Brain Adaptation and Hormesis

Dose-Response 2010

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A 24 year old soldier was transferred from Germany to Walter Reed Army Medical Center. The patient sustained a spinal cord injury after an IED exploded under his vehicle in Iraq. According to the patient, his buddies removed him from the vehicle because he was unable to move his lower and upper extremities. The patient was able to move all extremities by the time he reached Walter Reed but we were asked to determine residual spinal cord deficits. We were introduced to his family and they elected to stay in his room during the examination. Upon entering the room, the patient began to flirt with me. His wife, brother, mother and father were by his bed and remained stoic. In addition to the patient's childish mannerisms, he showed impairment of executive functioning. While I continued my examination to determine the extent of his deficits, the patient continued to flirt with me despite the presence of his wife and family. The patient lacked insight, goal-directed behavior, planning and was clearly disinhibited. The patient exhibited some signs of "organic driveness" as he exhibited brief but intense involvement in a meaningless activity. There was no evidence of astereognosia, extinction, neglect, constructional apraxia, finger agnosia, left-right confusion, acalculia or agraphia. The patient did have short-term memory problems. Motor examination showed no weakness but there were subtle long-tract signs (hyper-reflexia and bilateral upgoing toes). No other neurolgic abnormalities were found. Blood work, chest x-ray and EEG were normal. Brain MRI showed abnormal signal intensities in the cortex and deep white matter of the prefrontal cortex. After finishing my examination and leaving the room, the patient's brother came up to me and apologized profusely for his brother's behavior. He told me his brother was not like this prior to his injury.

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Question

- The question is what are the structural, cellular and molecular changes that underlie the clinical expression of this patient's brain injuries?
- While networks are clearly involved as every brain region is interconnected, we don't know precisely how physical forces (pressure waves etc from the blast) alter neuronal/glial circuitry, intrinsic cellular physiology and how the altered changes affect function in all types of cells in the brain.
- **Can we prevent or attenuate the alterations that occur from such injuries?**

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Intrinsic Neuronal Survival Pathways

- Low level activation of N-methyl-D-aspartate (NMDA) receptors protects vulnerable neurons against glutamate-mediated excitotoxicity via overactivation of NMDA receptors through the release and synthesis of brain-derived neurotrophic factor (BDNF) via an autocrine loop.
- This autocrine loop is an intrinsic neuronal survival pathway that exists in neurons.

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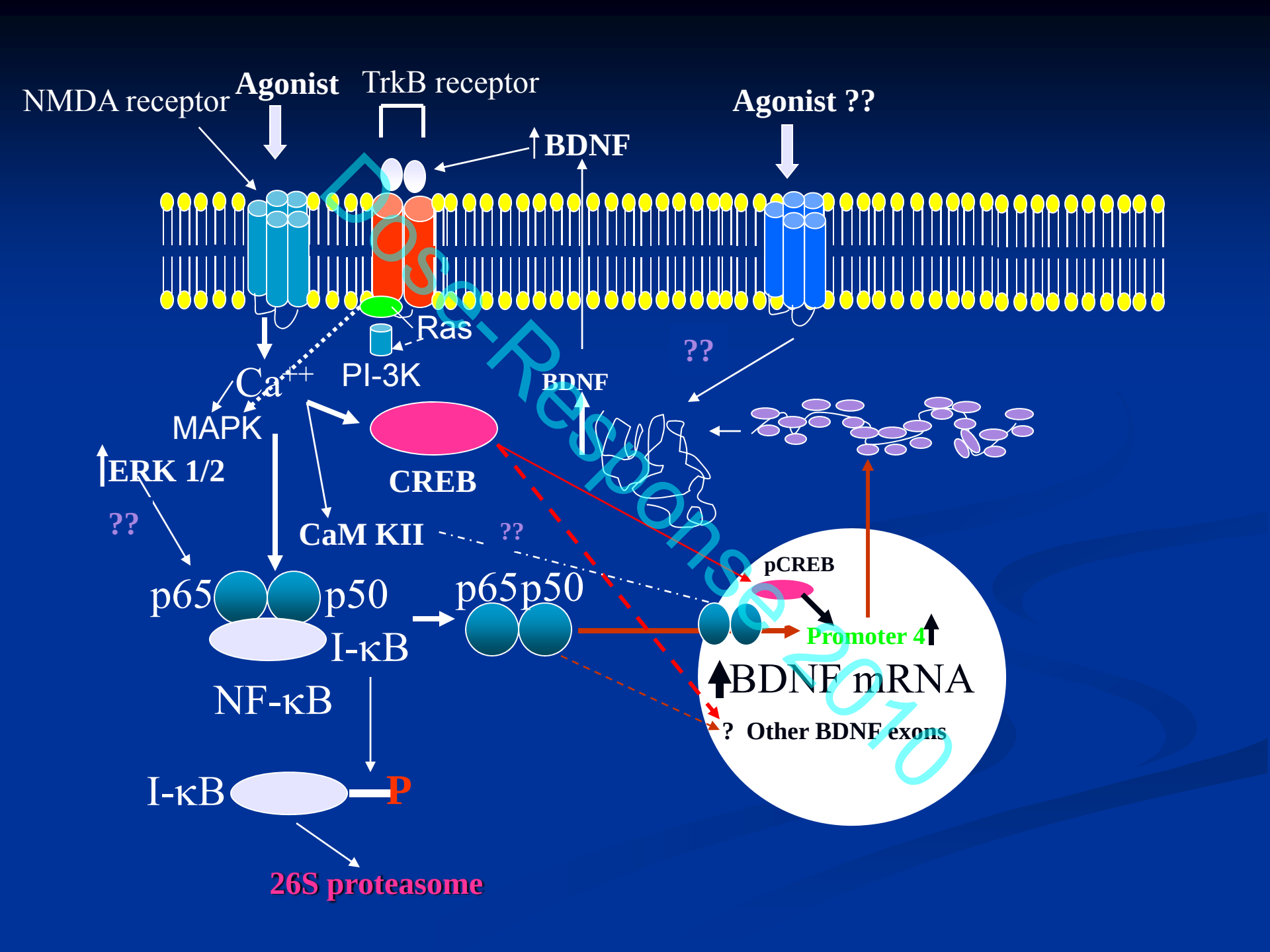
Intrinsic Neuronal Survival Pathways

- BDNF is a member of the neurotrophin family that plays a crucial role in neuronal survival, maintenance, neurogenesis and learning and memory.
- Delineating mechanisms that regulate BDNF expression may lead to new targets as pretreatments and therapies against traumatic brain injury and other neurodegenerative disorders.

Dose-Response 2013


NMDA receptor
Agonist
TrkB receptor
BDNF
Agonist ??
Ras
PI-3K
Ca++
MAPK
ERK 1/2
??
CREB
CaM KII
??
BDNF
??
p65
p50
I-κB
NF-κB
p65p50
pCREB
Promoter 4
BDNF mRNA
? Other BDNF exons
I-κB
P
26S proteasome
Cell-Response 2010

Central Hypothesis

- NMDA receptors regulate BDNF expression.

