CULA course 2024

Methods and tests to assess anxiety and cognition

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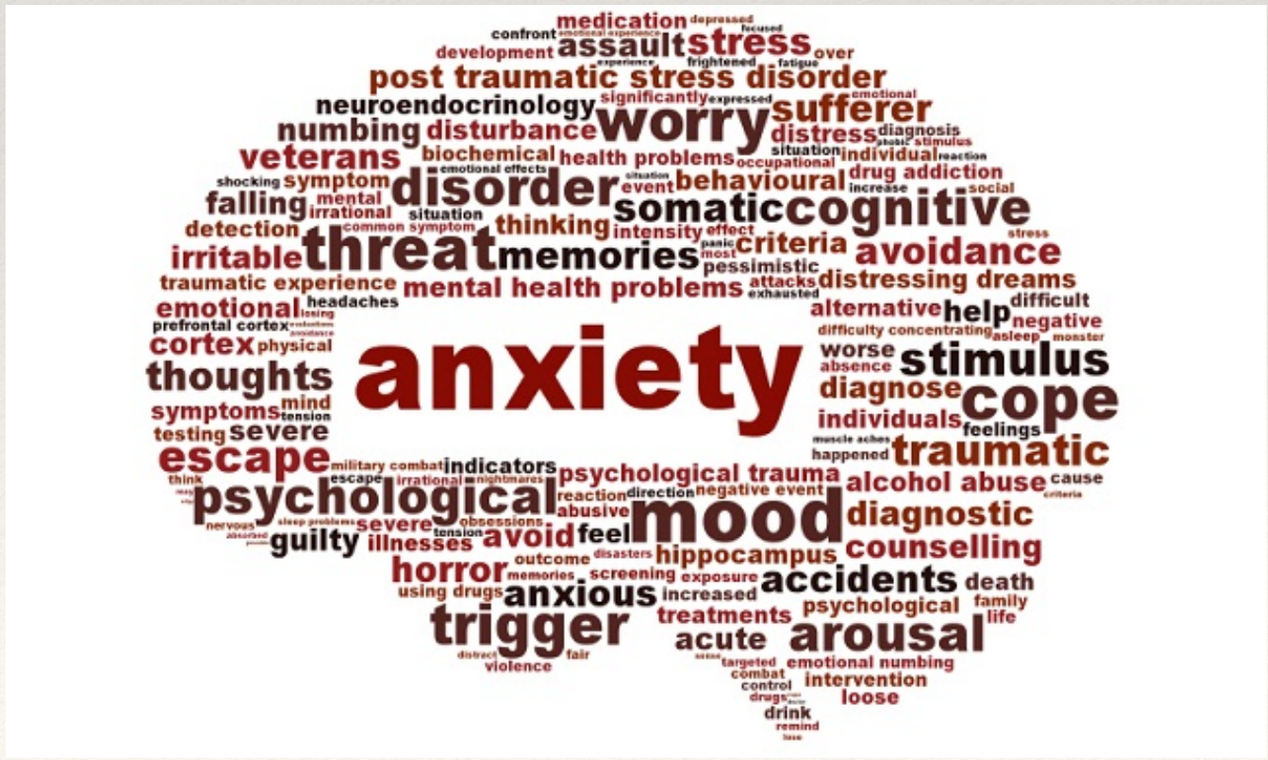
Neurophysiology and behavior lab

NBLab

Our research aims to understand the development of neurophysiological mechanisms underlying cognitive processes, such as working memory and long-term memory, to identify predictive changes in biophysical mechanisms in disease-states and test therapeutic regimens to prevent the emergence of neuropsychiatric symptoms.

Why is anxiety important?

- Disorders related to anxiety
  - Major depression
  - Post-traumatic stress disorder
  - Phobias
- co-morbidity or increased risk for other disorders
  - schizophrenia
  - autism
  - epilepsy
  - cancer

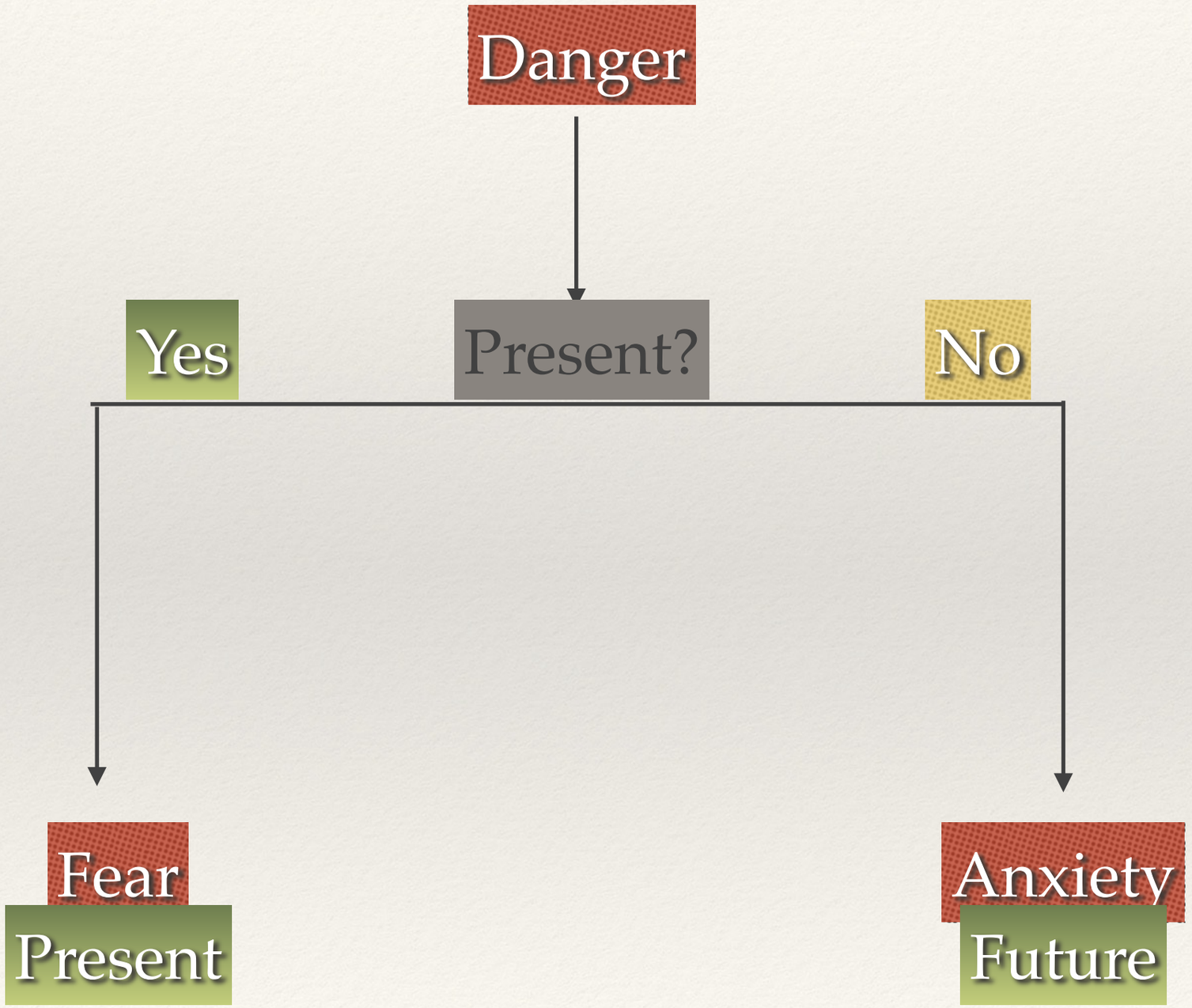


What is anxiety?

"psychological, physiological and behavioural state induced in animals and humans by a threat to well-being or survival, either actual or potential"

- Threat (Actual or potential)
- Coping strategies
  - Passive: Conservative withdrawal
  - Active: Flight or fight

Fear and anxiety

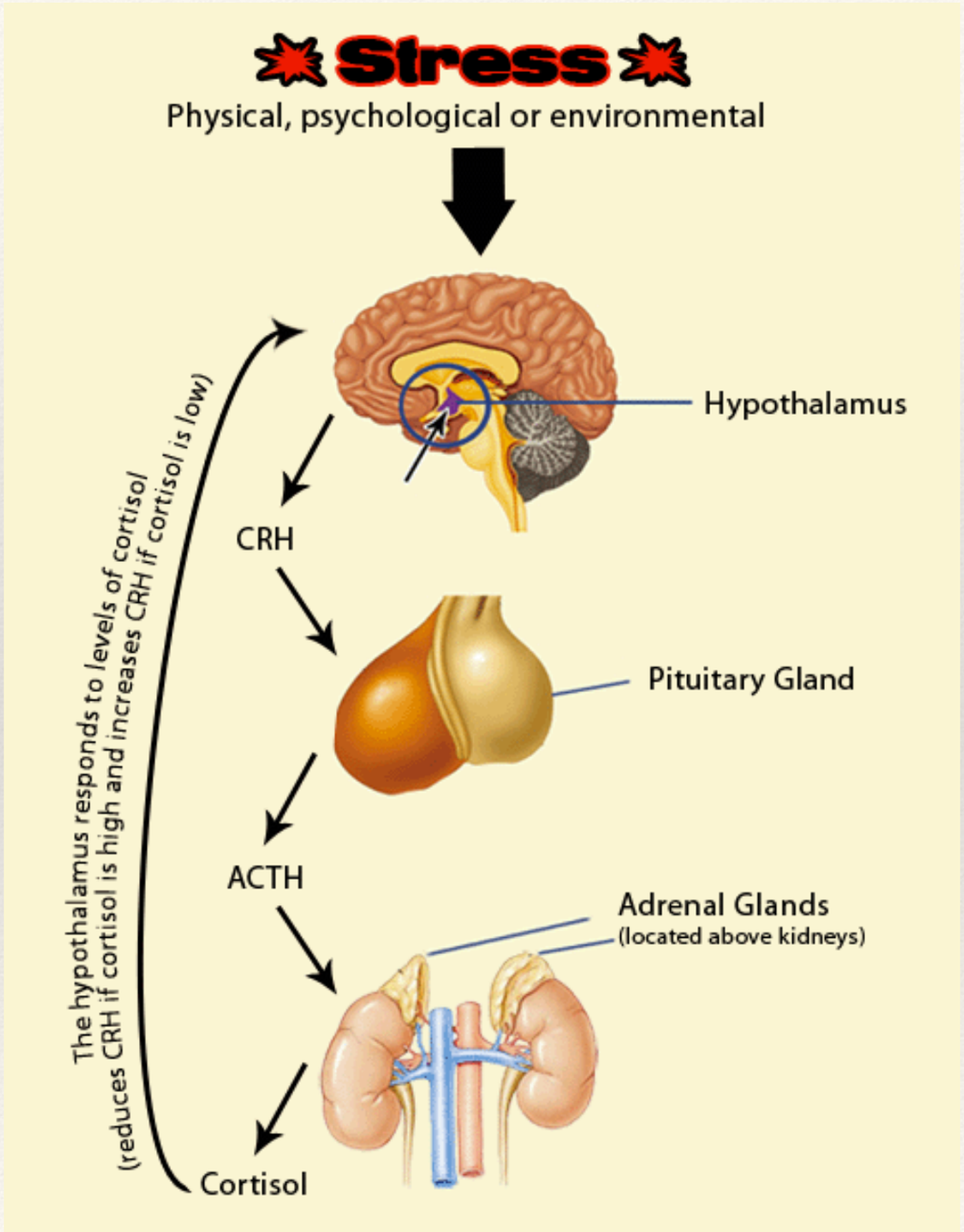


Trait vs State Anxiety

- State Anxiety: induced by anxiogenic factors
- Trait anxiety: innate characteristic of an organism
- Open for discussion: In order to assess trait anxiety, the organism needs to be exposed to anxiogenic stimuli

