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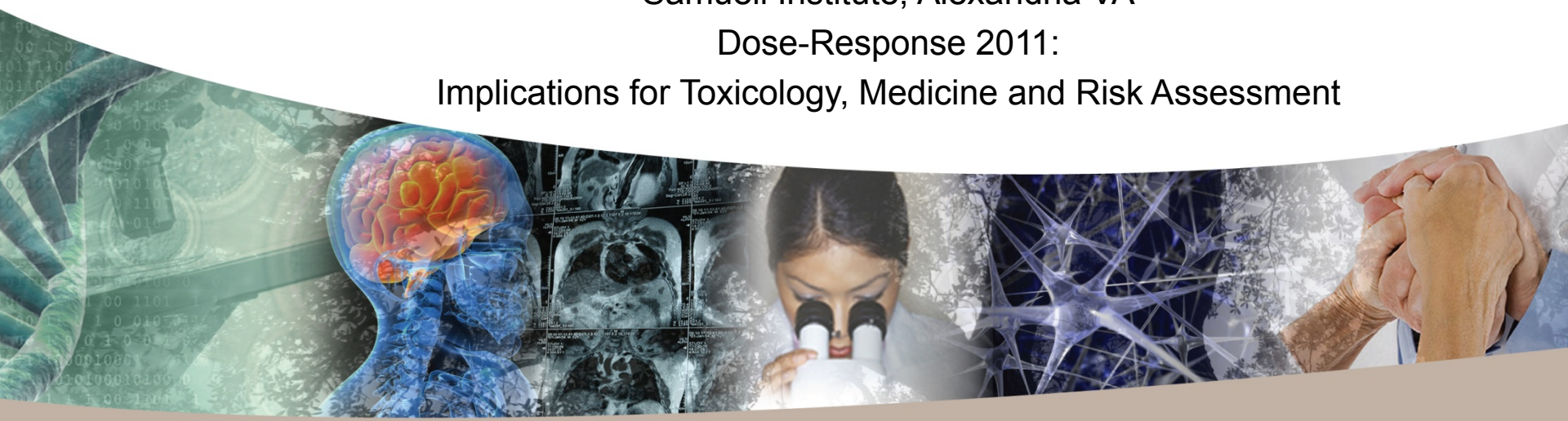
Therapeutic Implications of Hormesis

Wayne B. Jonas, MD
Samueli Institute, Alexandria VA

Dose-Response 2011:
Implications for Toxicology, Medicine and Risk Assessment



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Overview of Talk

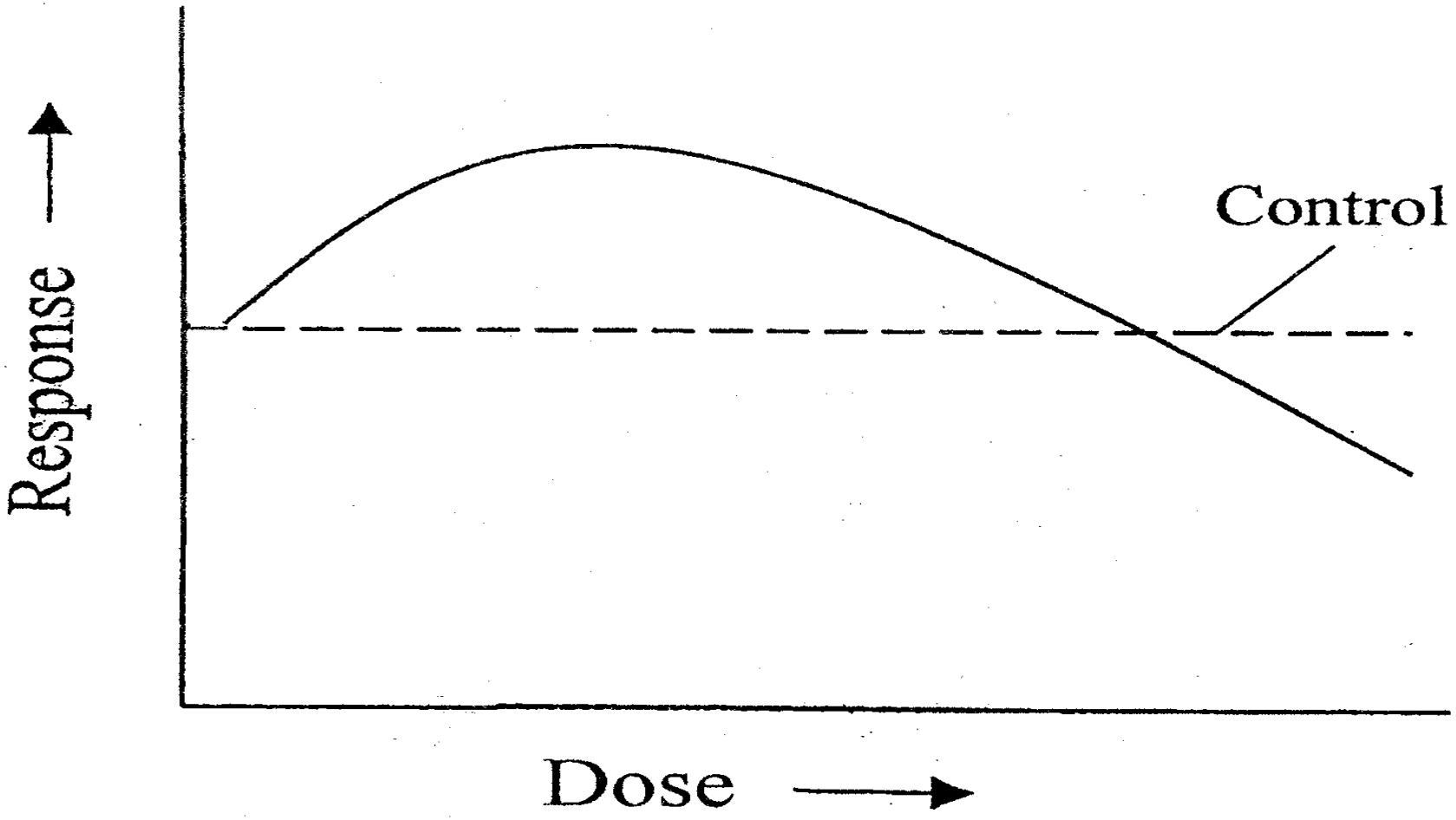
- Review the window demonstrated by hormesis where therapeutic opportunities exist.
- Explore some top agents and models for clinical application hormesis
- Explore some challenges for development of clinical hormesis
- Discuss conceptual and research needs to advance the clinical utility of hormesis
- Introduce the concept of Rapid Induction of Protective Tolerance (RIPT)

Systems and Cells

"This suggests that universal organizing principles apply to all networks, from the cell to the World Wide Web."

Oltvai ZN, Barabasi, AL. 2002. Life's complexity pyramid. Science 298: 763-765.

General form of Hormetic Dose-Response Relationship



Calabrese and Baldwin 2001c.

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Where might hormesis be used therapeutically?

- Radiation Protection
- Carcinogenesis
- Immunological conditions
- Brain Injury and Neurodegenerative Diseases
- Heavy Metal Toxicology

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- Radiation Protection
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- Immunological conditions
- Brain Injury and Neurodegenerative Diseases
- **Heavy Metal Toxicology**

Critical Review and Meta-Analysis of Serial Agitated Dilutions in Experimental Toxicology*

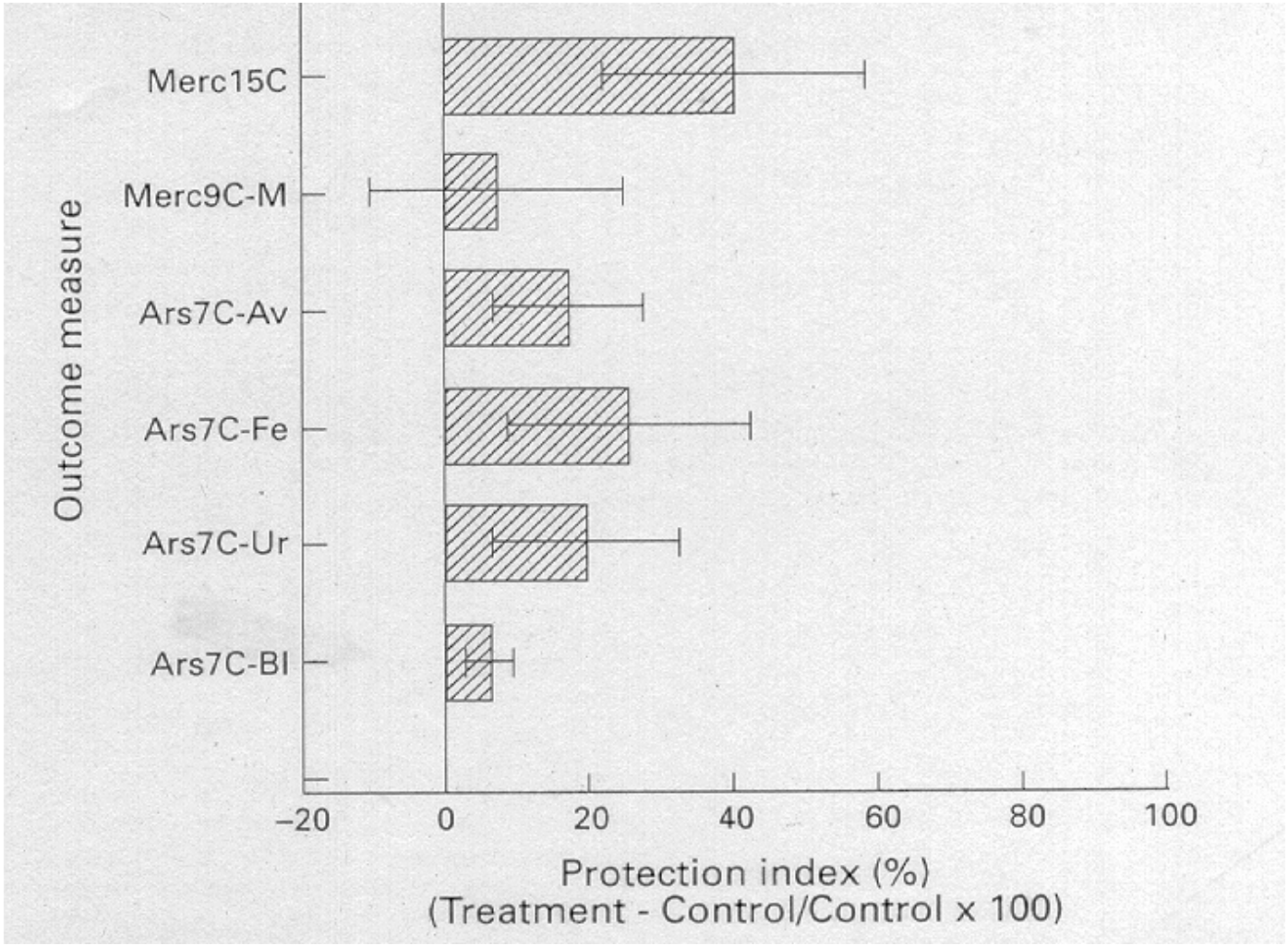
Klaus Linde,[1] Wayne B. Jonas,[2] Dieter Melchart[1] Felege Worku,[1] Hildebert Wagner[3] and Florian Eitel[4]

