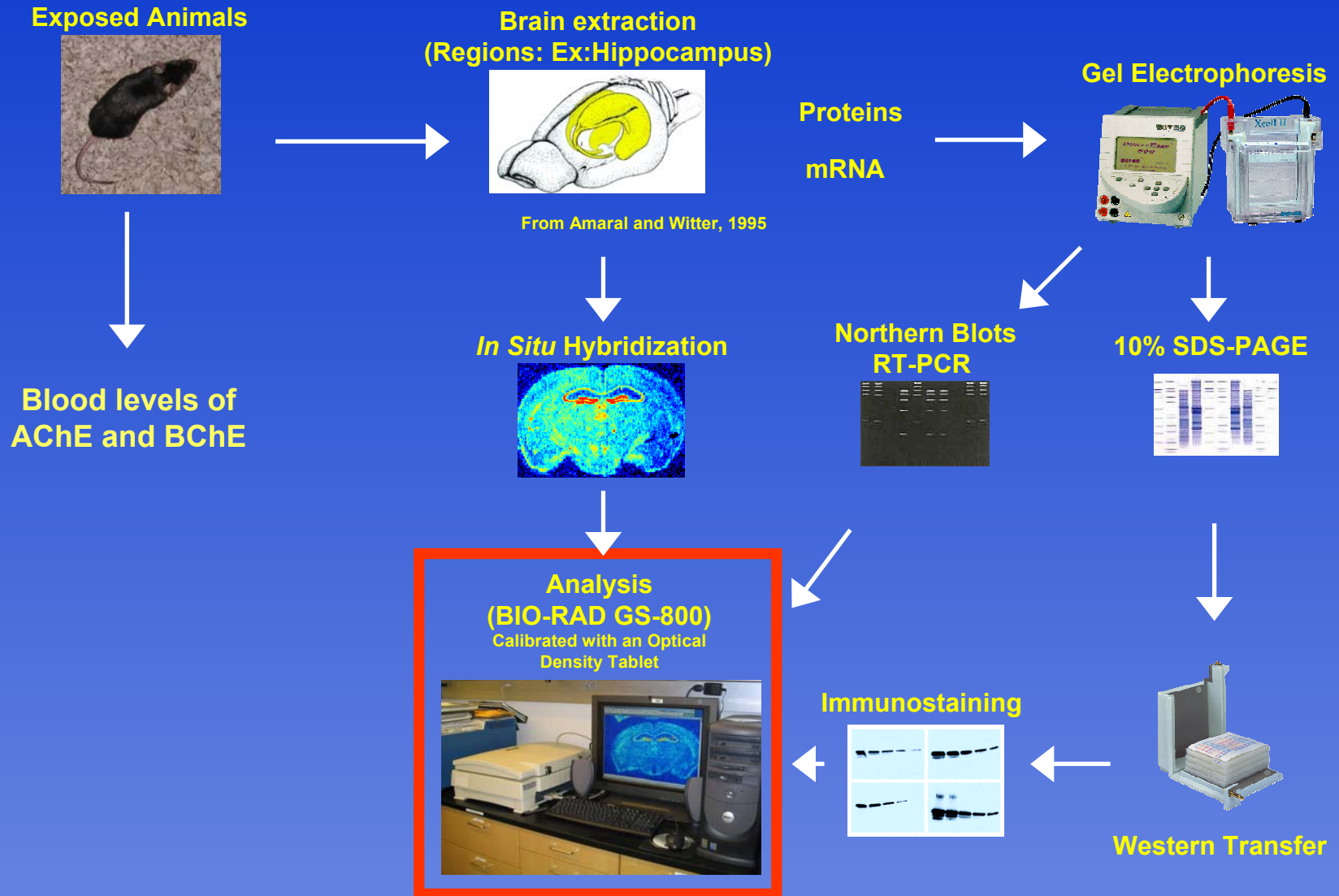
In situ Hybridization

- RNA probes for BDNF were generated by *in vitro* transcription using Strip-EZ™ RNA.
- For antisense ³³P-UTP labeled BDNF RNA probes, a plasmid containing a 460 bp BDNF
 - linearized with EcoRI
 - transcribed with T7 polymerase
- For sense ³³P-UTP labeled BDNF RNA probes
 - linearized with SalI
 - transcribed with SP6 polymerase

In situ Hybridization (cont.)