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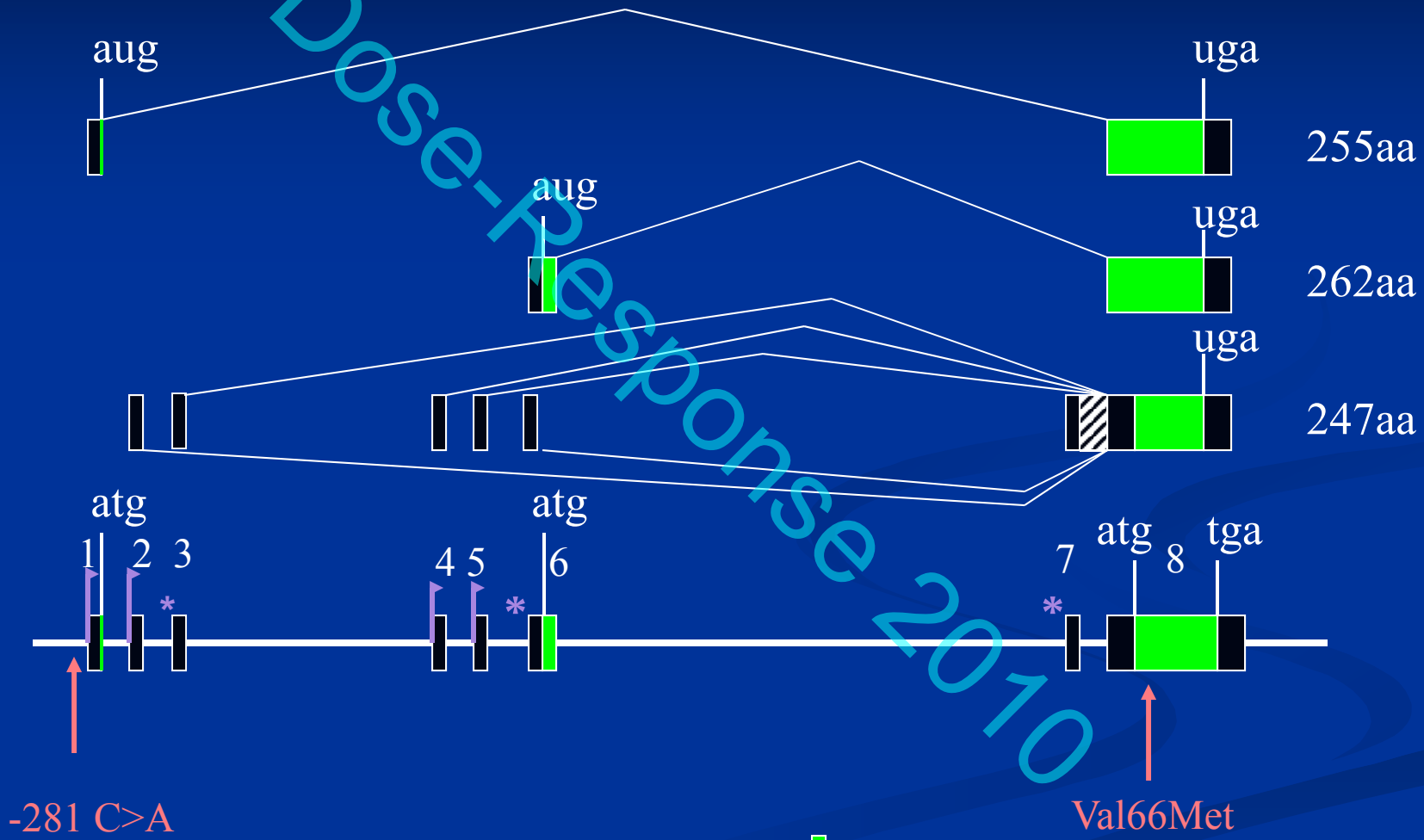
Functional *BDNF* Polymorphisms and Differential Promoter Usage Leading to Isoforms



Intrinsic survival pathways in neurons

- NMDA receptor-mediated neuroprotection against glutamate excitotoxicity requires exon 4-specific BDNF transcripts and activation of nuclear factor kappaB (NF-κB).
- Promoter IV is the major promoter that drives activity-dependent BDNF expression.

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Rat *BDNF* promoter 4

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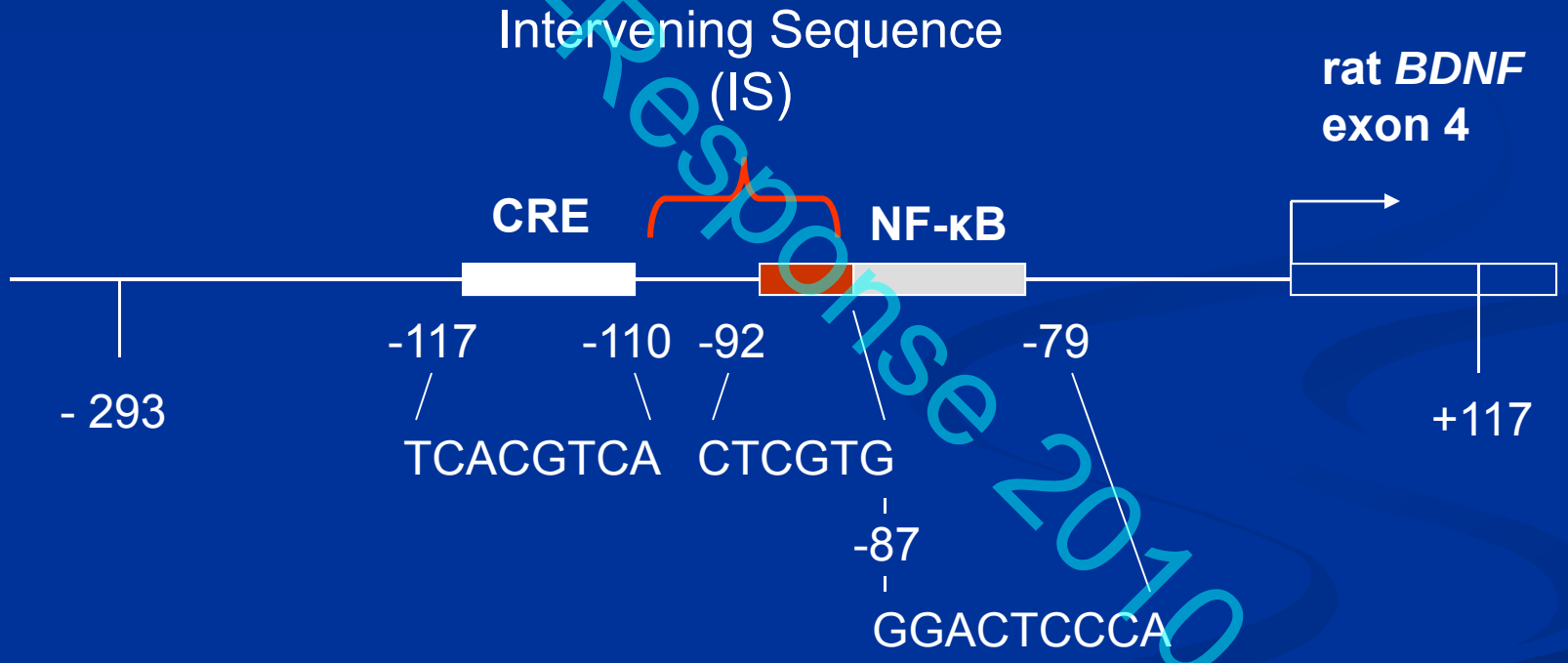

Promoter IV is the major promoter that drives activity-dependent BDNF gene transcription.

