# Methylene Blue Dose-Response

## Hormesis of Ehrlich's "Magic Bullet"

By

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# The Questions

1. Why is methylene blue a “magic bullet”, and what is it good for?
2. What is its unique molecular mechanism of action? What is an autoxidizable dye with electron cycling? Is that the magic?
3. How can it show hormetic dose-response effects at biochemical, physiological and behavioral levels?

# Methylene Blue and the Birth of Chemotherapy

- First synthetic chemical tested in human patients
- Synthesized by Caro in 1876 as an aniline dye
- Ehrlich injected into live rats in 1886, and used term "magic bullet" to describe its selective uptake by nerve tissue
- Ehrlich in 1891 showed was effective in malaria
- Wide range of uses: e.g., antidote against poisons, antimicrobial, supravital staining and diagnostic uses, medicinal photosensitizer, cancer chemotherapeutics, psychoactive uses in bipolar disorder, depression and psychosis.

# Revolutionary Progeny of Methylene Blue

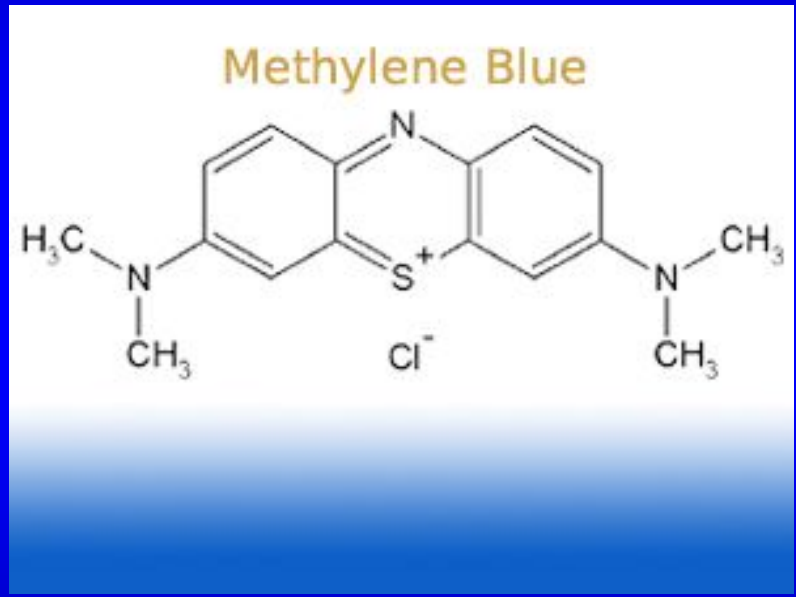
- First phenothiazine compound developed
- Derivatives spearheaded use of synthetic dyes in the industrial revolution
- Lead compound for first antimalarial drugs and antihistamines
- Lead compound for first antipsychotics, such as chlorpromazine, that brought about a revolution in the treatment of psychiatric patients (birth of psychopharmacology)

# A new revolution for the magic bullet in the 21st century?

- In the 2000's: metabolic-enhancing and antioxidant effects facilitate memory and neuroprotection
- Facilitation of psychotherapeutic memories in the treatment of anxiety disorders, e.g. phobias and PTSD
- Neuroprotective agent against neurodegenerative disorders, e.g. optic neuropathy and Alzheimer's disease

# Methylene blue (methylthioninium chloride)

- Redox compound
- **<u>Radical scavenging antioxidant</u>**
- Rapid NADH oxidation and electron donating/shuttling capacities
- FDA-approved for methemoglobinemia
- Experimental clinical use in chemotherapy induced encephalopathy and Alzheimer's disease