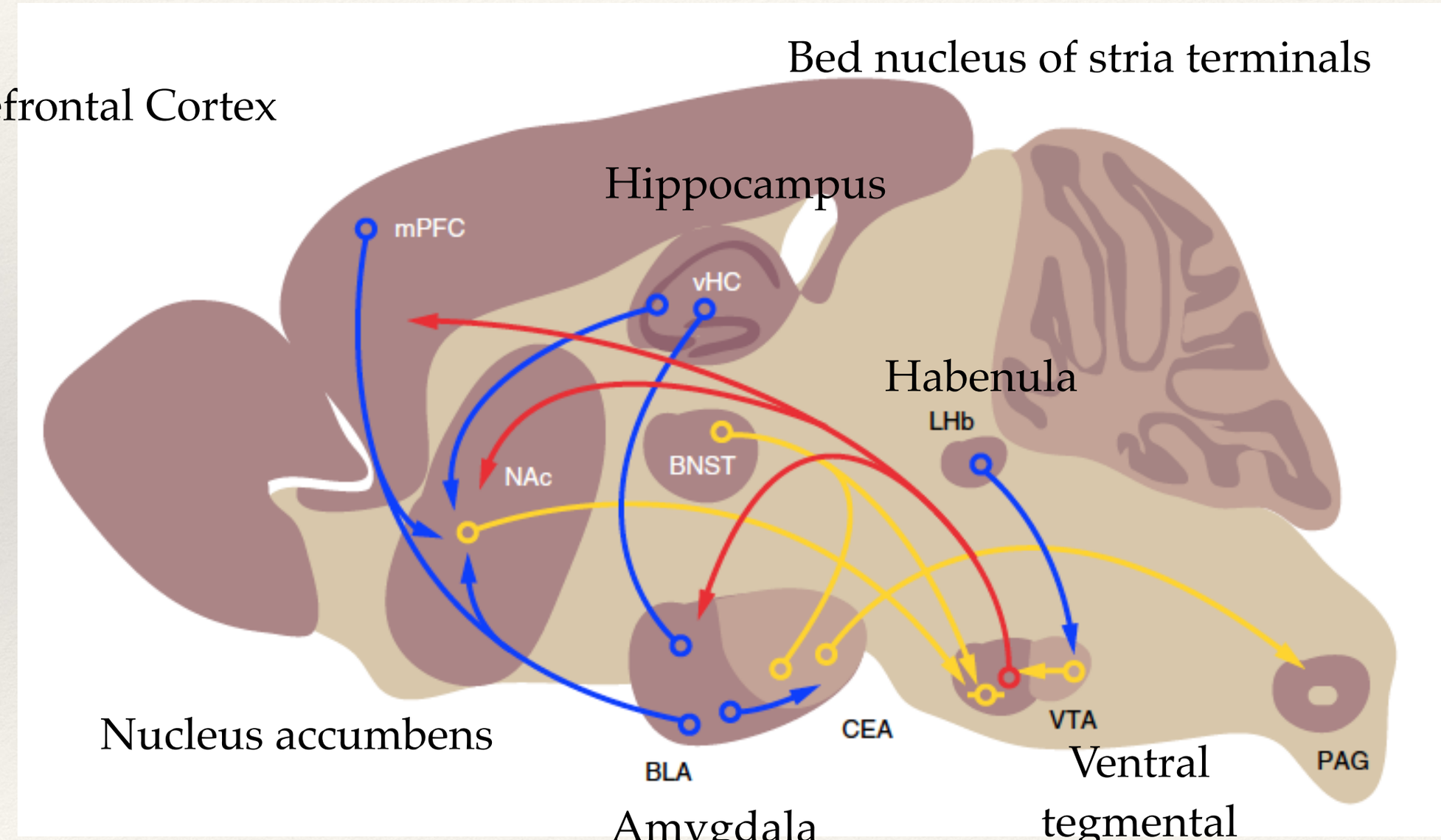
http://www.buzzle.com/img/articleImages/604761-4071-22.jpg

What systems underlie anxiety?



