Directional guidance of neuronal migration in the olfactory system by the protein Slit

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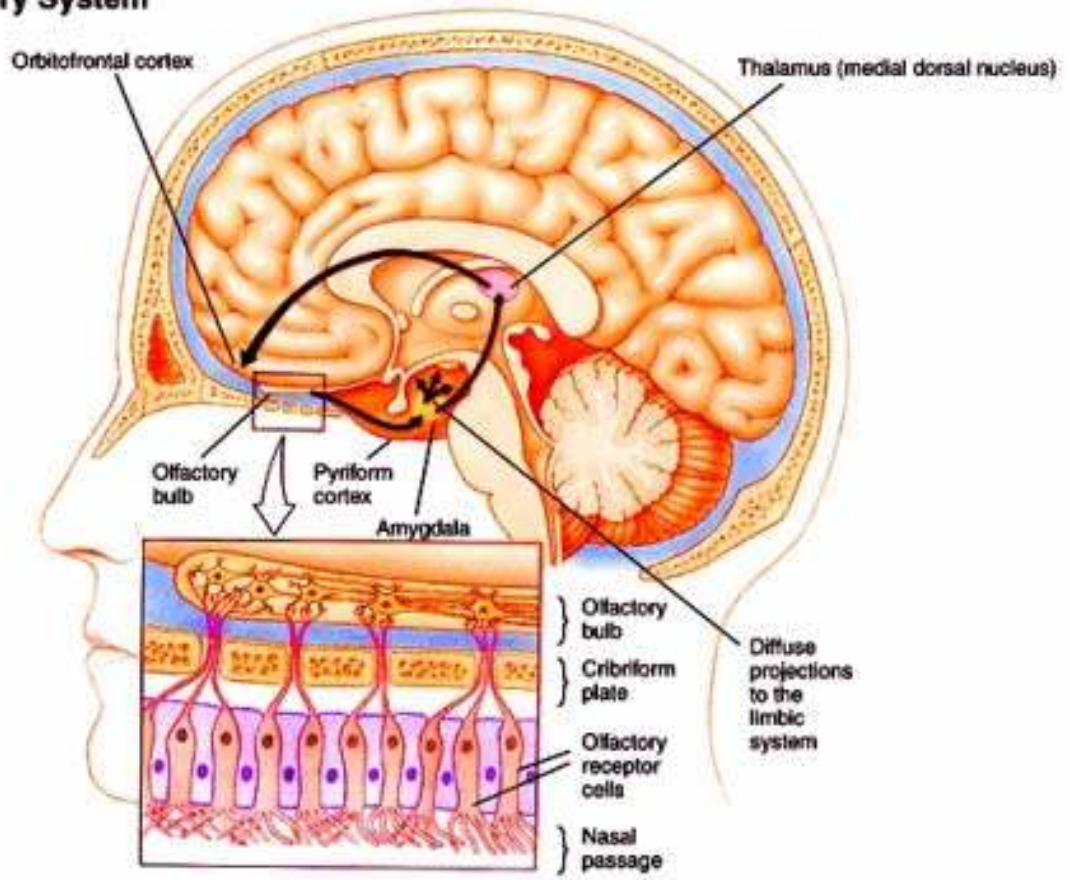
Introduction:

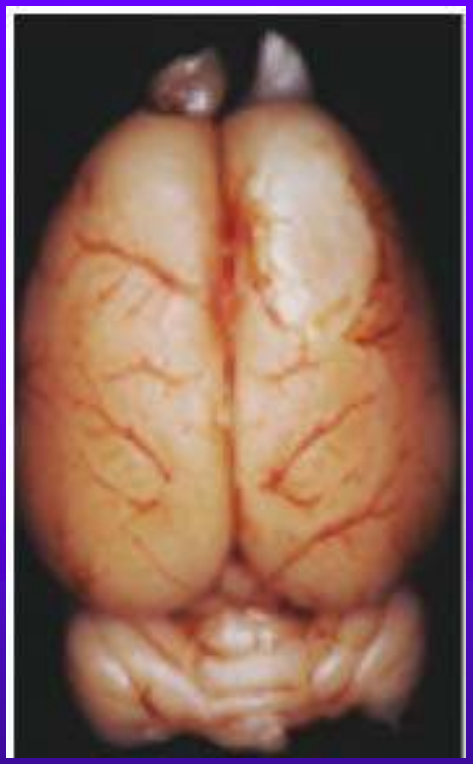
- In normal CNS formation, neuronal migration is crucial for the cells to be present at the proper positions. If not, cause diseases such as epilepsy, neuroblastoma.
- R.Y. Cajal, Hardesty and et al. inferred migration from their observations in 1900s.
- People thought that only the nuclei migrate due to the fact that early autoradiographic studies only traced nuclei.
- Some molecules were found to guide migrating neurons, but their exact function is unclear.