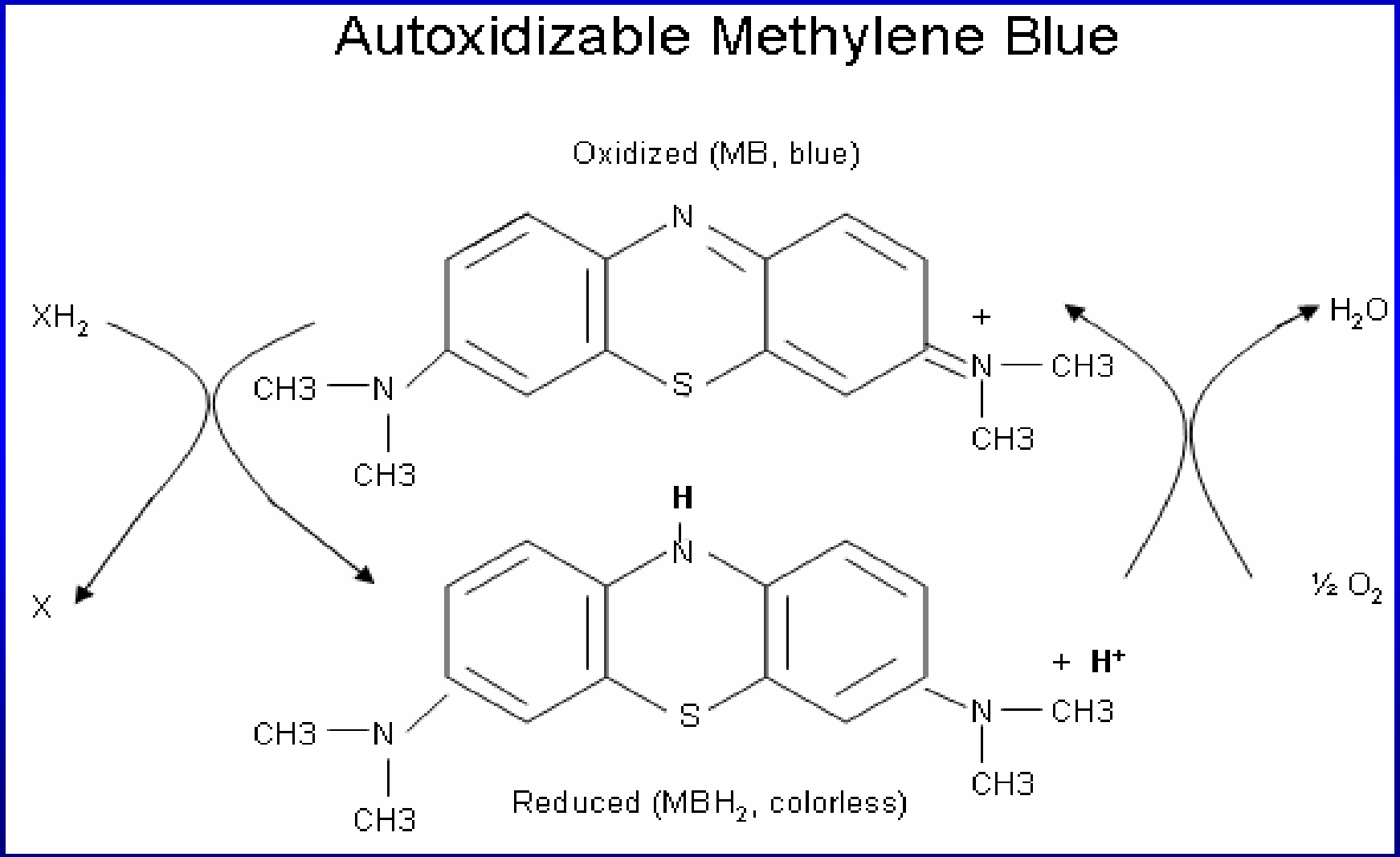

Visarius et al. 1997

Wainwright & Crossley, 2002

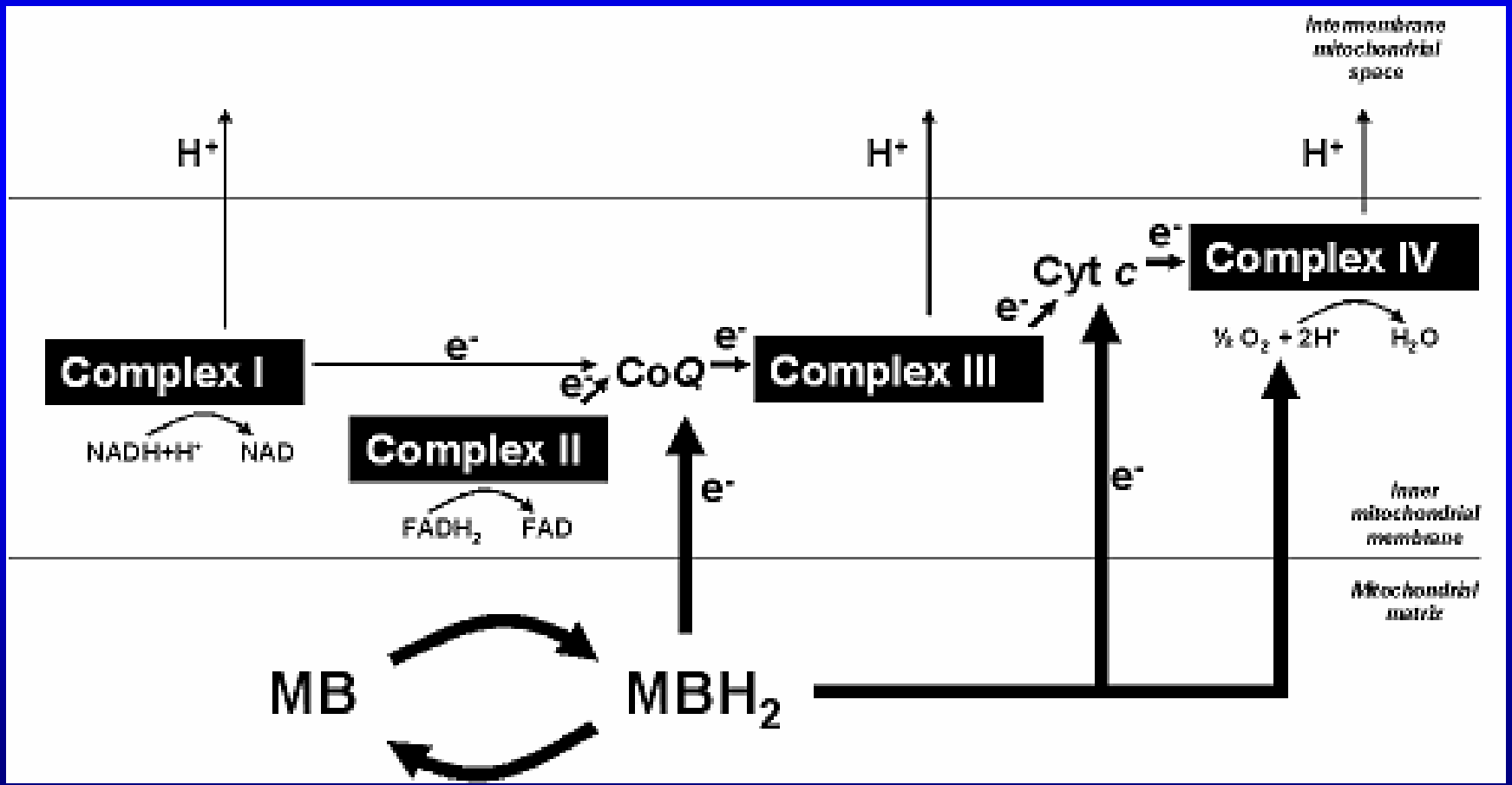
Riha et al. 2005

Bruchey & Gonzalez-Lima, 2008

# The Magic: Autoxidizing property of methylene blue for electron cycling



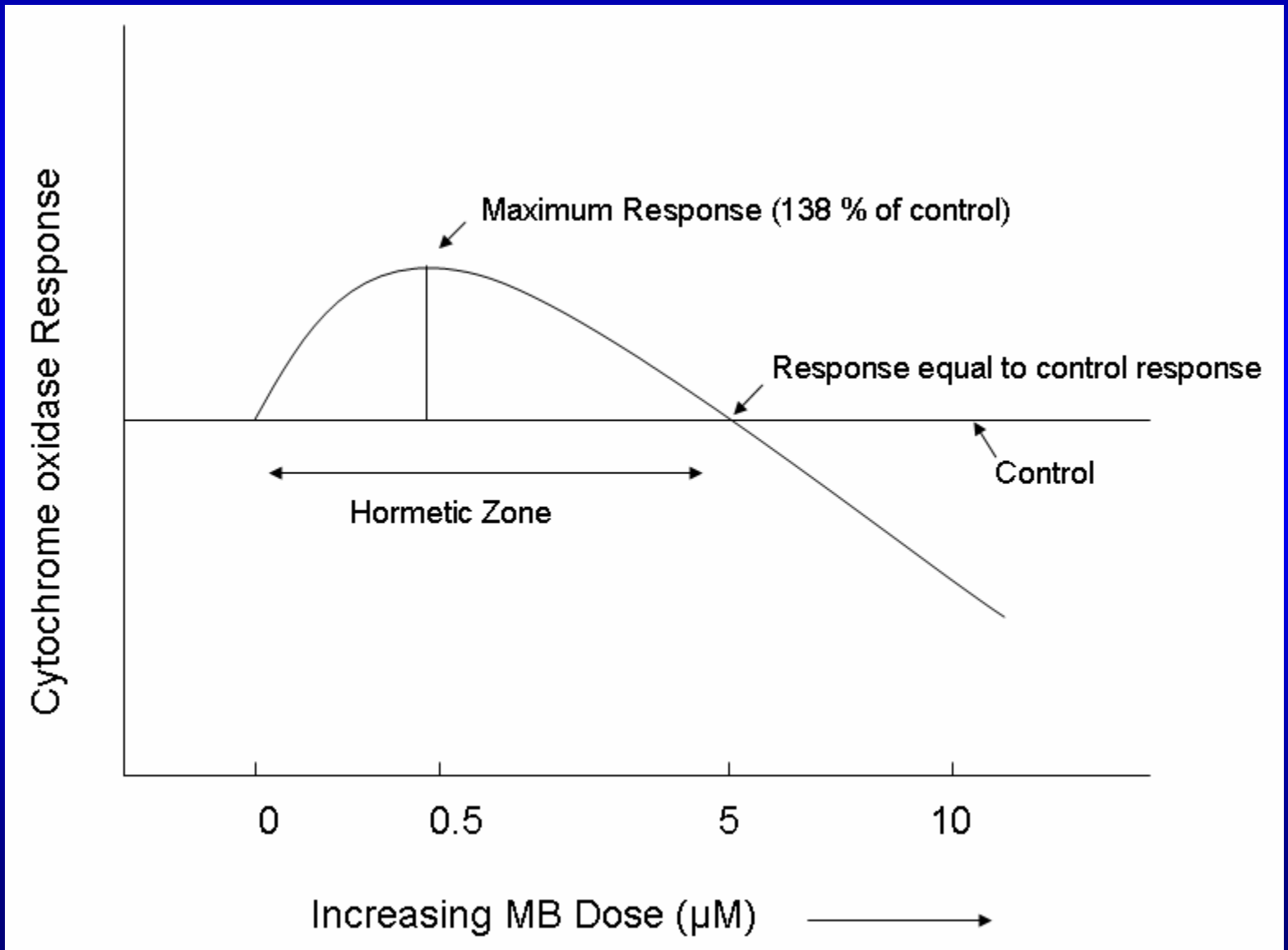
# Methylene blue as artificial electron donor to mitochondria electron transport chain complexes (increases energy) and to oxygen (prevents superoxide formation)



