BEHAVIOURAL TESTING IN RATS

Impact of Omega-3 Fatty Acids Supplementation and Exercise on Locomotor Activity, Exploratory Activity and Anxiety

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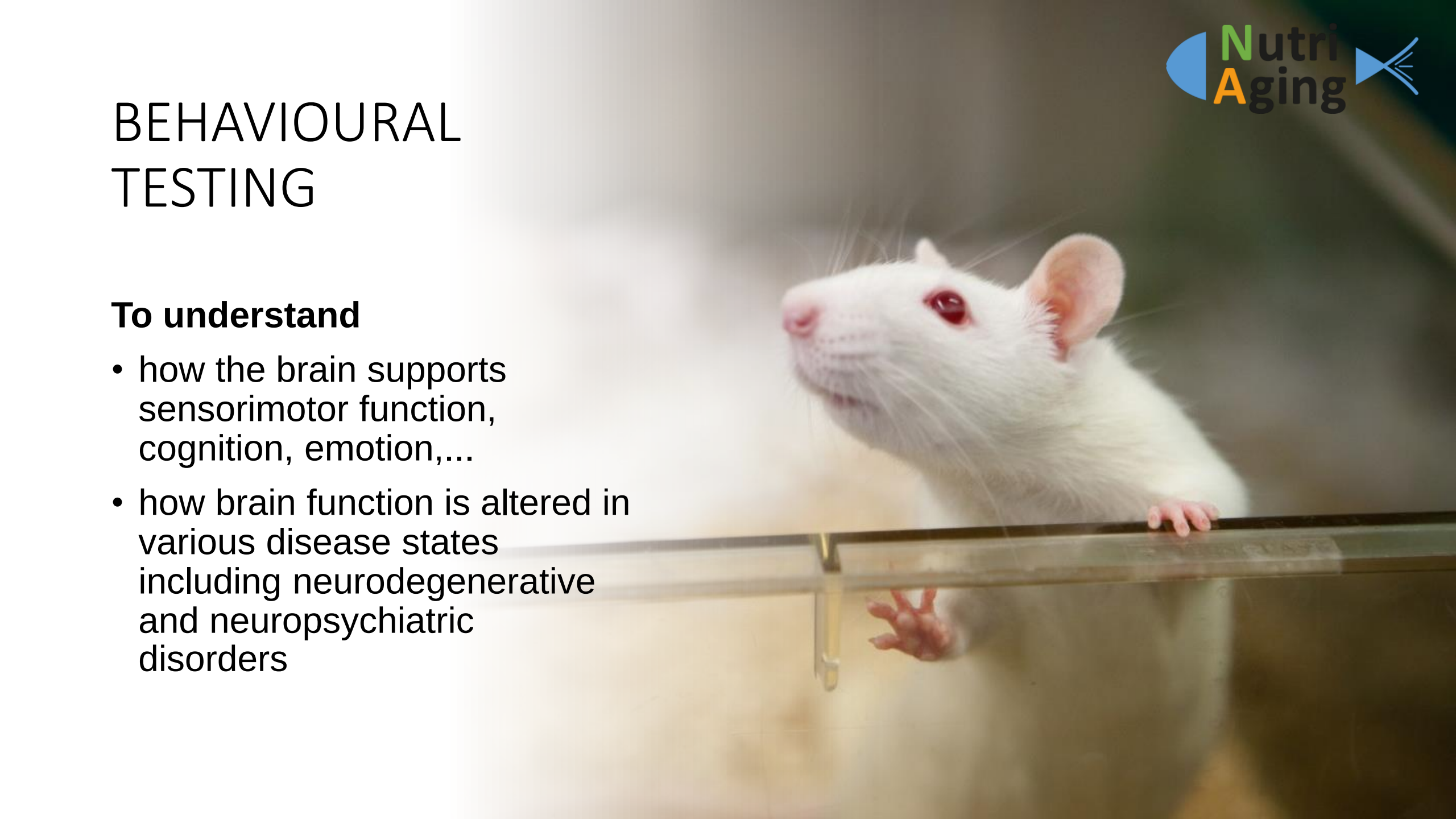
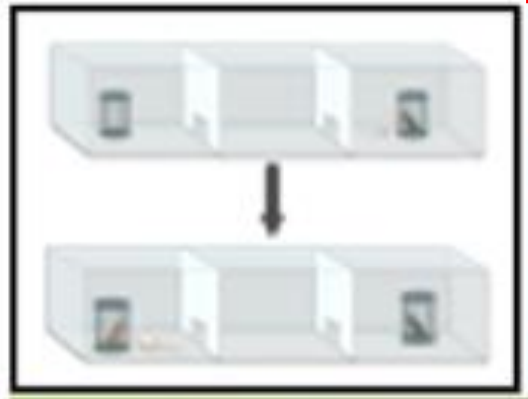
BEHAVIOURAL TESTING

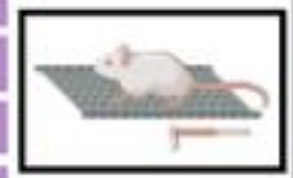
To understand

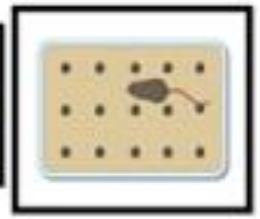
- how the brain supports sensorimotor function, cognition, emotion,...
- how brain function is altered in various disease states including neurodegenerative and neuropsychiatric disorders