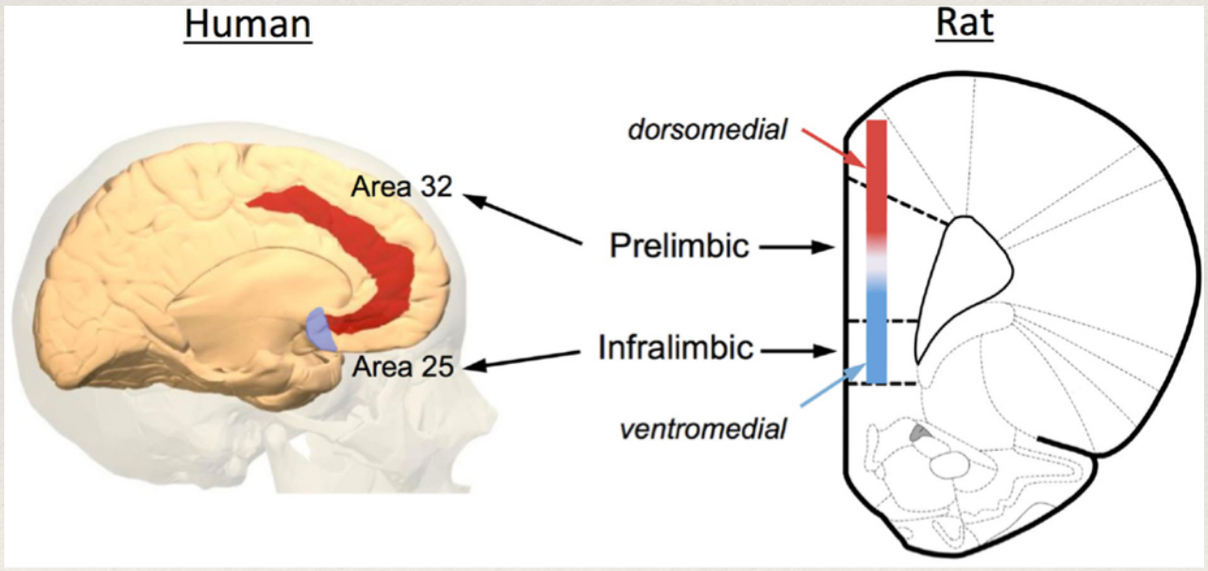
Stress & the HPA axis

Neurobiology of anxiety

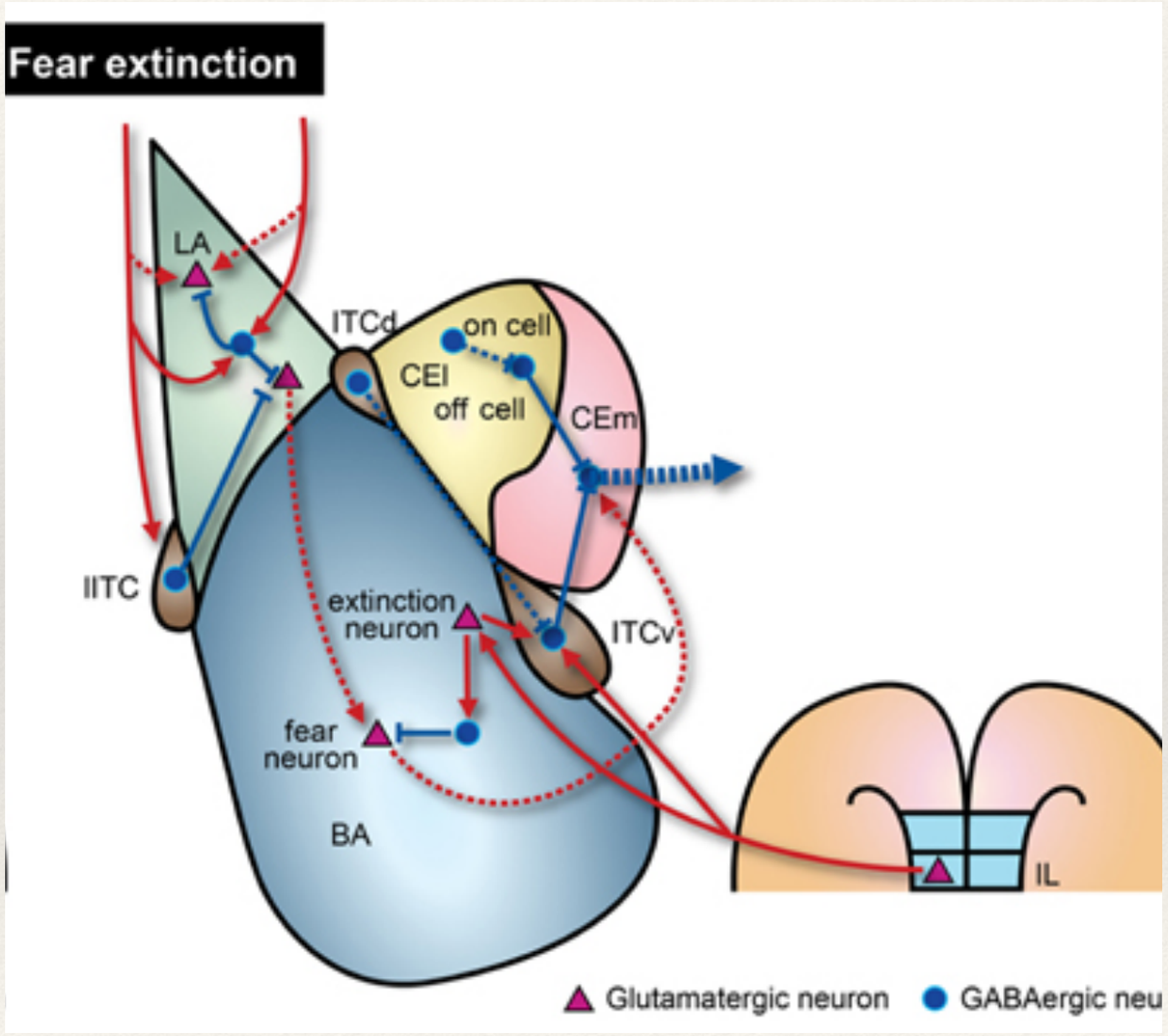


Brain areas involved in anxiety

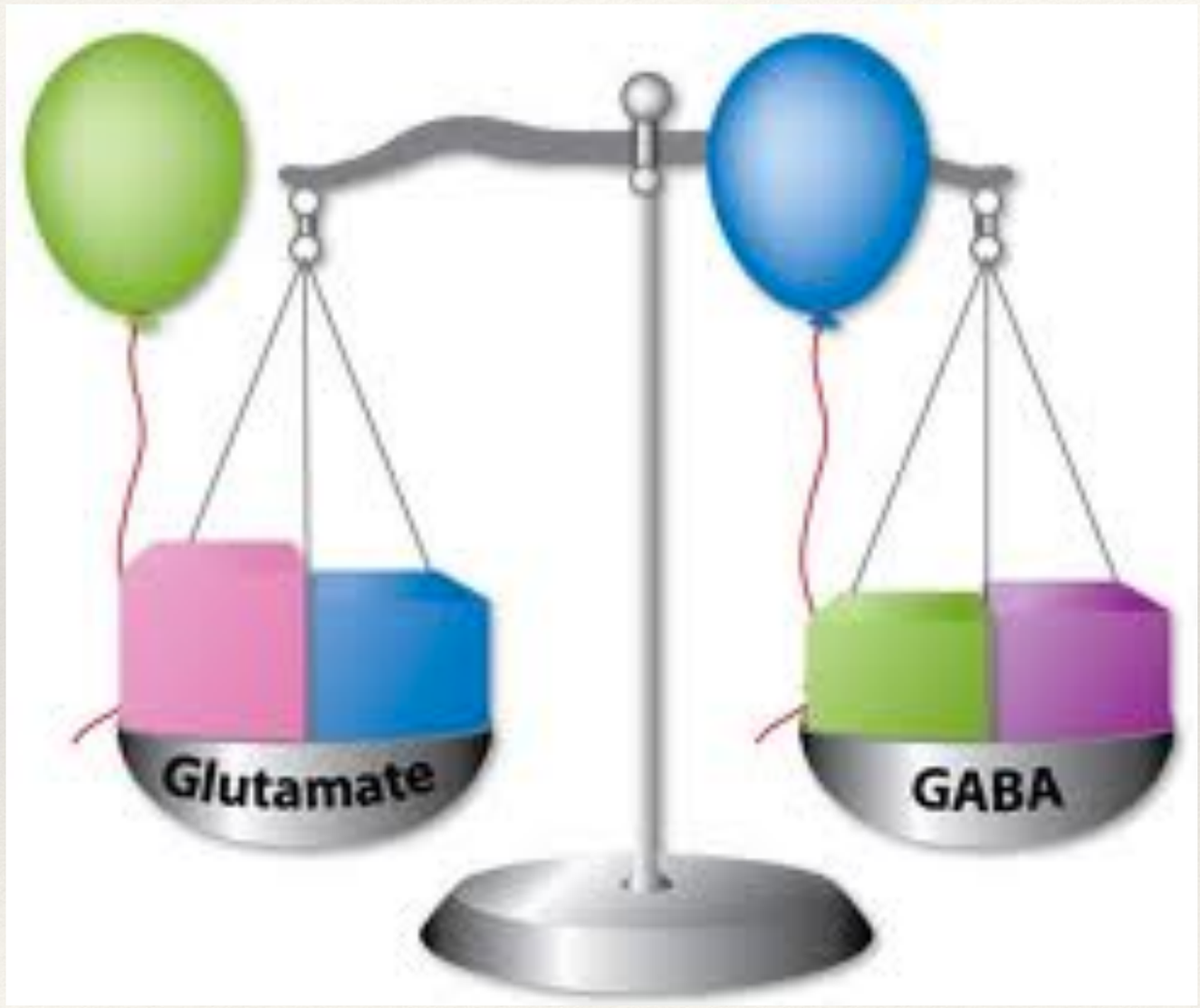
Prefrontal cortex

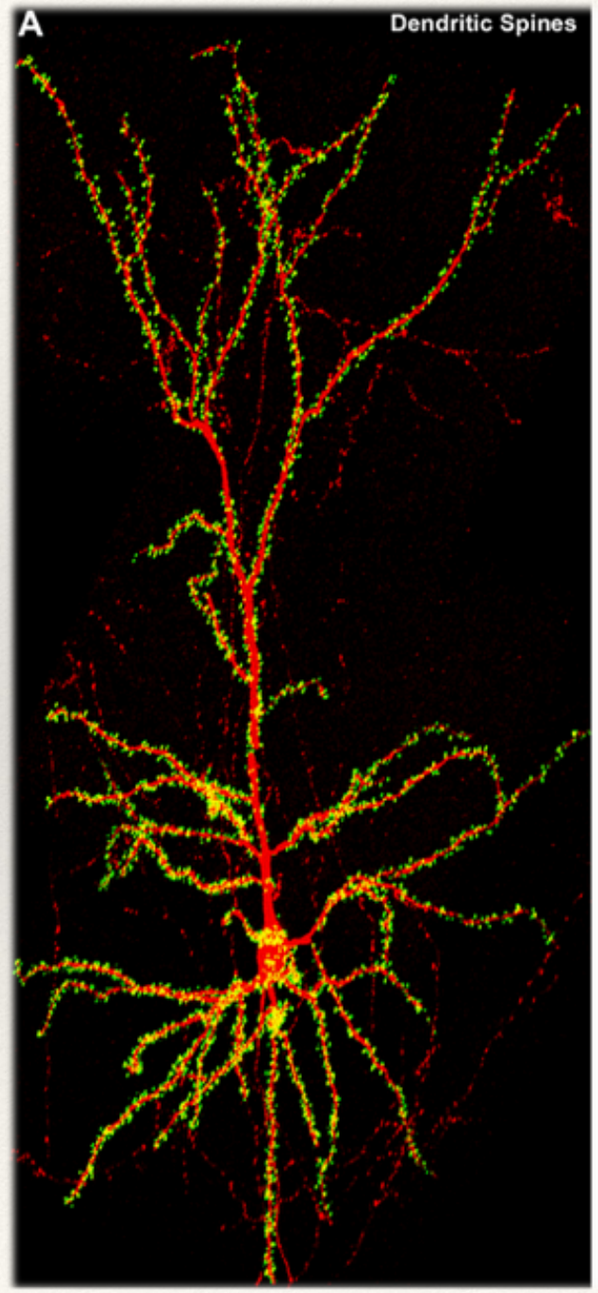


Amygdala



Neurotransmitters involved in anxiety





What is the anxiety response?

- Physiological characteristics
  - increased heart rate
  - sweating
  - increased breathing rate
  - decreased moving
  - freezing

Behavioural tests for anxiety in mice

Why mice?

- Small mammalian species
- Easily manipulated genome
- Predictable behavioral responses
- Strong corticosterone response
- Responds to known treatments

How do mice behave when they are anxious?

- They prefer closed or protected places, as opposed to open
- They are afraid of heights
- They prefer dark rooms, as opposed to light rooms
- They freeze, therefore, they move less, especially when they fear

Exploratory-behaviour based

- Open-field
- Elevated plus maze
- Elevated zero maze

Other anxiety tests

- Dark/light transition
- Social behaviour
  - stress-induced vocalisations
  - social interaction
- Predator response
  - Predator odor avoidance
- other
  - Hyponeophagia
  - startle response

Conditioned behavioral tests

- Require training
- Based on fear or threat memory
- Mouse performance depends on memory processes, as well
- Passive avoidance
- Active avoidance

Methodological considerations

- Test 1 animal at a time
- Always clean the device before each animal (usually use 70% ethanol)
- Same conditions across all animals tested
- Be aware of changes in noise or lighting
- Avoid causing any extra anxiety to the animal
- Do not stand in front of the device during an experiment.
- Always video-tape and analyse afterwards.

How to analyse behaviour

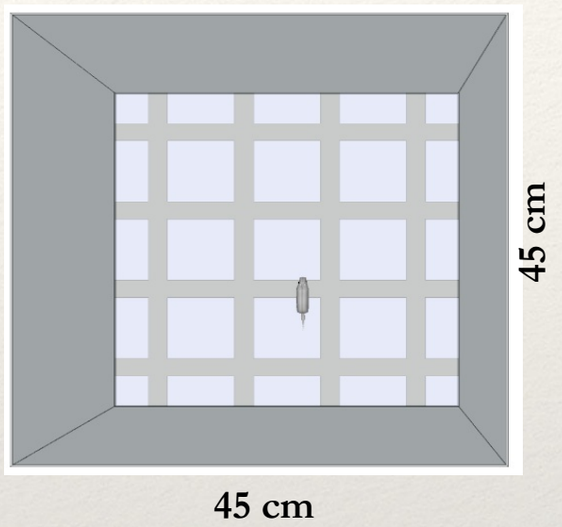
- Manually
  - Sometimes the human perception is superior to any computer program
- Semi-automatically
  - jwatcher
- Automatically using several programs
  - Noldus
  - Any-maze

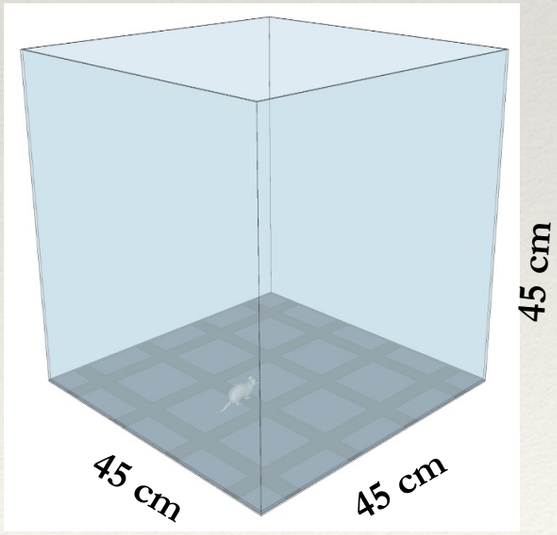
Trait anxiety tests

- Open-field test
- Elevated plus maze test
- Light-Dark Room Test

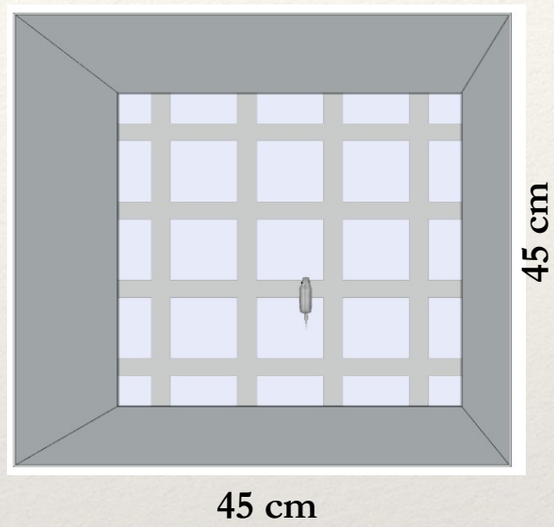
- **A conclusion is drawn from two or more of the above tests**

