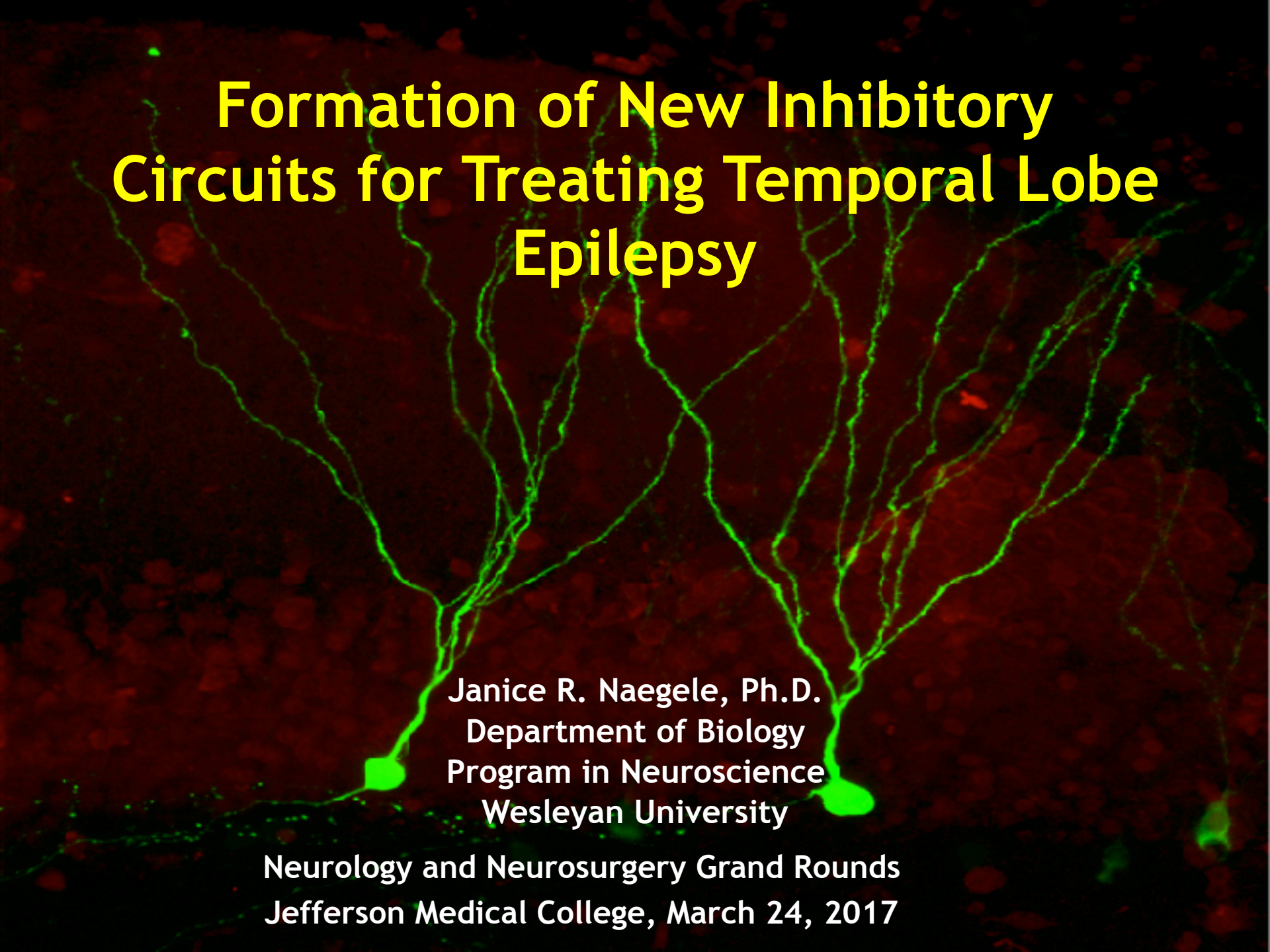
Formation of New Inhibitory Circuits for Treating Temporal Lobe Epilepsy



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Neurology and Neurosurgery Grand Rounds
Jefferson Medical College, March 24, 2017

NEUROSURGERY GRAND ROUNDS -- 3/24/2017

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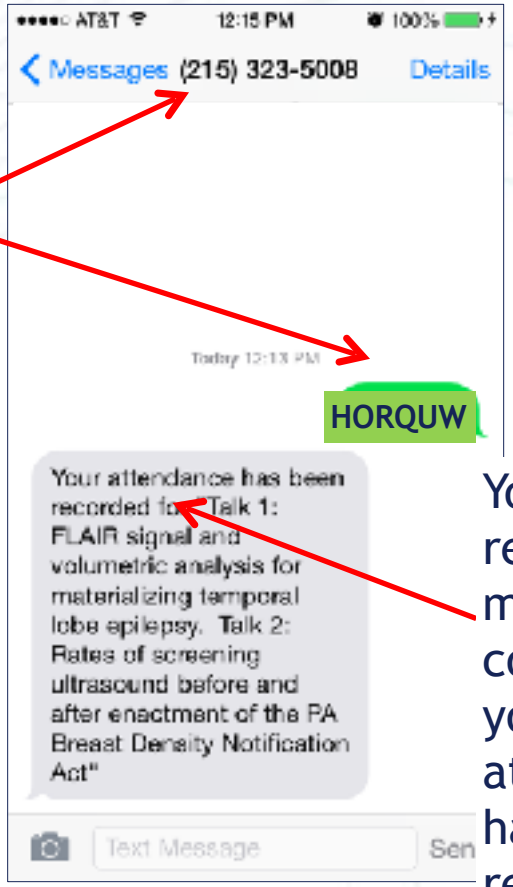

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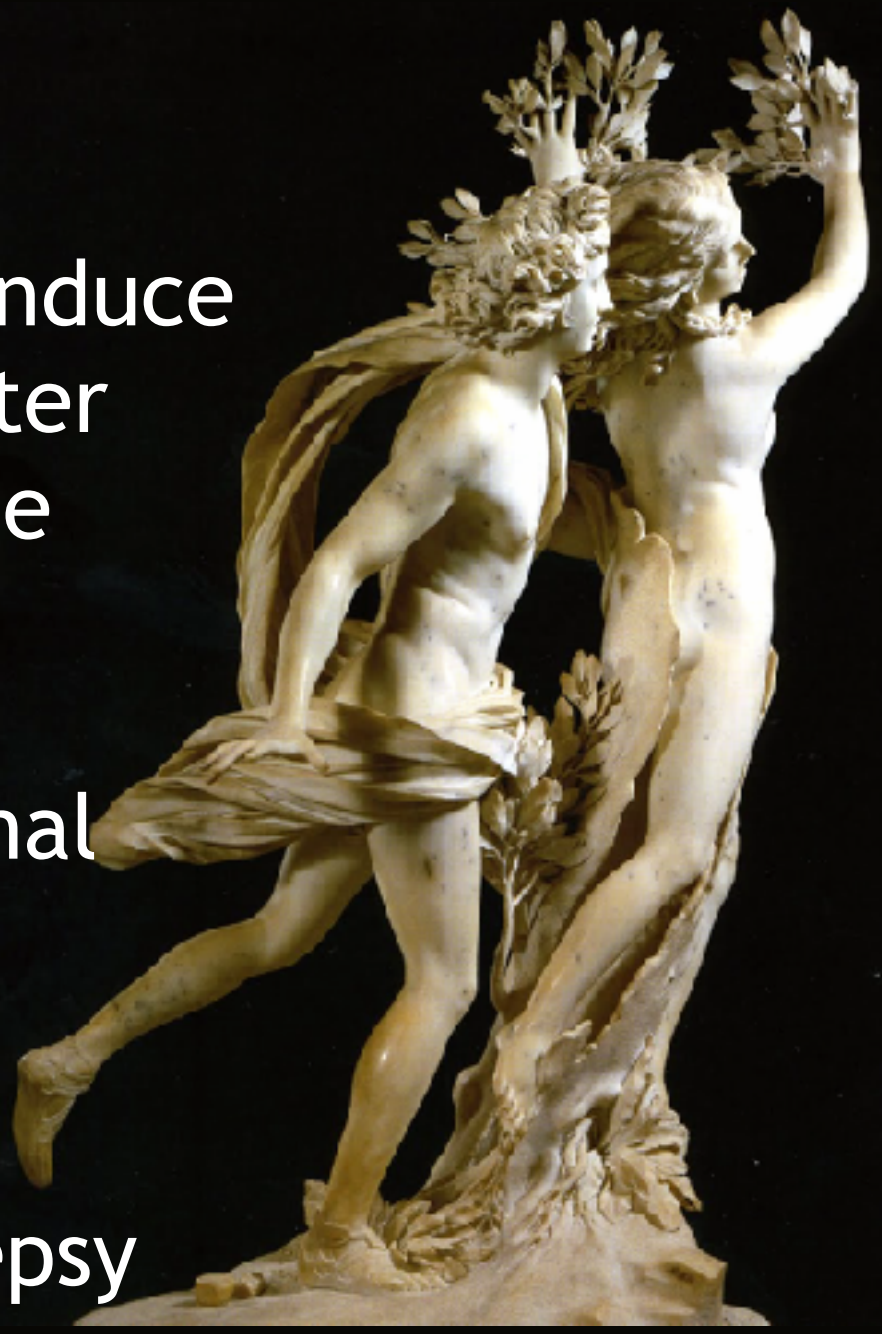
Take Home Message:

Stem cell transplants induce structural plasticity after transplantation into the Hippocampus.