[1]Projekt 'Münchener Modell', Ludwig-Maximilians-Universität, Pettenkoferstr. 8a, 8000 München 2, GFR, [2]Division of Medicine, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, USA, [3]Institut für Pharmazeutische Biologie, Ludwig-Maximilians-Universität, Karlstr. 29, 8000 München 2, GFR and [4]Theoretische Chirurgie, Chirurgische Klinik und Poliklinik Innenstadt, Ludwig-Maximilians-Universität, Nussbaumstr. 20, 8000 München 2, GFR

1 We conducted an overview and quantitative meta-analysis of all experimental literature on the protective effects of serial agitated dilutions (SADs) of toxin preparations.
2 Articles were systematically collected and evaluated for scientific quality using pre-defined methodological criteria and then independently analysed for validity.
3 We found 105 publications exploring the effects of SAD preparations in toxicological systems.
4 The quality of evidence in these studies was low with only 43% achieving one half of the maximum possible quality score and only 31% reported in a fashion that permitted reevaluation of the data.
5 Very few studies were independently replicated using comparable models.
6 Among the high quality studies, positive effects were reported 50% more often than negative effects.
7 Four of 5 outcomes meeting quality and comparability criteria for meta-analysis showed positive effects from SAD preparations.
8 Average percent protection over controls in these preparations was 19.7 (95%CI 6.2–33.2).
9 Further research with special attention to methodological detail and independent replication should be done.

Linde K, et al. Hum & Experiment Toxicol. 1994; 13: 485.

Mean protection index and 95% CI for outcome measures of 26 dilution tests



Linde K, et al. Hum & Experiment Toxicol. 1994; 13: 485.

Cross tolerance protection of low-dose metal exposure is from induced signature HSP and MT

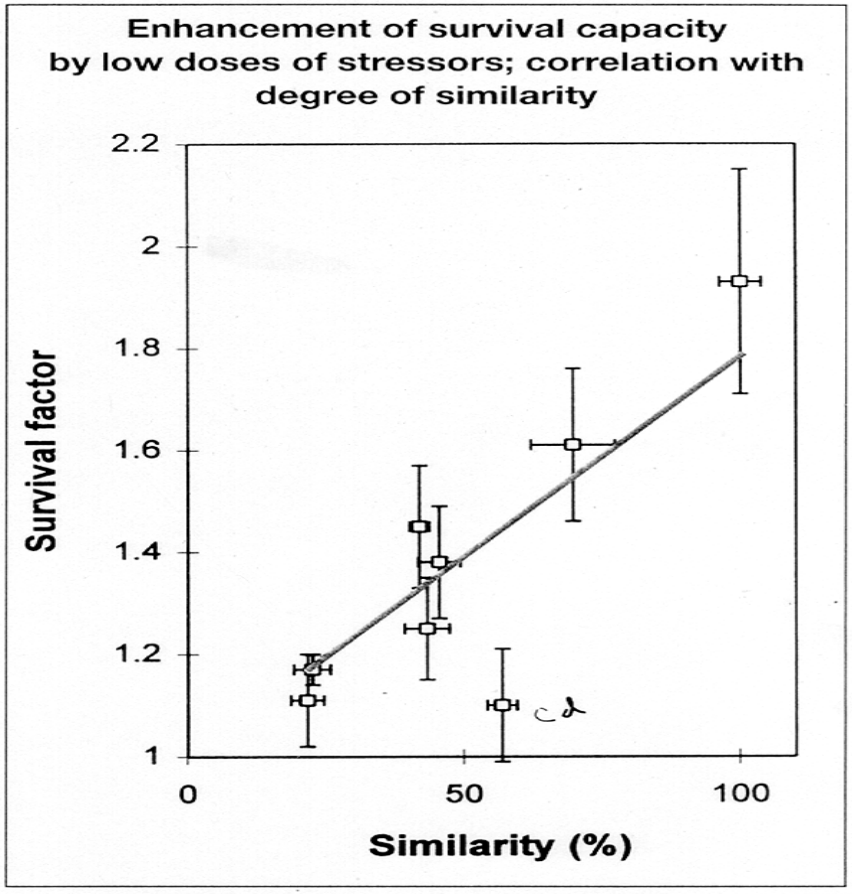


Fig. 6: Relationship between the survival factor and the percentage of similarity. The latter was obtained upon comparison of the heat shock-induced and the stressor treatment-induced protein patterns. Results are the mean of 2 experiments (± standard error).

Jan van Wijk ATHM, 1990.

Biphasic effect of cadmium on cell proliferation in human embryo lung fibroblast cells and its molecular mechanism

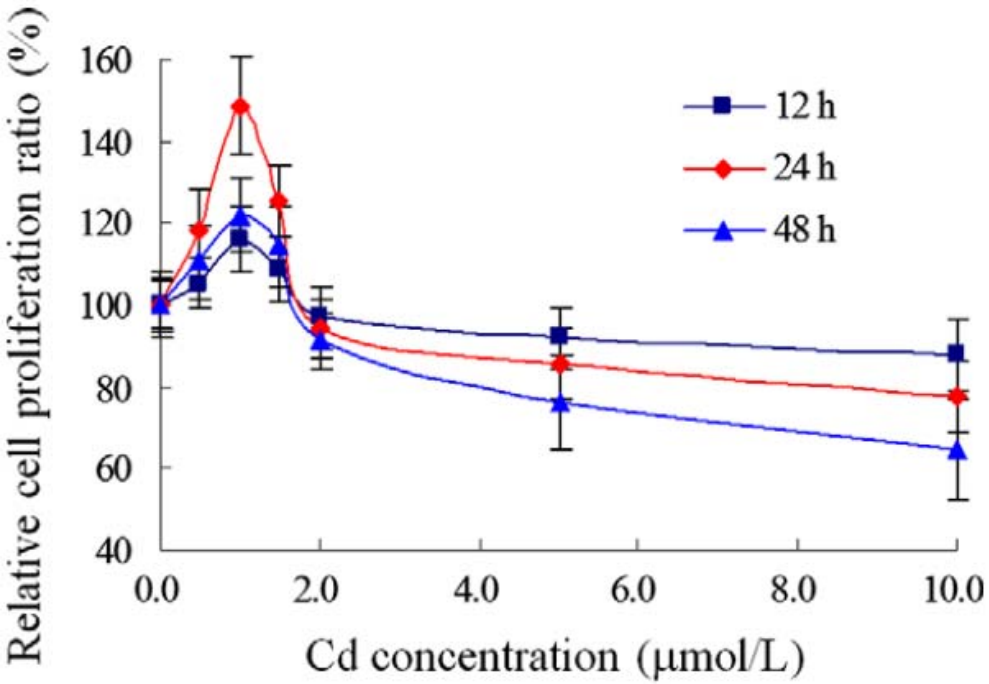


Fig. 1. Effects of Cd on HLF cells proliferation. Cells were treated with 0, 0.5, 1.0, 1.5, 2.0, 5.0 or 10.0 μmol/L Cd for 12, 24, and 48 h, respectively. Cell proliferation were tested by MTT assay and expressed as percentages of the value for the control cells, which is set at 100%. Data are presented as mean ± SD of triplicate experiments.

Jiang et al.*, 2009.* Biphasic effect of cadmium on cell proliferation in human embryo lung fibroblast cells and its molecular mechanism. *Toxicology in Vitro.* 23;973-978.

Long Duration Exposure and Biomarkers

Cadmium in Prostate Cells

Cadmium Doses:

Control	**0 M (Water)**
High Doses	**10^{-6} M**
	10^{-7} M
Low Doses	**10^{-18} M**
	10^{-21} M
Ultra low Doses	**10^{-32} M**
	10^{-36} M

