



# BEHAVIORAL AND SIGNALING EFFECTS OF BERRY FRUIT

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# What Is Aging?

- Aging results from a complex set of processes that renders the body less able to activate protective mechanisms to counteract stressors that can damage the body leading to a progressive dysregulation with advancing age.
- In the neuroscience world, aging is characterized by progressive losses in neuronal function accompanied by behavioral declines (decreases in motor and cognitive performance) in both humans and animals.



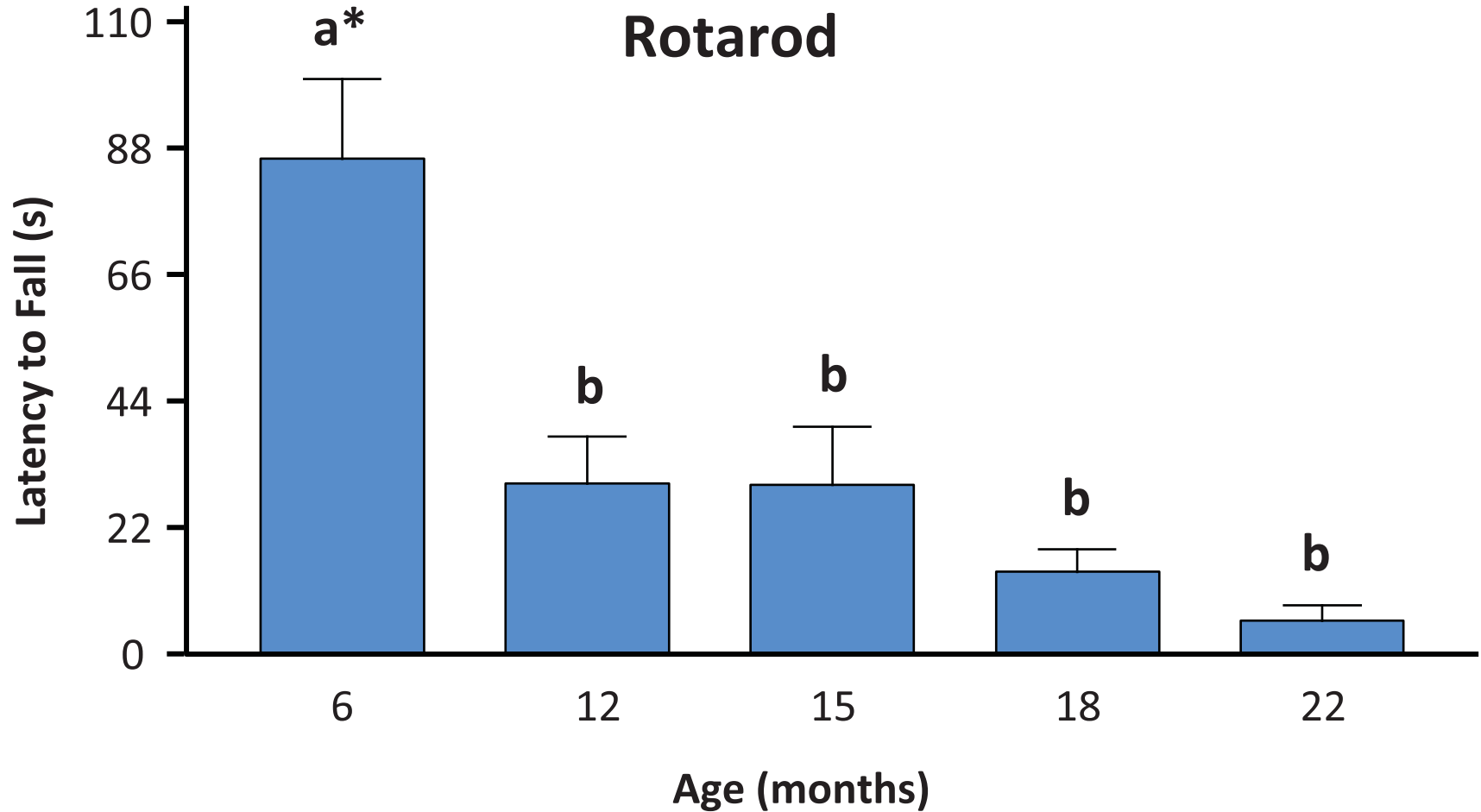
# The Four Stages Of Life

- 1) You believe in Santa Claus.
- 2) You don't believe in Santa Claus.
- 3) You are Santa Claus.
- 4) You look like Santa Claus.



# Rotarod Test

## Rat Age Study

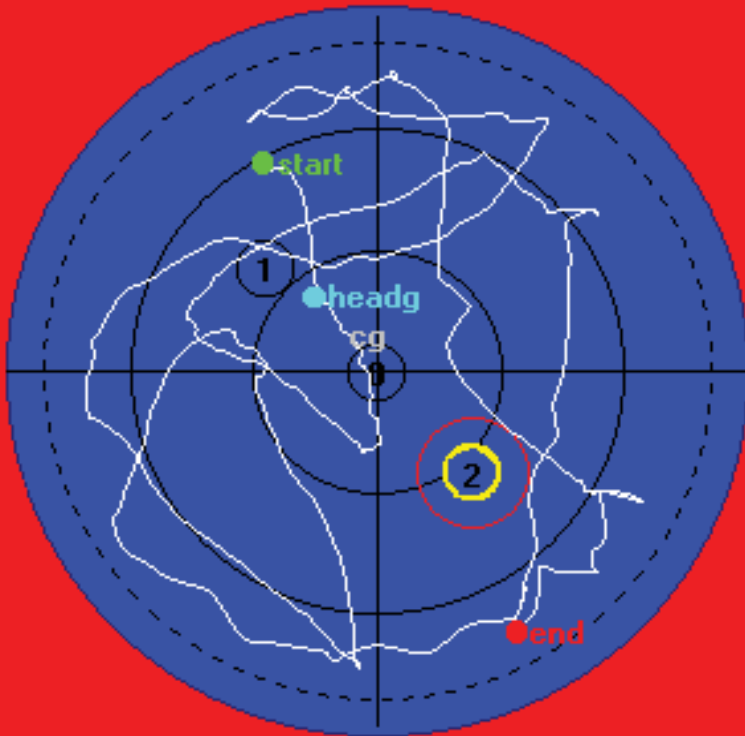


\*Means with different letters are significantly different from each other ( $P < .05$ ).

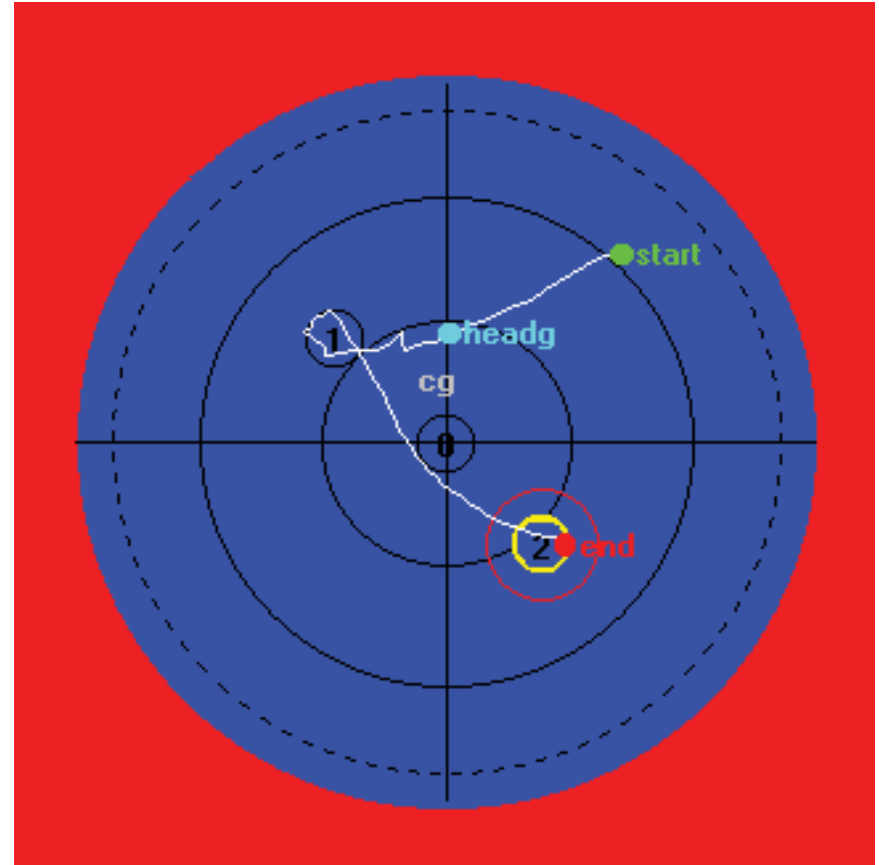
Shukitt-Hale et al. *Exp Gerontol.* 1998;33:615-624.

# Typical age-related loss of spatial memory seen in old rats in Morris Water Maze paradigm

Old rat



Young Rat



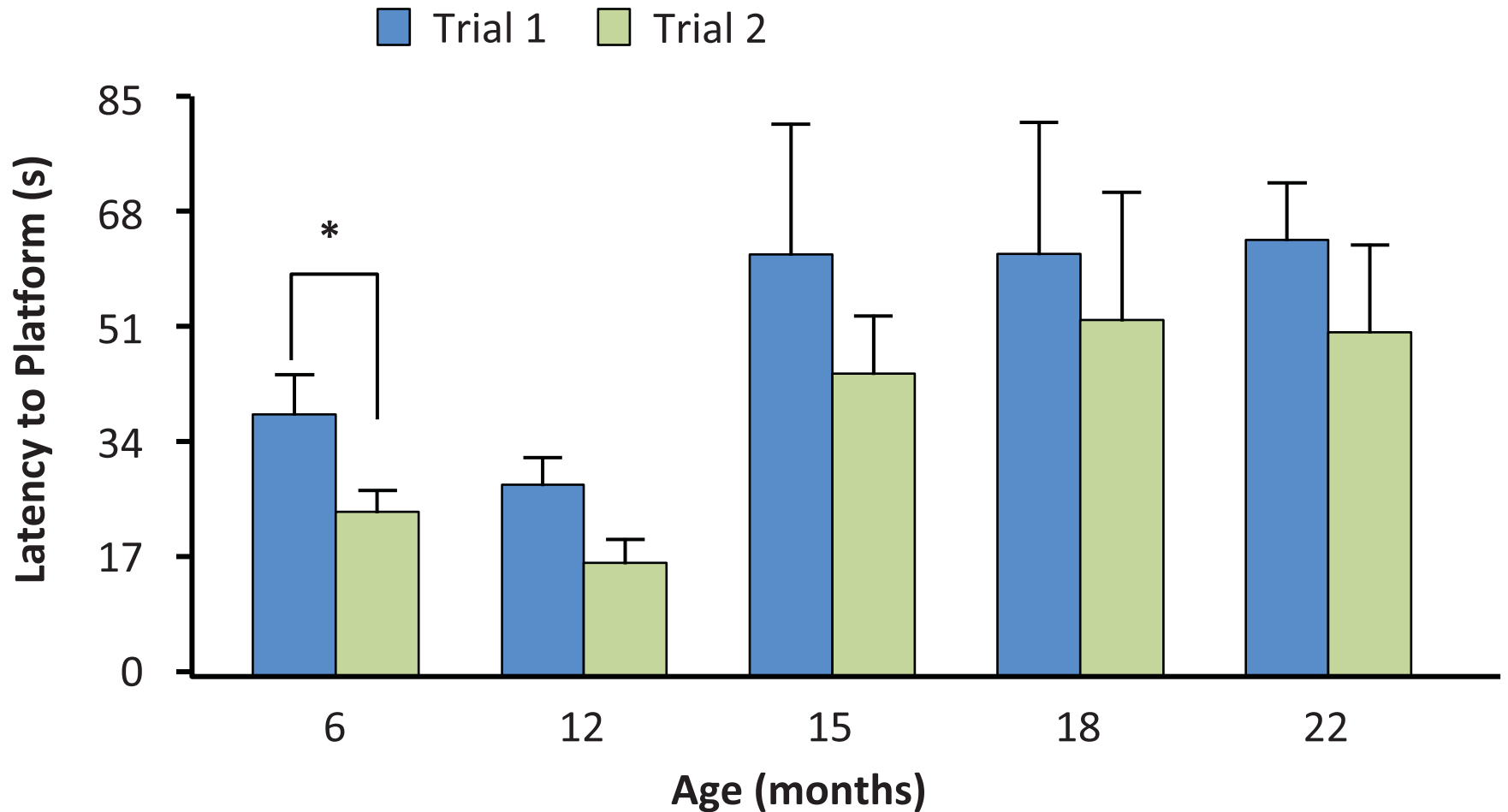
Reversal Test:

(1) represents original platform escape position on training days.

(2) represents reversed platform escape position on current test day.

# Morris Water Maze

## Rat Age Study



# Oxidative Stress And Inflammation In Brain Aging

- At rest the brain utilizes 20% of the body's oxygen.
- Markers to both inflammation and oxidative stress increase as a function of age and the brain becomes more sensitive to them.
- When coupled with genetic changes the brain becomes more vulnerable to diseases such as Alzheimer disease.



- We must find strategies to improve behavior, possibly by changing the neuronal environment by altering oxidative stress and inflammatory components.
- Research in our lab and others has shown that the behavioral deficits seen in aging can be retarded or even reversed by the polyphenolics in berry fruits, possibly by increasing antioxidant and/or anti-inflammatory levels.





# BEHAVIORAL STUDIES

# Procedures

- In all of our supplementation studies in aging, the rats were 19 months of age and supplemented for 8 weeks, typically at 2% of the diet.
- In the original study, an AIN 93 diet was used, and in all others an NIH-31 diet was used.
- In the purple grape juice and plum juice studies the animals drank the juice. For all others, the fruit, vegetable, or nut extract was added into the diet.



# Summary Of Findings

- Nutritional interventions can forestall age-related deficits in learning and memory, but did not affect motor behavior.
- Nutritional interventions can reverse deficits in learning and memory and declines in motor behavioral performance.
- The beneficial effects of berry fruit on behavioral performance were seen even when superimposed on an already well-fortified, healthy diet.



