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Repetitive transcranial magnetic stimulation in mice: evidence for anatomical and functional changes in neural circuits

Christina Mo
Sarah Dunlop
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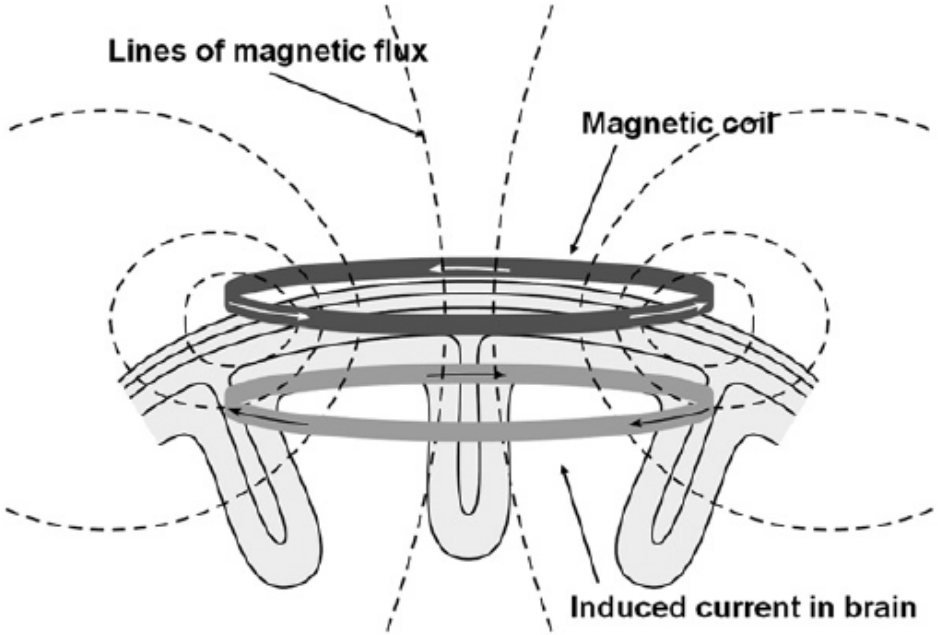

Brain function is underpinned by electrical activity inducing chemically mediated communication between cells.

Electrical and chemical communication are crucial in establishing normal brain structure and function during development and maintaining them throughout life.

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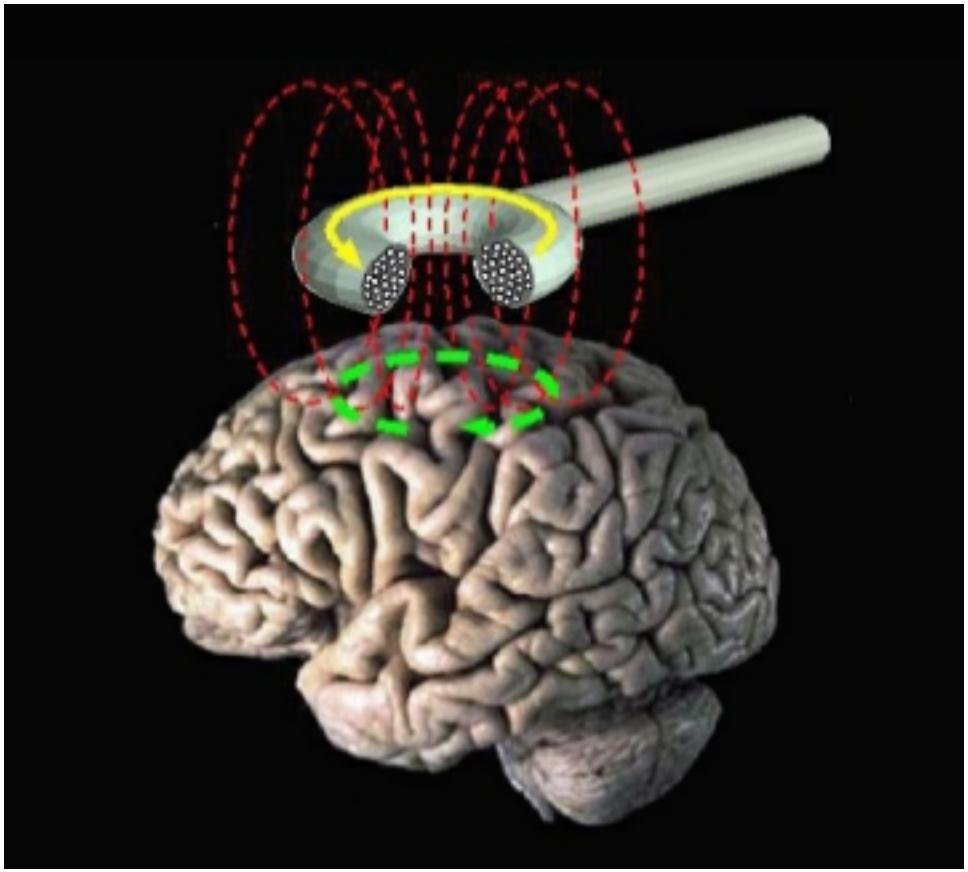
Transcranial magnetic stimulation

Repetitive transcranial magnetic stimulation (rTMS)



rTMS in the clinic

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- **Efficacy (up to 70% of cases) in treating:**
  Parkinson's disease, stroke, depression, obsessive compulsive disorder, schizophrenia, amblyopia, epilepsy…

- **Anecdotal and case-report evidence, inconsistent**

- **Specific cellular and molecular changes mostly unknown**

- **Animal studies mainly in normal animals, or in vitro**

- **Lack of consistency in rTMS parameters**

rTMS Mechanisms - 1

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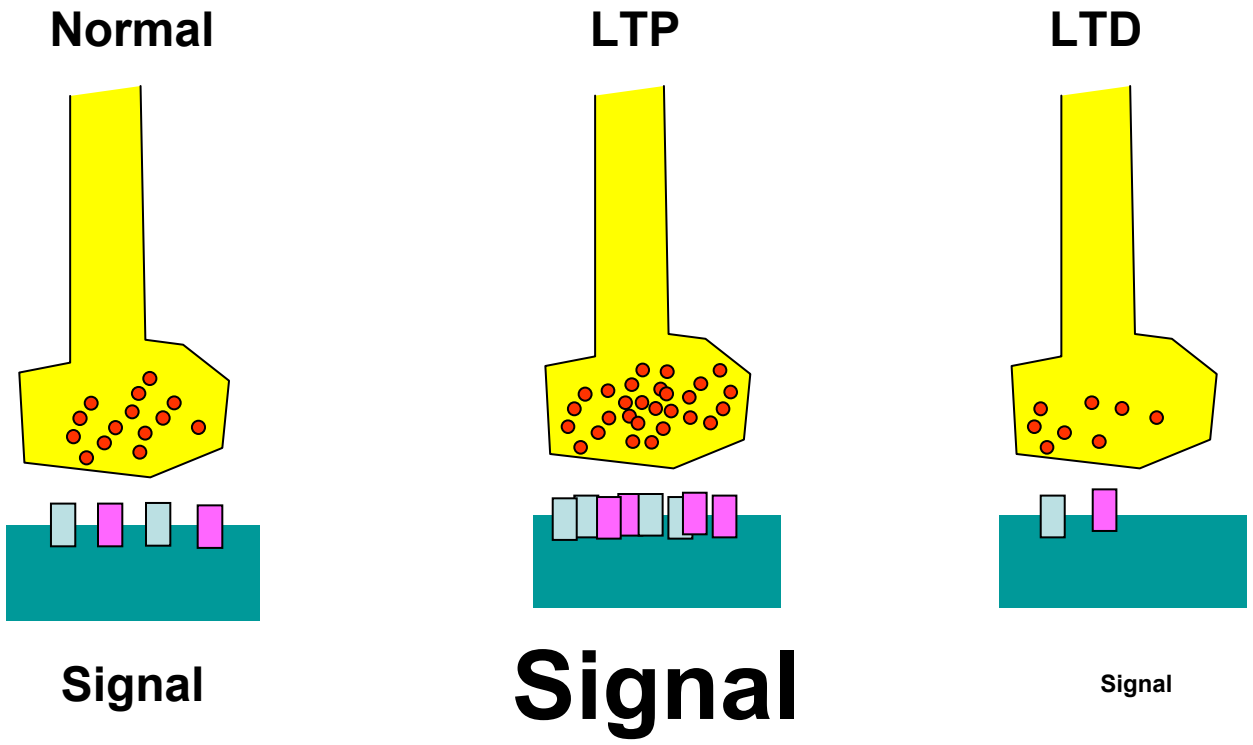
Low frequency rTMS: pulses at 1Hz or less.
Inhibitory: may induce LTD

