NX436 - Neuromodulation Project: Differentiation of itch and pain sensations in the anterior cingulate cortex through optogenetics-pharmacology combination.

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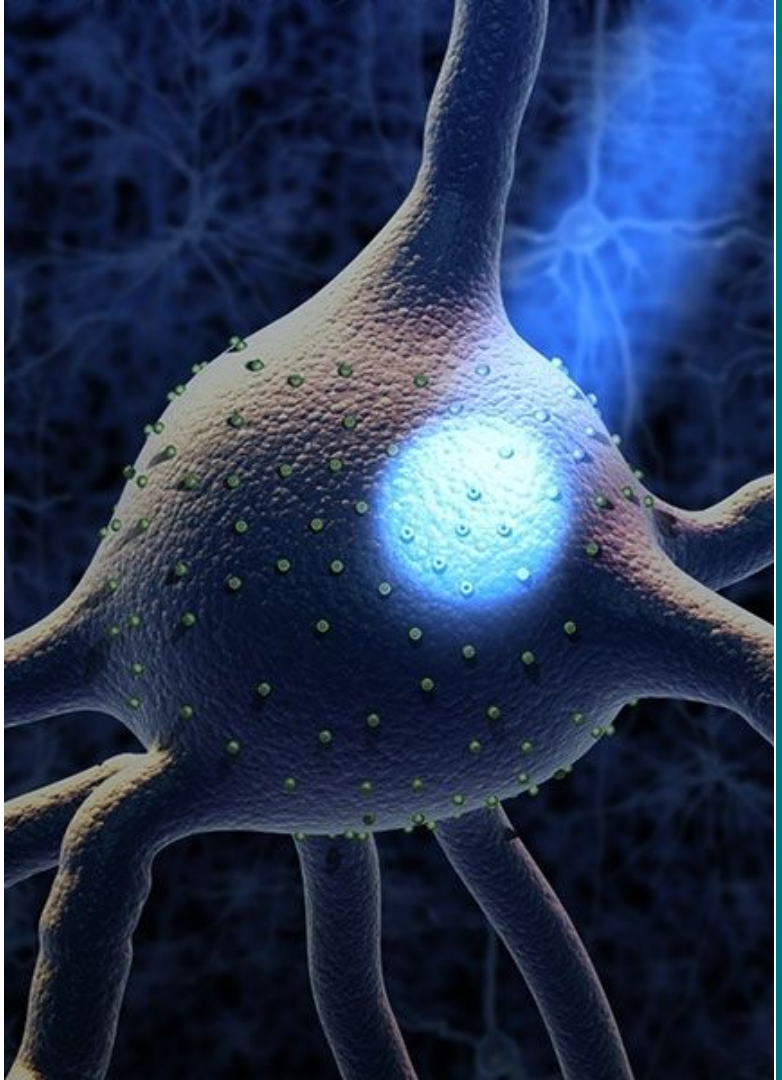

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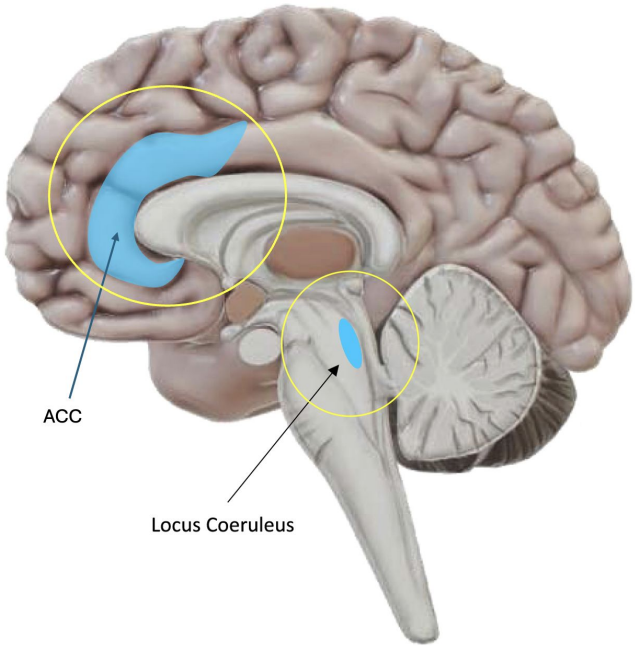
Pain and Itch differentiation : Why?

- Diseases such as Herpes, eczema and psoriasis
- 10% of global population
- Share similar mediator (Histamine, substance P, interleukins, and proteases)
- Differentiation in the brain never made
- Can give us insights about their mechanisms, and so possible development of treatments

Eczema plates

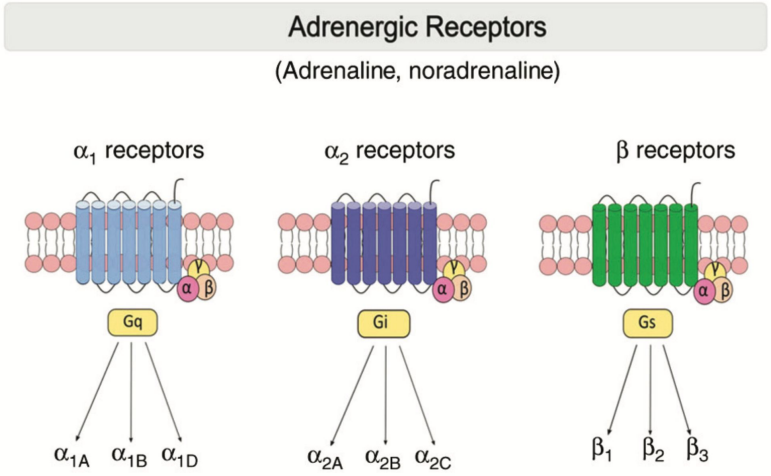
Which pathways?



- Locus Coeruleus (LC) is the main origin of noradrenergic (NA) neurons
- NA is a key neurotransmitter in CNS
- LC projects in dorsal spinal horn but also in the **brain**
- Anterior cingulate cortex (ACC) play a role in emotional response and chronic pain
- Ascending LC projection to the ACC

State-of-the-art

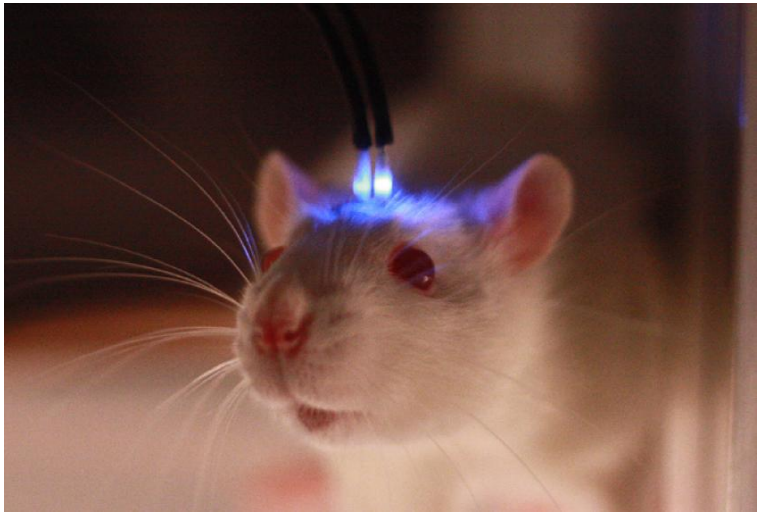
- In the ACC, NA facilitates presynaptic glutamate release to the pyramidal cells and enhances pyramidal cell membrane excitability, through 3 distinct subtype receptors (α_1, α_2, β_2)
- Unclear whether pain and itch information share a common pathway in the CNS
- Further study is needed for understanding the mechanism of how NA modulates these two different sensations in detail