BHLHB2

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- Rat enhancer of split-and hairy-related protein-2 is a member of a subfamily of bHLH proteins (Sharp-1 and Sharp-2).

- Mouse (Stra13) and human (DEC1) orthologs have been isolated that function as transcriptional repressors.

- BHLHB2 may be involved in the control of proliferation and differentiation.

- BHLHB2 is expressed in the developing rat brain (postnatal day 5) during a time when neurons are undergoing synaptogenesis and in adult brain (hippocampal CA1 field, the subiculum, cortex, and cingulate gyrus).

- BHLHB2 expression is induced by kainic acid *in vivo* and by in NGF-treated PC12 cells.

Proximal E-box Sequence in *Bdnf* Promoter 4 Functions as a Transcriptional Suppressor

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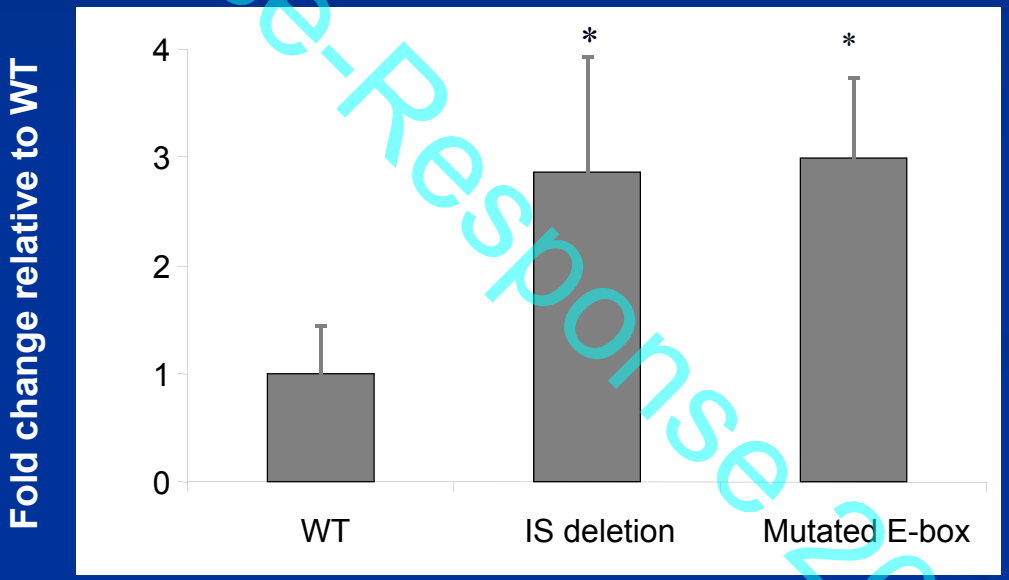