High frequency rTMS: pulses at 5Hz or more
Excitatory: may induce LTP



rTMS Mechanisms - 2

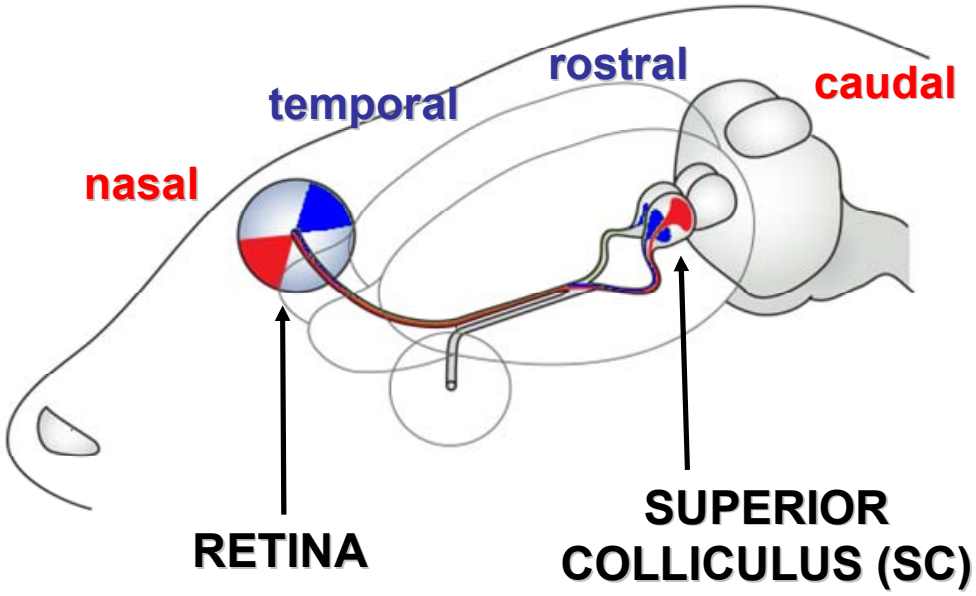
Increase in BDNF

If rTMS is having a long term effect on the human brain, it must be changing connectivity

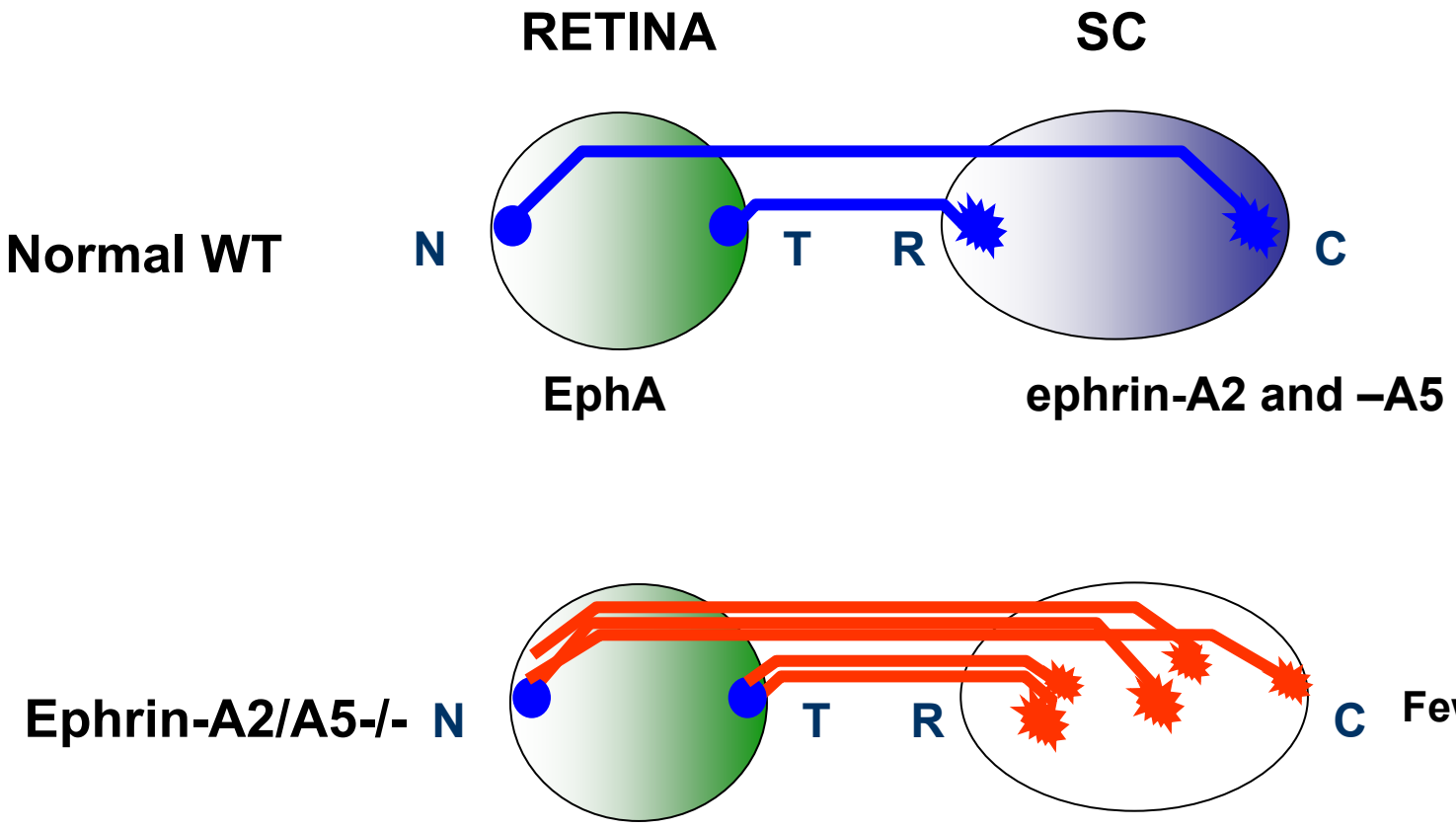
Hypothesis:
rTMS induces changes in brain connectivity

How can we look at this?

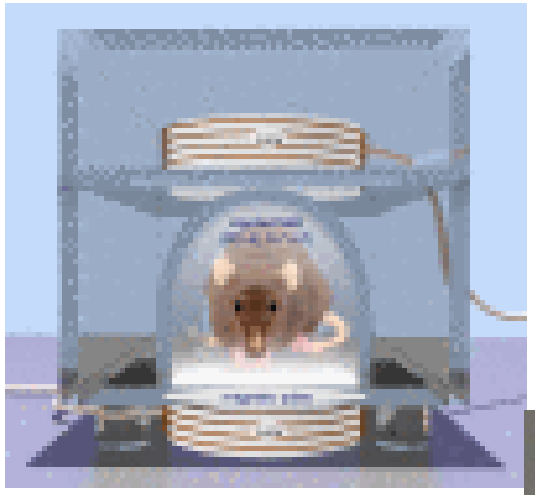
Simple connectivity – the visual system

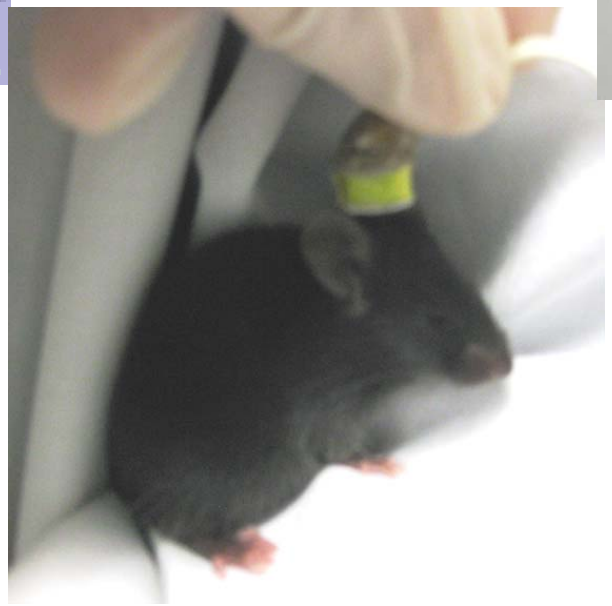


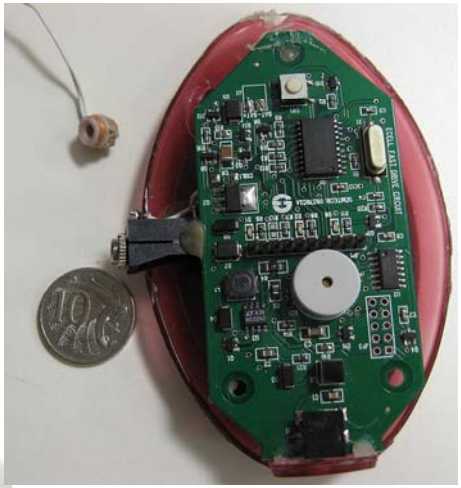
Abnormal connectivity: Ephrin-A knockout mice



rTMS on mice – a mouse coil?