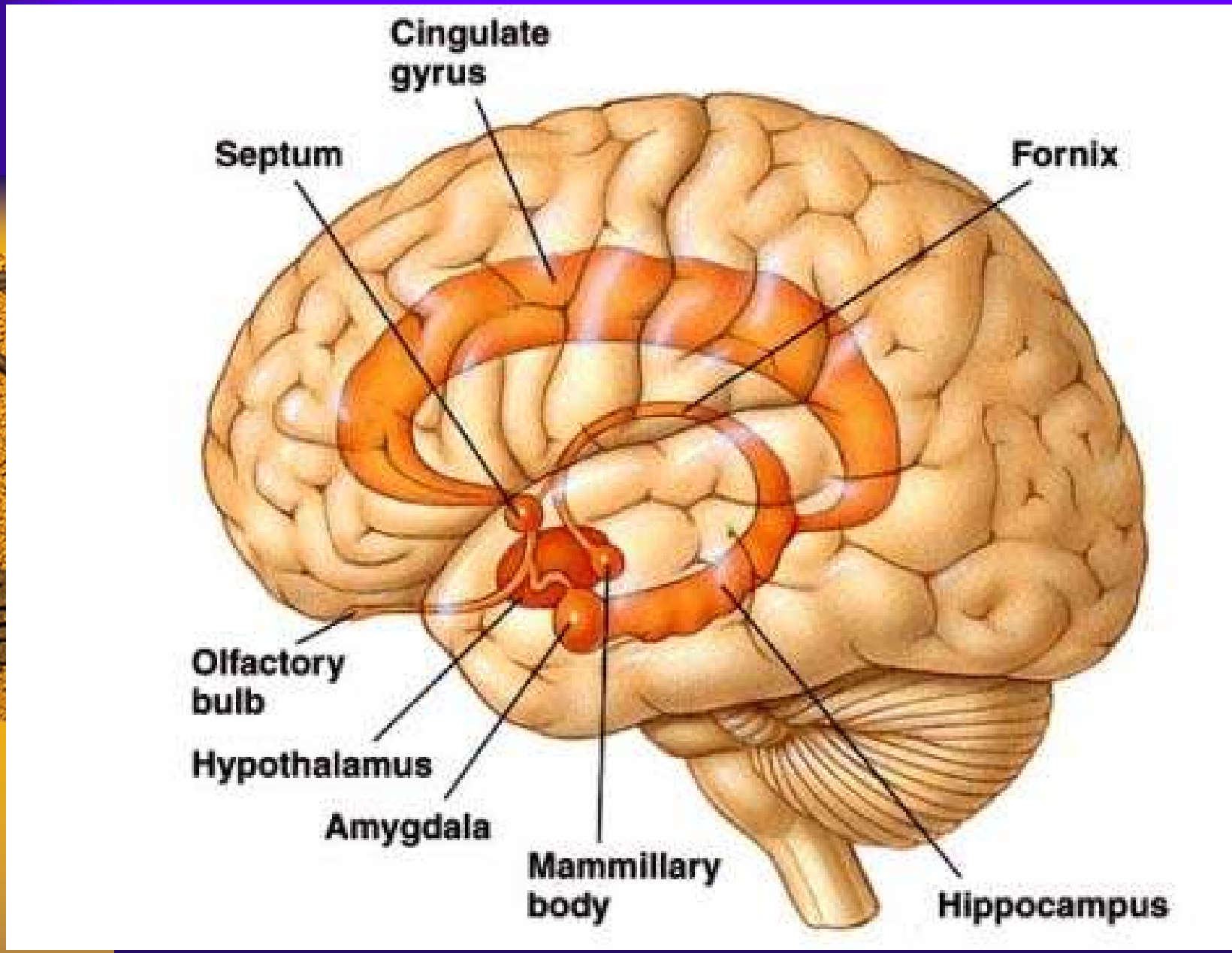
Olfactory system:

- Olfactory bulb relays information from olfactory epithelium to primary olfactory ctx.
- Interneurons in O.B. (granule and periglomerular cells) are made postnatally from anterior subventricular zone (SVZa) of the telencephalon in rodents.
- Neuronal progenitors migrate in the rostral migratory stream (RMS) from the SVZa to the olfactory bulb

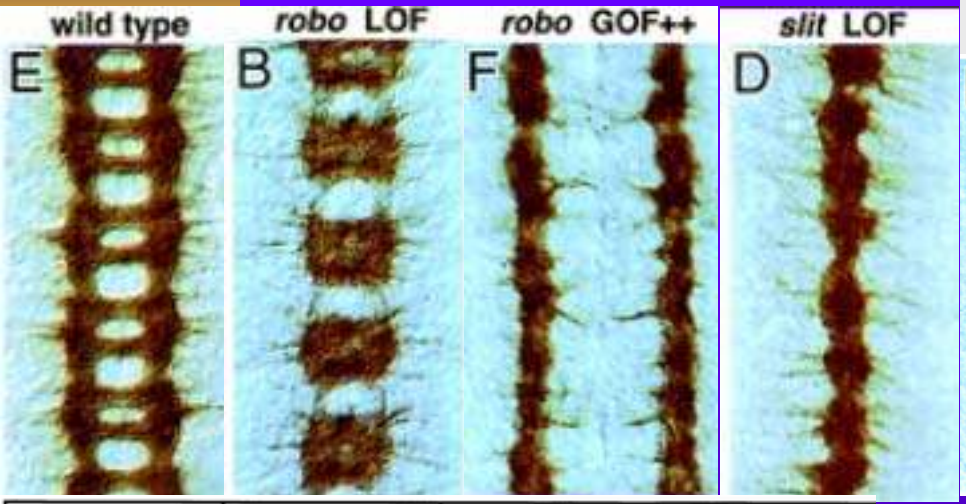
▶ Olfactory System



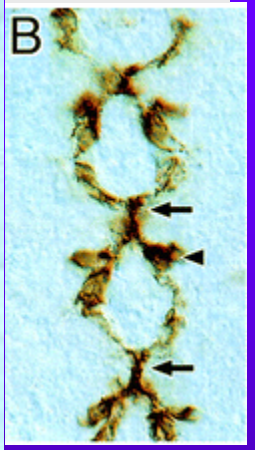


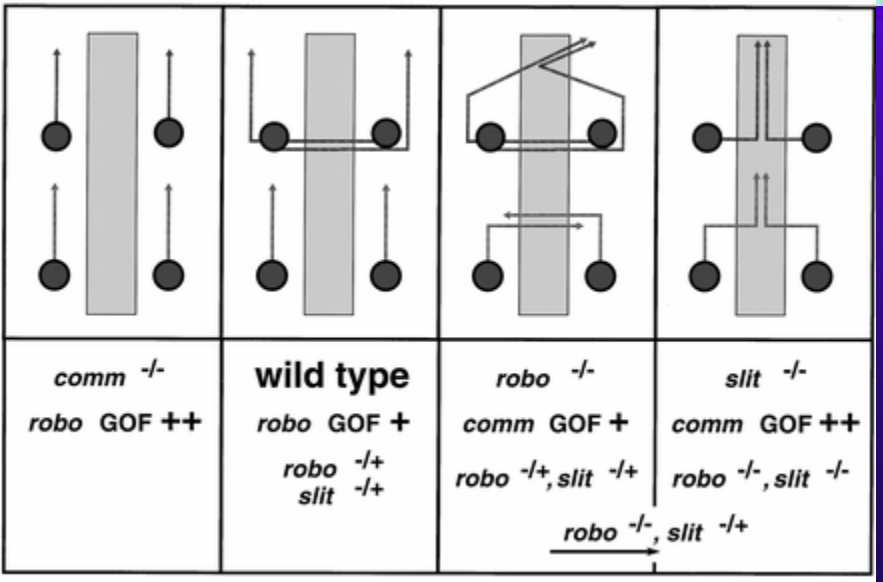

Cingulate gyrus
Septum
Fornix
Olfactory bulb
Hypothalamus
Amygdala
Mammillary body
Hippocampus

Previous studies found Robo and Slit and they bind to each to guide midline crossing.