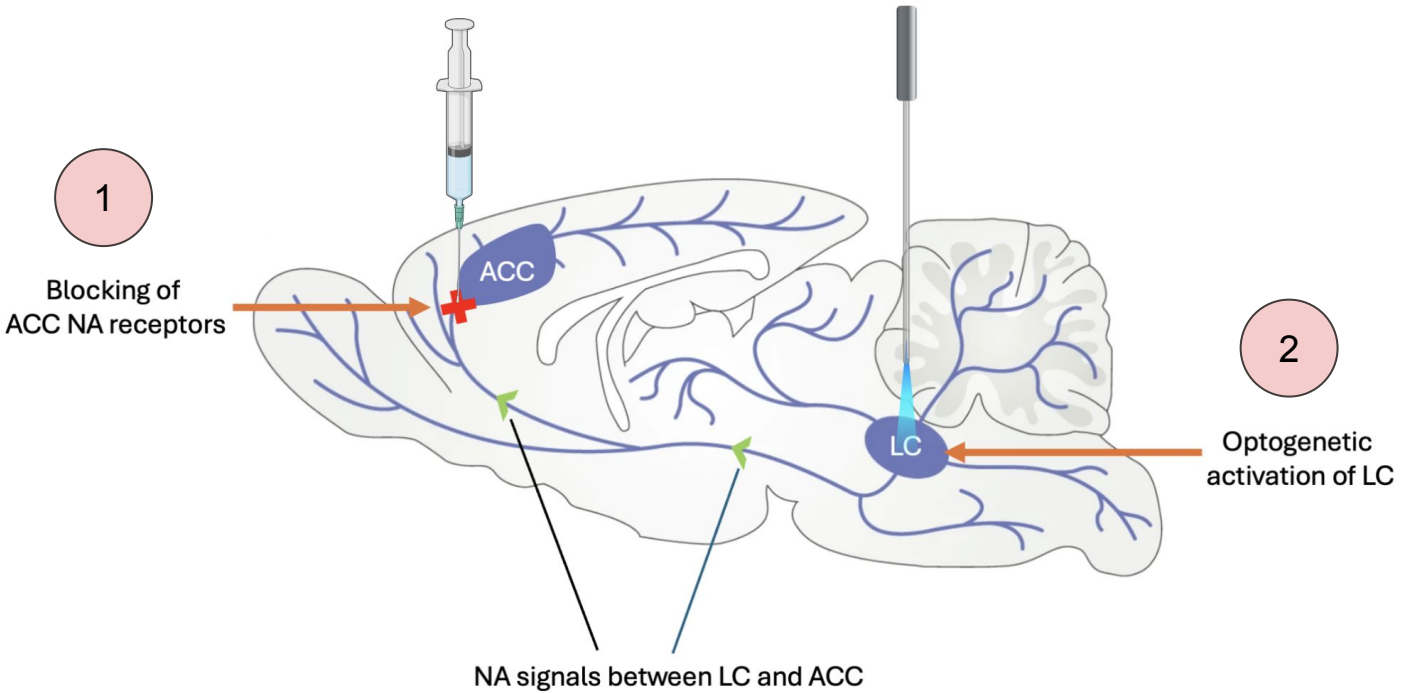
Ozdemir E., 2024

What tools had been used ?

- In vitro voltage/current patch clamp on ACC slices
- In vivo electrical stimulation of LC and sensing in ACC
- Immunostaining of pyramidal cells fibers and observation with electron microscopy
- Behavioral tests
- Optogenetics on LC



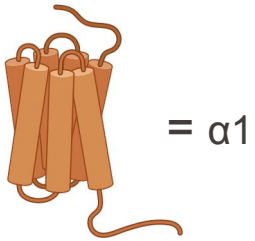
Concept


1
Blocking of
ACC NA receptors
ACC
LC
2
Optogenetic
activation of LC
NA signals between LC and ACC

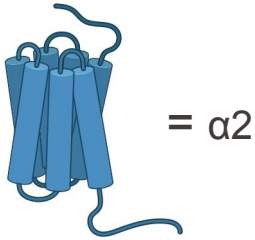
Hypothesis

- Coherent case (more likely) : all of these combinations induce different level of pain and itch

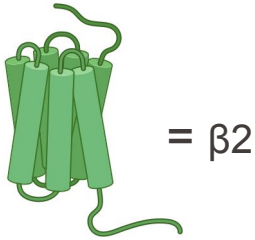
- Extreme case (unlikely) : one combination creates pain, one combination creates itch