# Reversals of Brain Aging Markers after Phytochemical Supplementation

## Dietary intervention improved behavior

- Morris water maze performance
- Motor behavioral performance (rod walking, accelerod)
- Motor behavioral learning
- Exploration of a novel environment

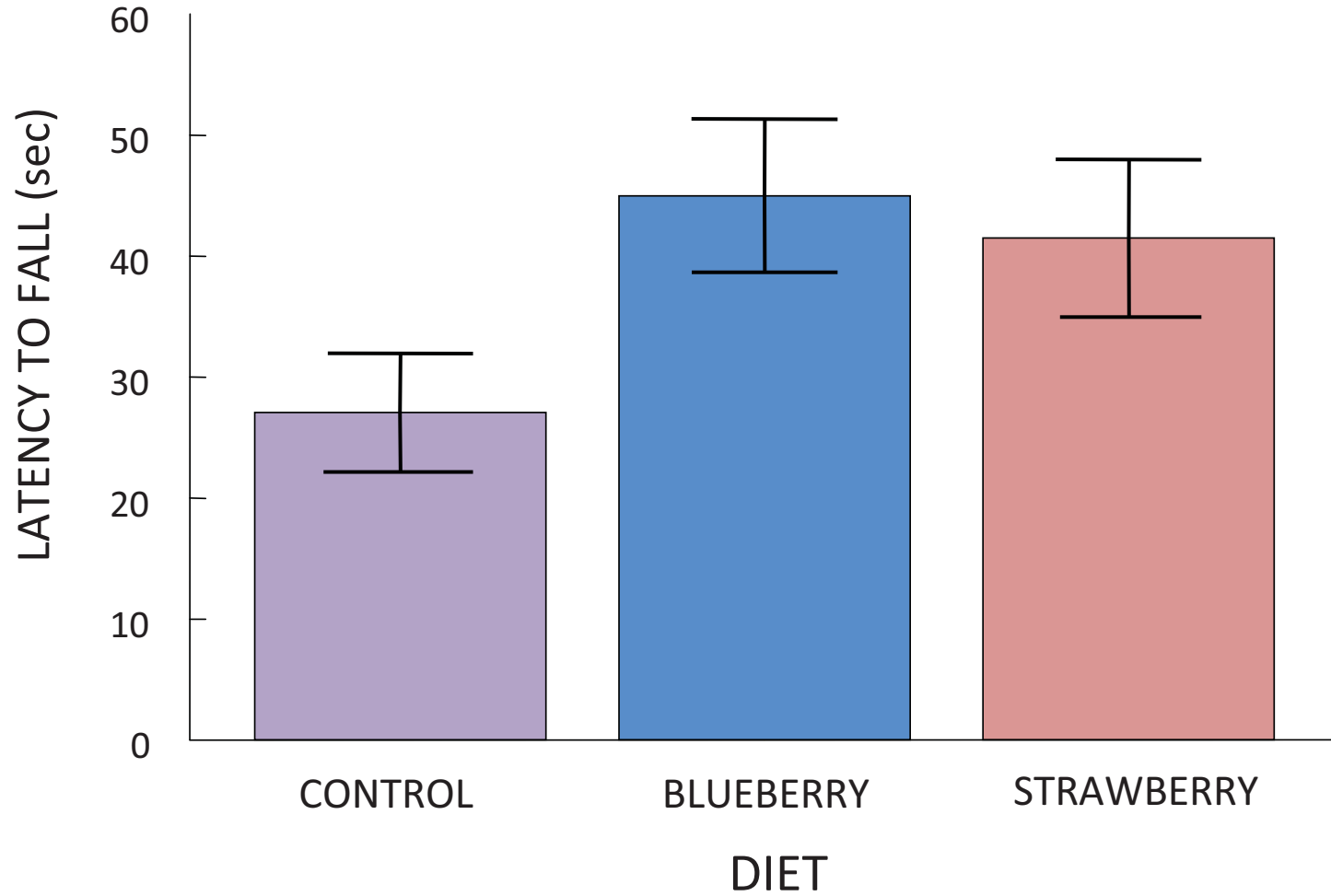
## Dietary intervention improved neuronal function

- Striatal calcium sequestration
- Cerebellar noradrenergic sensitivity
- Muscarinic receptor sensitivity

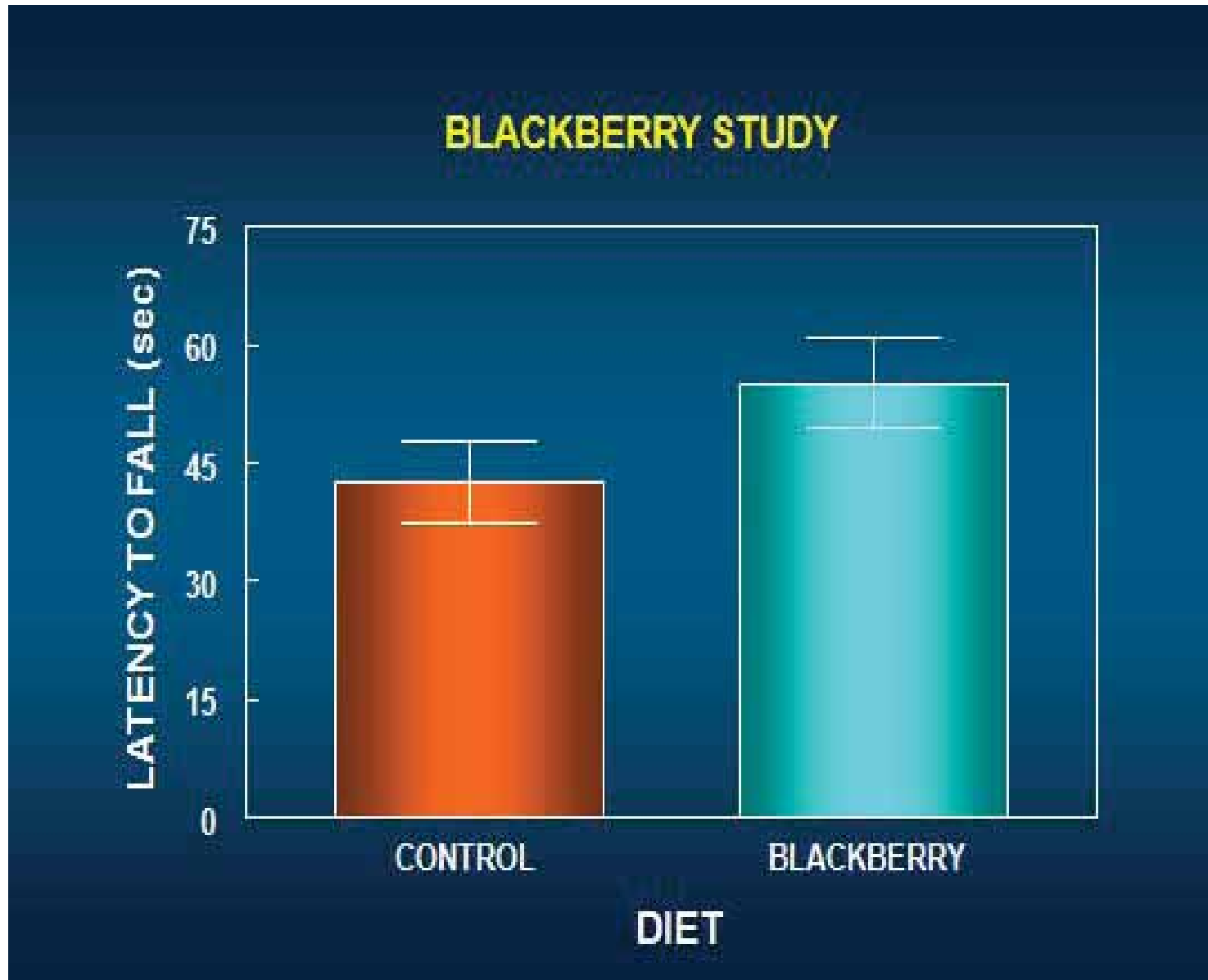
Localization of anthocyanins in the cortex & hippocampus was correlated with rat test performance.



# Rotarod

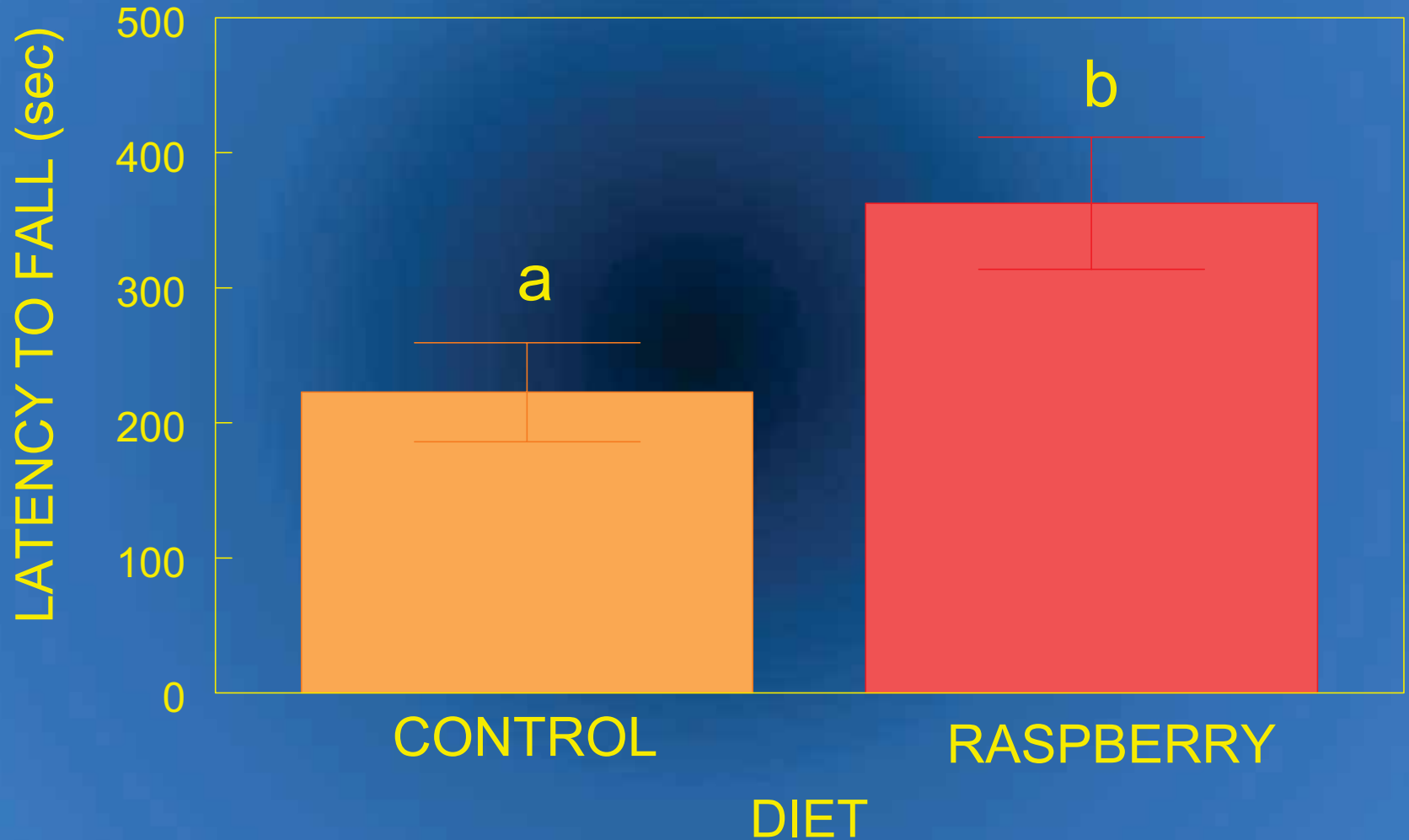


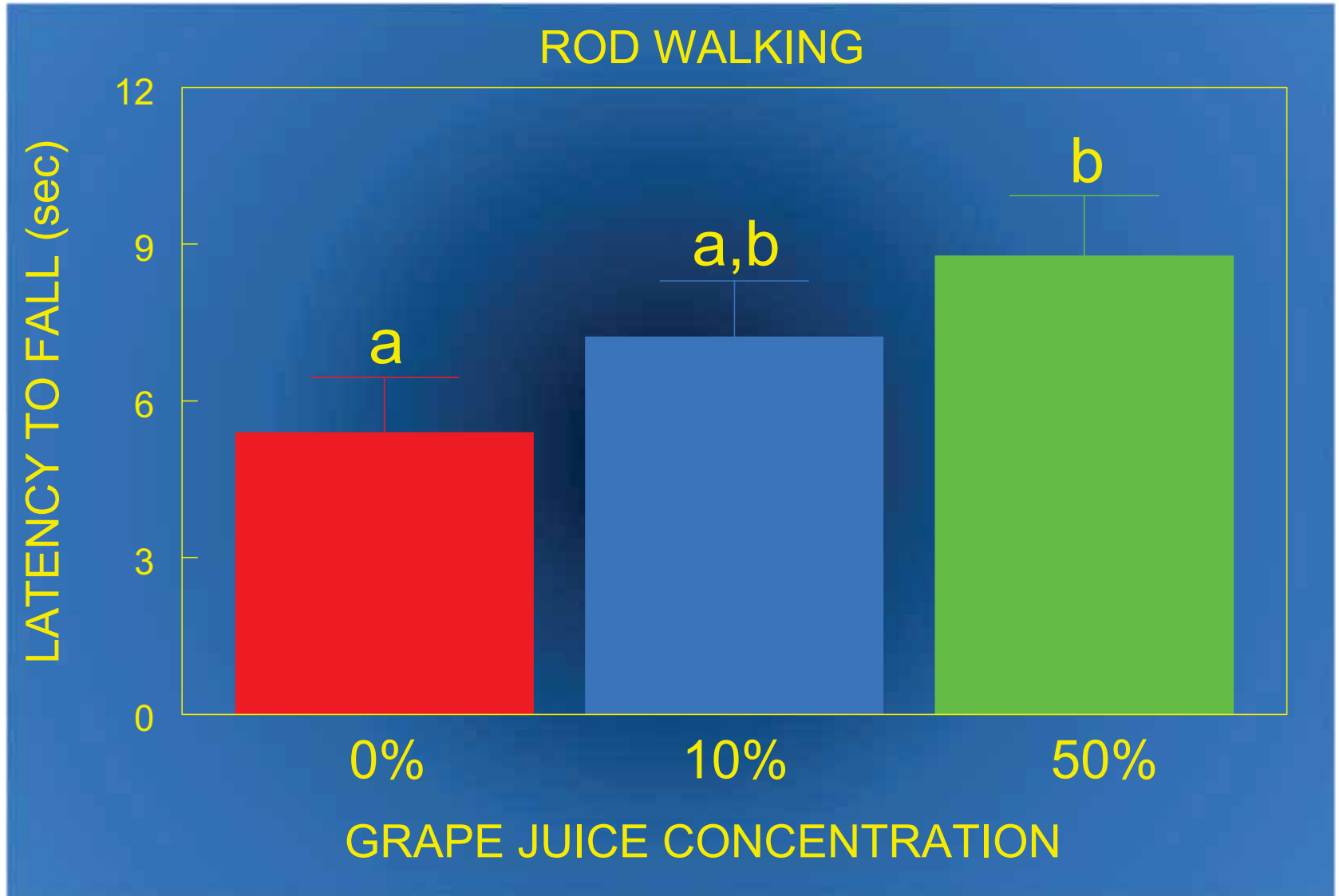
# Rotarod Performance



Shukitt-Hale et al. *Nutr. Neurosci.* 2009;12:135-140.

# RASPBERRY STUDY INCLINED SCREEN

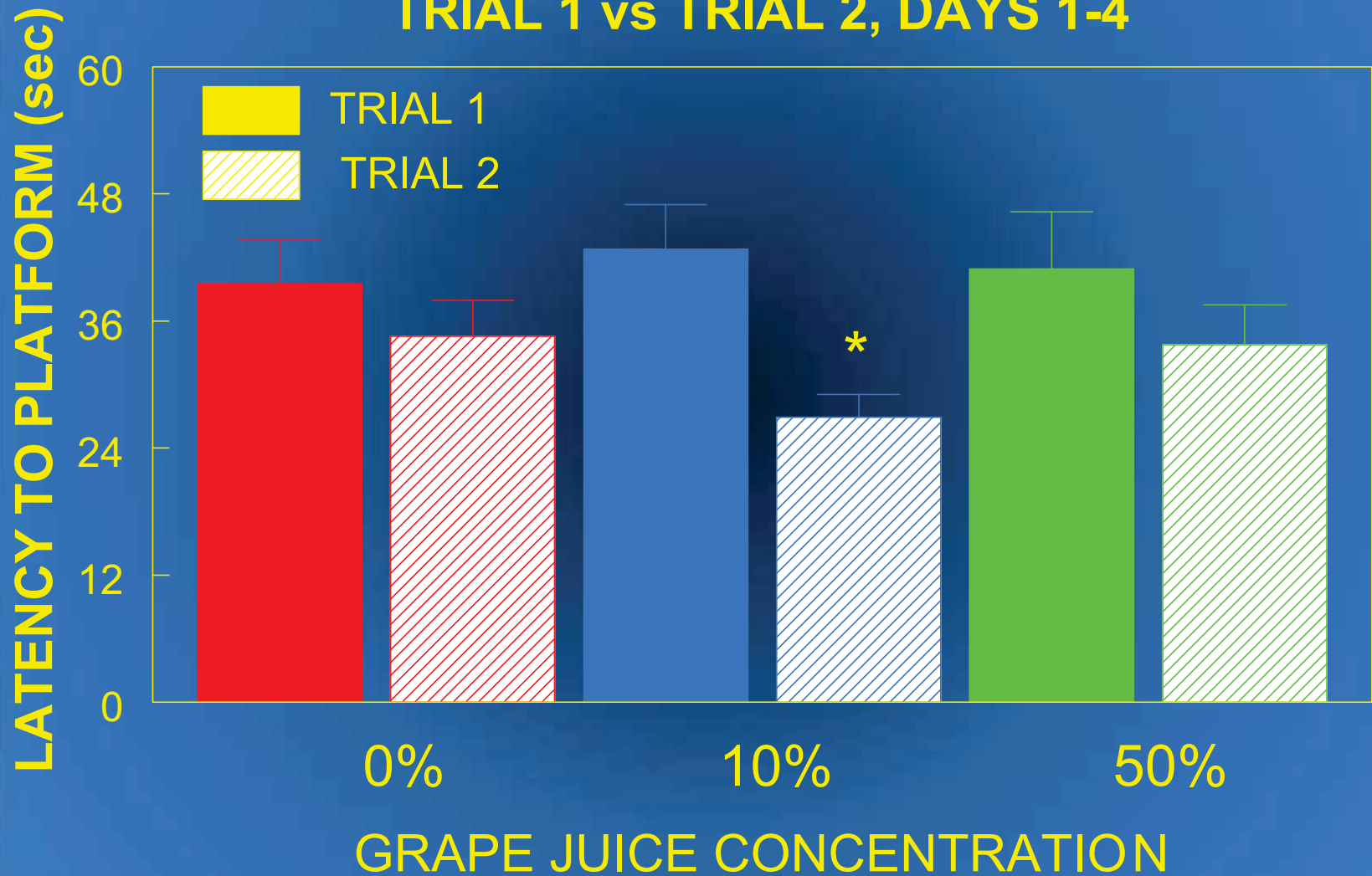




Shukitt-Hale et al. *Nutr.* 2006;22:295-302.