Slit mutant





- WT embryo: normal pattern of 2 commissures in each segment.
- *robo*^{4} null LOF allele: too many axons cross and recross midline
-) *slit*^{2} null LOF allele: axons enter but fail to leave the midline and instead run along it.

Con't….

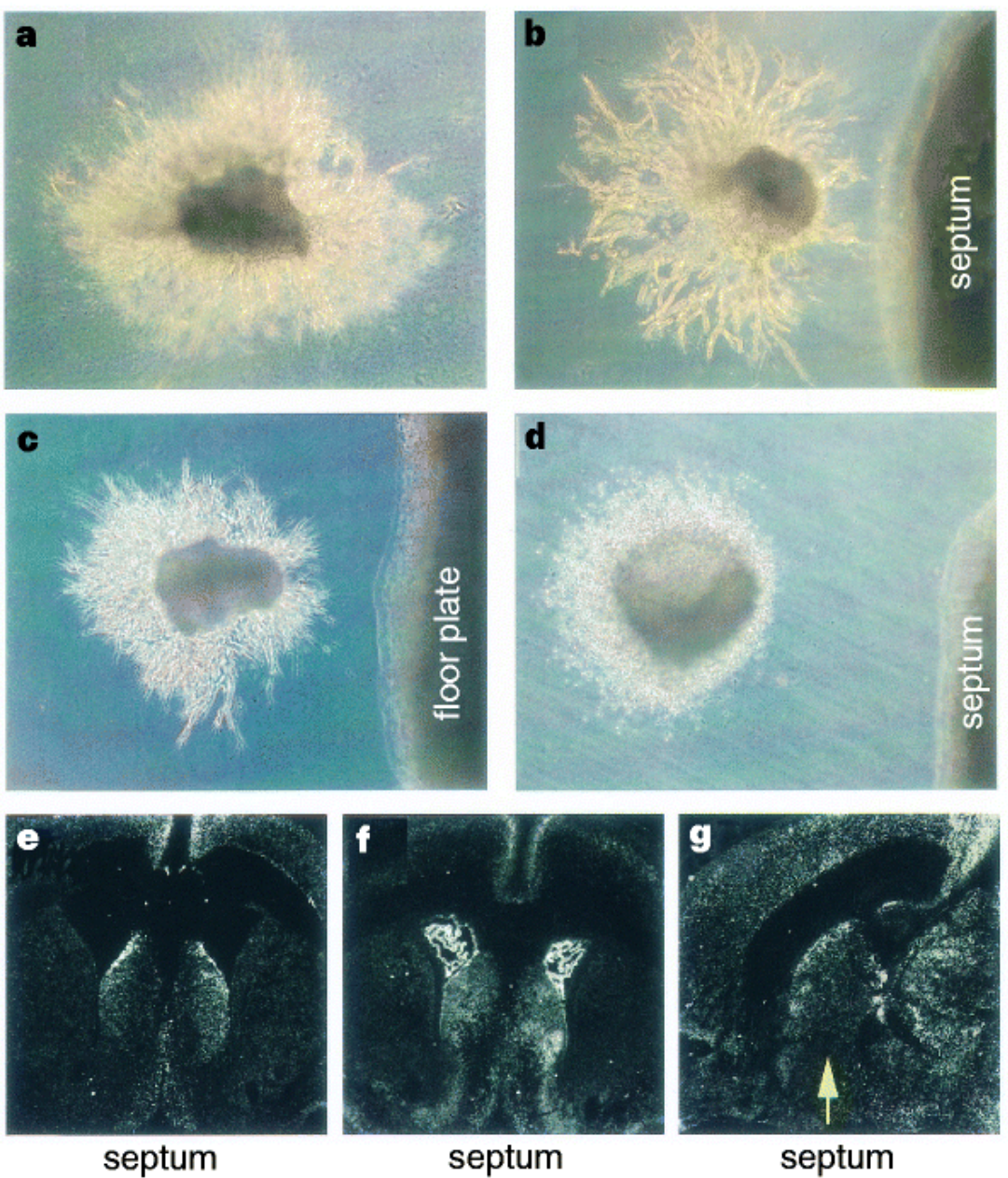
- Experiments involving a different culture method from collagen gel matrices using matrigel found no repellent activity in the septum for migrating SVZa cells.
- Matrigel allows neurons to migrate on top of each other, the chain migration, repulsive sue theory was not well accepted.
- Repellent in septum is unknown.