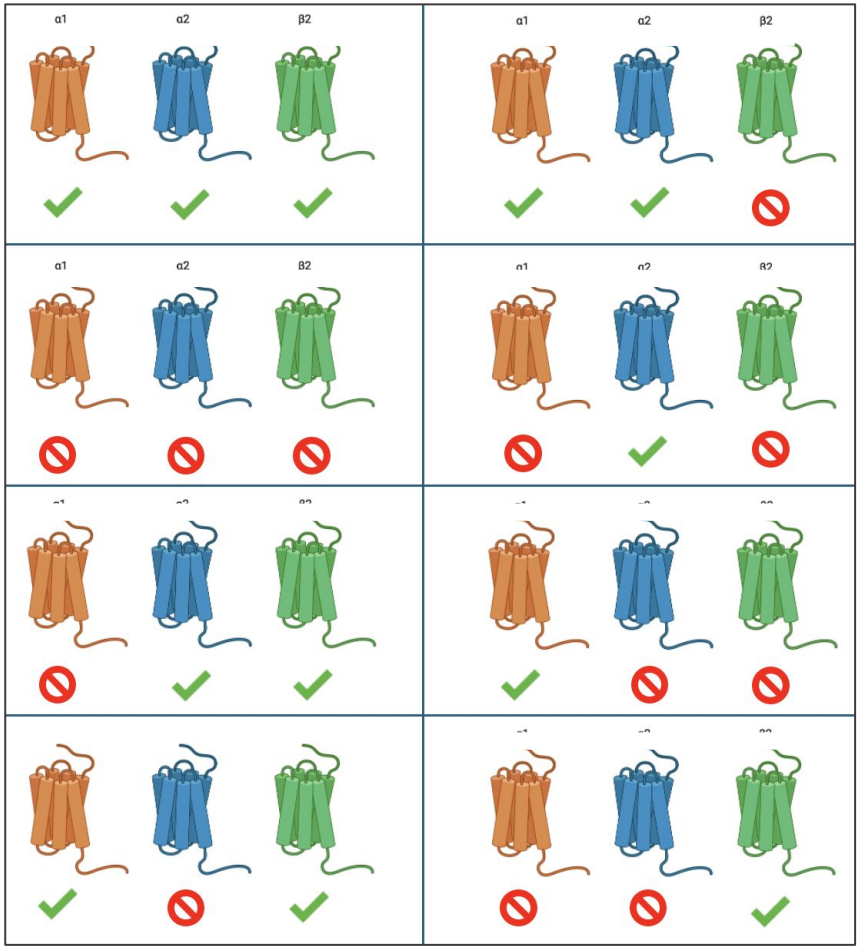
= α1

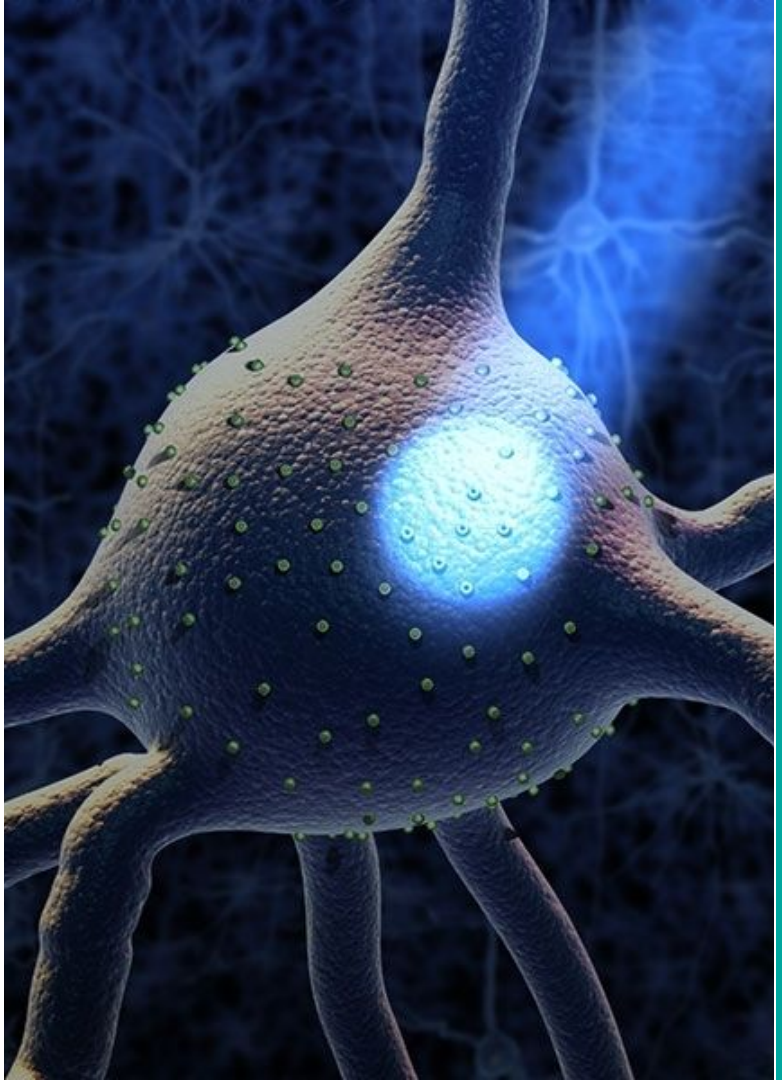


= α2



= β2





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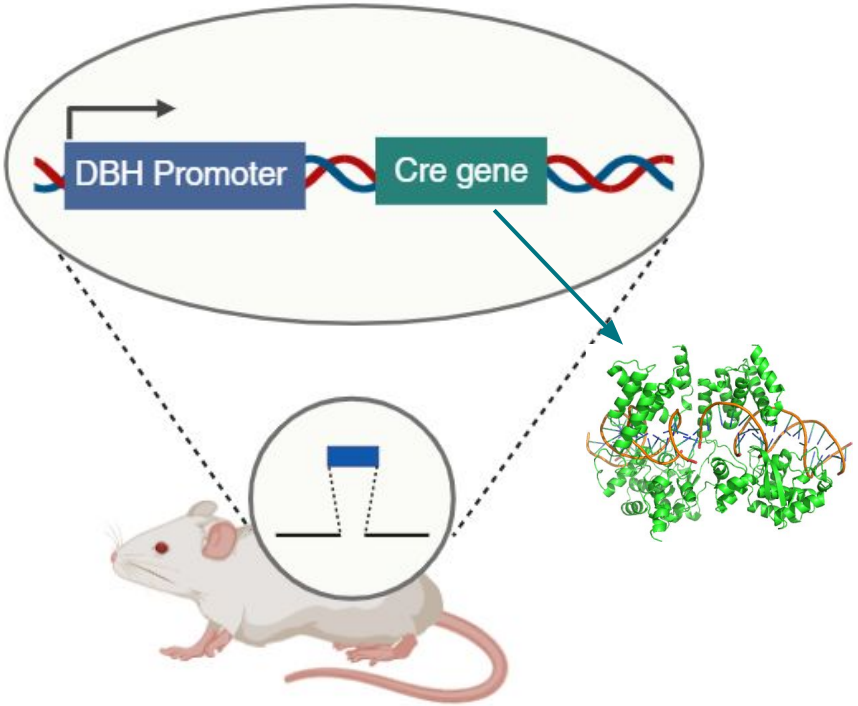
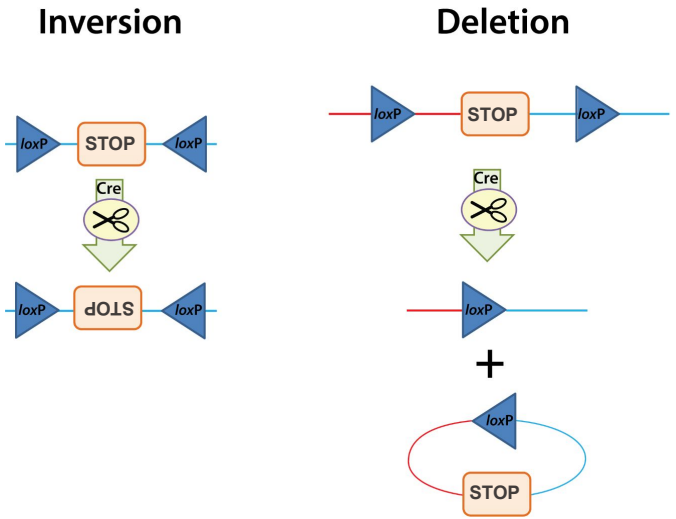
Animal model: DBH-Cre mice

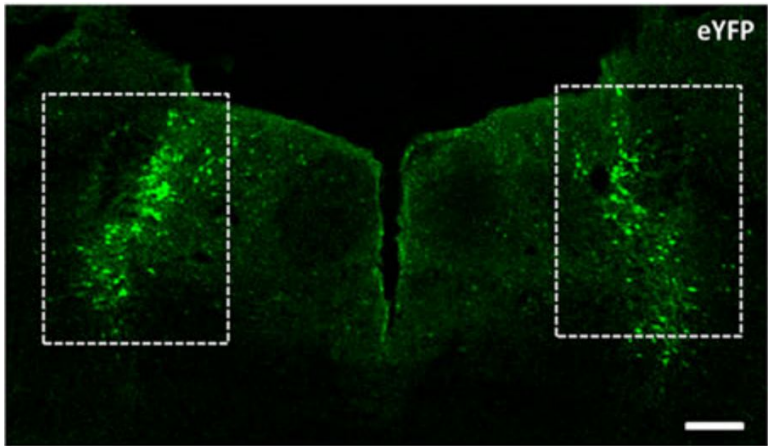
- Adult mice (8-12 weeks old)
- Why DBH-Cre?
  - Expression of Cre-recombinase only in DBH-positive neurons
  - DBH produces NA
  - Selective transfection of NAergic neurons of LC
- Commercially available



Viral vector: AAV-DIO-ChR2-eYFP

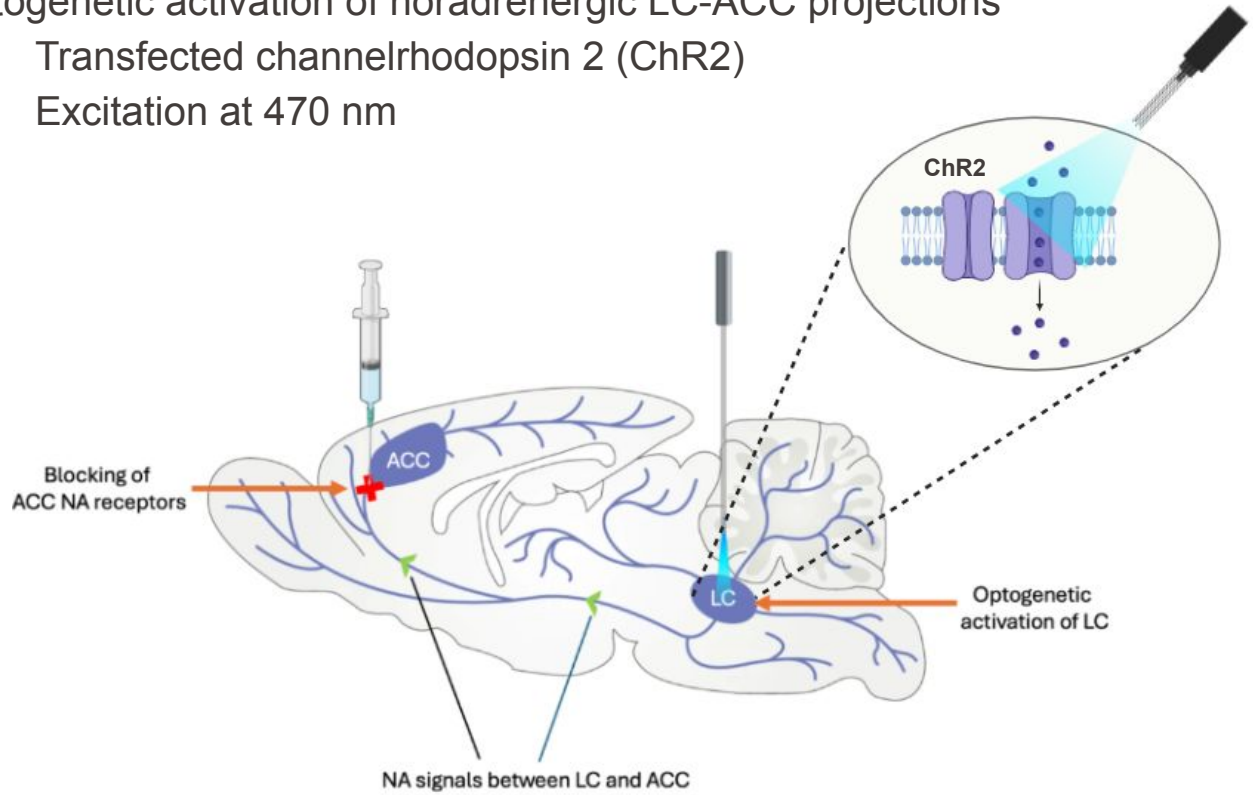
- Cre-dependent viral vector: STOP sequence flanked by loxP sites
- Co-transfection of enhanced Yellow Fluorescent Protein (eYFP) to visualize the viral infection
- Control sample: AAV-DIO-eYFP (only the fluorescent protein)





Optogenetic stimulation of the LC

- Optogenetic activation of noradrenergic LC-ACC projections
  - Transfected channelrhodopsin 2 (ChR2)
  - Excitation at 470 nm



Pharmacological blockage of NA receptors

- Dopamine metabolized in NE/NA by dopamine beta hydroxylase
- Reuptake of NE by NEtransporter (NET)
- Important for sleep/wake cycles, attention, orientation, mood, memory, pain and cardiovascular functions