The “e-cell” with custom mouse-size coil

Industry partners: Global Energy Medicine Pty Ltd

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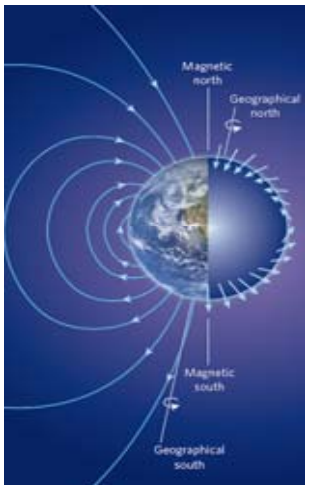
Our parameters:
High frequency (75Hz)
Low intensity (2mT; musculoskeletal repair)

But human studies have used lower frequency (25Hz) and higher intensity (1-2T)



Earth's magnetic field: 46 μT

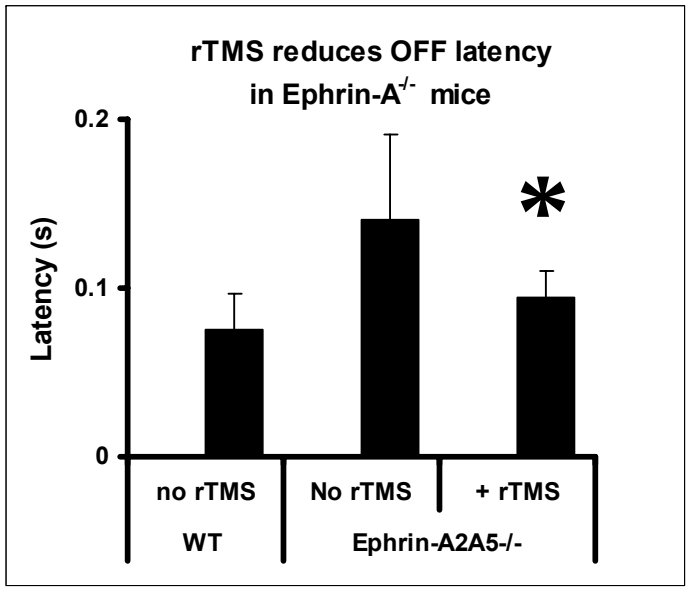
Pilot study:

Ephrin-A2-/- mouse prepared for electrophysiological recording

Electrode positioned in the brain and responses to light were recorded

1 minute of rTMS

Responses to light were recorded from the same location after 10 minutes



What was the question again?

rTMS is used to treat a wide range of neurological conditions, but noone really knows how it works.

Hypothesis: rTMS induces changes in brain connectivity

Aims:

To apply rTMS to knockout mice with abnormal connectivity in the visual system

To measure changes in connectivity

To look for evidence of molecular changes that might mediate these changes in connectivity

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Experimental groups:

WT	**rTMS**
	sham
Ephrin-A2A5-/-	**rTMS**
	sham

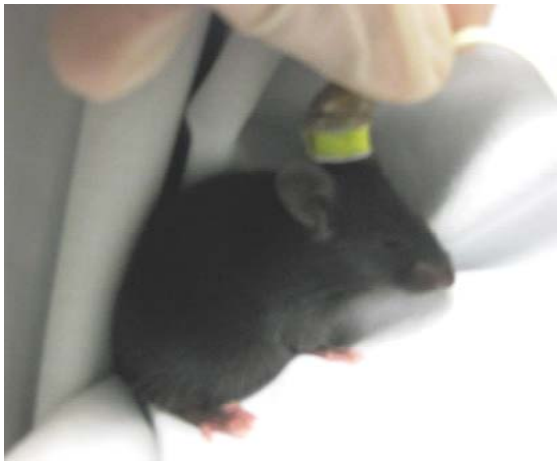
Timing:

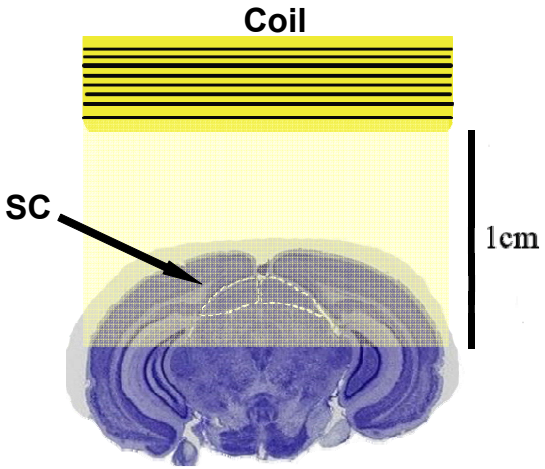
Chronic: 2 weeks of 10 minute daily stimulation (connectivity and molecules)

Acute: 10 minutes stimulation, sacrifice two hours later (molecules)

Methods

rTMS (or sham stimulation) applied daily for 10 minutes, for 14 days





Expected penetration of magnetic field and location of induced current in the brain

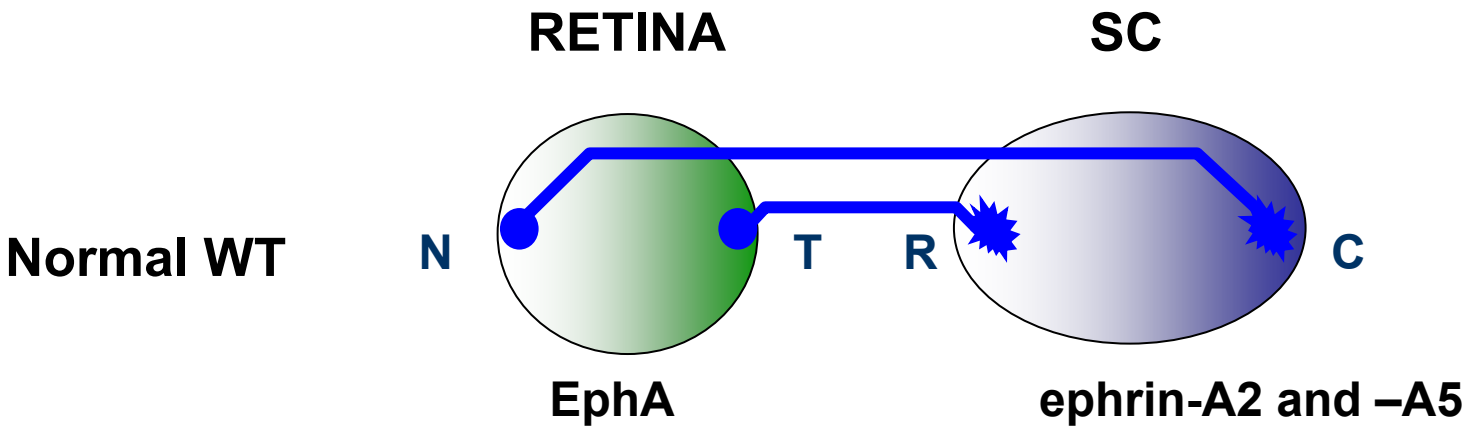
Topographic order of connections
Electrophysiology and anatomical tracing

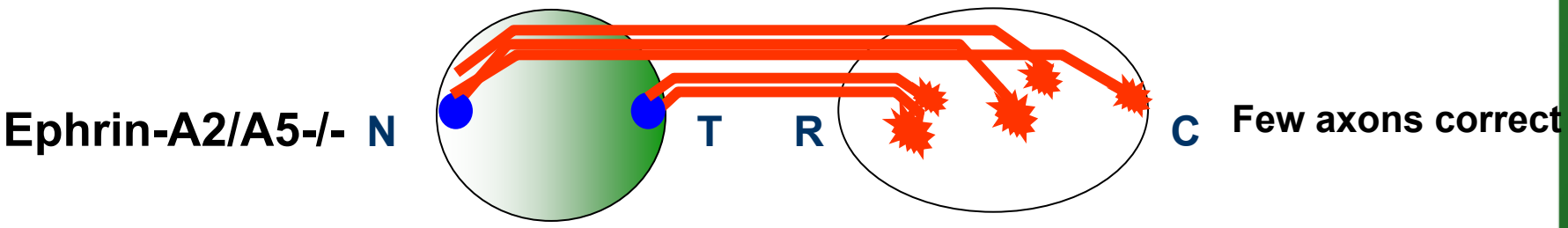
Receptive fields, Latencies of responses
Electrophysiology

Functional changes?
Visuomotor tracking behaviour

Molecular changes
ELISA (BDNF, GABA and NO)

Abnormal connectivity: Ephrin-A knockout mice





Functional vs silent projections

Anatomical tracing

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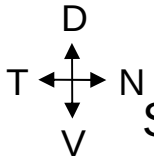
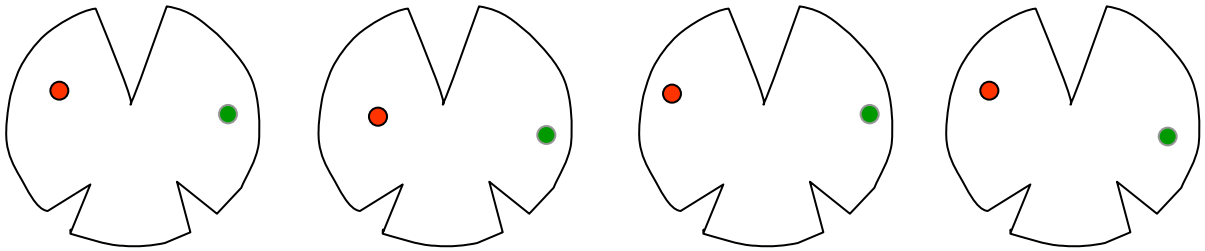
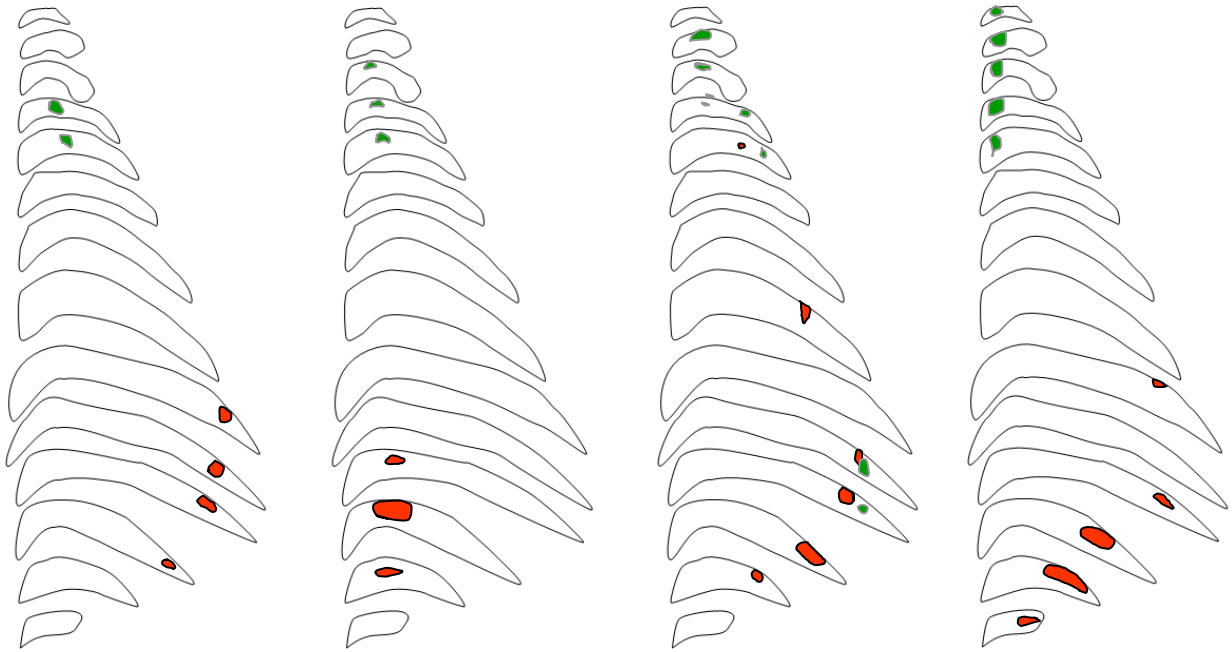
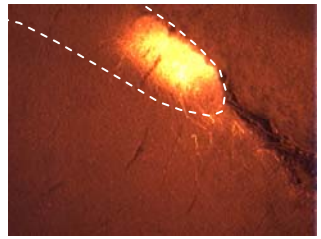