Methods:

- New born Sprague-Dawley (P3-7)
- In situ hybridization
- Co-culture of explants
- DiO, DiI labeling of postnatal
- Mutant Robo construct
- Epitope tagging (slit, robo,roboN)

Results:

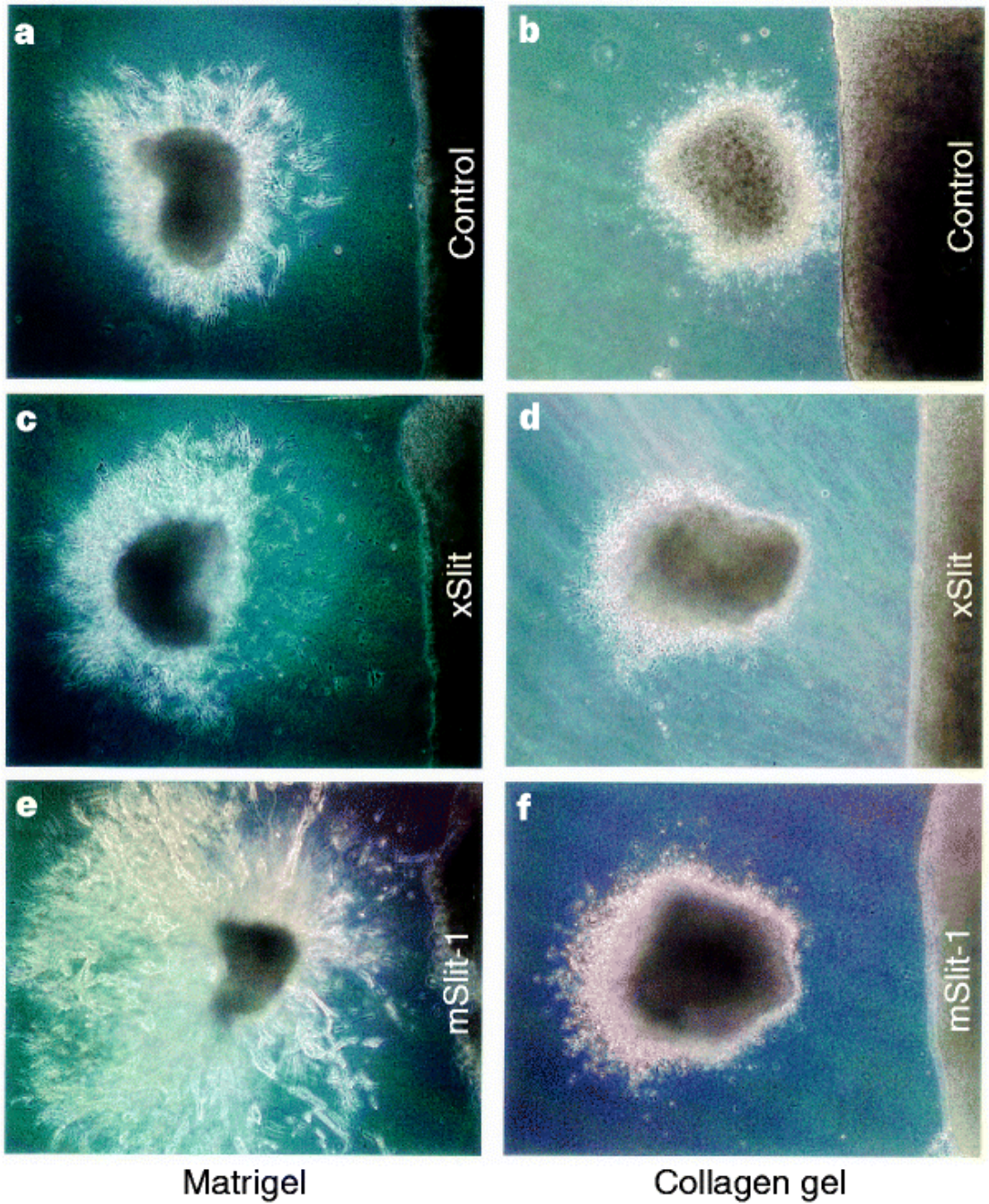
- Postnatal SVZa neurons are repelled by septum and floor plate, on matrigel and collagen gel matrices.
- slit-1&2 is expressed in septum and neocortex, and slit 2 in choroid plexus (secretes CSF)



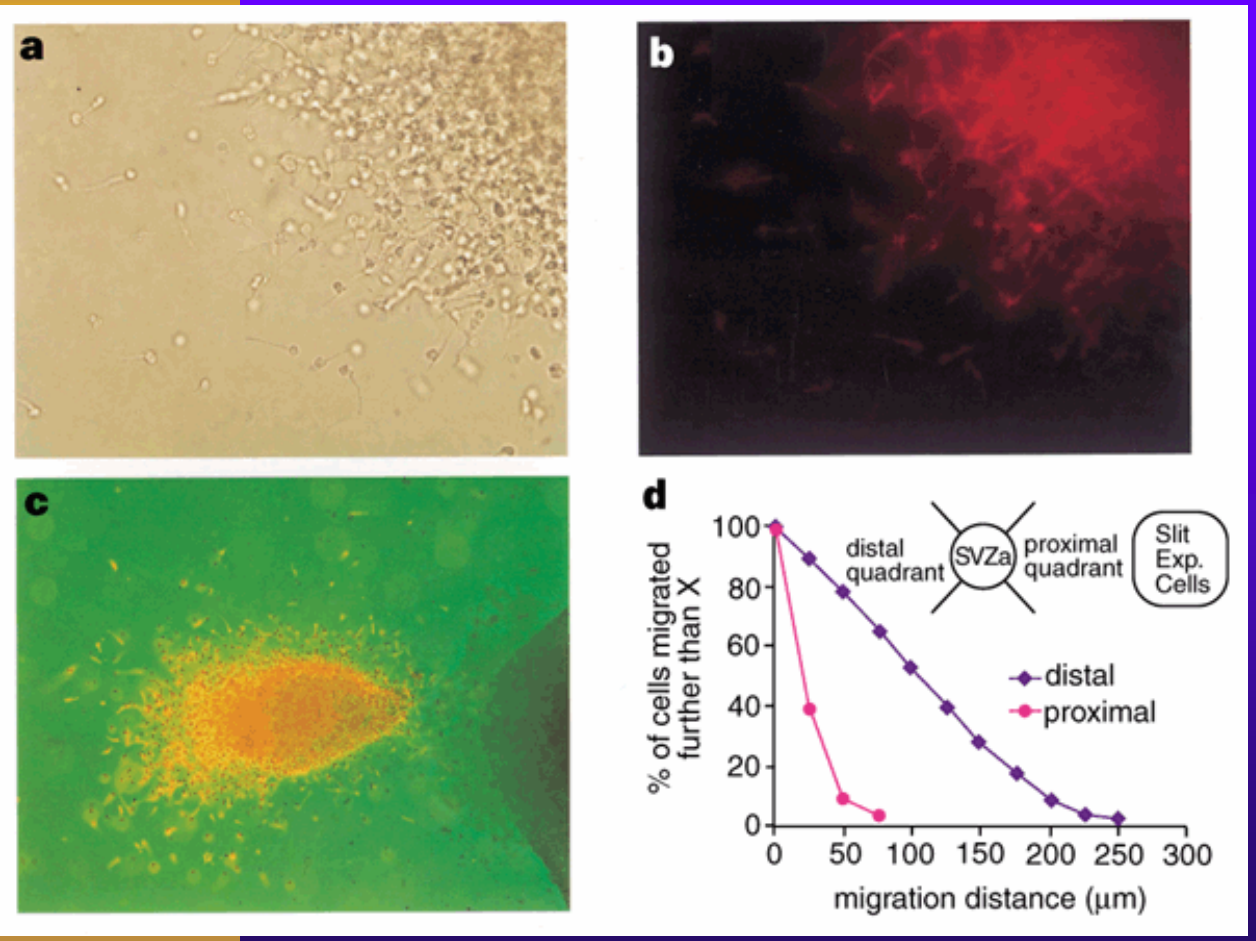
Slit proteins affect neuronal immigration?