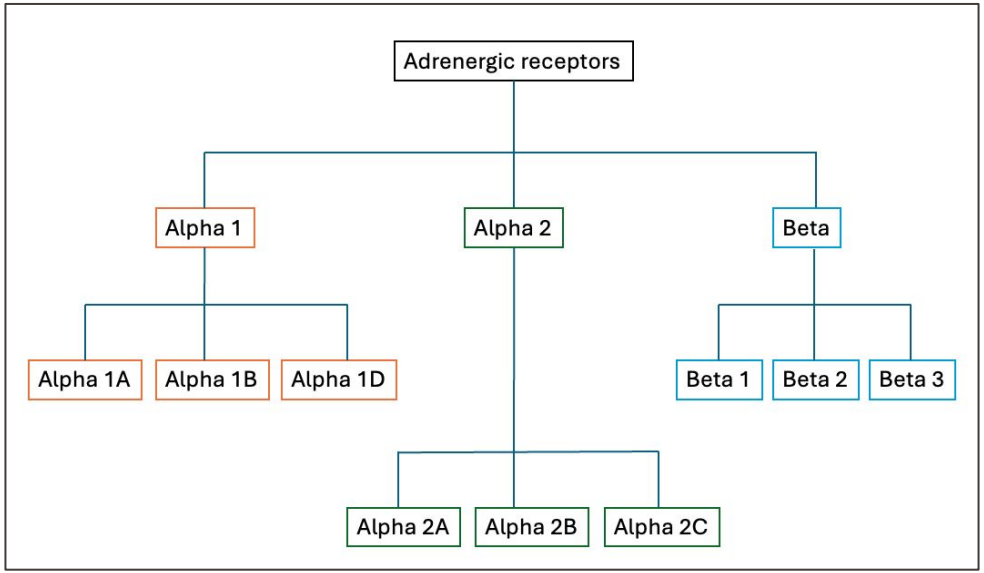
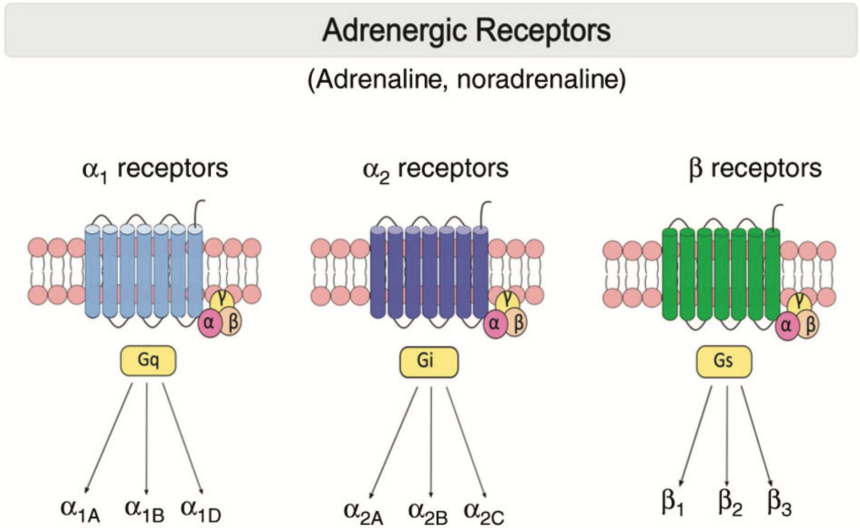


Figure : The families of NA receptors

Pharmacological blockage of NA receptors



α_1 NA receptor antagonists :

- Prazosin and tamsulosin are relatively selective for α_1 receptors and block α_2 and beta receptors only at high concentrations
- Phentolamine and phenoxybenzamine block both α_1 and α_2 adrenergic receptors with similar affinities

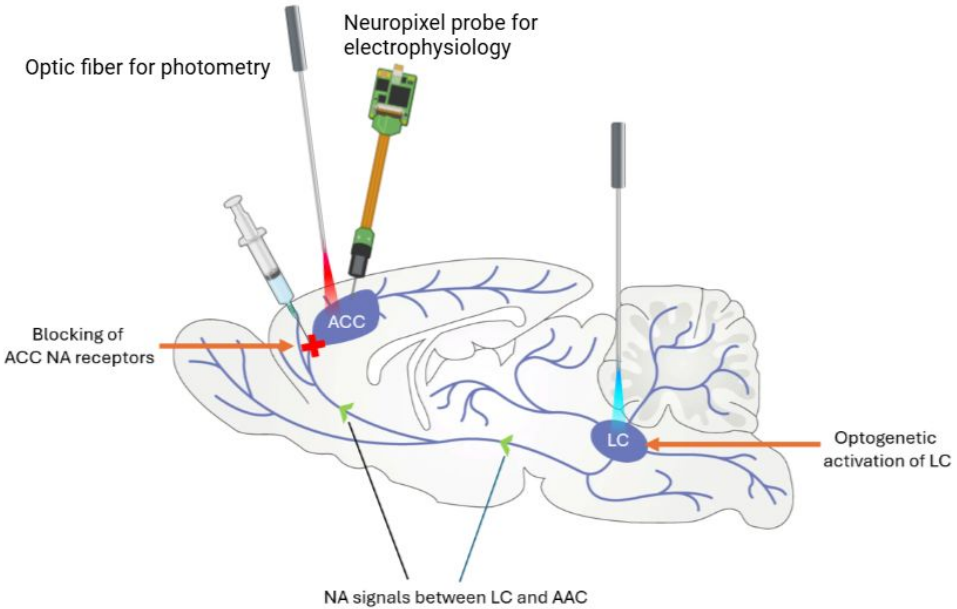
α_2 NA receptor antagonists :

- Yohimibine is selective for α_2 receptors and blocks α_1 and beta receptors only at higher concentrations

β NA receptor antagonist :

- Propranolol is the prototypic non-subtype-selective beta antagonist which has equal affinities at the β_1 and β_2 subtypes

Photometry and Electrophysiology in the ACC



Photometry : bulk monitoring of glutamate levels

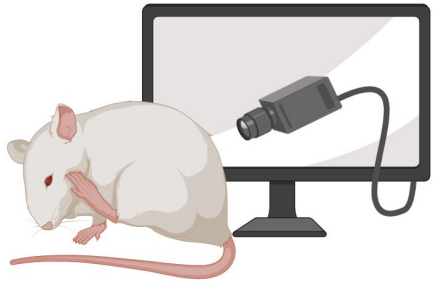
- Use a red-shifted version of iGluSnFR to avoid cross-talk with the stimulation ligth wavelength
- Monitor glutamate activity by measuring fluorescence changes

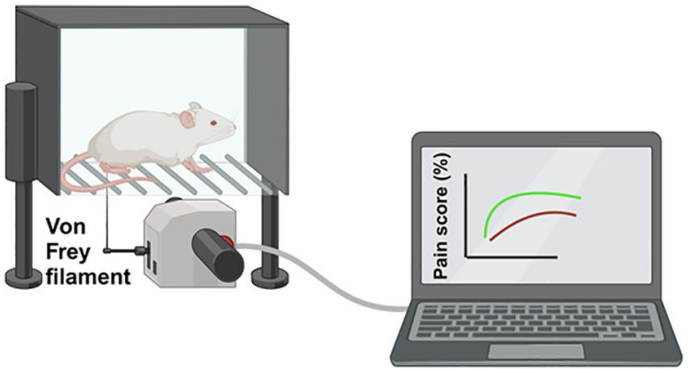
Electrophysiology : single-cell activity

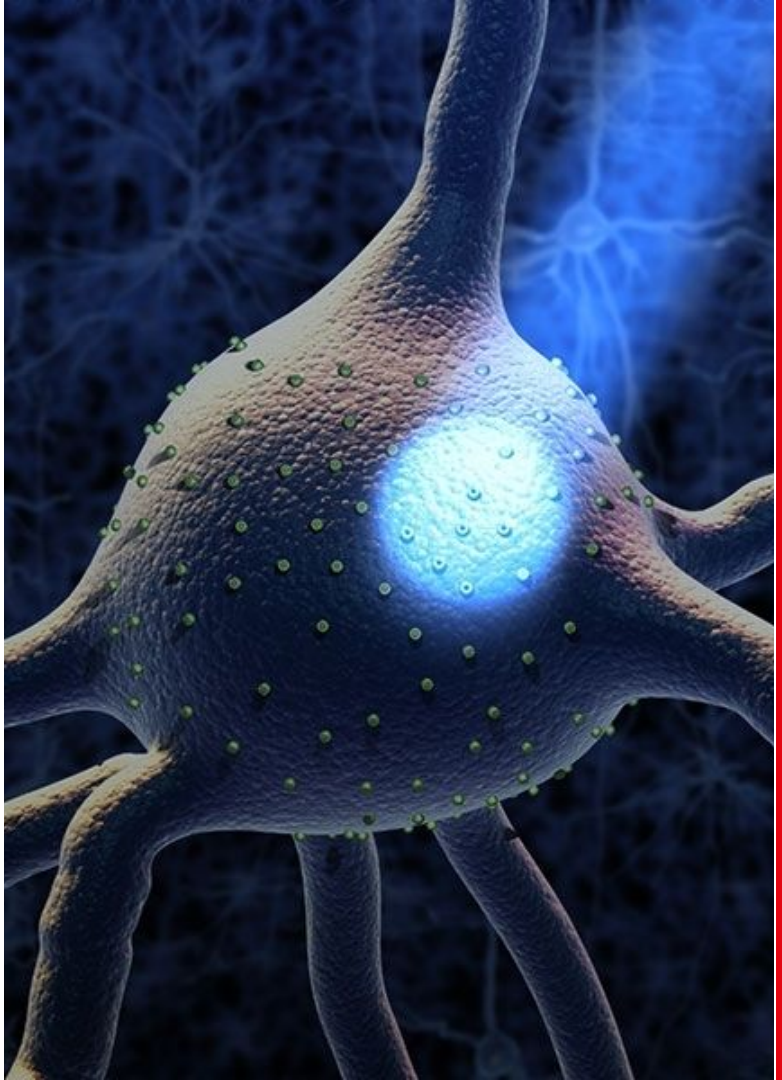
- Quantify changes in firing rates, synchrony, and burst patterns across the different testing conditions.