- Coronal sections (14 μm) were hybridized overnight with ^{33}P -UTP labeled cRNA probe.
- The sections were then exposed to a series of high temperature washes in various dilutions of SSC.
- The sections were then air-dried and autoradiographed on Kodak BioMax MS.
- The film was analyzed using the Model GS-800 Calibrated Imaging Densitometer and the Quantity One software.

Experimental Design



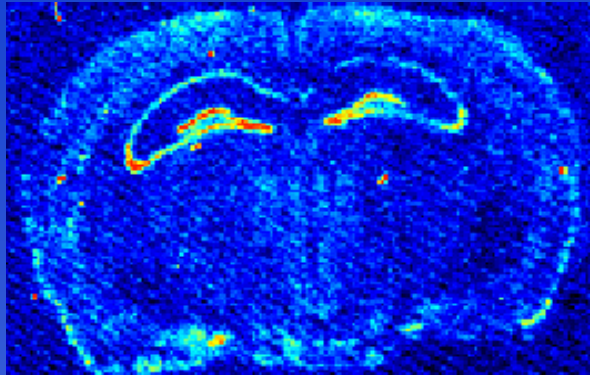
In situ hybridization cRNA controls

High



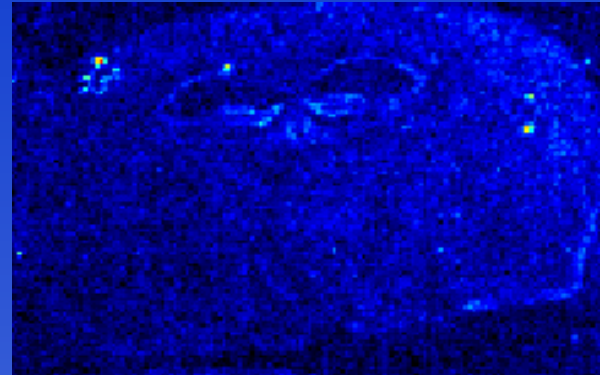
Low

A



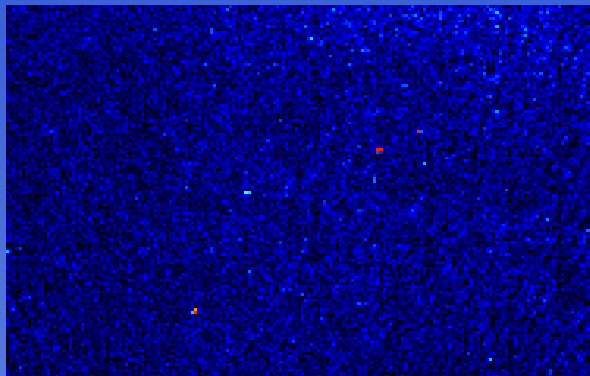
Control

B