necessary/sufficient?

- Day 5 SVZa explant co-cultured with HEK cells on matrigel and collagen gel.
- HEK expressing a control plasmid, symmetrically distributed.
- HEK with xslit and mslit, asymmetrically distributed.
- Collagen gel: slit can act on single cells.



Are the migrating cells neurons?



- Anti-n.s. ß-tubulin Ab, TuJ1, staining showed that they are neurons.
- Uneven TuJ1 staining in explant-> neurons migrate away from slit cells.
- More cells grow longer in distal quadrant.

From the findings….

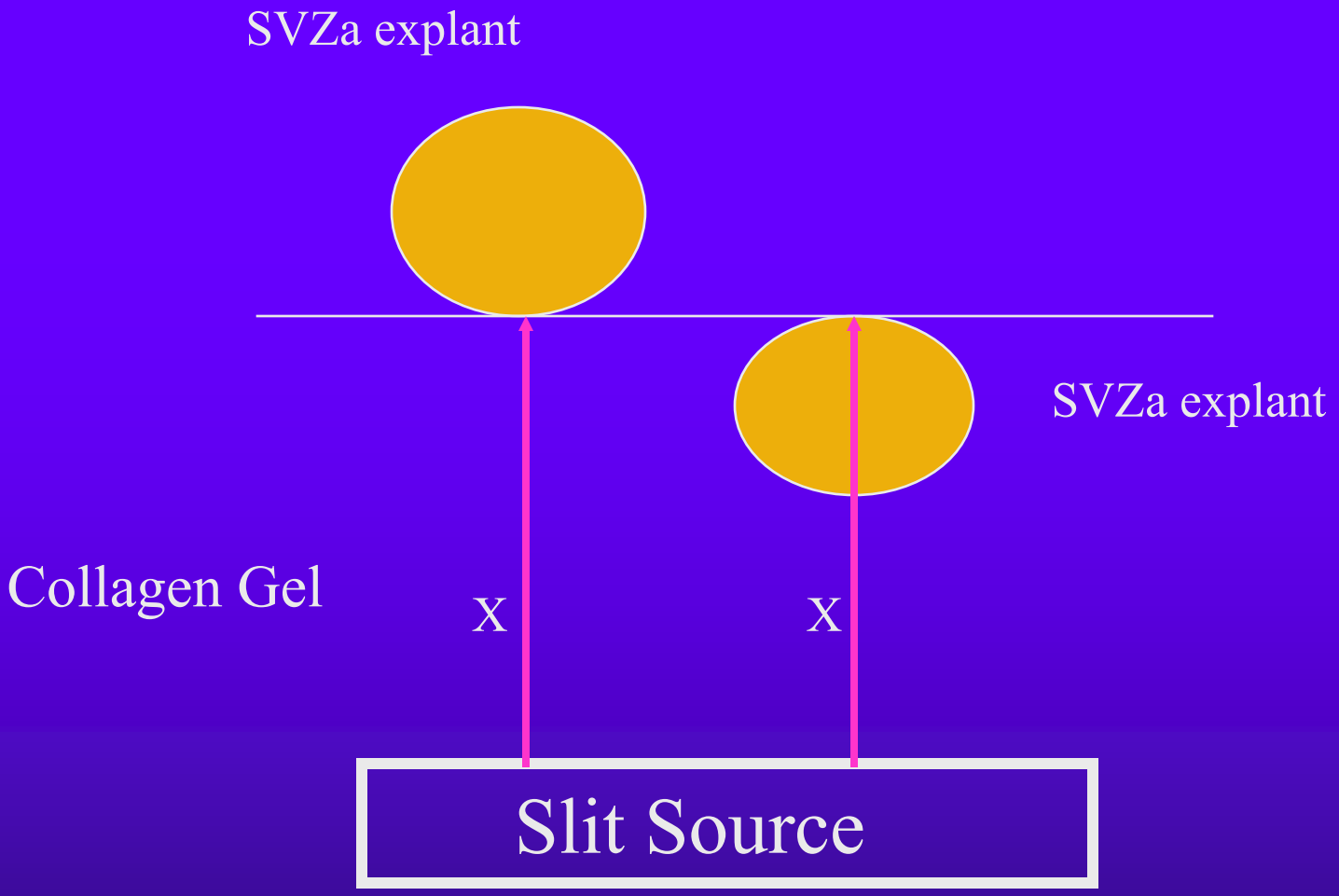
What conclusion(s) can you draw?

Slit inhibits migration?

Or

Slit repels neurons?