Open-Field Test





Open-Field Test



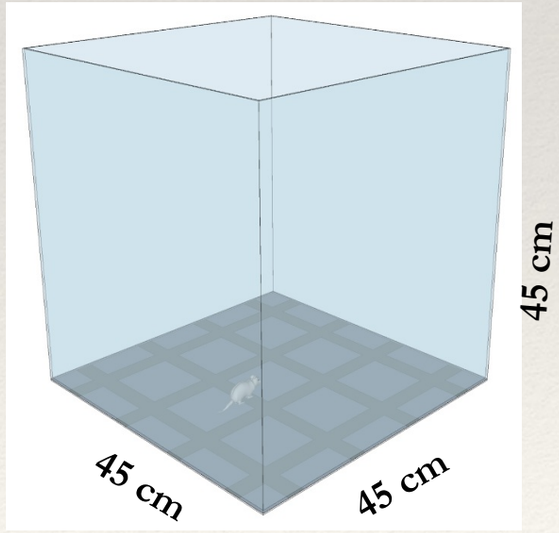
Locomotion

a. Mobility, in response to a novel environment (first 5-15 min)

b. Basal activity levels (after 1-2 hours

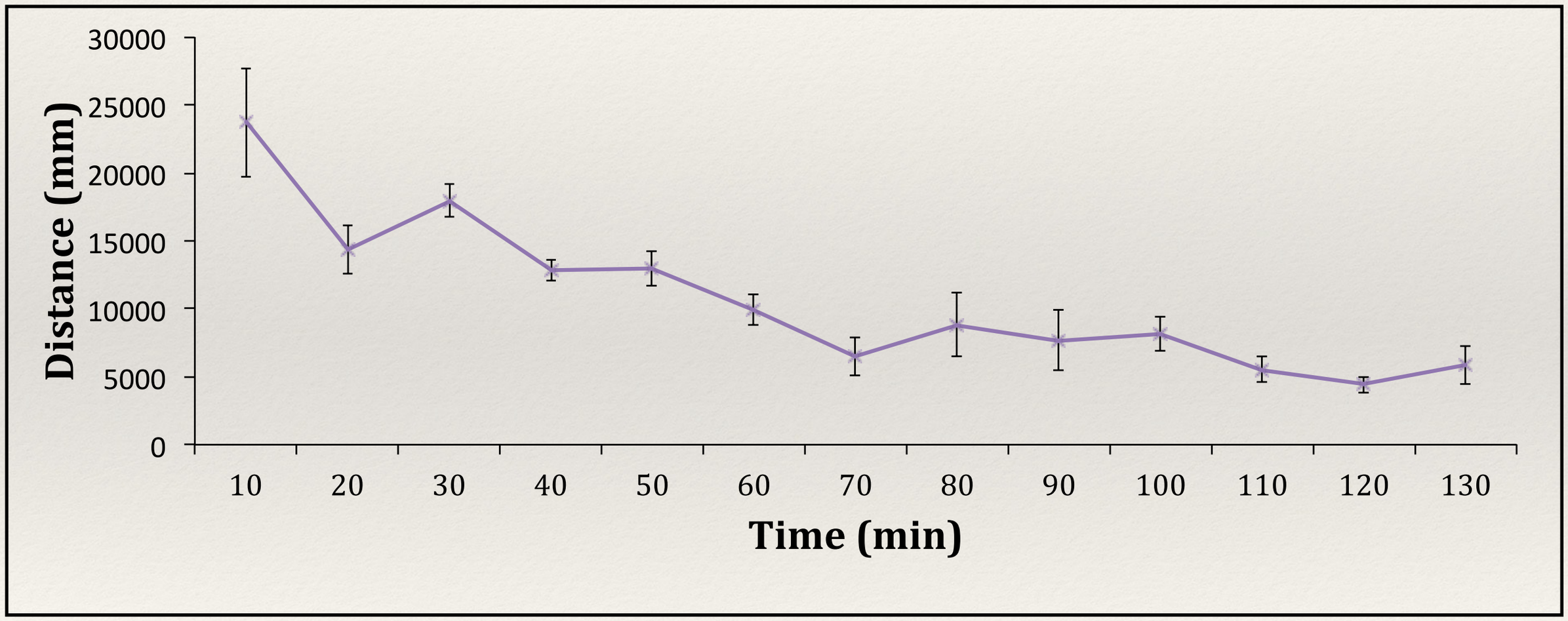
c. Horizontal movement

d. Vertical movement (rearing)



Decreased activity = increased anxiety

Locomotor habituation to the open-field



Open-Field Test

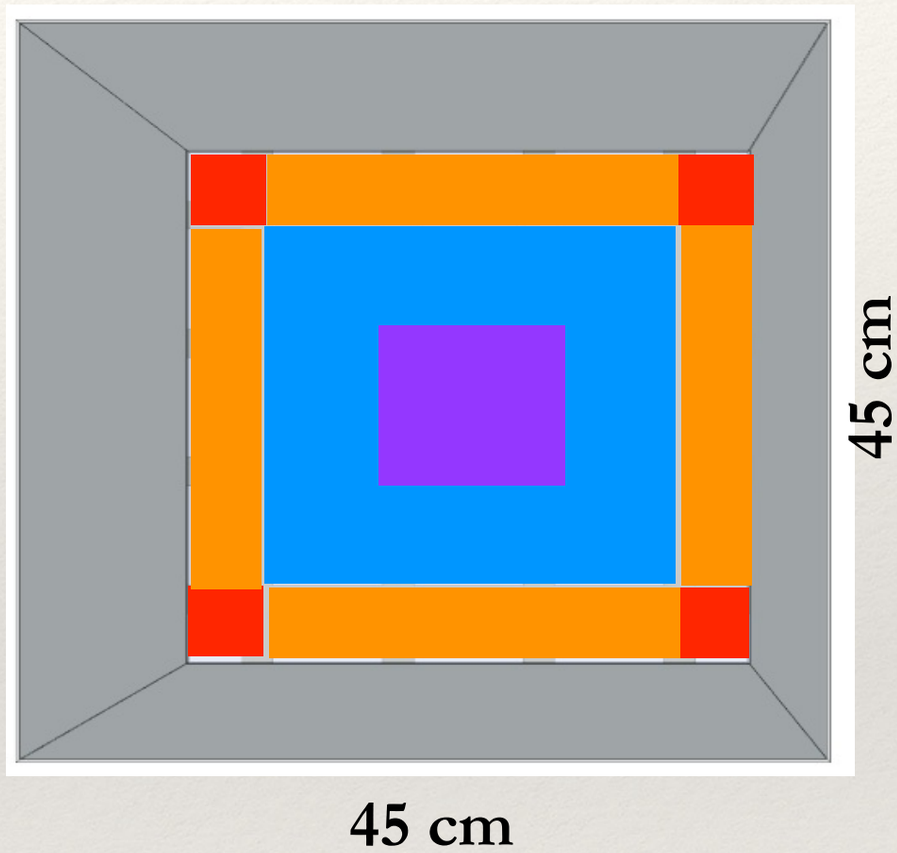
1. **Thigmotaxis**

   I. **Corners**

   II. **Walls**

   III. Periphery

   IV. Center



Thigmotaxis= $\frac{\text{Time spent in corners + walls}}{\text{Time spent in periphery + center}}$

Increased thigmotaxis = increased anxiety

Thigmotaxis

- Software analysis: Noldus, Any-maze

Open-Field Tests

1. **Grooming**

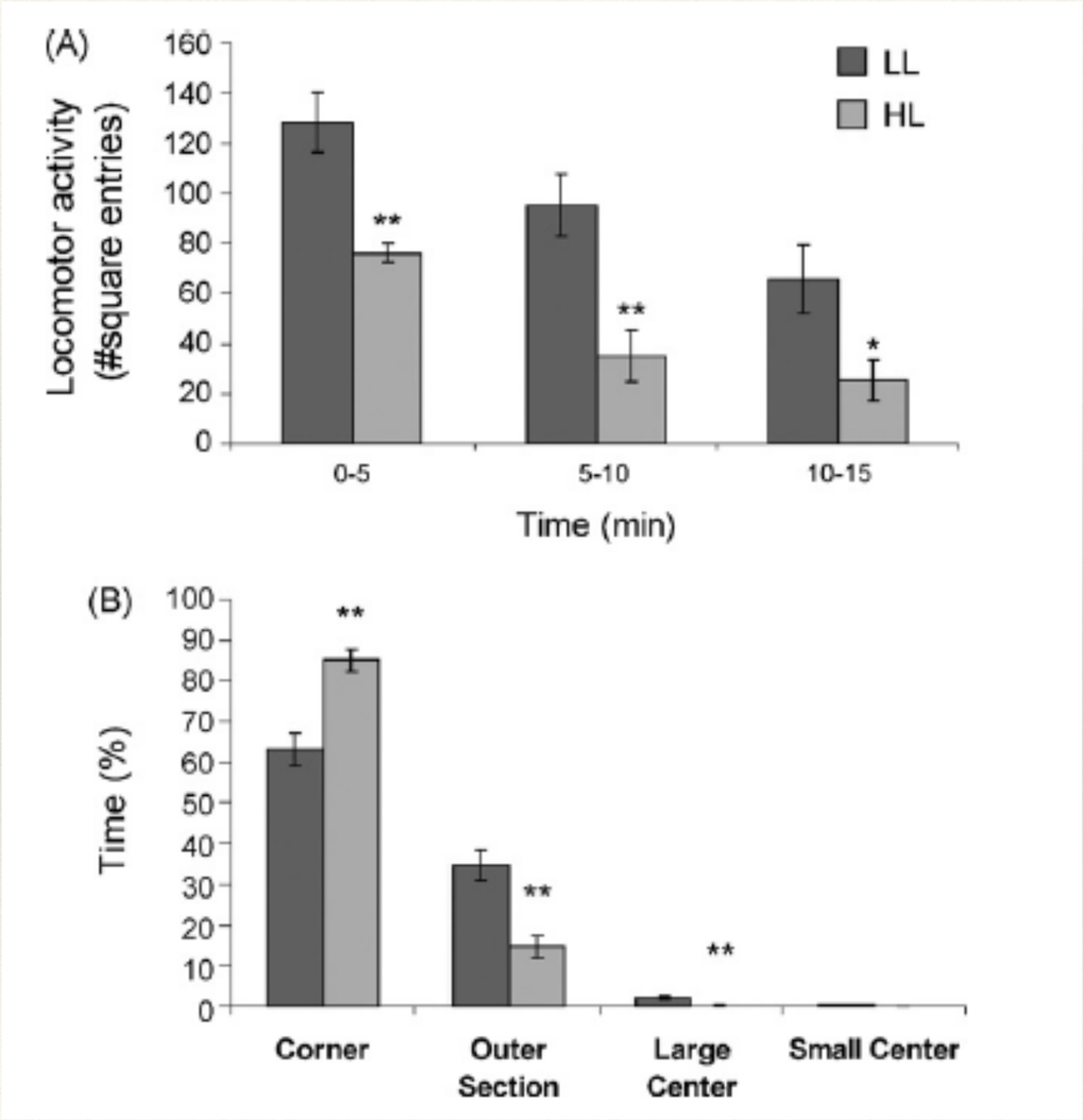
Increased grooming = increased anxiety (depends..)