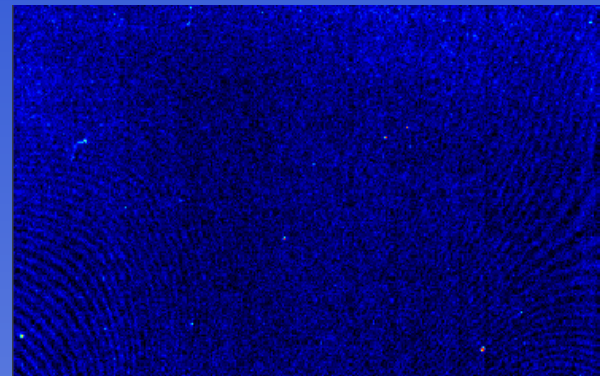
Sense

C



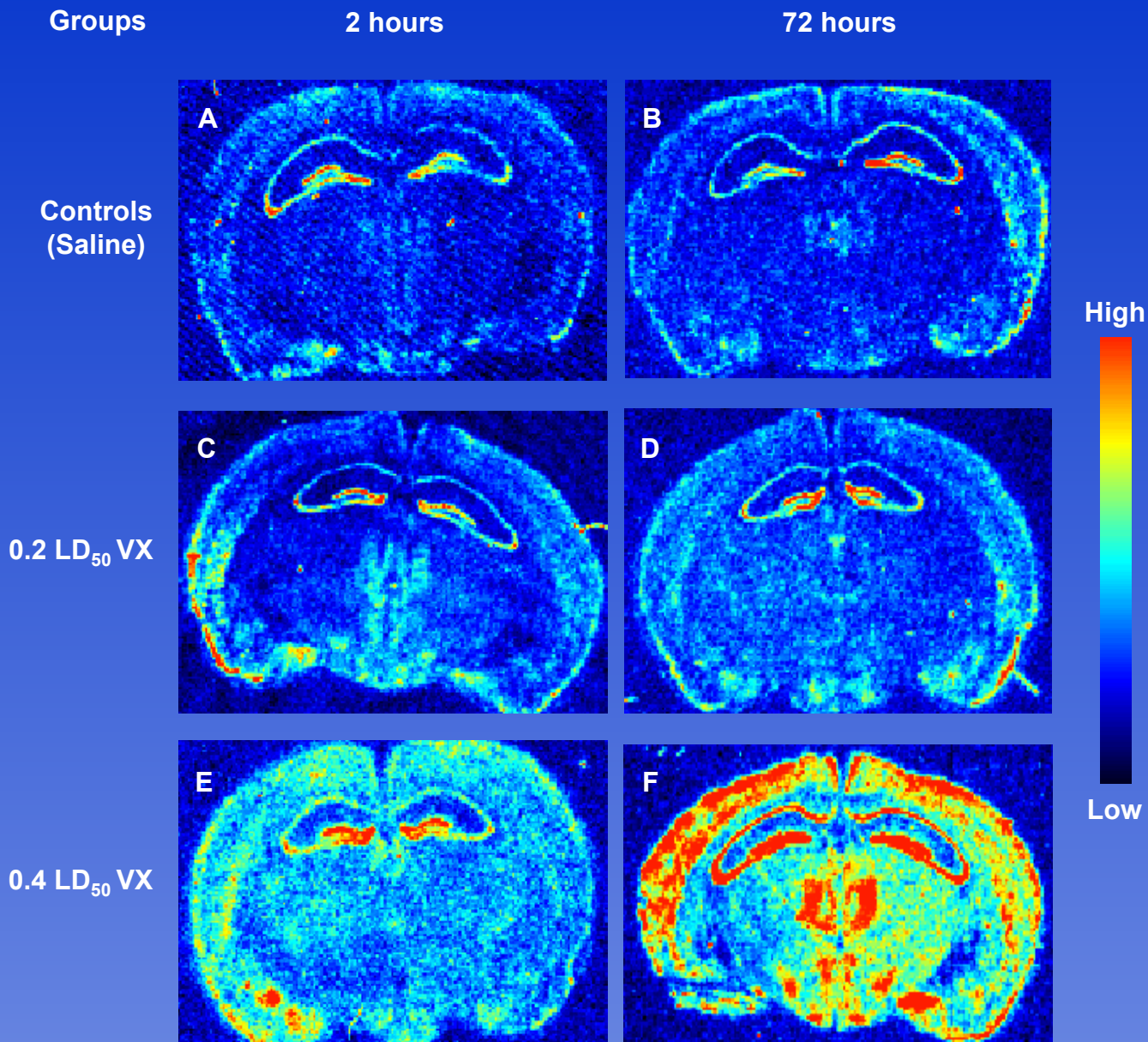
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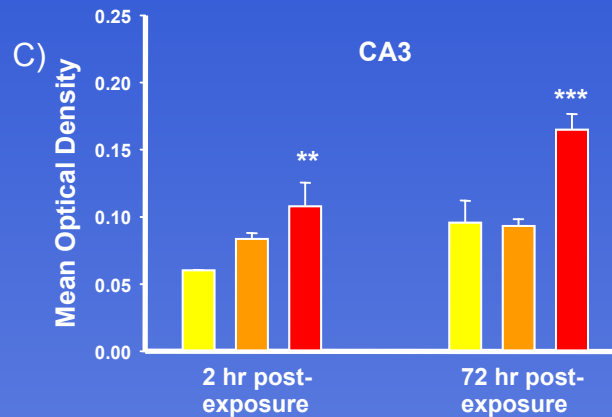
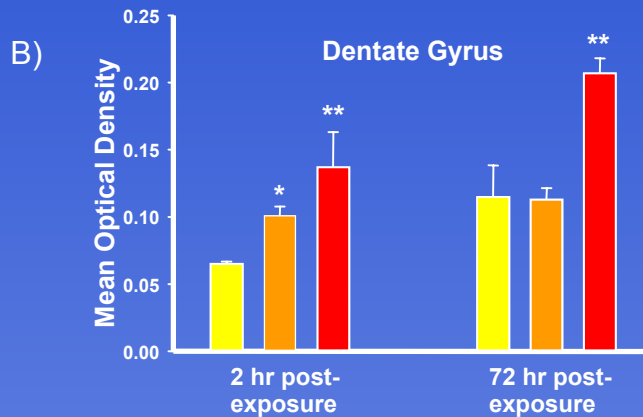
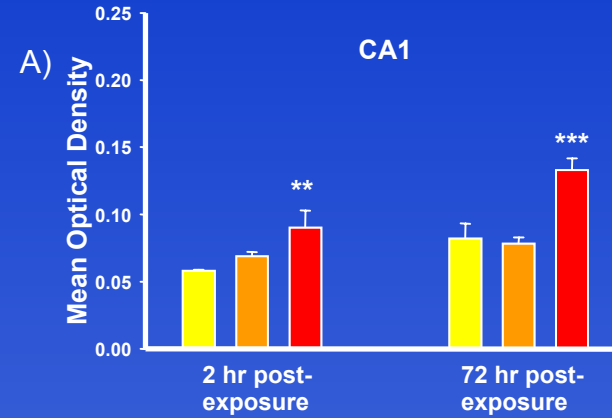
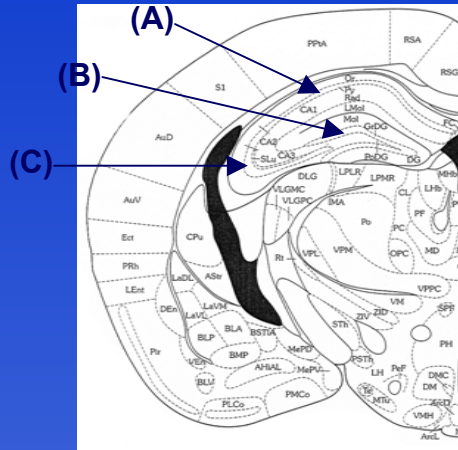


