Assessment of Gut Microbiome Role in Diet-Related Changes in Cognition

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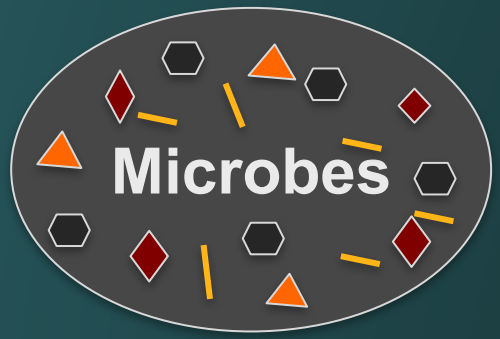
Introduction

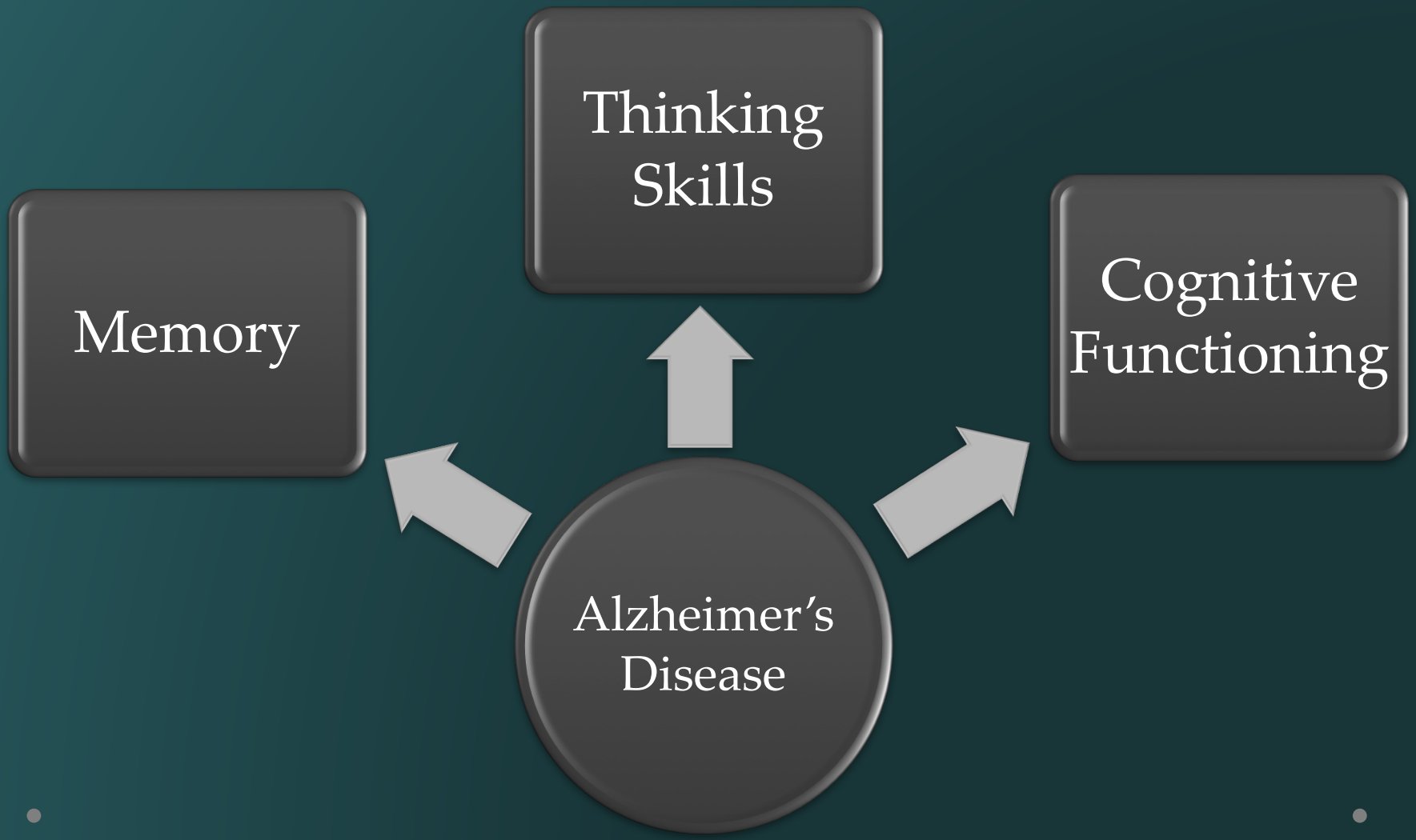


Gut Microbiome

- Community of microorganisms living in our intestines
- Interaction between the host diet and the gut microbiome
- Major player influencing the health status of the host

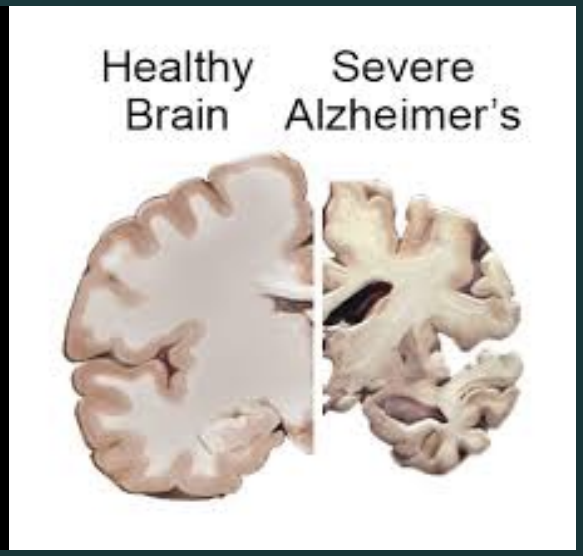
(Tsai et. al., 2009, Ghasias et. al., 2015)

High-sugar diets may lead to cognitive impairment



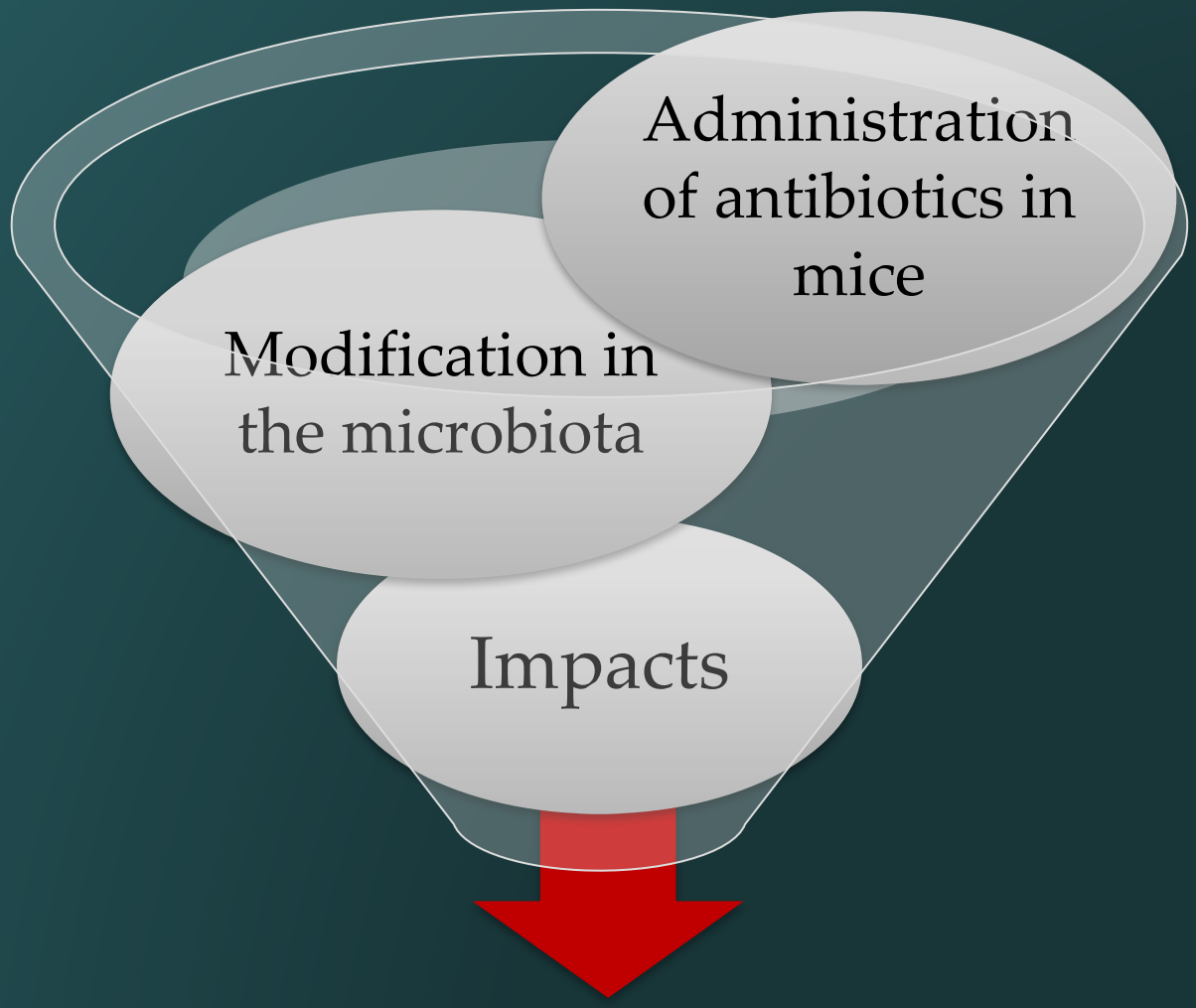
Sixth leading cause of death in the United States

No Cure → Treatments are only symptomatic → Combating high-sugar diets could reduce the possibility of Alzheimer's



(K.G Yiannopoulou et. al., 2011 & N.I et. al., 2017), http://cops.usdoj.gov & https://www.nia.nih.gov

Previous Studies



Behavior and Cognition

(M.G Gareau et. al., 2014)

Animal models are essential to research

- Mice are biologically very similar to humans and get many of the same diseases
  - 99% genes shared
- Mice can be genetically manipulated to mimic many human diseases or conditions

(N. Rosenthal et. al., 2007 and Four Main Resons, 2017), http://www.shzongyue.com/human.html

Purpose of Study

- Evidence that the microbiome influences cognition and the brain
- Evidence that high sucrose can cause changes in the gut bacteria and the mice show a loss of cognitive flexibility

http://discovermagazine.com/authors/kat-mcgowan

(Magnusson et. al., 2015)

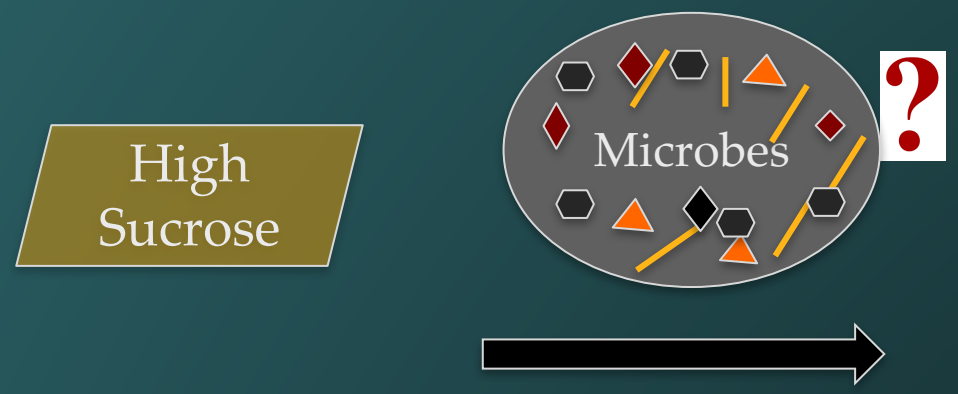
Hypothesis

The diet-induced changes in the microbiome cause changes in cognitive abilities.

Research objective

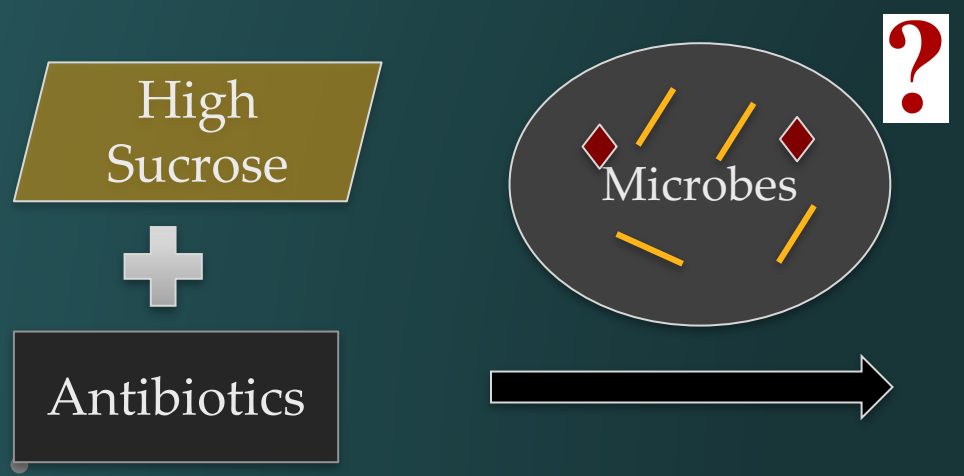
Investigate whether altering the microbiome via antibiotics, will change the behavioral results when animals are on a high sucrose only diet.