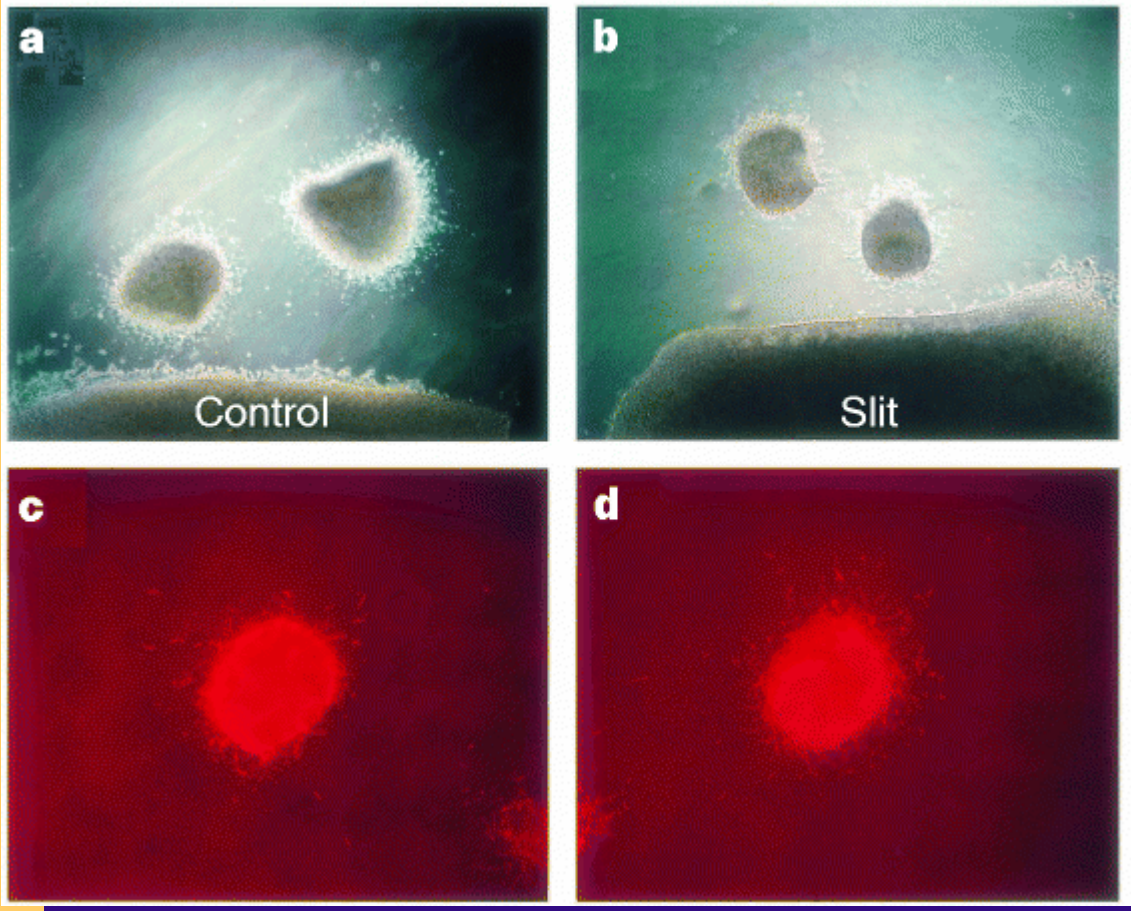
What would you do to test each possibility?



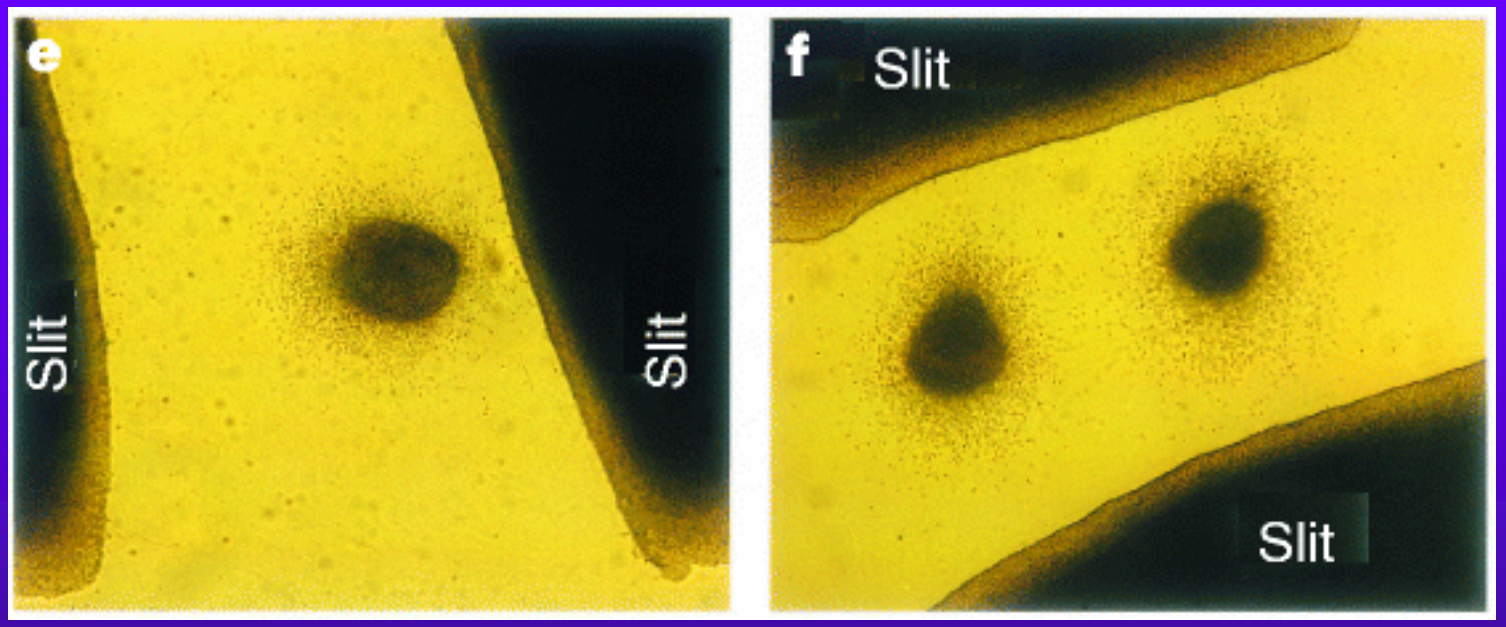
What happens if repelling/inhibition is true?