Operant Conditioning/Reward	Learning and Memory	Affective Behaviors	Sensorimotor Testing
Intravenous/Intracranial Self-Administration		Elevated Plus Maze	SHIRPA Primary Screen
Food Self-Administration		Light Dark Box	Rotarod
Water/Sucrose Self-Administration		Novelty Suppressed Feeding	Balance Beam
Operant Touch Screens		Forced Swim Test	Pole Test
Intracranial Self-Stimulation (coming soon)		Tail Suspension Test	Grip Strength
	T-Maze/Y-Maze	Marble Burying Test	Catwalk
	Morris Water Maze	Aggression	Hot Plate
	Barnes Maze	Social Interaction	Von Frey Test
	Radial Arm Maze	Passive Avoidance	Tail Immersion
	Novel Object Recognition	Open Field	
	Novel Object Location	Ultrasonic Vocalization	
	Social Recognition		
	Social Transmission of Food Preference		
	Fear Conditioning		
	Pre-Pulse Inhibition (startle response)		
	Taste Aversion		
Sucrose Preference Test			
Conditioned place preference/aversion			

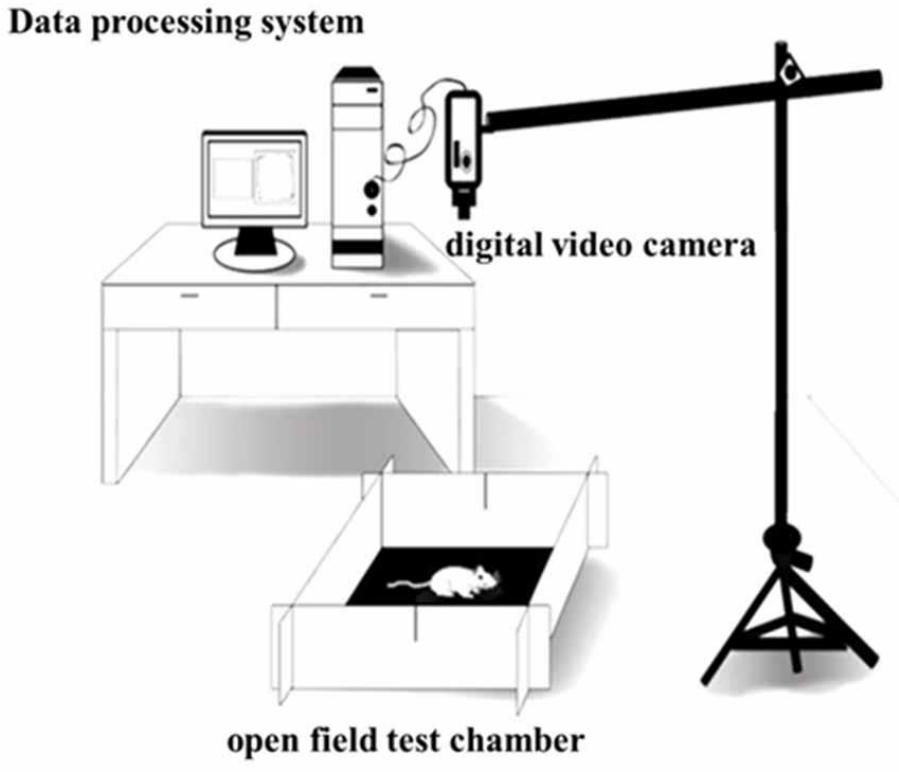






OPEN FIELD TEST

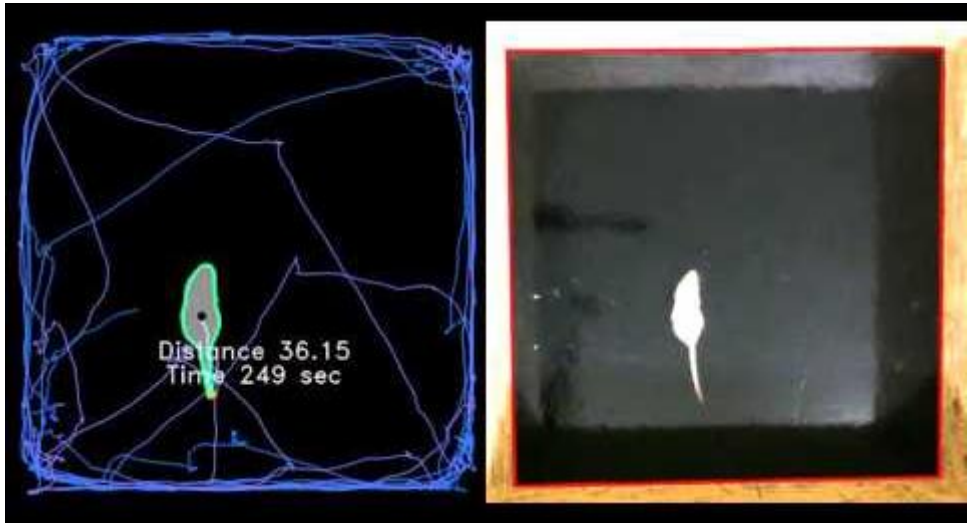
- based on natural exploratory instincts in a novel dark open field
- general locomotor activity levels, exploration, and anxiety



OPEN FIELD TEST

1. Habituation phase - very important!
2. Testing phase
   - 10 minutes - movement is recorded via EthoVision software

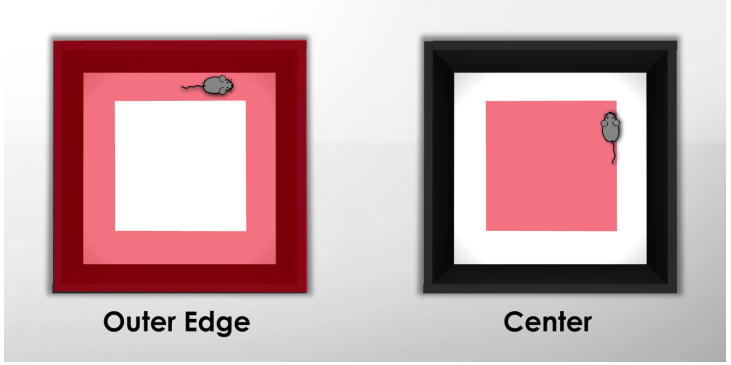
Apparatus



(100 cm × 100 cm × 40 cm)