Structural and functional changes may be the mechanism for seizure suppression in Temporal Lobe Epilepsy

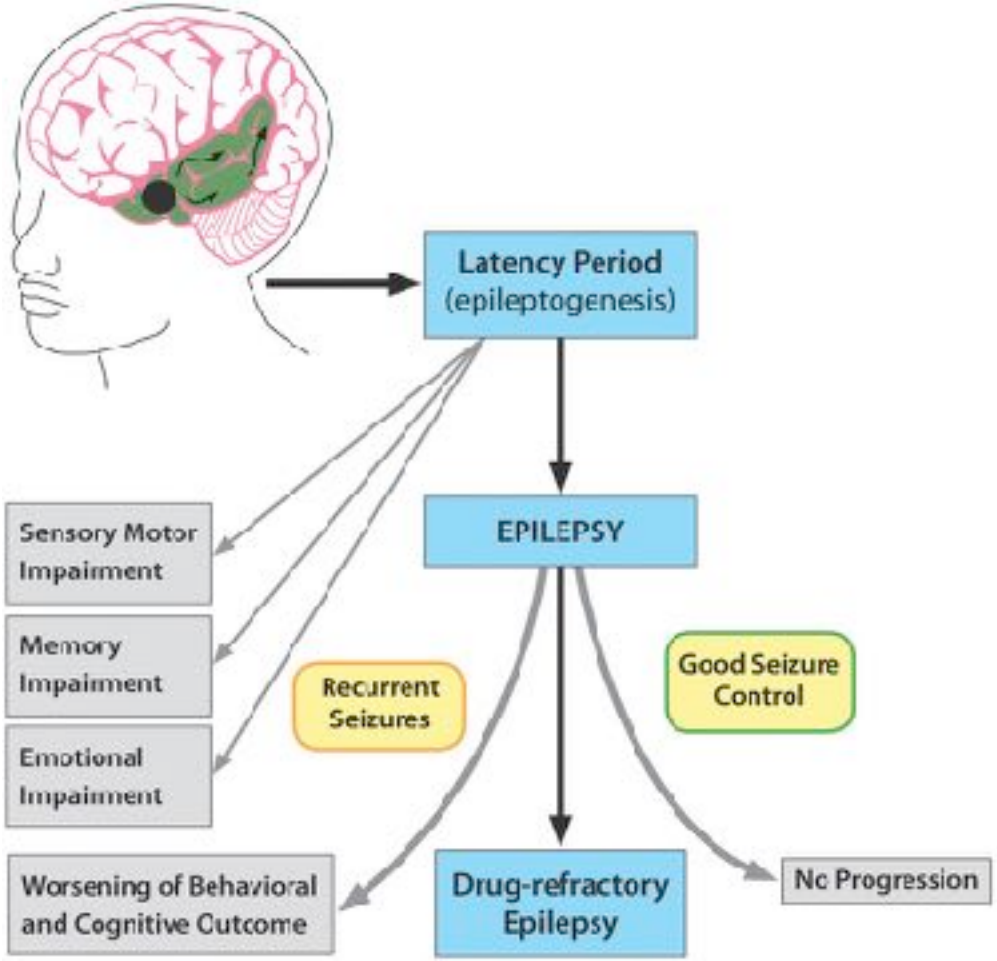


Imbalance between neuronal excitation vs. inhibition - unifying factor in seizure disorders

Temporal Lobe Epilepsy (TLE)

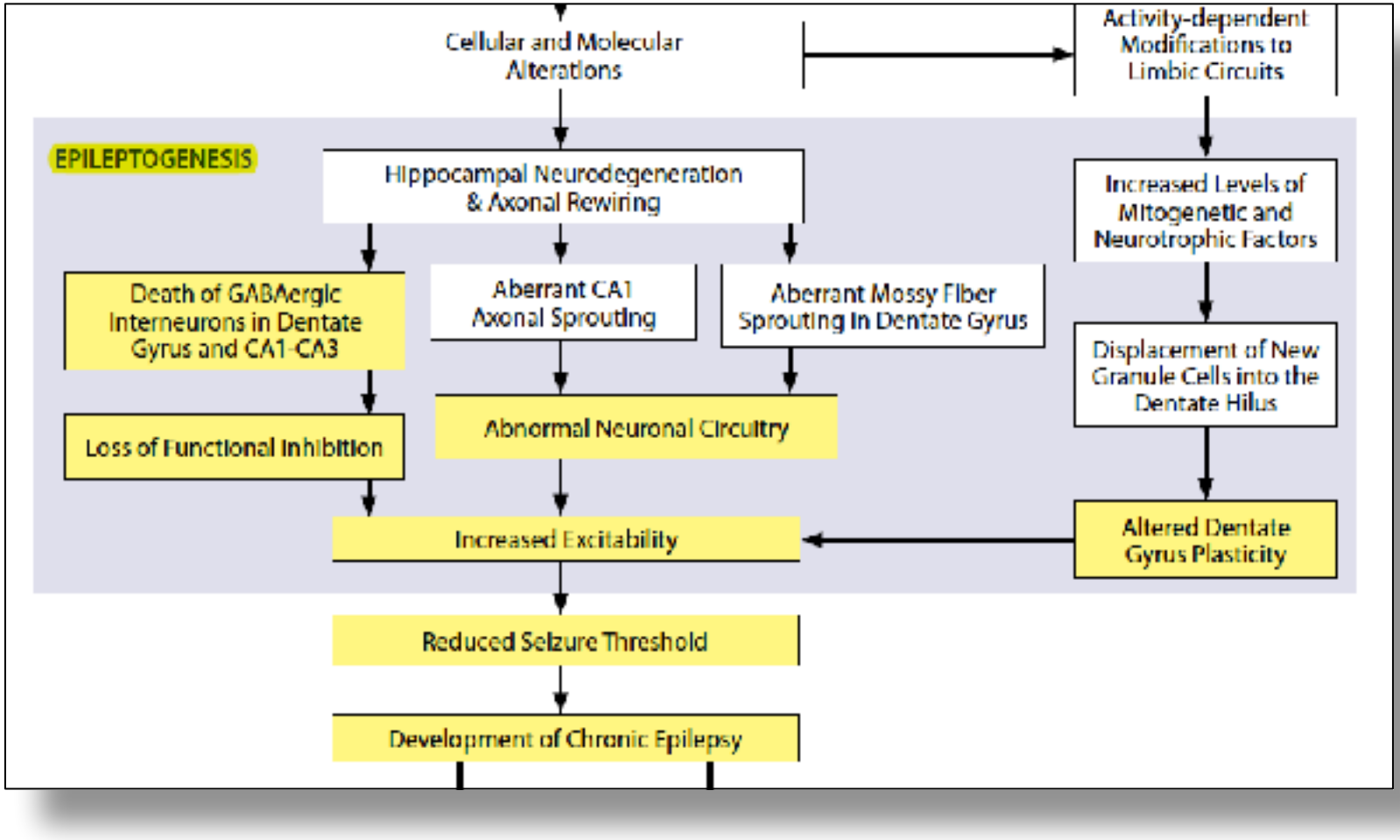
- Seizures cause cognitive and emotional impairments
- Seizures can be life threatening -*sudden death real concern*
- Surgical removal of epileptic brain regions may stop seizures
  - *But for some patients this approach cannot be used because of the critical role of the hippocampus in forming new memories*
- New treatments are needed
  - Deep brain stimulation
  - Gene therapy
  - *Neural stem cell transplantation*
    - *offers prospect of controlling seizures and repairing damaged regions of the brain (curing the underlying disease)*

Prolonged limbic seizures cause damage to vulnerable brain regions, including hippocampus



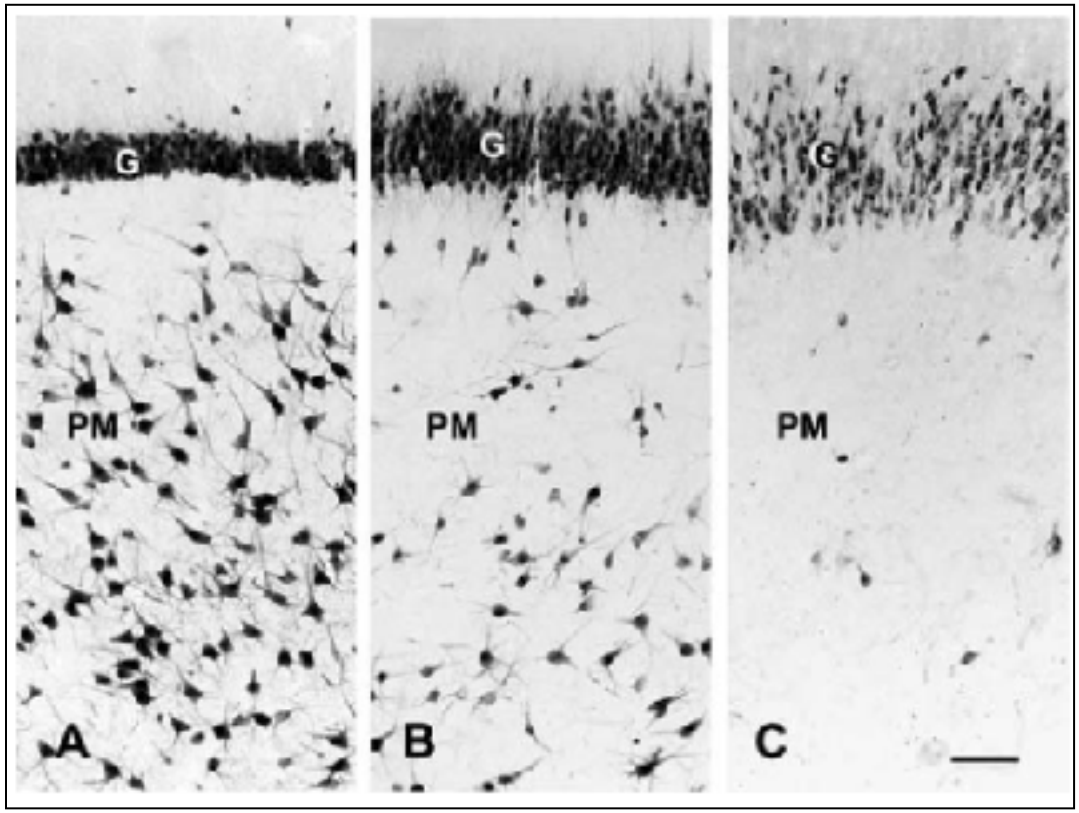
Naegele JR and Maisano X (2010) Gene and Stem Cell Therapies for Treating Epilepsy. ***Epilepsy: Mechanisms, Models, and Translational Perspectives.*** (CRC Press). . Rho JM, Sankar R, Stafstrom CE (Eds.). pp 589-608.

Many seizure-induced changes in TLE



Naegele JR and Maisano X (2010) Gene and stem cell therapies for treating epilepsy. ***Epilepsy: Mechanisms, Models, and Translational Perspectives***, Dekker M, Inc. (CRC Press). Rho JM, Sankar R, Stafstrom CE (Eds.). pp 589-608.

Patients with severe mesial temporal lobe epilepsy show loss of GABAergic interneurons in DG

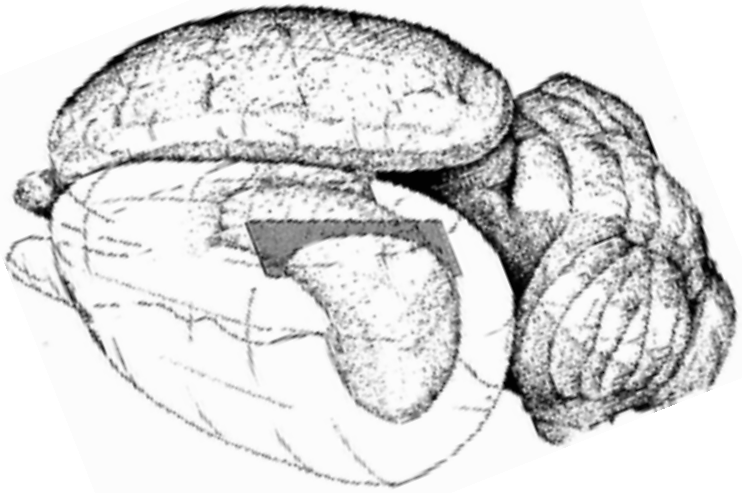


Swartz BE, Houser CR, Tomiyasu U, Walsh GO, DeSalles A, Rich JR, Delgado-Escueta A. Epilepsia. 2006 Aug;47(8):1373-82.