Locomotor differences confounds

- Sound
- Light
- Habituation
- circadian rhythms

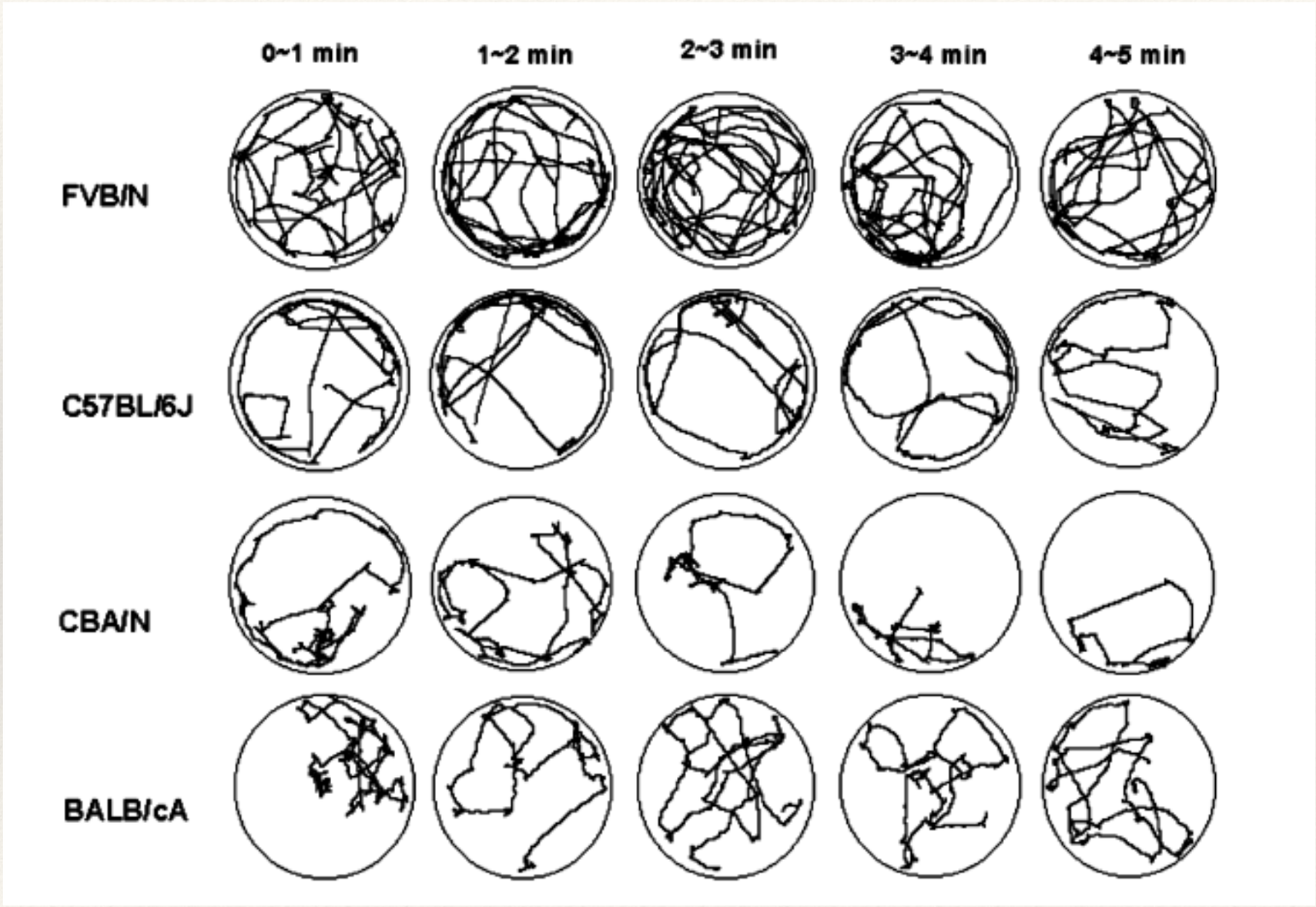
Open Field Test

Effects of illumination on open-field behavior



Bouwknecht et al, 2007

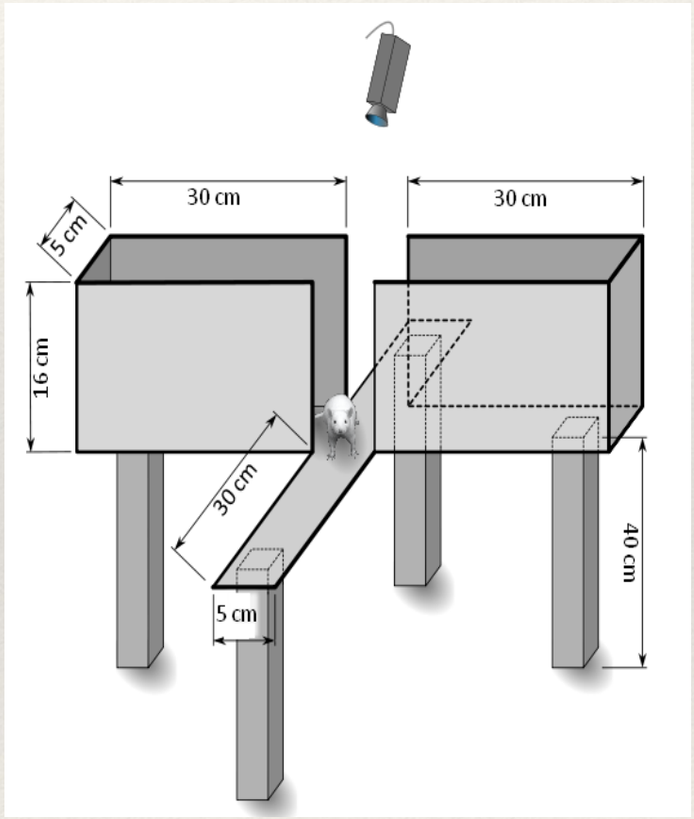
Open-field behavior in different strains



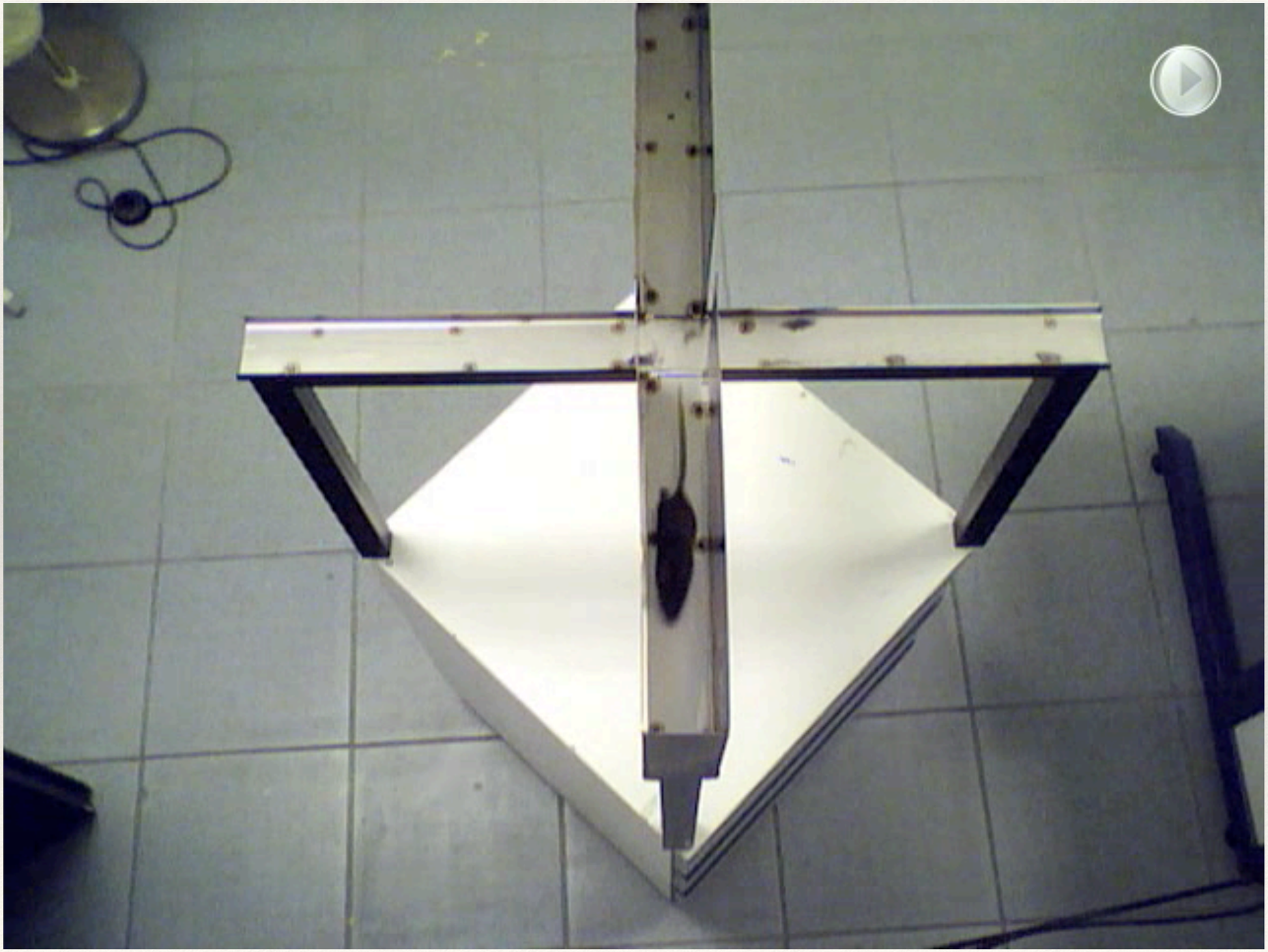
Kim et al, 2002

Elevated plus maze

- Place mouse in the intersection compartment
- Measure entries or time spent in the closed arms
- Measure entries or time spent in the open arms
- Risk-taking behaviour?