Duration: **20 weeks**

Cells: **RWPE-1 (Cells) Human Prostate**

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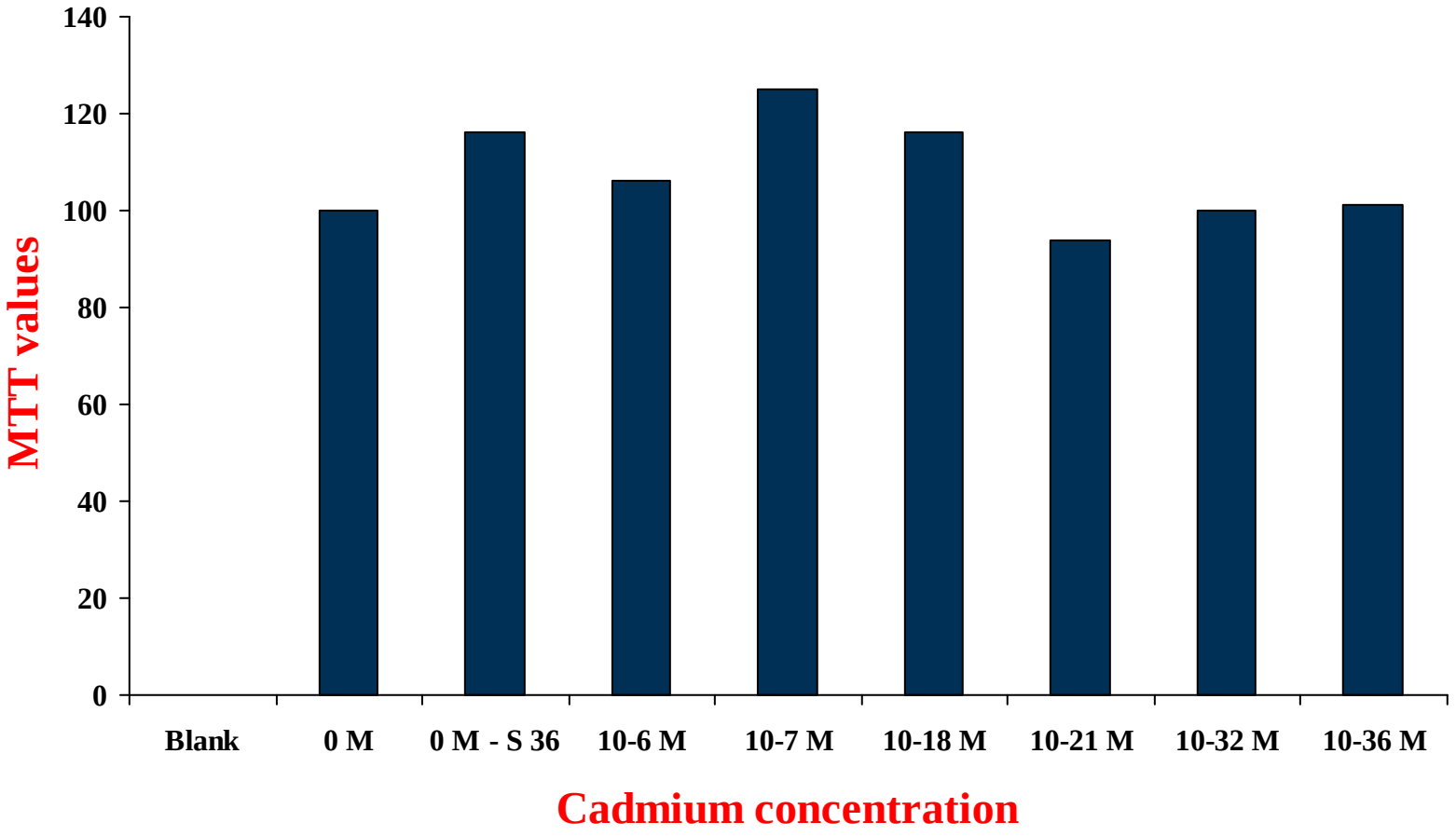
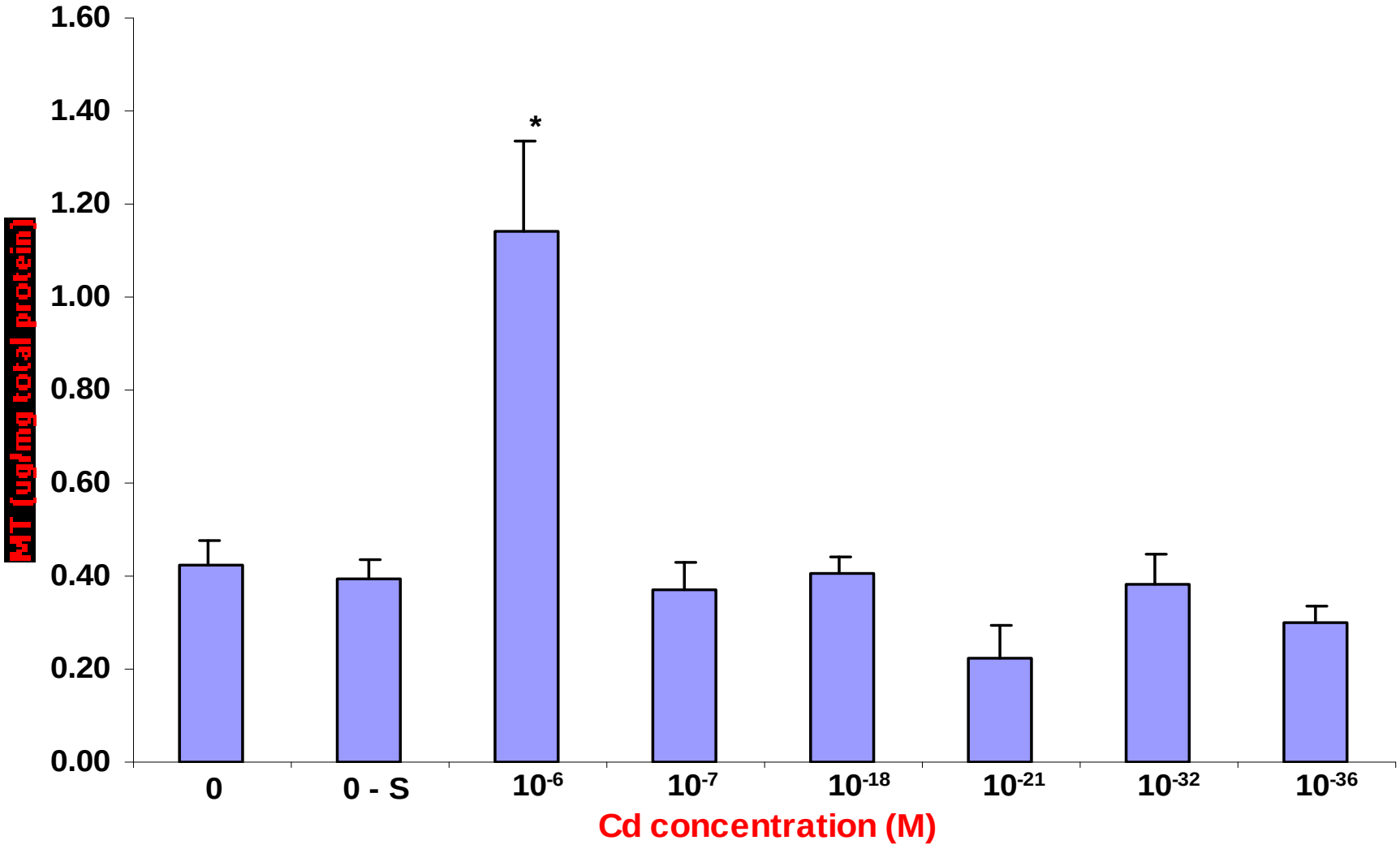
Effect of Low Level Cadmium Analyses

1. Growth and Toxicity assay
2. MT protein measurements
3. RNA expression analysis by RT-PCR and RPA
4. Cell morphology and transformation

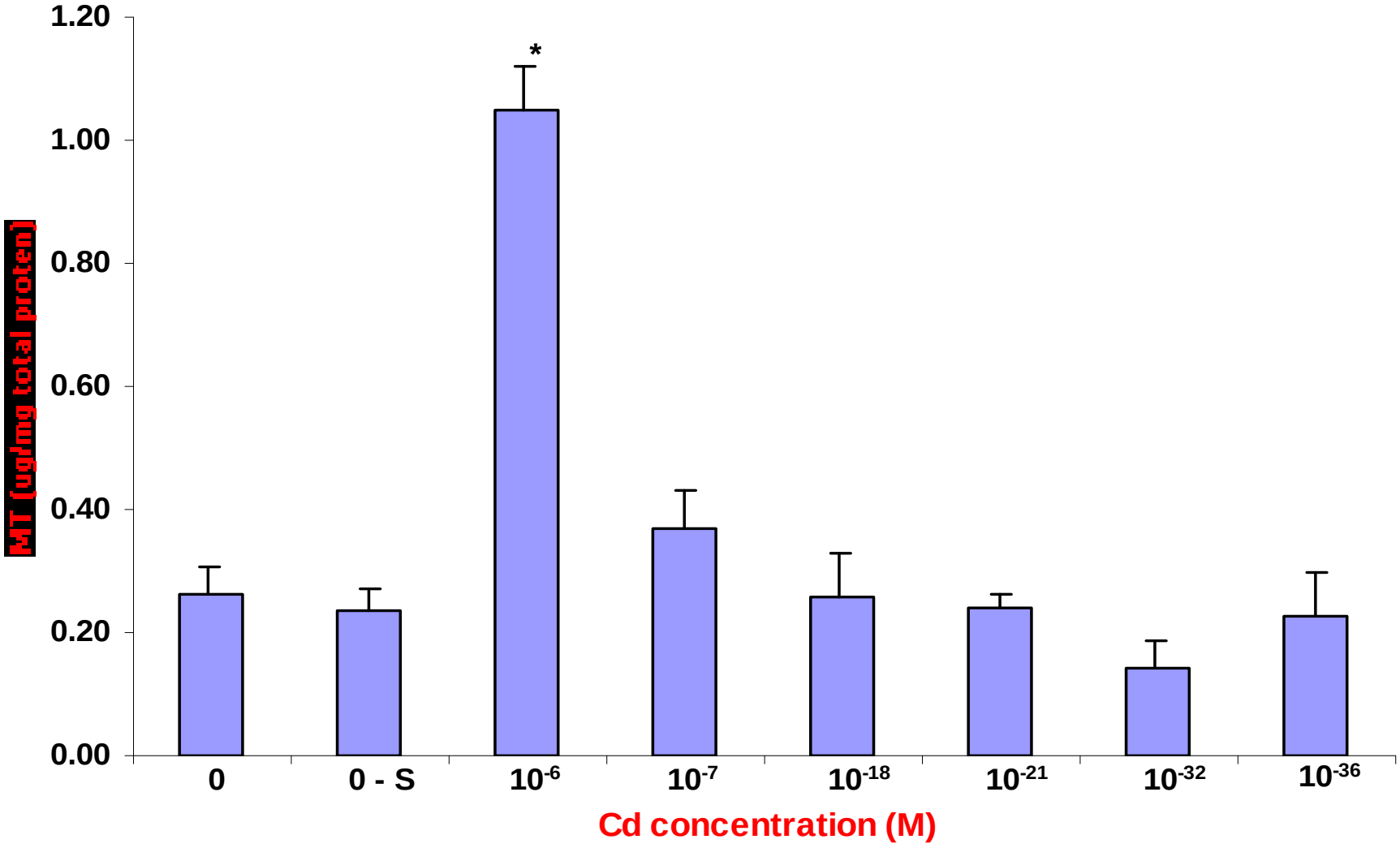
Cadmium Cytotoxicity



MT Protein Levels in RWPE-1 Cells (3 weeks)

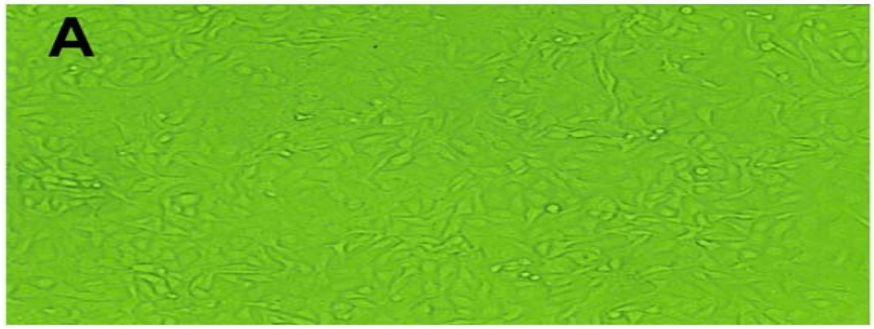


MT Protein Levels in RWPE-1 Cells (20 weeks)