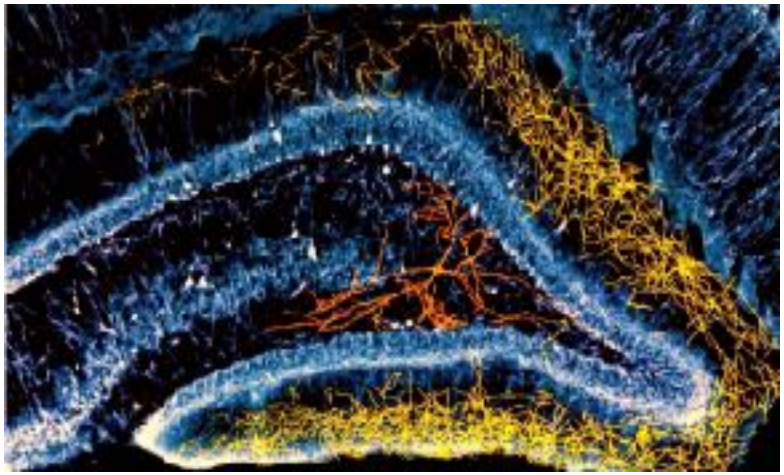
Dentate Gate Hypothesis
Neural gate preventing run-away excitation

Death or dysfunction of GABAergic interneurons disrupts the gate, allowing seizures to propagate

The rodent brain and dentate gyrus in hippocampus

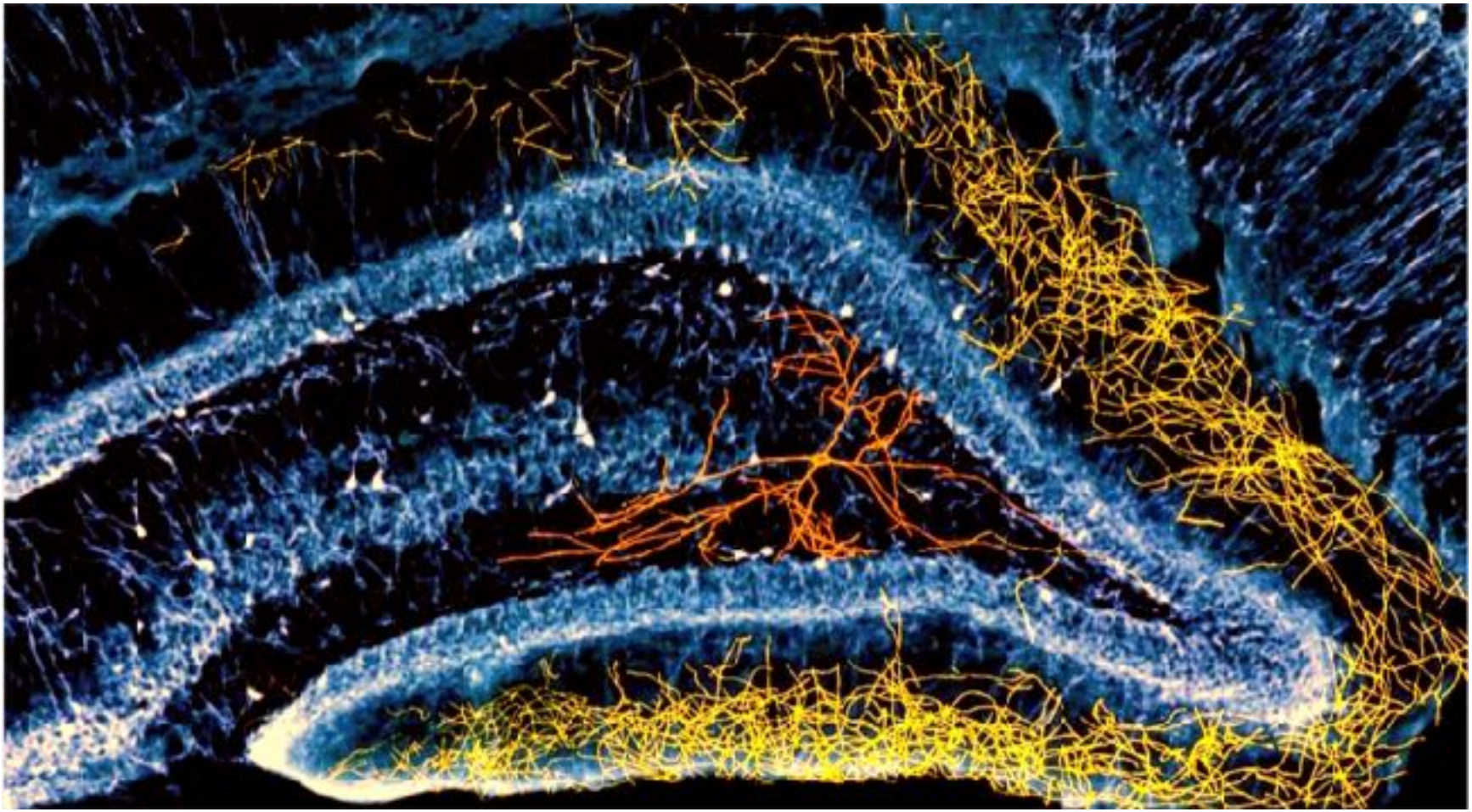


Rodent brain viewed from above
With slice through hippocampus



- Microscopic view of the dentate gyrus, a region of the hippocampus *with adult neurogenesis*
- Granule cell layer of dentate gyrus (blue)
- GABAergic interneuron (red)
- Axon (yellow) – note the broad expanse covered by a single cell!

Prolonged status epilepticus results in loss of hilar inhibitory interneurons



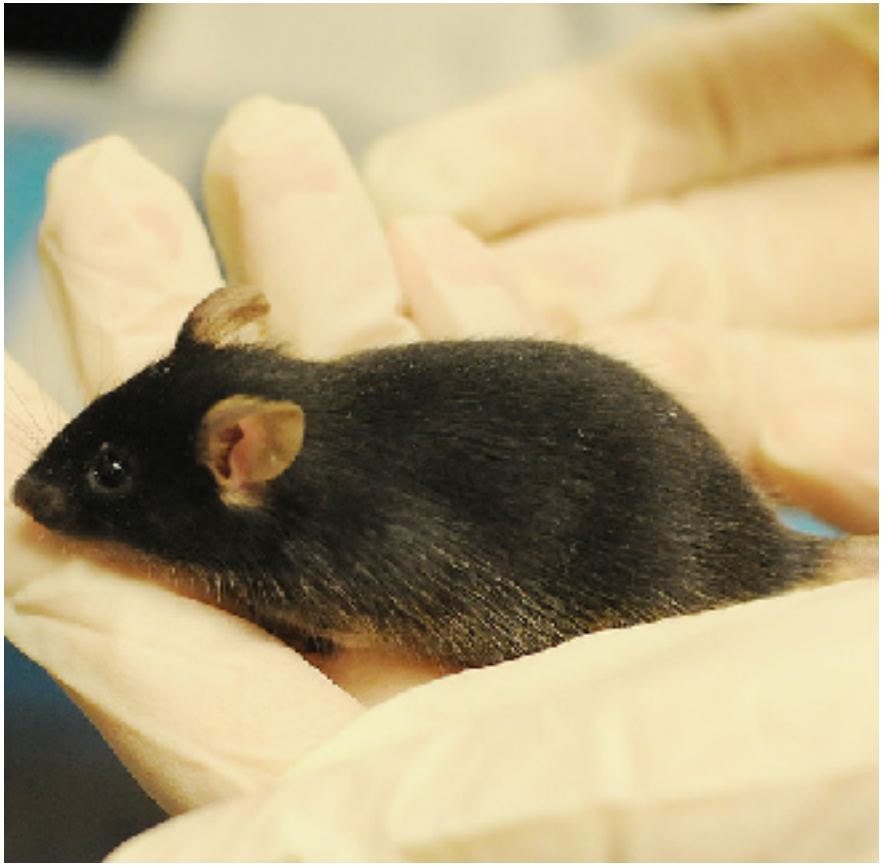
HIPP GABAergic Interneuron in hilus

Image courtesy of Gyorgy Buzsaki

Testing novel stem cell therapies for intractable epilepsy requires animal models

Translational studies use acute and chronic TLE models in rats and mice

Pilocarpine model of TLE in mice



Block peripheral muscarinic AChRs (methyl scopolamine, 30 min)

↓

Induce seizures (pilocarpine, 40 min)

↓

Status epilepticus (60 minutes)

↓

Attenuate seizures (midazolam; hours)

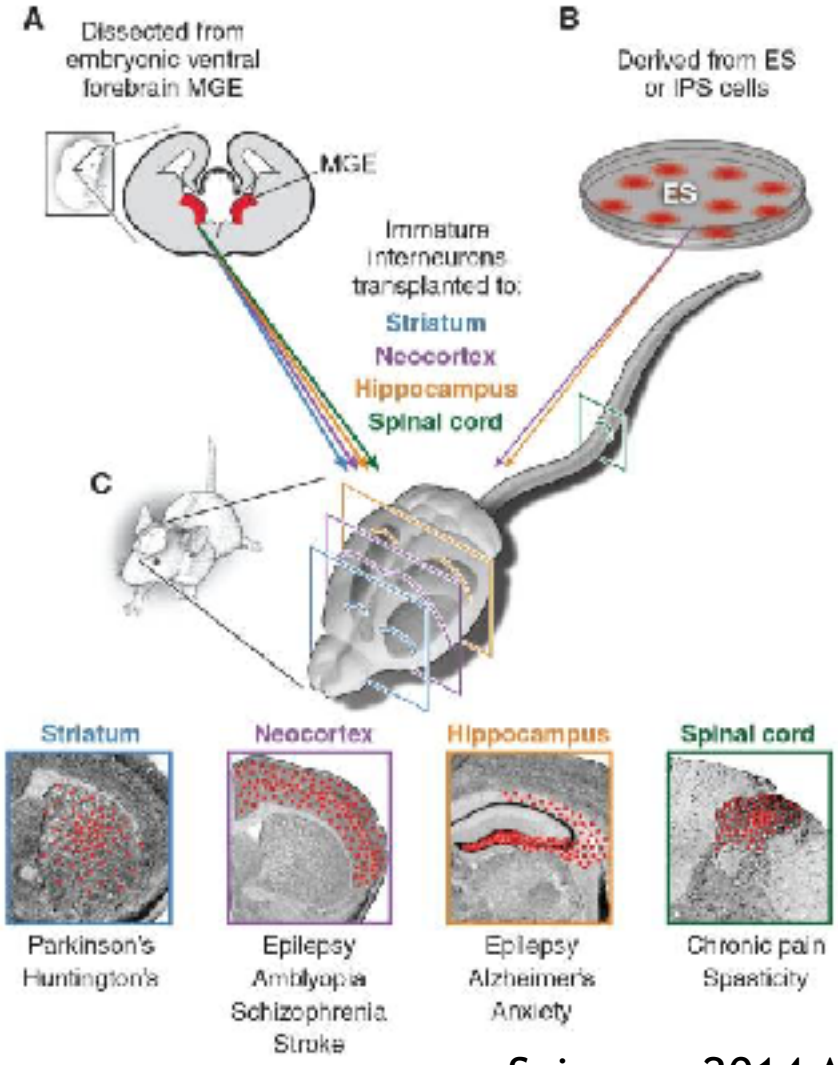
↓

Development of spontaneous Seizures (days)