Previous studies



Poor Cognitive flexibility
Poor memory

Objective



Change?
If so,
Improvement?
Worsening?

Treatments

Control defined diet

VS

High-sucrose diet

	Control defined diet	High-sucrose diet
Percent of kcal provided by:		
Fat	13.0	11.8
Protein	25.0	17.7
Carbohydrates (CHO)	61.9	70.4

2x2 factorial design

	Control defined diet	High-sucrose diet
Water	6	6
Antibiotics	6	6

C57BL/6

http://www.criver.com/products-services/basic-research/find-a-model/c57bl-6-e-mouse

Water Treatments

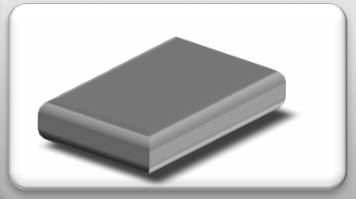
Antibiotic cocktail in water	
Ampicillin (1gm/L)	Used to treat infections of both negative and positive bacteria
Vancomycin (.5 gm/L)	Effective against gram positive
Neomycin Trisulfate (1gm/L)	Effective against gram negative bacteria & partial activity against gram positive bacteria
Metronidazole (1gm/L)	For treating anaerobic infections and protozoa

Water (control)
Sterile and filtered Water

Timeline for 8 week old male mice

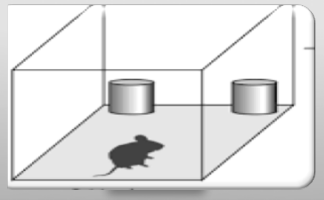
	Control diet	High sucrose diet	Antibiotics	Behavior testing	Fecal collection
Week -3	Start on control diet				
Week -2				Step down latency, novel object, and marble burying	Swap Feces end of week
Week -1			Start antibiotics cocktail		Antibiotic feces end of week
Week 0		Start high-sucrose diet			
Week 2					2 week post-diet change end of week
Week 5					Collect feces end of week
Week 7				Step down latency, novel object, and marble burying	
Week 8				Morris water maze	

Behavioral Testing



Step-down latency

Marble burying



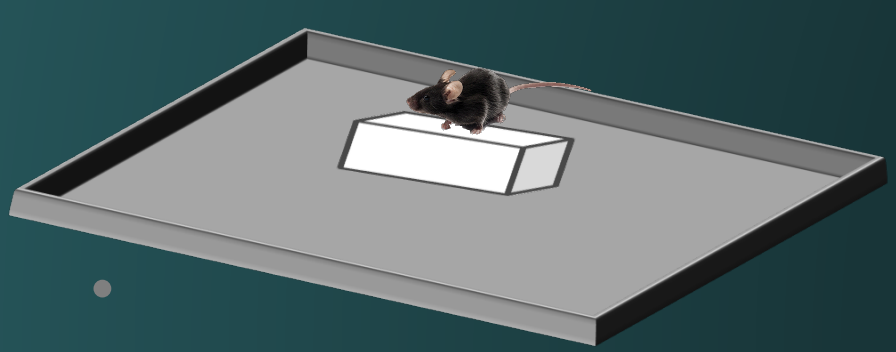
Novel object recognition

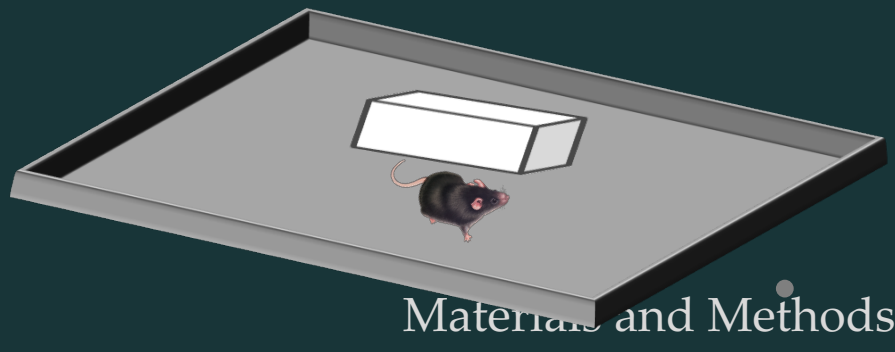
Morris water maze

Step-down latency

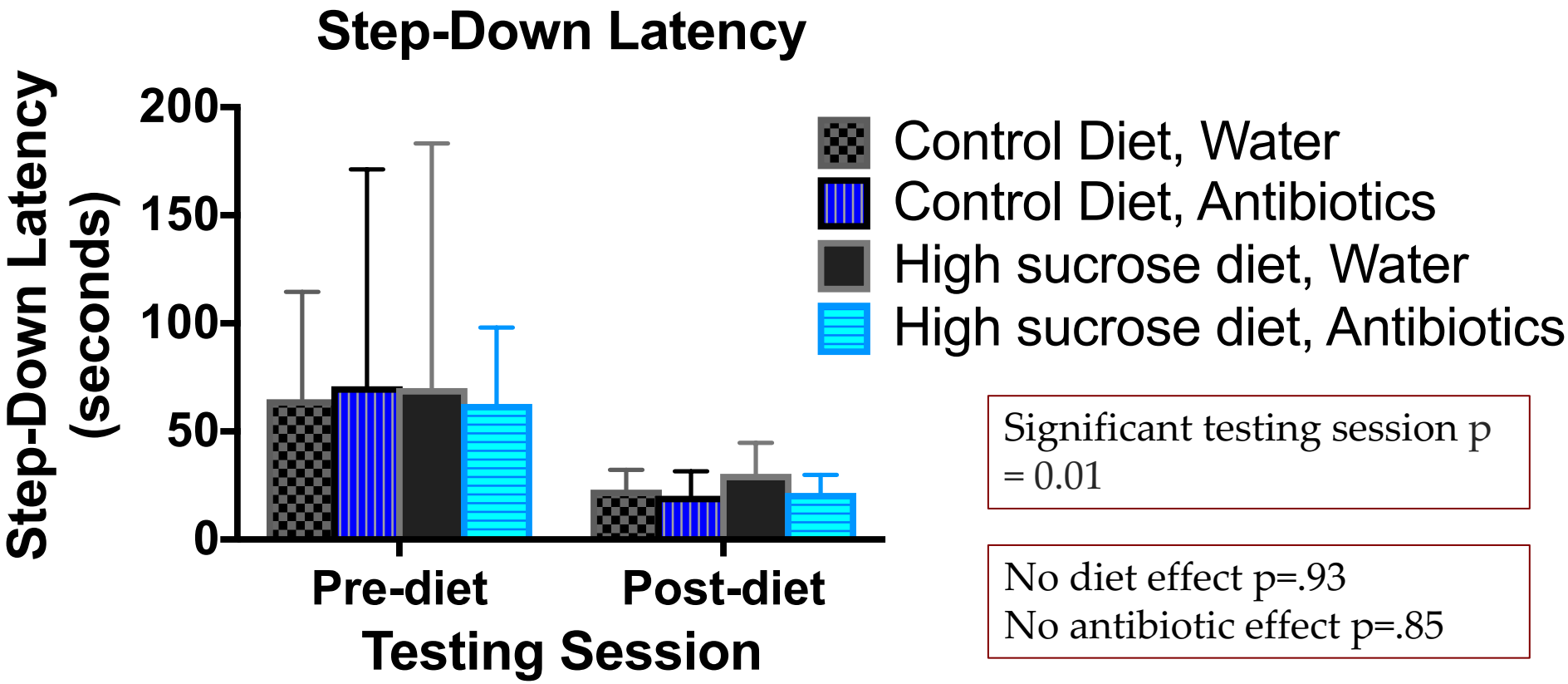
Impulsiveness and anxiety

- Placed on a 12.5 x 8.5 cm platform elevated 4 cm from a surgical table in dimly-lit room
- 3 trials with 5 minute maximum; 1 minute interval
- Duration of time, compared to control animals, indicates anxiety (increased latency) or impulsiveness (decreased latency)





Main effect of testing session for step-down latency



Significant testing session p = 0.01

No diet effect p=.93
No antibiotic effect p=.85

Novel object recognition

Memory and cognitive ability

- Plastic box arenas12”W x 18”D x 10”H
- Consisted of four sessions with 5 minutes in duration
- Time recorded each time they approached an object

Novel object recognition trials

Habituation

- Open field behavior conducted for percentage time in center zone, exploration distance, and speed