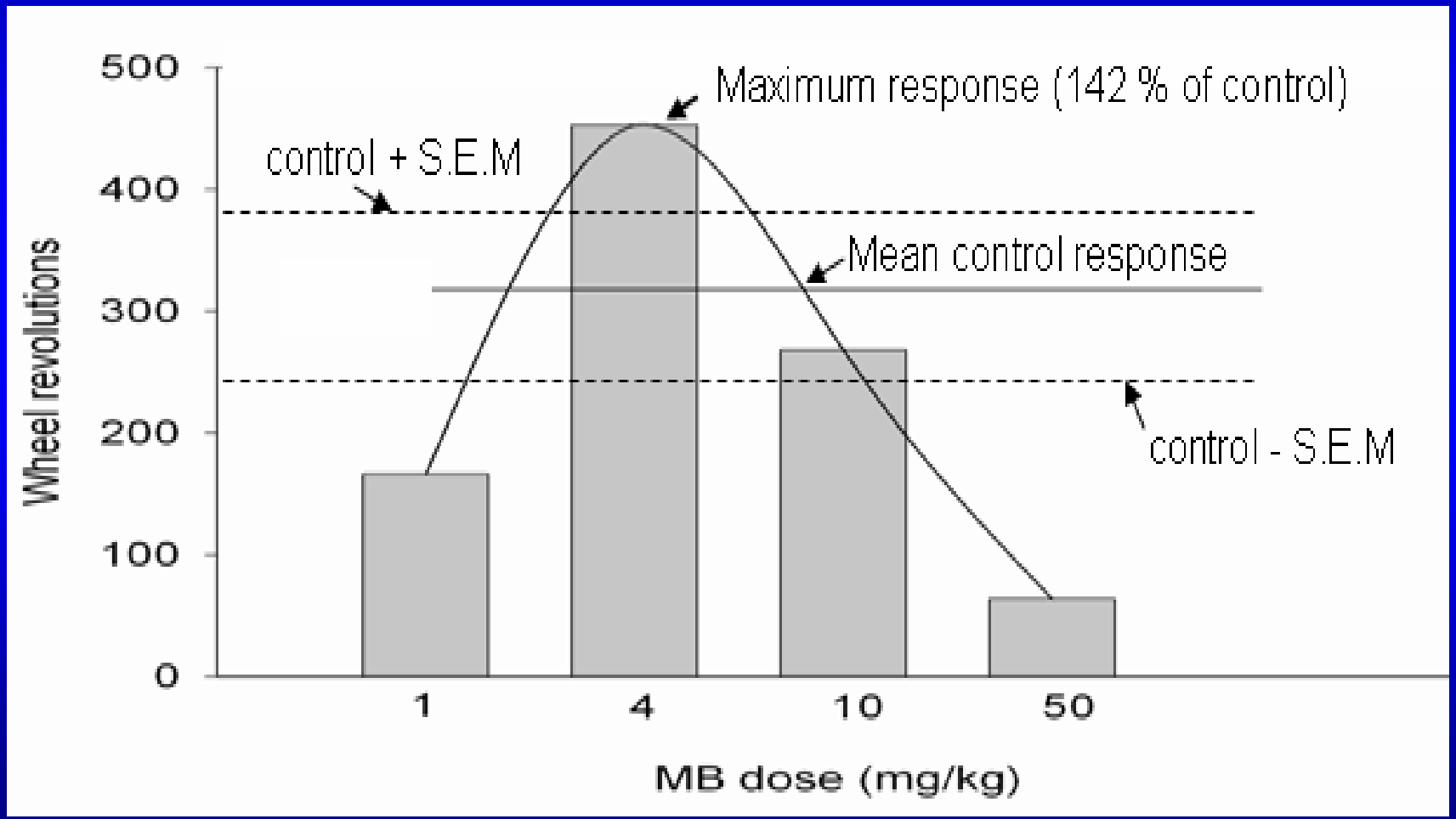
# Autoxidizable Mechanism in Hormesis

- To enter cytosol, MB is reduced to lipophilic form MBH2 at cell surface
- Inside cell, MBH2 is re-oxidized to MB by heme proteins
- At *low* doses, there is MB-MBH2 equilibrium (electron cycling) and MBH2 can *donate electrons* to ETC complexes and oxygen, leading to enhanced energy metabolism and decreased superoxide formation
- At *high* doses, equilibrium is impaired and MB can *take electrons* away from ETC complexes, leading to decreased activity of these complexes and more oxidative stress

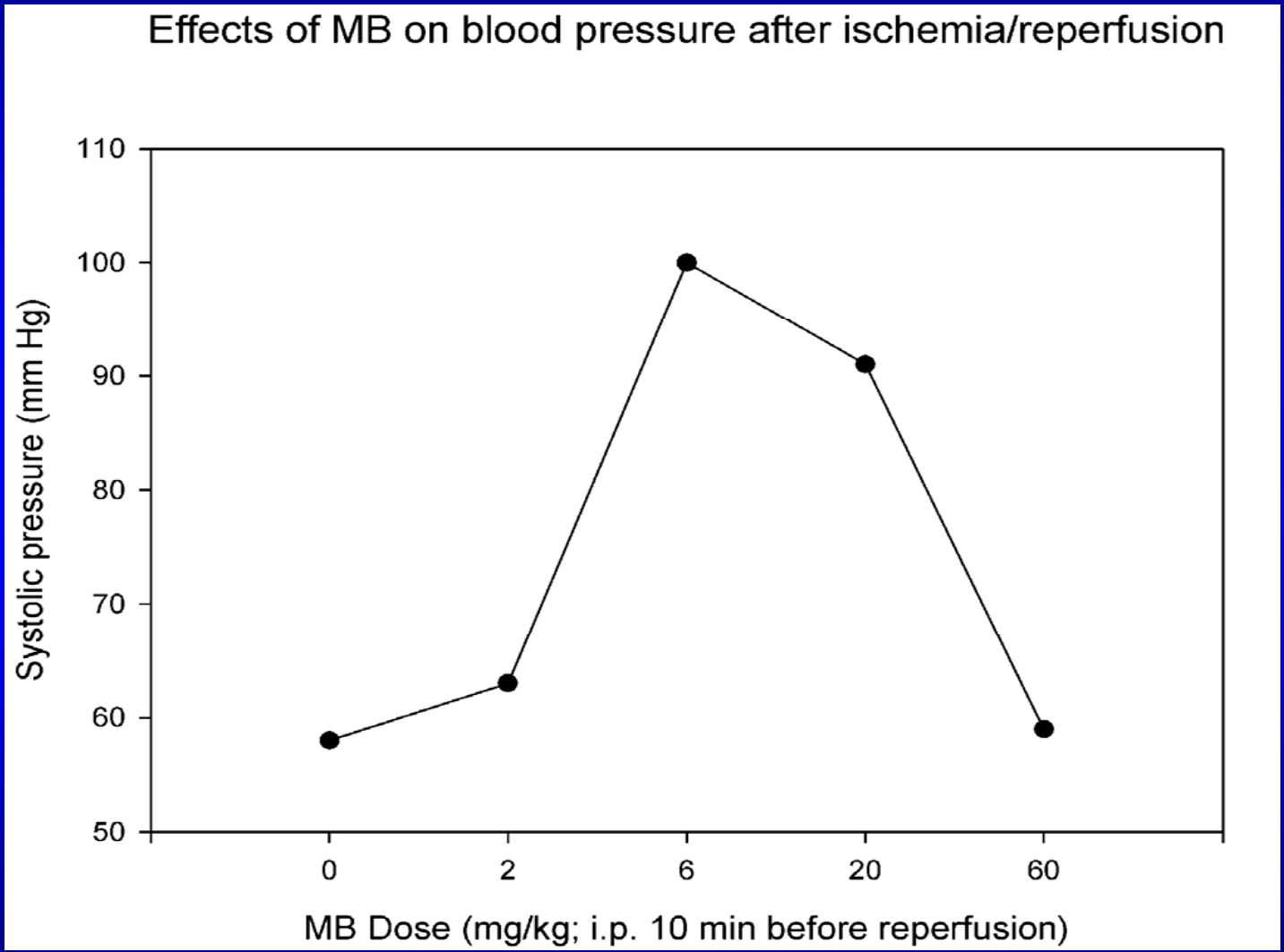
# Biochemical: MB hormetic effects on brain cytochrome oxidase activity (complex IV)