Recording of behaviors

- Video recording of behaviors before and after LC stimulation for each testing condition
- Von Frey Test for additional pain sensitivity measurements







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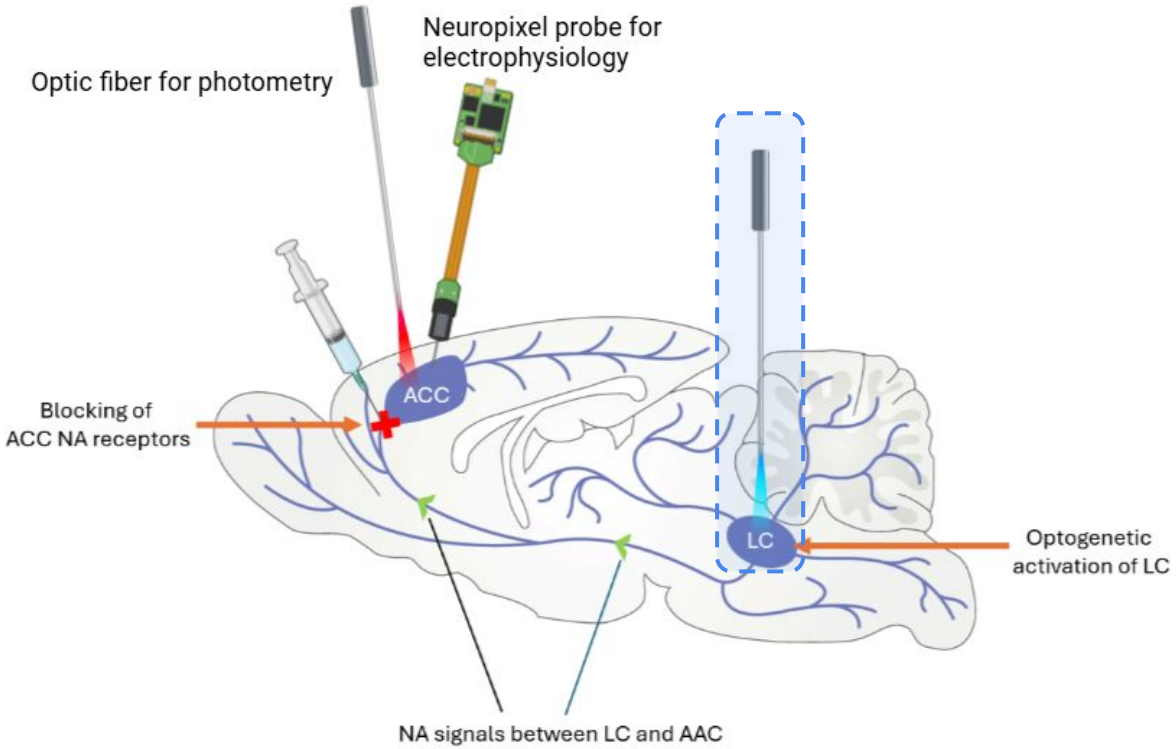
Surgical plan

- Access to the LC:
  - AP: -5.3 mm,
    ML: ±0.8 mm,
    DV: -4.0 mm

- Viral injection: 10^{13} concentration, 200 nL per site
- Wait 4-5 weeks for gene expression