# GRAPE JUICE STUDY - MWM

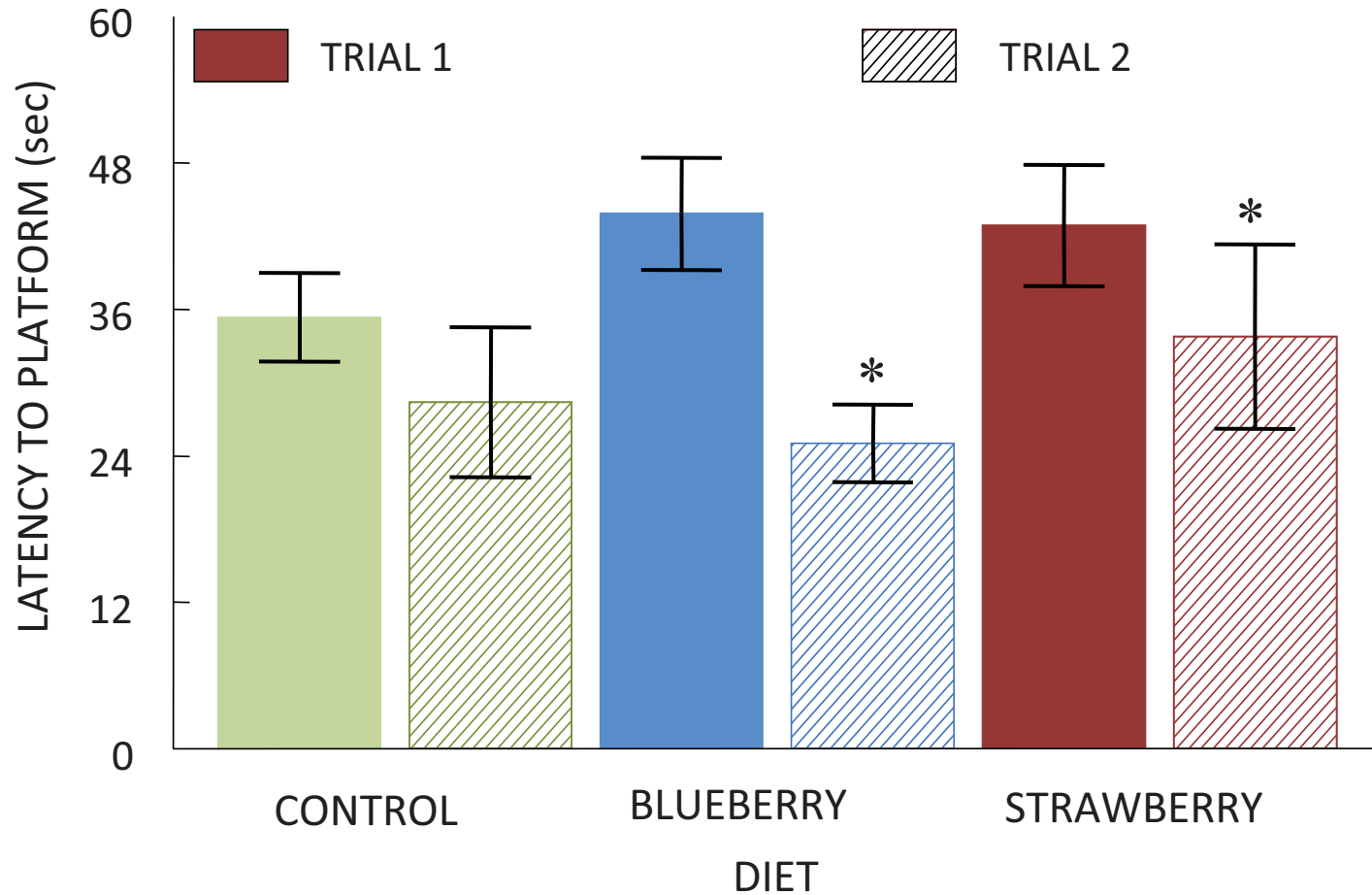
TRIAL 1 vs TRIAL 2, DAYS 1-4



Shukitt-Hale et al. *Nutr.* 2006;22:295-302.

# MORRIS WATER MAZE

TRIAL 1 vs TRIAL 2, DAYS 3-4



\*=different than Trial 1

# It's not just fruits and vegetables that are good for your brain

## WALNUTS



- Polyphenols
  - twice as much as almonds, peanuts, hazlenuts<sup>1</sup>
  - syringic acid, juglone, ellagic acid, proanthocyanidins<sup>2</sup>
- PUFAs, Melatonin, folate, gamma tocopherol<sup>3</sup>



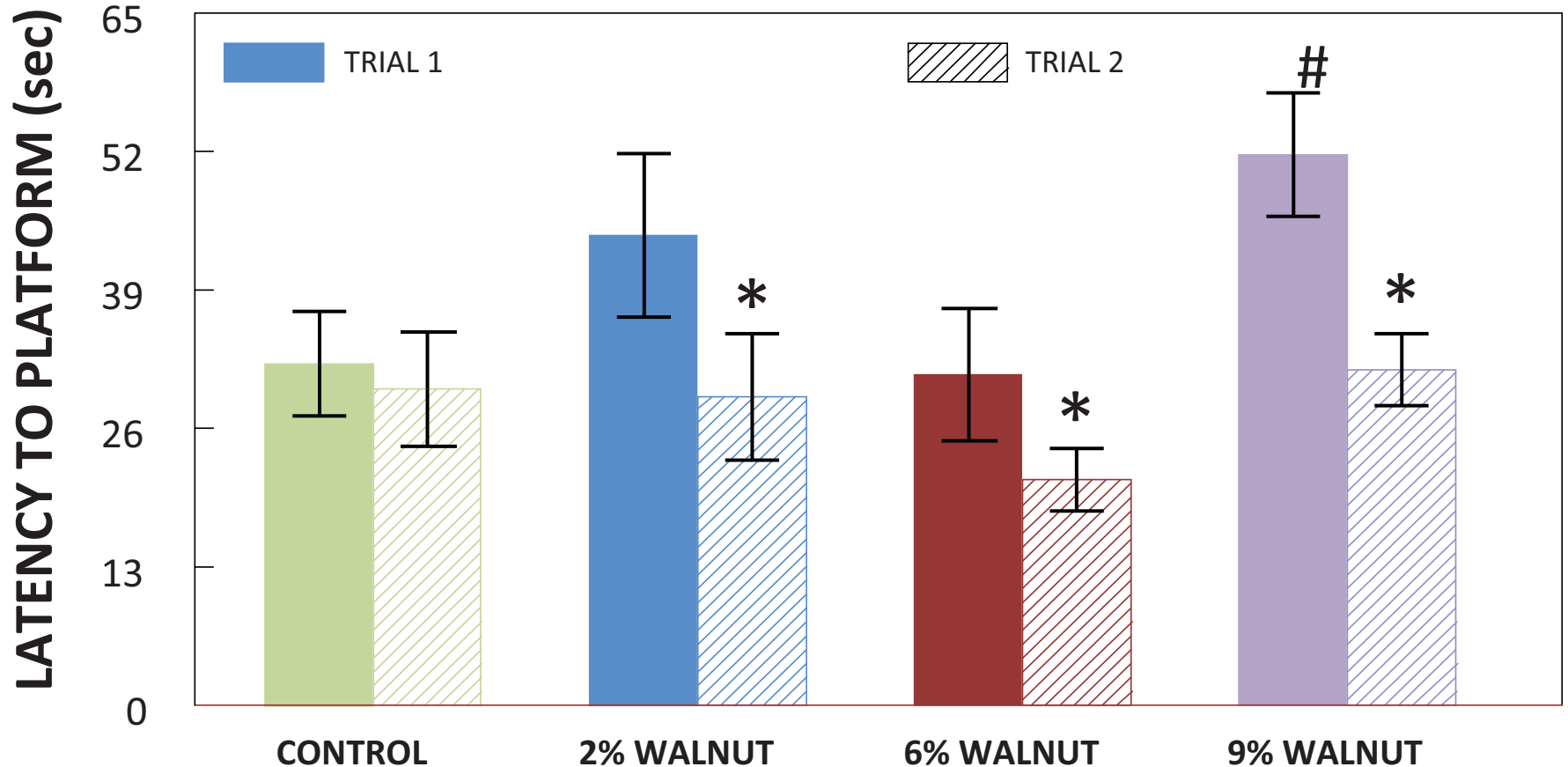
1 Macfarlane, B.J. 1988 Am J Clin Nutr; 47:270-274

2 Colaric M. 2005. J Agric Food Chem; 53:6390-6396

3 USDA National Nutrient Database, 2005

# Walnut Studies

## TRIAL 1 vs TRIAL 2, DAYS 3-4



Willis et al. *Brit. J. Nutr.* 2009;101:1140-44.



## Neurological Benefit

	Memory	Motor
Blackberries	X	X
Black currant	X	
Blueberries	X	X
Concord grape juice	X	X
Cranberries	X	X
Plum Juice	X	
Raspberries		X
Spinach	X	
Strawberries	X	X
Walnuts	X	X
Coffee	X	X



# MECHANISMS

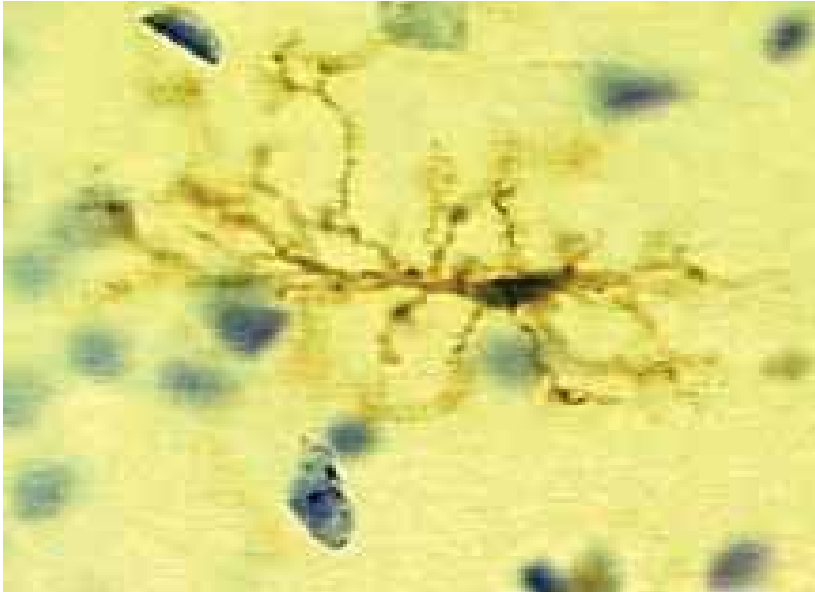
# Possible Mechanisms in the Beneficial Effects of the Polyphenolics

- Functional antioxidant/anti-inflammatory effects
  - Decreased sensitivity to oxidative stress
  - Decreased sensitivity to neurotoxins and inflammatory agents
- Direct effects on the brain
  - Increased calcium clearance
  - Membrane effects
  - Alterations in signaling**
    - **Decreased oxidative stress/inflammatory signaling**
    - **Increased protective signaling**
    - **Signaling in learning and memory**
  - Increased neurogenesis
  - Increased arborization
  - Increased autophagy (natural house-keeping)