Familiarization

- 2 similar objects

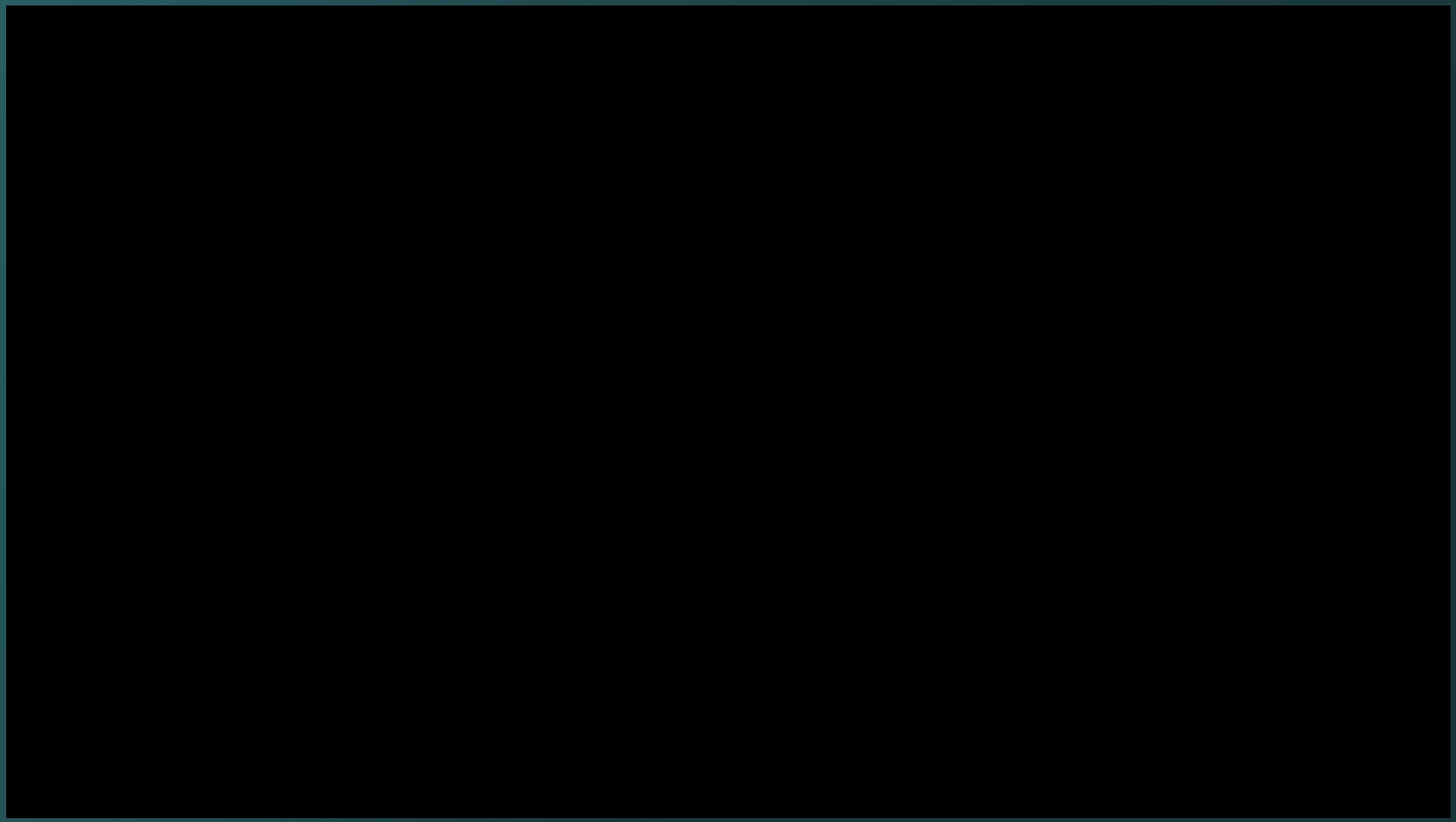
1-hour delay

- 1 replaced with novel object

24-hour delay

- Previous novel object replaced with new novel object

Novel object recognition video

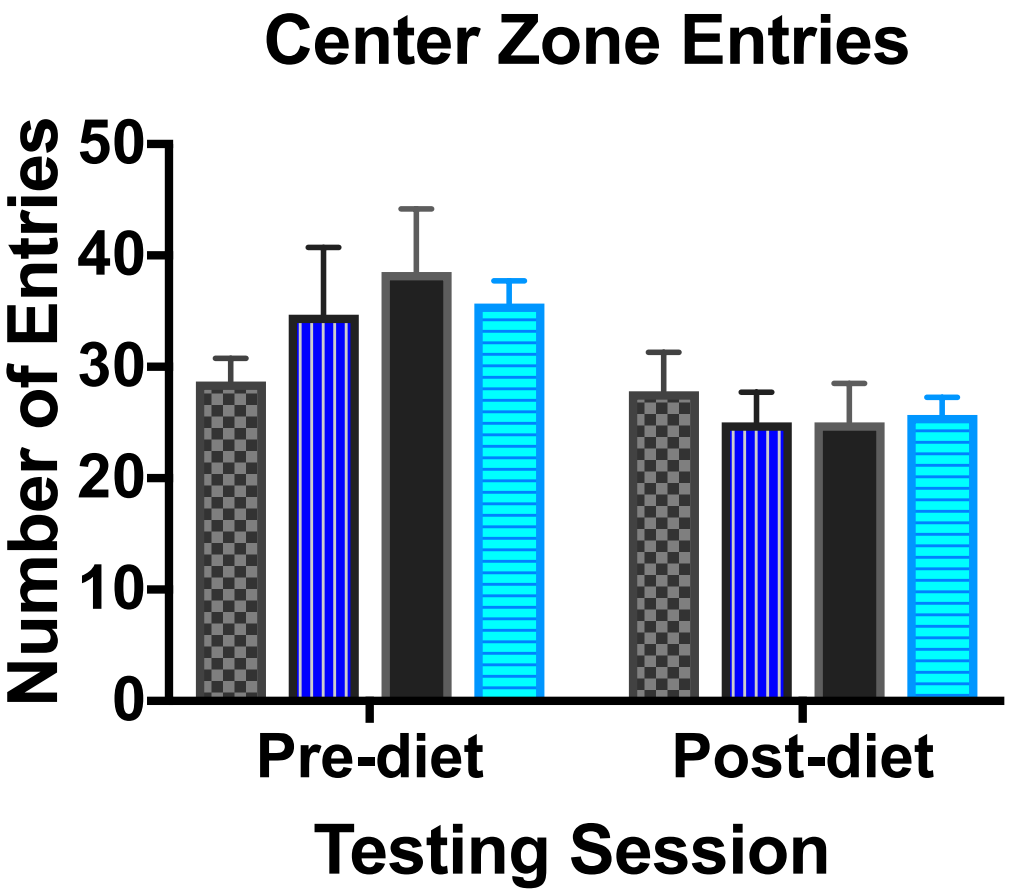


Novel object recognition trial

Habituation

- Open field behavior conducted for percentage time in center zone, exploration distance, and speed

Significant effect of testing session in center zone entries

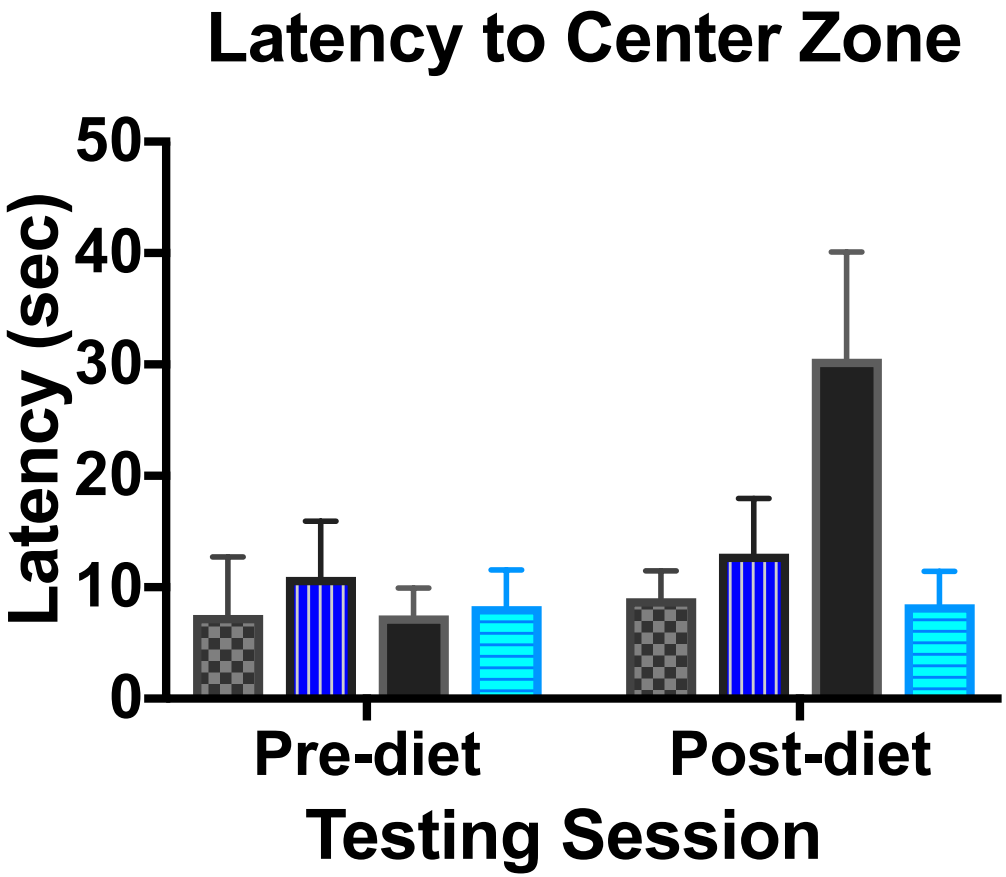


- Control Diet, Water
- Control Diet, Antibiotics
- High Sucrose, Water
- High Sucrose, Antibiotics

Significant effect of testing session $p < .01$

No diet effect $p = .36$
No antibiotic effect $p = .92$

Near significant effect of testing session in latency to center zone

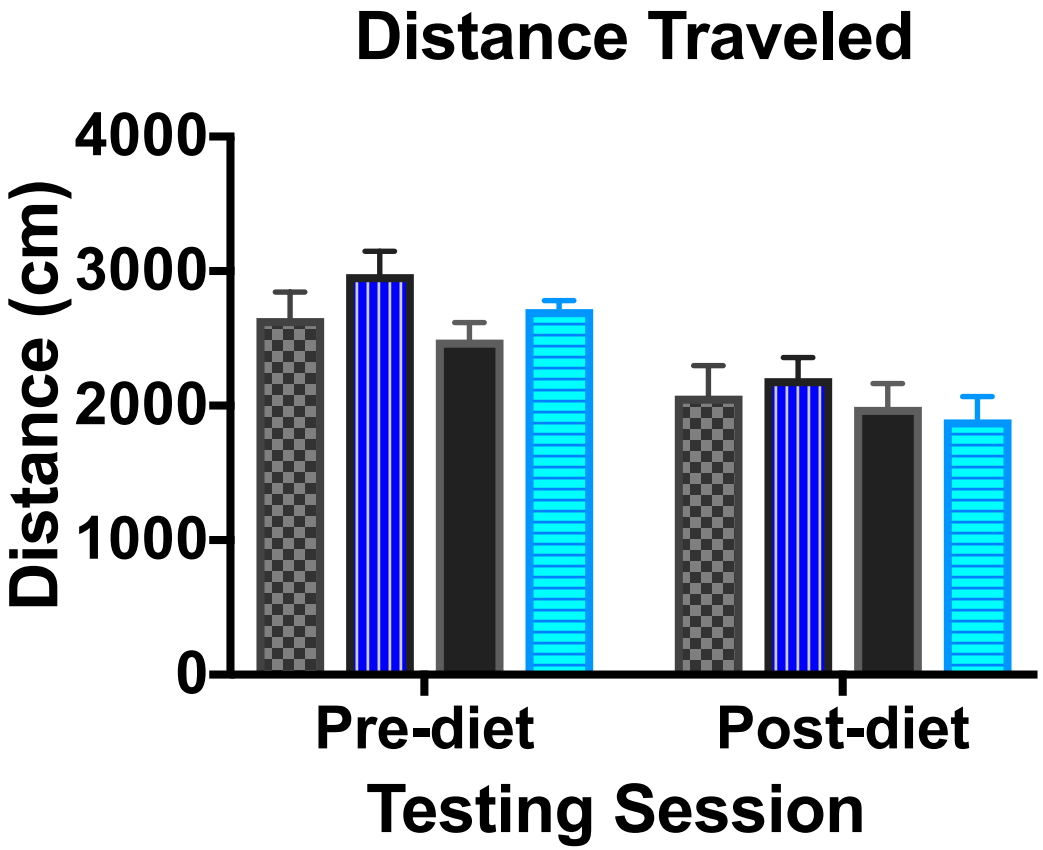


- Control Diet, Water
- Control Diet, Antibiotics
- High Sucrose, Water
- High Sucrose, Antibiotics

Near significant effect of testing session p = .06

No diet effect p = .35
No antibiotic effect p= .37

Significant effect of testing session in distance traveled zone

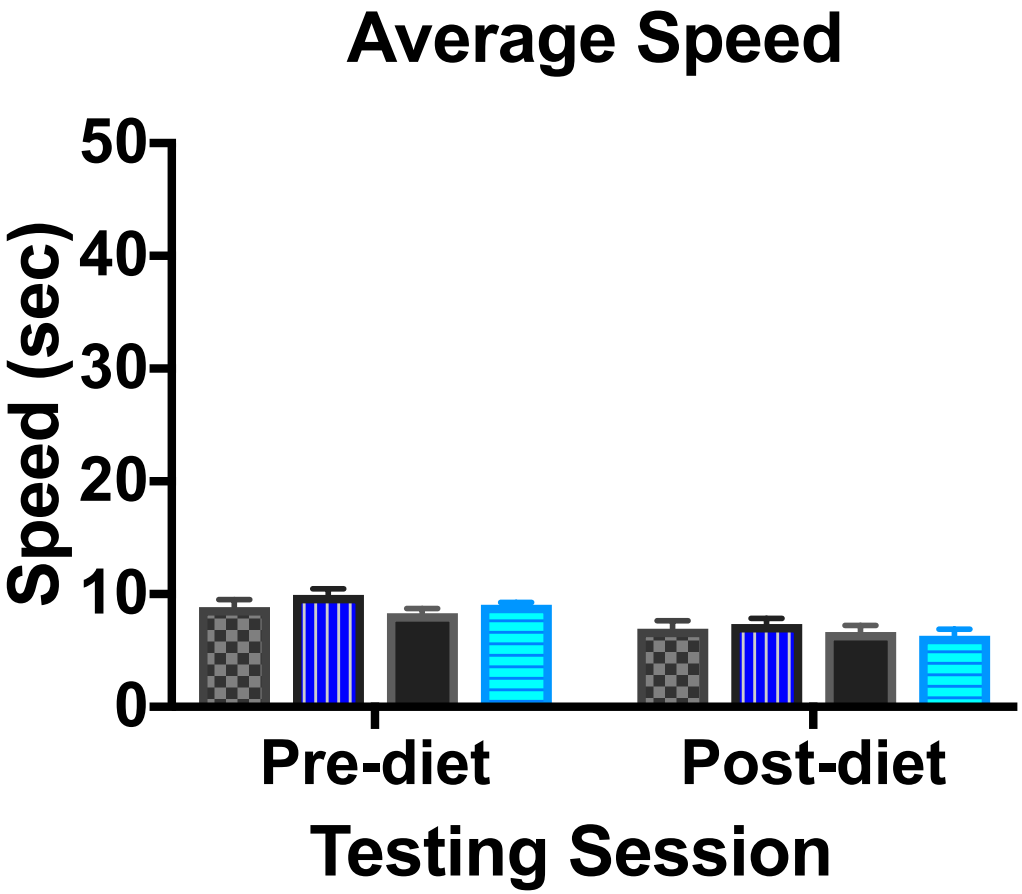


- Control Diet, Water
- Control Diet, Antibiotics
- High Sucrose, Water
- High Sucrose, Antibiotics

Significant effect of testing session $p < .01$

No diet effect $p = .15$
No antibiotic effect $p = .29$

Significant effect of testing session in speed



- Control Diet, Water
- Control Diet, Antibiotics
- High Sucrose, Water
- High Sucrose, Antibiotics