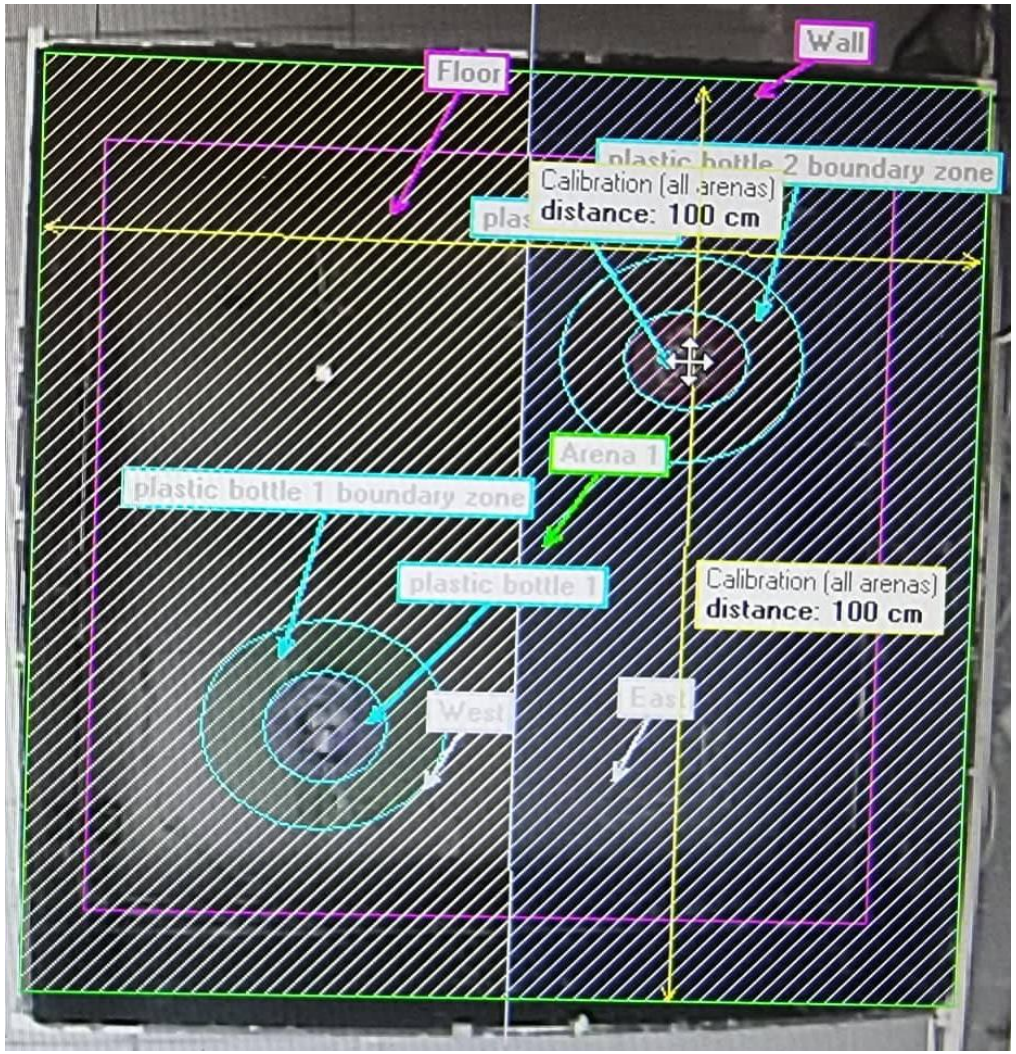
Outputs

- **distance moved** – represents exploration activity and general locomotor activity level
- **cumulative time and frequency to center** – represent anxiety levels

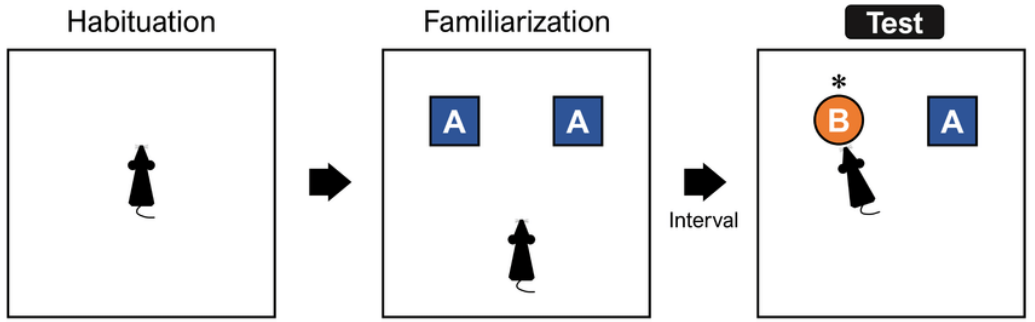


NOVEL OBJECT RECOGNITION TEST

- uses reaction to a novel object as an avenue to evaluate dorsal hippocampal function
- rats should investigate the novel object



NOVEL OBJECT RECOGNITION TEST



3 separate phases on 3 consecutive days

1. Habituation phase
   - exposure to the open field for 10 minutes
2. Familiarization phase
   - exposure to 2 identical objects for 10 minutes
3. Testing phase
   - exposure to a familiar object from the previous phase and a novel object for 10 minutes

