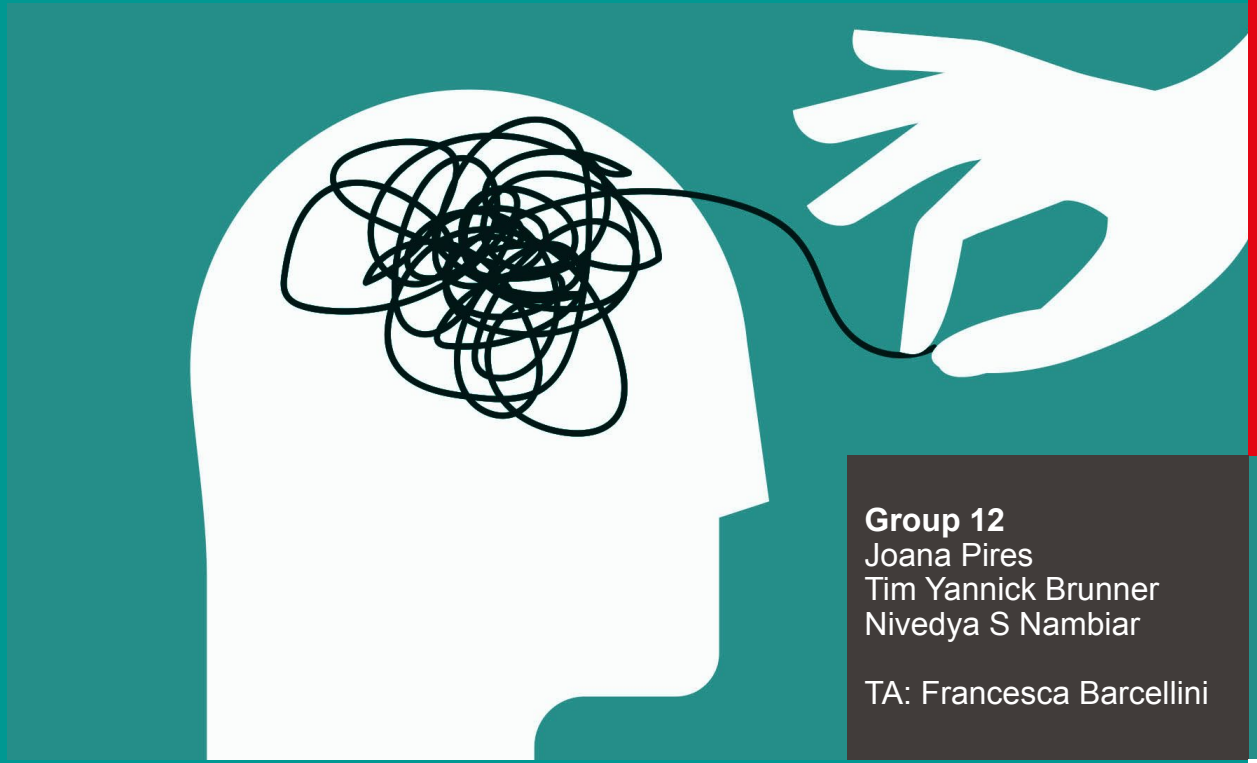


Investigation of Dorsal Raphe Nucleus Role in SSRI resistant Mice

Group 12
Joana Pires
Tim Yannick Brunner
Nivedya S Nambiar

TA: Francesca Barcellini

NX - 436

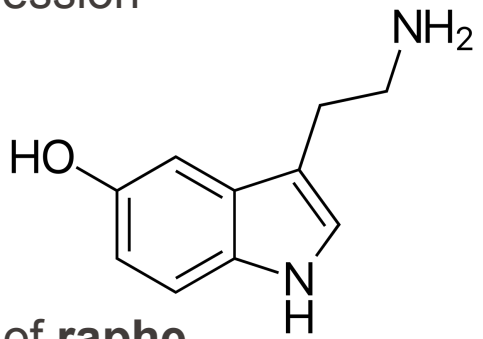
Major Depressive Disorder (MDD)

image from shutterstock

- Most common **psychiatric disorder**, affecting >250 million people worldwide[1]
- Characterized by hopelessness, low self-esteem, feelings of guilt
- Treatments: **antidepressants (like SSRI)**, psychotherapy or a combination of both[2]