Spontaneous seizures in the pilocarpine model in mice

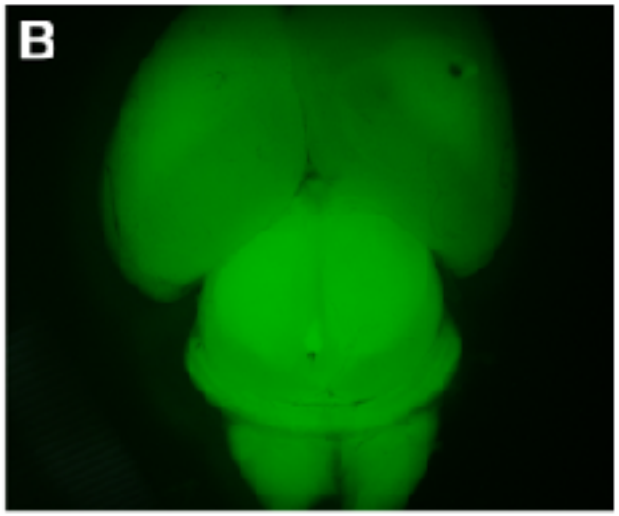
Racine Stage 5

Sources of immature GABAergic interneurons

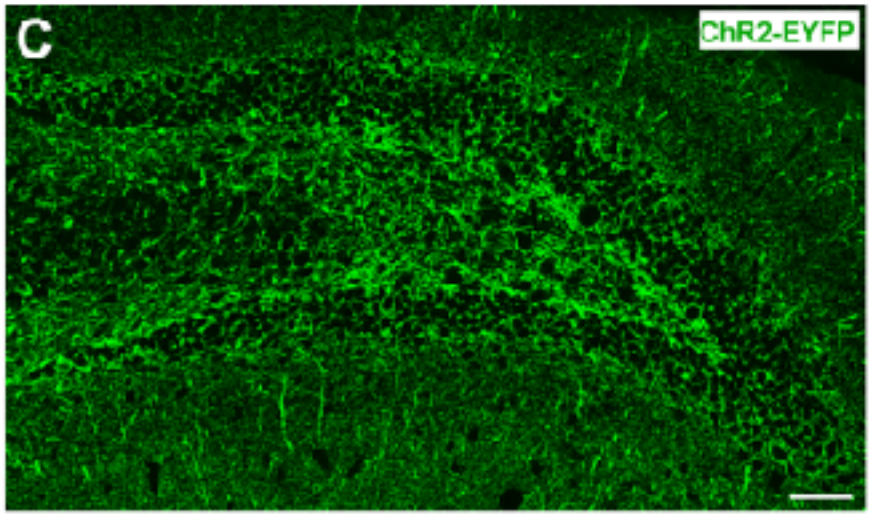


Science. 2014 Apr 11; 344(6180): 1240622.
doi: 10.1126/science.1240622

Donor cells from VGAT-ChR2(H134R)-EYFP transgenic mouse embryos

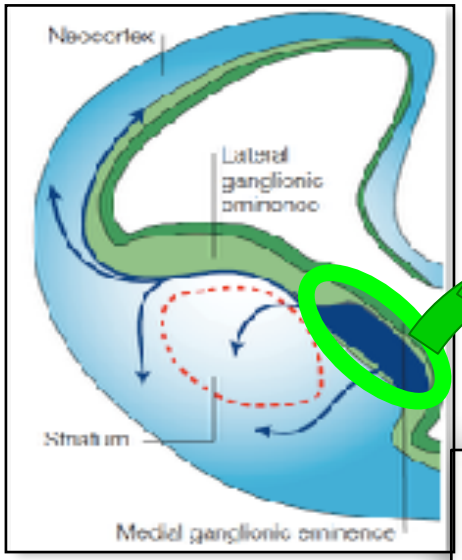


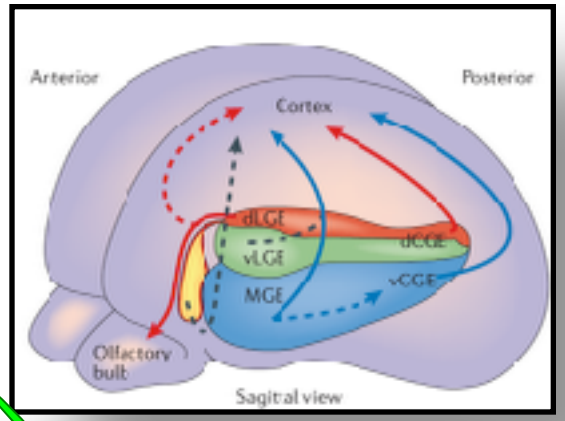
Embryonic day 13.5 brain

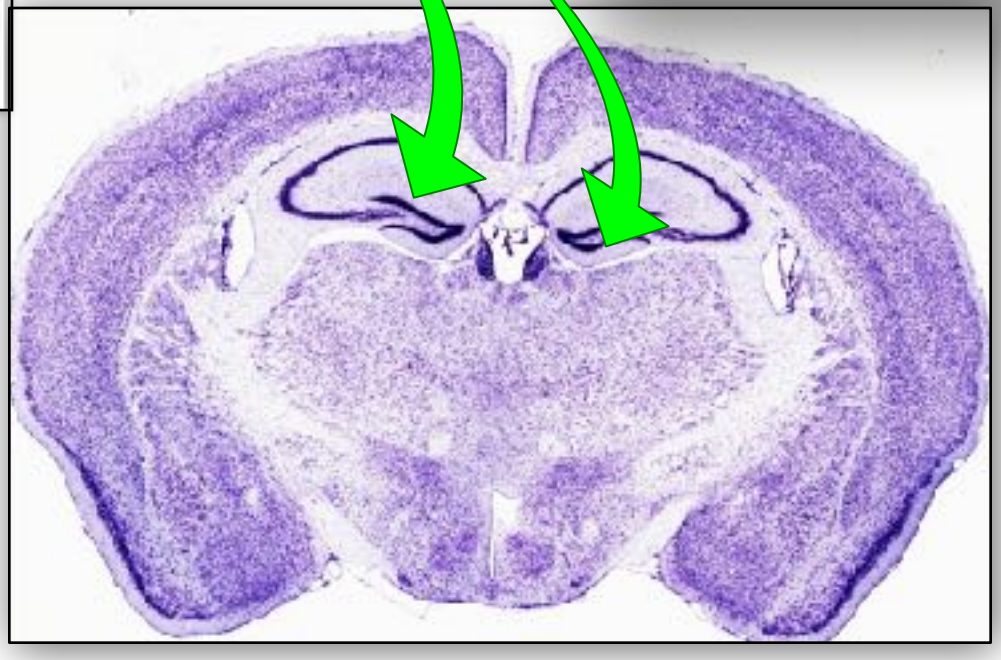


Dentate gyrus of adult transgenic mouse with the endogenous VGAT-ChR2-EYFP GABAergic interneurons

Bilateral MGE cell TX into adult dentate gyrus







Transplant parameters

- deposited bilaterally
- dentate gyrus
- 14-21 days post-SE
- Multiple sites
- ~100,000 cells/side

Sources of E13.5 MGE cells

- VGAT-Venus embryos
- VGAT-ChR2-eYFP embryos
- wt embryos; CAG-eYFP electroporation

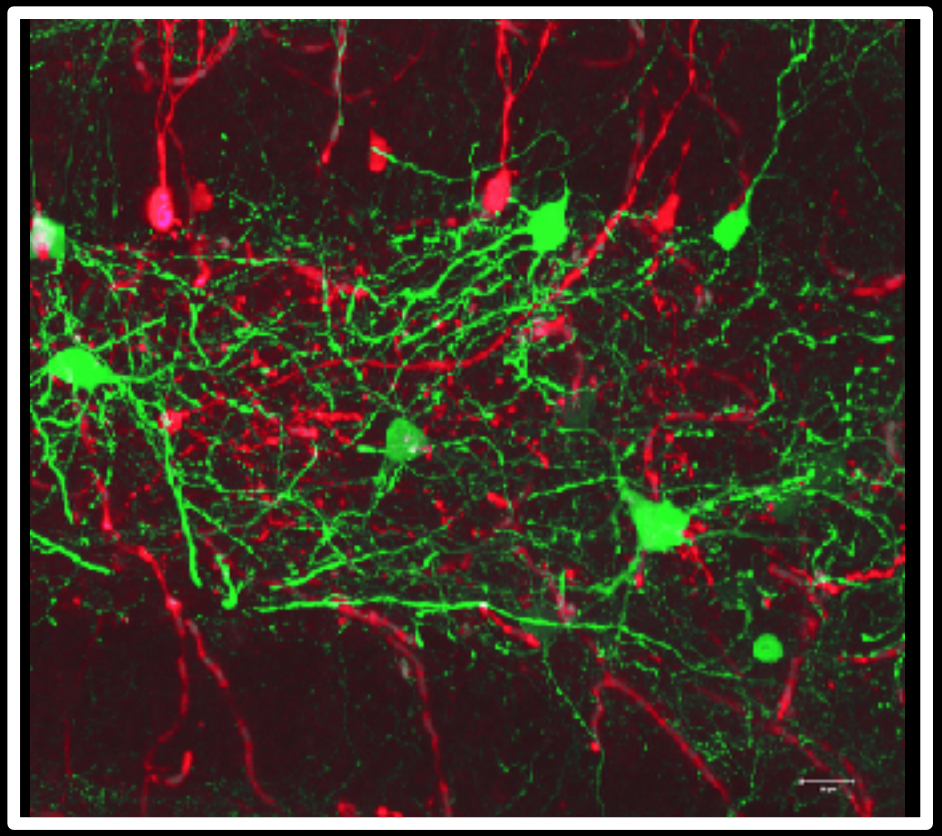
The big questions

Do GABAergic progenitor transplants into the dentate gyrus mice with temporal lobe epilepsy integrate and form functional synapses?

Are these neural stem cell transplants effective for suppressing seizures?