More cells in distal quadrant in both explants -> repel

Explants on top of slit source, neurons still migrate out -> not inhibition of migration

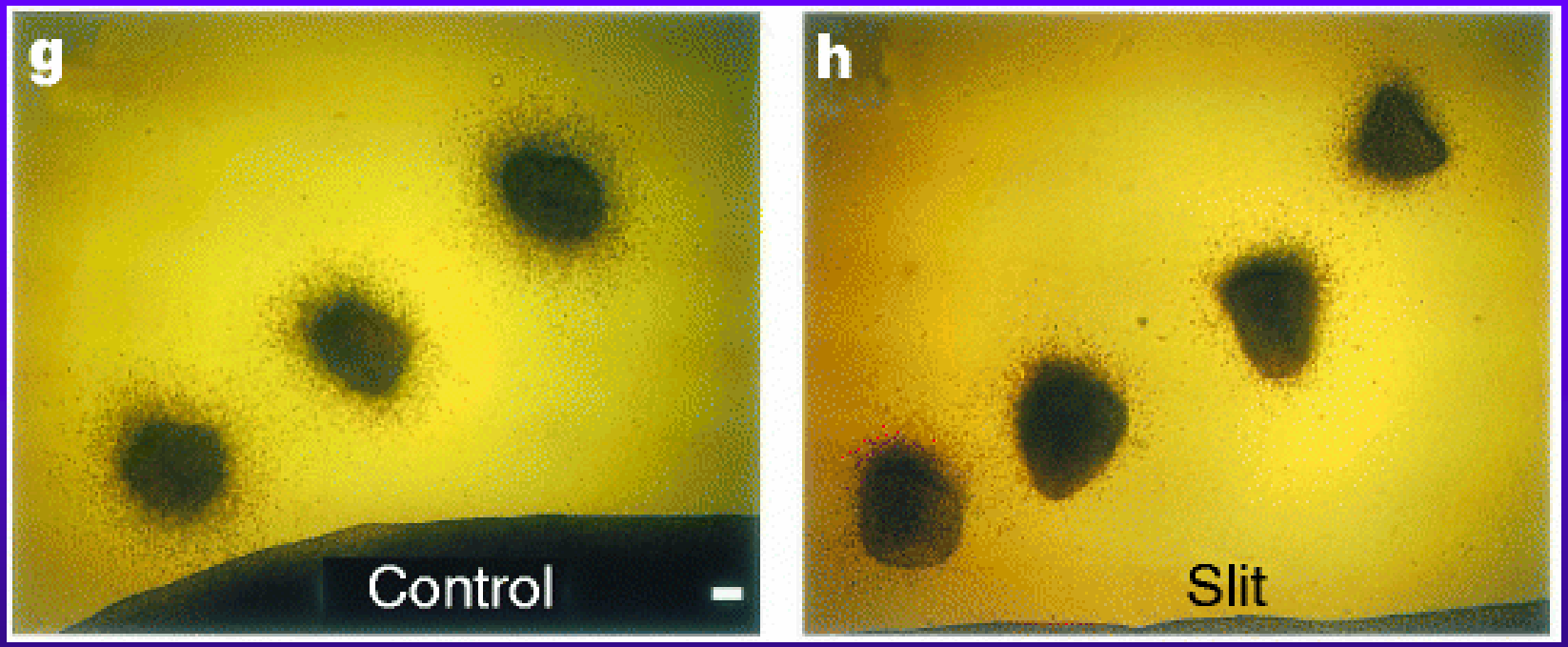


Is the presence or gradient of slit important in neural migration?



- One SVZa explant placed between the two slit sources, closer source more influential.
- Placed at an equal distance results in symmetric migration of neurons.
- Another good prove of NOT inhibition.

How closer should the slit source be in order to influence migration of neurons from SVZa?