# Behavioral: MB hormetic effects on spontaneous locomotion in rats (24 hours)

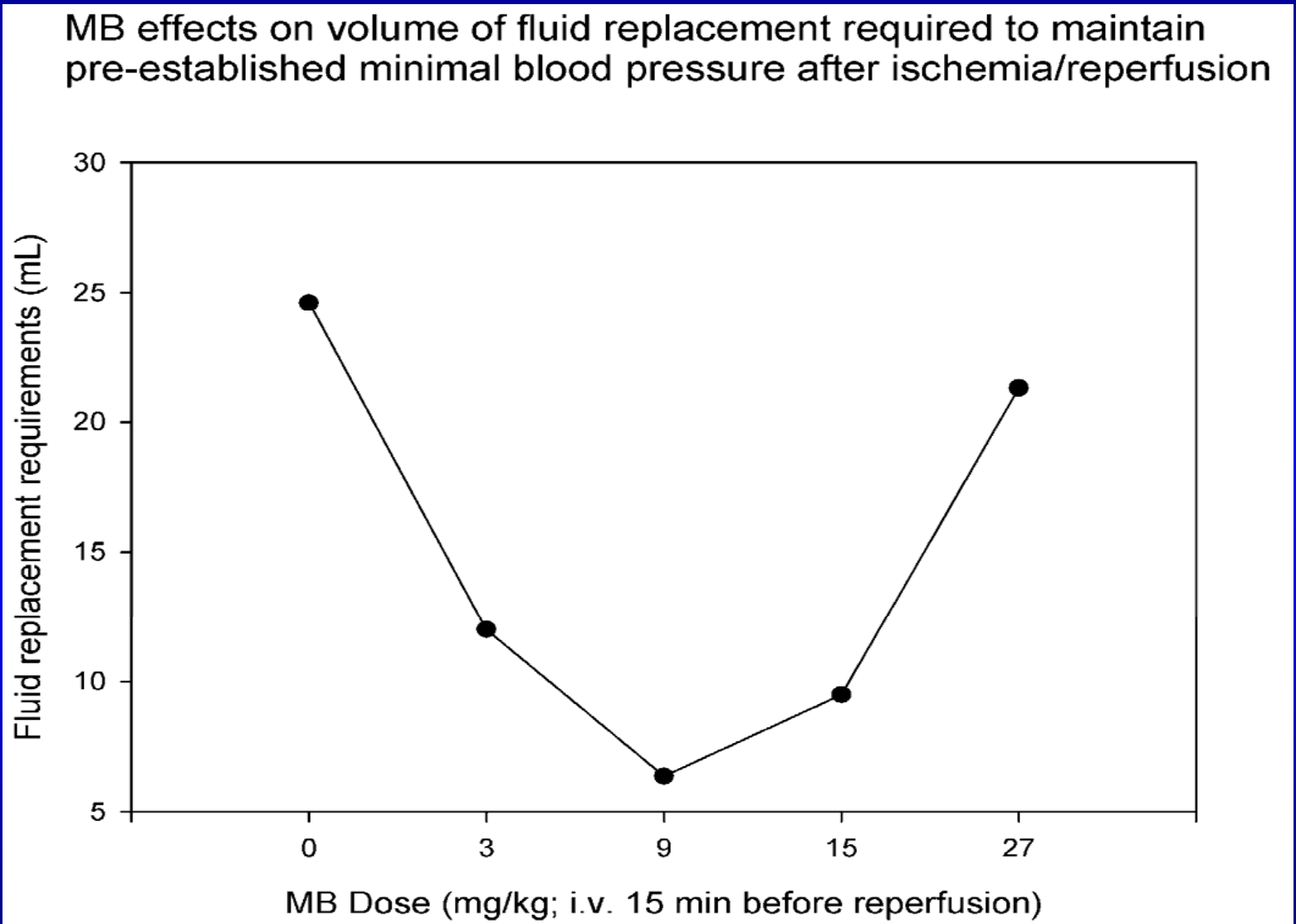


# Physiological: MB prevents ROS-induced loss of aortic tone



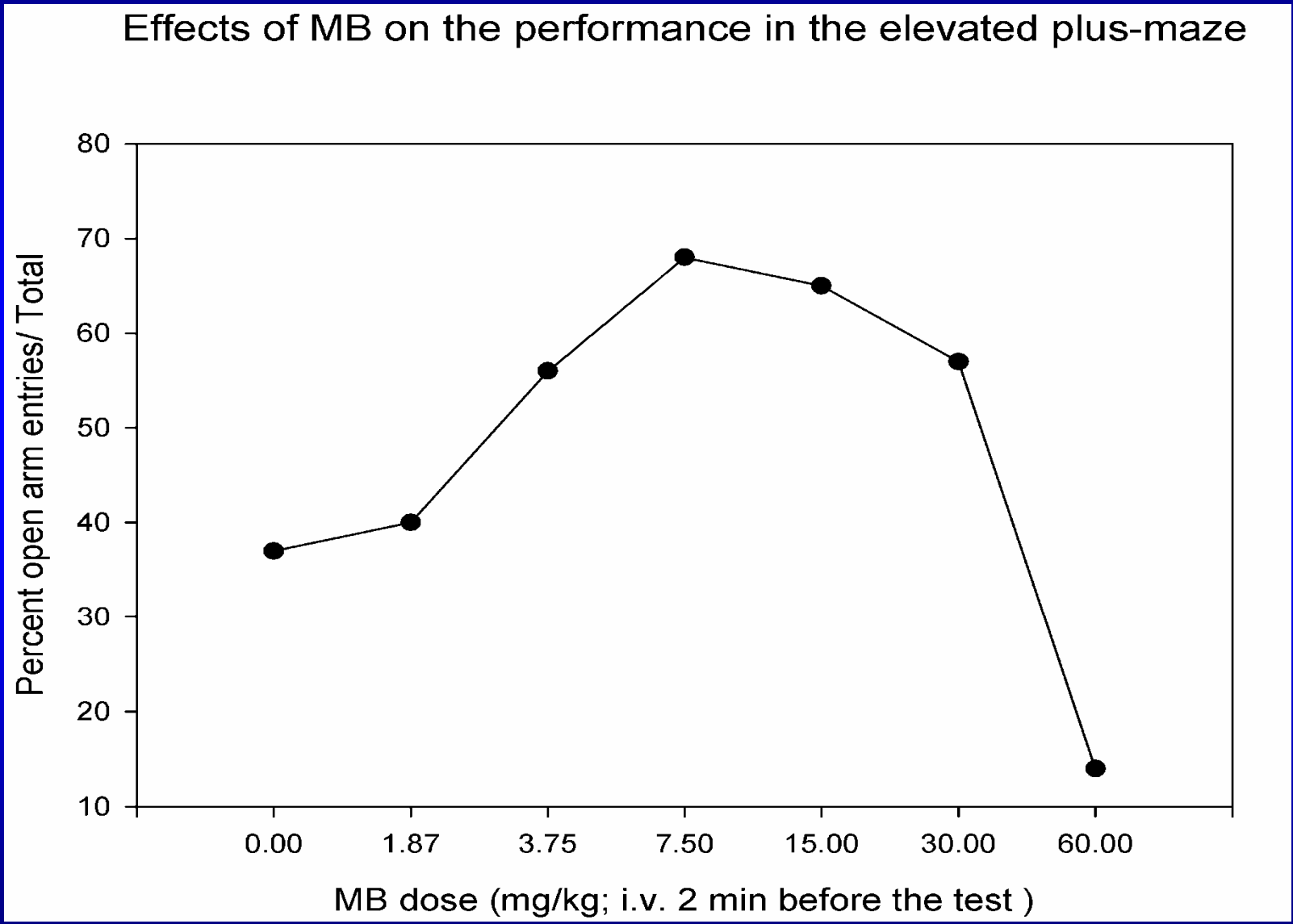

**Modified from Weinbroum et al. (2002). Methylene blue in preventing hemodynamic and metabolic derangement following superior mesenteric artery