MDD and Serotonin

- **Serotonin hypothesis** - 5-HT as the key player in depression[3]
- 5-HT deficiency → psychological and physical effects[4]
- 5-HT excess → leads to serotonin syndrome[5]
- Almost exclusively produced in **serotonergic neurons** of **raphe nuclei** located in midbrain[6]



The serotonin theory of depression: a systematic umbrella review of the evidence

Joanna Moncrieff, Ruth E. Cooper, Tom Stockmann, Simone Amendola, Michael P. Hengartner & Mark A. Horowitz

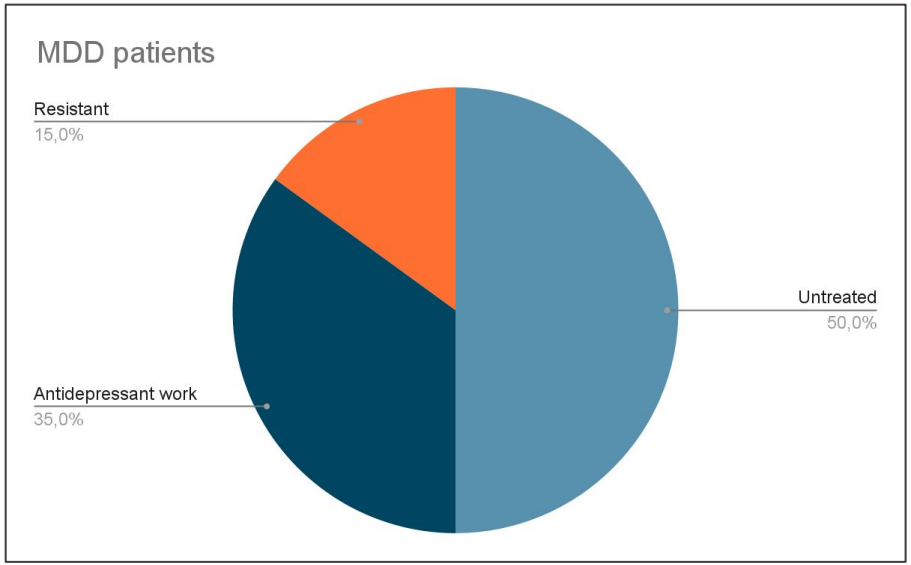
Selective Serotonin Reuptake Inhibitors (SSRI)

- Most prescribed **antidepressant** drug to treat depression
- Mechanism - increase level of serotonin by inhibiting reabsorption by serotonin transporter[7] [8]