Significant effect of testing session $p < .01$

No diet effect $p = .15$
No antibiotic effect $p = .28$

Novel object recognition trials

Familiarization

- 2 similar objects

1-hour delay

- 1 replaced with novel object

24-hour delay

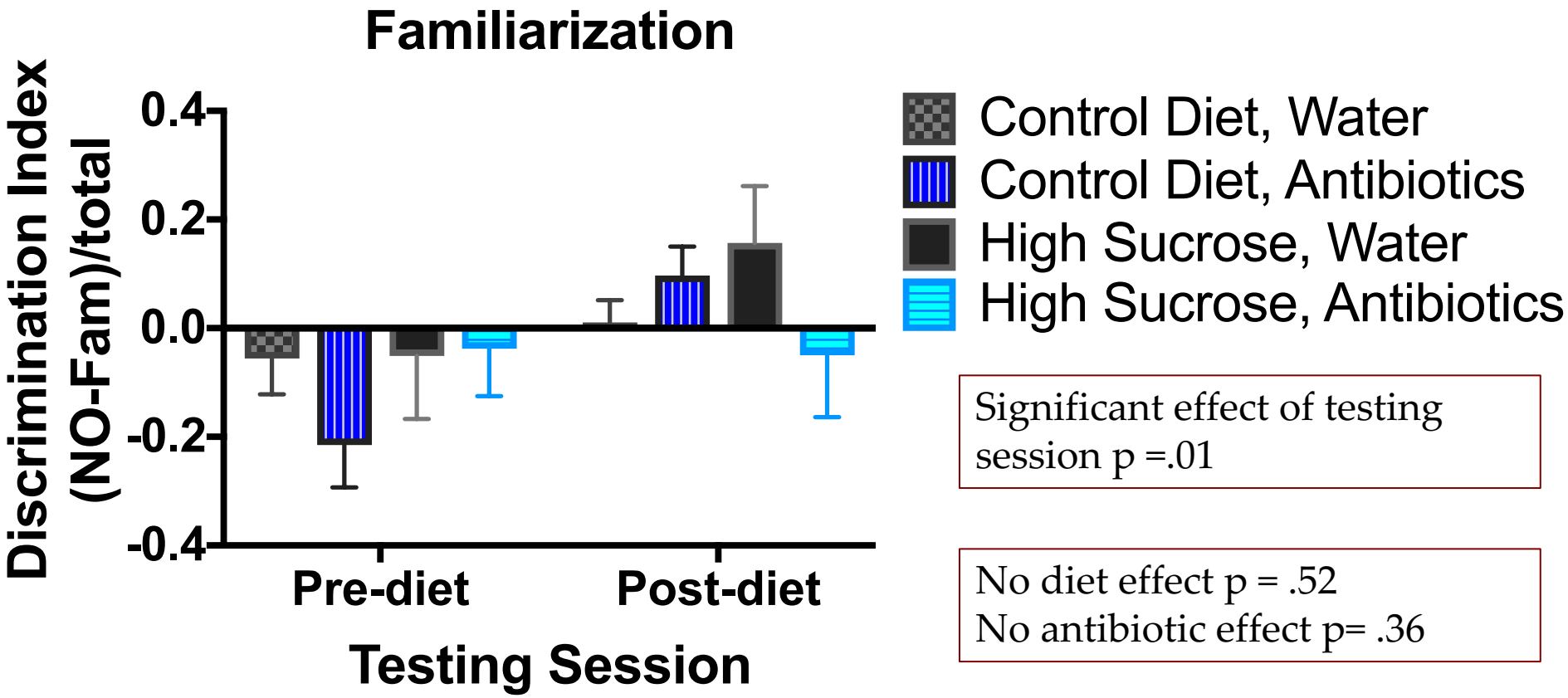
- Previous novel object replaced with new novel object

Novel object recognition calculation

- For novel object tasks, exploration time for each object was obtained by manual timing and a discrimination index was calculated by

$$\frac{Exploration\ time\ for\ novel\ object - Exploration\ time\ for\ familiar\ object}{Total\ exploration\ time}$$

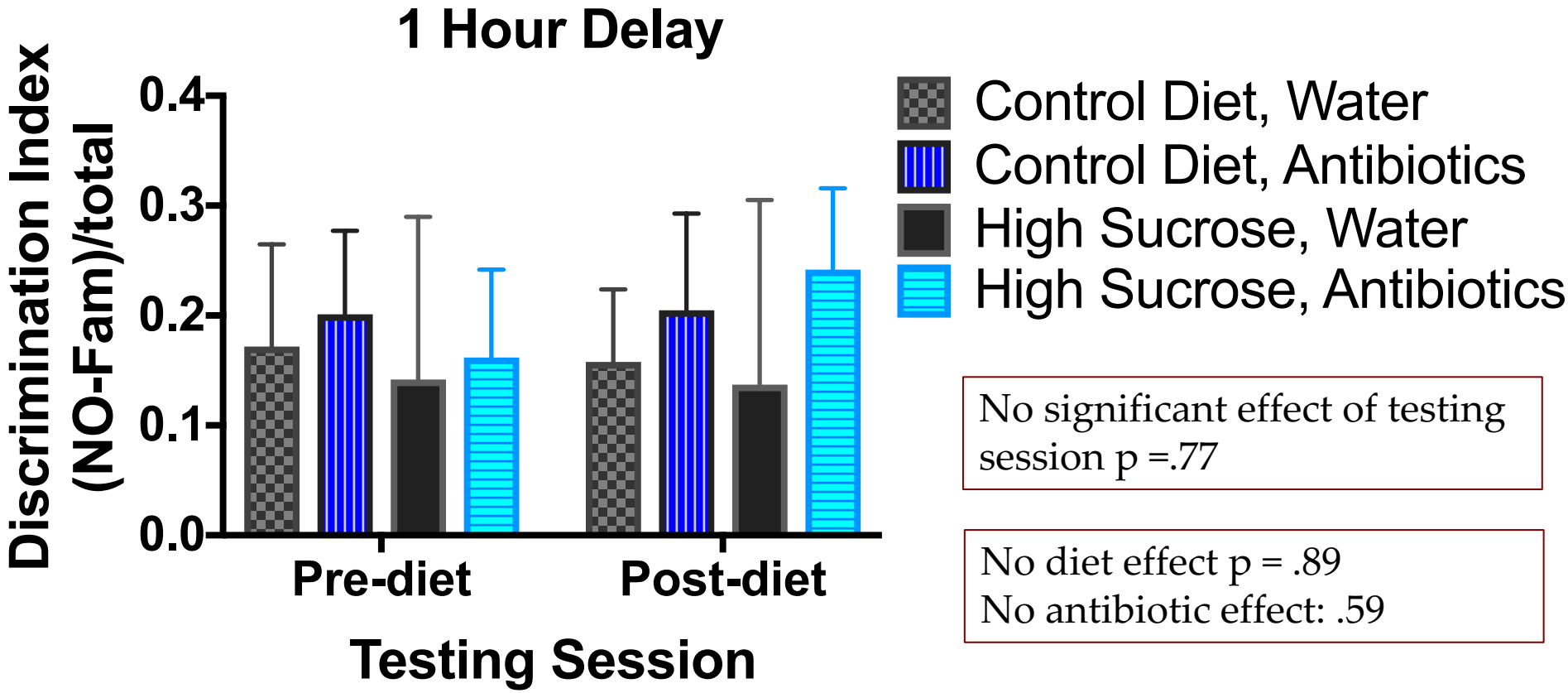
Main effect of testing session on familiarization



Significant effect of testing session p =.01

No diet effect p = .52
No antibiotic effect p= .36

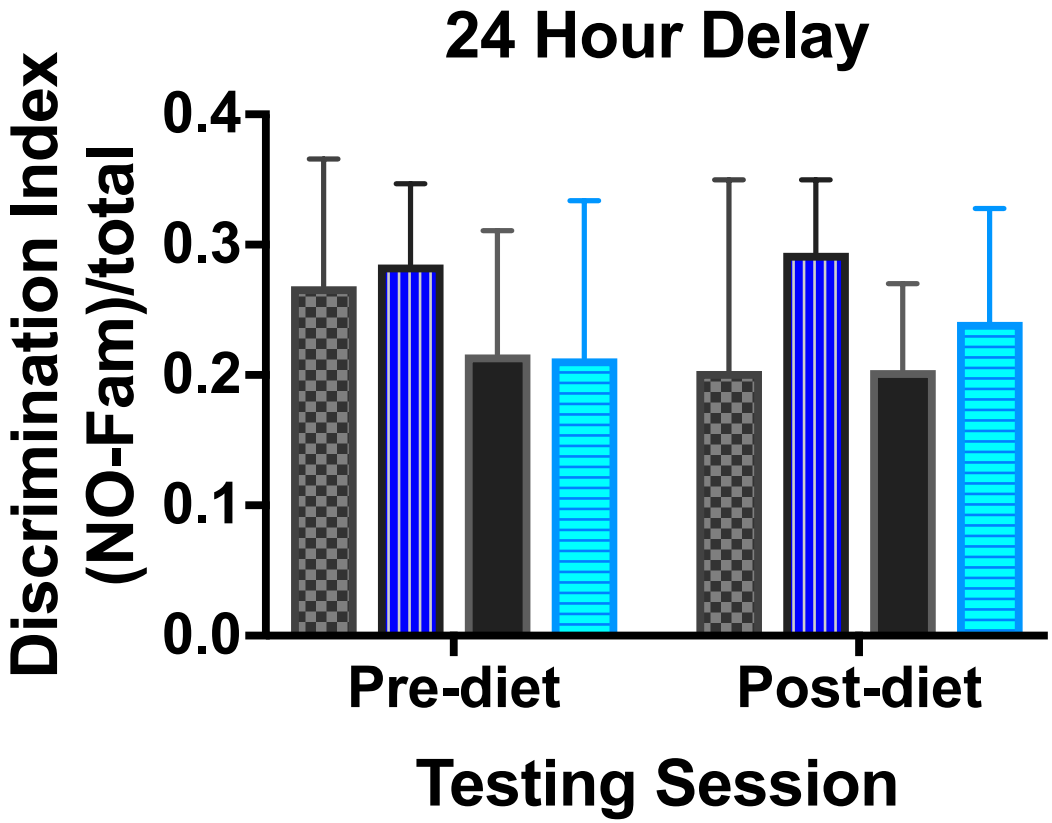
No diet or antibiotic effect on 1-hour delay memory



No significant effect of testing session p =.77

No diet effect p = .89
No antibiotic effect: .59

No diet or antibiotic effect on 24-hour delay memory



Control Diet, Water
Control Diet, Antibiotics
High Sucrose, Water
High Sucrose, Antibiotics

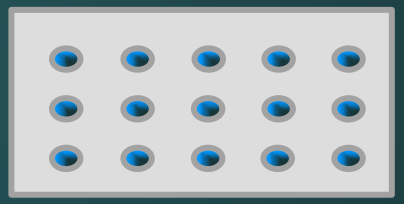
No significant effect of testing session p =.83

No diet effect p = .60
No antibiotic effect p = .67

Marble Burying

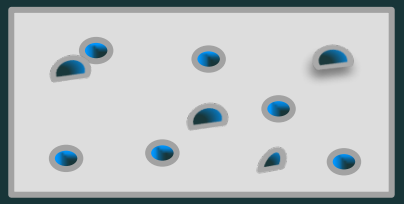
Compulsiveness and anxiety

- Marbles provide an effective unconditioned stimulus provoking burying
- 15 glass marbles arranged in an array of 3 x 5 on flattened bedding
- Marbles displaced, partially buried, fully buried, and the total number of marbles buried



Before

30 minutes

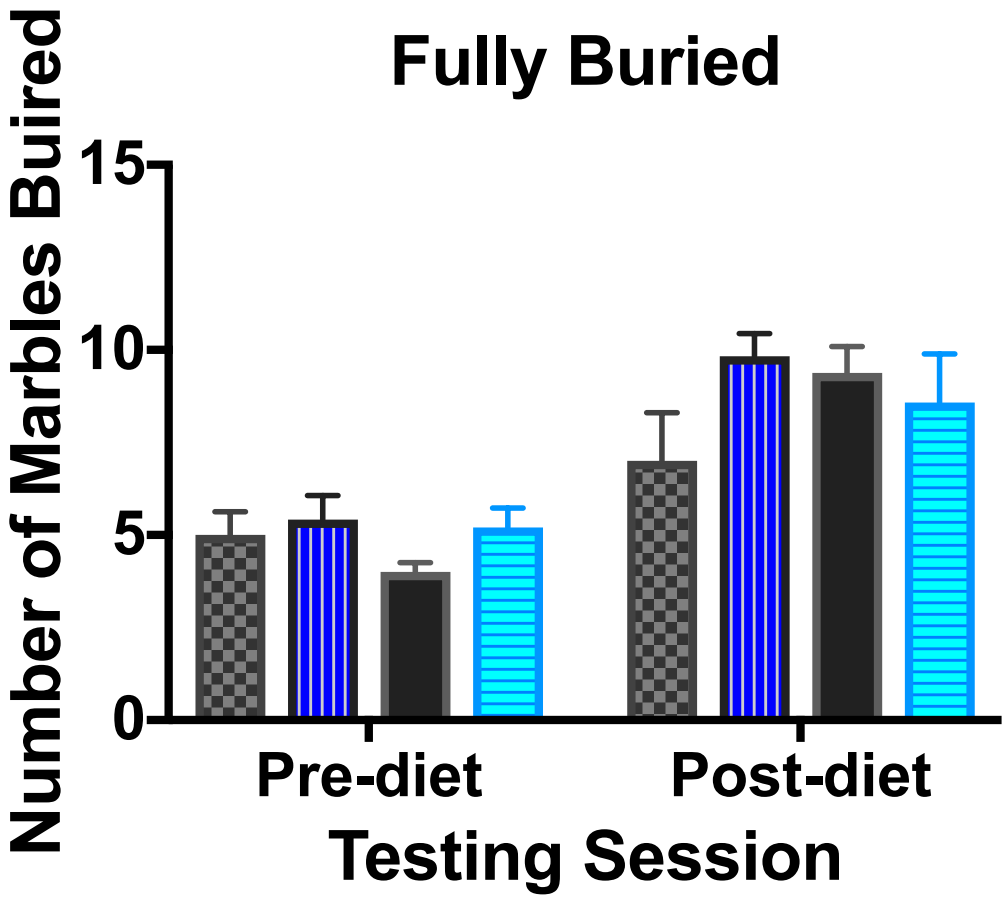


After

Marble Burying video

http://genestogenomes.org/tagc16-shorts-keeping-histone-marks-leads-to-losing-marbles/