clamping/unclamping: An intrathecal vs. intraperitoneal dose-response study. *Shock,* 17, 372-376**

# Physiological: MB prevents ROS-induced loss of blood volume



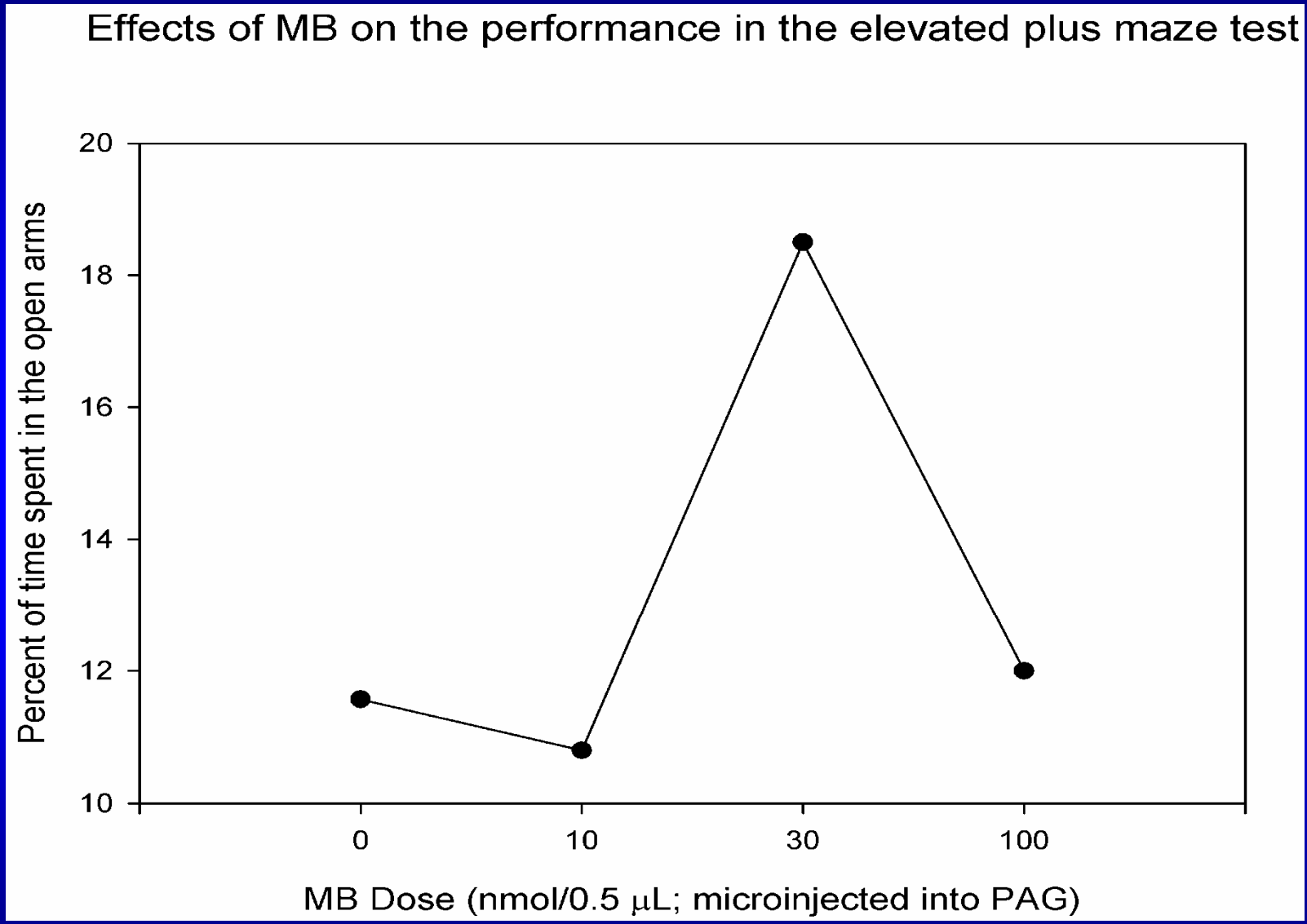

**Modified from Weinbroum, A.A. (2004). Methylene blue attenuates lung injury after mesenteric artery clamping/unclamping. *Eur. J. Clin. Invest*., 34, 436-442**