$*p < 0.05$

- Consensus E-box sequence
- Wild Type intervening sequence
- Mutated E-Box

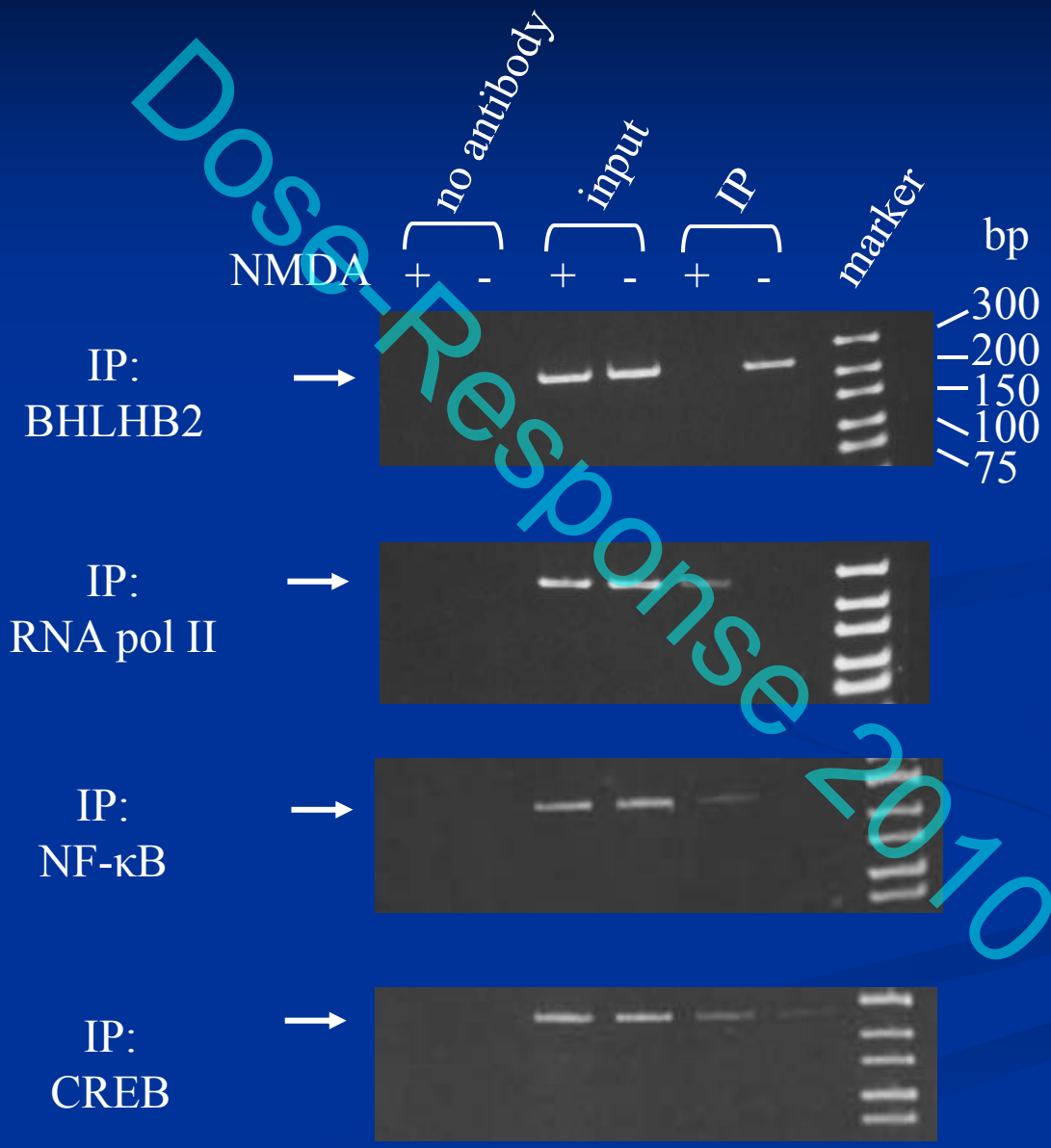
CACGTG

-106 CAGCGTGGAGCCCTCtCGTGGA -85

CAGCGTGGAGCCCTCTacTGGA

Jiang et al., *J. Neurosci.*, 2008

BHLHB2 is displaced from *BDNF* promoter 4 following NMDA receptor activation



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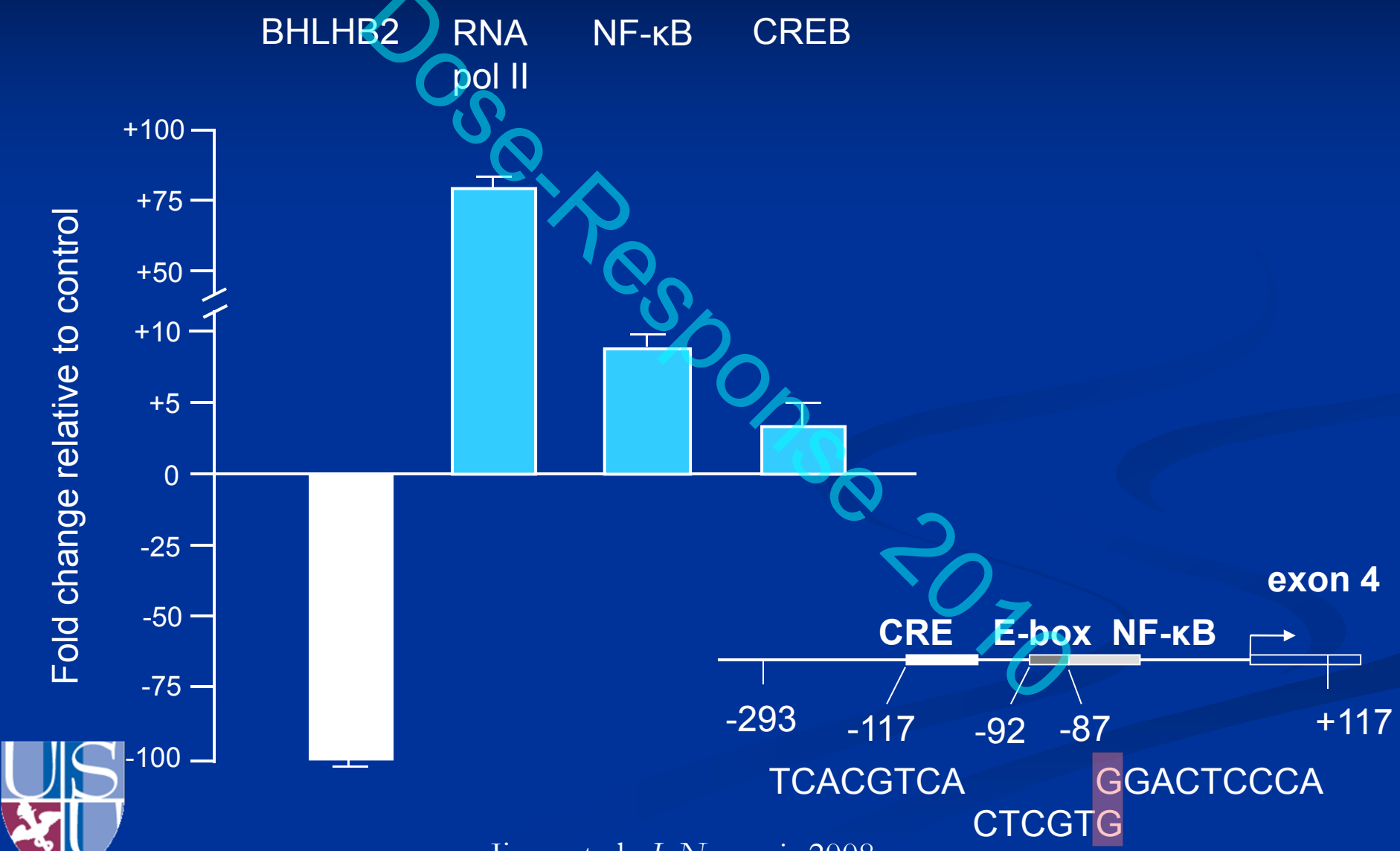
Jiang et al., *J. Neurosci.*, 2008

Quantitative ChIP Assay Showing *Bdnf* Promoter 4 Occupancy by Transcription Factors Following NMDA Receptor Activation



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Jiang et al., *J. Neurosci.*, 2008