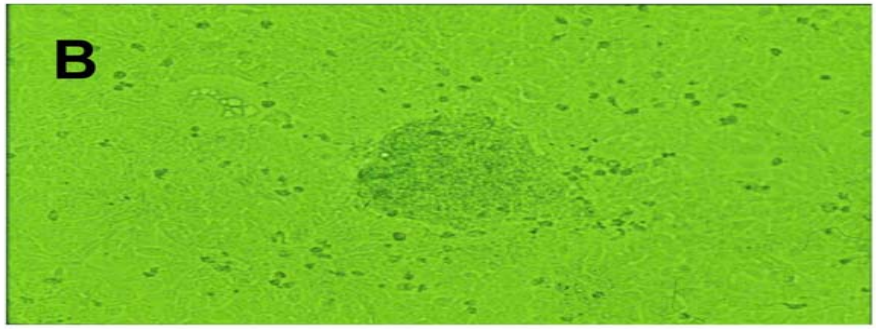


Reduced Cell Transformation from LD Cd Pretreatment

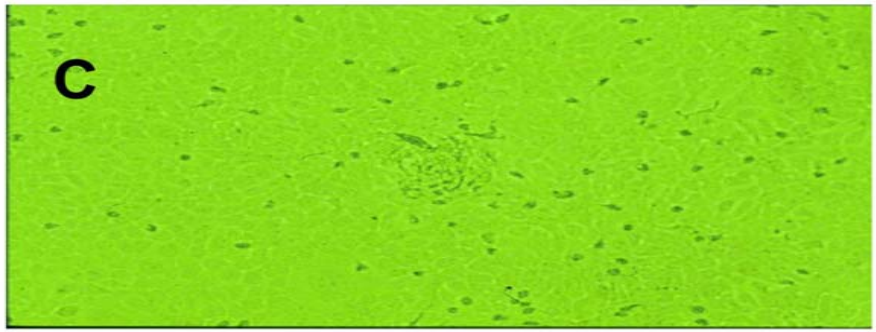
NL Cells



No Cd pretreatment



Cd 10^{-18}M pretreatment



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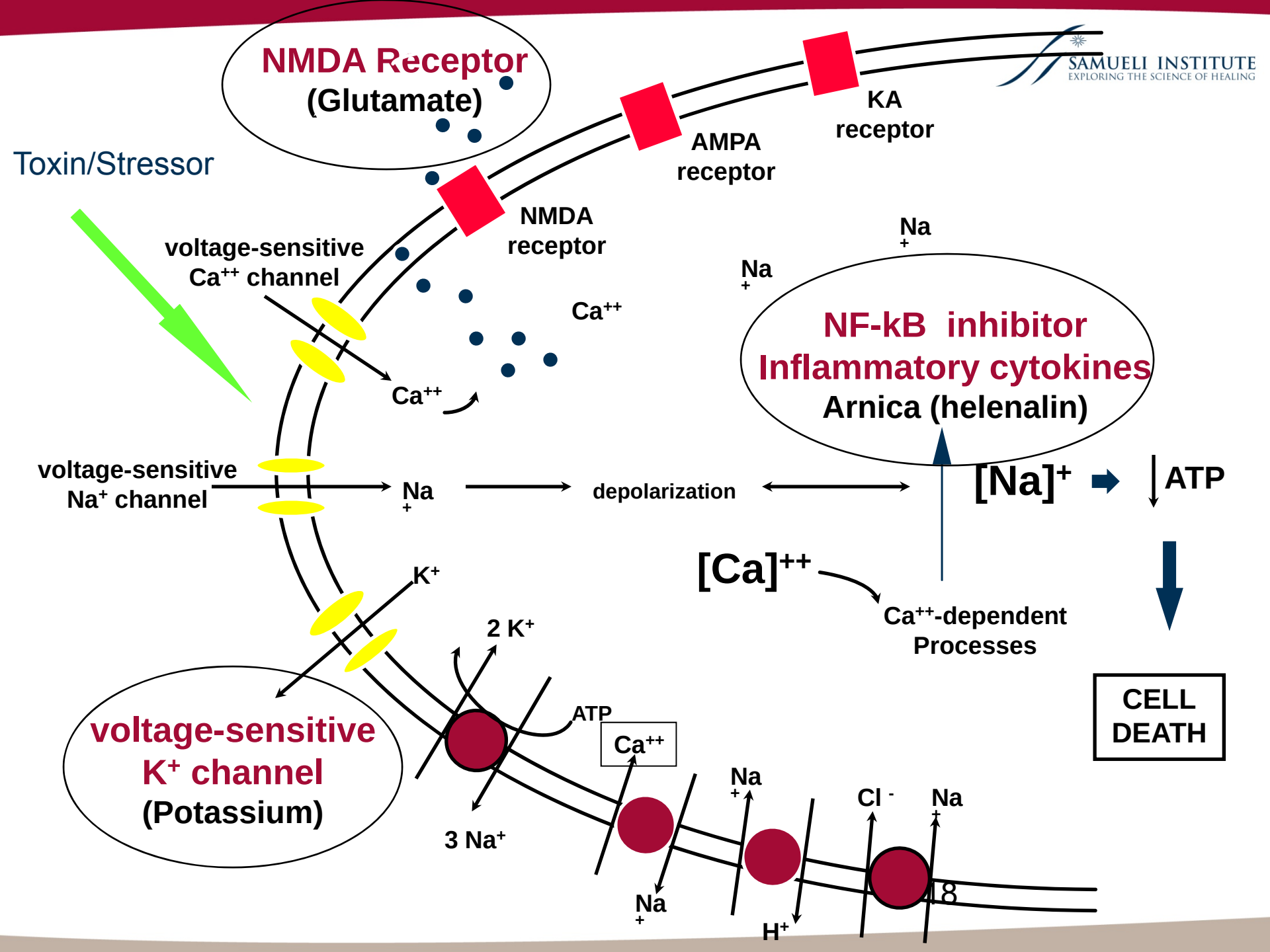
Where might hormesis be used therapeutically?

- Radiation Protection
- Carcinogenesis
- Immunological conditions
- **Brain Injury and Neurodegenerative Diseases**
- Heavy Metal Toxicology


NMDA Receptor
(Glutamate)
Toxin/Stressor
KA
receptor
AMPA
receptor
NMDA
receptor
voltage-sensitive
Ca++ channel
Ca++
Ca++
Na+
Na+
NF-kB inhibitor
Inflammatory cytokines
Arnica (helenalin)
voltage-sensitive
Na+ channel
Na+
depolarization
[Na]+
ATP
[Ca]++
Ca++-dependent
Processes
CELL
DEATH
K+
2 K+
voltage-sensitive
K+ channel
(Potassium)
ATP
Ca++
Na+
Cl -
Na+
3 Na+
Na+
H+

Subtoxic concentrations of N-methyl-D-aspartate (NMDA) protect neurons against glutamate-mediated excitotoxicity in hippocampal cultures

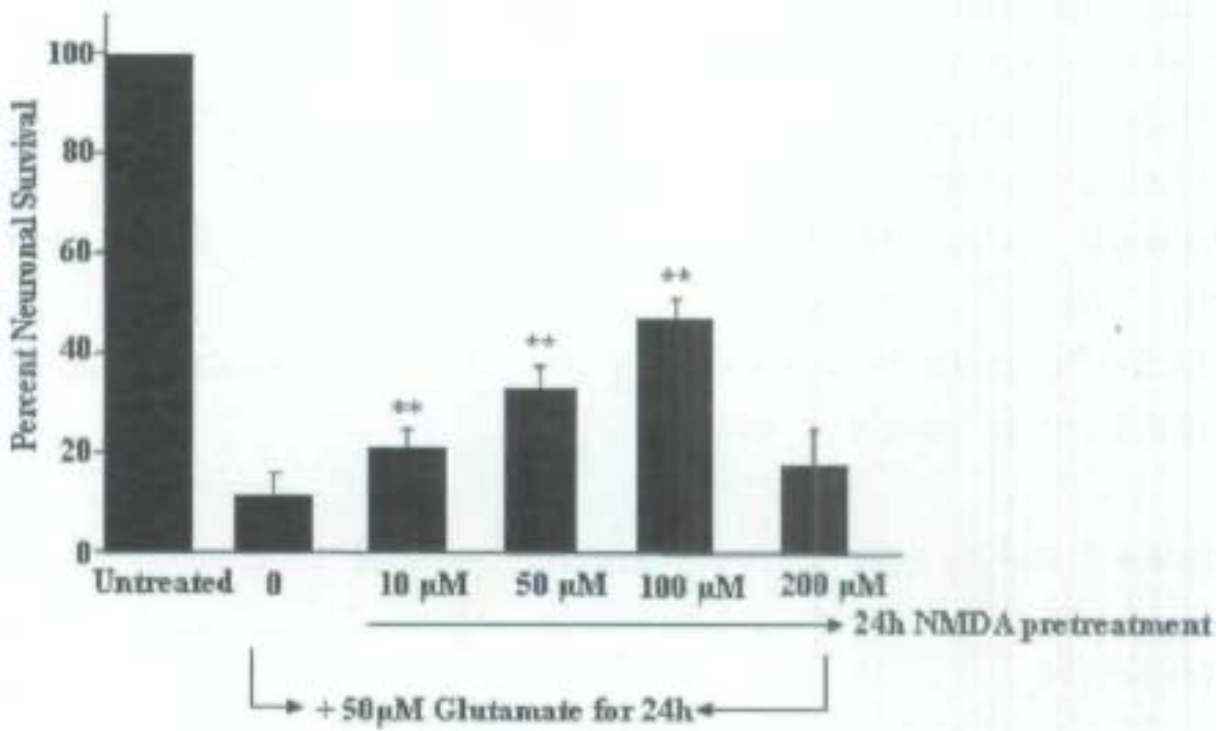
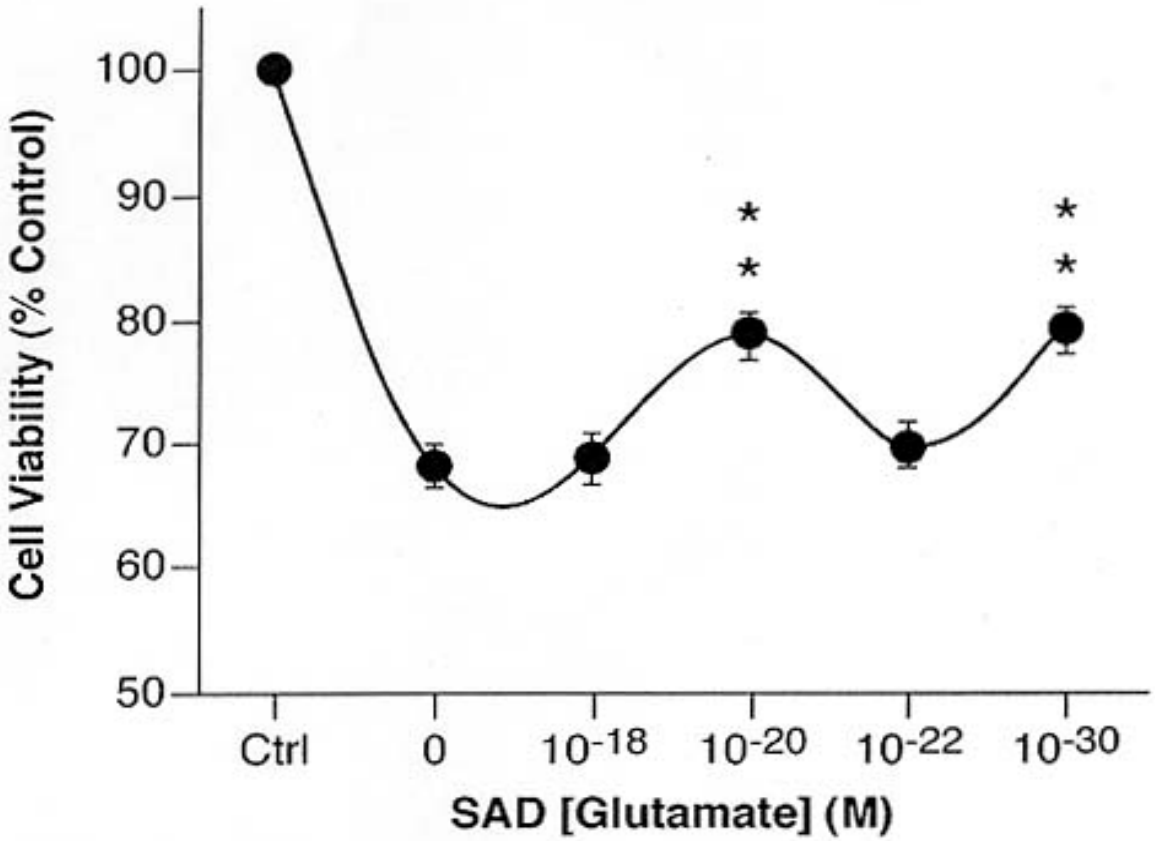