Main effect of testing session

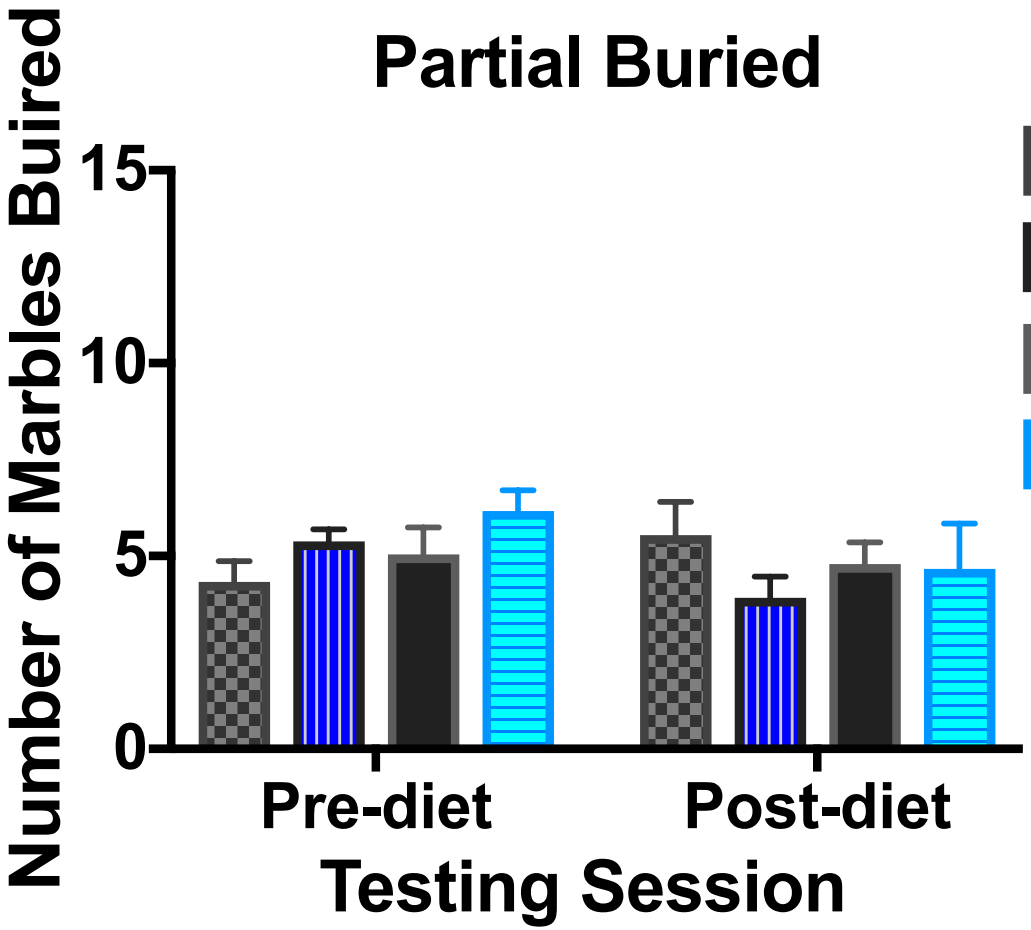


- Control Diet, Water
- Control Diet, Antibiotics
- High Sucrose, Water
- High Sucrose, Antibiotics

Main effect of testing session
p= <.01

No diet effect p = .97
No antibiotic effect .10

Interaction between testing session and antibiotic treatment



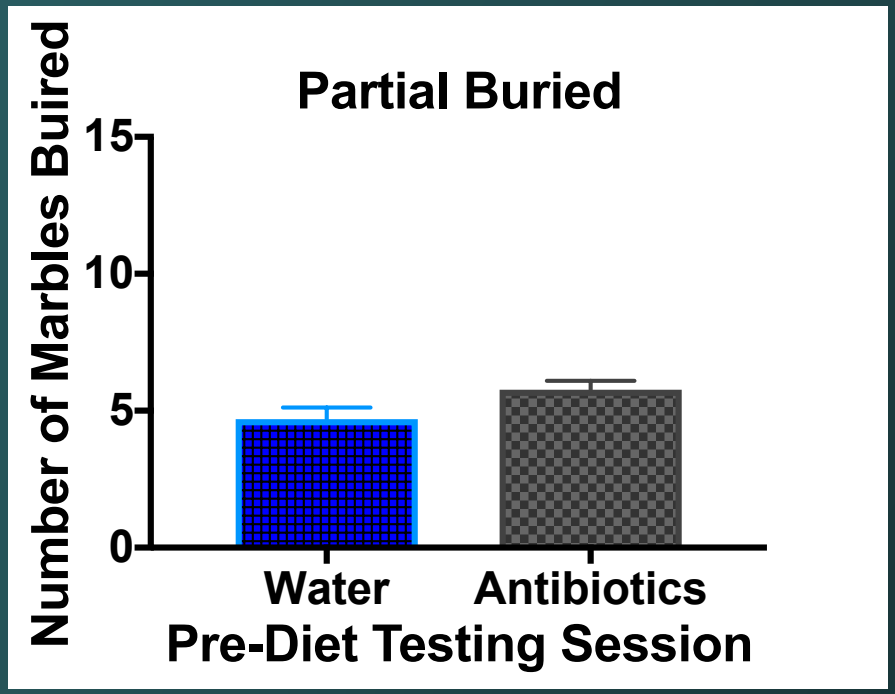
- Control Diet, Water
- Control Diet, Antibiotics
- High Sucrose, Water
- High Sucrose, Antibiotics

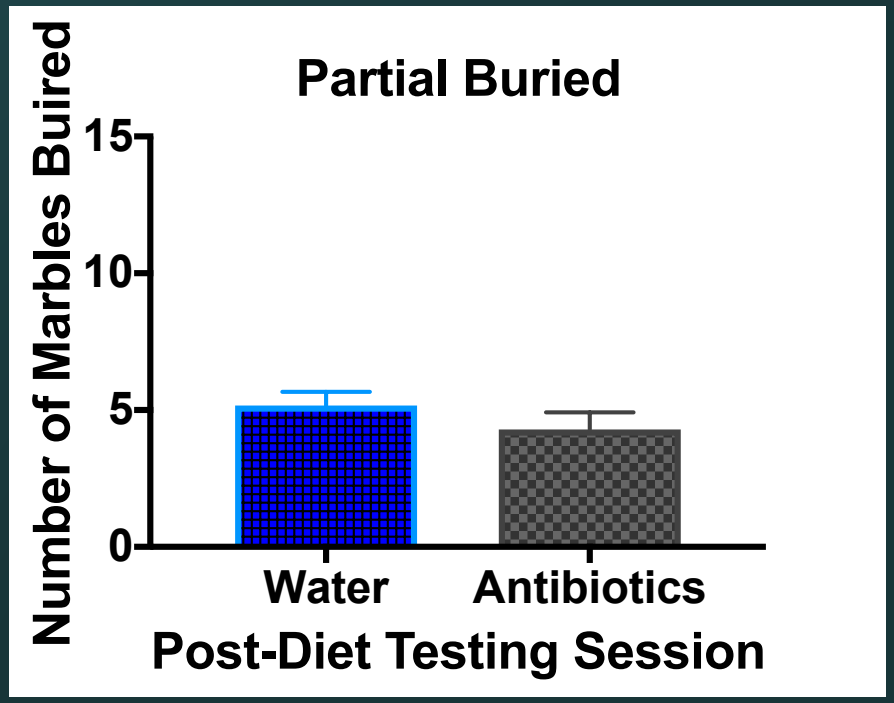
No Significant effect of testing session p =.28

No diet effect p = .49
No antibiotic effect p = .85

Testing session and antibiotic treatment interaction p = .04

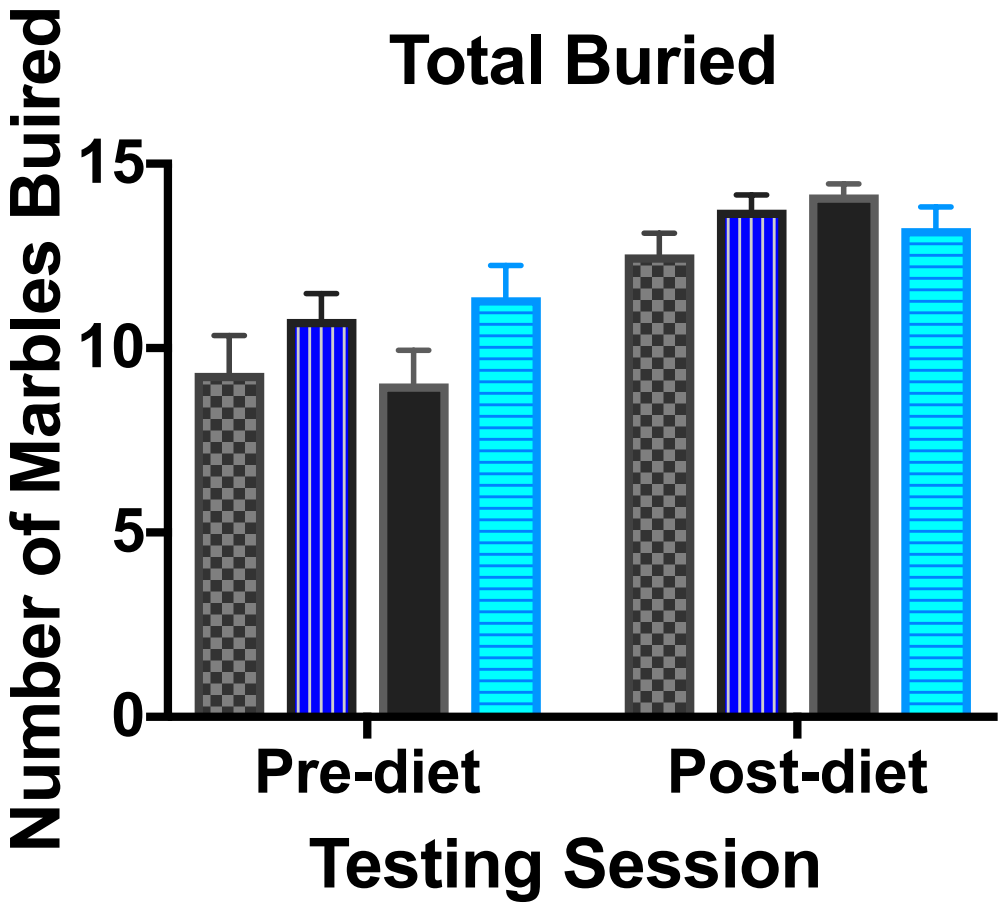
Number of partially buried marbles with data collapsed across diet





Trend for antibiotics groups partially burying more during pre-diet change testing session p = .06. No significant effect in post diet change session p = .29

Significant main effect of antibiotic treatment

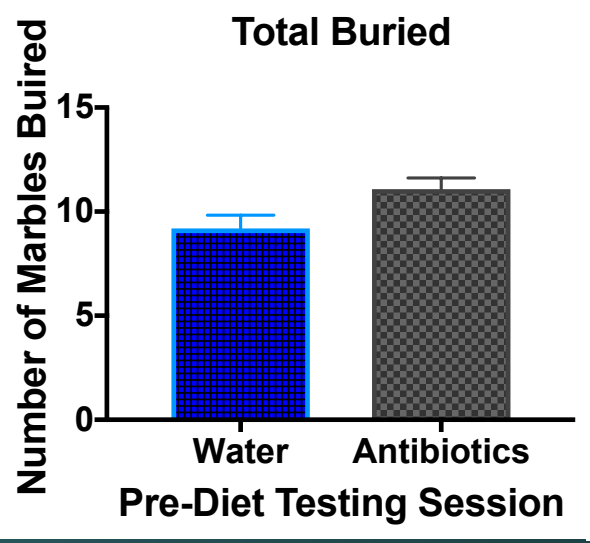


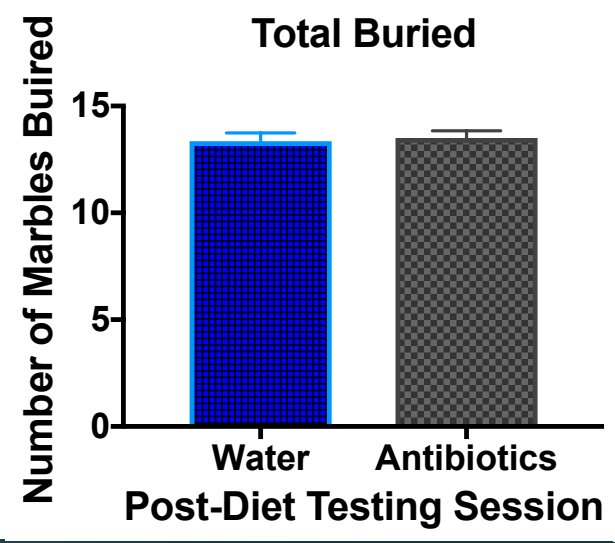
- Control Diet, Water
- Control Diet, Antibiotics
- High Sucrose, Water
- High Sucrose, Antibiotics