https://www.britannica.com/science/antidepressant

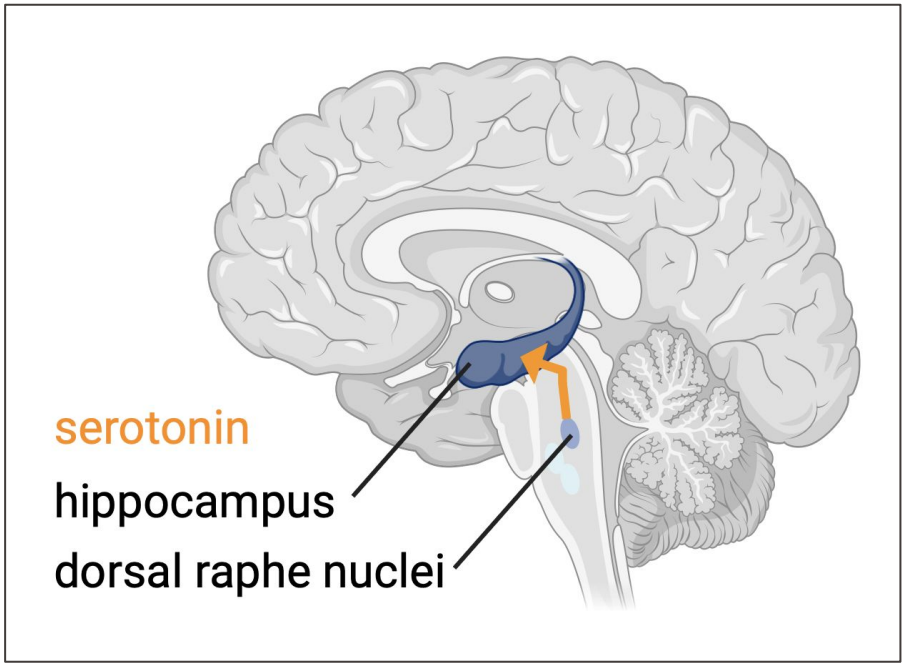
SSRI-Resistance

- 15% of MDD patients have SSRI resistance[10]
- Hypothesis: auto-negative regulation of serotonergic neuron of dorsal raphe nucleus[11]
- DRN is the largest serotonergic nucleus



Hippocampus and Dorsal Raphe Nucleus (DRN)

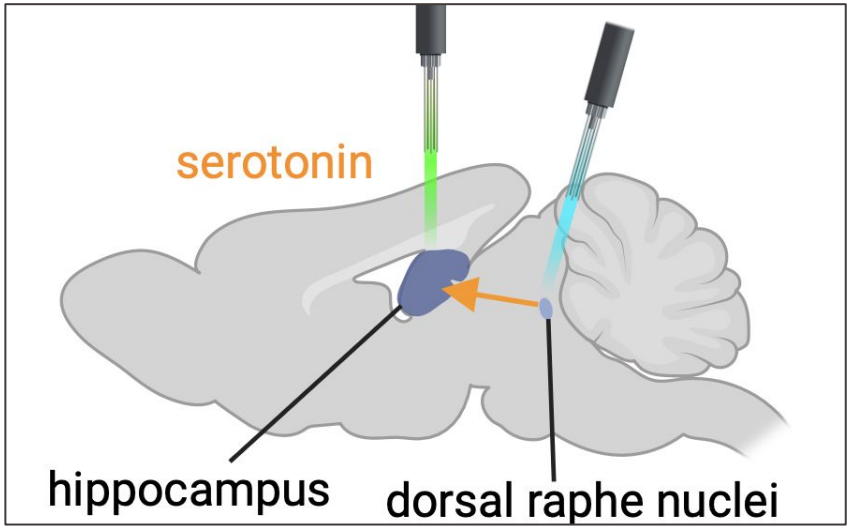
- **Serotonin sensitive neurons** in hippocampus => Neurogenesis
- Neurogenesis => antidepressant[14]
- **Serotonergic neuron in DRN** project to the hippocampus[12]
- Indirect activation of **hippocampus**[13]



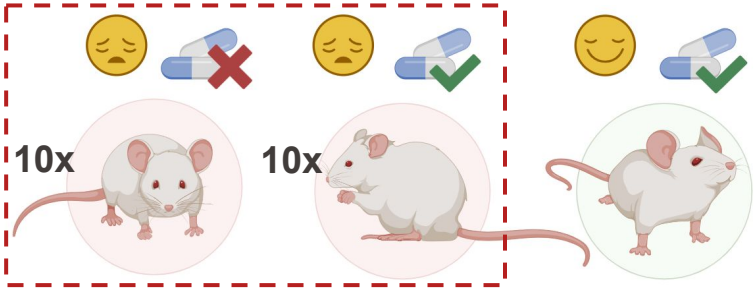
Study Overview:

Use optogenetic to investigate the role of dorsal raphe nucleus in SSRI resistant mice

- Stimulate **serotonergic** neurons in dorsal raphe nucleus
- Record activity of **serotonin sensitive neuron** in hippocampus