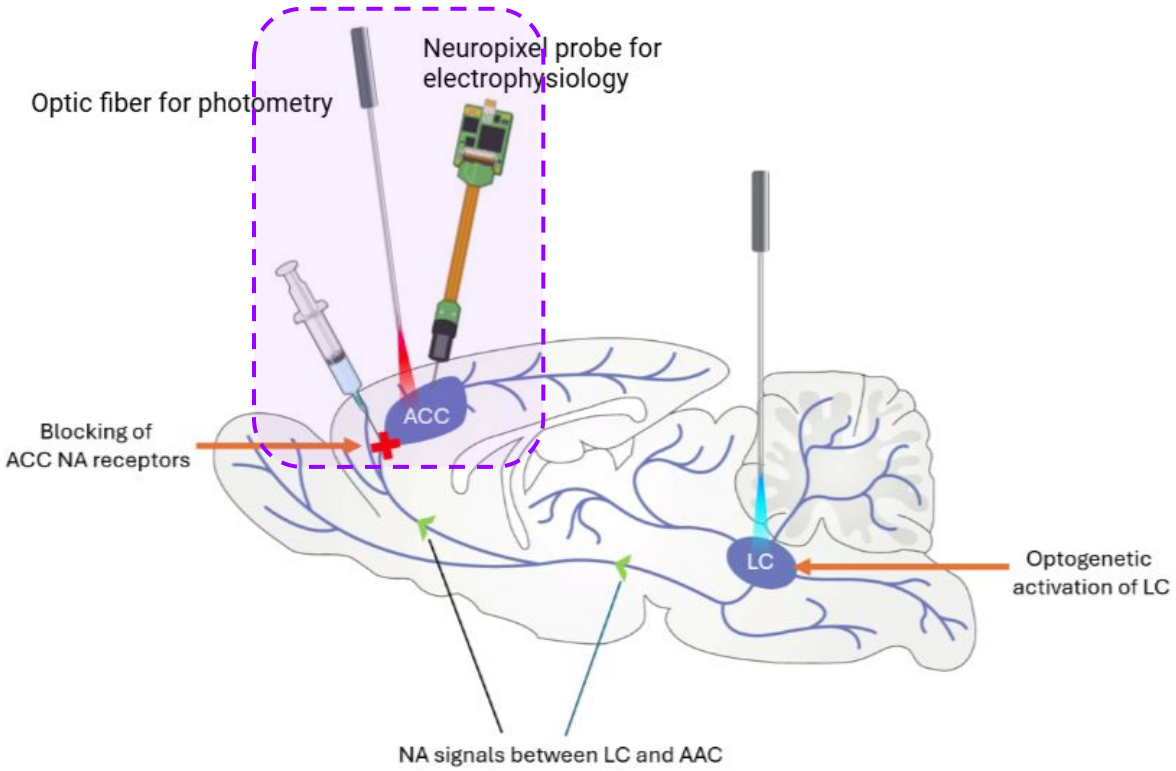


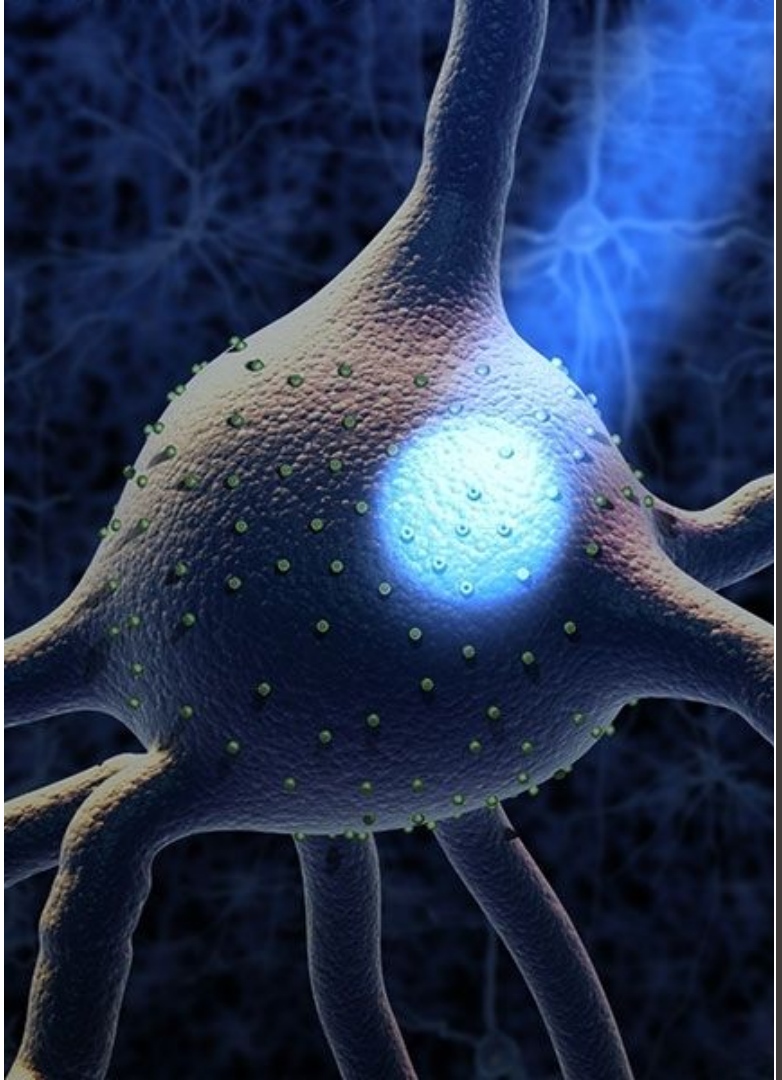
Surgical plan

- Access to the ACC:
  - AP: +1.0 mm,
    ML: ±0.3 mm,
    DV: -1.1 mm

- iGluSnFR-R → wait 4-5 weeks

- NA blockers → 10 minutes before stimulation





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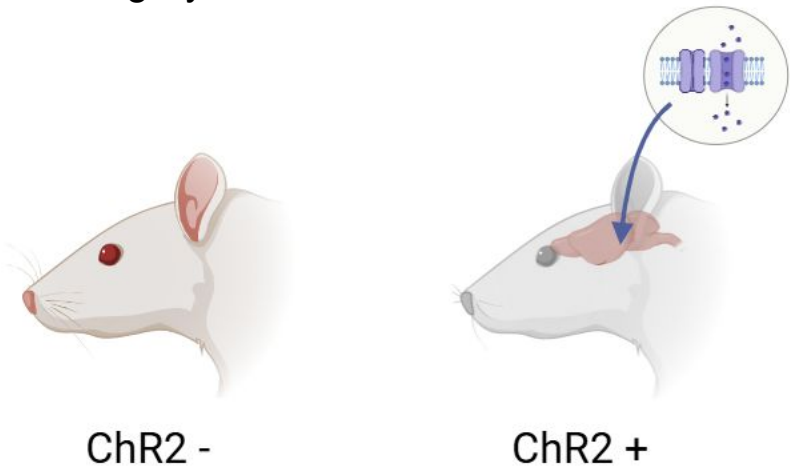
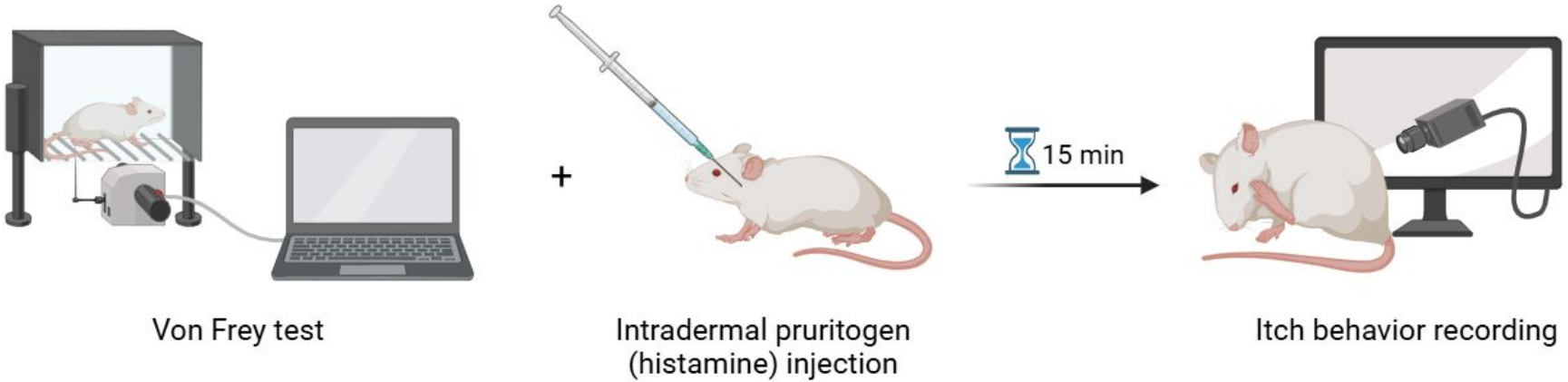
Study groups

- All blockers combination can be tested on the same mice
- 2 groups: ChR2 negative and positive control
- 8 mice per group → 16 mice
  - Time-intensive surgery



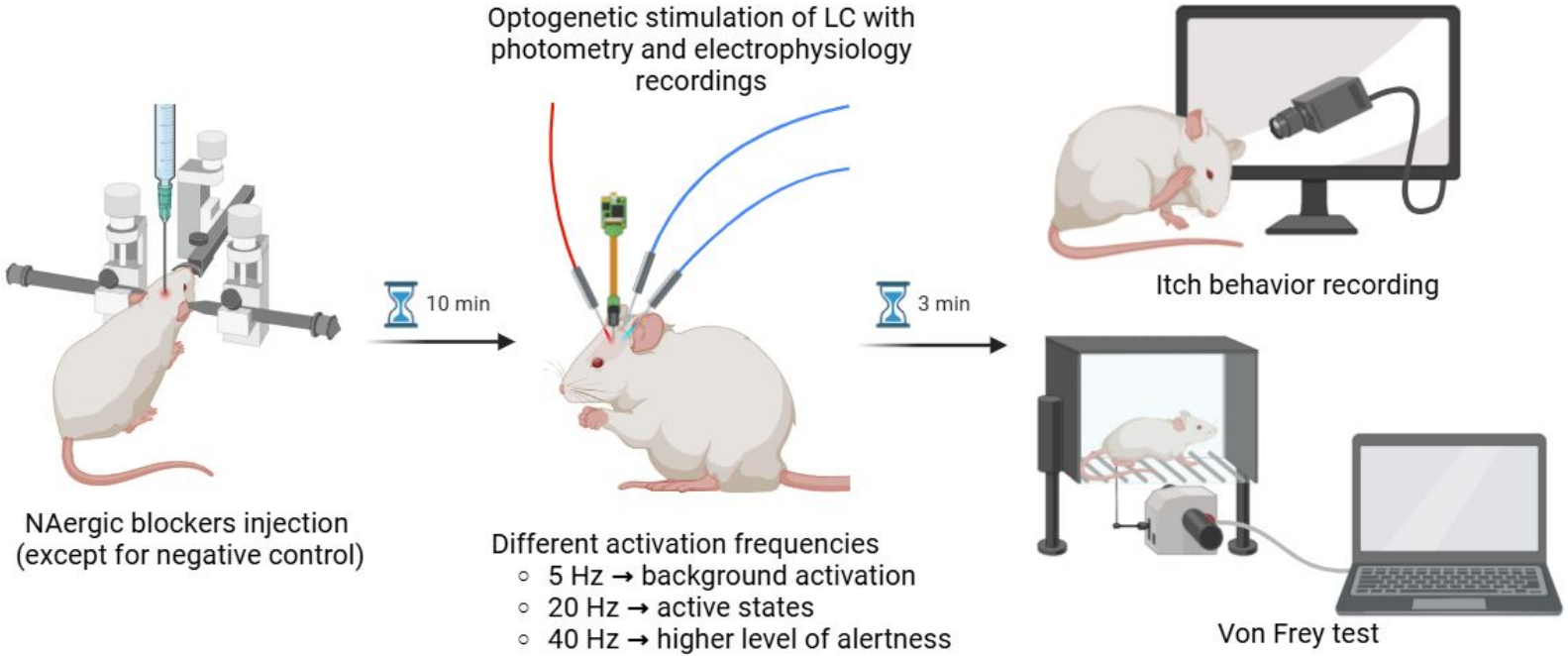
Experiment pipeline

- Acquiring baseline measurements before optogenetic stimulation

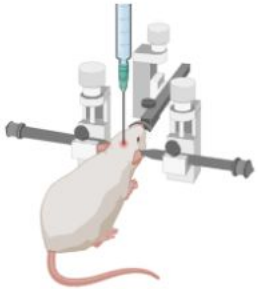


Experiment pipeline

- Blocking, stimulating and recording



Study timeline



Viral injections:
- AAV-DIO-ChR2-eYFP in LC for optogenetic stimulation
- iGluSnFR-R in ACC for photometry

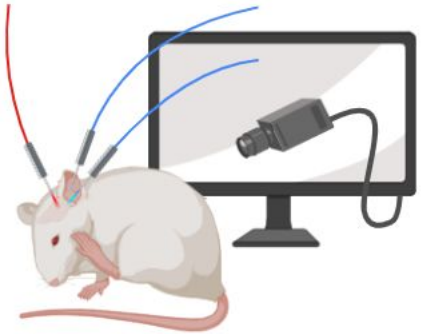
Day 1

4 weeks

Surgical procedure:
- Optic cannulae implantation on LC and ACC
- Neuropixel probe implantation in ACC