# Anxiety: MB intravenous before test



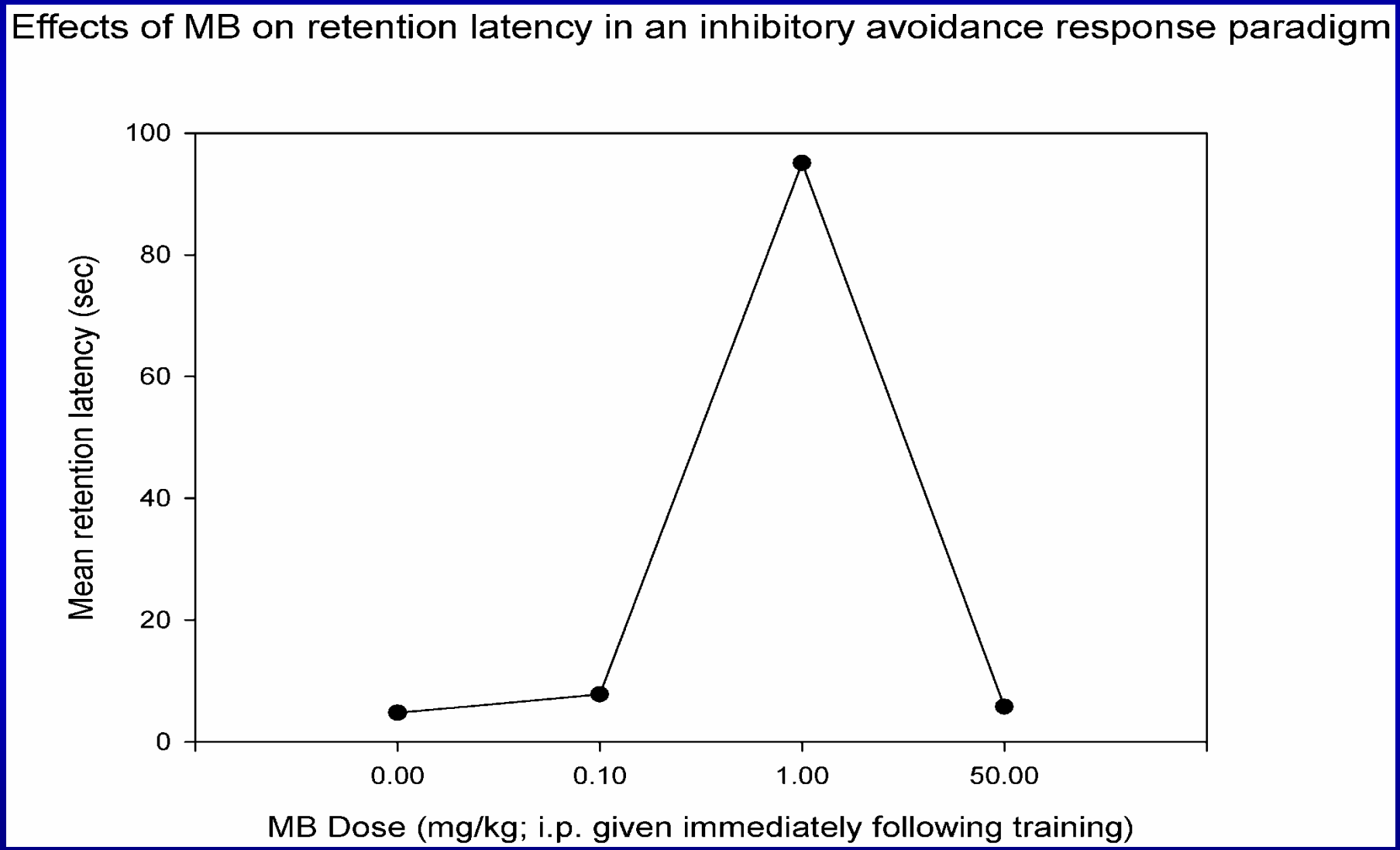

**Modified from Eroglu and Caglayan (1997). Anxiolytic and antidepressant properties of methylene blue in animal models. *Pharmacol. Res.* 36, 381-385**

# Anxiety: MB intracerebral administration




**Modified from De Oliveira and Guiaraes (1999). Anxiolytic effect of methylene blue microinjected into the dorsal periaqueductal gray matter. *Brazilian J. Medical Biol. Res.* 32, 1529-1532**

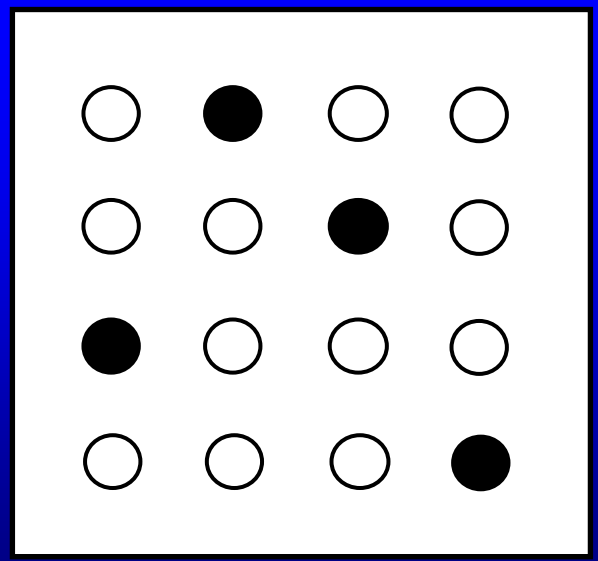
# Memory: Hormetic effects of post-training methylene blue



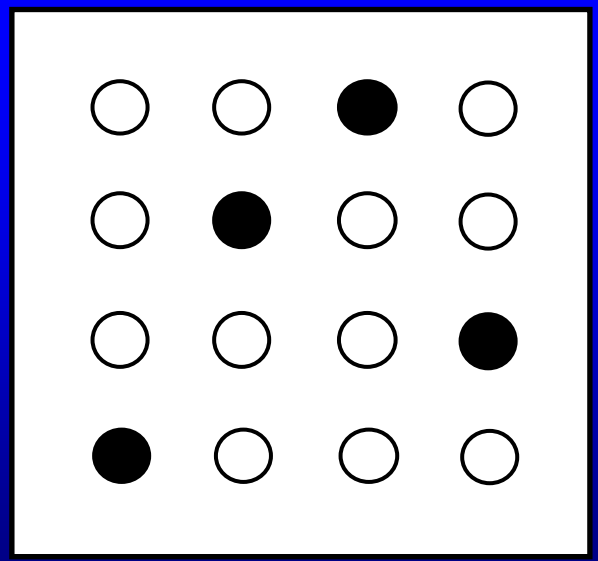

**Modified from Martinez Jr. et al. (1978). Methylene blue alters retention of inhibitory avoidance responses. *Physiological Psychology* 6(3), 387-390**

# Holeboard Memory Test:

Solid circles (●) correspond to holes baited and open circles (○) to holes unbaited in the baiting patterns used in training trials. The first baiting pattern (BP1) was used during the five days of training in the first run. The unbaited probe test on the sixth day was scored according to this same pattern. Fifteen days following the first run, the second run began using the second baiting pattern (BP2/reversal) for the five days of training trials. The unbaited probe test on the sixth day of the second run was scored according to BP2/reversal. To avoid any difference in difficulty between the two patterns, BP1 and BP2 were mirror images.