Outputs

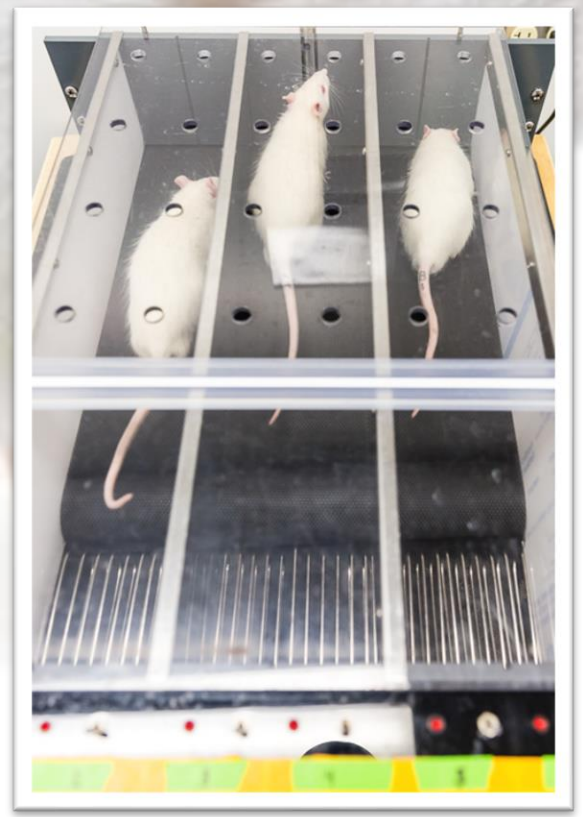
- **total exploration activity -** time spent interacting with both objects
- **time of interaction with novel object** - evaluation of memory and learning

ANIMALS

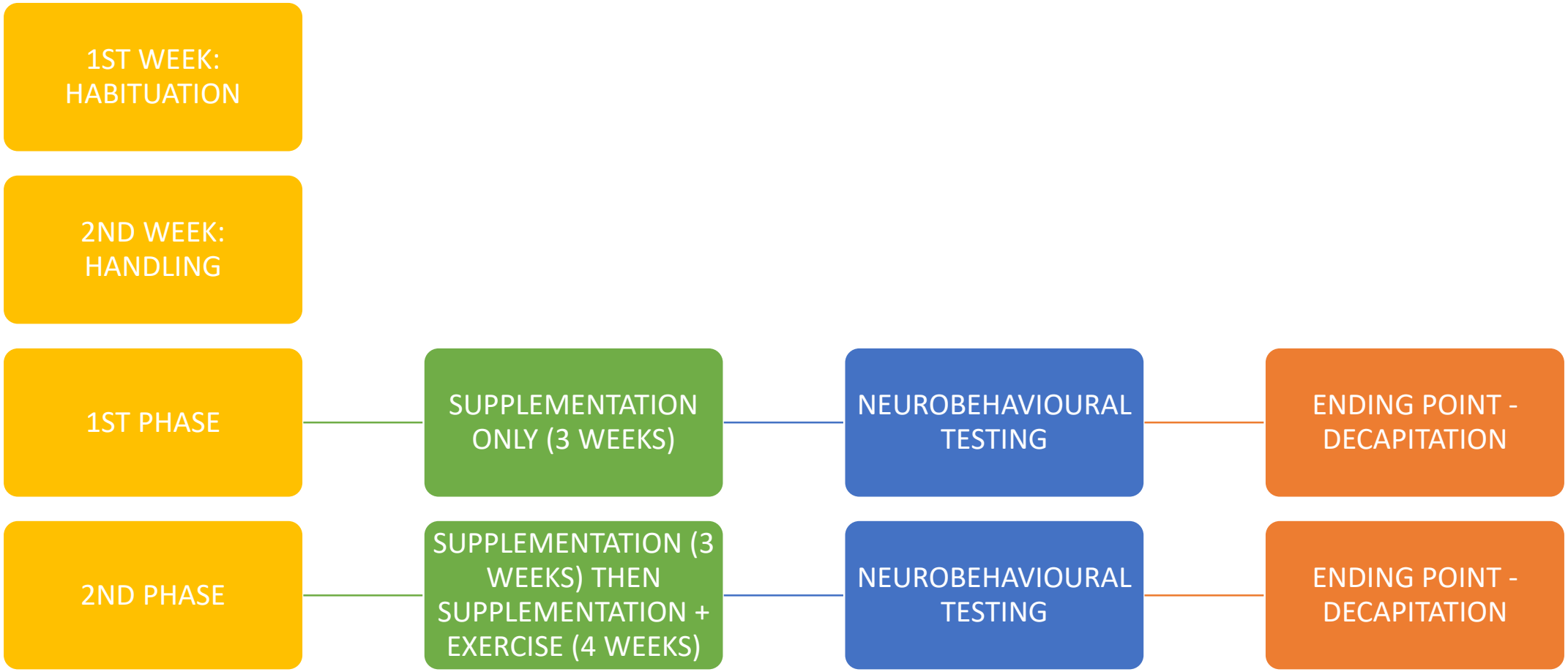
Male Wistar rats (300-700g) randomly allocated to groups (6 rats/group)

- 2 age groups
  - 1. adult rats **(AR)** (9-10 months) (n=42)
  - 2. old rats **(OR)** (24-25 months) (n=40)

- omega-3 FAs supplementation
  - *160 mg/kg, or 320 mg/kg of rat weight*
- exercise
  - 5 times/week for 4 weeks