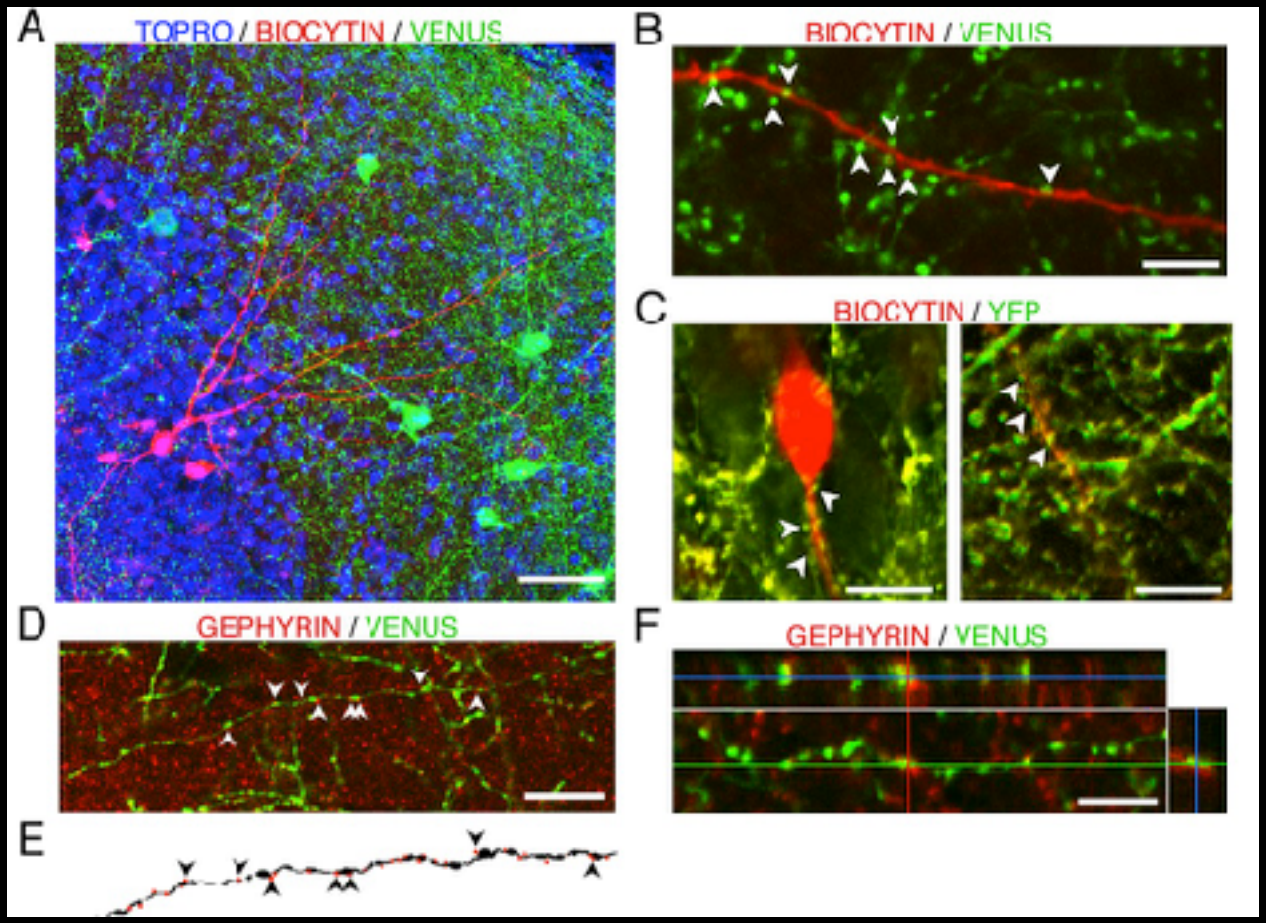
What are the underlying mechanisms?

Transplanted cells wire up with granule neurons several weeks after transplantation



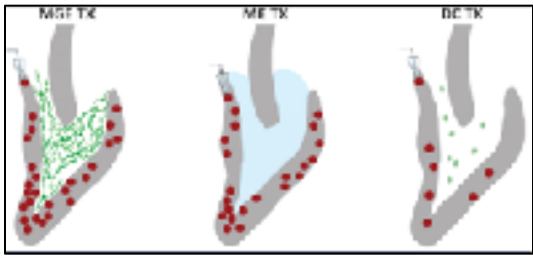
Transplanted GABAergic interneurons/pRubi retrovirus-labeled GCs

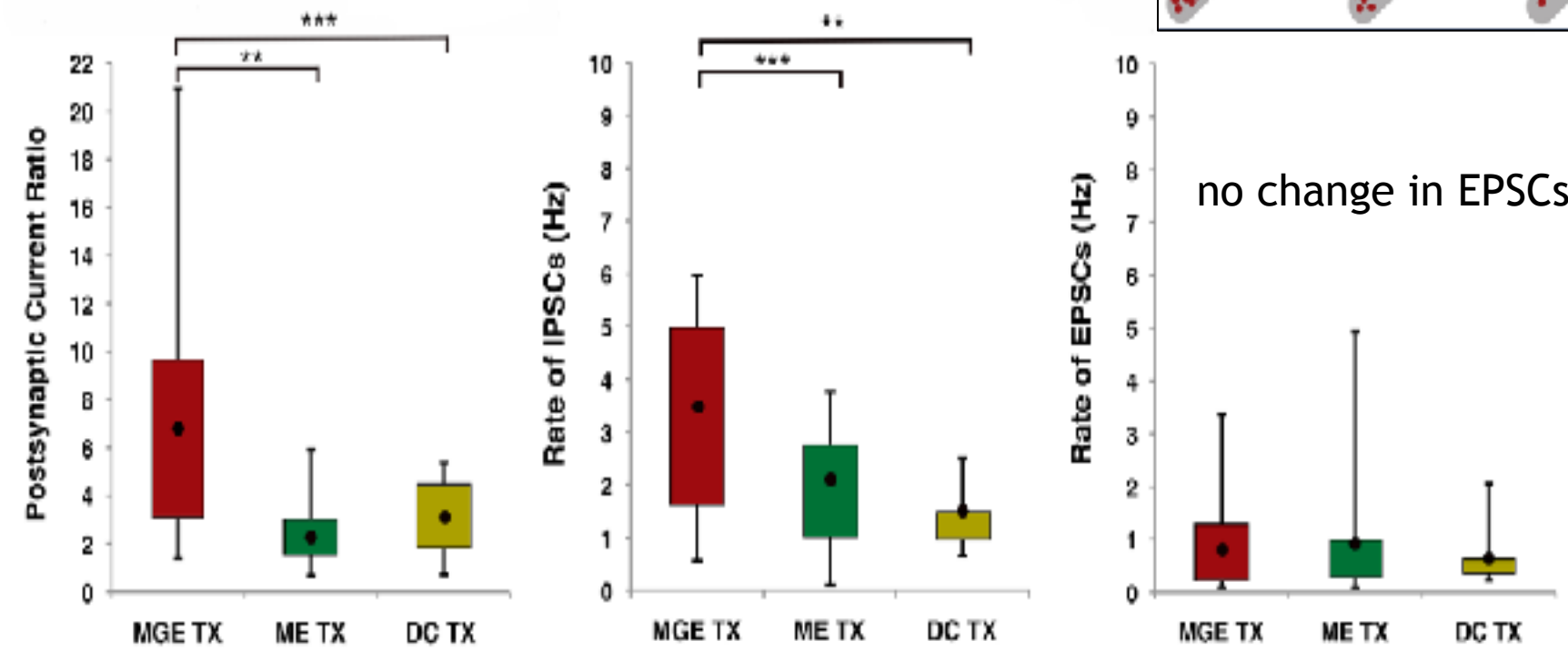
Transplanted GABAergic interneurons induce clustering of molecular components of inhibitory synapses postsynaptically



Henderson, Gupta et al. J. Neuroscience 2014

Electrophysiological recordings show increased spontaneous inhibitory postsynaptic currents after transplantation