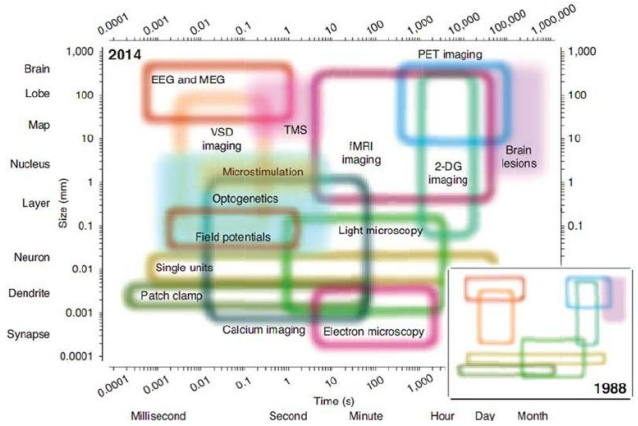
Study Design

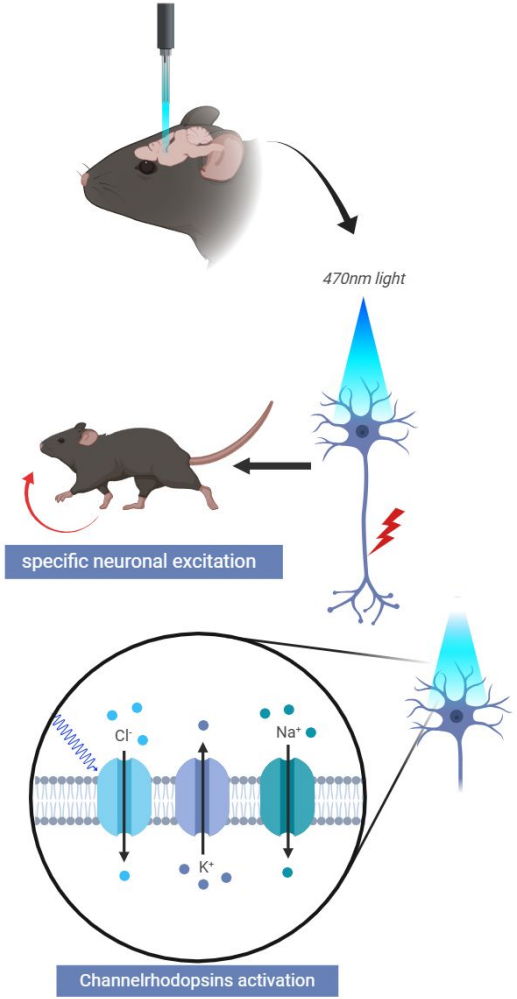


Mouse Models	Inducing Depression[23]	Inducing SSRI Resistance[21]	Assessment Steps		
			Baseline	SSRI administration	SSRI + optogenetic stim.
Group 1: Depressed SSRI-resistant mice	**Chronic Mild Stress (CMS)**	Adrenocortico-tropic hormone (ACTH)	depressed	depressed	reduced resistance (similar to group 2) less depressed?
Group 2: Depressed non-resistant mice	Learned Helplessness (LH)	*none*	depressed	less depressed	less depressed (Sham)

Optogenetics

- Neuron transfected with viral vector
  - Integration of channelrhodopsin
  - Excitability by specific wavelength
- Why optogenetics: Very precise excitation
  - Specific promoter





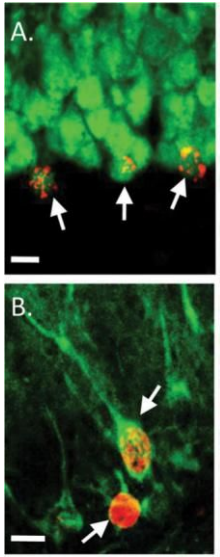
Viral Vector for Serotonergic Neurons

pAAV-TPH2

- Adeno Associated Virus (AAV)[24]
  - commonly used in dorsal raphe nucleus
- TPH2 promoter
  - targets serotonergic neurons
- chR2
  - makes the cells sensitive to blue light

Recording the Effect of the Optogenetic Stimulation[15]

- Behavior Test
  - Tail Suspension Test[21]
  - Increased immobility time in depression
- Analyse serotonin concentration in the hippocampus region
  - Photometry[16]
- Analysis of the hippocampus to verify plasticity
  - Histology