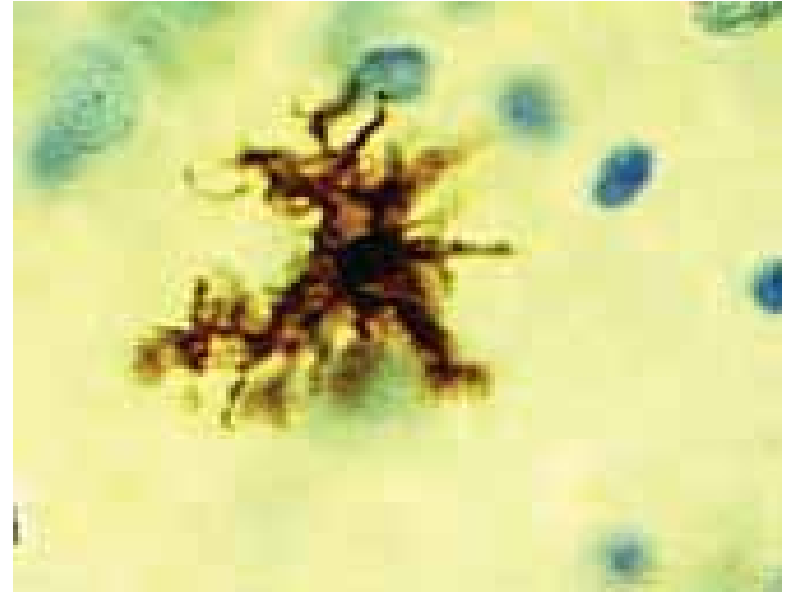




# Microglial Cells



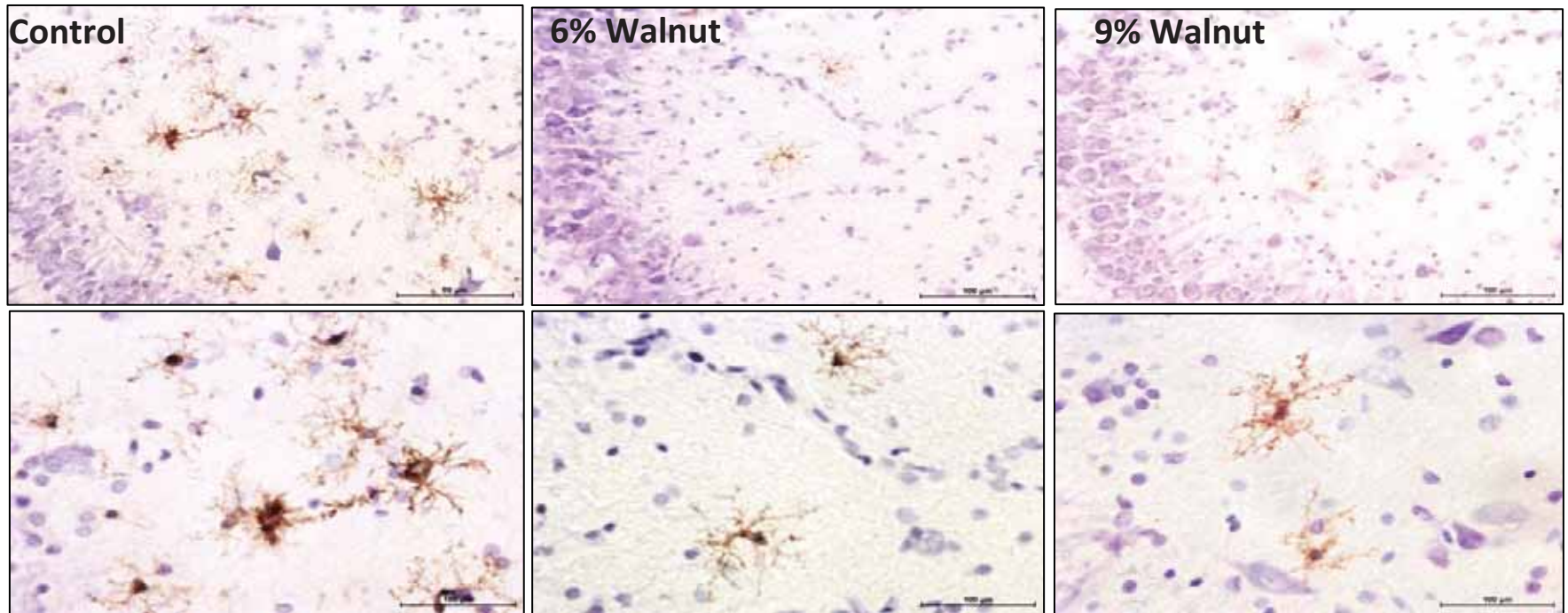
Slightly activated



Very activated

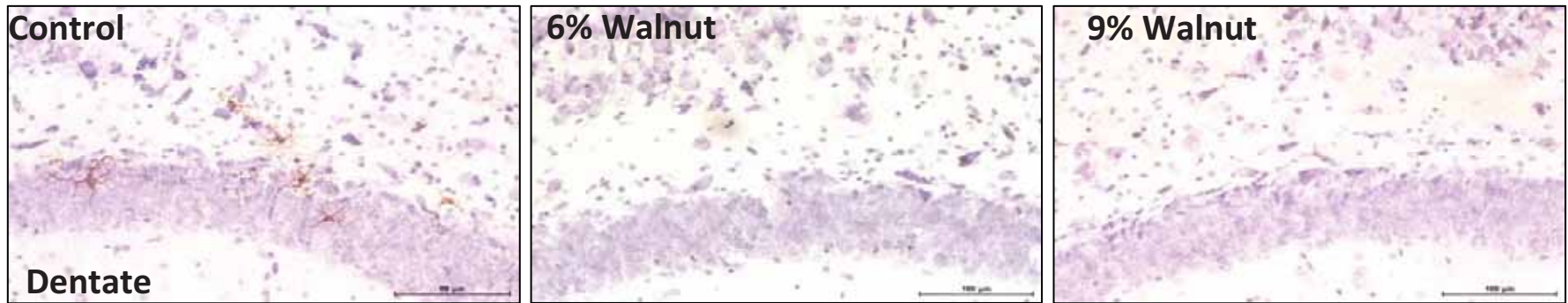
Microglial cells are the brain's immune cells and become activated in response to inflammatory challenges. These cells are in the hippocampus of aged rats.

# Activated Microglia in the CA3 Hippocampal Region



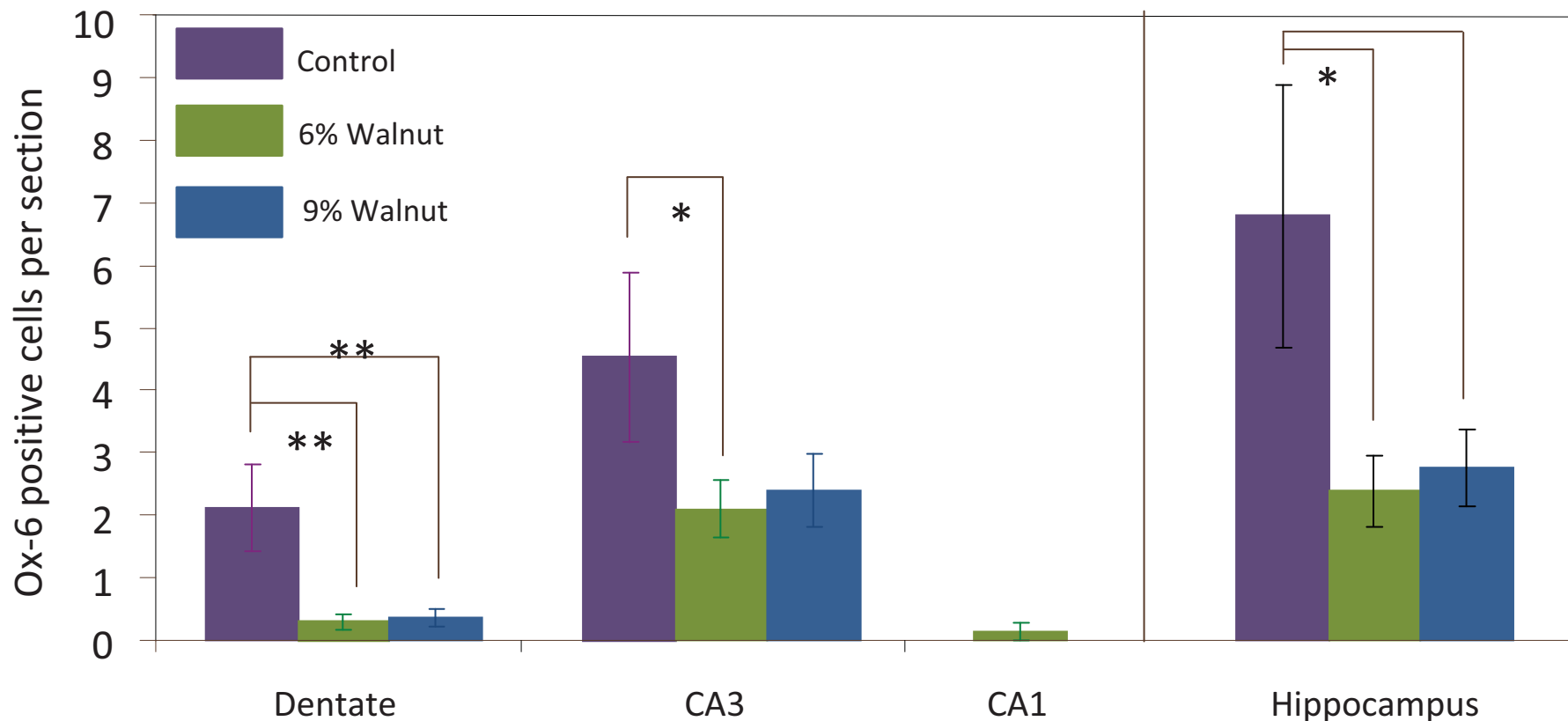
Immunohistochemistry for Ox-6 (brown) demonstrates the number of activated microglia in the CA3 region hippocampus of aged animals.

# Activated Microglial in the Dentate



Activated microglia are also found in the dentate gyrus; animals that consumed a walnut supplemented diet appear to have fewer Ox-6 positive cells in the dentate.

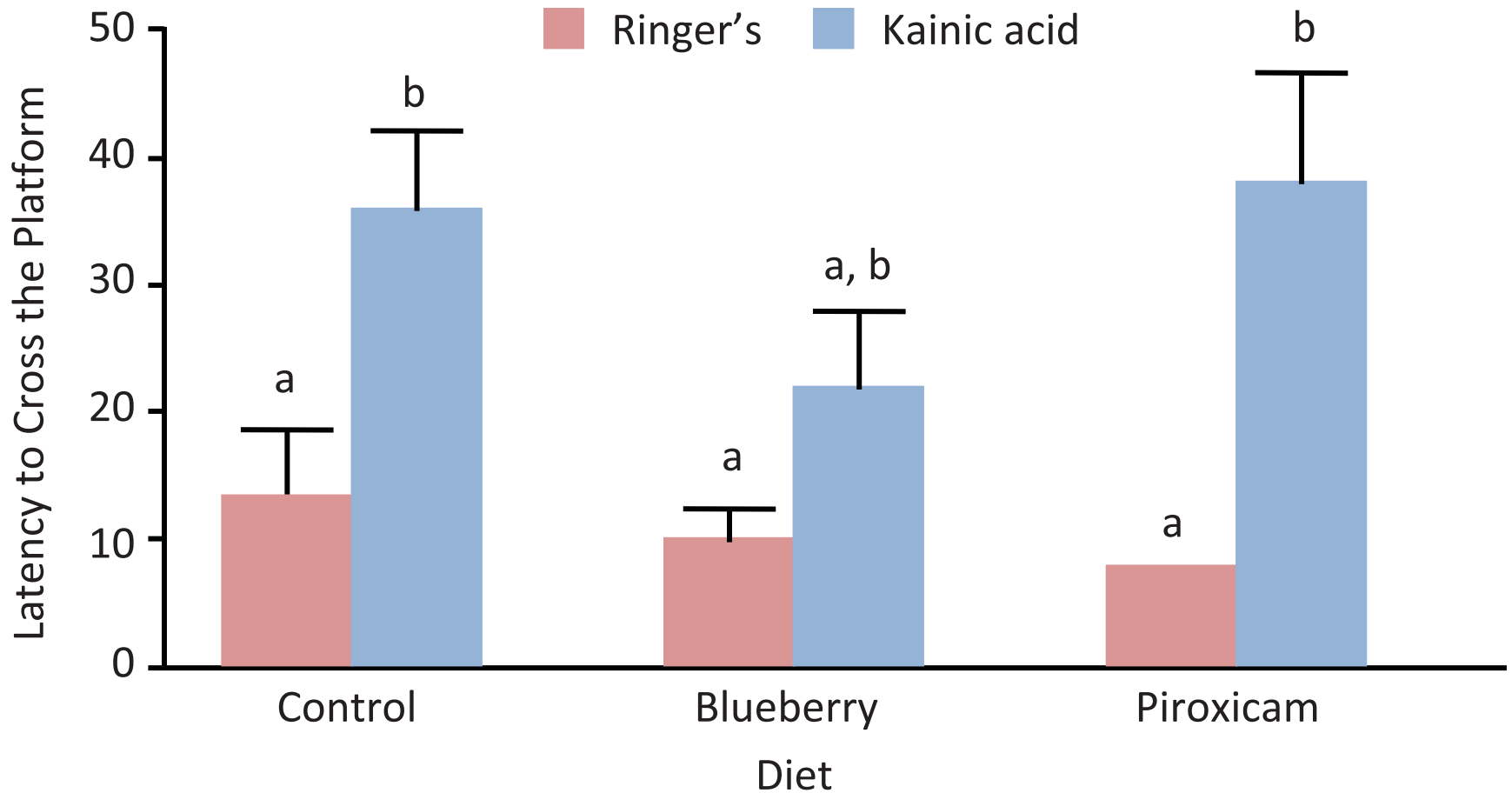
# Walnuts Decrease the Number of Activated Microglia in the Aged Hippocampus



Quantification of Ox-6 positive microglia reveals that dietary walnut supplementation is associated with significantly fewer activated microglia in the dentate, CA3, and the hippocampus as a whole. (mean  $\pm$  SEM; n=5 control, 7 for 6% walnut, and 6 for 9% walnut; \*p<.05, \*\*p<.01)

# Kainic Acid Diet Study

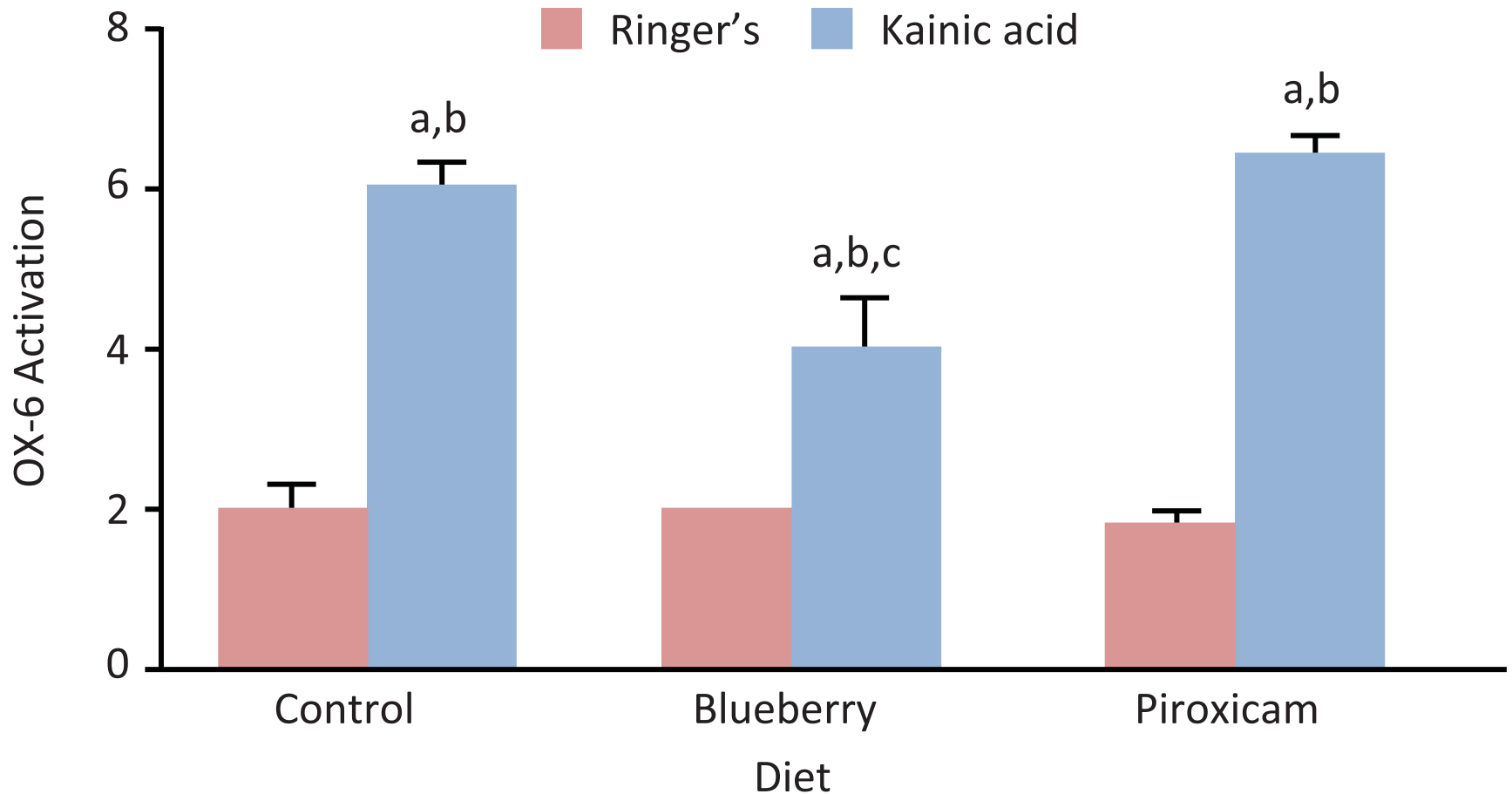
## Morris Water Maze-Day 3 Probe



a=KA different than matched diet Ringer's group; b=different than control Ringer's group;