RNase

Spatio-Temporal Distribution of BDNF mRNA

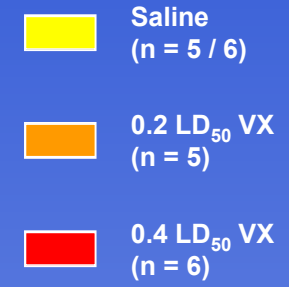
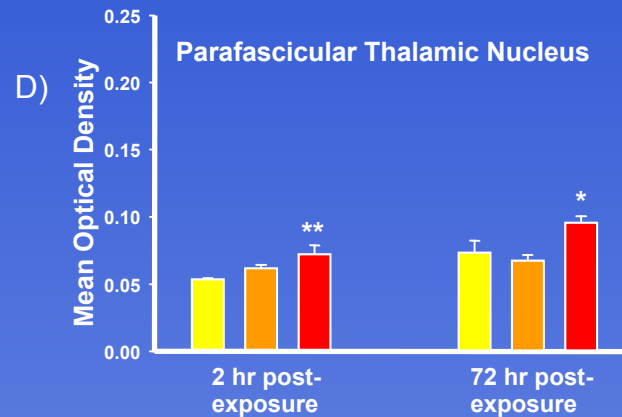
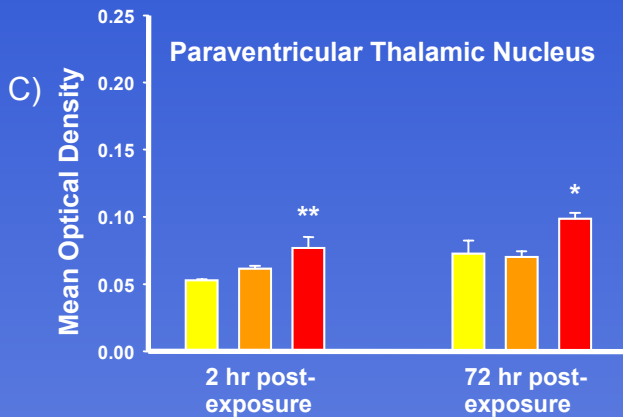
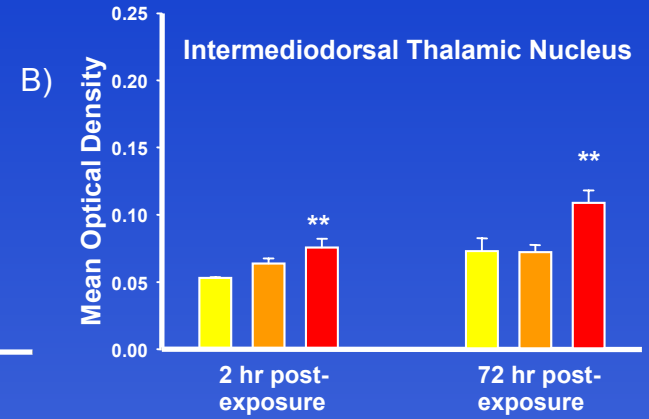
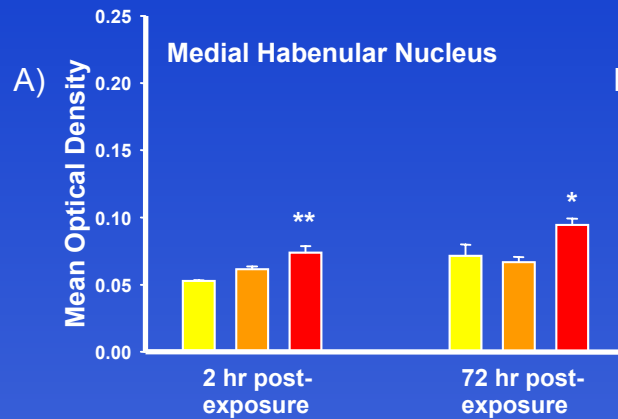
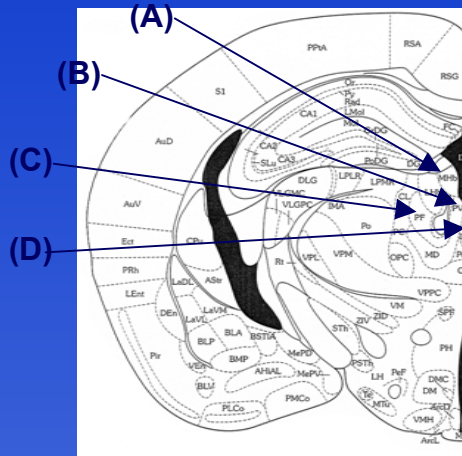


BDNF mRNA expression in the hippocampal region following repeated exposure to low levels of VX