Recording Technique: Photometry

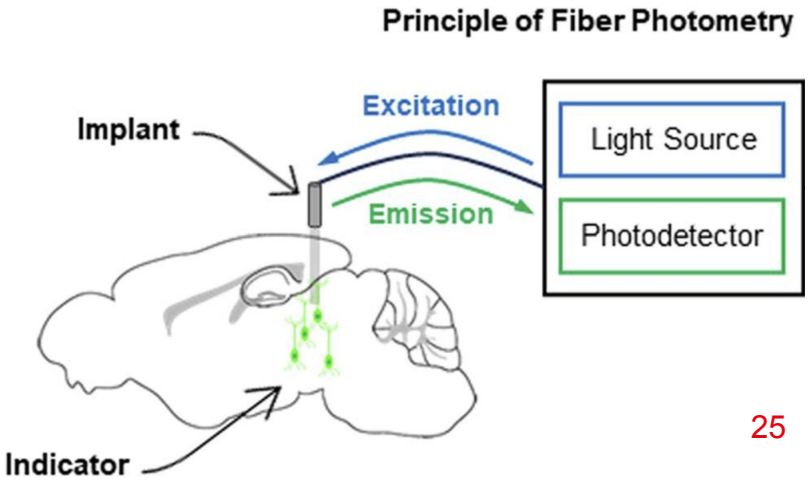
- Fluorescence activity in vivo
- Neurotransmitter sensors transgenically introduced into neurons
- Neural activity modifies fluorescence intensity[17]
  - Compared to baseline

\+ **chronic**

\+ **specific brain regions**

\- **spatial resolution: overlap**

\- **lacks single-cell resolution**



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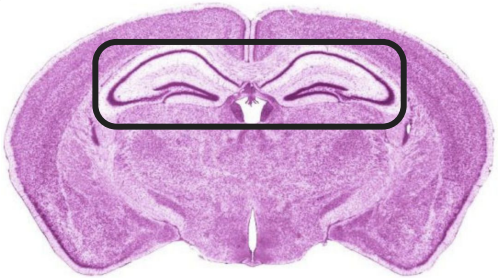
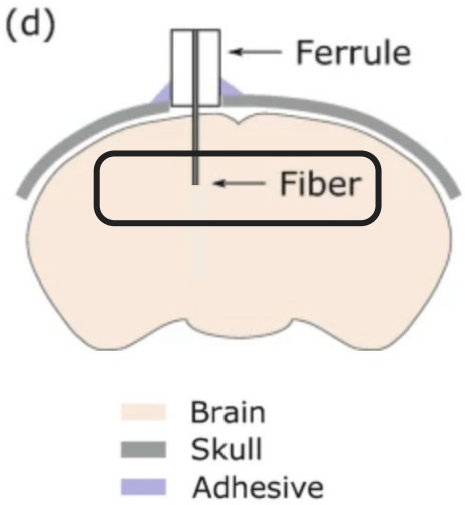
Recording Technique: Photometry

- Serotonin darkening 5-HT1A receptor (sDarken)[17]
  - **reduction** of **green fluorescence** upon serotonin binding
- Fluorescence baseline
  - serotonin release by **sugar**





Yu, L., Murari, K. (2021). Functional Monitoring and Imaging in Deep Brain Structures. In: Thakor, N.V. (eds) Handbook of Neuroengineering. Springer, Singapore. https://doi.org/10.1007/978-981-15-2848-4_135-1