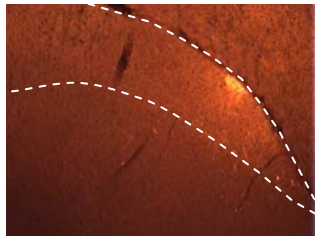
Sham WT | Active WT | Sham Ephrin | Active Ephrin



N=4 | N=7 | N=7 | N=5







X^2: $p<0.05$

0% | 0% | 71% | 43%

% ectopic projections

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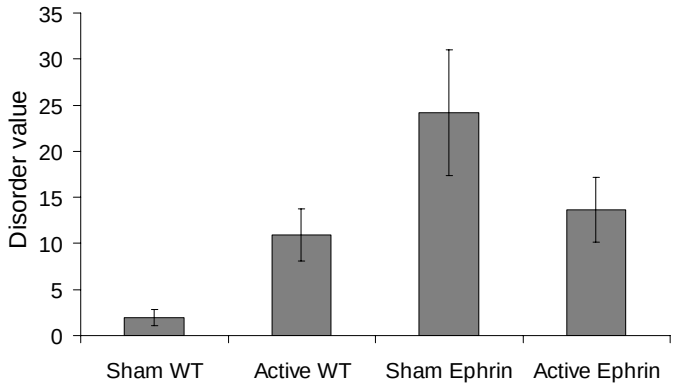
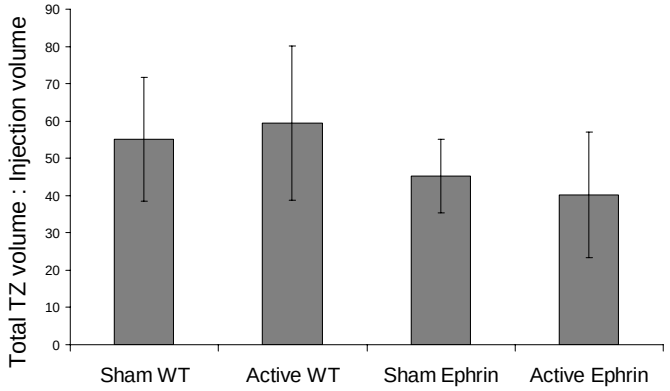
Topographic disorder



Volume of axon terminals



No significant effect of rTMS

Abnormal connectivity: Ephrin-A knockout mice

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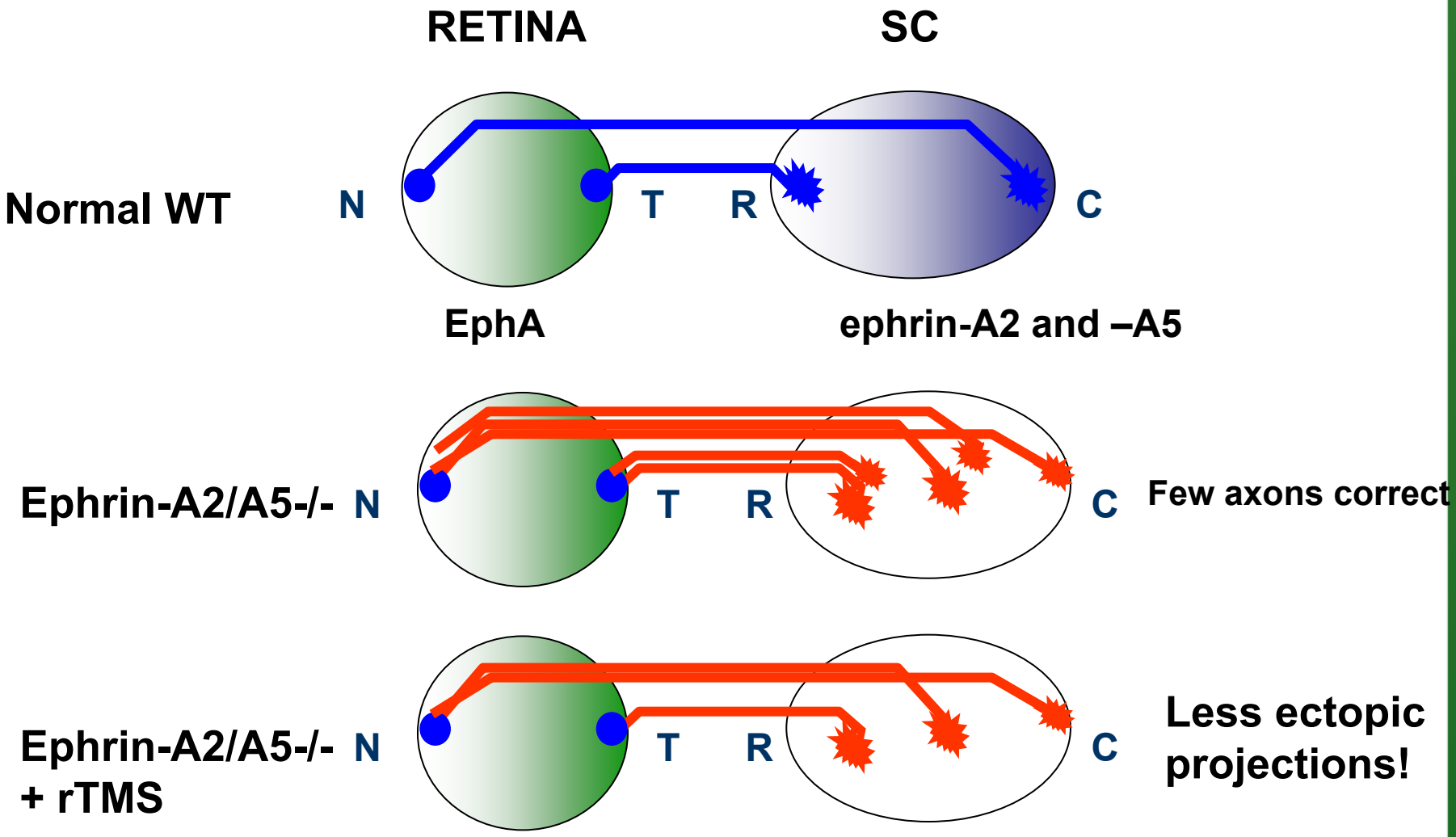