BHLHB2 regulates kainate-induced *BDNF* promoter 4 transcription *in vivo*

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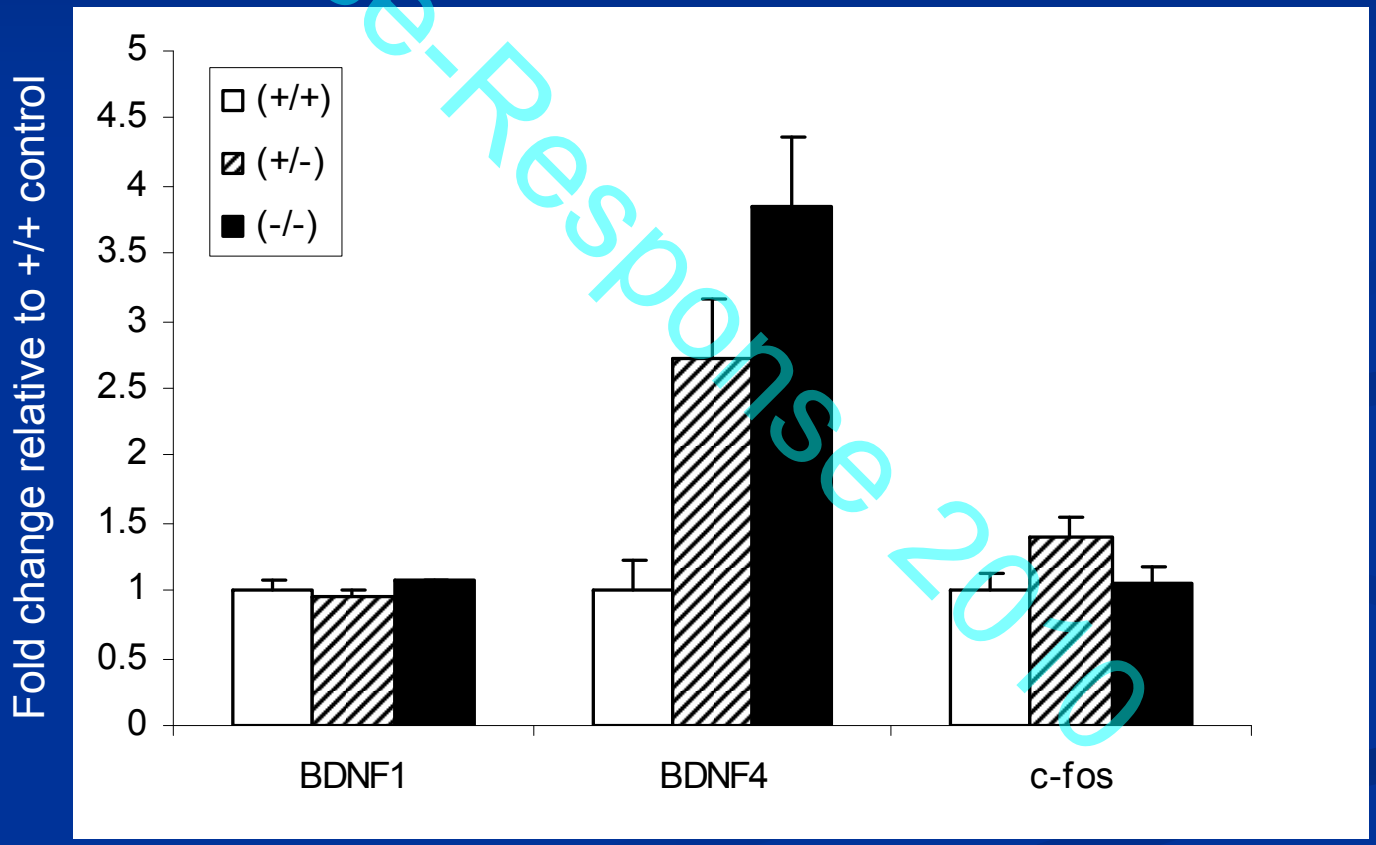
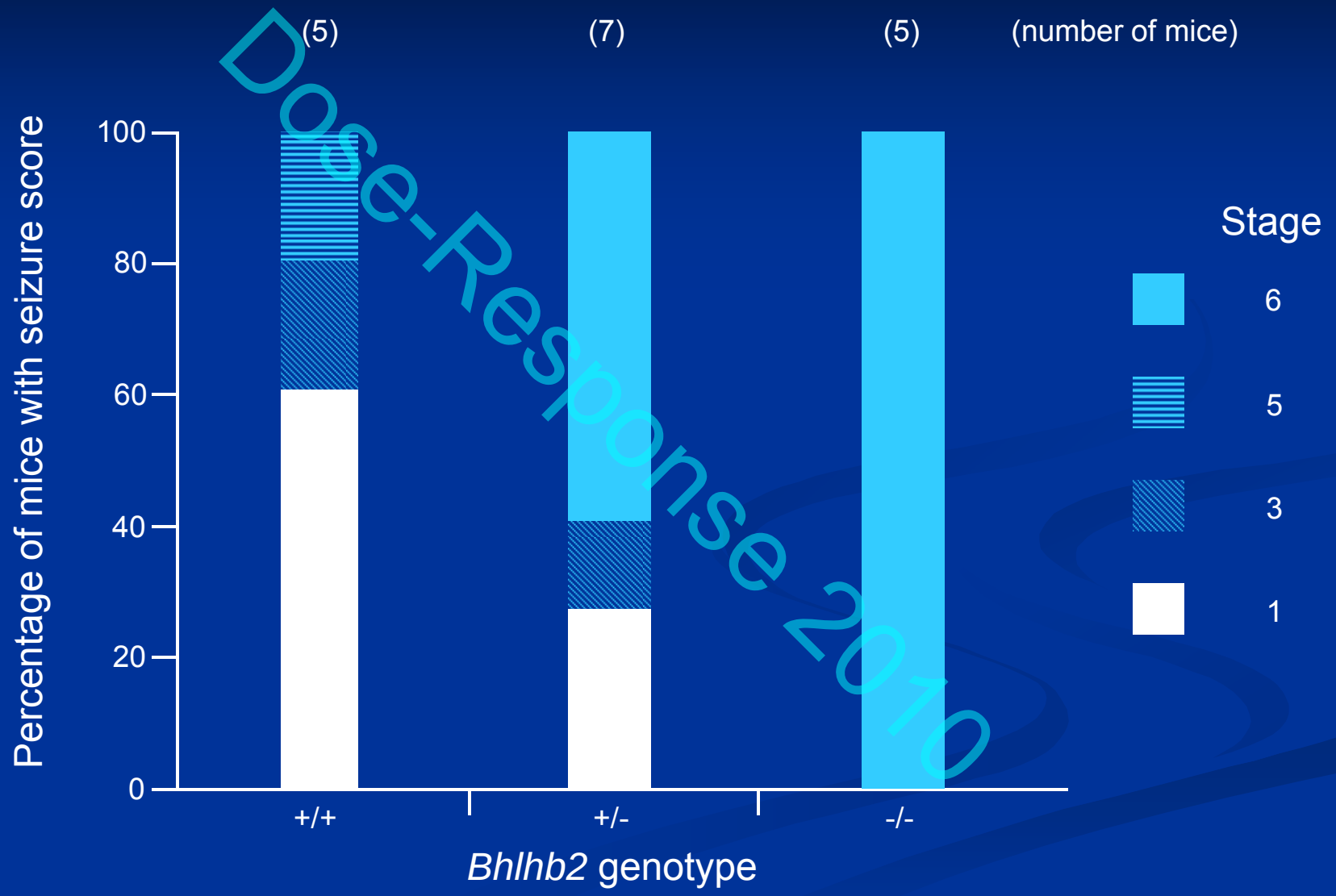


Jiang, Lipsky, *J. Neurosci.*, 2008

Susceptibility of *Bhlhb2* mutant mice to kainate-induced seizures



F(2,14) = 5.304; *P* = 0.019

Jiang et al., *J. Neurosci.*, 2008

BHLHB2 is localized to the nucleus in rat CA1 neurons

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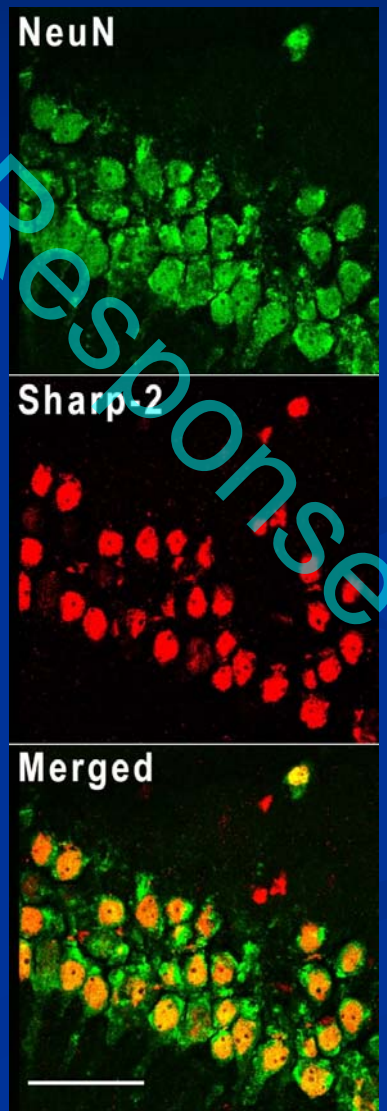

Summary

- NMDA receptor activation protects neurons against glutamate excitotoxicity through a BDNF autocrine loop (hippocampus and cerebellum).
- Promoter IV of the bdnf gene contains a class B E box that binds BHLHB2.
- BHLHB2 is a transcriptional repressor of promoter IV.
- NMDA receptor activation reduces BHLHB2 occupancy on promoter IV and increases BDNF exon 4 mRNA levels.
- BHLHB2 KO mice show increased seizure susceptibility suggesting that BHLHB2 regulates neuronal excitability.
- BHLHB2 is expressed in the hippocampus.