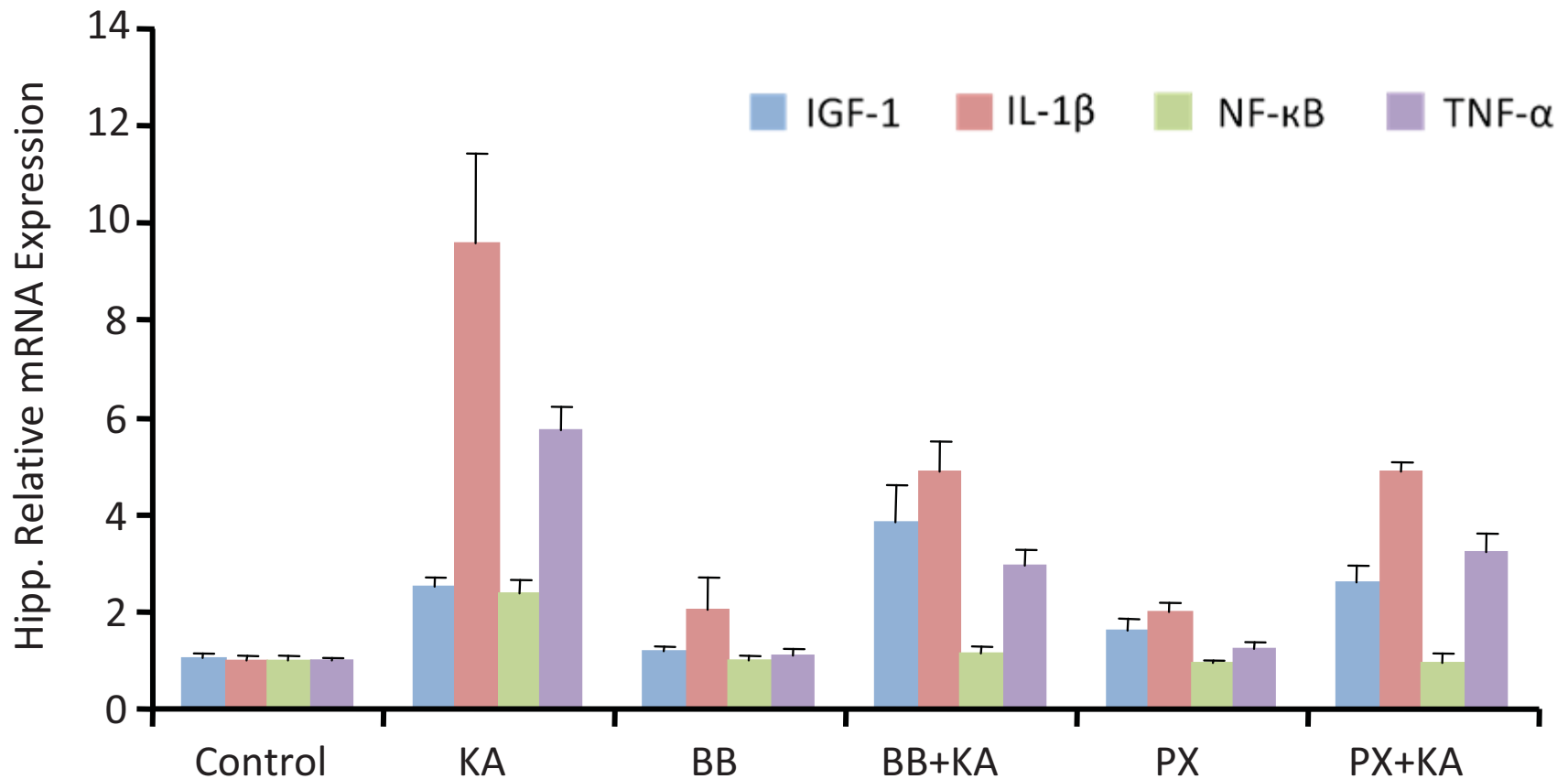
Shukitt-Hale et al. *Nutr. Neurosci.* 2008; 11:172-82.

# The Effects of Kainic Acid on OX-6 Activation



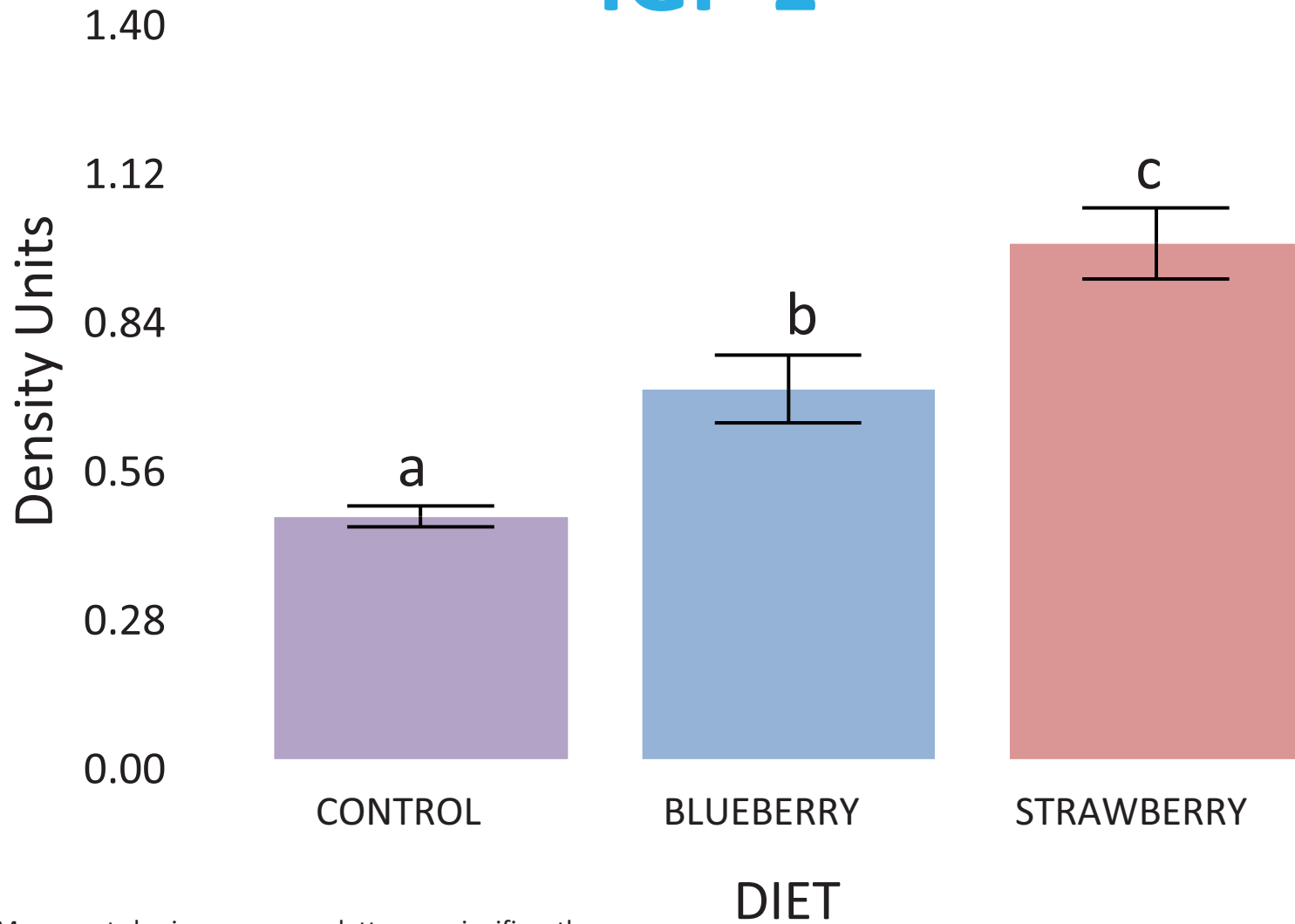
a=KA different than matched diet Ringer's group; b=different than control Ringer's group; c=different than control KA group.

# Inflammatory and Protective Signals in Hippocampus in Control, BB or PXM-Treated Animals Given KA or Vehicle



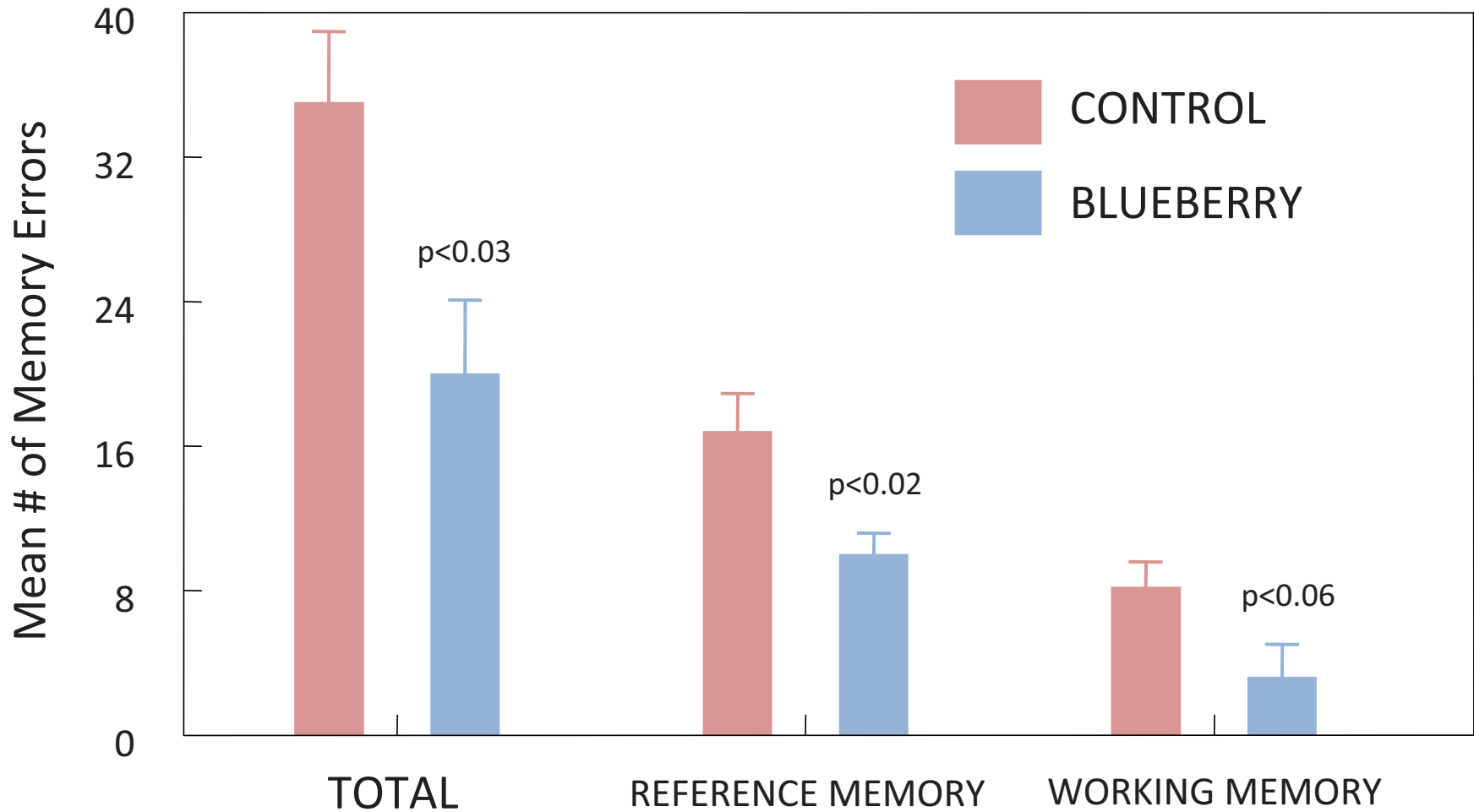
Shukitt-Hale et al. *Nutr. Neurosci.* 2008; 11:172-82.

# IGF-1



Means not sharing a common letter are significantly different from each other ( $p < 0.05$ ; Fisher's LSD)

# Errors In The Radial Arm Water Maze



# Correlations Between Neurogenesis, IGF-1, and RAWM Day 4 Performance In Old Control Fed and Blueberry Supplemented Rats

- Total errors (any wrong arm entrance) and neurogenesis  
 $r = -0.677, p < 0.05$
- Reference memory errors (entering an arm that does not contain the platform) and neurogenesis  
 $r = -0.654, p < 0.04$
- Working memory errors (reentries into an arm) and neurogenesis  
 $r = -0.646, p < 0.05$
- IGF-1 increased by blueberry supplementation ( $p < 0.05$ ) and total errors and IGF-1 were correlated  $r = -0.626, p < 0.05$



Therefore, polyphenolics can have direct effects on the brain, i.e., not mediated through oxidative stress or inflammatory pathways, by directly increasing signaling and neurogenesis. Additionally, berry fruits may protect neurons by activating protein kinase signaling cascades.



In addition to neutralizing free radicals, berries may prevent the deleterious effects of inflammation and oxidative stress by blocking the stress signals whose generation results in downstream production of cytokines.