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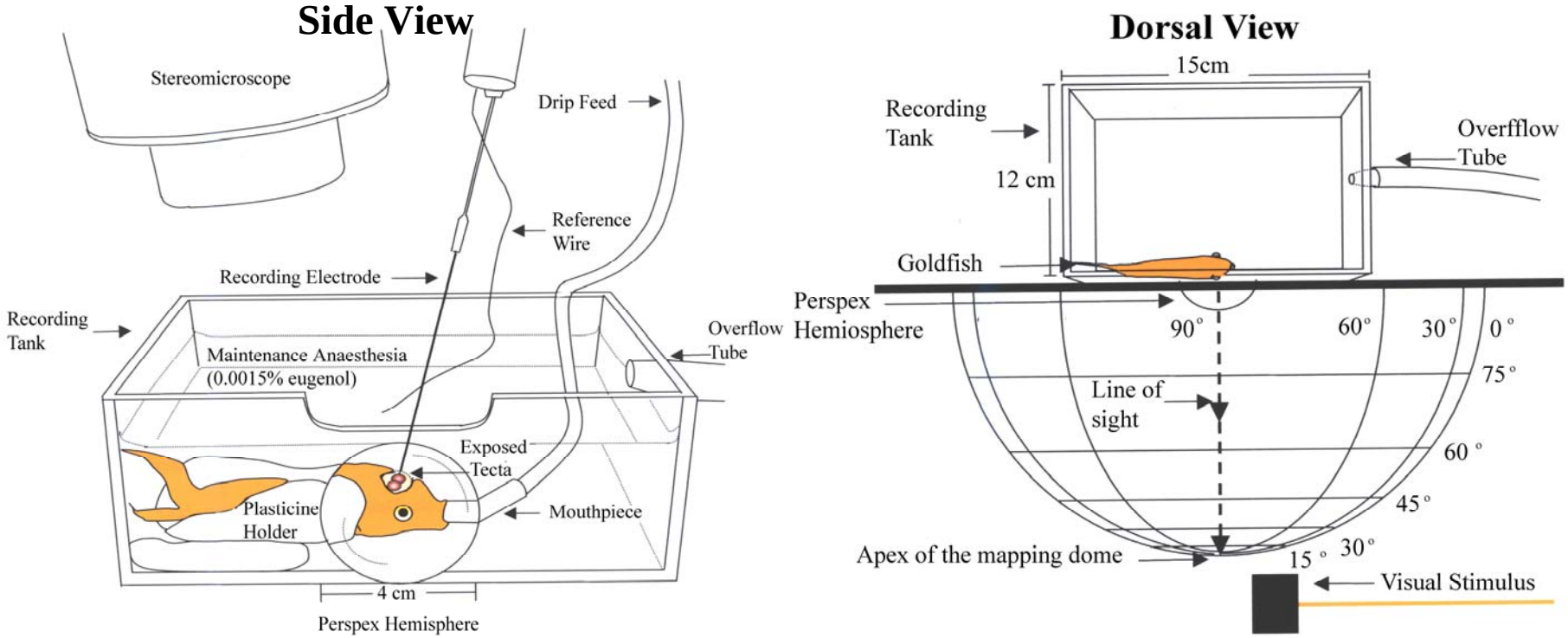
Electrophysiology



Electrophysiology

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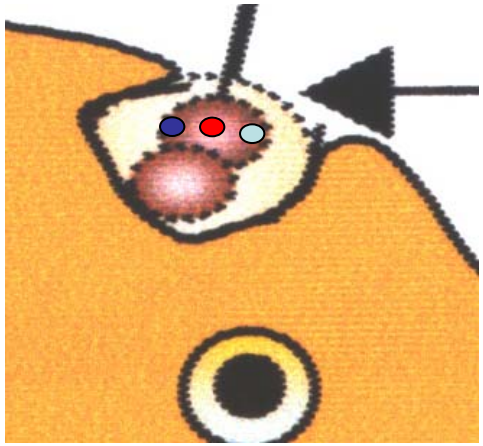
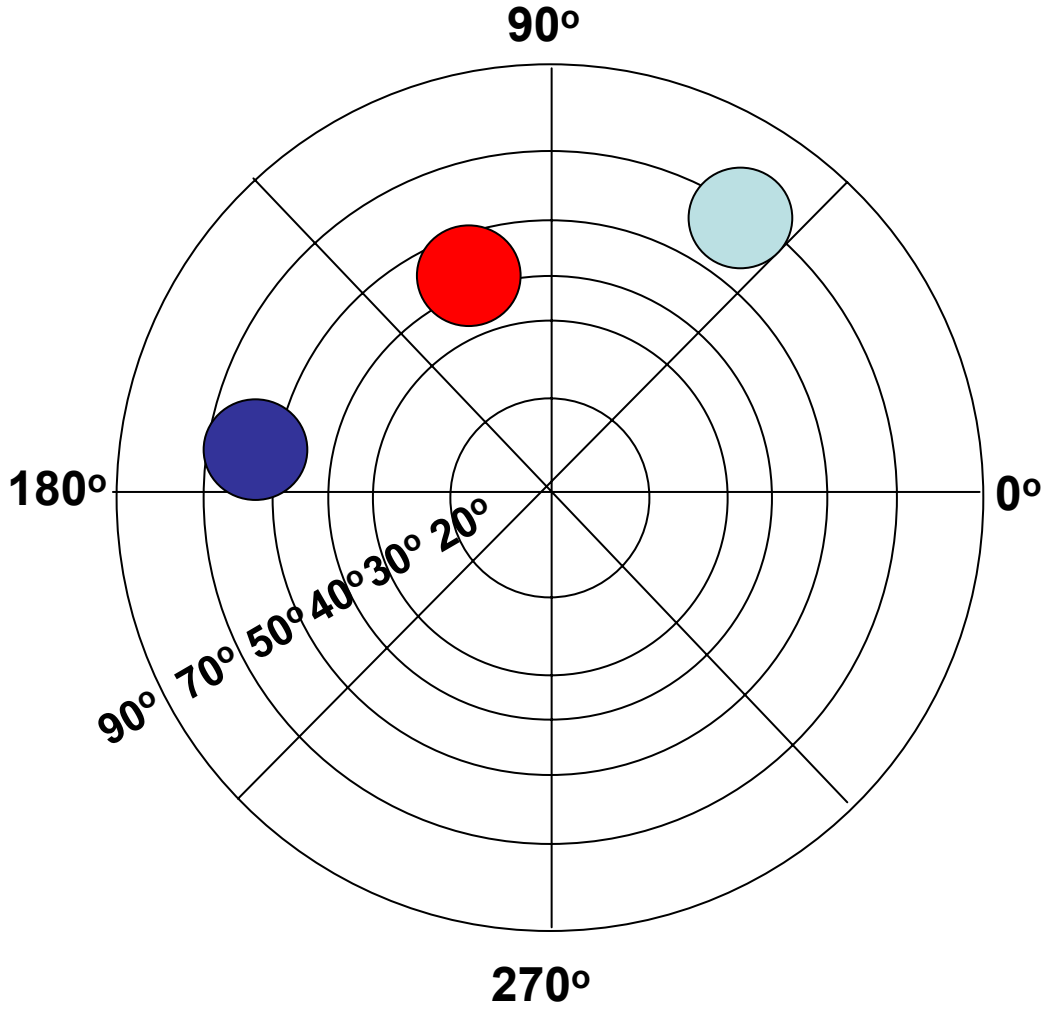


Goldfish brain (optic tectum)



Goldfish visual field

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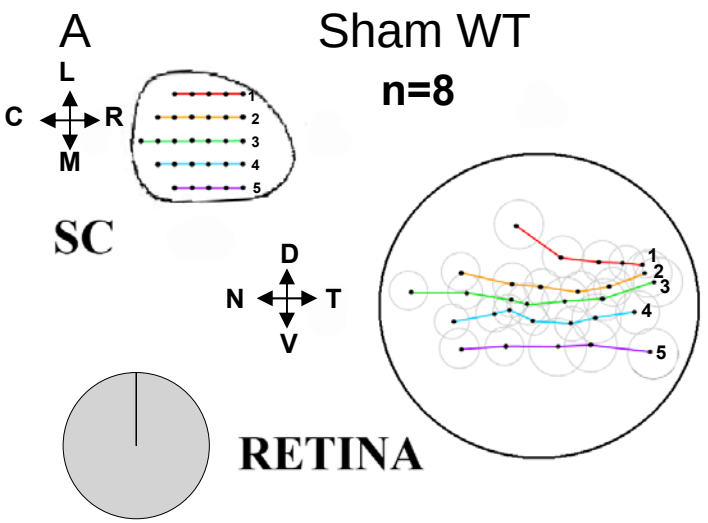