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Palytoxin

- Palytoxin (PTX) is a large water-soluble polyalcohol that was originally isolated from marine soft corals.
- Palytoxin exhibits robust toxicity in mammals and constitutes a serious public health risk to humans.
- Palytoxin contamination has been confirmed in tropical areas but is now spreading to temperate waters and has been detected in Europe.
- Intoxication occurs through the ingestion of comtainated seafood and via toxic aerosols. Symptoms include rhinorrhea, cough and fever.
- Domoic acid (DOM), a marine toxin, is a tricarboxylic amino acid structurally related to kainic acid and activate both AMPA and kainate receptors with low and high affinity respectively. DOM induces excitotoxicity via the low affinity activation of AMPA receptors in cultured cerebellar granule cells.

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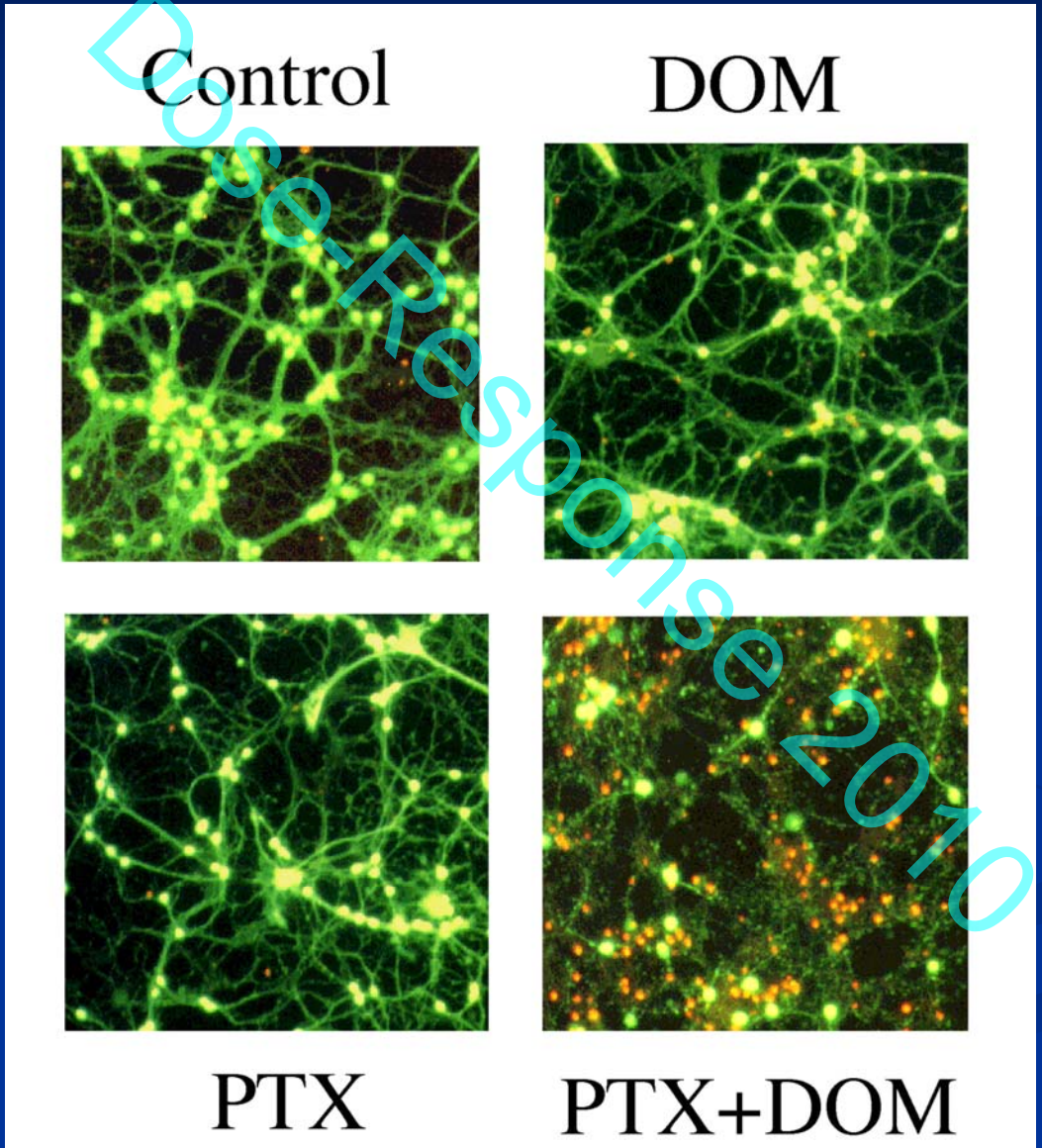
Neurotoxic synergism between domoic acid and palytoxin



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Perez-Gomez et al., 2010, *in press*

A subtoxic concentration of domoic acid becomes toxic in the presence of a subtoxic concentration of palytoxin