Fig. 5. Subtoxic concentrations of NMDA protect neurons against glutamate-mediated excitotoxicity in hippocampal cultures. Cultures were incubated with various concentrations of NMDA (10-100 mm) on DIV 7 for 24h, followed by the addition of an excitotoxic concentrations of glutamate (50 μm) and neuronal viability was quantified 24h later. **p < 0.01 versus glutamate (50 μm) alone by ANOVA plus Tukey.

Jiang et al.*, 2005.* The excitoprotective effect of N-methyl-D-aspartate receptors is mediated by a brain-derived neurotrophic factor autocrine loop in cultured hippocampal neurons. *Journal of Neurochemistry:* 94;713-722.

Figure 4



Effect of SAD pre-treatment on glutamate toxicity in cerebellar neurons

● Effects on moderate glutamate toxicity (n > 33)

Jonas, W.B., Lin, Y., Tortella, F. *NeuroReports*. 2001; 12: 335-339.

Middle Cerebral Artery Occlusion Model

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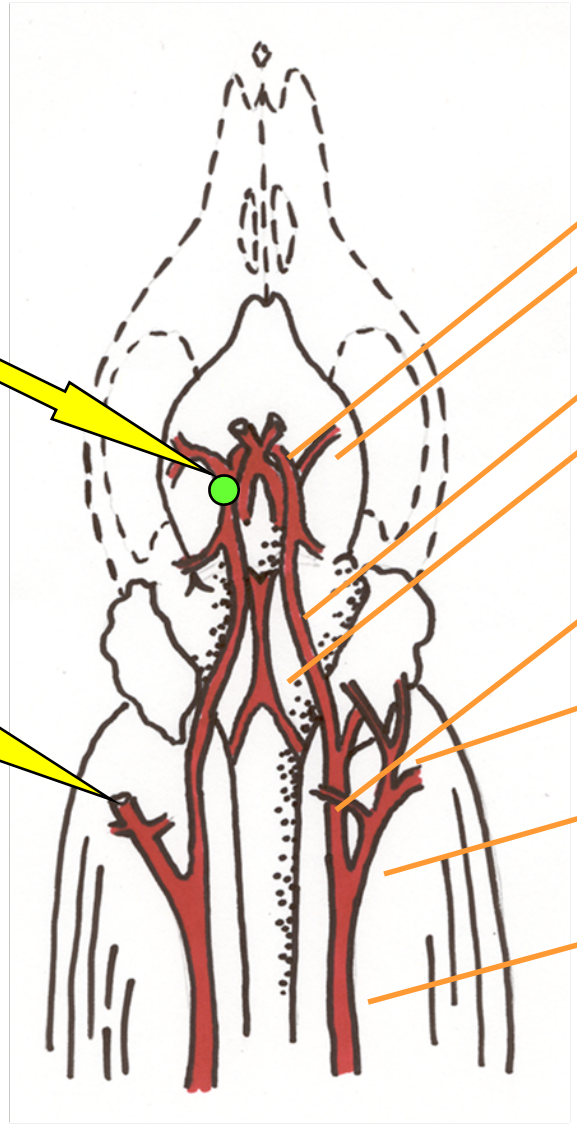

Point of Occlusion

Filament
(inserted into external carotid and advanced to the middle cerebral artery)

anterior cerebral artery

middle cerebral artery

internal carotid artery

basilar artery

occipital artery

superior thyroid artery

external carotid artery

common carotid artery

Middle Cerebral Artery Occlusion

- Intraluminal Filament Method (MCAo)
- Male Sprague-Dawley Rats (275-325 g)
- 2 h Occlusion → 24 h Reperfusion & Recovery
- Treatment Protocol:
  - -- Post-Injury Treatments
  - -- Variable Dosing Schedule (Potency & Therapeutic Window)
  - -- I.V. (bolus), 2ml/kg @ 0.5, 2, 4, & 6 hrs post MCAo
- Parameters Studied:
  - -- Infarct Analysis (TTC)
  - -- Neurological Function (Clinical Neurological Exam)
  - -- Brain Function (Cortical EEG Analysis)

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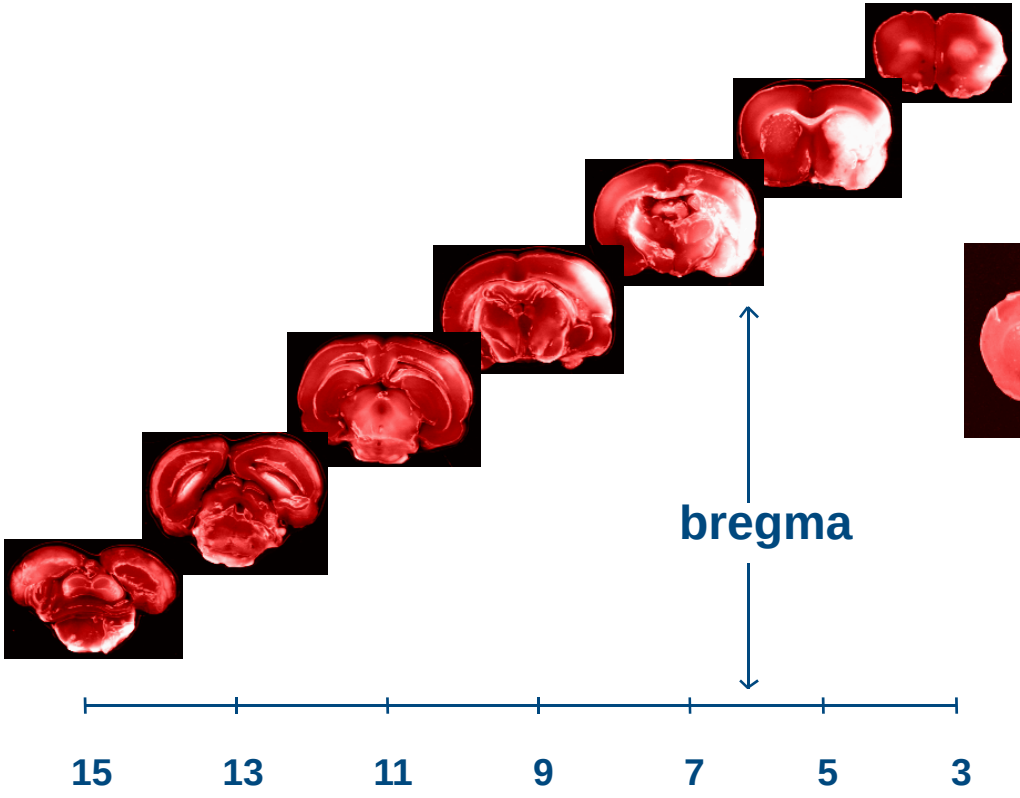
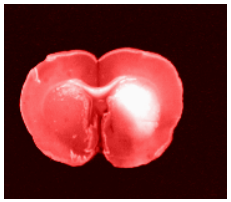
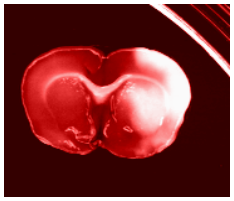
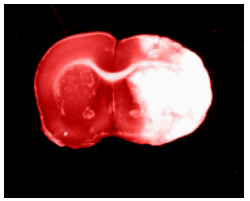
Temporary MCAo & Reperfusion Model in Rats