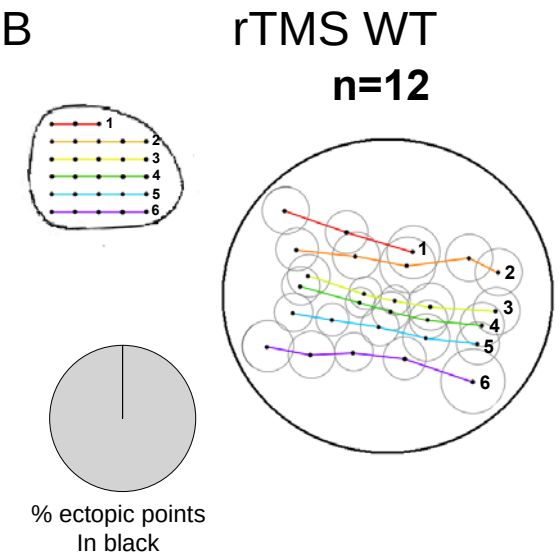

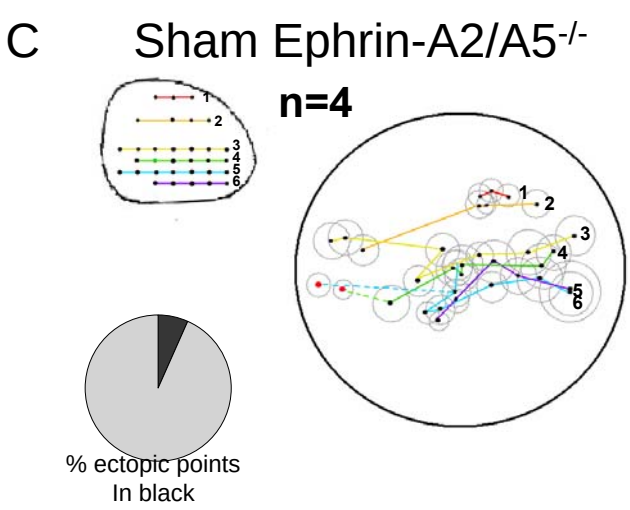

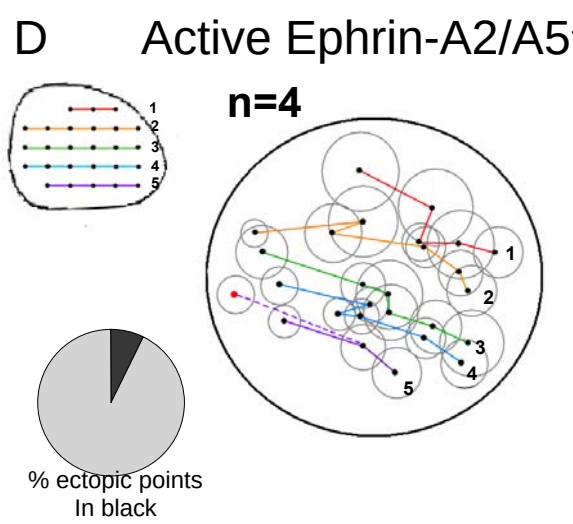

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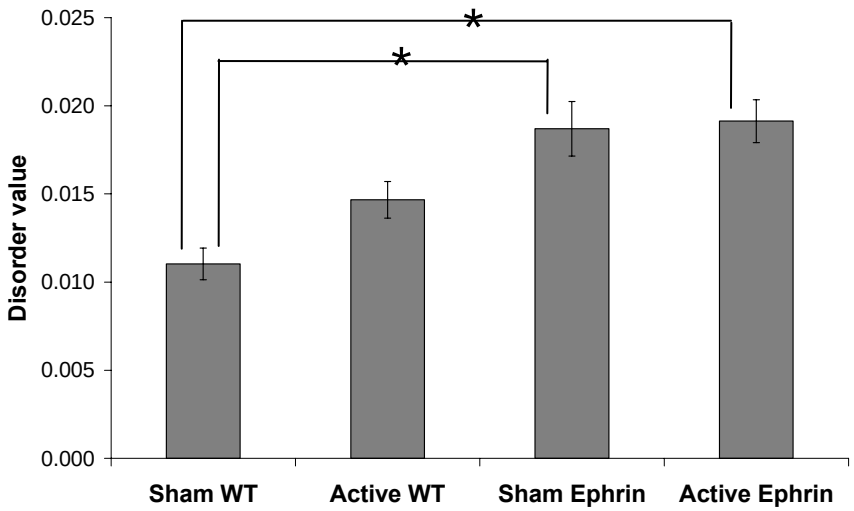
Topographic disorder



Following rTMS in ephrin-A2A5-/- mice….

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Anatomical tracing labels fewer ectopic projections

But, the number of <u>functional</u> ectopic projections is the same

rTMS is removing the “silent” ectopic projections

Abnormal connectivity: Ephrin-A knockout mice

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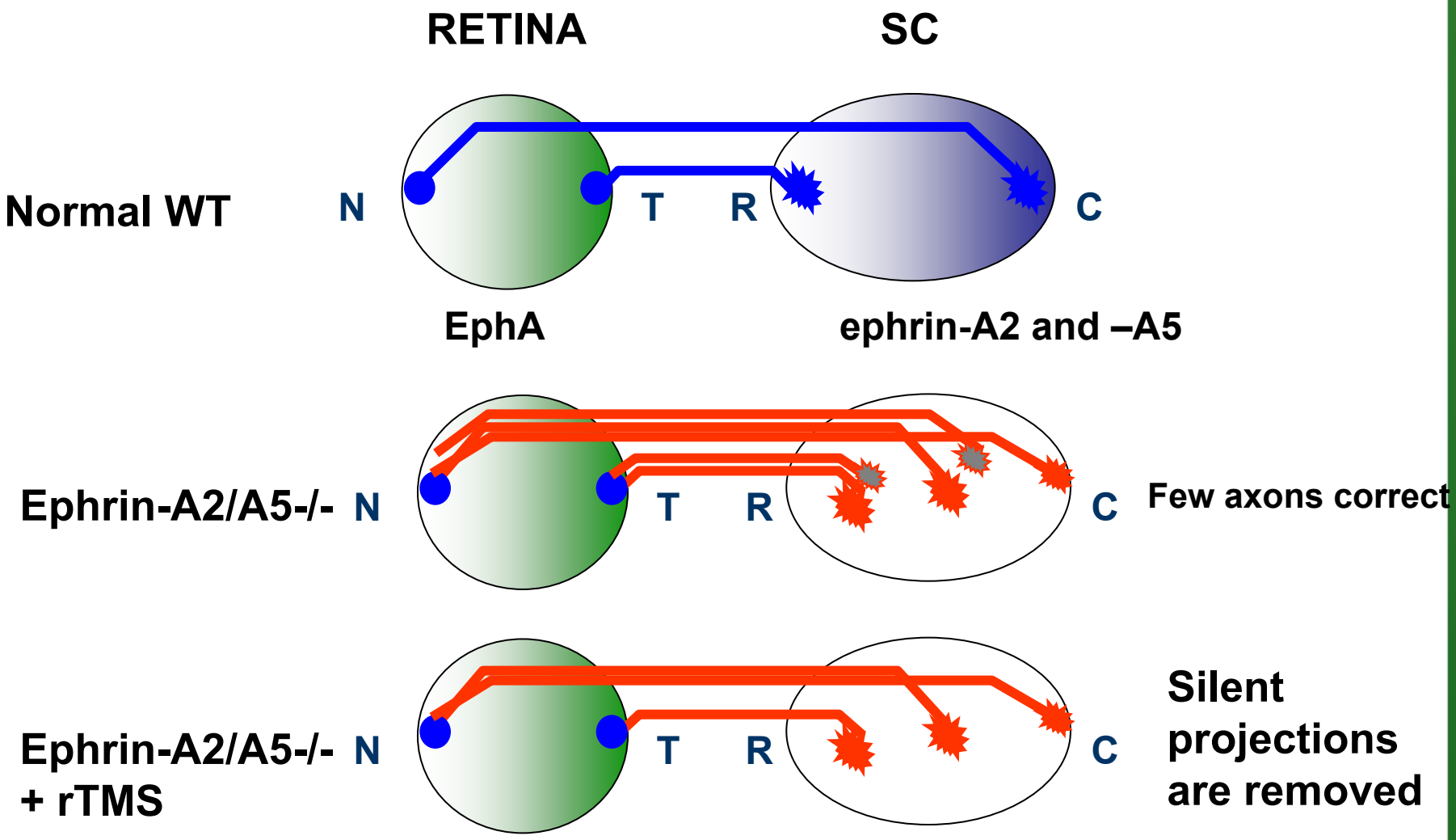

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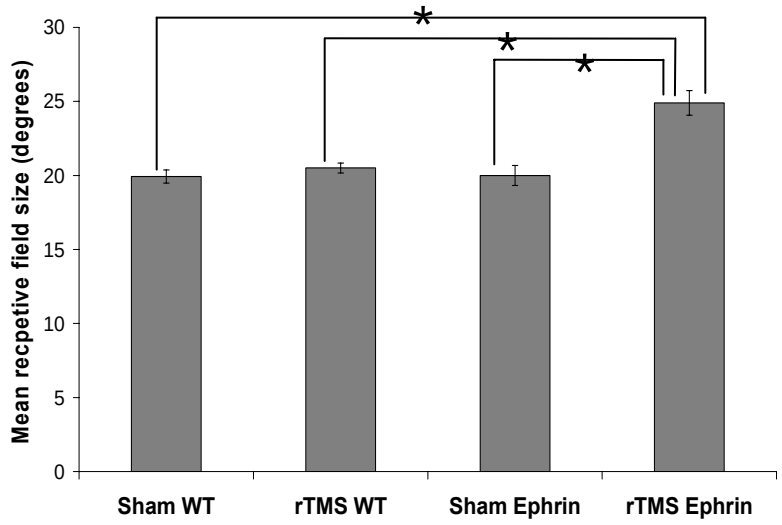
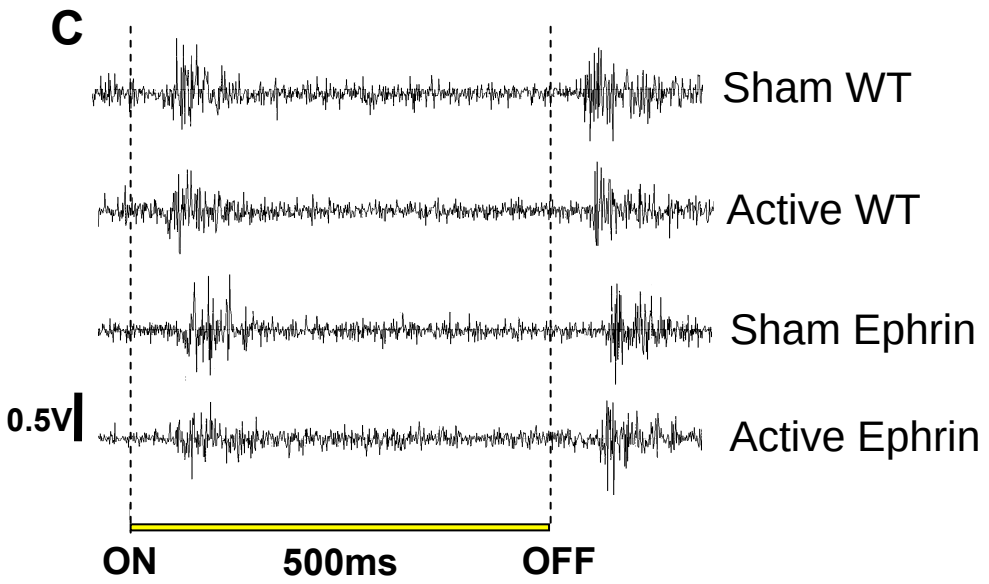
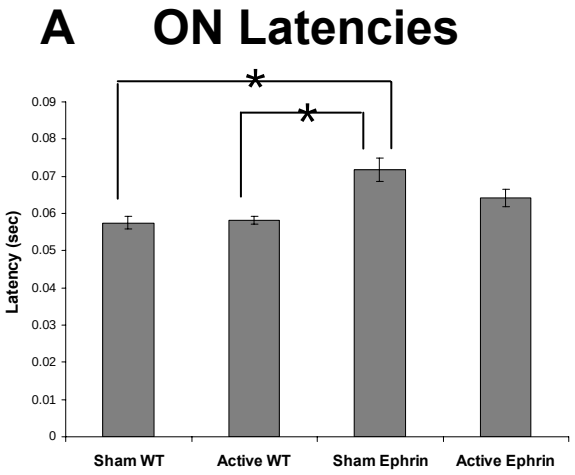
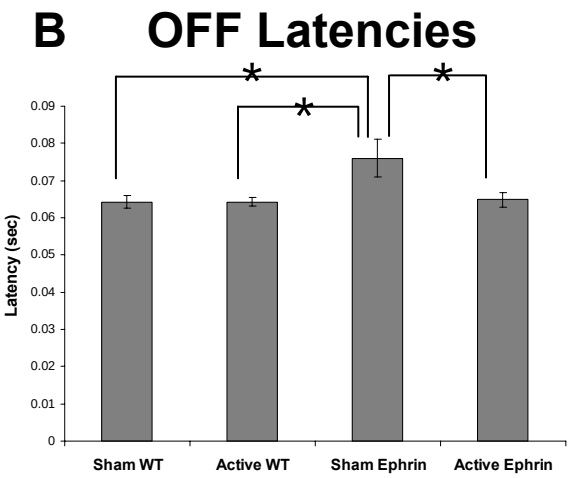
Receptive field size