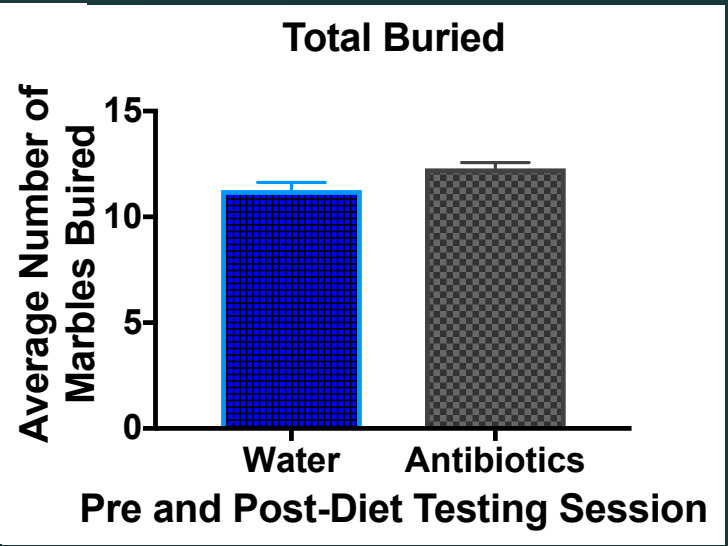
Main effect of testing session
p = < .01

No diet effect p = .45
Significant antibiotic treatment effect p= .04

Number of total marbles buried with data collapsed across diet







No difference between antibiotic treatments in the post-diet change session P = .78

Morris water maze

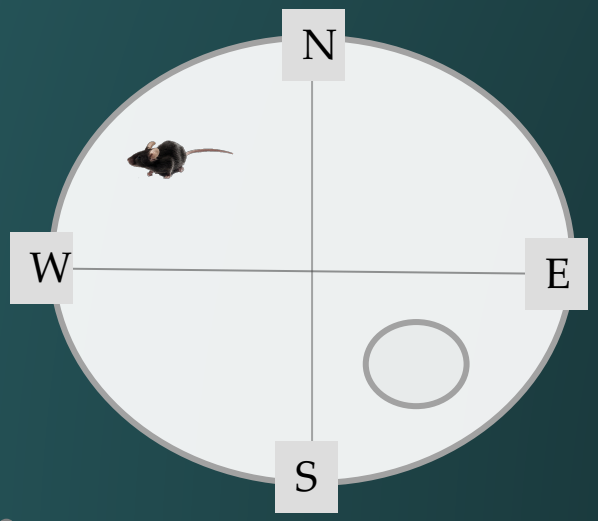
Spatial long-term memory & Cognitive flexibility

Morris water maze

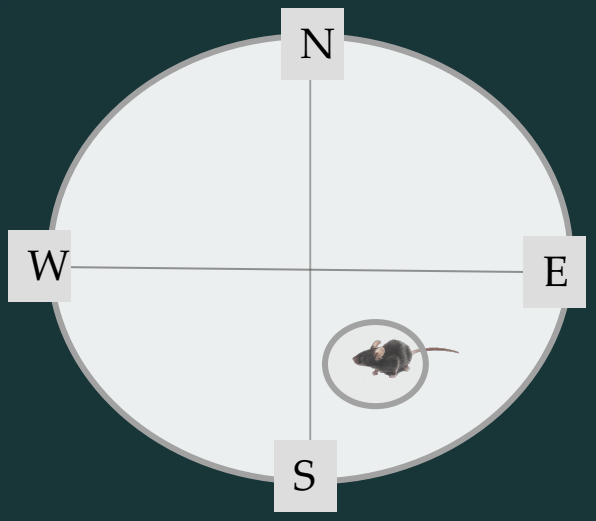
Spatial long-term memory & Cognitive flexibility

- 4 foot diameter plastic tank filled with water ((16-18 °C) 1 cm above platform level
- Removable circular platform

Before Learning

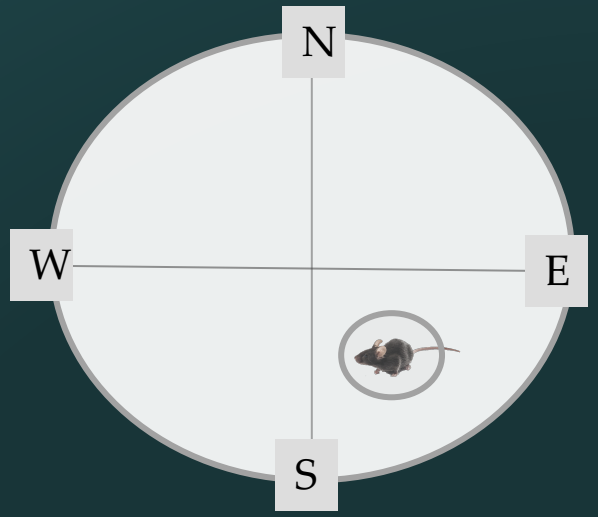


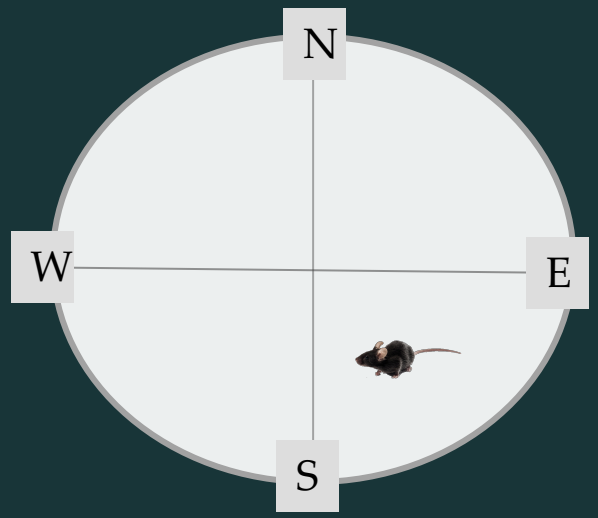
After Learning



Morris water maze trials

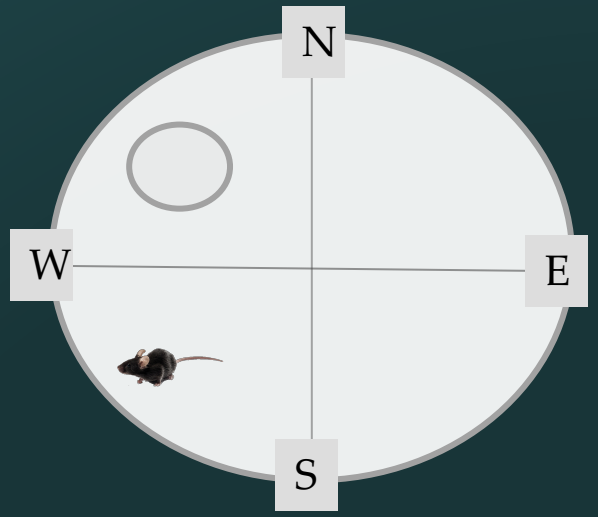
- Acclimation - 2 days
  - Experience and Training
- Place Trials - 4 days
  - Spatial long-term memory
  - 8 trials per day
  - Platform in SE quadrant.
- Probe Trials – 4 days
  - Determine spatial bias
  - Initial (PR0)and 1 per day
  - Platform not present (30 sec.)

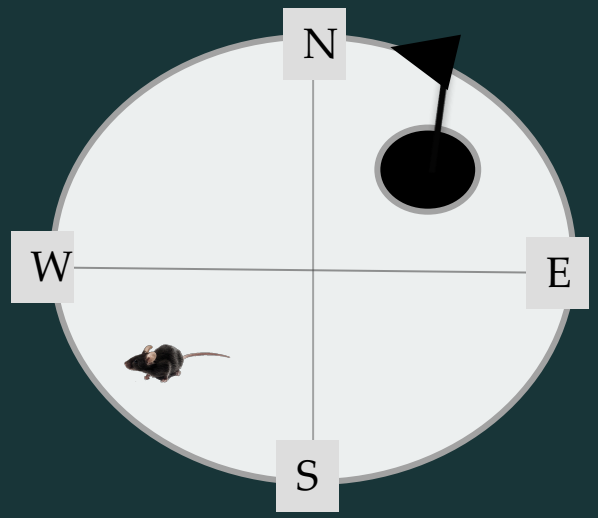


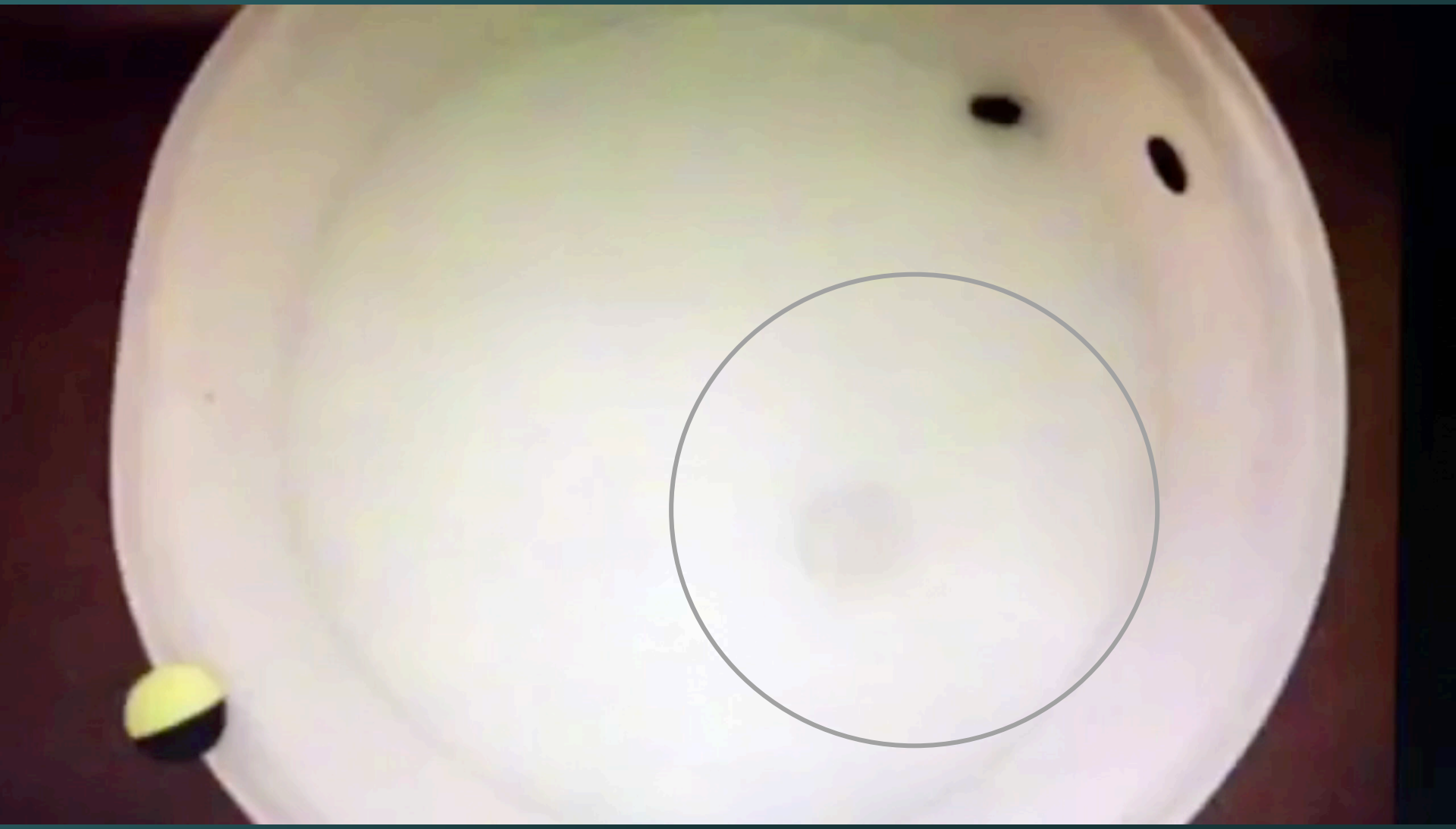


Morris water maze trials cont.