Elevated plus maze video



Light-Dark test

- Place mouse in the dark compartment
- Measure latency for the mouse to exit the dark compartment
- Measure time spent in the dark and light compartments

Light-Dark Test

Light-Dark Test

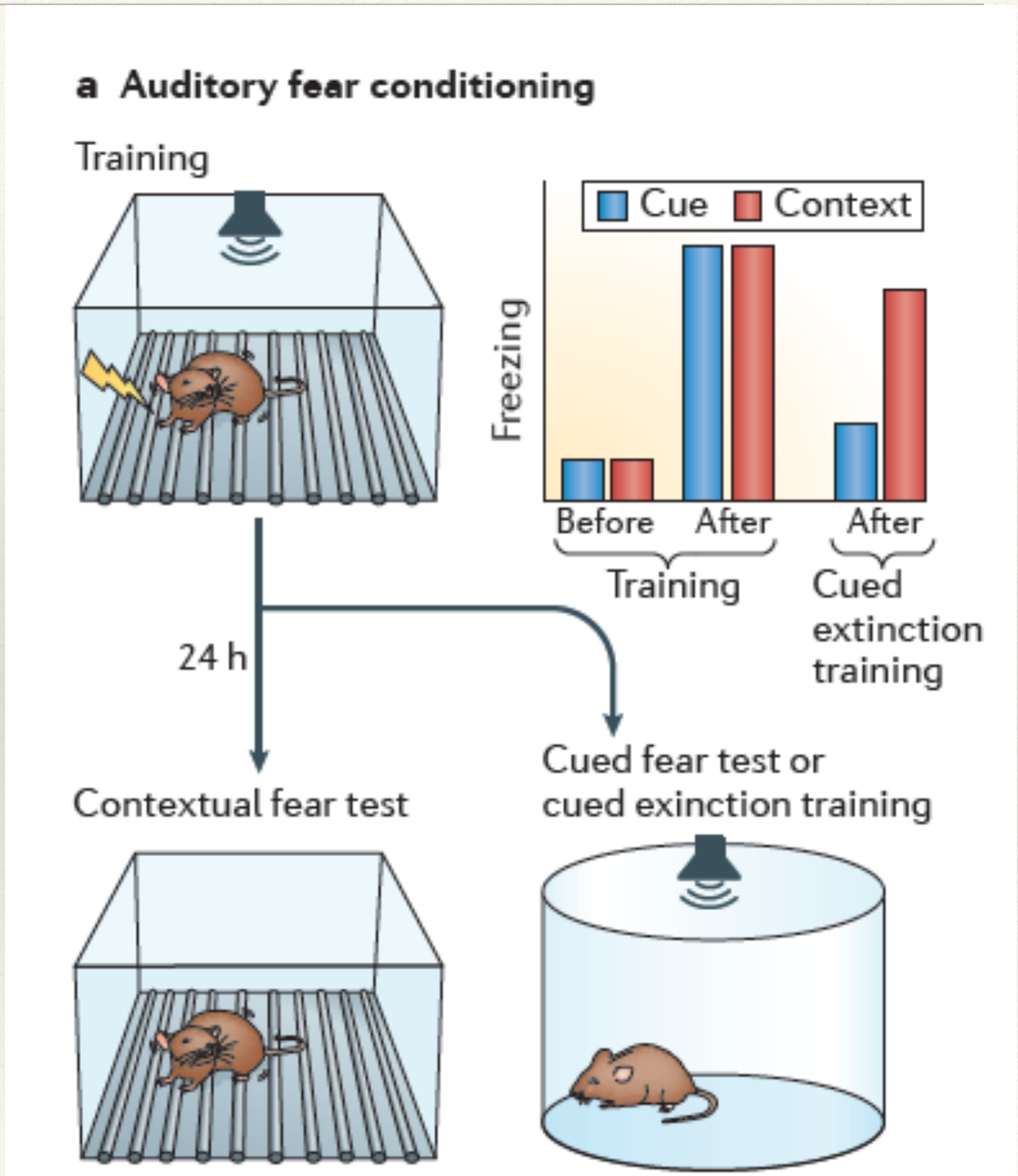
Testing Memory

Testing memory

- Based on negative feelings, such as fear
  - Easy to train
- Based on exploration and curiosity
  - Requires animal handling to reduce stress and anxiety to the animals
  - Object recognition
- Based on reward
  - Requires food or water restriction

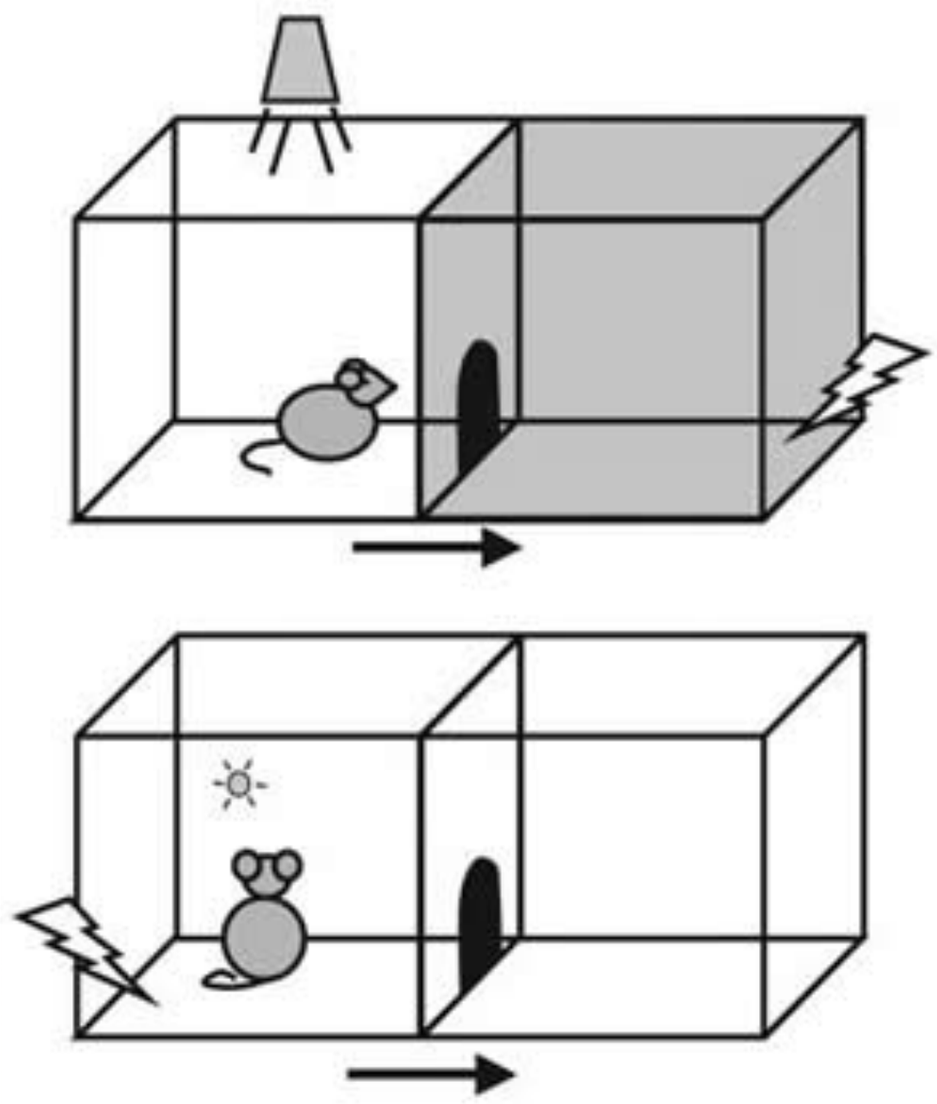
Fear conditioning

- Threat: Electric shock
- Day 0: Training
- Day1: Testing long-term memory
- Unconditional stimulus: shock
- Conditional stimulus: environment/ sound
- Unconditional response: Freezing



Contextual fear conditioning

Conditioned behavioural tests



Passive Avoidance
Exploits a natural tendency of mice to enter dark environments.

Unidirectional: mouse goes from light to dark chamber.

Active Avoidance
Mouse learns to avoid shock based upon the presentation of a light cue.

Unidirectional: mouse is always shocked in the same chamber/location.

Object recognition tasks

Object recognition tasks

- Require handling
- Require habituation to the open-field
- Require low illumination
- Novel object recognition task
- Object-to-place
- Temporal order object recognition task

Memory tests based on reward

- Requires food or water restriction to increase motivation
- Requires handling
- Requires habituation to the room and equipment
- Left-right discrimination
- Delayed alternation task in the T-maze