Day 30

1 week

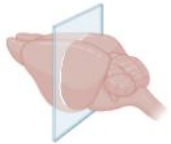


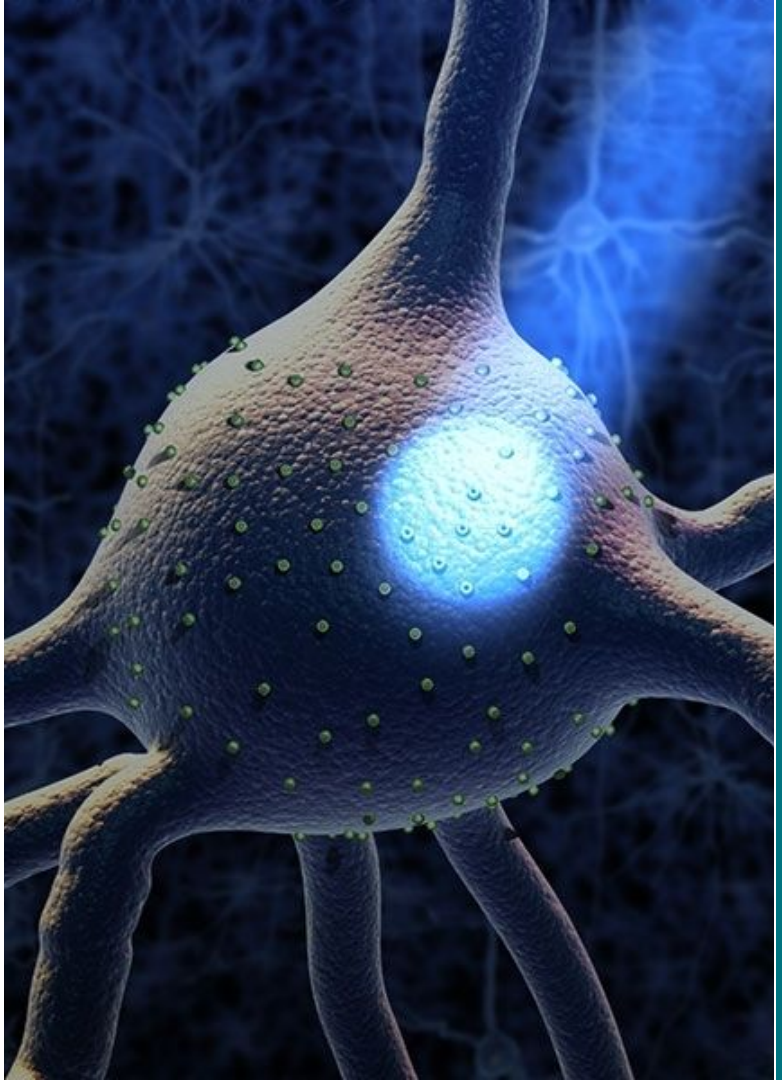
Days 37-44

Day 44

Experiment sessions:
- Inject a NA receptor blocker combination (a different one each day)
- Induce pain/itch behaviors
- Optogenetic stimulation of LC (3 patterns)
- Recors results

Brain extraction and histology to verify transfection





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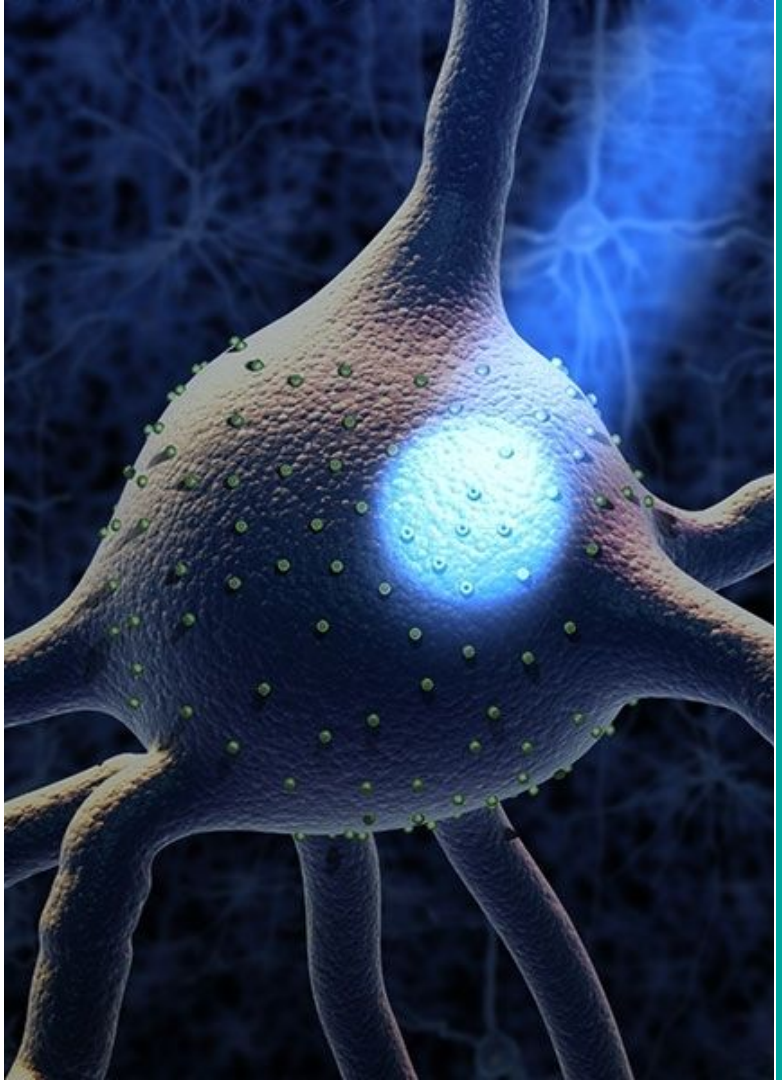
- **Targeted therapeutics**: receptor-specific drugs (β-blockers for chronic pain, α_1-antagonists for chronic itch for example).
- **Neuromodulation protocols**: refine non-invasive methods (TMS or tDCS) to selectively modulate ACC activity for pain or itch relief.
- **Expanding to comorbid conditions**: improve therapies for sensory and emotional disorders (anxiety, depression) often linked to chronic pain or itch.



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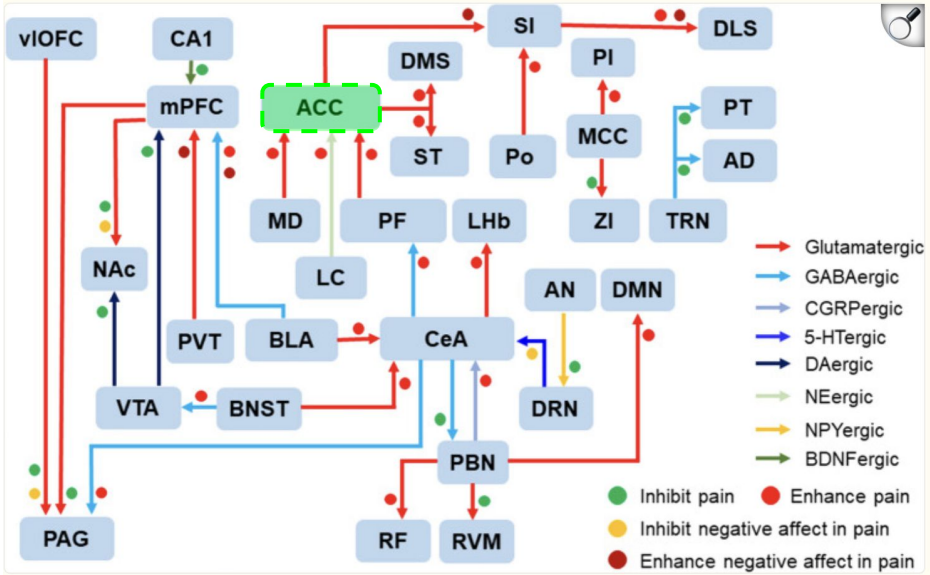
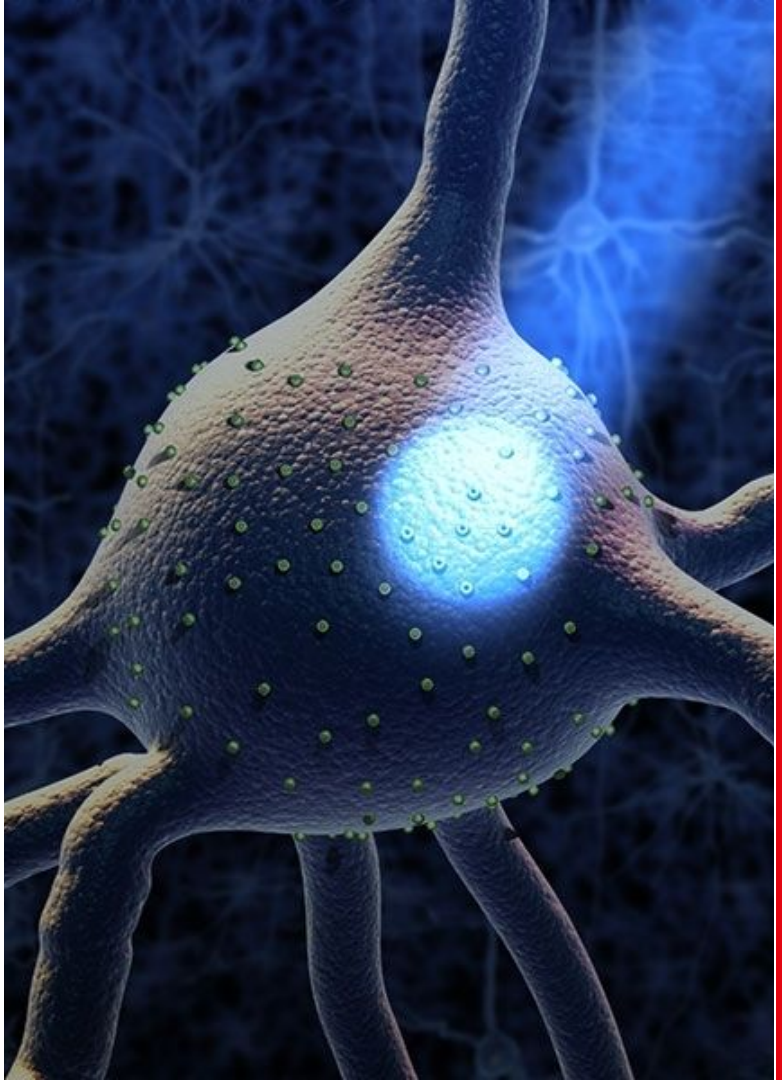
Limitations and Challenges