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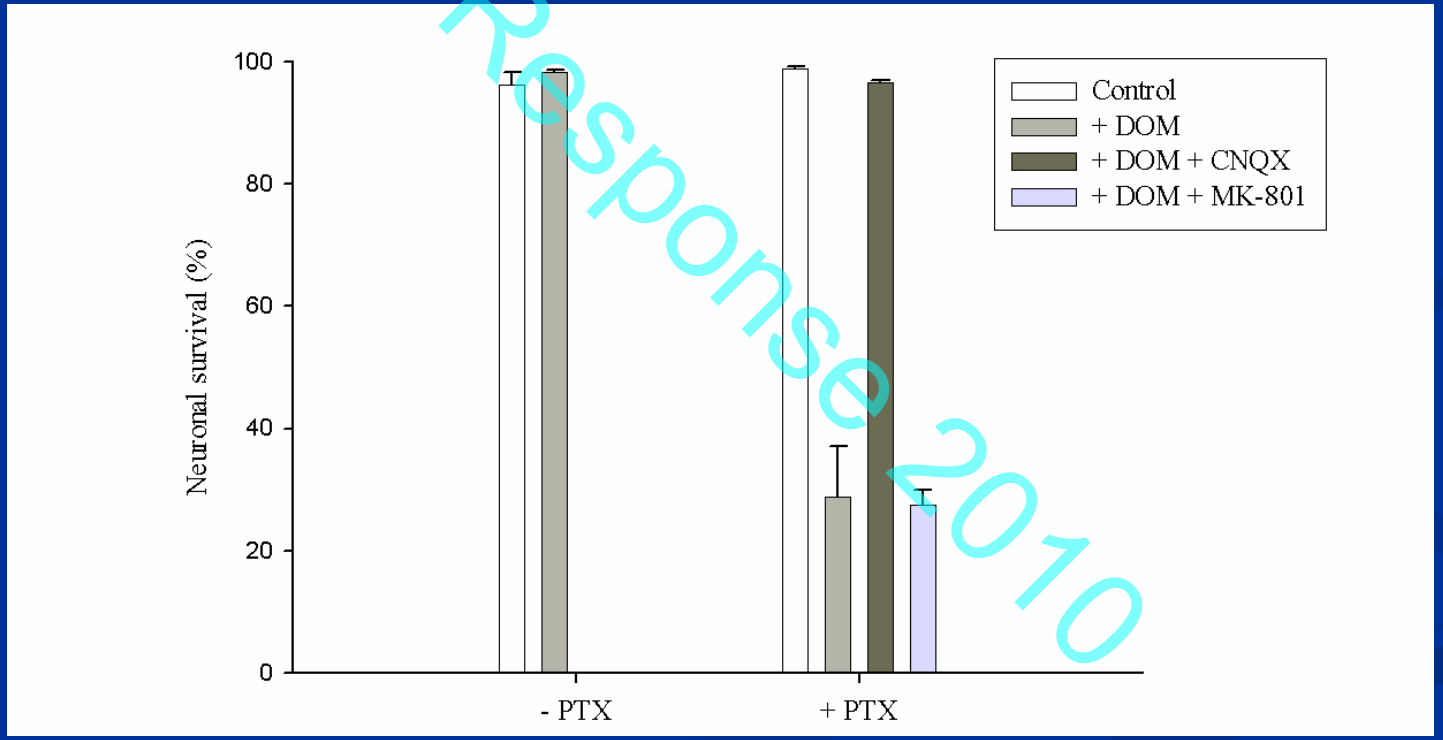

Picomolar concentration of palytoxin enhances domoic acid-induced excitotoxicity

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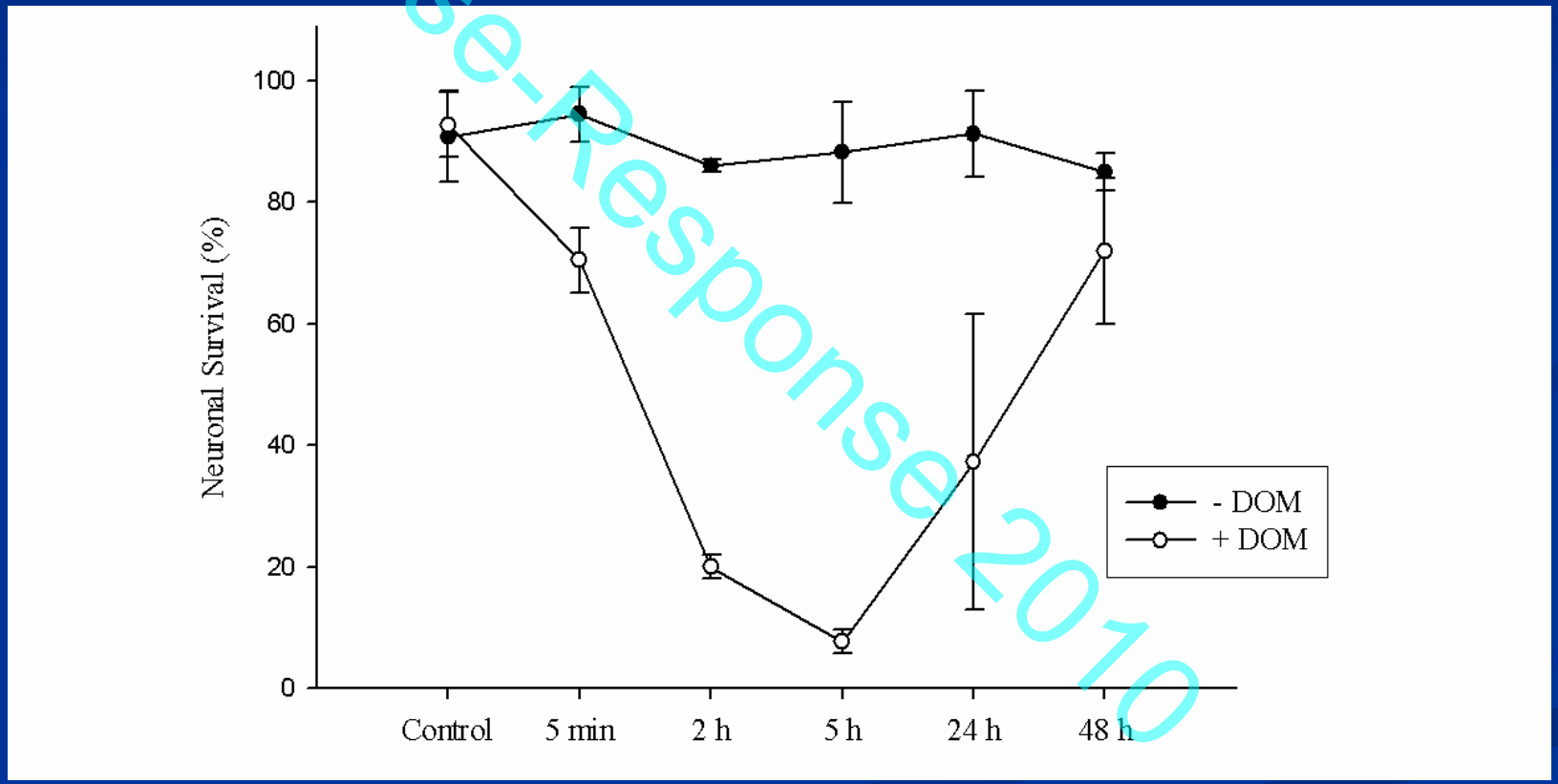
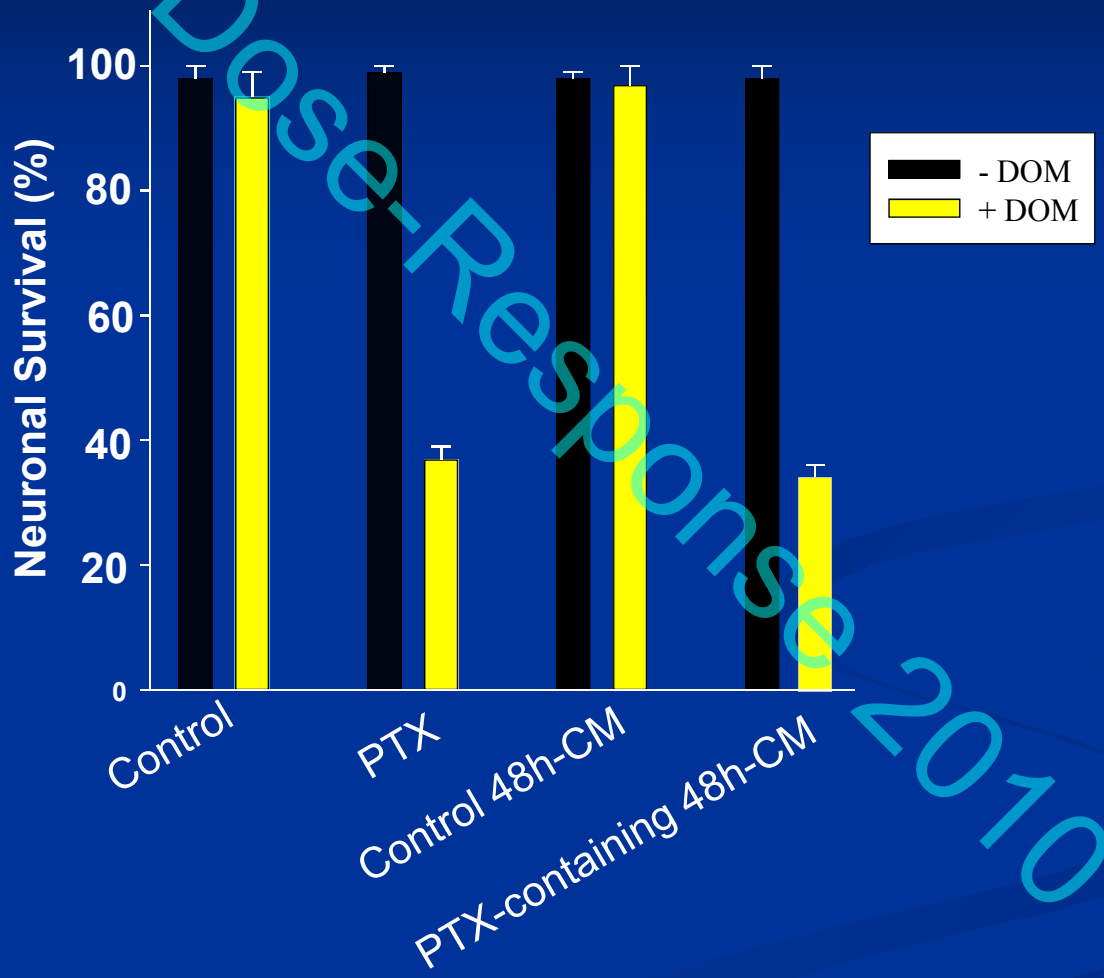