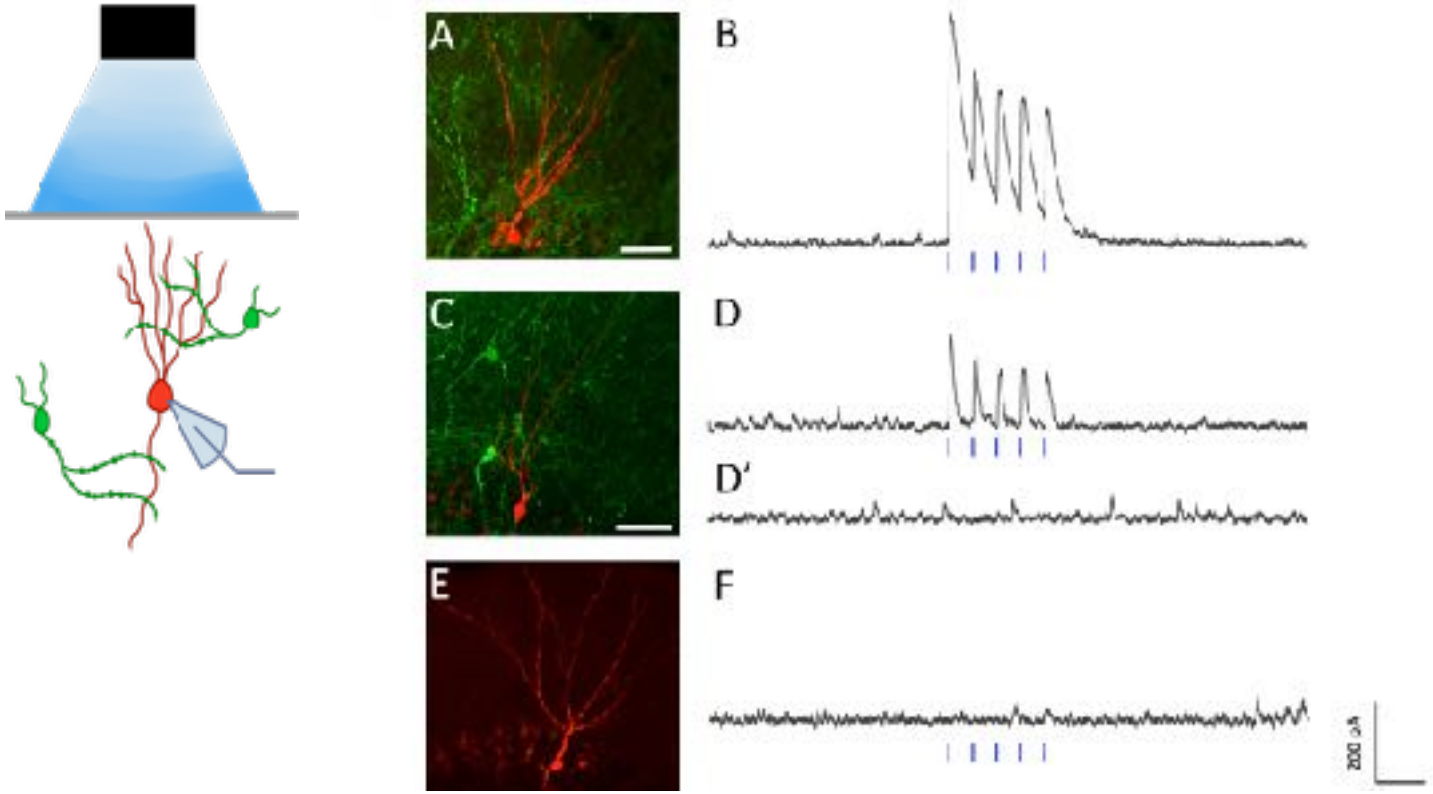




** p<0.01, *** p<0.05

Henderson, Gupta et al. J. Neuroscience 2014

Optogenetic demonstration of functional inhibitory synaptic connections



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Our research questions

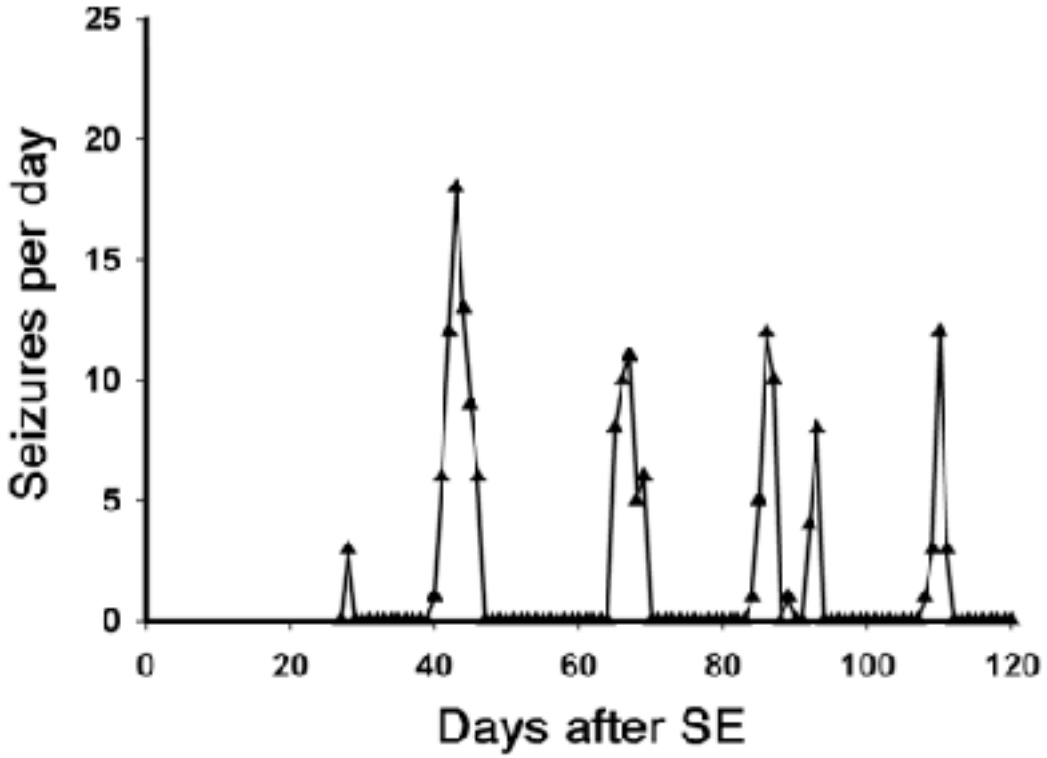
Do GABAergic progenitor transplants into the dentate gyrus mice with temporal lobe epilepsy integrate and form functional synaptic connections?

yes.

Are these neural stem cell transplants effective for suppressing seizures?

What are the underlying mechanisms?

Typical pattern of clustered seizures in pilocarpine model of TLE in C57Bl/6 mice

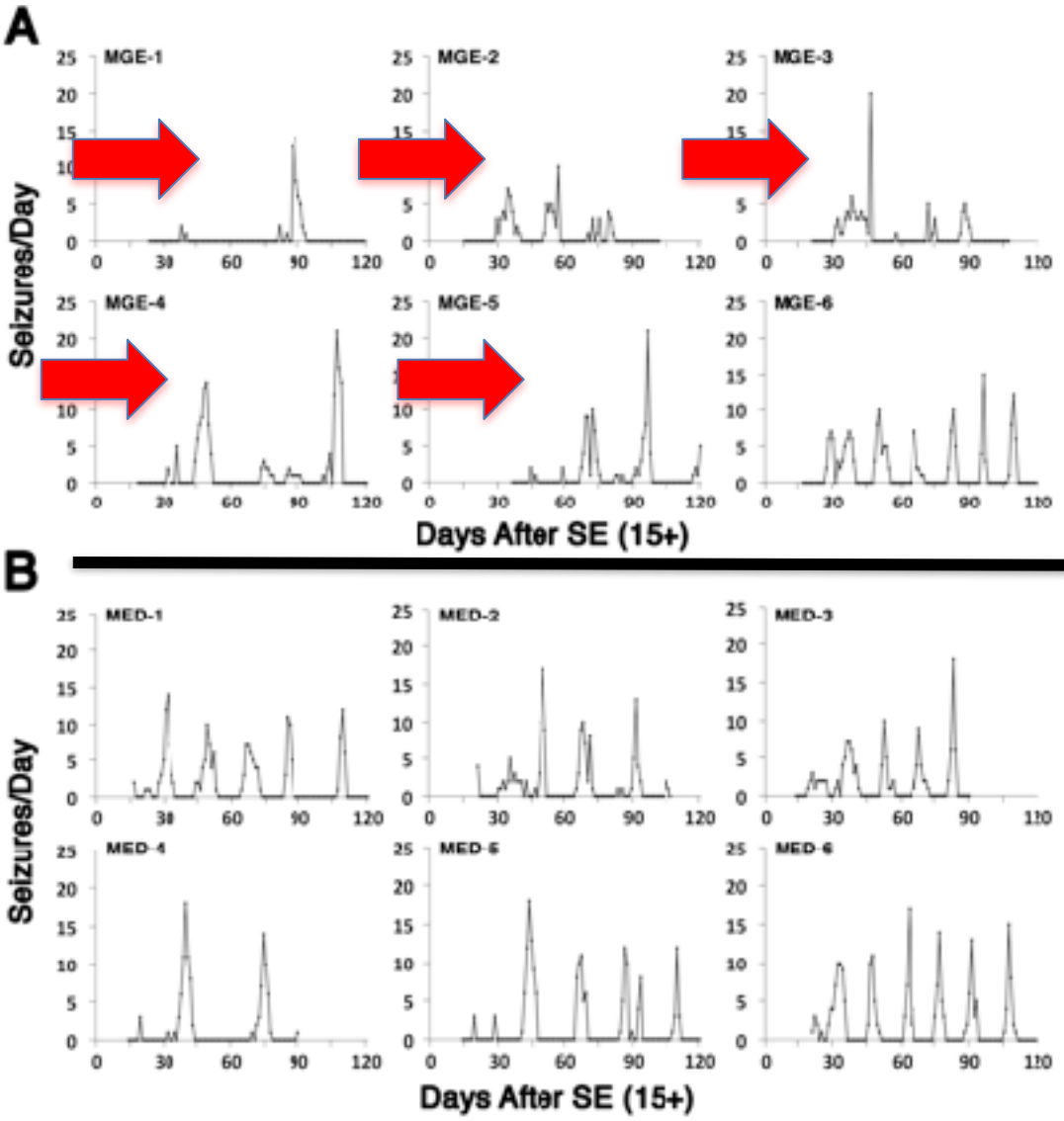


- Clusters: seizures occur in bouts or clusters (>3 seizures/day for 3-5+ days)
- Seizure-free intervals: ~ variable, often 10-20 day-periods between clusters
- Incidence/Frequency in clusters: ~ 5-30 seizures/day

Spontaneous seizures reduced by GABAergic progenitor grafts in dentate gyrus

Transplants

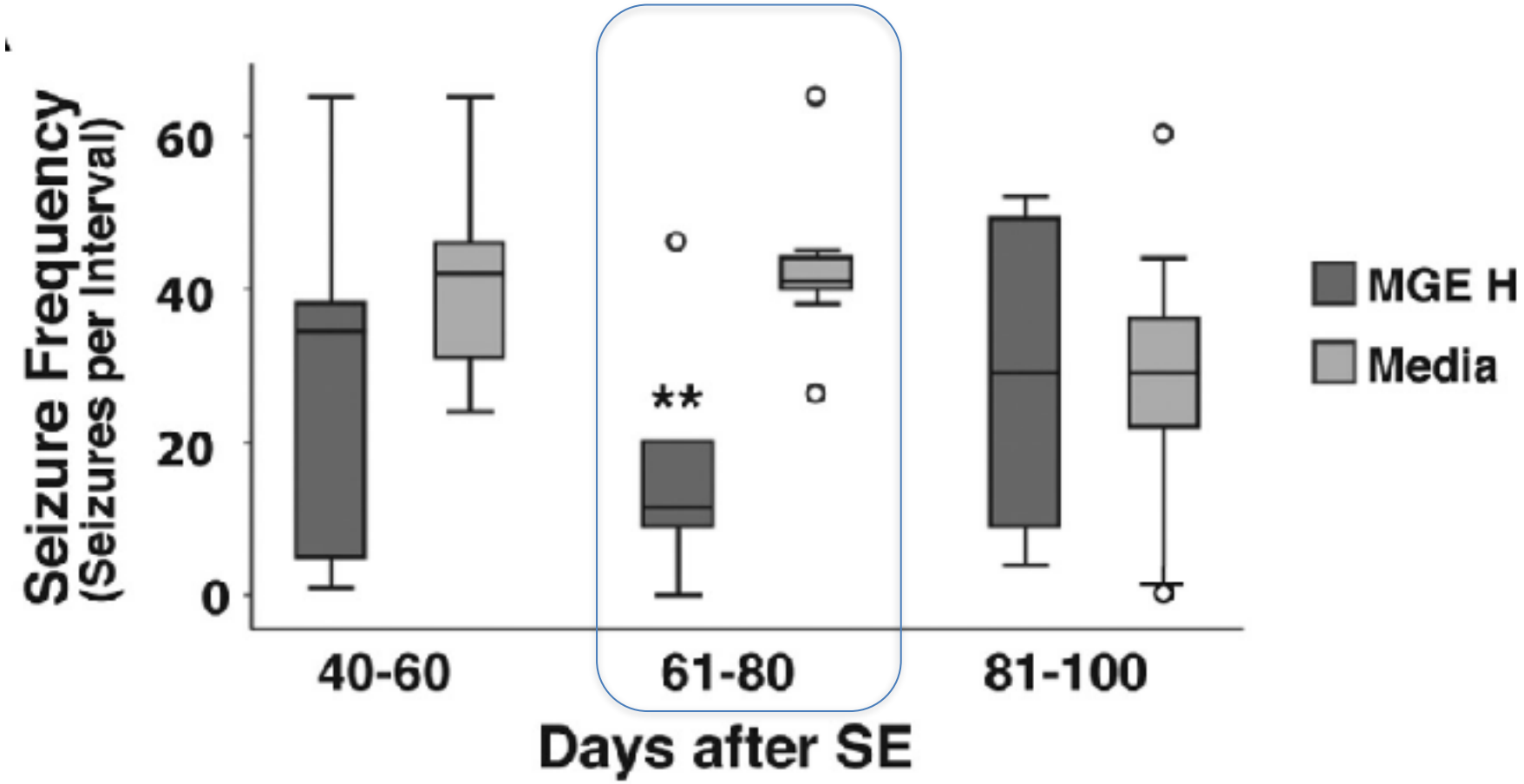
Media Controls



Overall, 64% fewer total seizures after 90 days of EEG recording, compared with mice injected with media

Henderson, Gupta et al. J. Neuroscience 2014

Significant seizure suppression ~ 2-3 months after transplantation



Henderson, Gupta et al. (2014) *J. Neuroscience*

Our research questions

Do GABAergic progenitor transplants into the dentate gyrus mice with temporal lobe epilepsy integrate and form functional synaptic connections?