- Reversal Trials – 1day
  - cognitive flexibility
  - 8 trials
  - Platform in NW quadrant
- CUED Trials – 1 day
  - Control memory task (flag on top of platform)
  - 6 trials (S, Center, NE, W, SE, SW)
  - Test motivation, visual, and physical ability

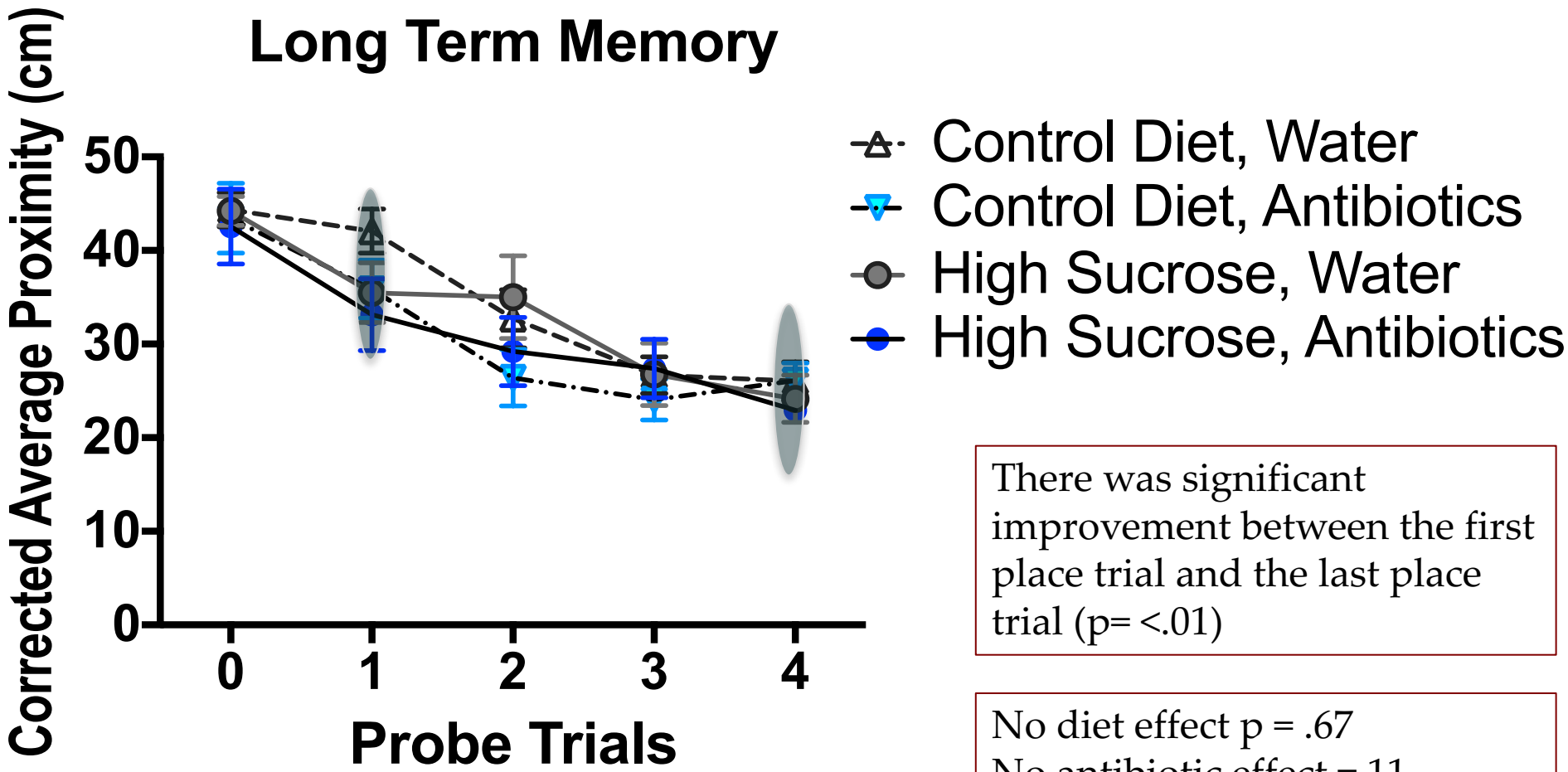






Materials and Methods

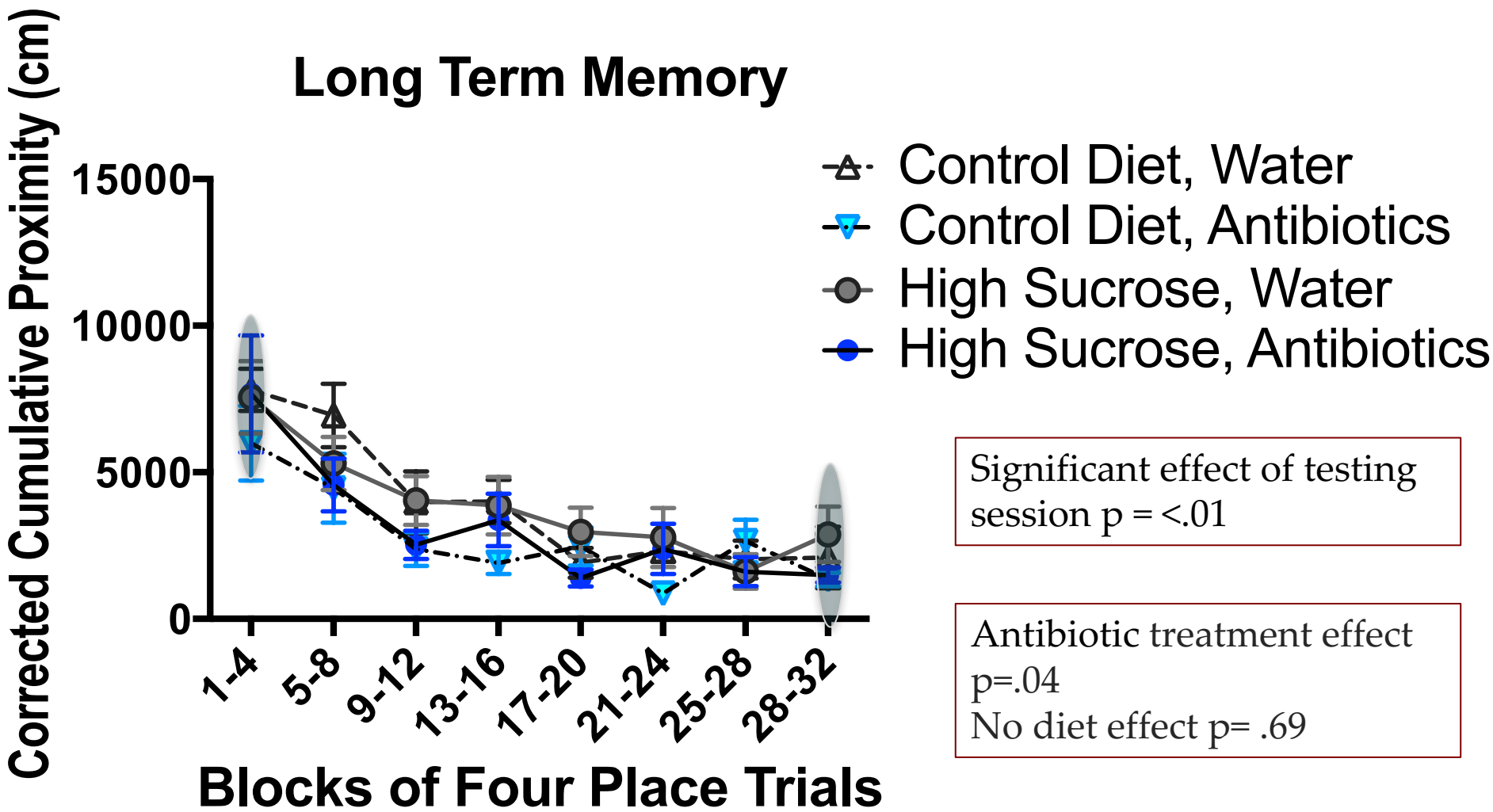
Probe trial results showed no treatment effect on development of spatial bias



There was significant improvement between the first place trial and the last place trial (p= <.01)

No diet effect p = .67
No antibiotic effect =.11

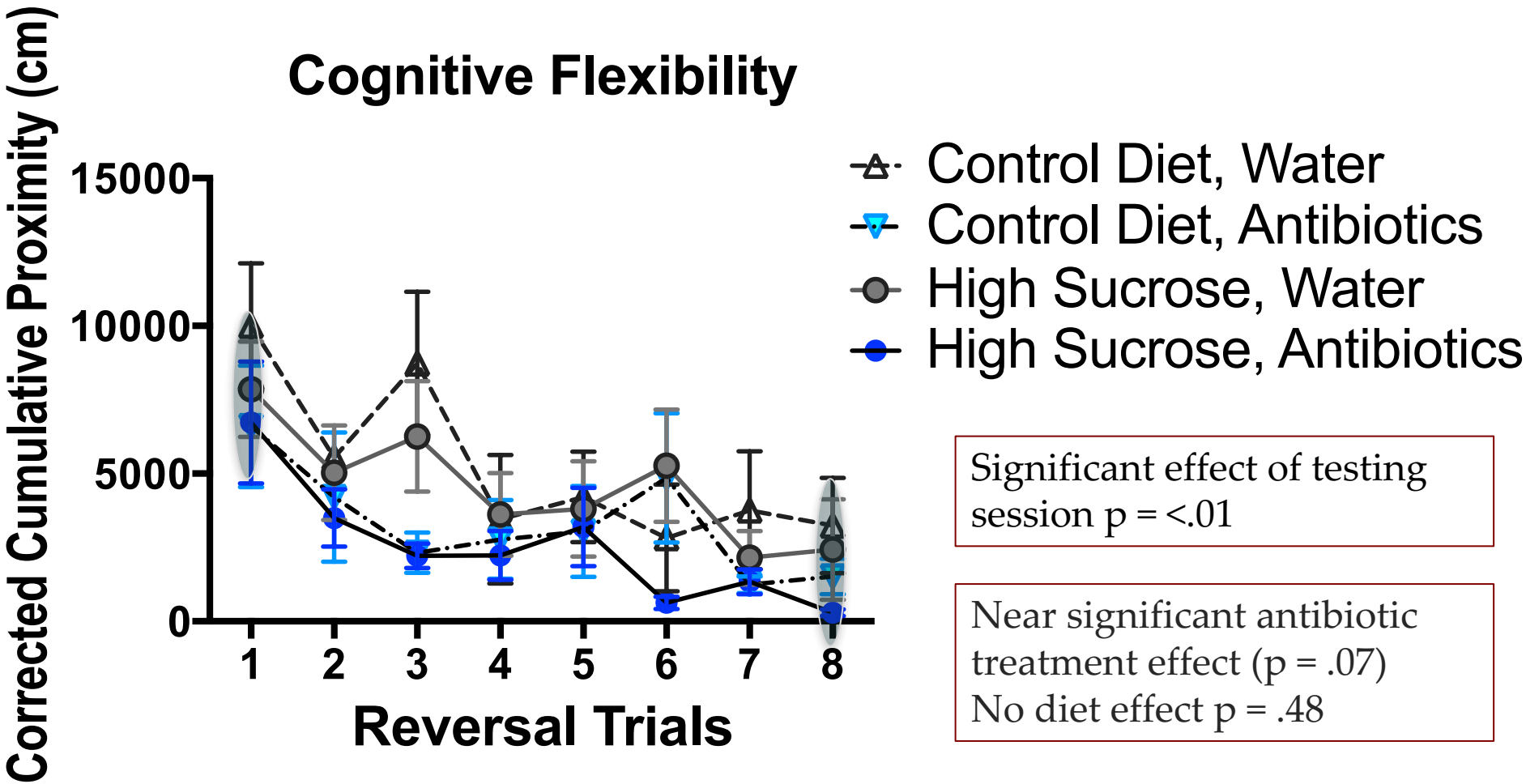
Blocks of 4 place trials showed antibiotic treatment effect on long term memory



Significant effect of testing session p = <.01

Antibiotic treatment effect p=.04
No diet effect p= .69

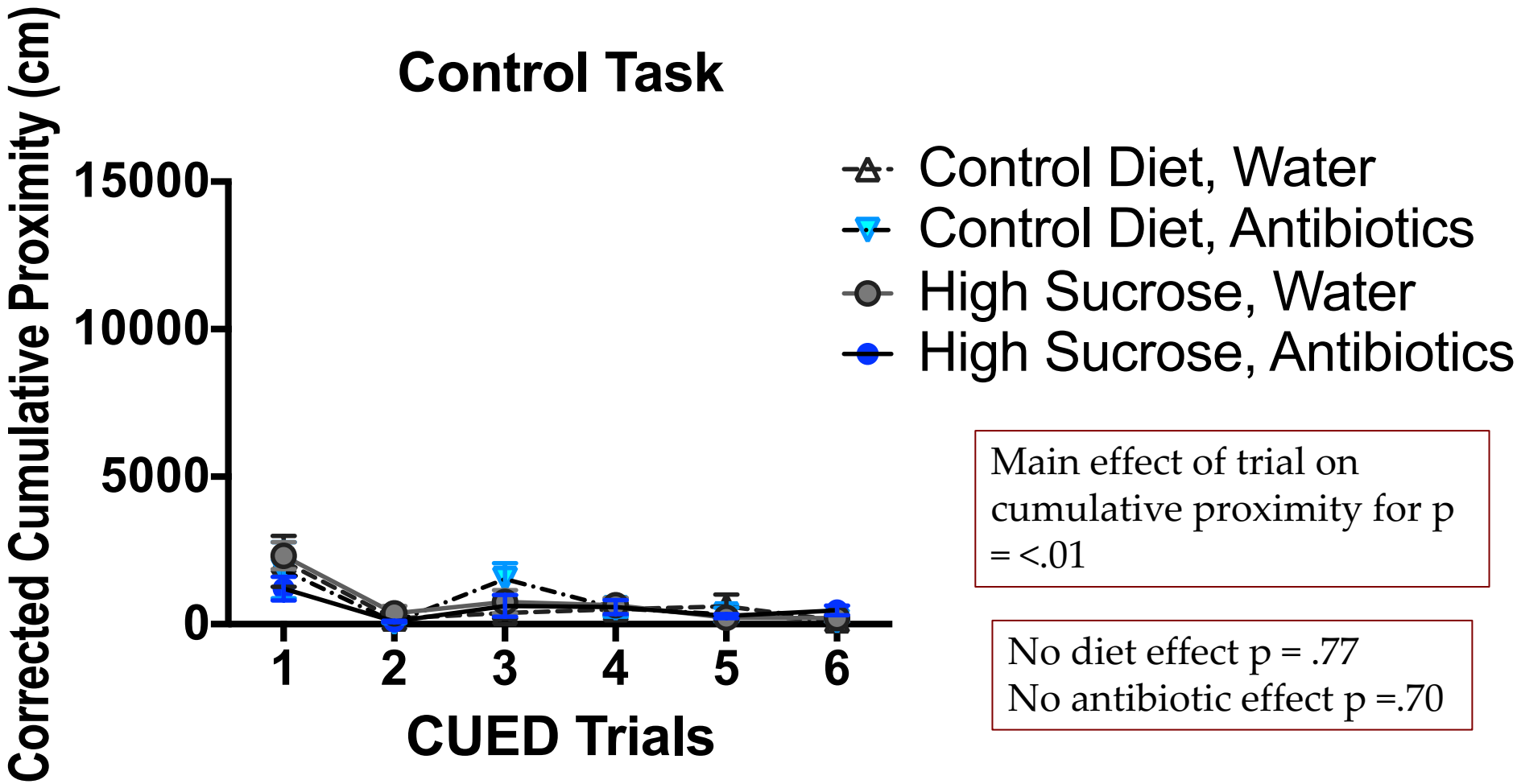
Reversal trials showed near significant antibiotic treatment effect on cognitive flexibility