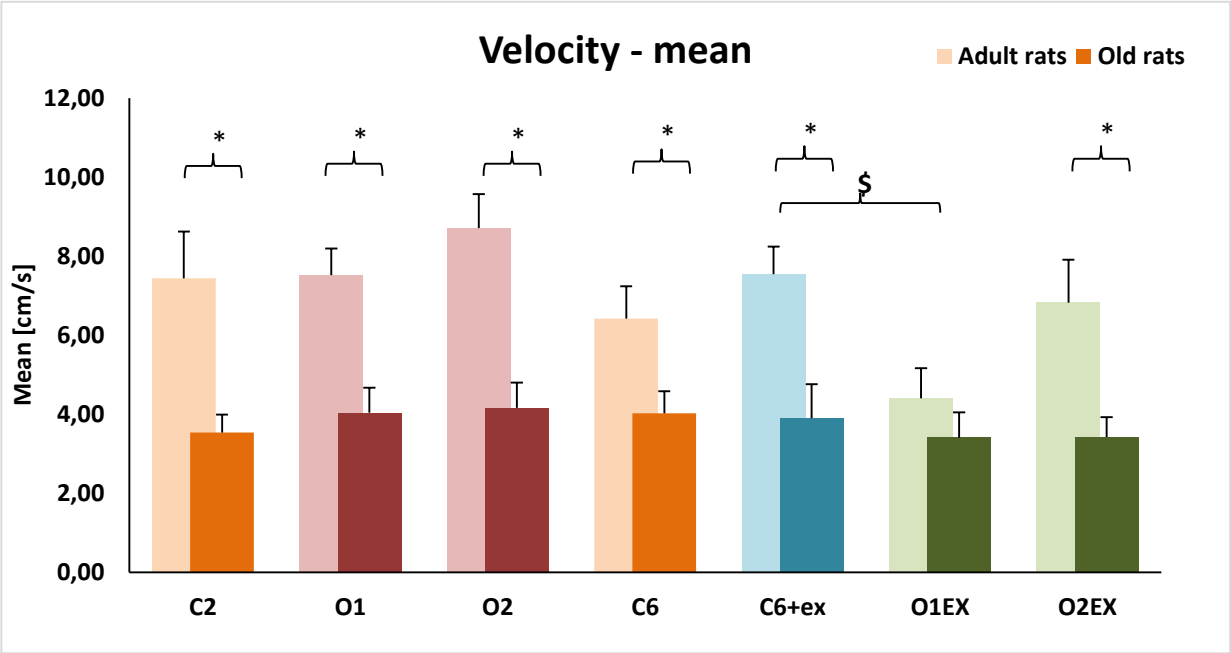


TREATMENT


1ST WEEK: HABITUATION
2ND WEEK: HANDLING
1ST PHASE
SUPPLEMENTATION ONLY (3 WEEKS)
NEUROBEHAVIOURAL TESTING
ENDING POINT - DECAPITATION
2ND PHASE
SUPPLEMENTATION (3 WEEKS) THEN SUPPLEMENTATION + EXERCISE (4 WEEKS)
NEUROBEHAVIOURAL TESTING
ENDING POINT - DECAPITATION

RESULTS
OPEN FIELD TEST

- significantly lower in almost all groups of old rats
- no significant changes across treatments, only significant decrease in O1EX vs. C6+ex



Graph 1: Velocity - mean

C2 – placebo/daily (3w),
O1 – ω-3 FA - 160 mg/kg body weight (BW) daily (3w),
O2 – ω-3 FA - 320 mg/kg BW daily (3w),
C6 – placebo /daily (7w),
C6+Ex – placebo/daily (3w), then placebo/daily + exercise (4w)**,**
O1Ex – ω-3 FA - 160 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w),
O2Ex - ω-3 FA - 320 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w); ***p<0.05; $ p<0.05**

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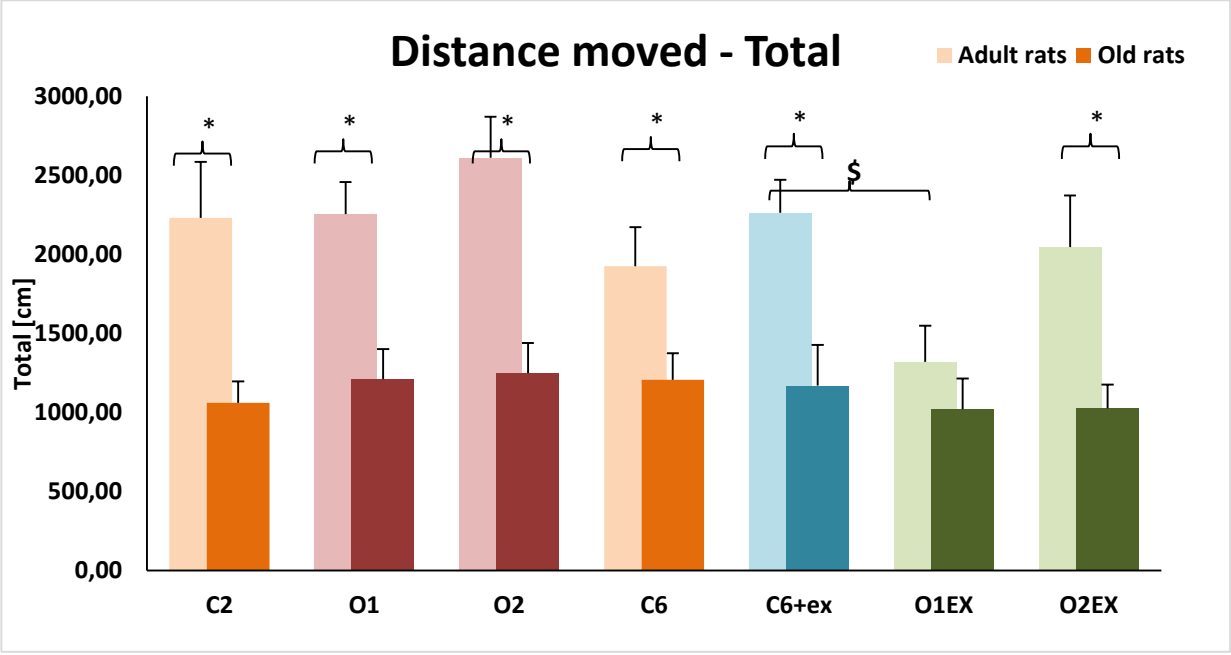