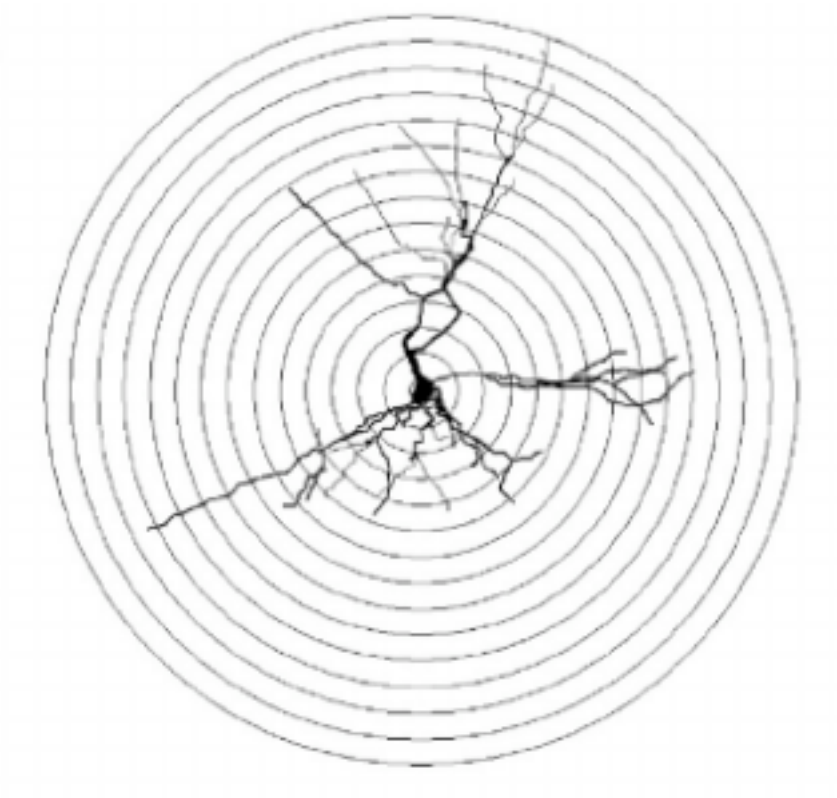
Yes.

Are these neural stem cell transplants effective for suppressing seizures?

Yes.

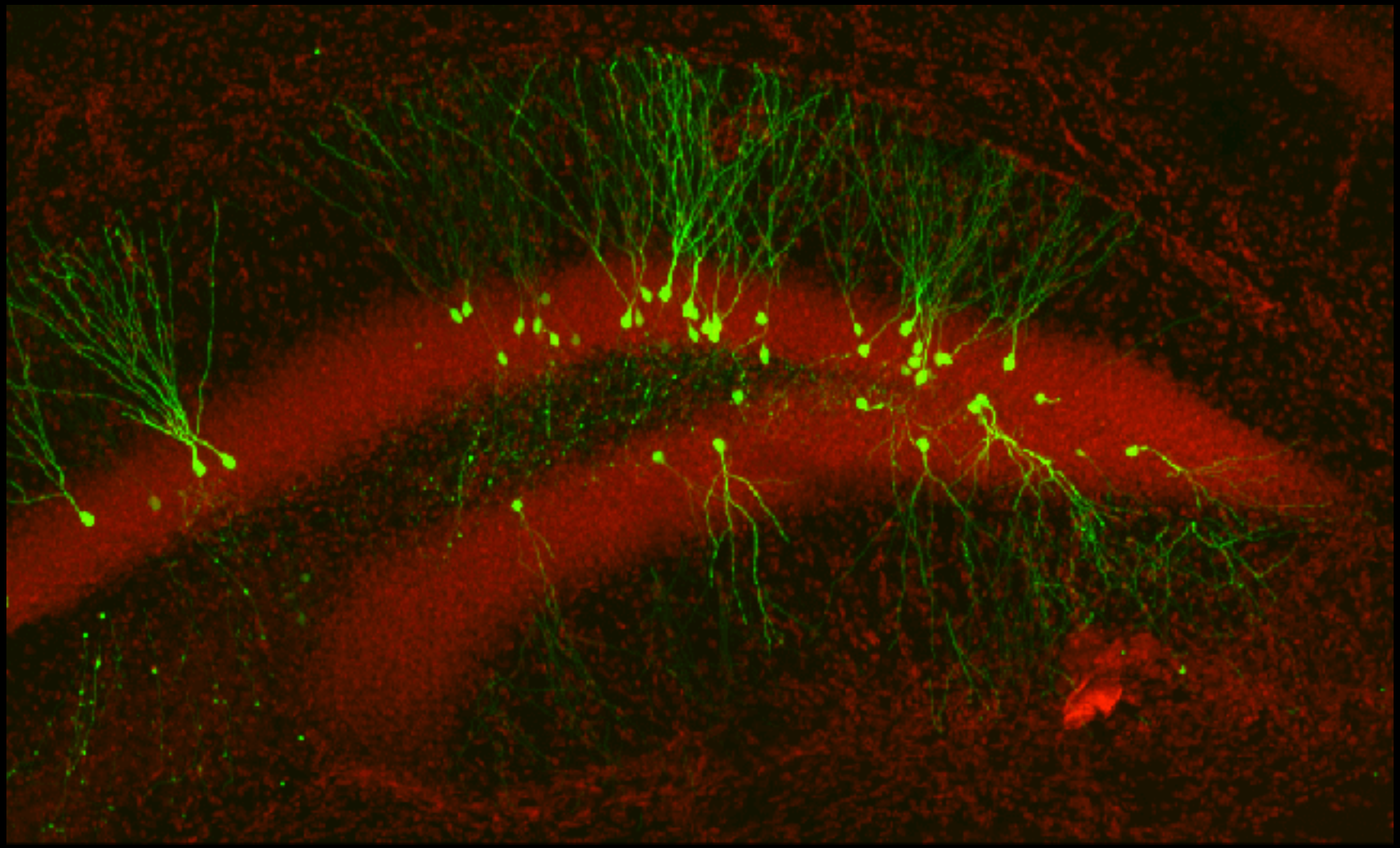
What are the underlying mechanisms?

Do the transplants alter the structural (and functional) properties of adult-born neurons?