Electrophysiology - latency







Following rTMS in ephrin-A2A5-/- mice….

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Receptive field size is increased

Latency is reduced

Faster and more efficient neurotransmission?

No behavioural changes

What was the question again?

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rTMS is used to treat a wide range of neurological conditions, but no one really knows how it works.

Hypothesis: rTMS induces changes in brain connectivity

Aims:

To apply rTMS to knockout mice with abnormal connectivity in the visual system

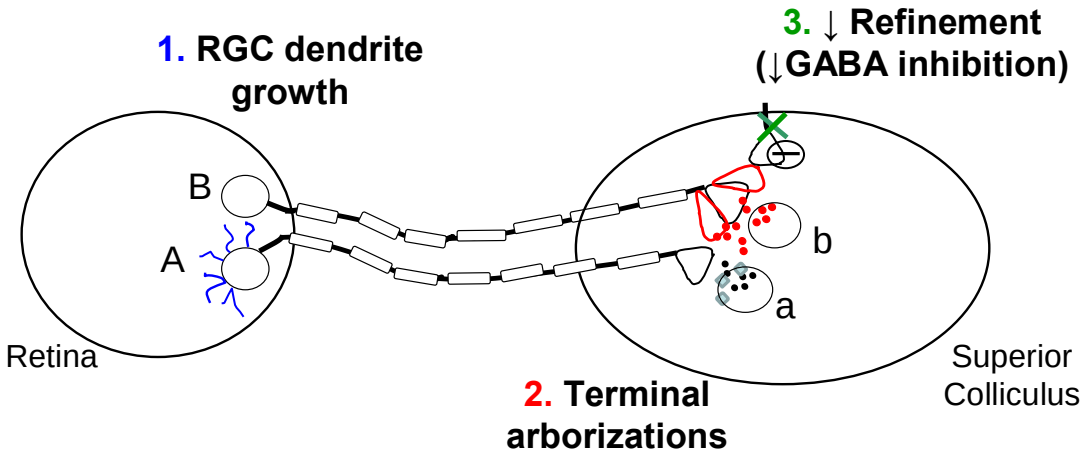
To measure changes in connectivity

To look for evidence of molecular changes that might mediate these changes in connectivity

rTMS induces changes in connectivity:

- **Abnormal projections are removed**
- **Receptive fields are increased**
- **Latencies are reduced to normal levels**

How does this happen?


1. RGC dendrite growth
3. ↓ Refinement (↓GABA inhibition)
B
A
b
a
Retina
Superior Colliculus
2. Terminal arborizations

Candidate molecules: BDNF, Nitric oxide and GABA

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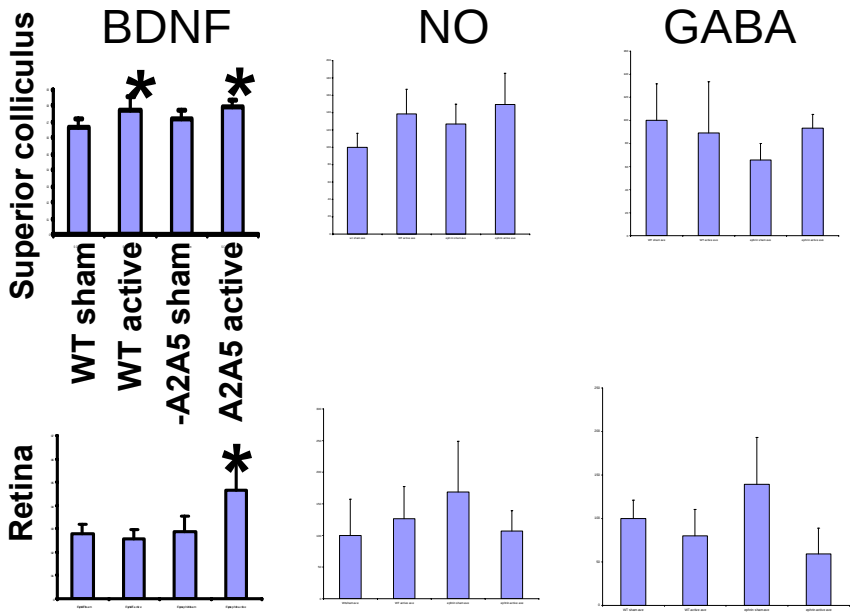
BDNF: promotes arborisation (dendritic and axonal), branching, LTP

NO: required for LTP/LTD (retrograde messenger)

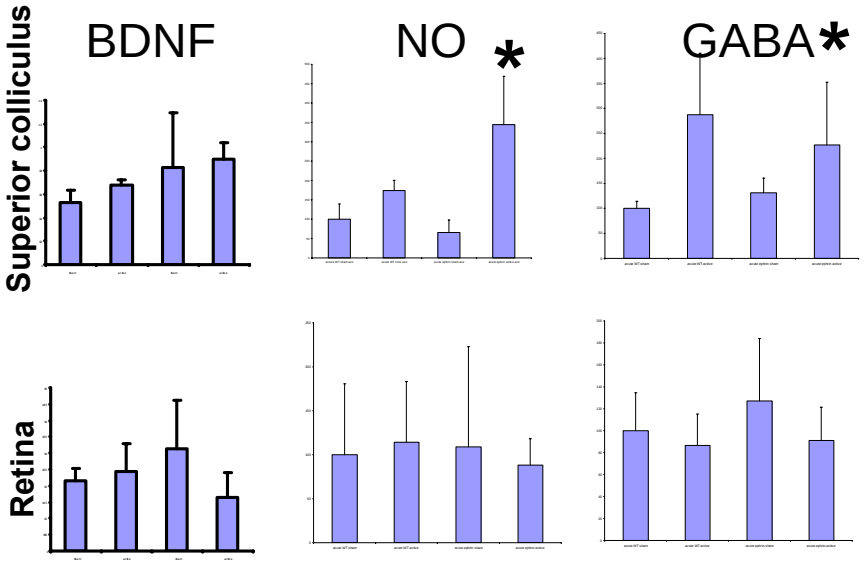
GABA: inhibitory neurotransmitter (silencing)

Chronic rTMS (daily for 2 weeks, 24 hours after last stimulation)



N=4-7 per group

Acute rTMS (single stimulation, 2 hours after)



N=3 per group

Loss of silent ectopic projections

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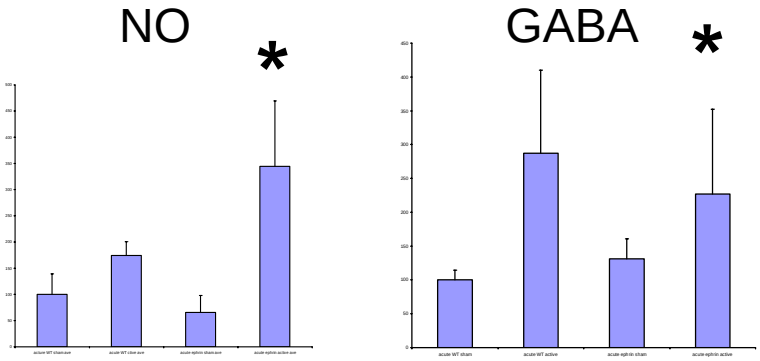
Ephrin-A2/A5-/-