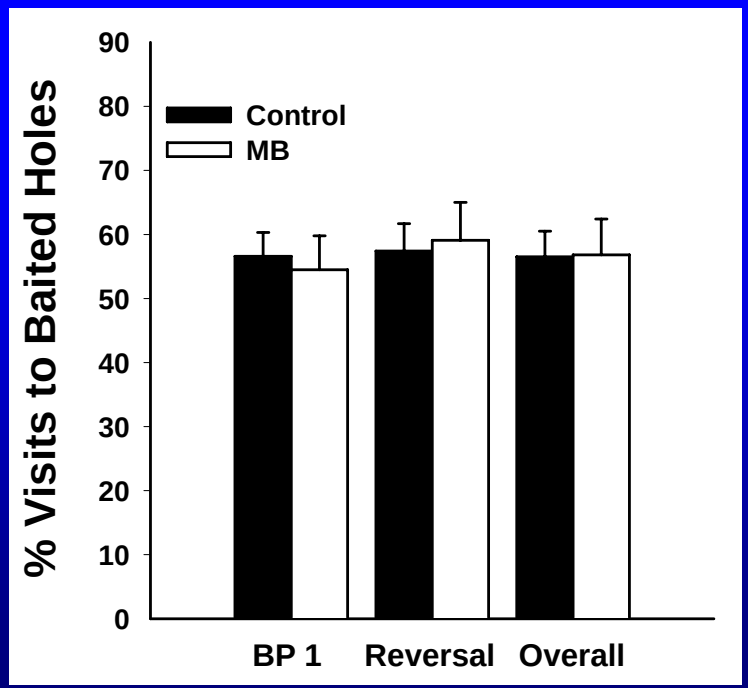
**BP1**



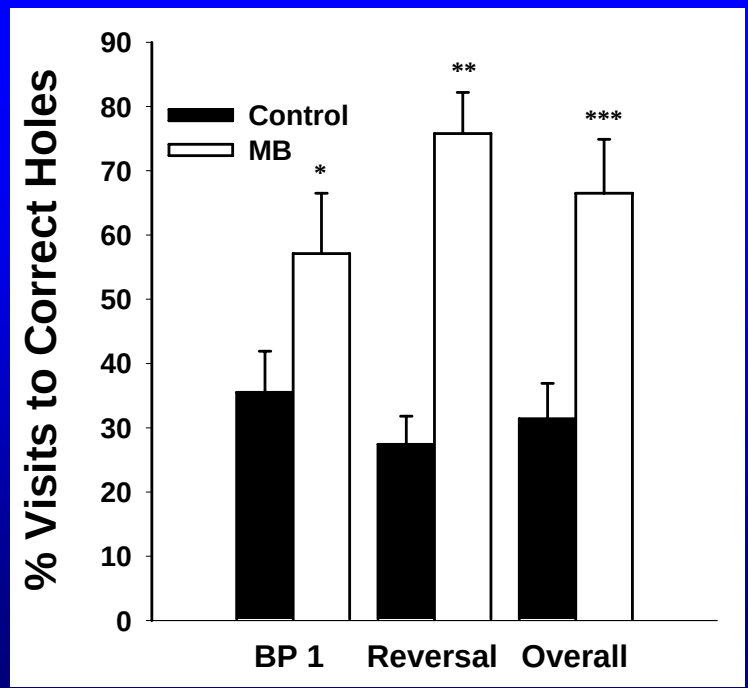
**BP2/Reversal**

NORMAL RATS: Mean ± standard error bars for spatial learning performance (% visits to baited holes) in training trials (A) and memory retention scores in probe trials (B) in groups of control and methylene blue (MB)-treated subjects. The percentage score is shown for the first baiting pattern (BP1), the second baiting pattern (BP2/reversal), and the averaged total (overall).
*p = 0.037, **p = 0.000014, ***p = 0.00017.