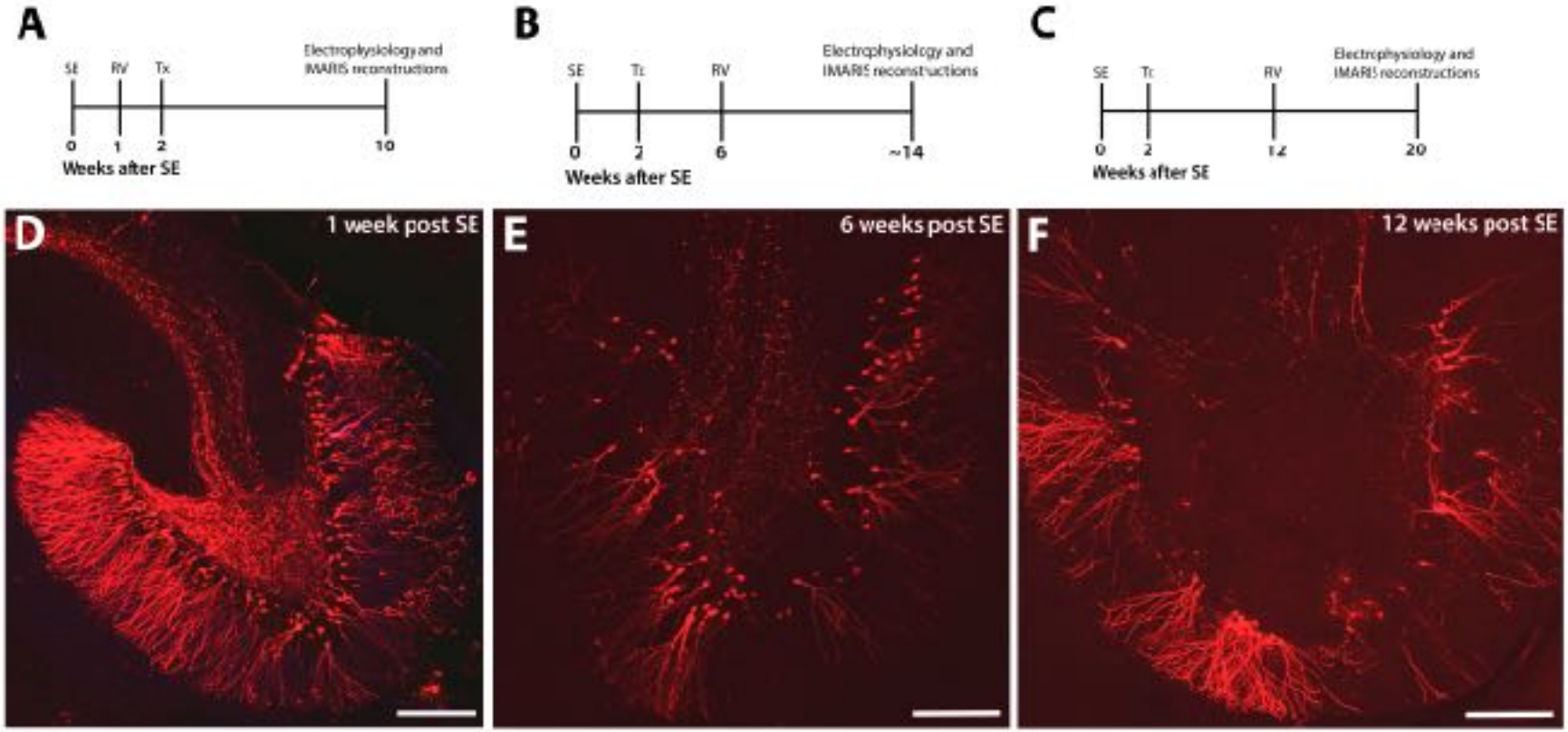


Sholl Analyses:
Structural analyses of branches in 3D

pRubi-EYFP expressing retroviral labeling of adult-born granule cells in TLE

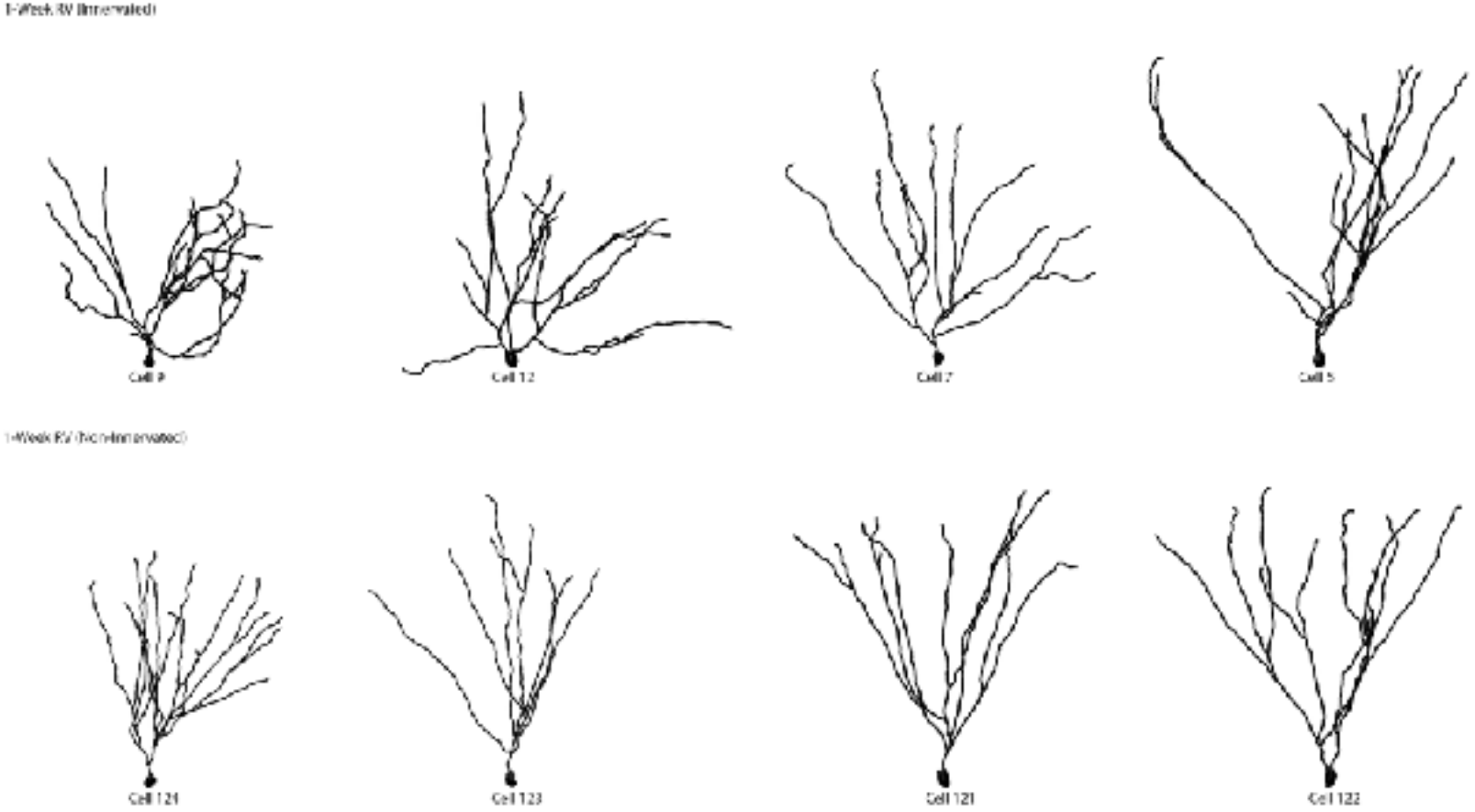


Experimental time line to study adult-generated granule cell structural plasticity



Helping to identify new neurons that become hyperexcitable

Structural analyses of dendritic arbors:



Shall analyses of adult-born granule cells: GABAergic transplants restrain dendritic overgrowth

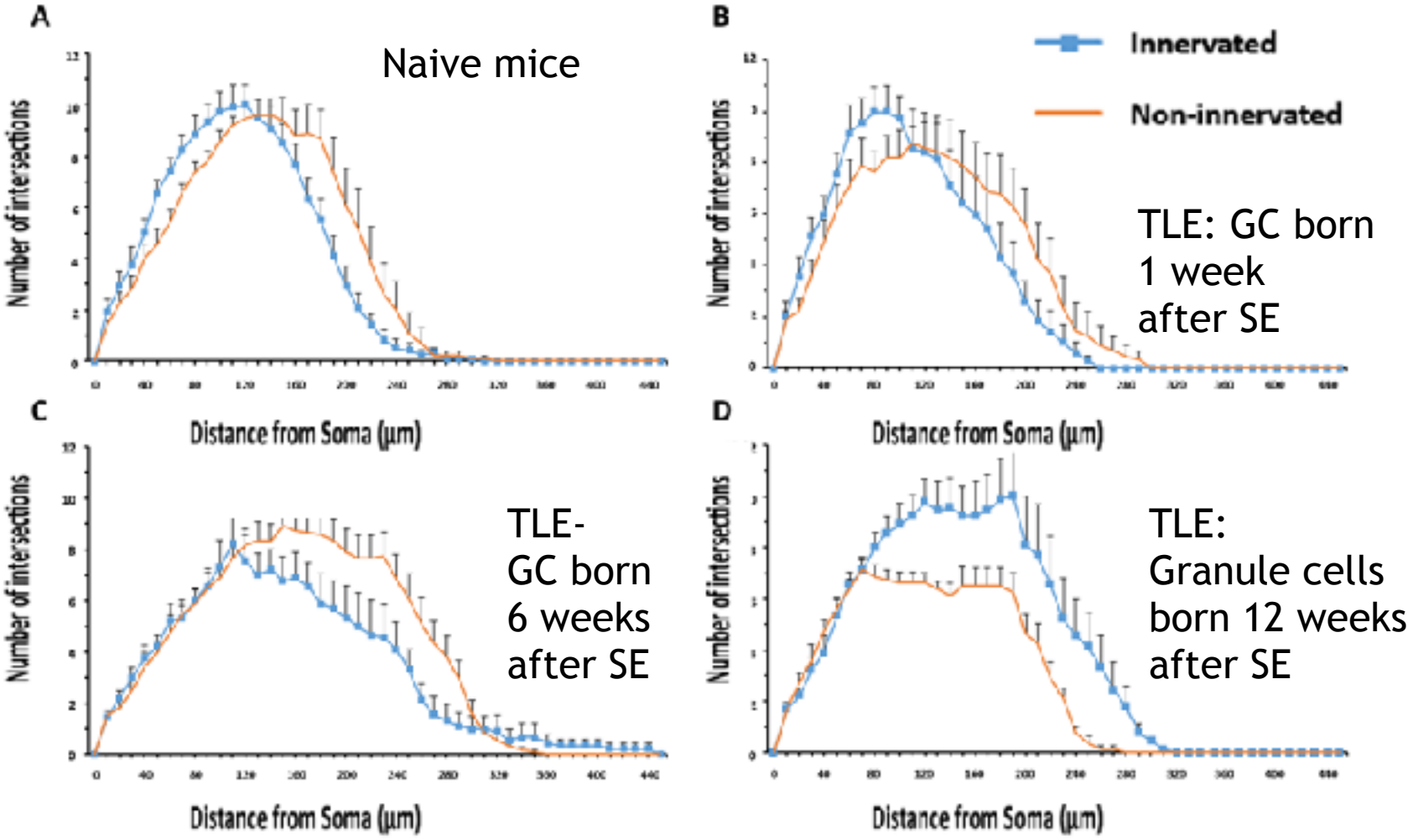
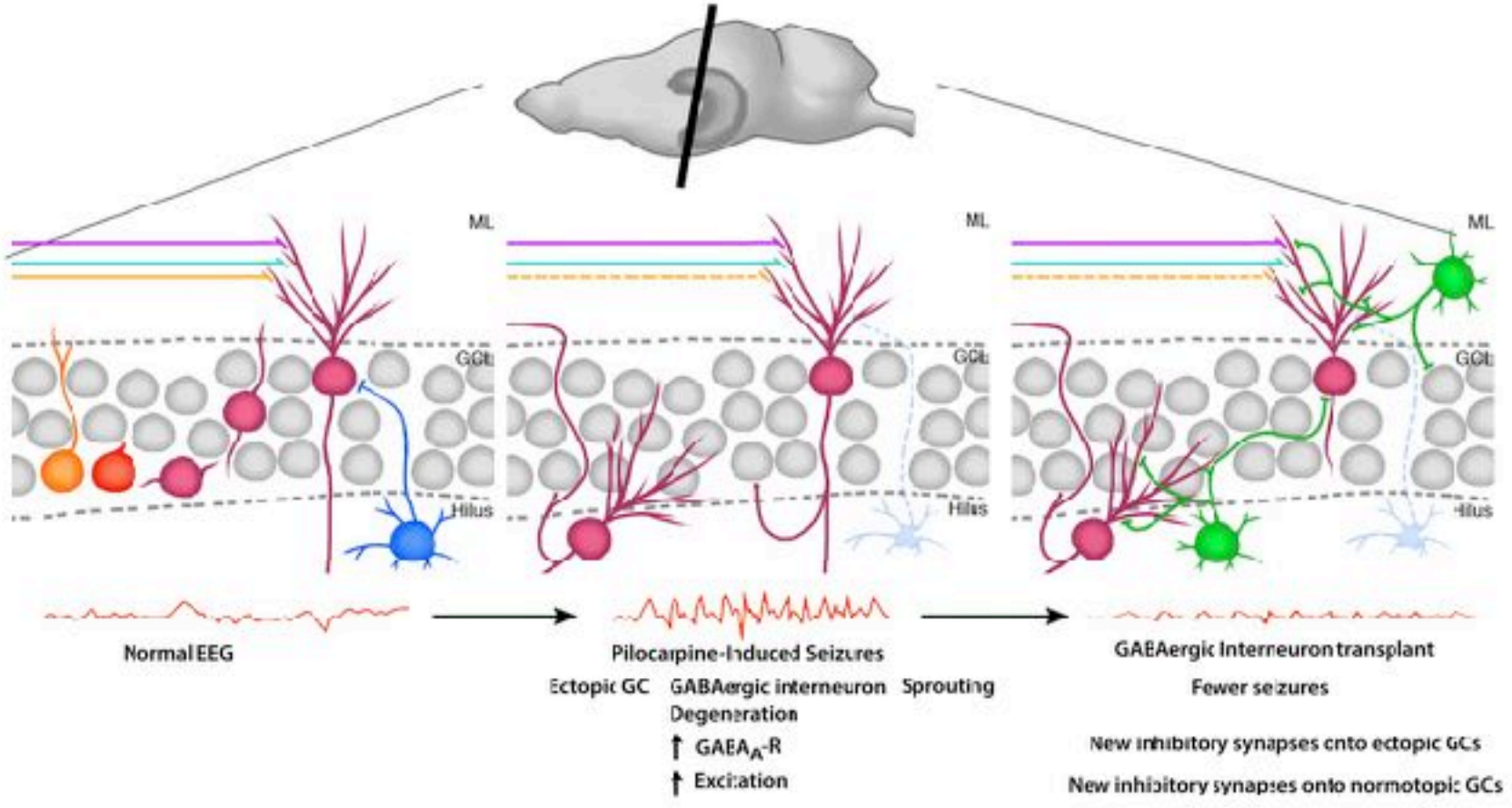


Fig 4. Sholl analysis of dentate granule cells. Average number of intersections made between dendrites, and concentric spheres measured at 30µm intervals from the soma, for naive cells (A), and epileptic cells born 1 wk (B), 6 wks (C), and 12 wks (D) post induction of SE.

Model of seizure suppression by transplantation of GABAergic progenitors



Summary

Transplants of MGE derived GABA progenitors:

- Transplanted GABAergic interneurons form functional inhibitory synapses with GCs in the dentate gyrus
- Adult-born hippocampal neurons in epileptic mice undergo hypertrophy and form too many branches
- Wiring up with fetal transplants restrains overgrowth of adult-born granule cell dendrites
- Restraining excessive dendrite growth and branching may reduce excitability and correct cognitive impairments.

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