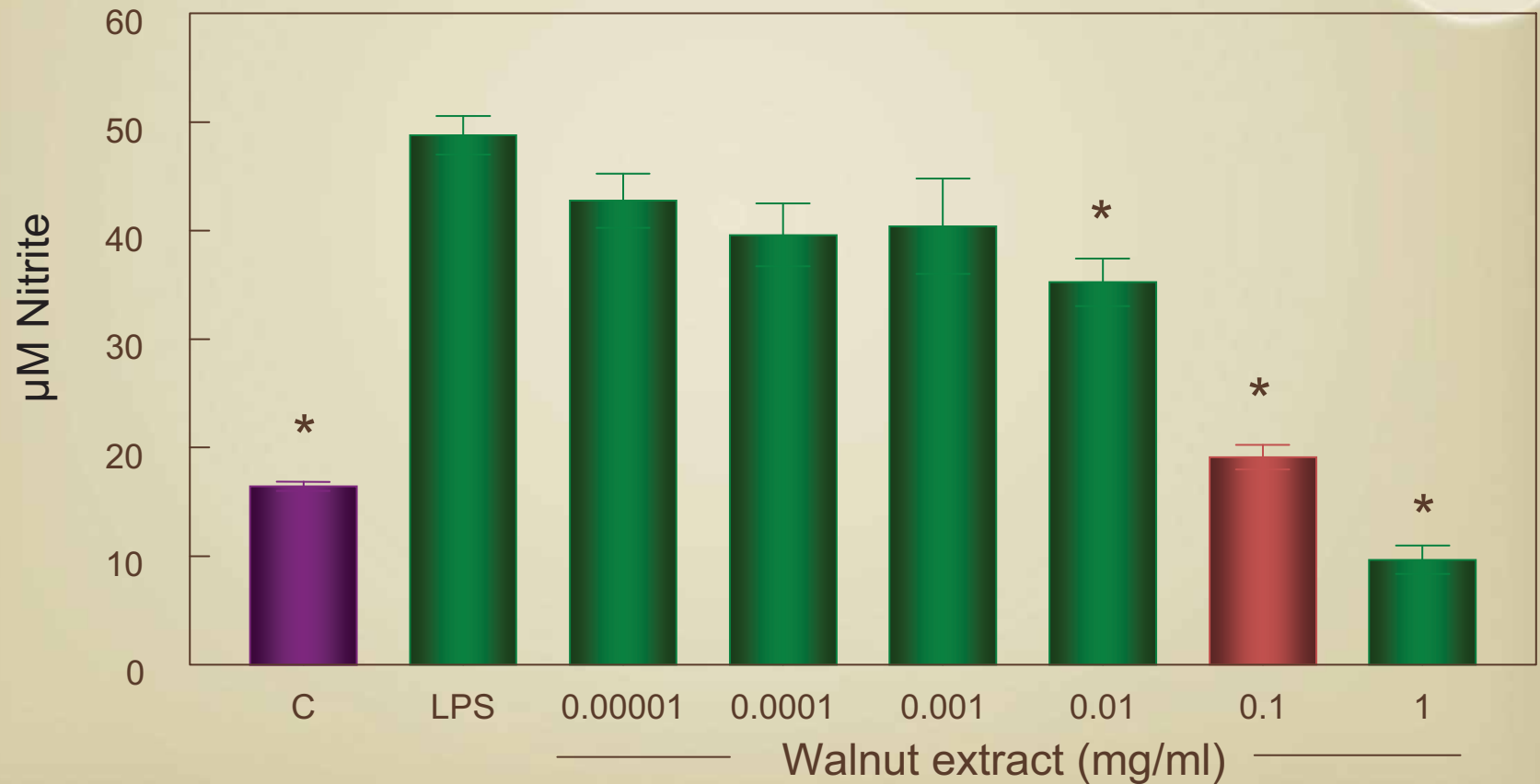


# CELL STUDIES

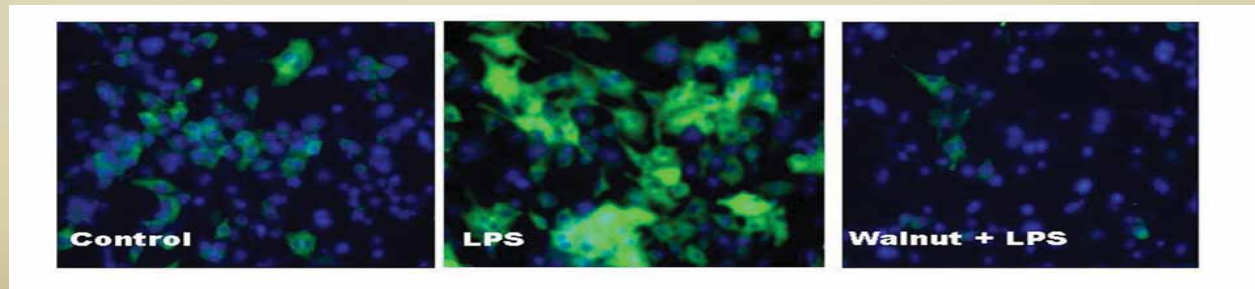
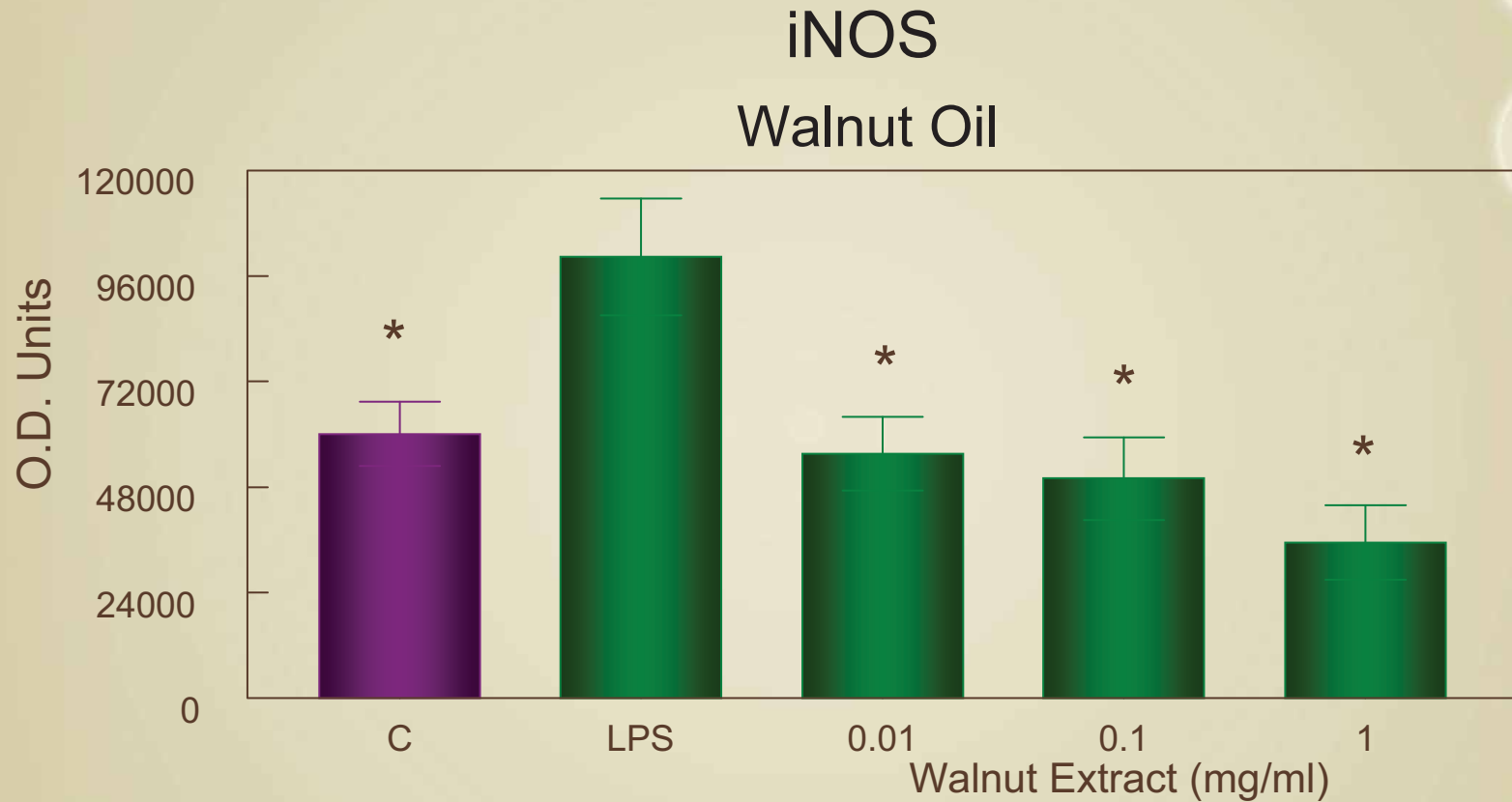
# Effects of Walnuts on LPS-Activated BV2 Microglia: NITRIC OXIDE RELEASE

NITRITE

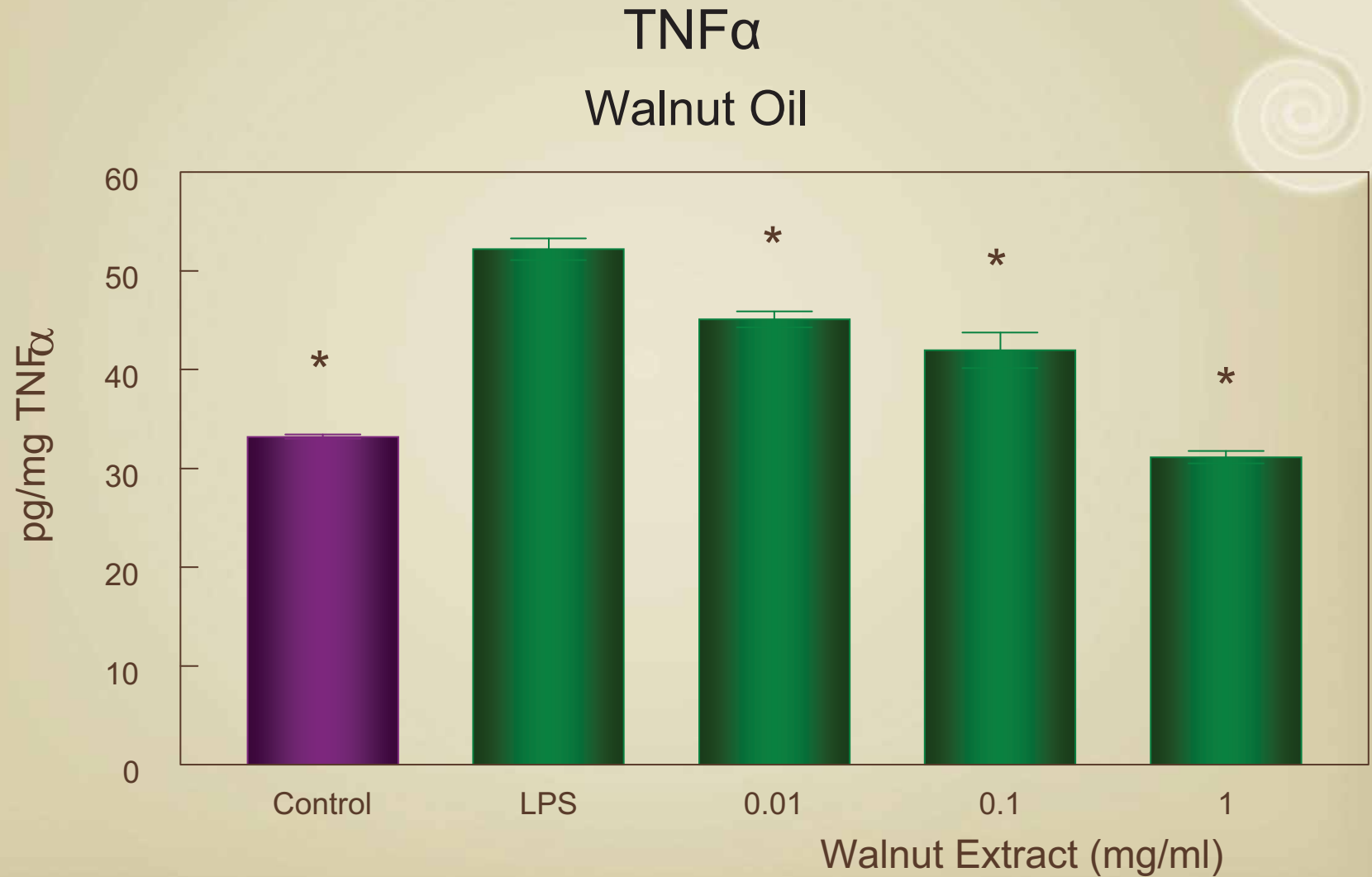
Walnut Oil



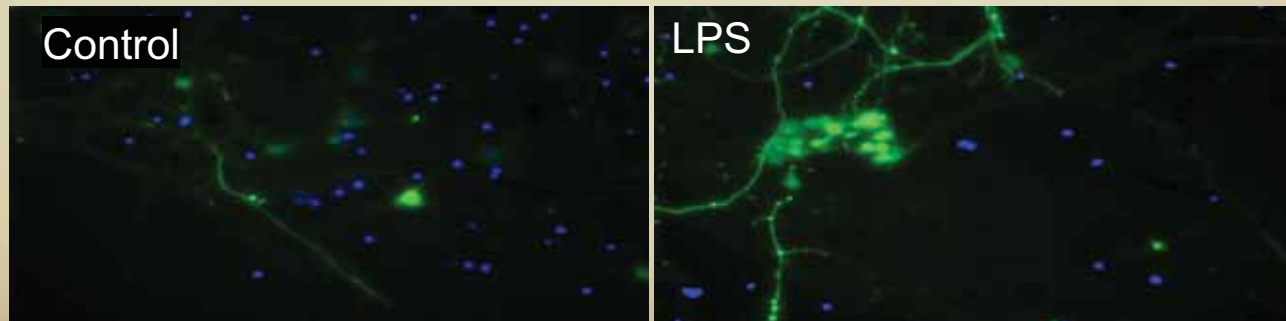
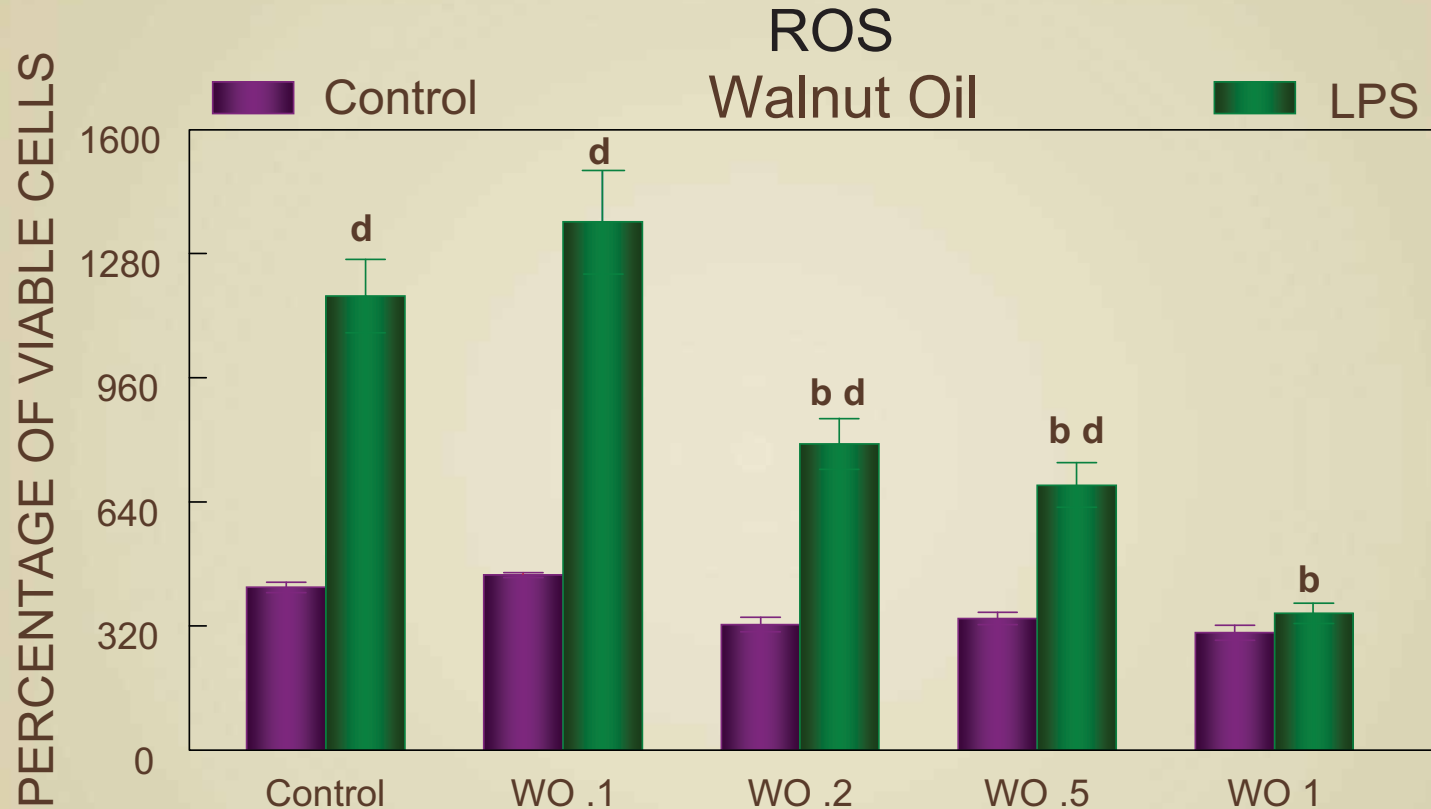
# Effects of Walnuts on LPS-Activated BV2 Microglia: iNOS EXPRESSION