Surgical Plan

Dorsal raphe nucleus at 40°

AP: 6.25 * BL/4.35 mm

ML: 0mm

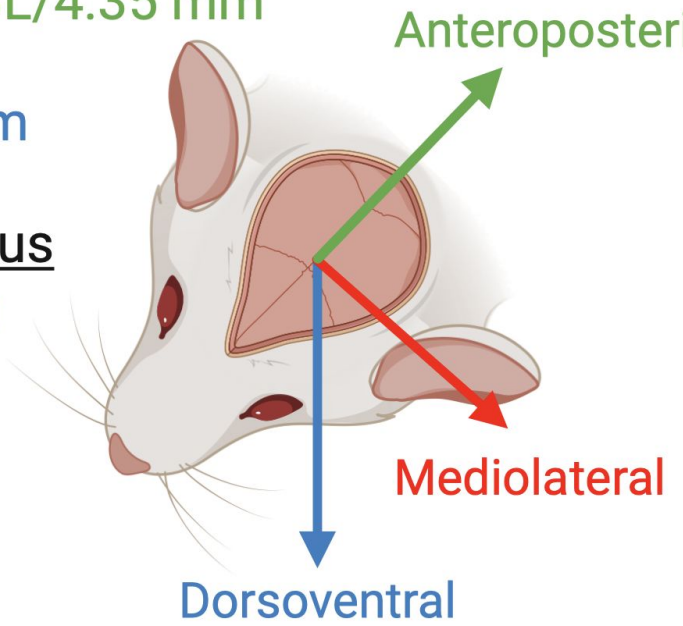
DV: -3.33mm

Hippocampus

AP: -1.7mm

ML: 1.6mm

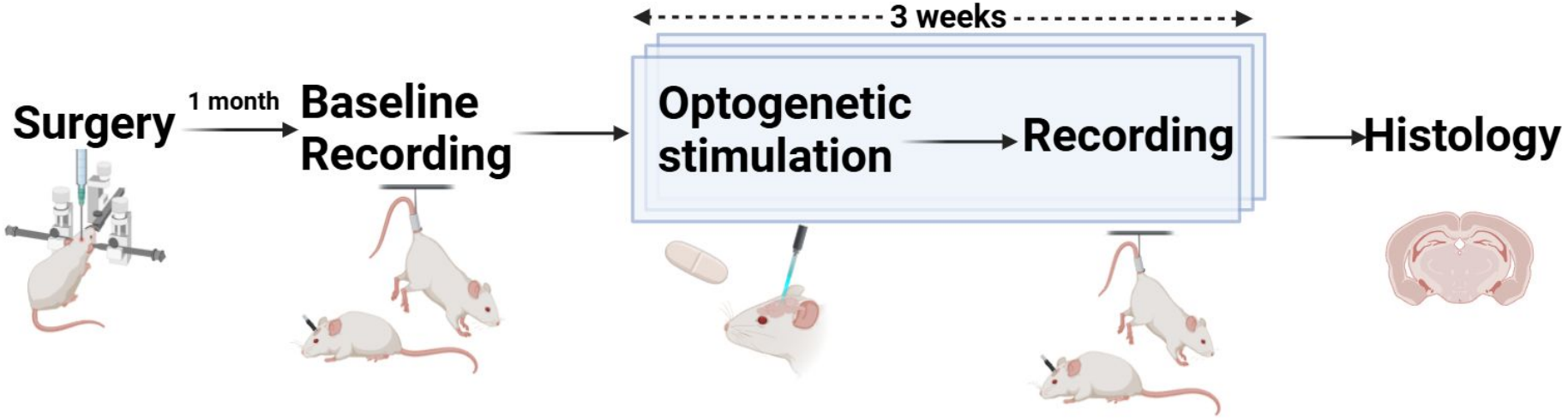
DV: 1.9mm



Coordinates[19, 20]:

- Injection of virus
- Implantation of optic fiber

Timeline of study



Result Interpretation

During **SSRI administration**, if we stimulate more **serotonin release** and observe **changed mouse behavior**:

→ **Does it affect the hippocampus structure ?**

Expected Symptoms before Optogenetics	Depressed SSRI-resistant Mice	Depressed nonresistant Mice
Behavior Test	Increased immobility time	Decreased immobility time
Photometry (serotonin concentration)	Higher fluorescence (low serotonin)	Lower fluorescence (high serotonin)
Histology (hippocampus plasticity)	Low or absent neurogenesis	Neurogenesis detectable

Expected Symptoms after Optogenetics	Depressed SSRI-resistant Mice	Depressed nonresistant Mice
Behavior Test	Decreased immobility time	Decreased immobility time
Photometry (serotonin concentration)	Lower fluorescence (low serotonin)	Lower fluorescence (high serotonin)
Histology (hippocampus plasticity)	Neurogenesis detectable	Neurogenesis detectable

Expected assimilation between the two mouse strains

Limitations and Challenges

- Artificially depressed mice
- Artificially introduced SSRI resistance in mice
- Volumetric spread of serotonin might cause side effects
- Chronic vs acute effect

Translatability

- DBS

Deep brain stimulation of the dorsal raphe induces anxiolytic and panicolytic-like effects and alters serotonin immunoreactivity