- α_1 antagonist is not fully specific, it can block α_2 receptors with a lower specificity (same for α_2 antagonist)
  - ➢ Could induce bias in the observed results
- Itch and pain sensations might be intrinsically correlated in NAergic modulation
- Further research about downstream glutamate networks may be needed





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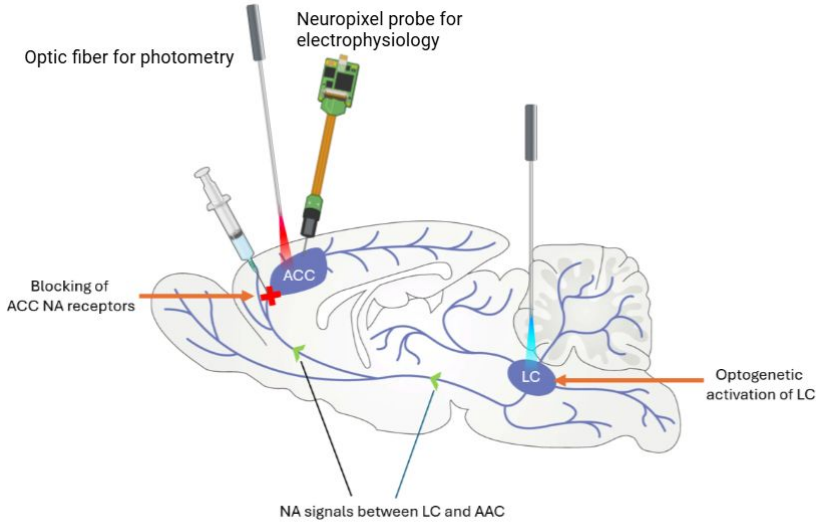
- We aim to differentiate itch and pain modulation in the ACC by NAergic projections from LC.
- Combination of optogenetic stimulation, pharmacological blocking and behavior recording.
- Potential for clinical application like receptor-specific blockers, even if further research might be needed.



Thank you!
Questions?

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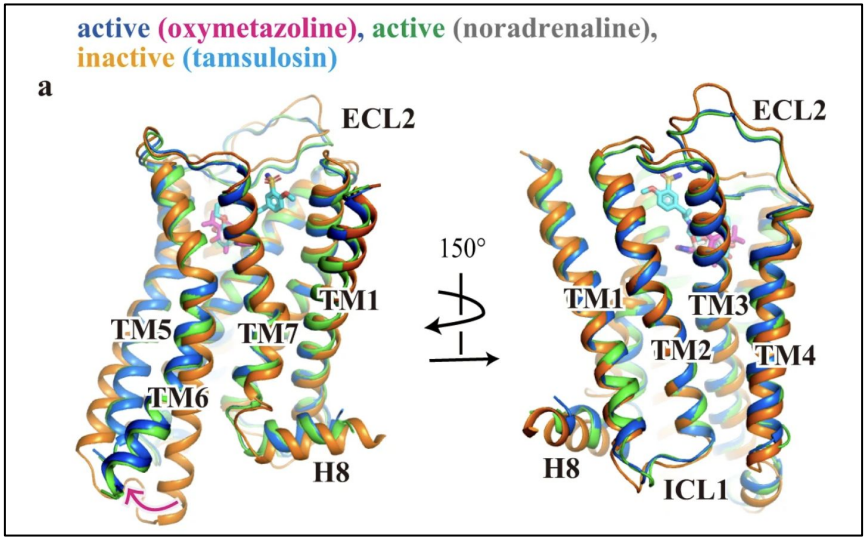
Pharmacological blockage of ACC NA receptors

α1 Noradrenaline Receptor



Structure :

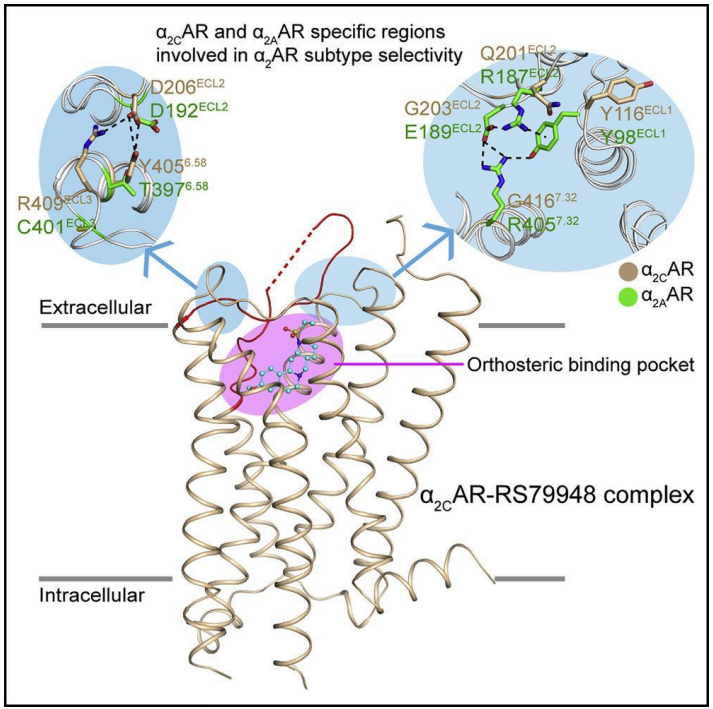
- span the membrane seven times
- N-terminus extracellular and the C-terminus intracellular
- long C terminal tail, short third intracellular loop

Antagonist :

- prazosin and tamsulosin are relatively selective for alpha-1 receptors and block alpha-2 and beta receptors only at high concentrations
- phentolamine and phenoxybenzamine block both alpha-1 and alpha-2 adrenergic receptors with similar affinities

Experiment design - Pharmacological blockage of NA receptors

α2 Noradrenaline Receptor



Structure :

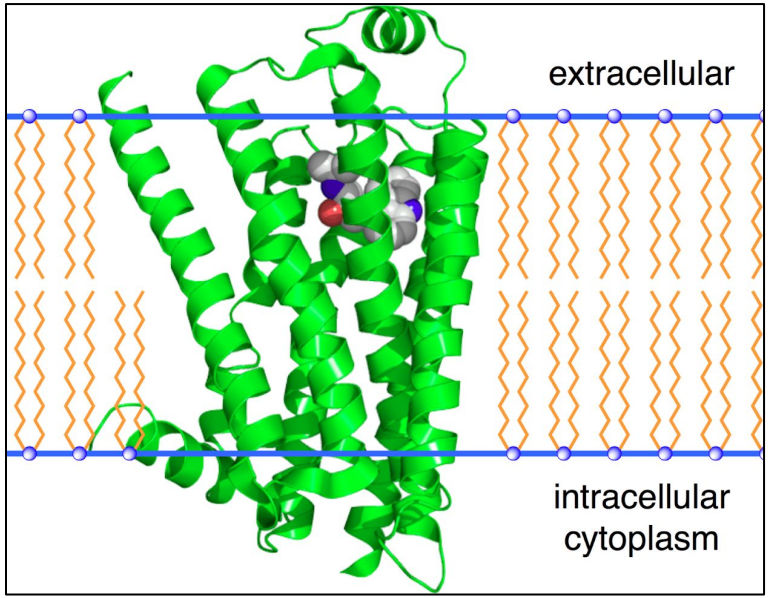
- Third intracellular loops have multiple sites of phosphorylation

Antagonist :

- antagonist yohimibine is selective for alpha-2 receptors and blocks alpha-1 and beta receptors only at higher concentrations

Experiment design - Pharmacological blockage of NA receptors

β Noradrenaline Receptor



Structure :

- It has the same c terminal tails and third intracellular loop as a1
- similar overall structure
- differences in the binding surface

Antagonist :

- propranolol is the prototypic non-subtype-selective beta antagonist which has equal affinities at the beta-1 and beta-2 subtypes