Graph 2: Distance moved - total

C2 – placebo/daily (3w),
O1 – ω-3 FA - 160 mg/kg body weight (BW) daily (3w),
O2 – ω-3 FA - 320 mg/kg BW daily (3w),
C6 – placebo /daily (7w),
C6+Ex – placebo/daily (3w), then placebo/daily + exercise (4w)**,**
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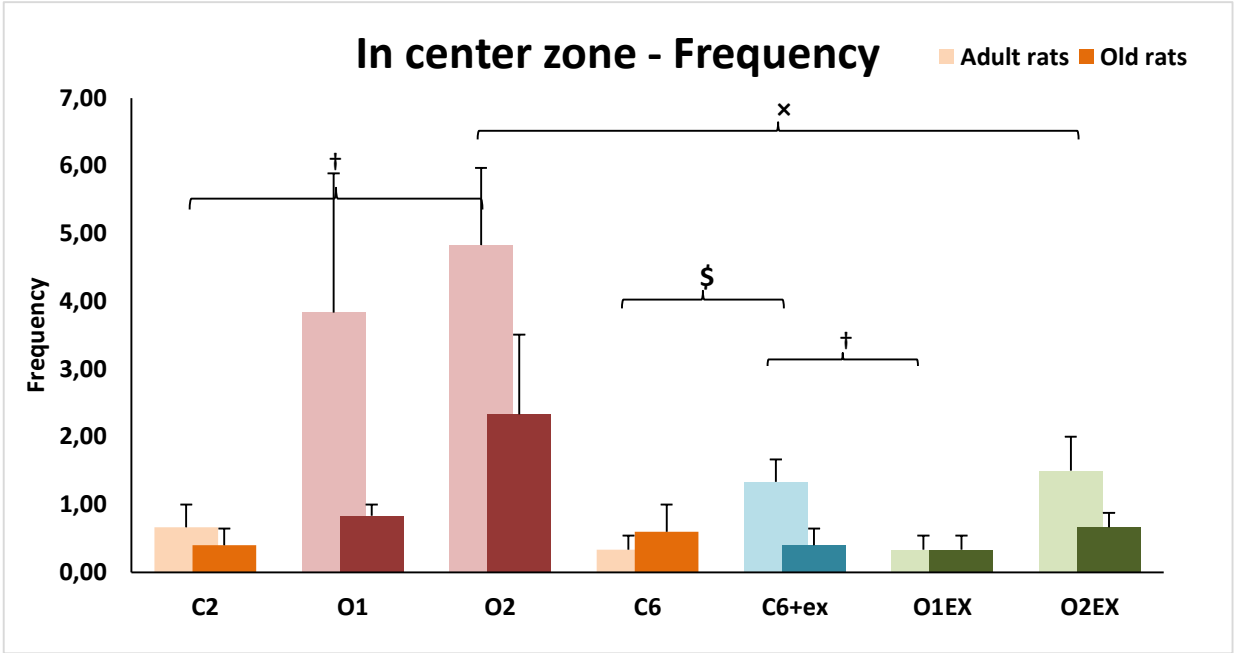
O2Ex - ω-3 FA - 320 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w); ***p<0.05; $ p<0.05**

Nutri Aging

- no significant differences old rats vs. adult rats
- O2 significantly higher in adult rats vs. C2, also vs. O2EX

- C6+EX significantly higher frequency in adult rats vs. C6, but also vs. O1EX

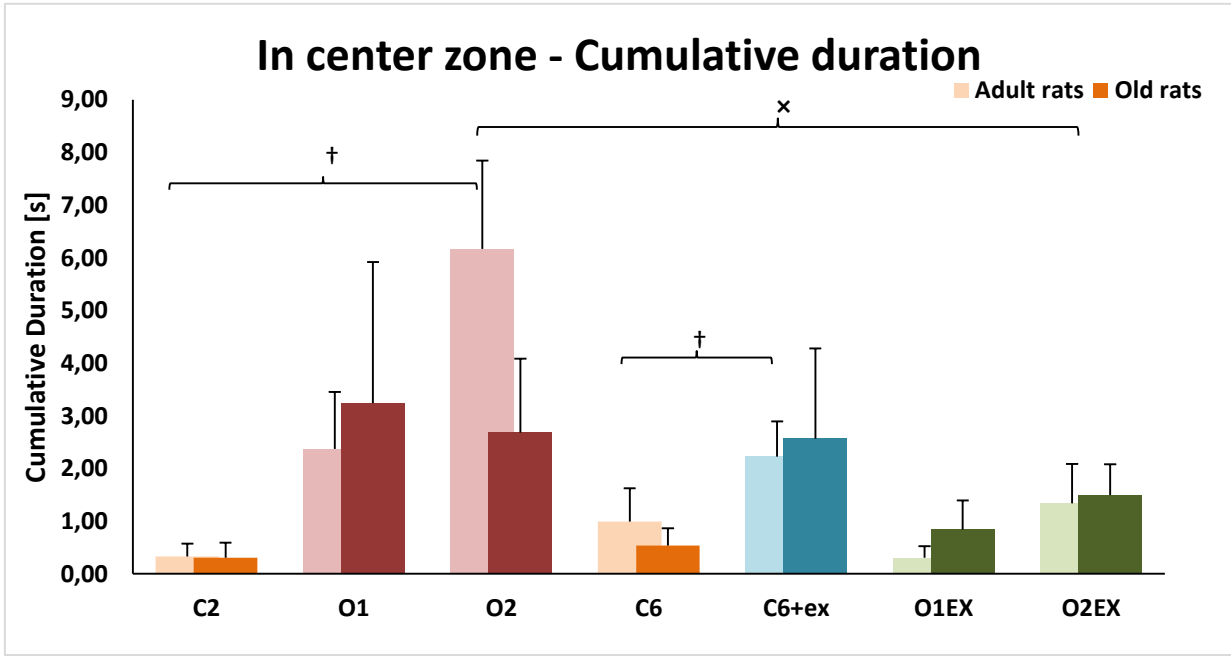


Graph 3: In center zone - Frequency

C2 – placebo/daily (3w),
O1 – ω-3 FA - 160 mg/kg body weight (BW) daily (3w),
O2 – ω-3 FA - 320 mg/kg BW daily (3w),
C6 – placebo /daily (7w),
C6+Ex – placebo/daily (3w), then placebo/daily + exercise (4w)**,**
O1Ex – ω-3 FA - 160 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w),
O2Ex - ω-3 FA - 320 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w); ***p<0.05; $ p<0.05**