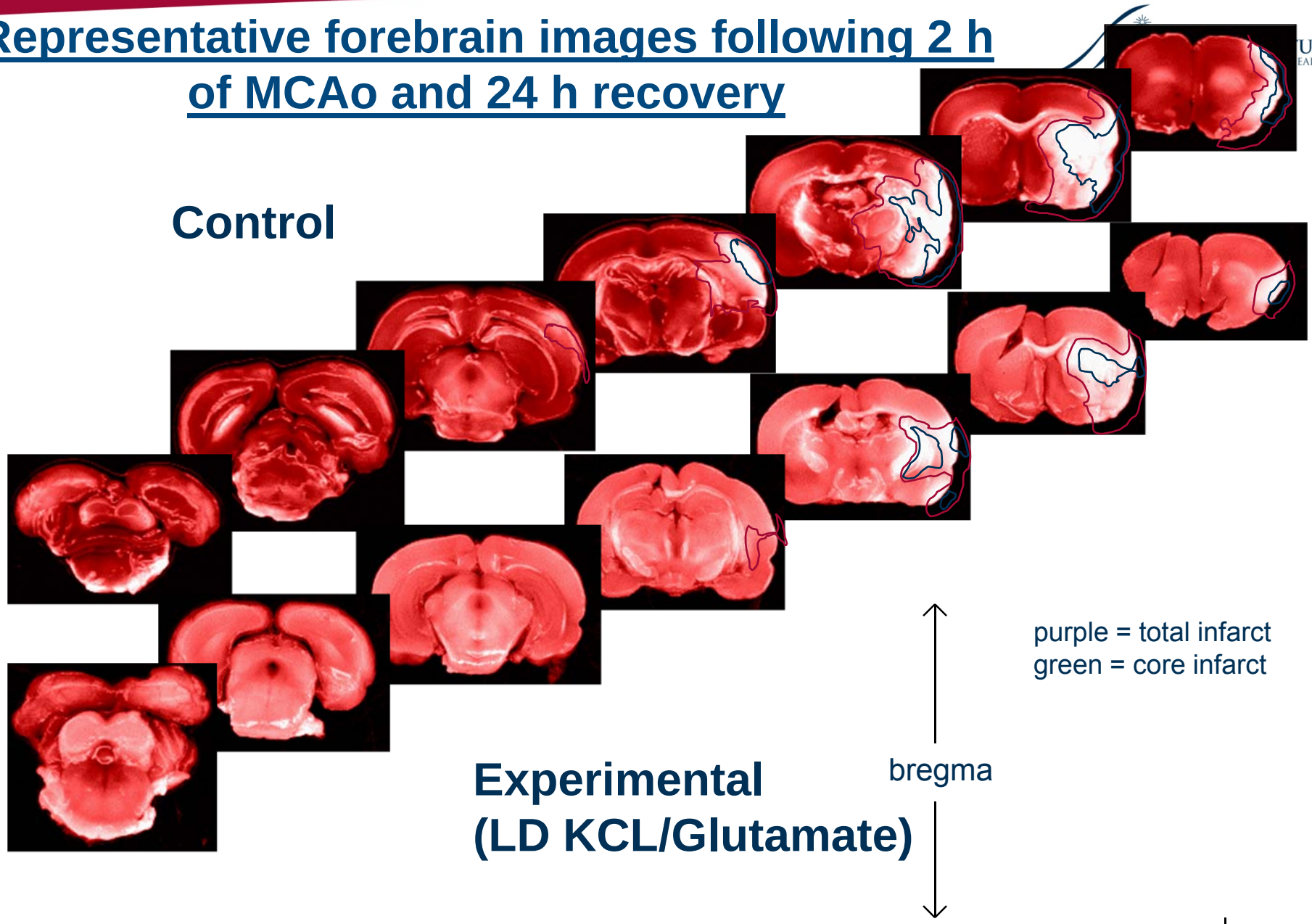



6 h


24 h


72 h

Representative forebrain images following 2 h of MCAo and 24 h recovery



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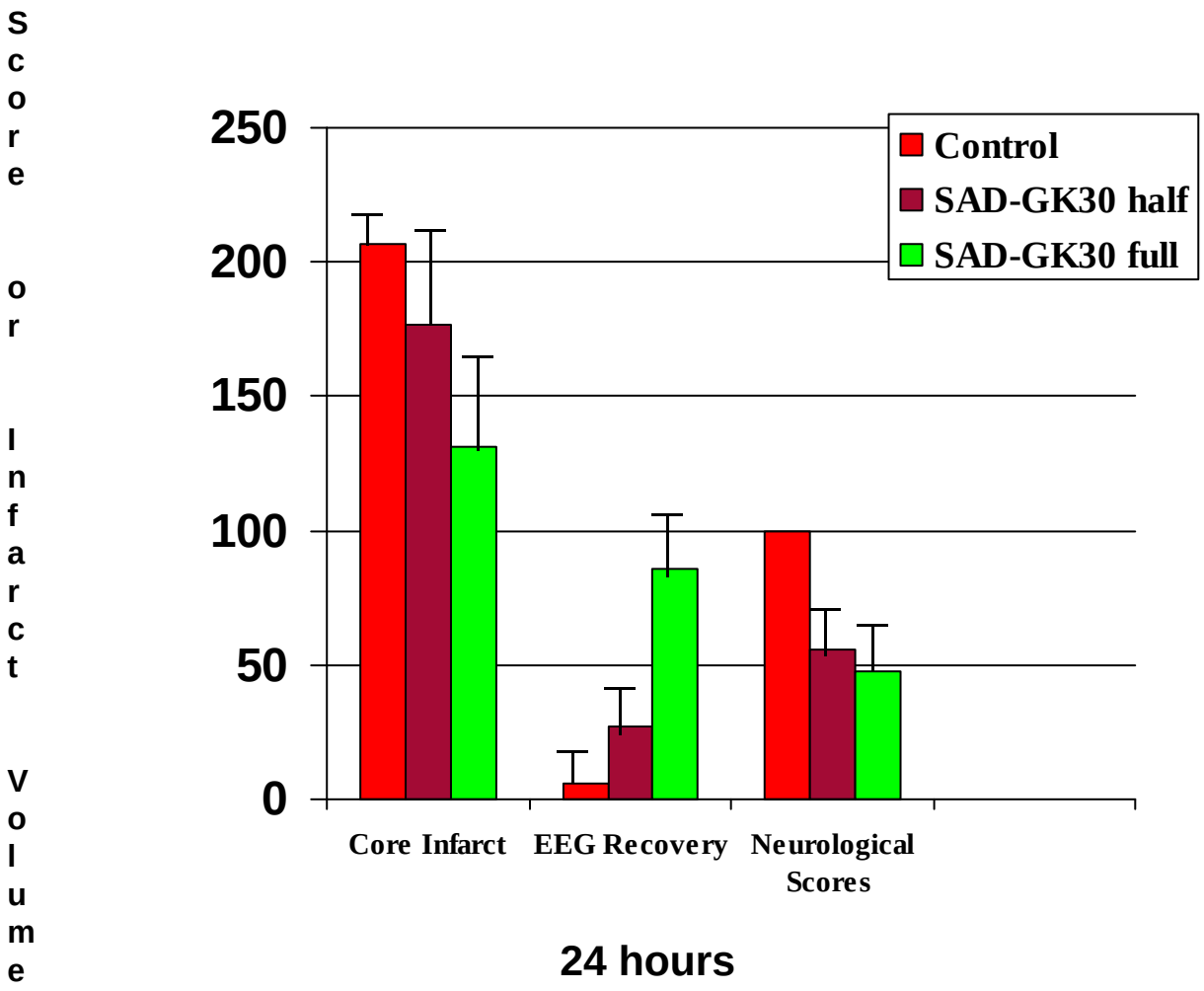
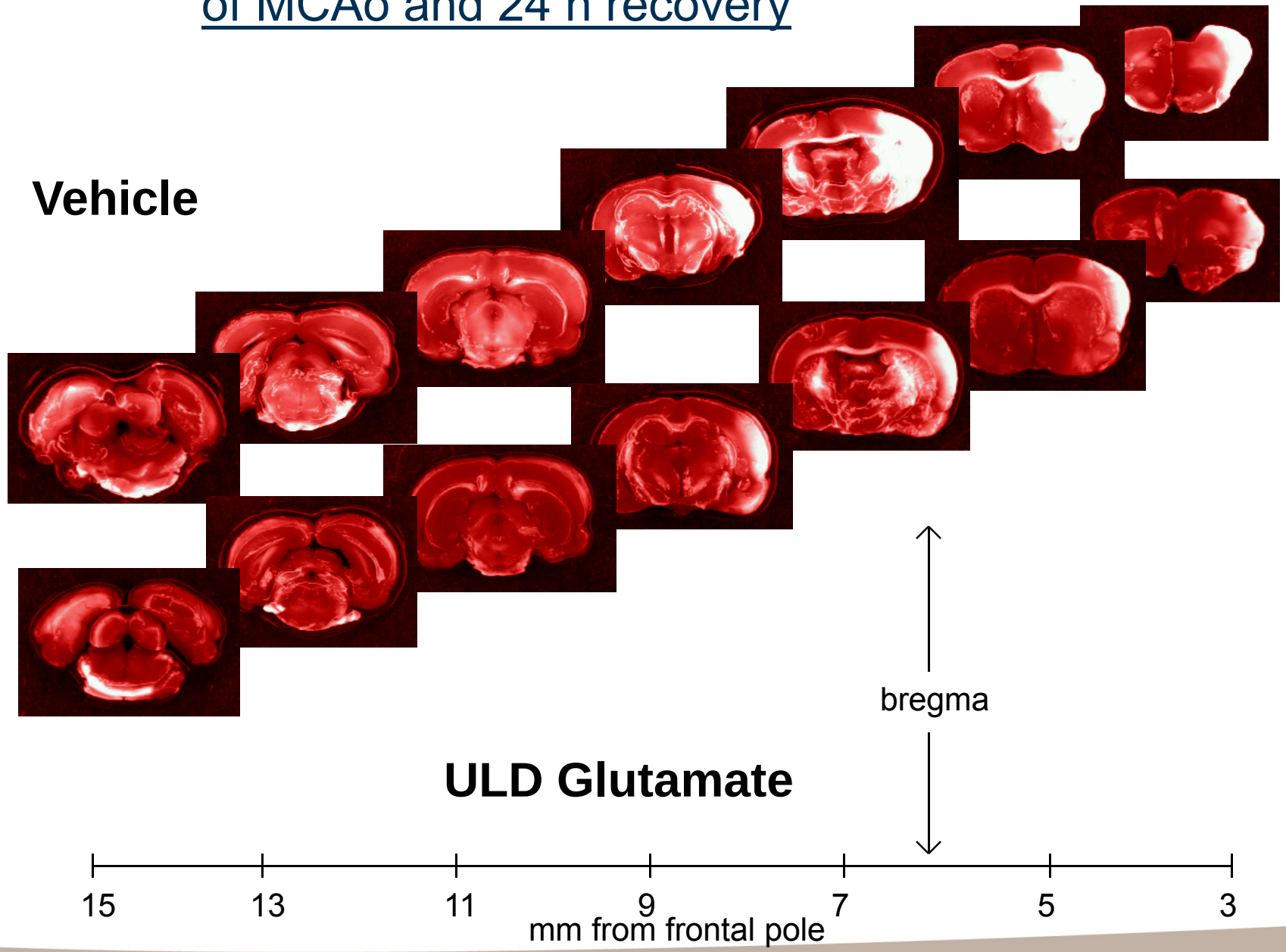
Neuroprotection from Low-Dose GK30