Significant effect of testing session p = <.01

Near significant antibiotic treatment effect (p = .07)
No diet effect p = .48

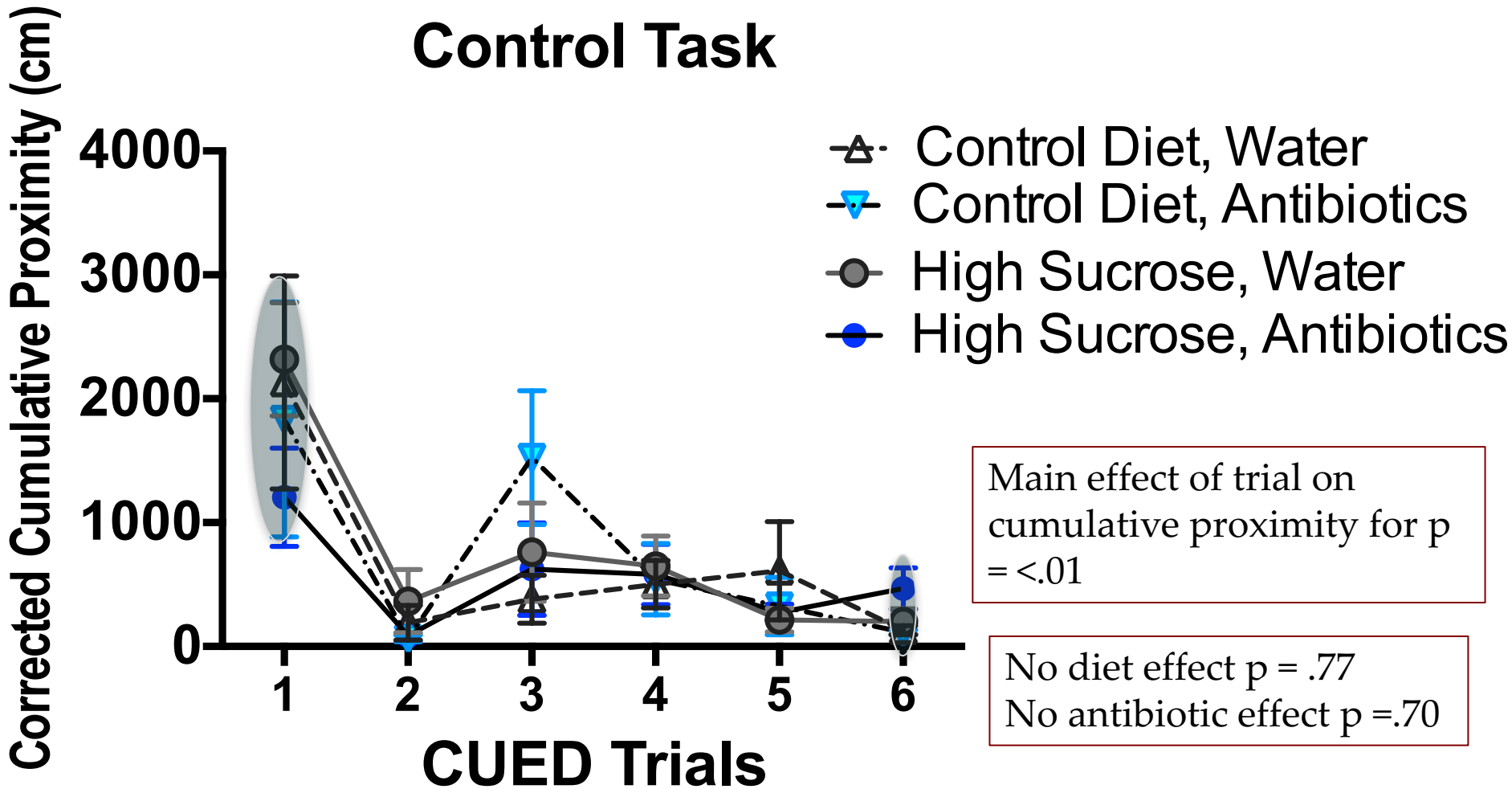
Main effect of trials on cumulative proximity



Main effect of trial on cumulative proximity for p = <.01

No diet effect p = .77
No antibiotic effect p =.70

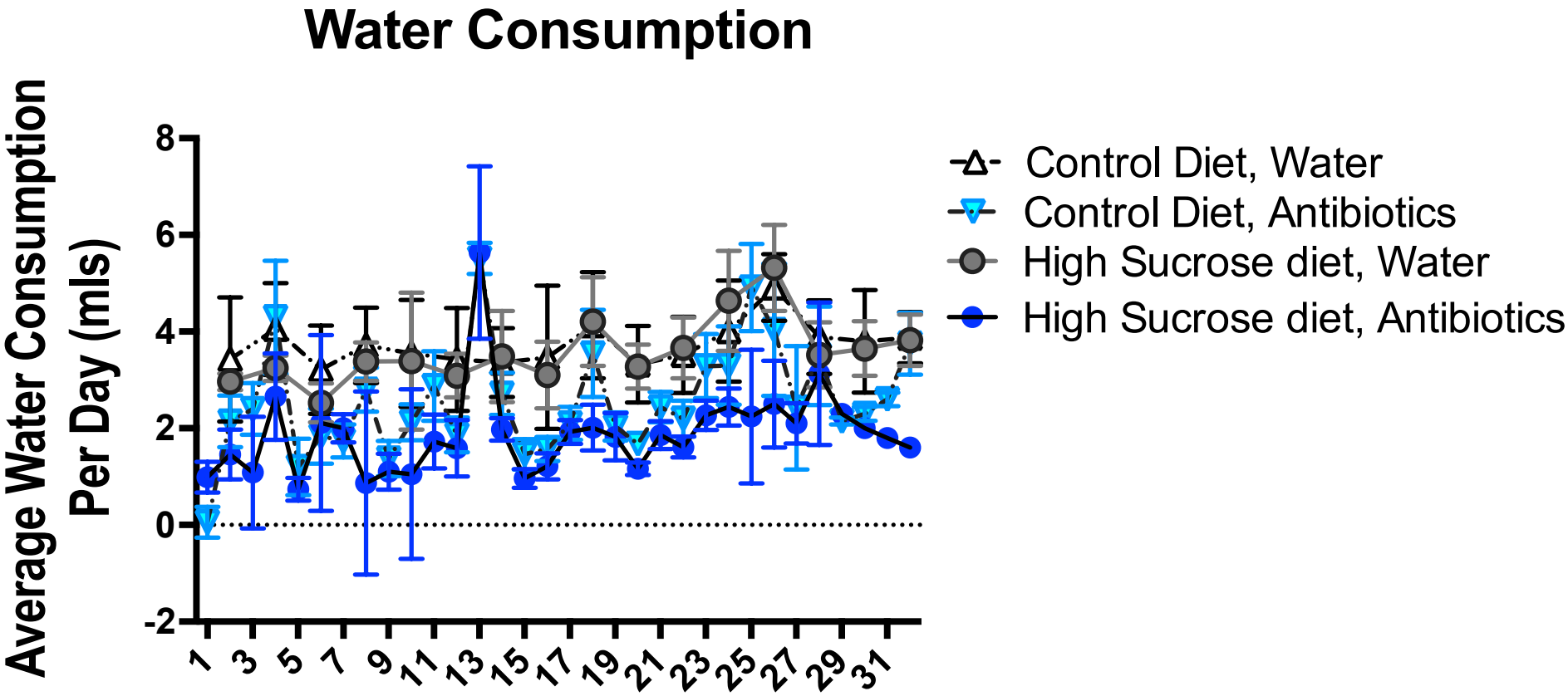
Main effect of trials on cumulative proximity



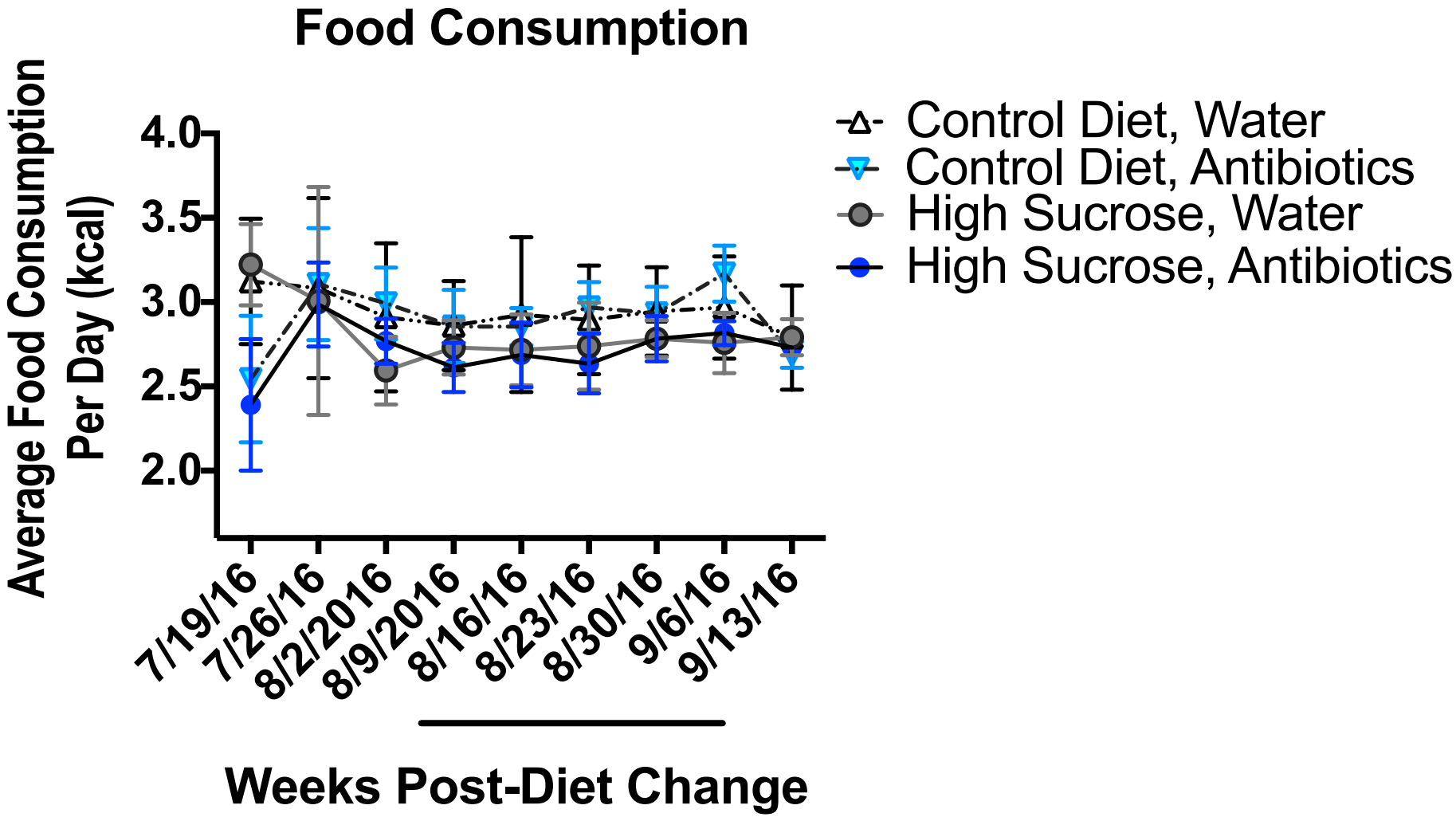
Main effect of trial on cumulative proximity for p = <.01

No diet effect p = .77
No antibiotic effect p =.70

Water consumption was monitored



Food consumption was monitored



Summary

- Antibiotic treatment effects
  - Blocks of 4 place trials – long term memory
- There were no high-sucrose diet effects, as compared to a defined control diet, for the behavioral tests conducted
- Significant testing session changes
  - total buried marbles
  - fully buried marbles
  - familiarization for novel object
  - step-down latency

Discussion

- The significant antibiotic treatment effect suggests that the gut microbiota influences behavioral outcomes.
- The lack of effect of the high sucrose diet may be due to using a non-chow, defined diet as the control.
- This will be further investigated through microbiome analysis
- Bacterial content from fecal collection will demonstrate the bacteria present and or reduced from antibiotics and any differences between diets

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