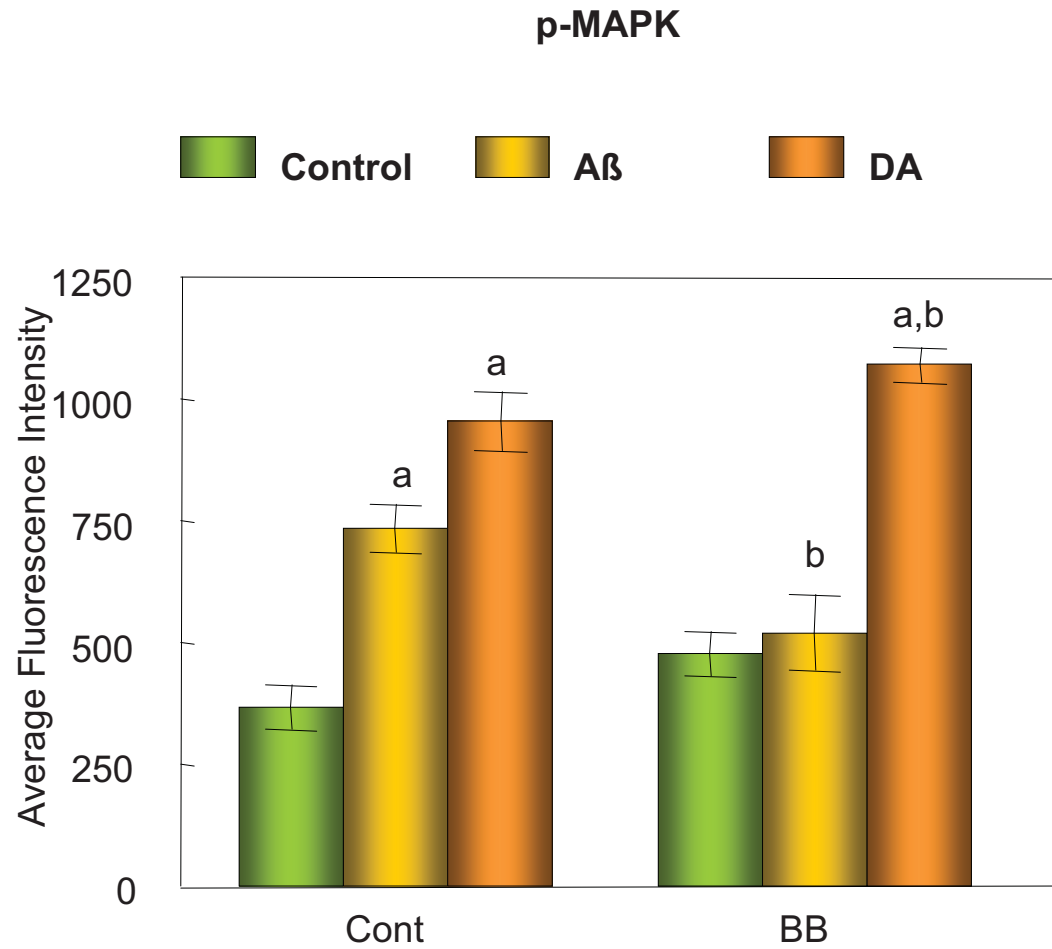


# Effects of Walnuts on LPS-Activated BV2 Microglia: TNF $\alpha$ EXPRESSION

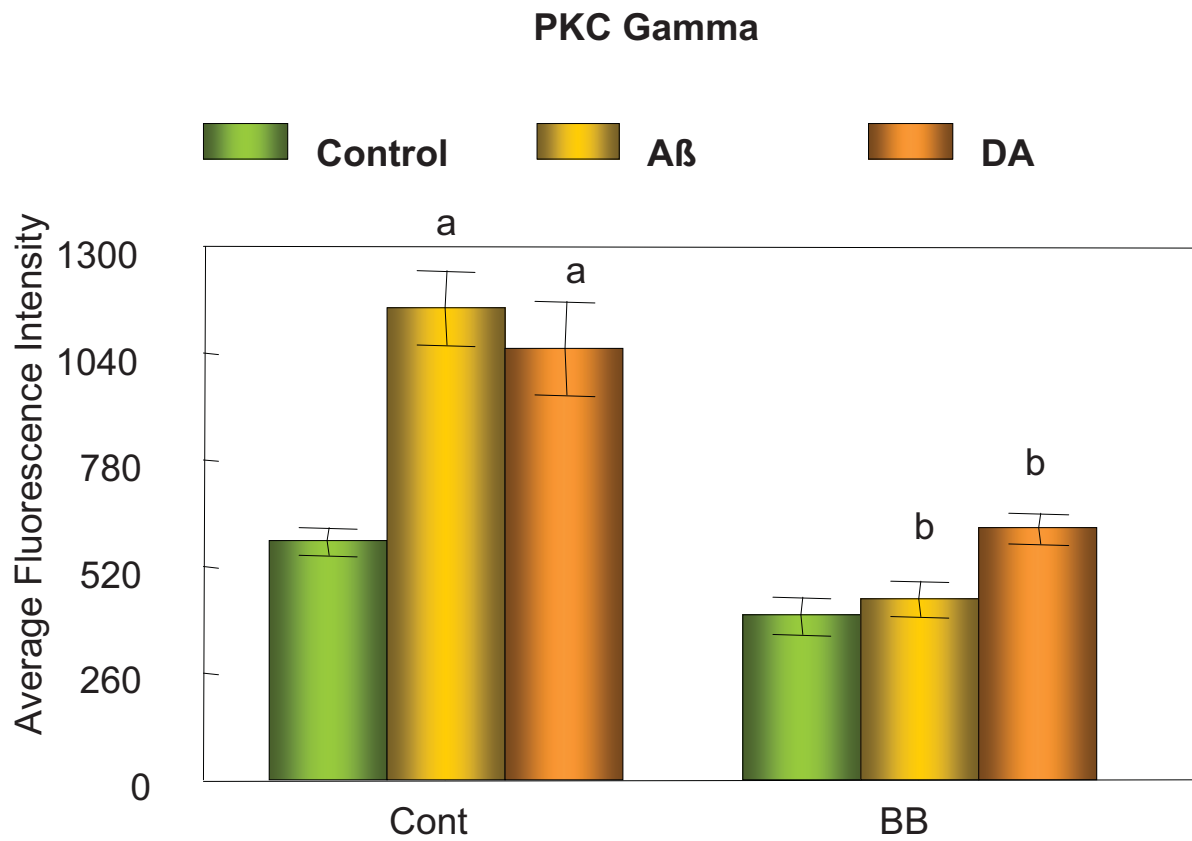


# Effects of Walnuts on LPS-Activated Hippocampal Neurons: ROS ACTIVATION

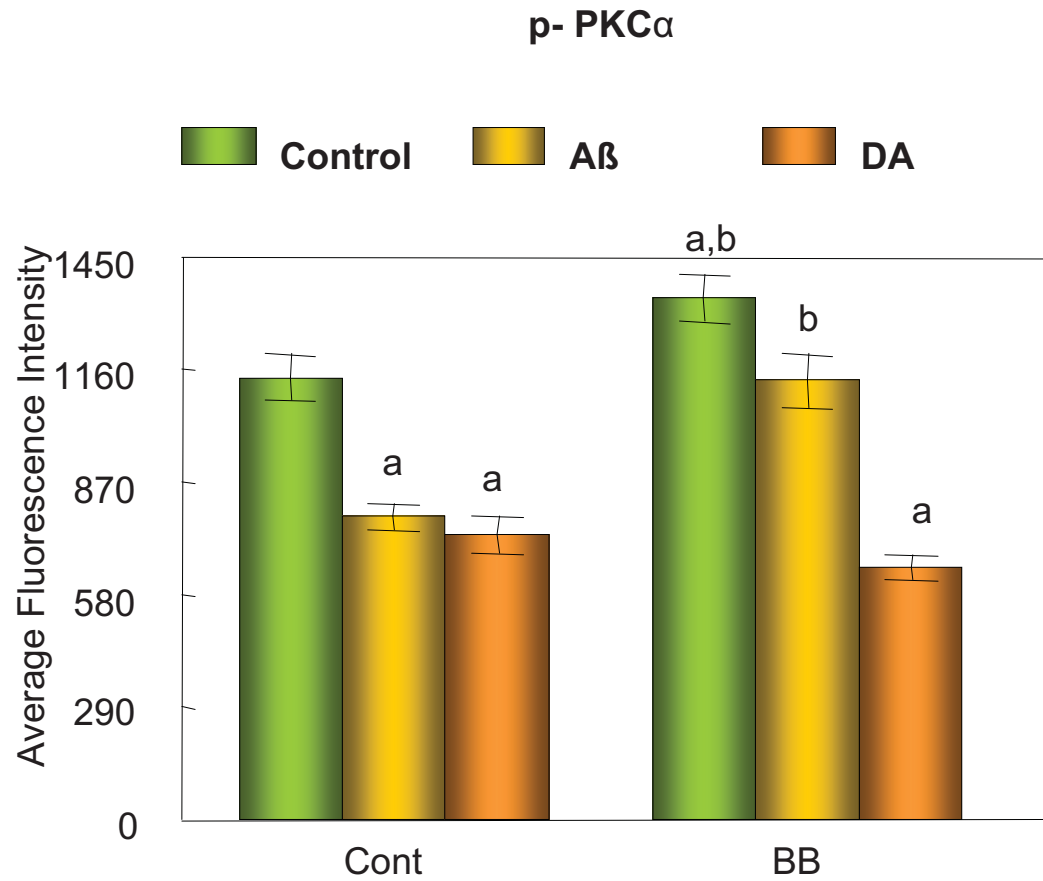




From: J.A. Joseph et al. / Journal of Alzheimers Disease 2007, 11(4), 433-446.



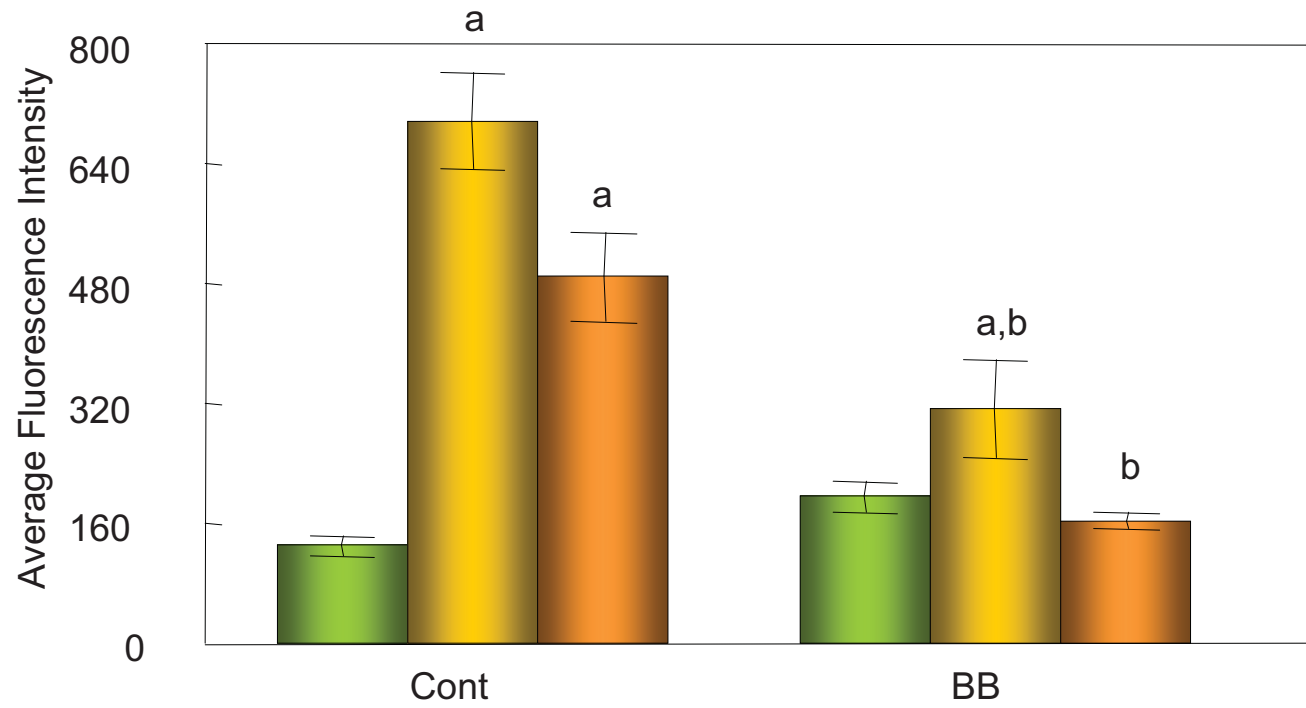
From: J.A. Joseph et al. / Journal of Alzheimers Disease 2007, 11(4), 433-446.

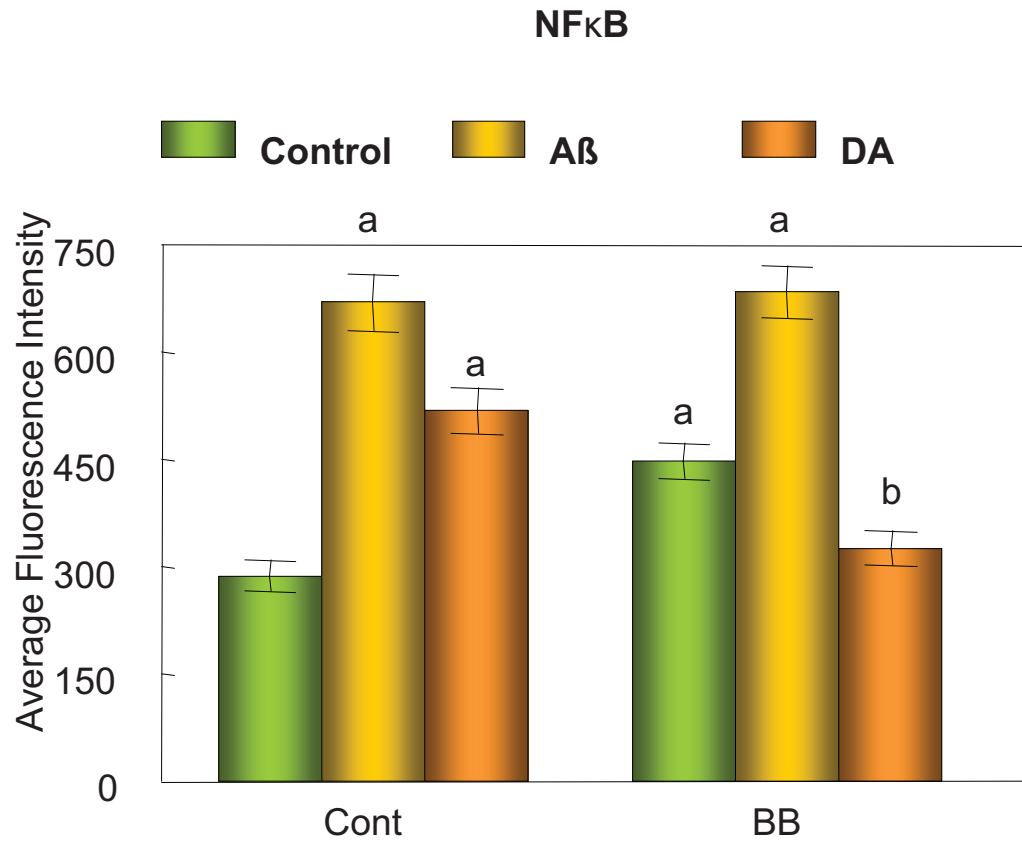


From: J.A. Joseph et al. / Journal of Alzheimers Disease 2007, 11(4), 433-446.