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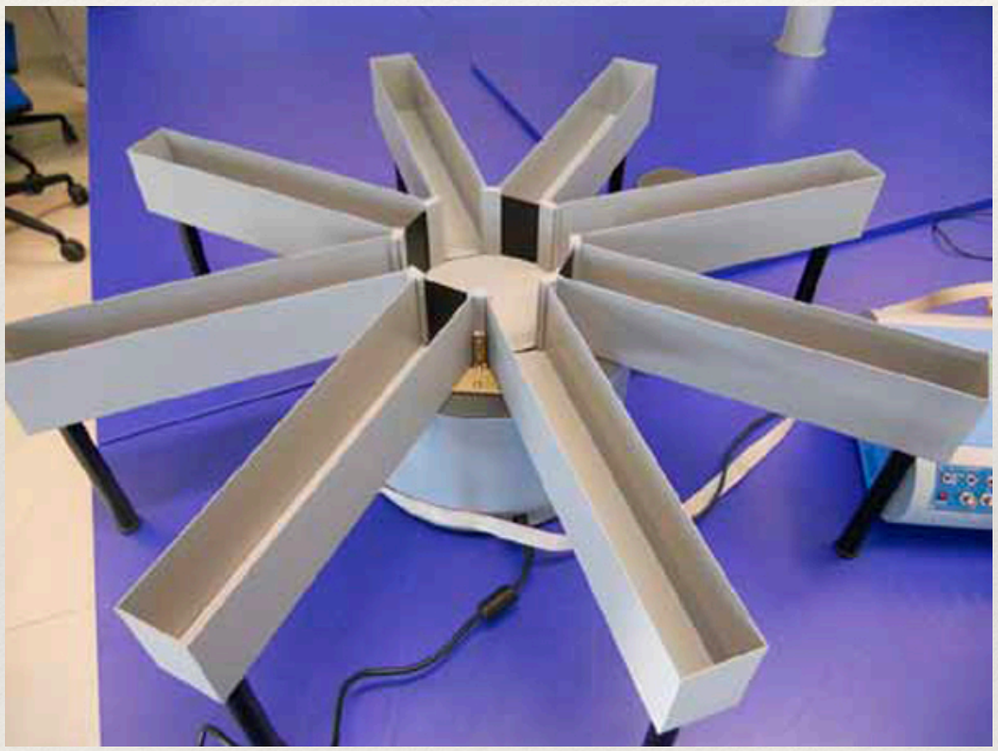
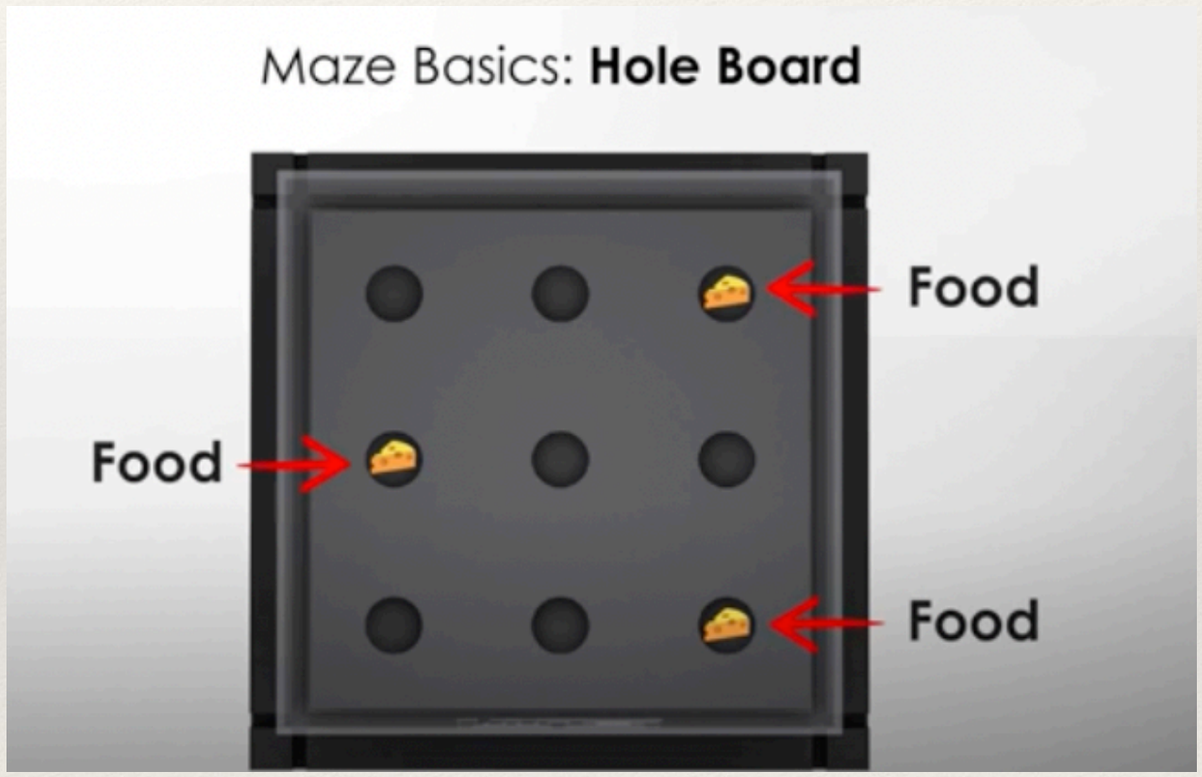
Other mazes used





Behavioural tests for anxiety and cognition in zebrafish

Kyriaki Sidiropoulou

Why zebrafish?

- Smaller animal compared to mice
- Not mammals
- Shorter breeding times
- Genetic manipulations
- Strong cortisol response

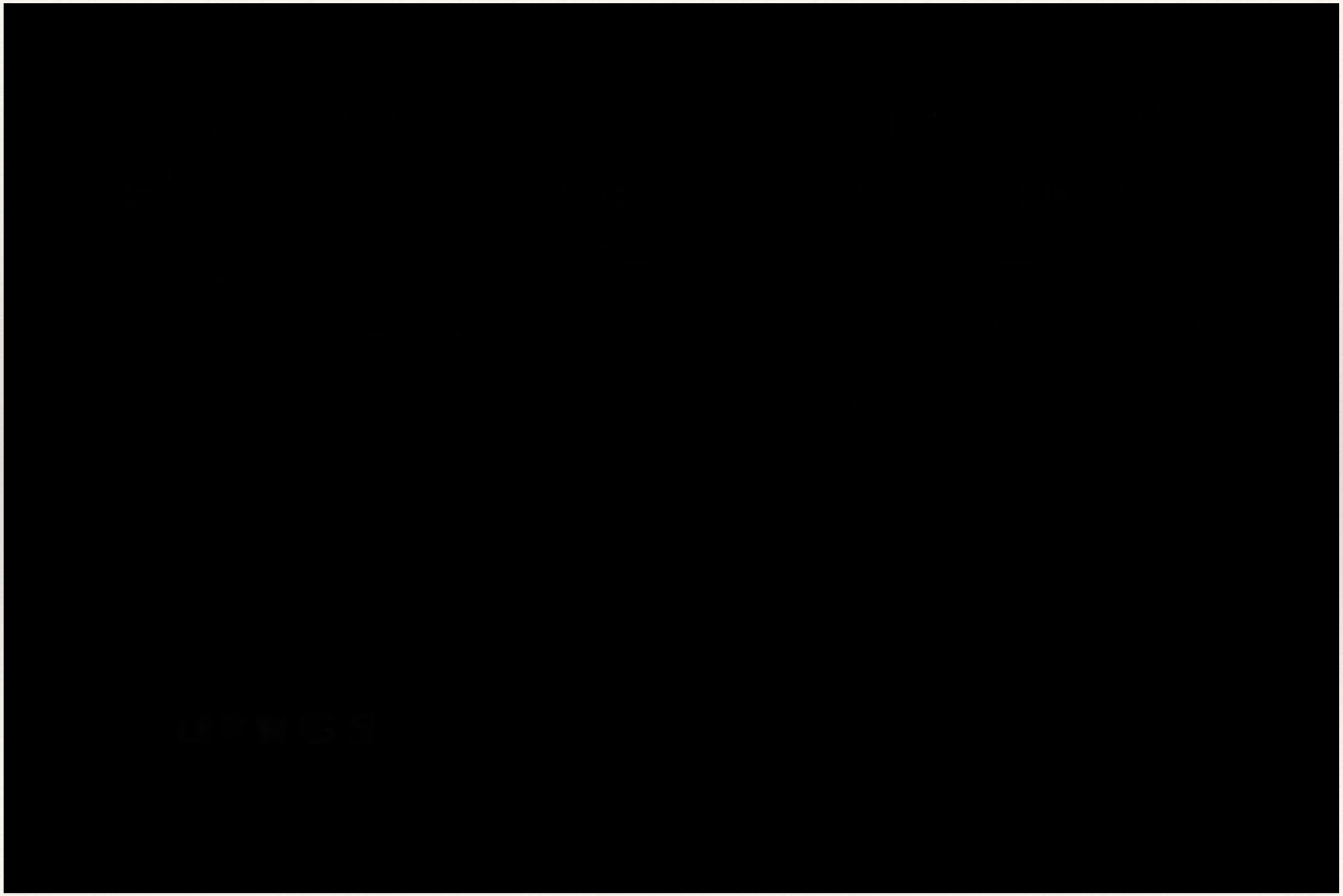
Zebrafish is good model for anxiety

- Robust cortisol response
- Sensitivity to drug treatment
- Behavioural strain differences