J A Lemes [1], M S C F Silva [1], B S M Gonçalves [1], I C Céspedes [2], M B Viana [3]

- Focal non-invasive methods - TI, FUS

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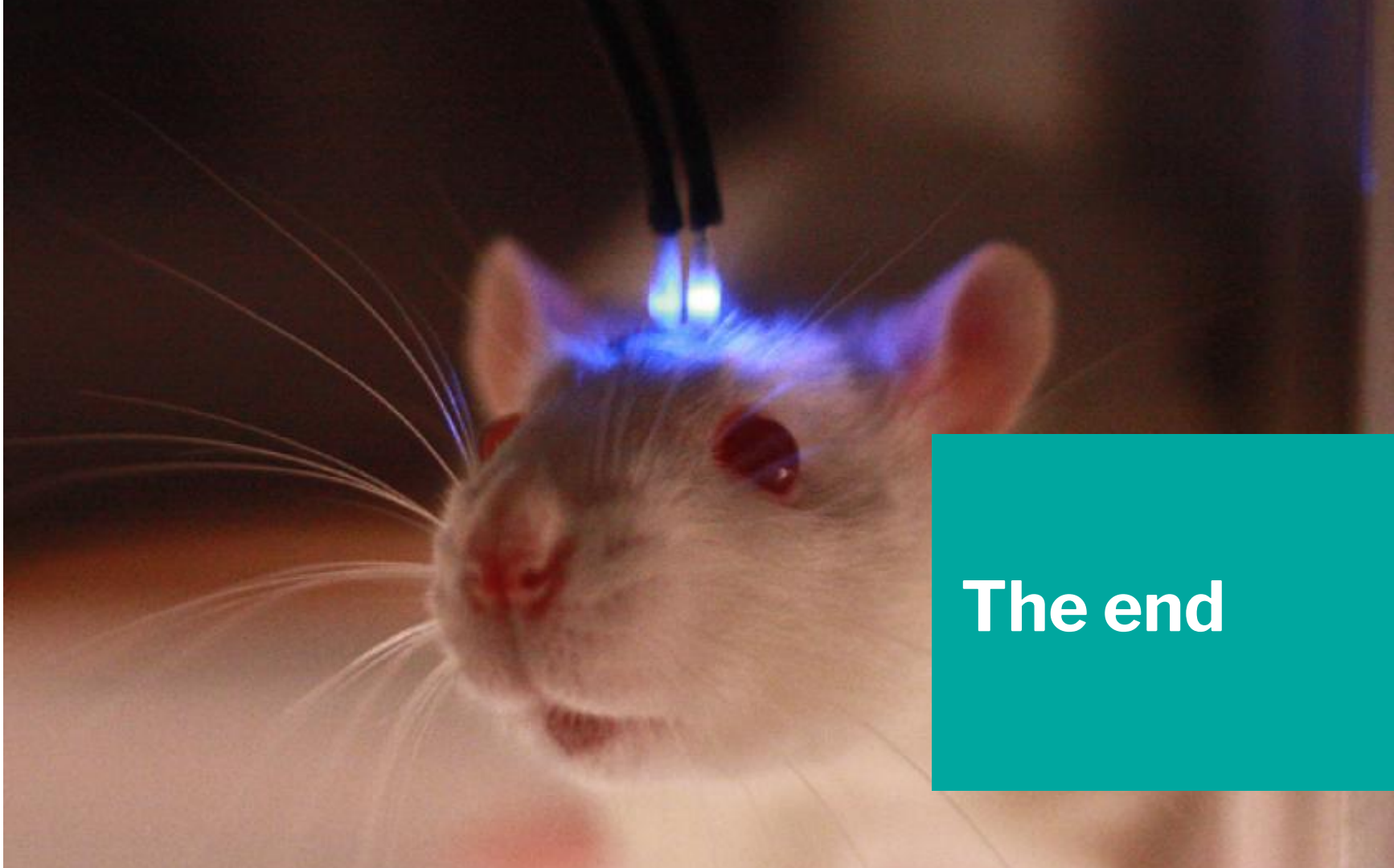

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