- O2 significantly higher in adult rats vs. C2, also vs. O2EX

- C6+EX significantly higher amount of time in center zone in adult rats vs. C6



Graph 4: In center zone – Cumulative duration

C2 – placebo/daily (3w),
O1 – ω-3 FA - 160 mg/kg body weight (BW) daily (3w),
O2 – ω-3 FA - 320 mg/kg BW daily (3w),
C6 – placebo /daily (7w),
C6+Ex – placebo/daily (3w), then placebo/daily + exercise (4w)**,**
O1Ex – ω-3 FA - 160 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w),
O2Ex - ω-3 FA - 320 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w); ***p<0.05; $ p<0.05**

Nutri Aging

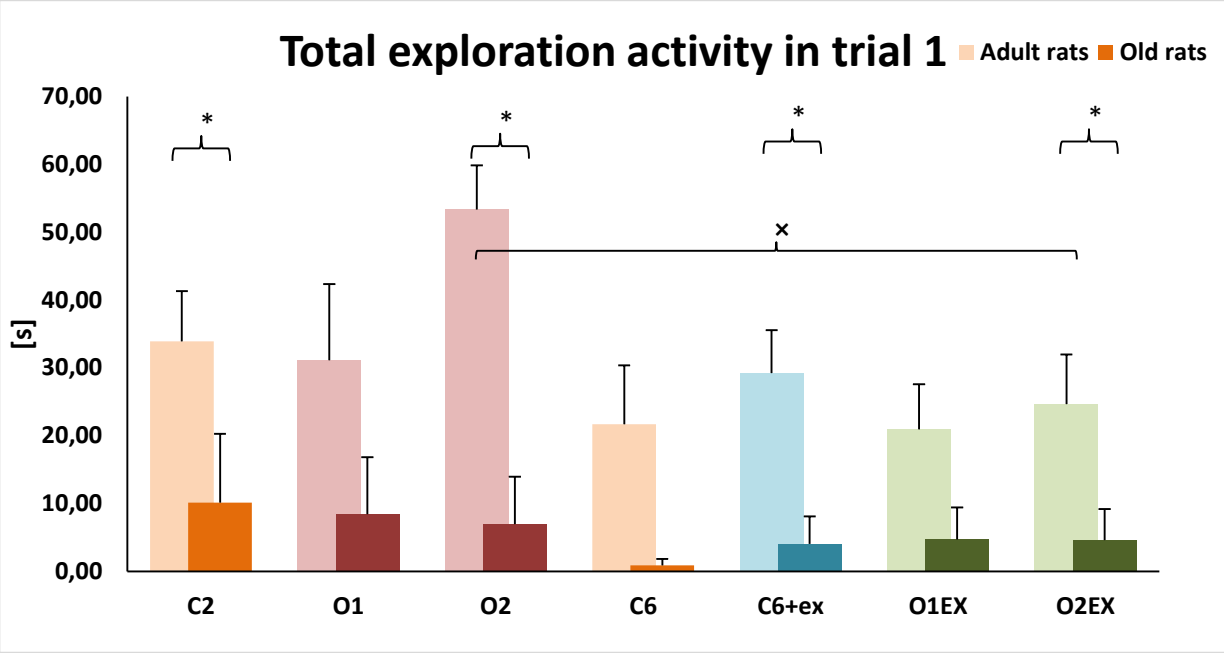
SUMMARY

- age significantly reduces locomotor activity
- omega-3 FA supplementation seem to have positive effect on anxiety in adult rats, but not in combination with exercise
- exercise alone significantly improves anxiety in adult rats

RESULTS

NOVEL OBJECT RECOGNITION TEST

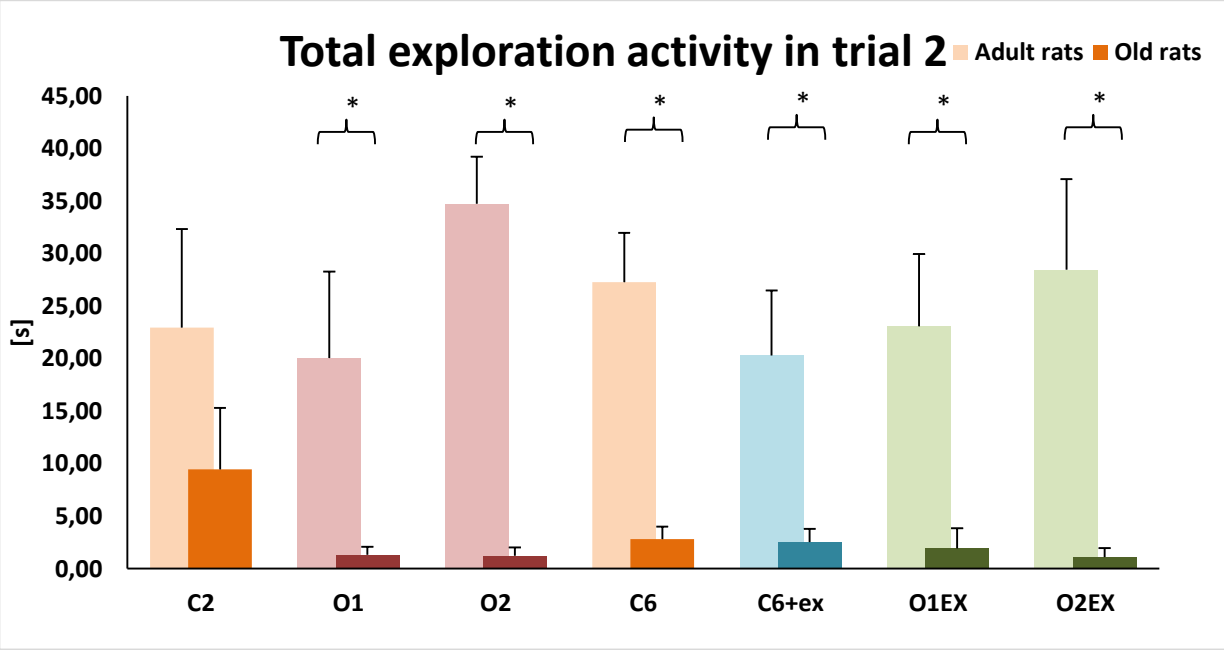
- old vs. adult – significantly lower in C2, O2, C6+ex, O2EX
- O2 vs. O2EX