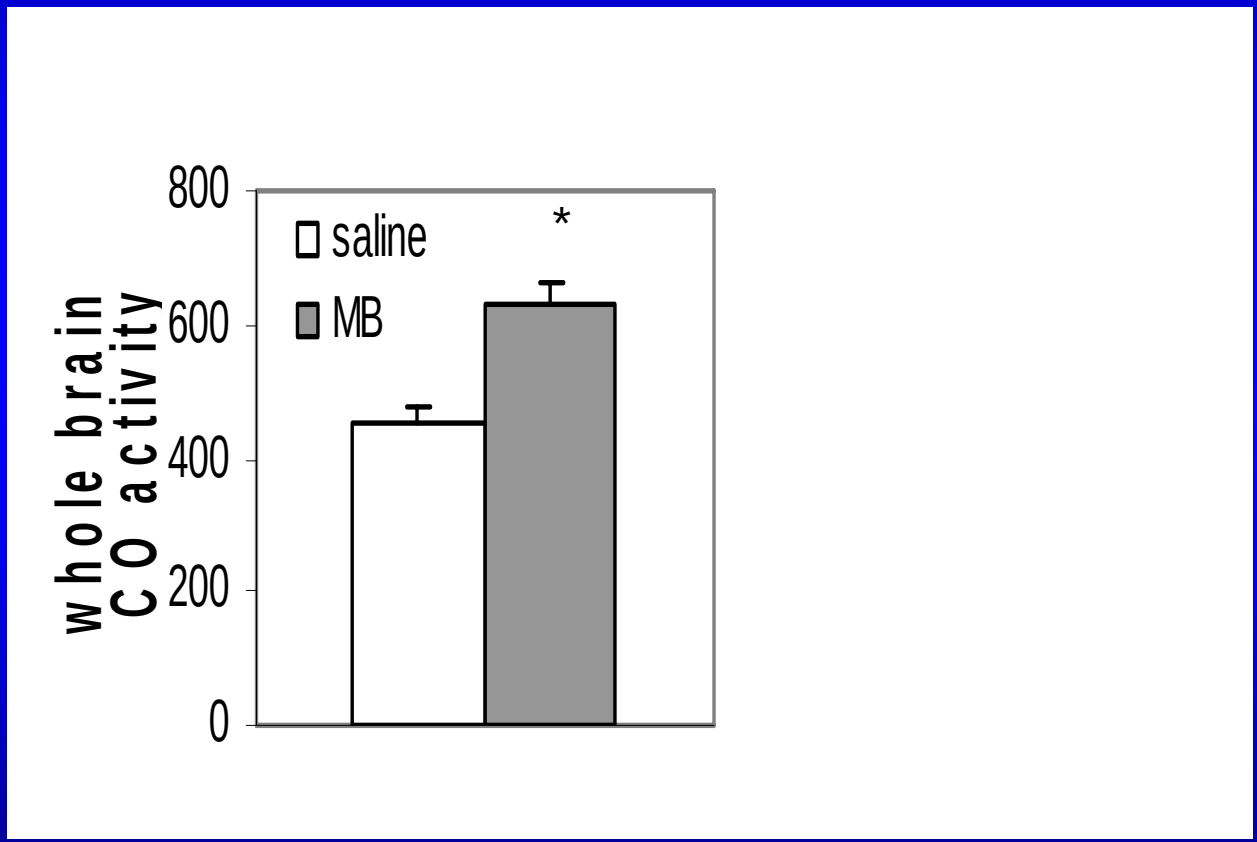
**A. Performance in Training Trials**



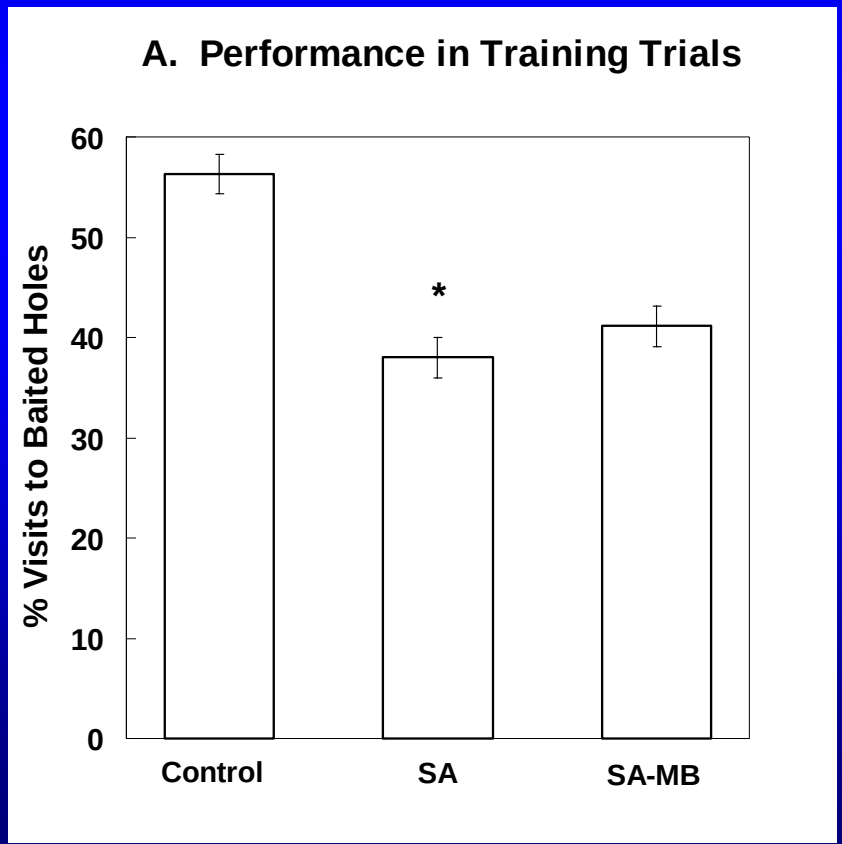

**B. Memory Retention in Probe Trials**

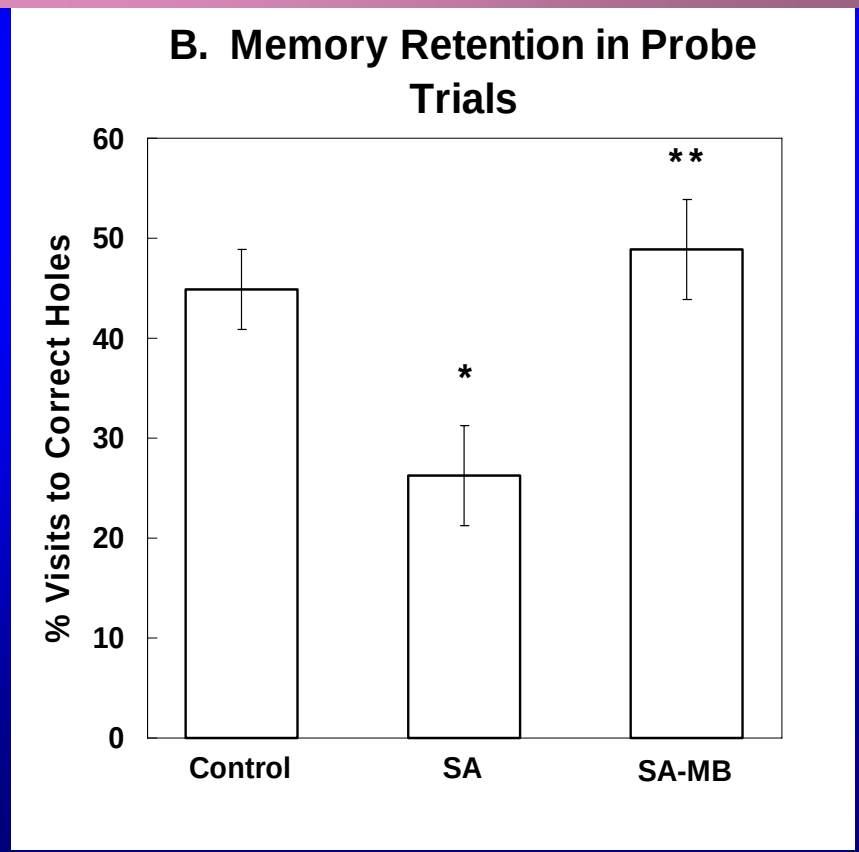


# MB increases brain cytochrome oxidase activity 38% by 24 hrs after 5 daily 1 mg/kg injections

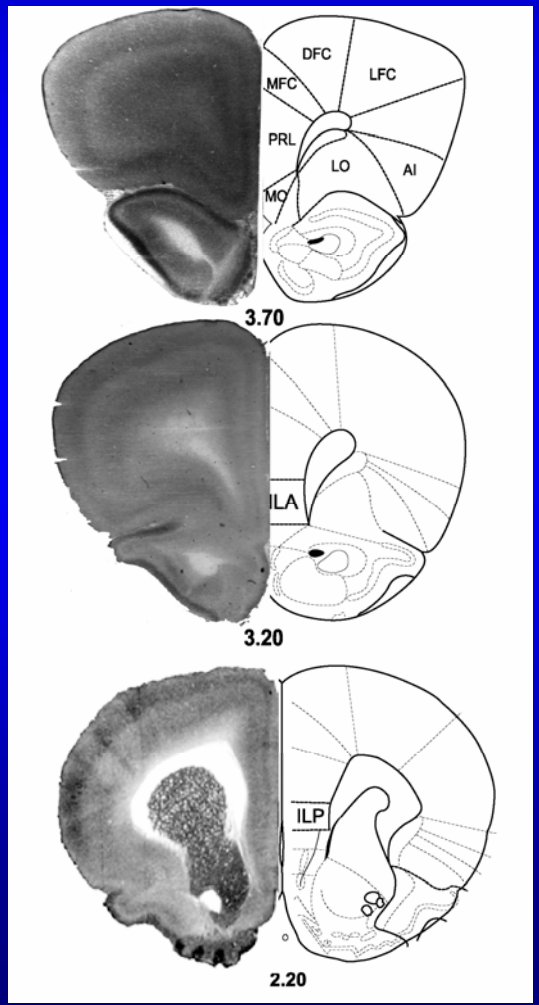


IMPAIRED RATS: Mean ± standard error (SE) bars of spatial learning performance in training trials (A) and memory retention in probe trials (B) in groups of control rats (C) and rats treated with sodium azide (SA) or sodium azide plus post-training methylene blue (SA-MB).
*C vs. SA, $p < 0.05$, Bonferroni-corrected. **SA vs. SA-MB, $p < 0.05$, Bonferroni-corrected.



# MB (4 mg/kg) increases cytochrome oxidase metabolism of brain regions mediating memory retention




Brain-behavior correlations between cytochrome oxidase activity and post-extinction freezing scores

| Region | | r | p |
|---|---|---|---|
| Dorsal Frontal Cortex* | (DFC) | -0.69 | 0.005 |
| Medial Frontal Cortex* | (MFC) | -0.66 | 0.008 |
| Anterior infralimbic cortex* | (ILA) | -0.65 | 0.009 |
| Prelimbic frontal cortex | (PRL) | -0.60 | 0.018 |
| Lateral orbital cortex* | (LO) | -0.68 | 0.006 |
| Whole brain homogenate | (WB) | -0.4 | 0.125 |

# Conclusions

- Methylene blue shows hormetic dose-responses at biochemical, physiological and behavioral levels
- Low and high doses produce opposite effects
- Methylene blue's autoxidizable property promotes electron cycling in mitochondria at *low* doses, which may be the molecular basis for its general "magic" actions *in vivo*:

  1. Energy production by electron transport chain, and
  2. Antioxidant scavenging of reactive oxygen species

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