Few axons correct

Ephrin-A2/A5-/- + rTMS

Silent projections are removed

Acute rTMS



Bigger receptive fields
= larger RGC dendritic arbors

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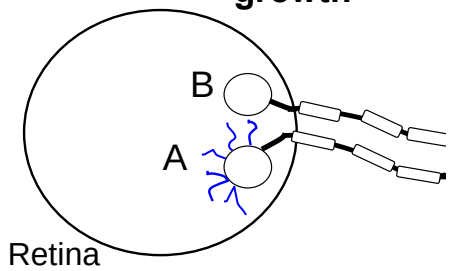
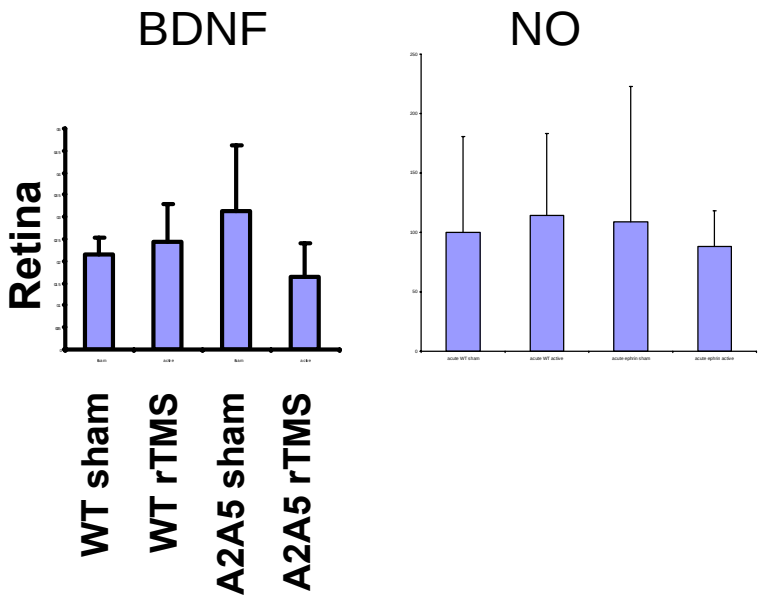
1. RGC dendrite growth

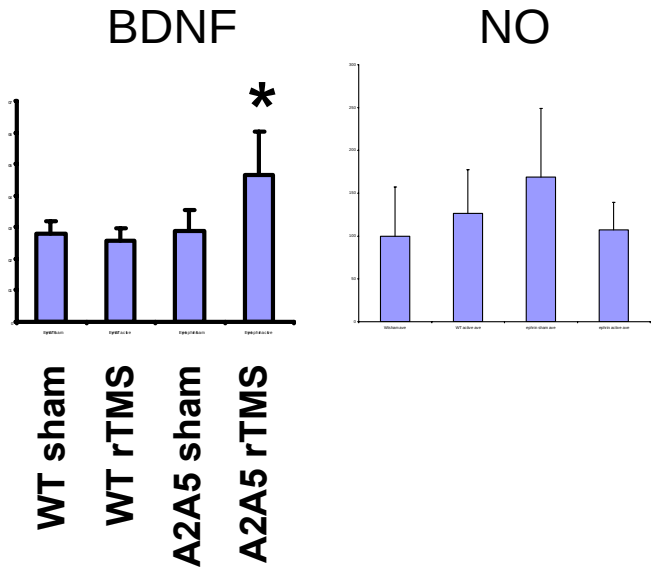


- **Retina not stimulated directly**
- **Retrograde signal – NO, BDNF**

Acute rTMS



Chronic rTMS



Bigger receptive fields – changes in the SC

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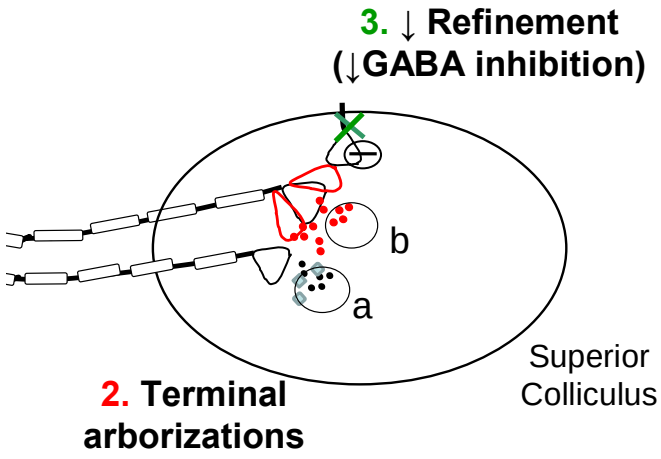
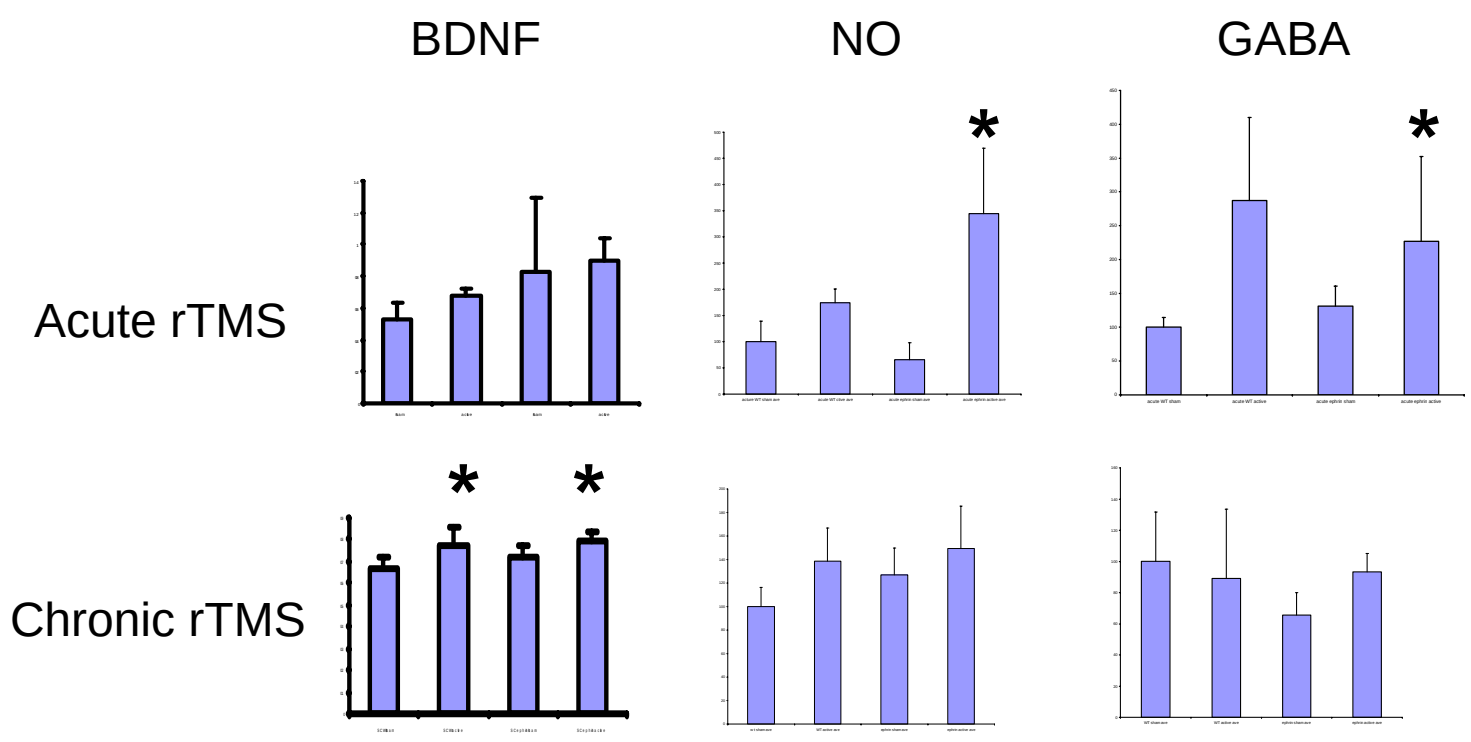


BDNF – increases terminal arborisations

NO – required for LTP

GABA – silences and refines terminals



Shorter response latencies

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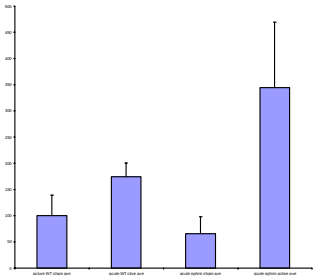
Rapid onset: 10 minutes after rTMS (pilot study)

= Unlikely to be changes in retinal processing or myelination

NO enhances correlated firing of synaptic inputs:

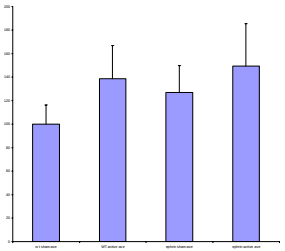
Acute rTMS

NO *



Chronic rTMS

NO



Long term (chronic) changes may involve structural modifications

rTMS induces changes in connectivity:

- **Abnormal projections are removed**
  GABA+NO in the SC

- **Receptive fields are larger**
  Increased BDNF in the retina

- **Latencies are reduced**
  Increased NO in the SC

rTMS induces changes in connectivity…

But not in normal WT mice!

Good outcome – will not affect "normal" parts of the brain, i.e. no side effects!

Is it specific to ephrin-A2A5-/- mice?

- **Normal levels of BDNF, NO and GABA**
- **Abnormal connectivity is found in most neurological conditions – appropriate model.**

Where to next?

Testing rTMS parameters (ARC linkage)

Homeostatic changes

Cerebellum – behavioural change (NHMRC)

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