Graph 5: Total exploration activity in trial 1

C2 – placebo/daily (3w),
O1 – ω-3 FA - 160 mg/kg body weight (BW) daily (3w),
O2 – ω-3 FA - 320 mg/kg BW daily (3w),
C6 – placebo /daily (7w),
C6+Ex – placebo/daily (3w), then placebo/daily + exercise (4w)**,**
O1Ex – ω-3 FA - 160 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w),
O2Ex - ω-3 FA - 320 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w); ***p<0.05; $ p<0.05**

- old vs. adult – significantly lower in O1, O2, C6, C6+ex, O1EX, O2EX

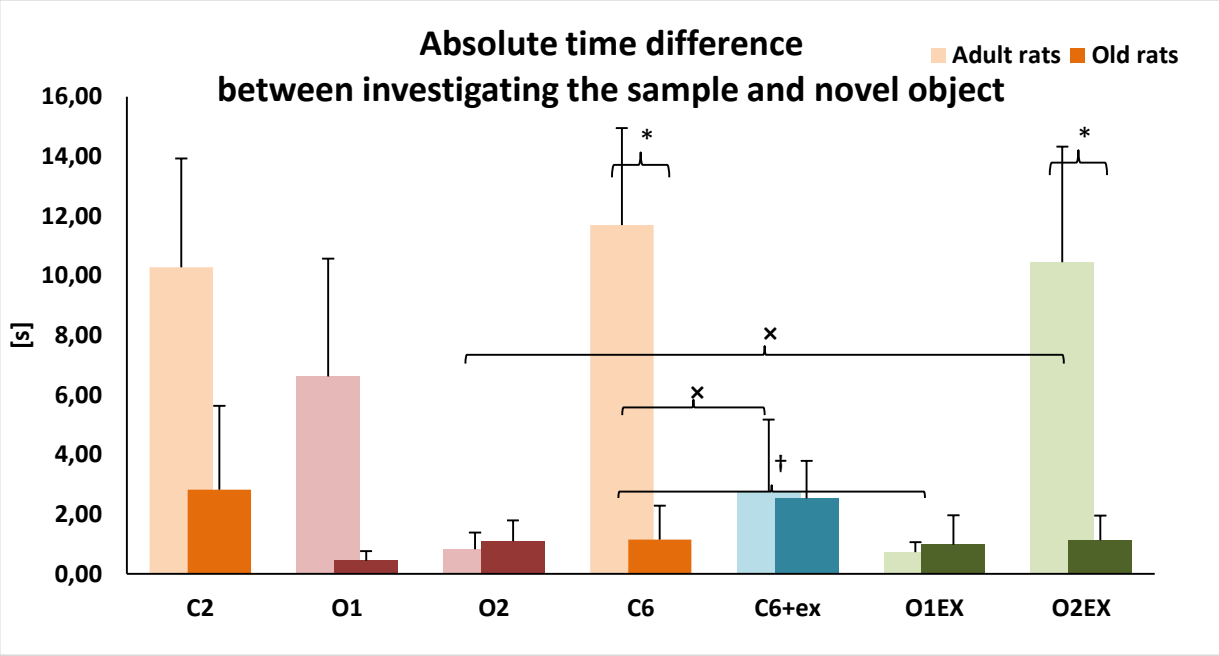


Graph 6: Total exploration activity in trial 2

C2 – placebo/daily (3w),
O1 – ω-3 FA - 160 mg/kg body weight (BW) daily (3w),
O2 – ω-3 FA - 320 mg/kg BW daily (3w),
C6 – placebo /daily (7w),
C6+Ex – placebo/daily (3w), then placebo/daily + exercise (4w)**,**
O1Ex – ω-3 FA - 160 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w),
O2Ex - ω-3 FA - 320 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w); ***p<0.05; $ p<0.05**

Nutri Aging

- Old vs. Adult – C6, O2EX
- O2EX spent significantly more time investigating the novel object than O2
- C6 - significantly more time investigating the novel object than C6+ex and O1EX



Graph 7: Absolute time difference between investigating the sample and novel object

C2 – placebo/daily (3w),
O1 – ω-3 FA - 160 mg/kg body weight (BW) daily (3w),
O2 – ω-3 FA - 320 mg/kg BW daily (3w),
C6 – placebo /daily (7w),
C6+Ex – placebo/daily (3w), then placebo/daily + exercise (4w)**,**
O1Ex – ω-3 FA - 160 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w),
O2Ex - ω-3 FA - 320 mg/kg BW daily (3w), then ω-3 FA in the indicated dose + exercise (4w); ***p<0.05; $ p<0.05**

the higher the time difference, the more time the rat spent exploring the new object

SUMMARY

- age decreases exploration activity, significantly in some groups
- effect on memory is not definite

CONCLUSION

- ageing affects locomotor activity and exploration activity, but not anxiety
- effect of omega-3 FAs supplementation – potential to positively affects anxiety, but not memory
- effect of combined intervention – potential to positively affects memory, but not anxiety

THANK YOU FOR YOUR ATTENTION!