Neurological Scores x 10

Representative forebrain images following 2 h of MCAo and 24 h recovery



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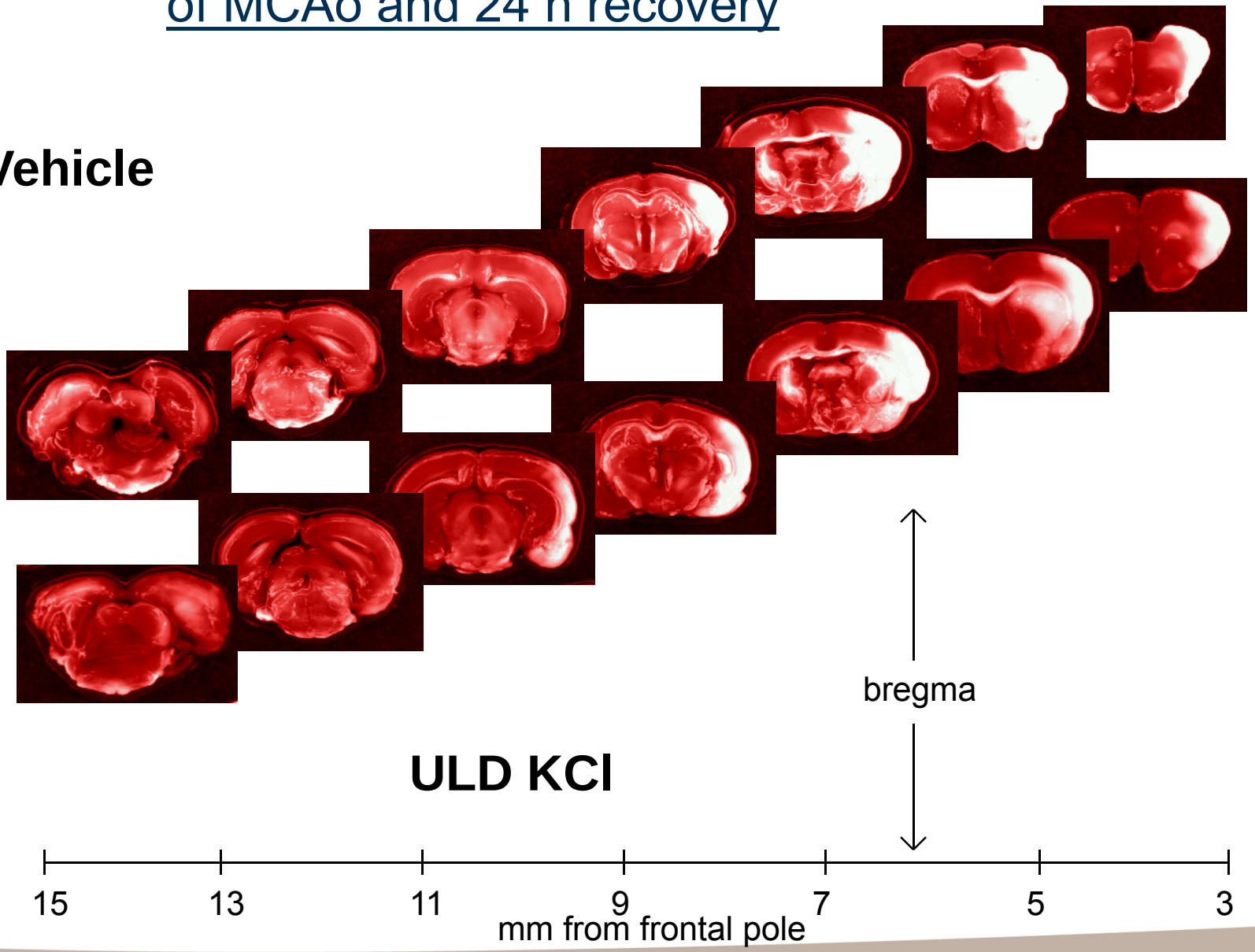

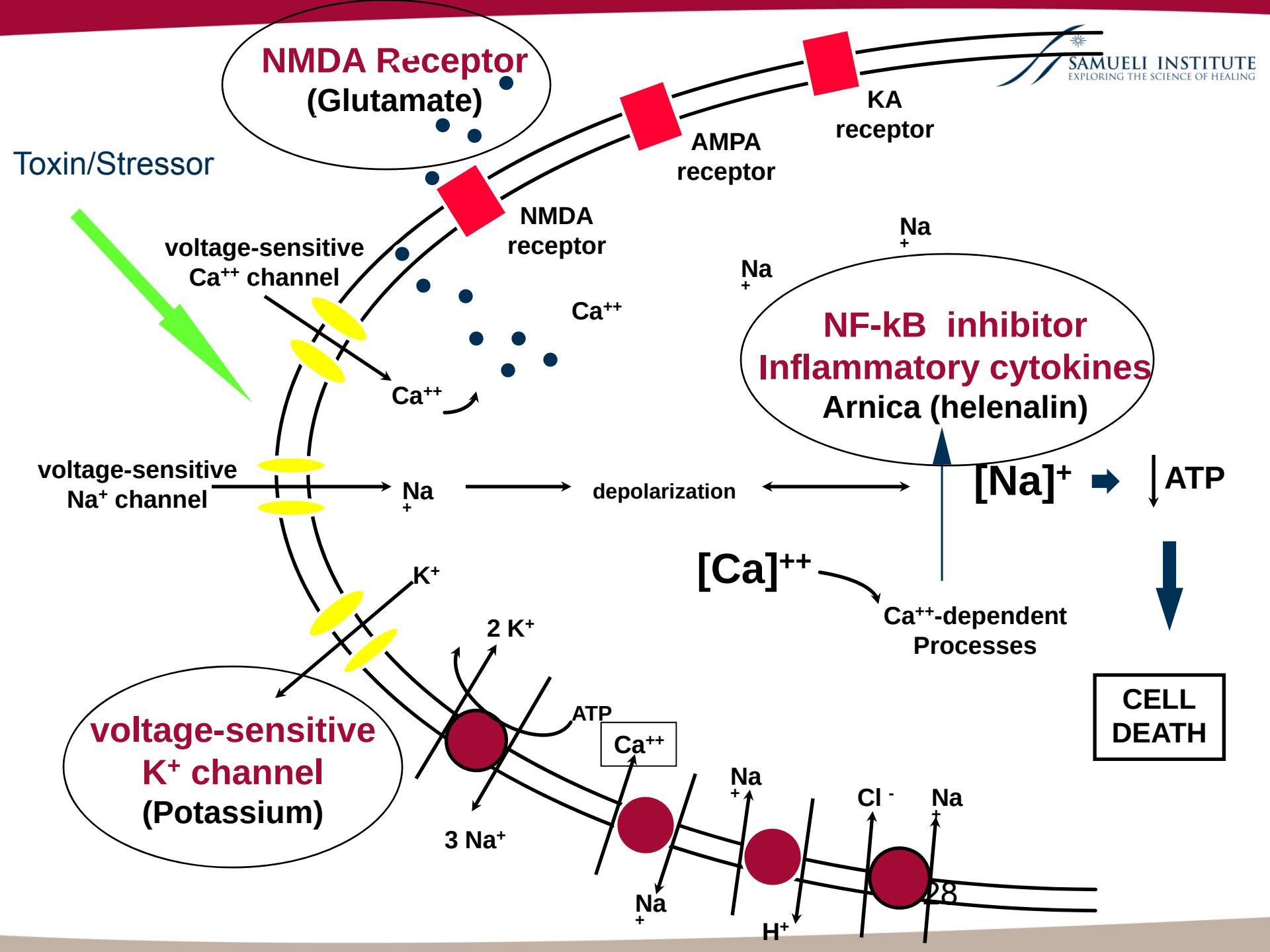
NMDA Receptor
(Glutamate)
Toxin/Stressor
KA
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AMPA
receptor
NMDA
receptor
voltage-sensitive
Ca++ channel
Ca++
Ca++
Na+
Na+
NF-kB inhibitor
Inflammatory cytokines
Arnica (helenalin)
voltage-sensitive
Na+ channel
Na+
depolarization
[Na]+
ATP
[Ca]++
Ca++-dependent
Processes
CELL
DEATH
K+
2 K+
voltage-sensitive
K+ channel
(Potassium)
ATP
Ca++
Na+
Cl -
Na+
3 Na+
Na+
H+

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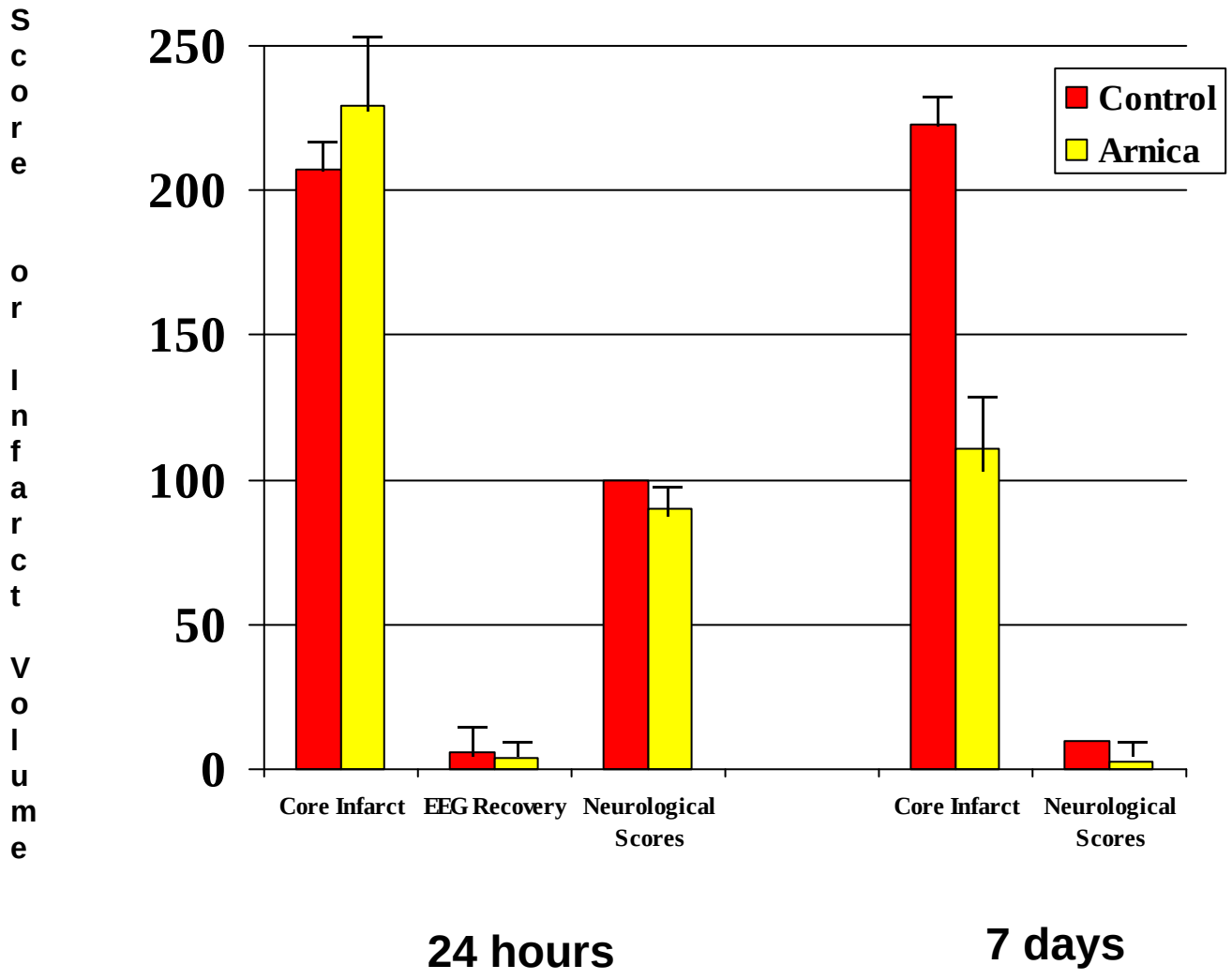
Neuroprotection from Low-Dose *Arnica*



Neurological Scores x 10

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Where might hormesis be used therapeutically?