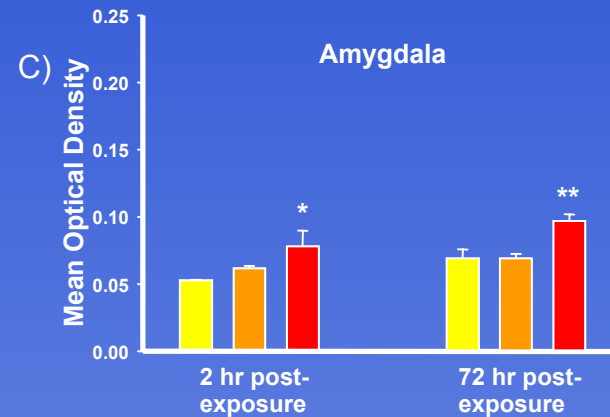
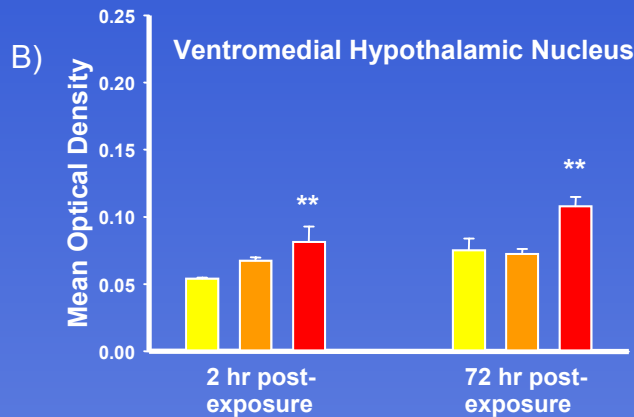
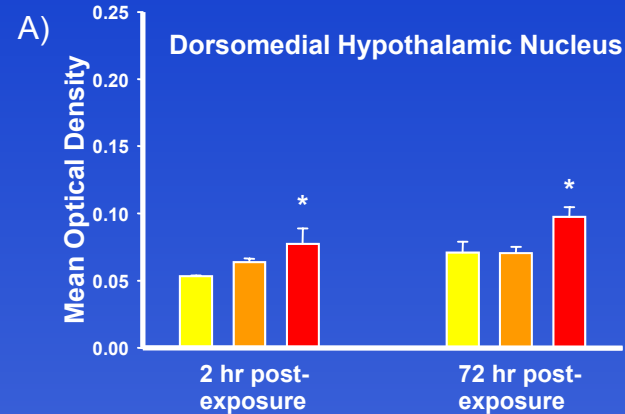
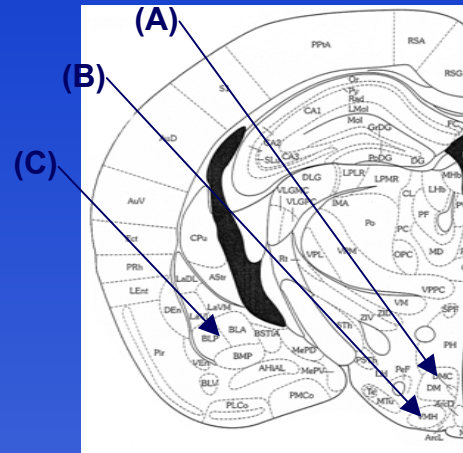


Saline (n = 5)
 0.2 LD₅₀ VX (n = 5)
 0.4 LD₅₀ VX (n = 6)

BDNF mRNA expression in the thalamic region following repeated exposure to low levels of VX



BDNF mRNA expression in the hypothalamic and amygdaloid regions following repeated exposure to low levels of VX



Saline (n = 5)
 0.2 LD₅₀ VX (n = 5)
 0.4 LD₅₀ VX (n = 6)

Summary

- We examined the temporal profile of BDNF mRNA expression at 2 hr and 72 hr after repeated injections of either saline, 0.2LD₅₀ VX, or 0.4LD₅₀ VX using *in situ* hybridization analysis.
- Animals that were injected with 0.4 LD₅₀ VX showed elevated levels of BDNF mRNA expression 2 hr and 72 hr later, in areas of the hippocampus, lateral amygdala, cortex, hypothalamus and thalamus.
- In some brain regions, 0.2 LD₅₀ VX also increased BDNF expression at 2 hr, but not at 72 hr, post-exposure.

Acknowledgment

- WRAIR

- Lumley LA
- Saviolakis GA
- Bah MJ
- Alagappan A
- Chang WE
- Shah JD
- Robison CL
- Meyerhoff JL
- Dawood MY
- Karp A

- USAMRICD

- CPT Midboe
- Cerasoli D
- Clark C
- SPC D'Ambrozio

This research was funded by
Task Area N (Low Dose CWNA)