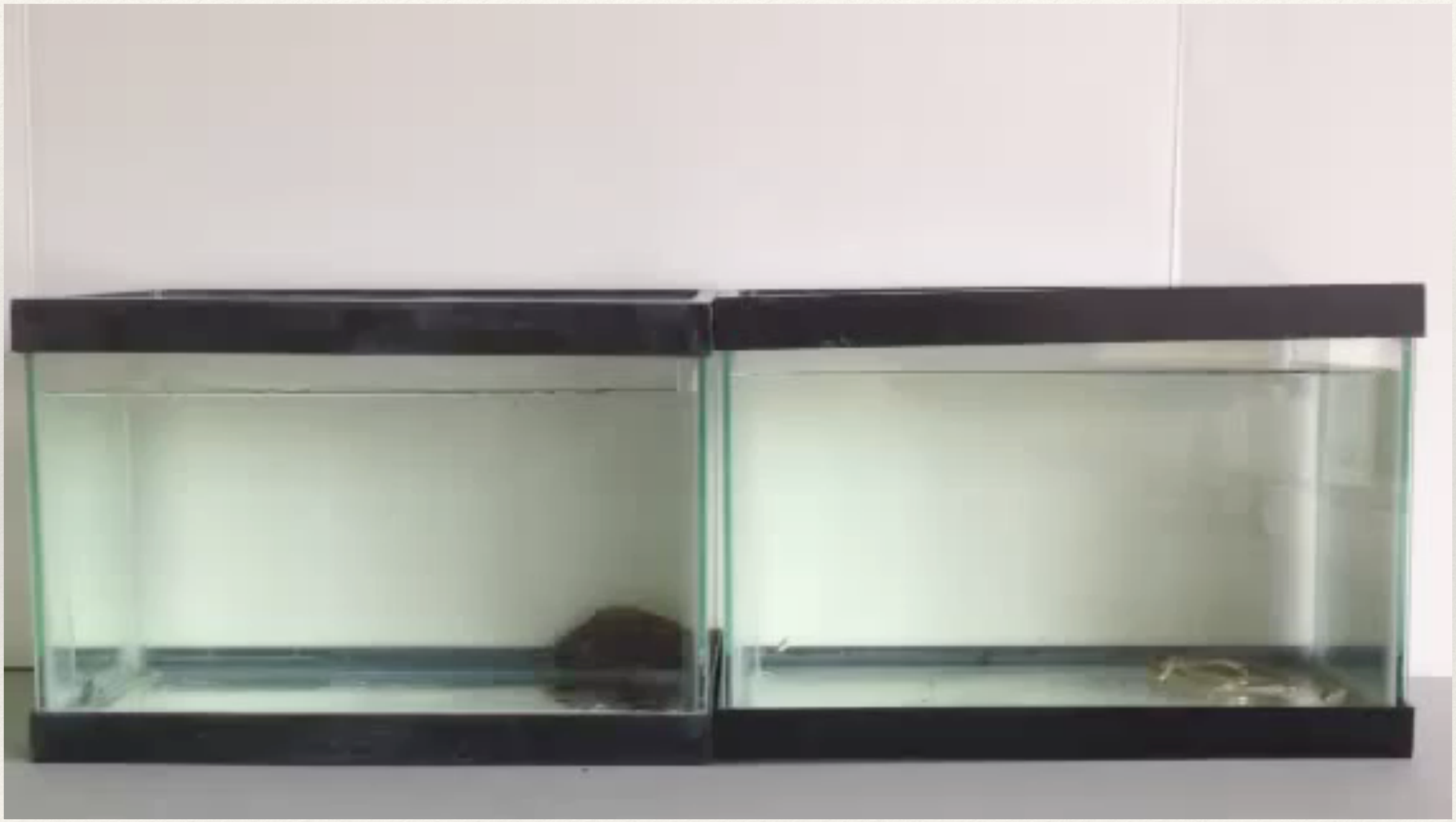
Anxiety behaviour in zebrafish

- Swim at the pool bottom
- Decreased and erratic movements

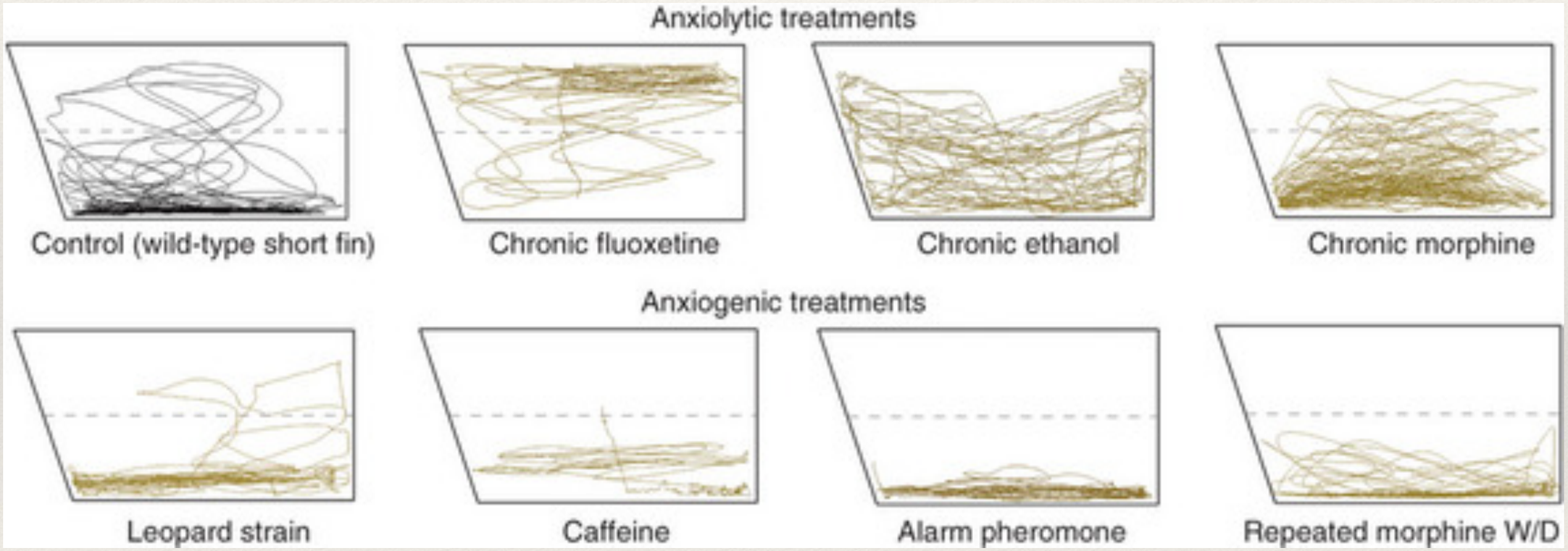
Exploration in zebrafish



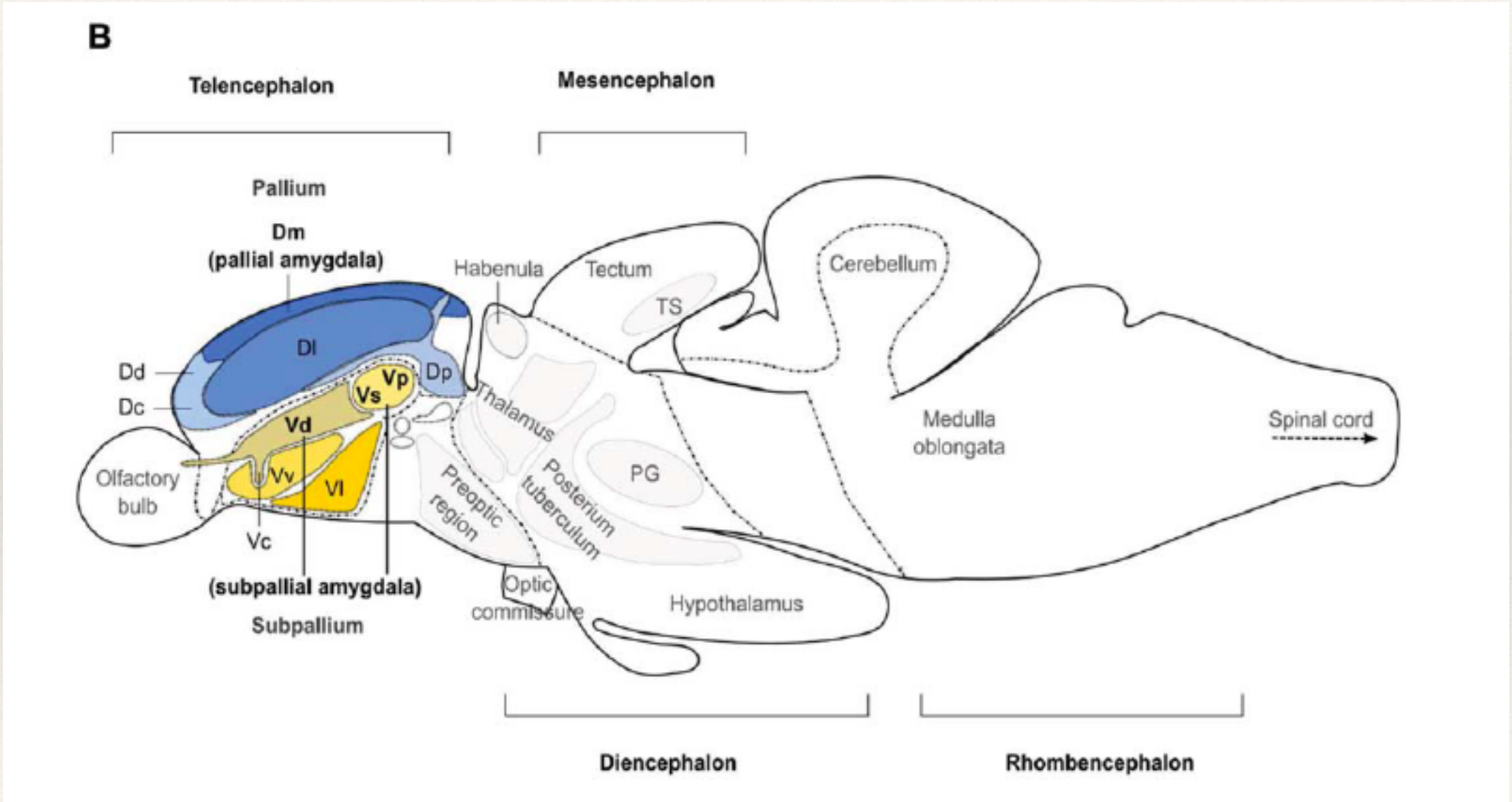
Predator-induced anxiety



Zebrafish: Response to different treatments



Do zebrafish have an amygdala?

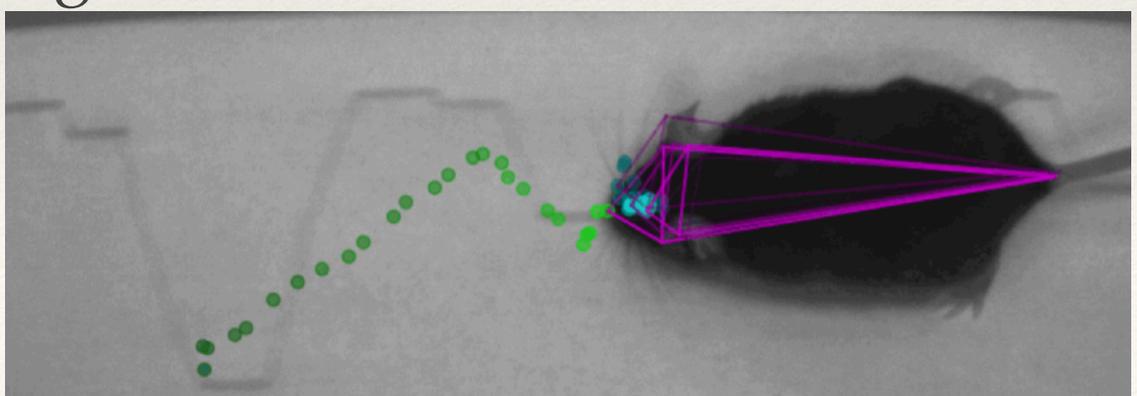


Memory tests in zebrafish

- There is a need to develop appropriate and high-throughput memory tests in zebrafish
- Associative tests are difficult to develop in zebrafish
- Object recognition is different in zebrafish; They prefer the familiar object unlike the rodents
- Non-associative tests might be best for zebrafish

Behavioral analysis

- Human observation
  - Very accurate but time-consuming
- Commercially available software
  - Noldus
  - Anymore
- DeepLabCut
  - Based on deep learning algorithms



Bibliography

- La-Vu, Tobias and Adhikari (2020) To approach or avoid: An introductory overview of the study of anxiety using rodent assay, Frontiers in Behavioral Neuroscience, 14:145
- Kim, S., Lee, S., Ryu, S., Suk, J.-G., & Park, C. (2002). Comparative analysis of the anxiety-related behaviors in four inbred mice. *Behavioural Processes*, *60*, 181–190
- Perathoner, S., Cordero-Maldonado, M. L., & Crawford, A. D. (2016). Potential of zebrafish as a model for exploring the role of the amygdala in emotional memory and motivational behavior. *Journal of Neuroscience Research*, *94*(6), 445–462
- May et al. (2016) Object recognition memory in zebrafish, Behavioural Brain Research, 296: 199-210
- Gerlai (2016) Learning and memory in zebrafish (Danio rerio), Methods in Cell Biology, 134: 551-586
- Cachat et al. (2010) Measuring behavioral and endocrine responses to novelty stress in adult zebrafish, Nature Protocols, 5 (11) 1786-1799
- Walf and Frye (2009) Using the Elevated Plus Maze as a Bioassay to Assess the Effects of Naturally Occurring and Exogenously Administered Compounds to Influence Anxiety-Related Behaviors of Mice, Chapter in “Mood and Anxiety Related Phenotypes in Mice”, Humana Press.