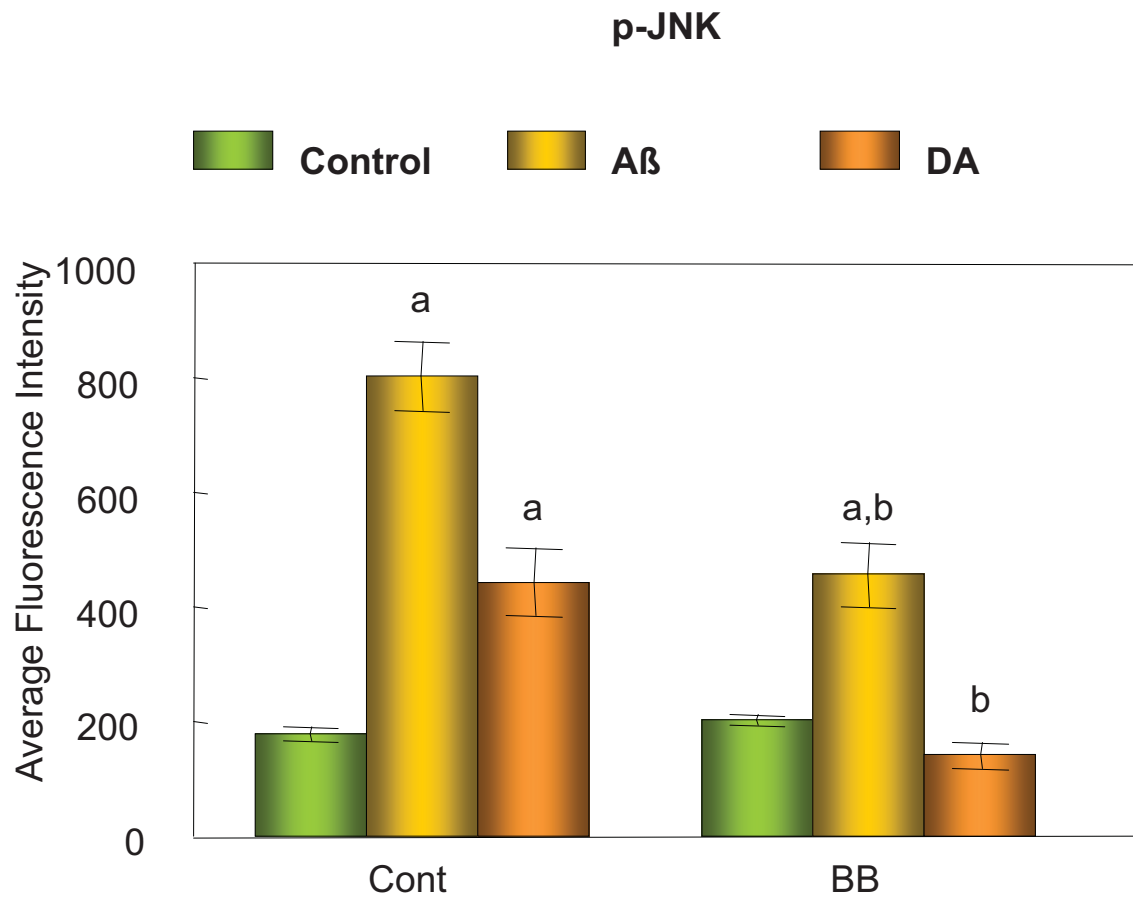
p-p38

Control A $\beta$  DA

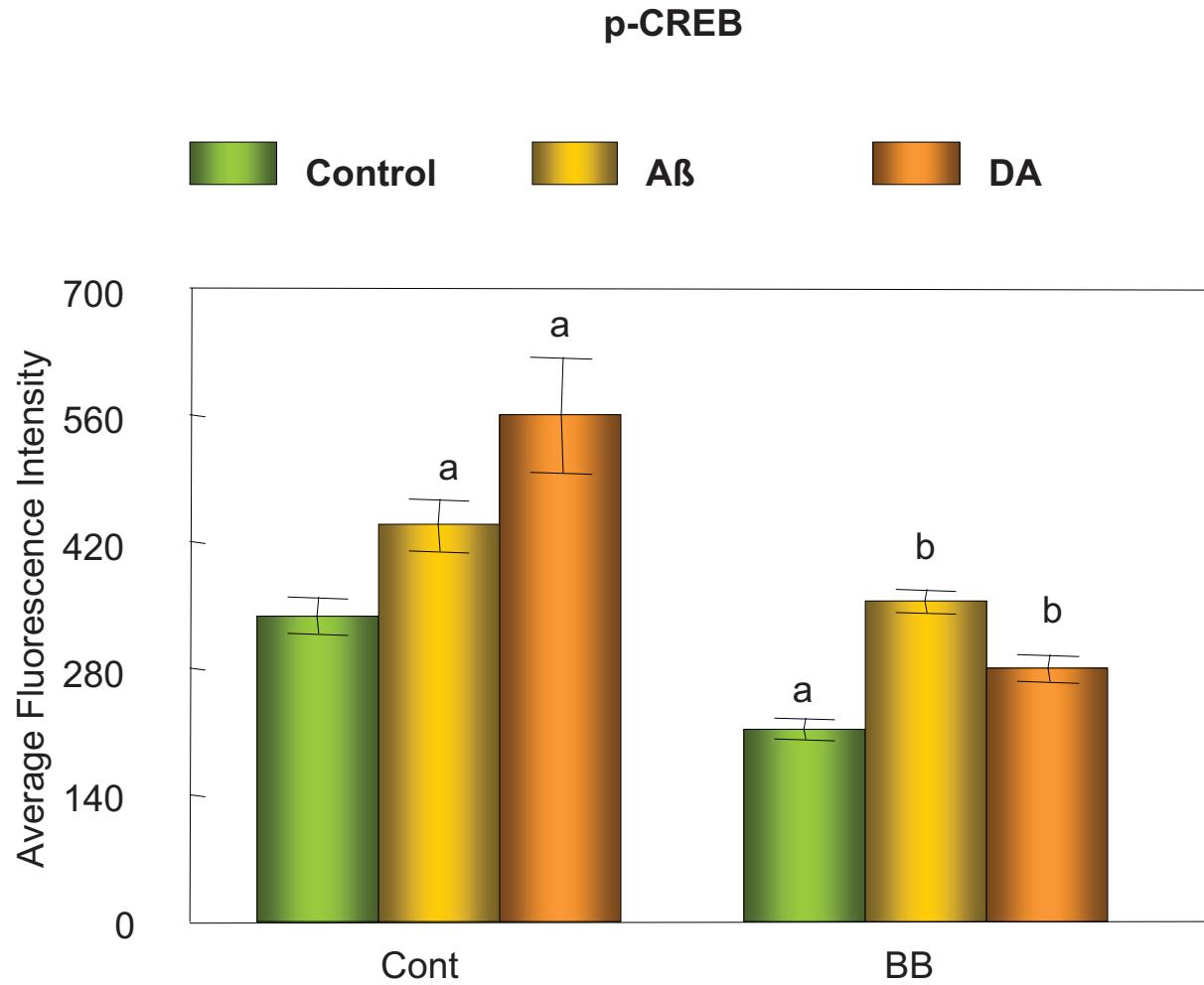




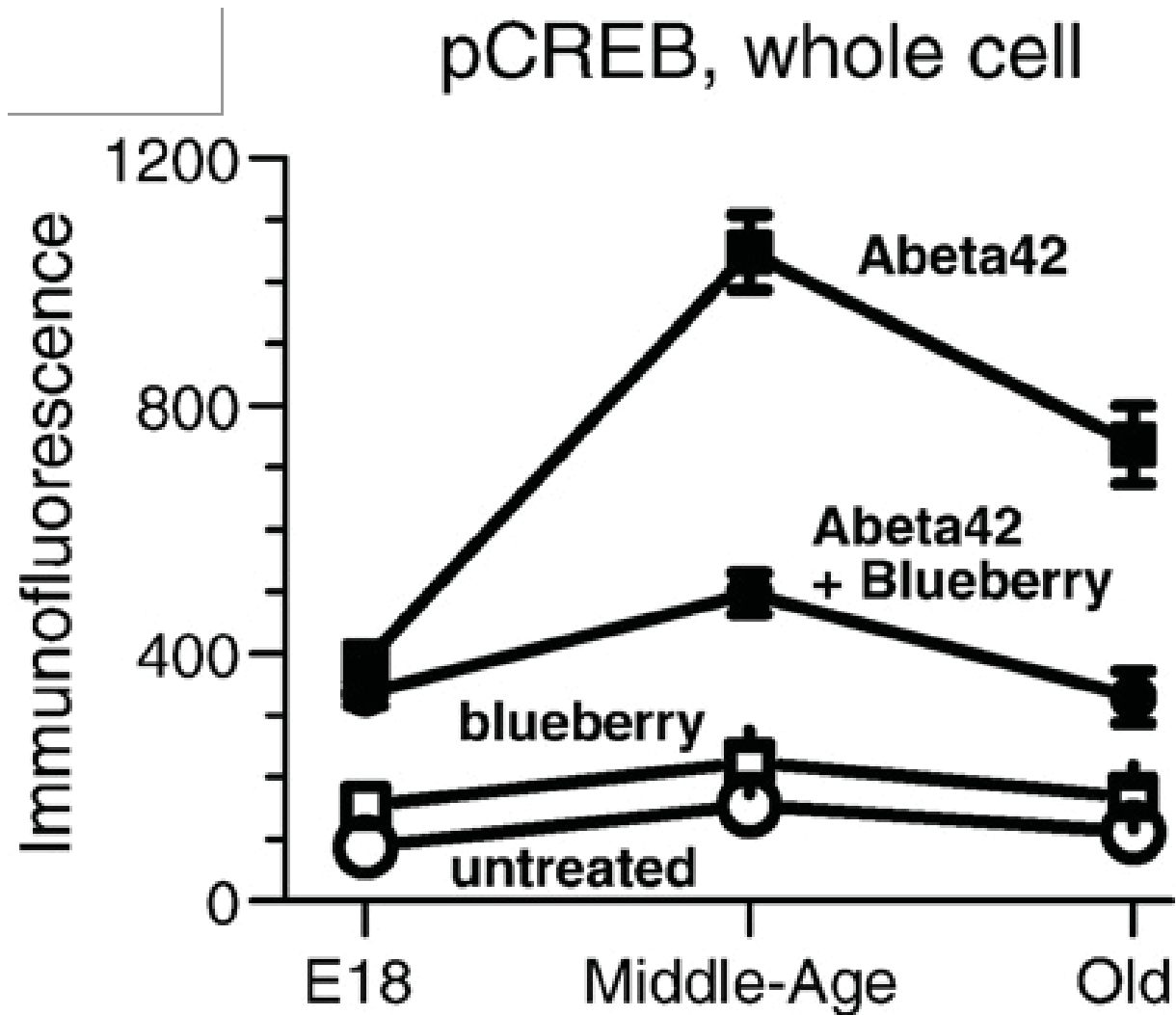
From: J.A. Joseph et al. / Journal of Alzheimers Disease 2007, 11(4), 433-446.



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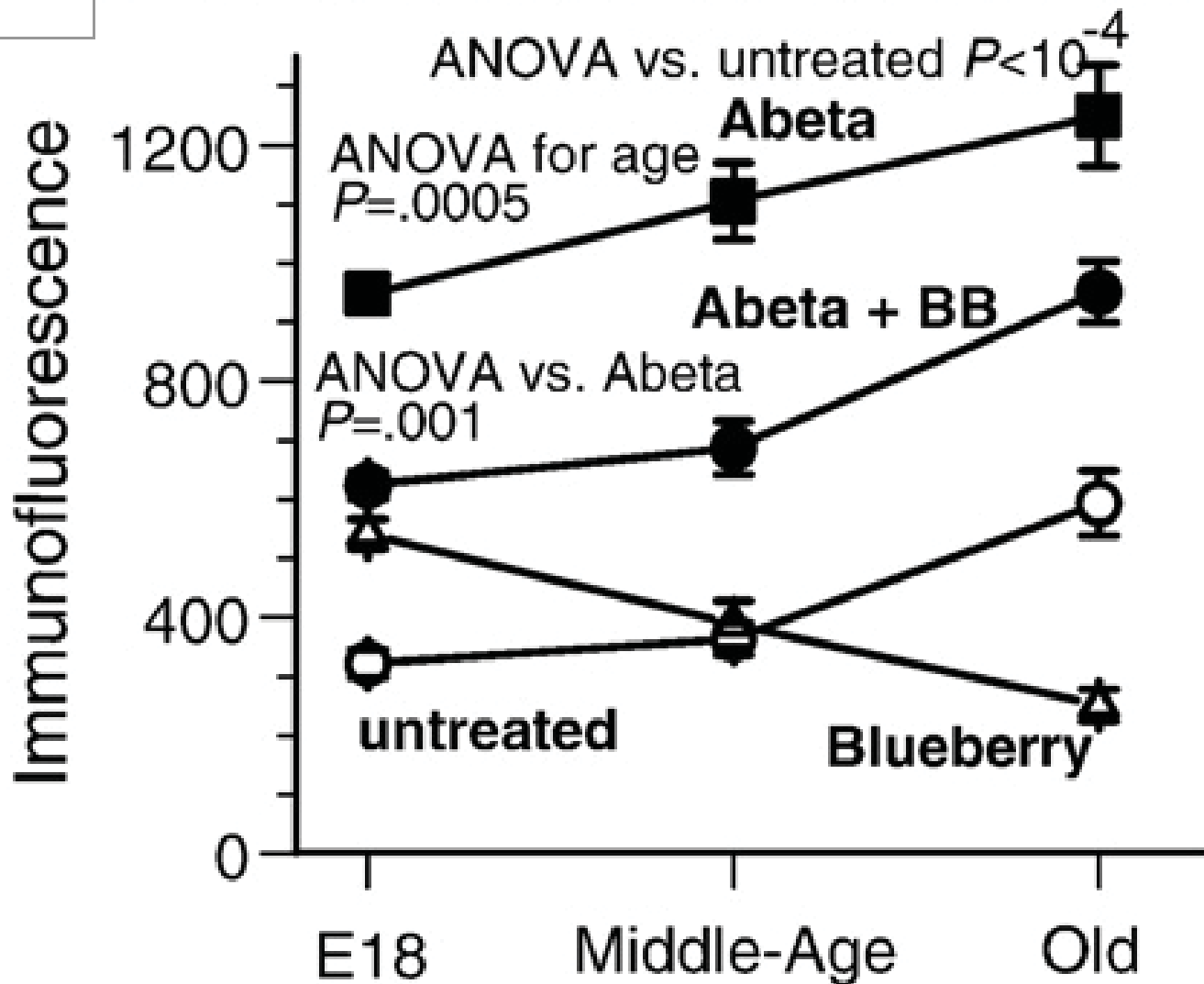


From: J.A. Joseph et al. / Journal of Alzheimers Disease 2007, 11(4), 433-446.



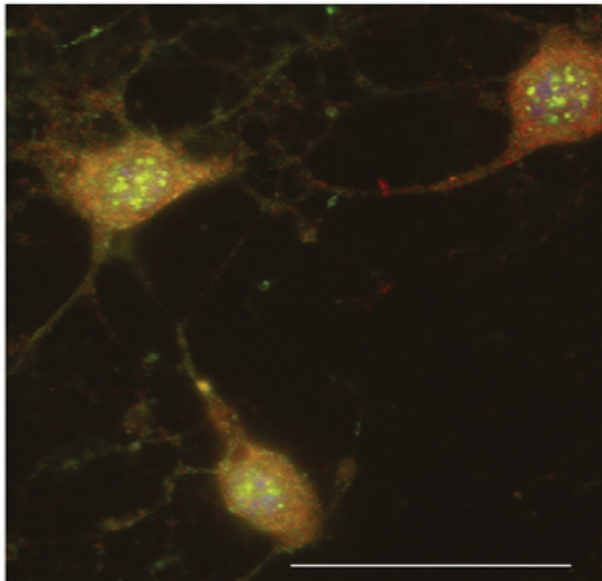
From: G.J. Brewer et al. / Journal of Nutritional Biochemistry 21 (2010) 991–998

# pERK Immunofluorescence with age

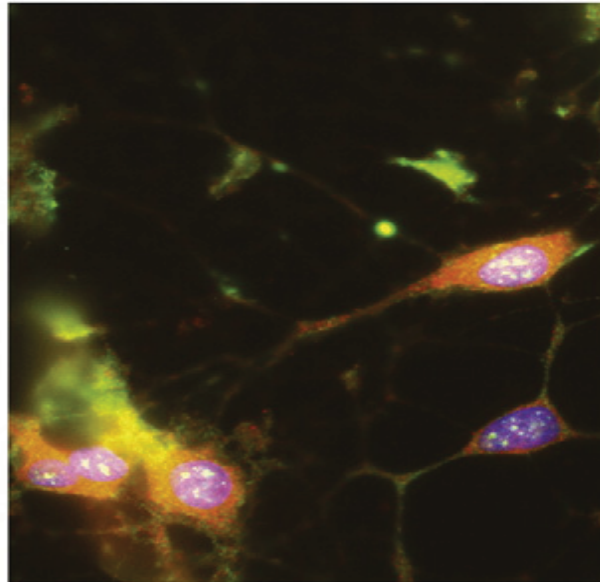


pCREB  
pERK 1/2  
nuclear DNA

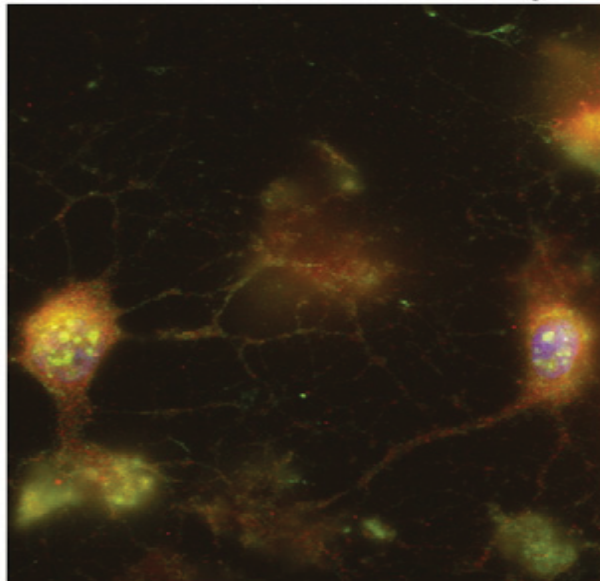
**A** untreated



**B** A-beta



**C** A-beta + blueberry



# Conclusions

- Eating berries, grapes and walnuts may help prevent or reverse age related declines in cognition and brain functioning.
- This may be due to the activity of polyphenols and other components such as omega fatty acids.
- These components may act to inhibit or reduce inflammation and oxidative stress and/or enhance protective mechanisms.
- Polyphenolics can have direct effects on the brain by directly increasing neuronal signaling cascades and neurogenesis.



Therefore, it appears that the significant effects of polyphenolics on motor and cognitive behavior are due to a multiplicity of direct and indirect actions, the former involving effects on neuronal communication and the latter involving antioxidant and anti-inflammatory activity.



**In loving memory of**



**Dr. Jim Joseph**

## Neuroscience Lab

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L. Willis, Ph.D.

A. Martin, MD, Ph.D.

C. Andres-Lacueva, Ph.D.

I. Cantuti-Castelvetri, Ph.D.

R. Galli Ph.D.

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L. Simon, H. Stellwagen

A. Szprengiel, V. Cheng