Toxicity-no toxicity-toxicity



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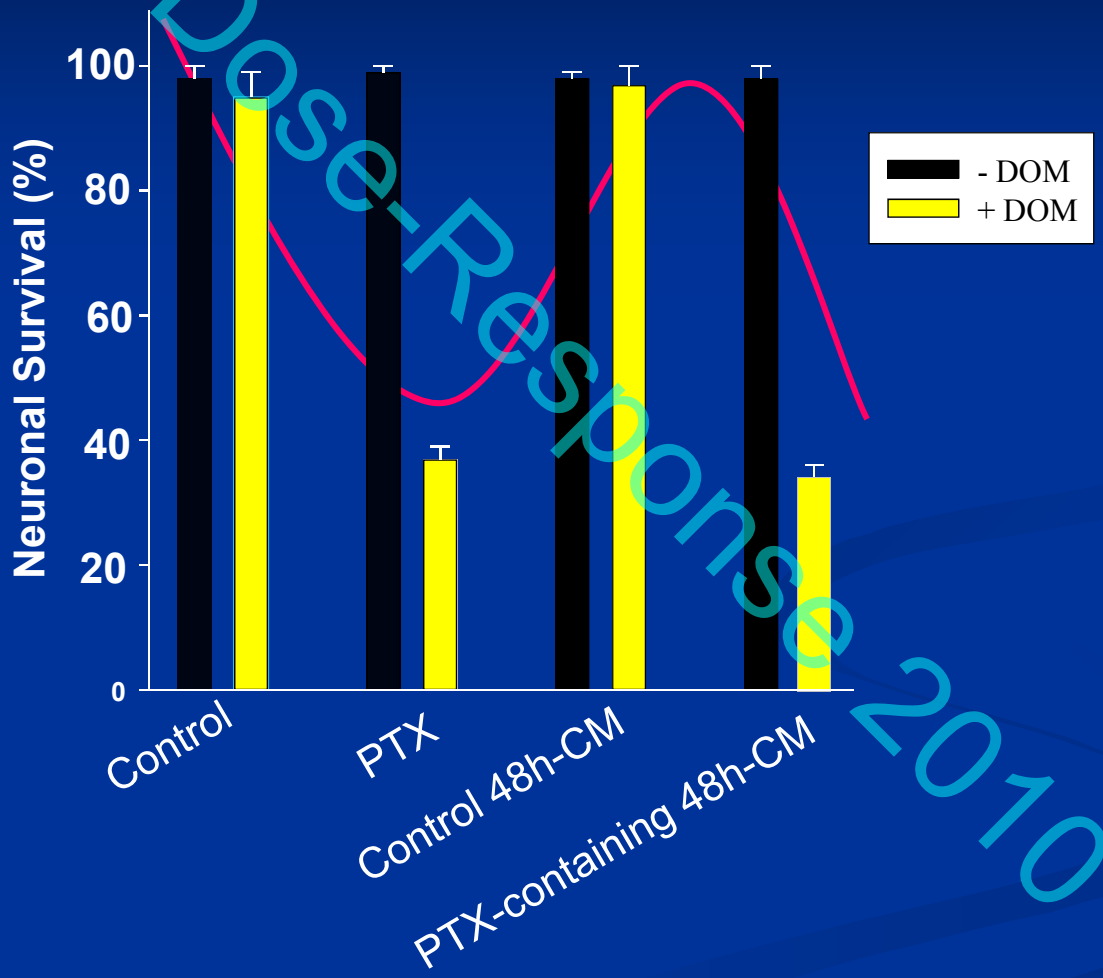
Toxicity-no toxicity-toxicity



Summary

- A subtoxic concentration of domoic acid or palytoxin does not affect neuronal survival.
- Neurotoxicity occurs when a subtoxic concentration of palytoxin and domoic acid are combined in culture.
- Low dose effects of toxins added in combination can result in neuronal cell death.
- Thus, low dose effects can occur when toxins in combination block key components in a pathway required for proper neuronal cell function.

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ACKNOWLEDGEMENTS

NIAAA, NIH
Xueying Jiang
Feng Tian
Ke Xu
Heather Kinney

USUHS
Jun Chen
Hongna Pan
Xianzhang Hu
Michael Lee
Cynthia Chen

CNRS, Valbonne, France
Nicolas Blondeau

NIA, NIH
Mark P. Mattson

NCI, NIH
Yang Du
Neal Copeland
Nancy Jenkins
Lino Tessarollo

Fairfax INOVA, George Mason University
Krasnow Institute
Robert H. Lipsky

University of Toronto
Sean Egan

University of Oviedo, Spain
Antonello Novelli
Maite Fernandez-Sanchez
Anabel Perez-Gomez

University of Oxford, UK
Adrian Harris
Helen Turley

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