- Radiation Protection
- Carcinogenesis
- Immunological conditions
- Brain Injury and Neurodegenerative Diseases
- Heavy Metal Toxicology

ANIMAL MODELS FOR THE STUDY OF LOW-DOSE PROTECTIVE EFFECTS

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EXPERIMENTAL MODEL	TREATMENT	EFFECT
Arsenic intoxication (Wurmser 1955, Cazin 1987-1991)	Arsenic -7M or -17M	Increase of urinary arsenic excretion
Mercury intoxication (Cambar 1983, Guillemain 1984)	Mercury -9M or -15M	Protection
Liver toxicity (CCl_4) (Bildet 1975-84, Palmerini 1993)	CCl_4 -7M or Phosphorus -7M, -15M, -30M	Protection
Experimental hepatocarcinoma (De Gerlach e Lans 1991)	Phenobarbital -9M	Decrease of tumor growth
Skin UVinflammation (Bastide, Poitevin, Bildet 1975-90)	Apis -7M or -9M	Protection
Adjuvant arthritis (Conforti 1995)	Intraperitoneal adjuvant	Protection
Bursectomised chicken embryos (Bastide 1993-1994)	Bursin -30-40M in ovo	Recovery of immune function

MODELS FOR THE STUDY OF LOW-DOSE PROTECTIVE EFFECTS

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EXPERIMENTAL MODEL	TREATMENT	EFFECT
Liver cancer	Phenobarbital 1ppm	Reduced foci
Liver cancer	DDT .01 ppm	Reduced foci
Multiple tumors	mistletoe lectin 1 ng/ml to 30 pg/ml	Increased thymidine uptake
Prostatitis (McLane and McMichael)	chorionic gonadotropin	Improved symptoms
Brian plasticity/memory (Diamond)	DHEA	Increased
T-cell function (Sharp)	deltorphin -8M -14M	Biphasic effects
Aging (Rattan)	Mild heat stress	Anti-aging effects
Diabetes (Cai)	Zinc MT	Prevention

Challenges in development of clinical hormesis

Challenges:

- Conceptual challenges
- Terminology
- Laboratory and toxicology focus
- Variability
- Relevance and utility

Needs:

- Need sound mechanistic basis for study of low-dose stimulation
- Need to optimize low-dose effects
- Need to exam the entire dose-response range for various agents
- Need to better communicate hormesis

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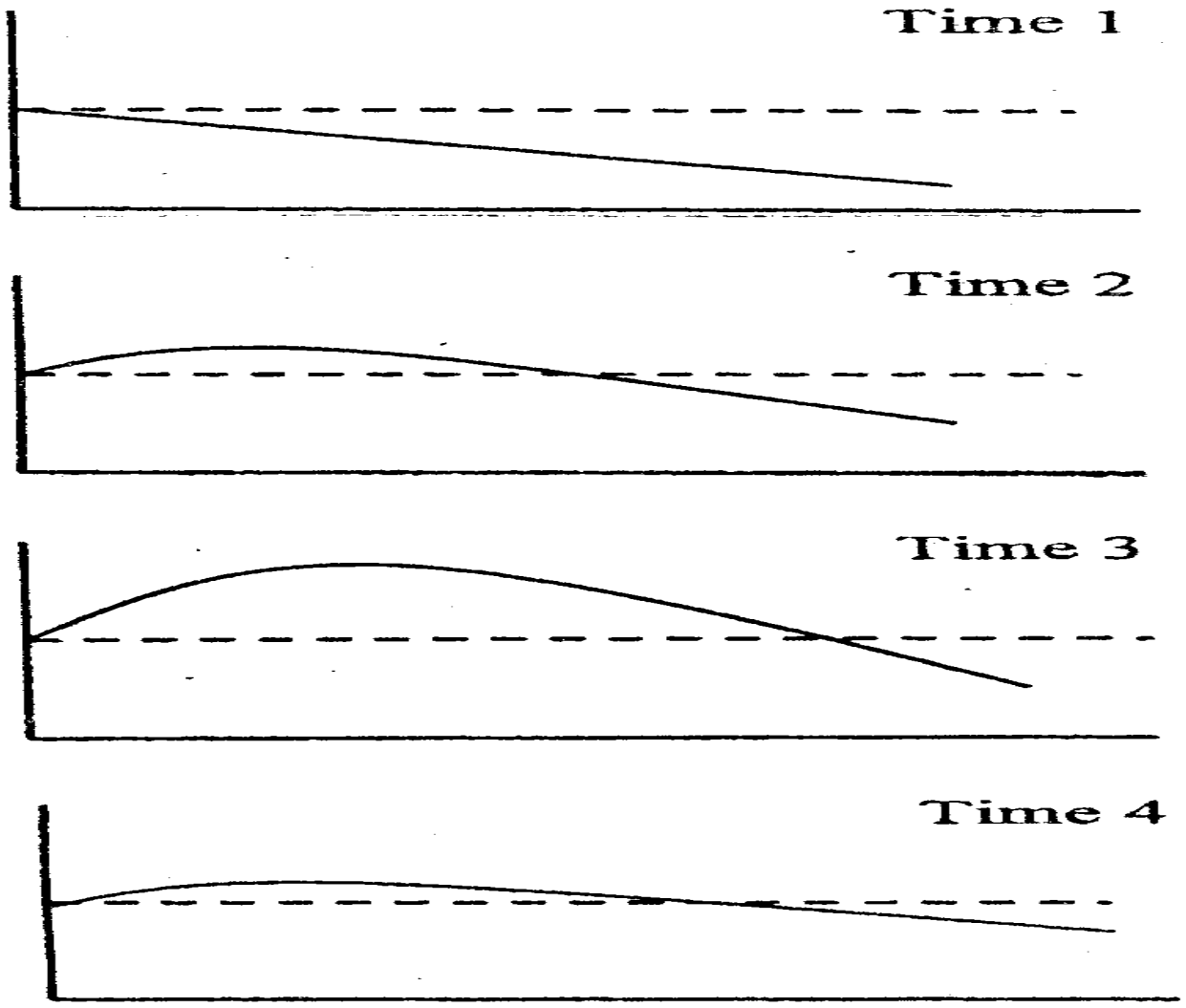
Challenges to Development of Clinical Hormesis

http://www.creators.com/comics/speed-bump/17088.html

Terms Used for Hormesis

- Non-linear
- Paradoxical
- Adaptive
- Stimulatory
- Threshold
- Bidirectional
- Biphasic
- BELLE
- Non-monotonic
- U/J-shaped
- Tolerance inducing
- Reparative
- Beneficial

Temporal Sequence of Hormetic Dose-Response Relationship



Calabrese and Baldwin 2001c.

Variability of Protection from Low-dose Toxic Agents

- NMDA ++
- Cyclohexamide +
- PLA_2 -
- Con-G -
- MMP -
- Arnica ±
- CN +
- Glutamate +++
- K –
- Silica +
- Mercury/Arsenic ++
- Tularemia +

Very few exposure-protection studies of most agents that matter clinically

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Language	**English**	**5**
Country	**Finland**	**1**
	U.S.	**4**
Toxin	**Chemical agent -Sarin**	**3**
	- Soman (1 combined with Sarin)	**2**
	Biological agent - Tularemia	**1**
Model	**Animal – rats/mice**	**4/1**
	Central nervous system	**2**
	Ex-vivo – spinal cord	**1**
Outcomes	**Physiologic**	**2**
	Mortality	**1**
	Multiple	**2**
Administration	**Perfusion**	**1**
	Injection	**3**
	Ingestion	**1**
Dilution Ranges	**Low dose - 0.003 nM to 1 μM sarin**	**1**
	- 1 – 100 μg/kg sarin	**1**
	- 12.5 & 50 μg/kg sarin	**1**
	- 4 & 20 μg/kg soman	**1**
	- 60 μg/kg soman	**1**

"I believe I have a new approach to psychotherapy, but like everything else, it first has to be tested on mice."

RAPID INDUCTION OF PROTECTIVE TOLERANCE (RIPT)

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VISION & OBJECTIVES

- A rapid protective countermeasure for CBNR threats?
  - Reduce CBNR mortality and injury by induction of cellular/whole organism hormetic tolerance.
  - Target agents are cyanide, botox, phosgene, tularemia, VEE, Athrax and radiation.
  - May be effective for emerging infections such as flu and the effects of environmental toxins

CONCEPT

Agent	Current Approach
Anthrax	Vaccine - 6 doses
*Tularemia	Antibiotics – 6 weeks
*Cyanide	Hyperbaric Oxygen
*Brain Trauma	ICU Surgery
Mustard	Barrier Methods
Radiation	Potassium Iodide
*Cadmium	HE-2100

RIPT Approach

1 dose

2 X day

During threat situations

***Tested**

Result
LD_{50} increased 3X
LD_{50} increased 3X
Reduce mortality 30%
40% reduced injury
50% reduced damage
60% reduced damage

Exposure to sub-toxic doses of toxic compounds can confer protection to or modulation damage from higher doses of same or similar harmful agents.

A Cellular

Hormetic Bioshield?

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Publications

1. Diane Marotta, D.,Marini, A., Banaudha, K., Maharaj, S., **Jonas, W. B.** Non-linear effects of glutamate and KCL on glutamate toxicity in cultured rat cerebellar neurons. *International J. Neuroscience.* 2003.
2. **Jonas, W.B.,** Kaptchuk, T., Linde, K. A critical overview of homeopathy. *Annals of Internal Medicine.* 2002 (in press).
3. Marotta, D., Marini, A., Banaudha, K., Maharaj, S., Ives, J., Morrissette, C.R., **Jonas W.B.**, Non-linear Effects of Cycloheximide in Glutamate-treated Cultured Rat Cerebellar Neurons. *Neurotoxicology*. 2002; (in press).
4. **Jonas, W.B**., Anderson, R.L., Crawford, C.C., Lyons, J.S. A systematic review of the quality of homeopathic clinical trials. *BMC Complementary and Alternative Medicine*. 2001, 1: 12 (31 December 2001). URL: http://www.biomedcentral.com/browse/medicine.
5. **Jonas, W.B.,** Lin, Y., Tortella, F. Neuroprotection from glutamate toxicity with ultra-low dose glutamate. *NeuroReports*. 2001; 12: 335-339.
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