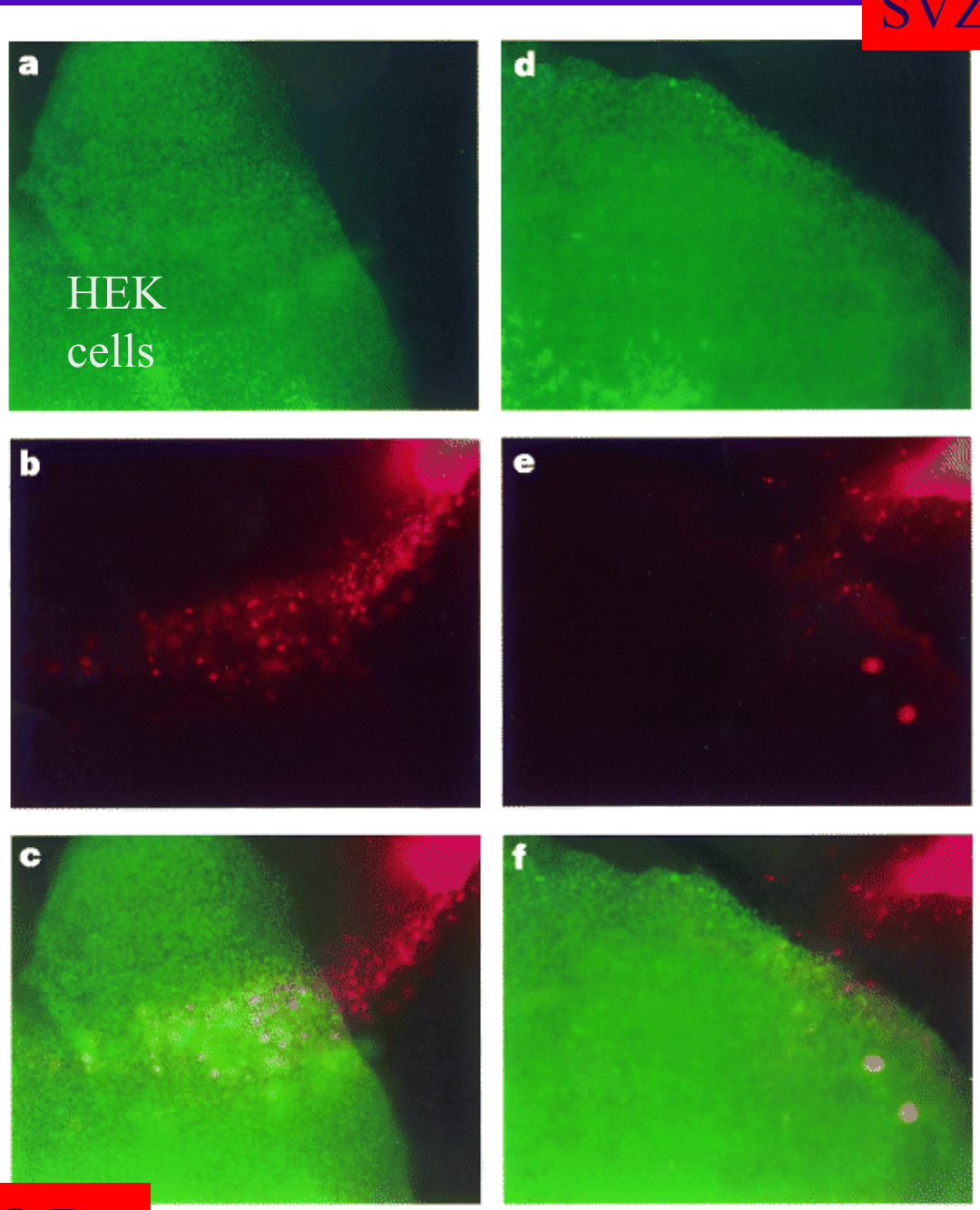


The effective distance of slit is ~1 mm.

Yeah…but can Slit guide migration in natural pathway?

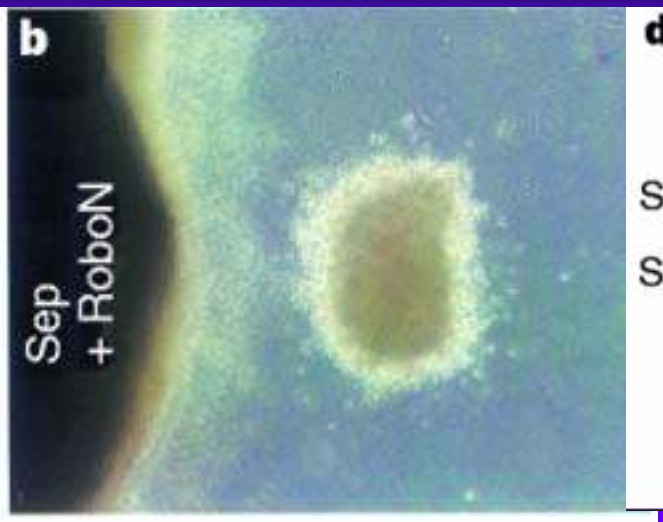
- Sagittal sections of postnatal brains containing SVZa, RMS, and the Olfactory bulb were isolated and cultured.
- Prelabel HEK cells (expressing control vector or Slit) by lipophilic dye, DiO and place on top of the RMS.
- Prelabel neuronal precursors in SVZa with DiI and observe the migration 24 h later.

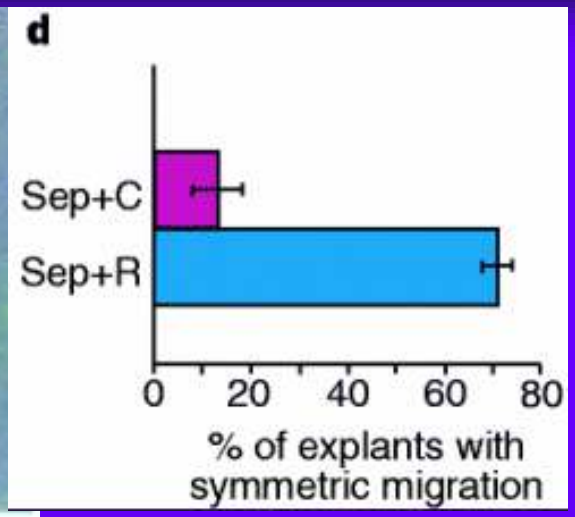
- Control cells don't repel SVZa neurons from migrating into RMS
- Slit cells only allows very few neurons to migrate into RMS.



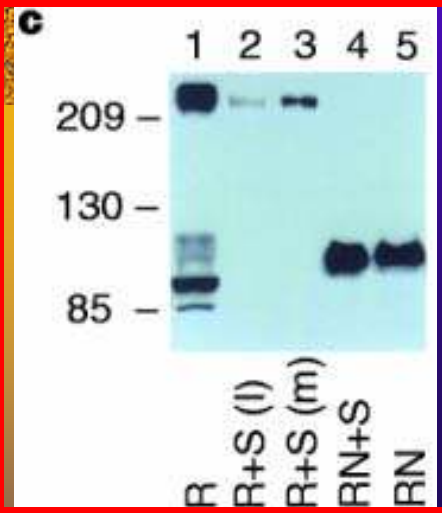
Does endogenous Slit contribute to repulsive activity in septum?

- Robo is the receptor for Slit. Full length 1660 aa.
- RoboN, the extracellular domain of Robo (Robo718aa-HA), was constructed to be secreted into medium and bind to Slit.
- Serve a purpose of inhibiting Slit-Robo signaling, like that of 3FGFR3c.
- Placed explants of septum on top of HEK cells expressing control or RoboN Plasmid and co-culture with SVZa explant.





- S+Control: few % of SVZa have symmetric migration -> effective in repelling neurons
- S+RoboN: mostly symmetric migration.

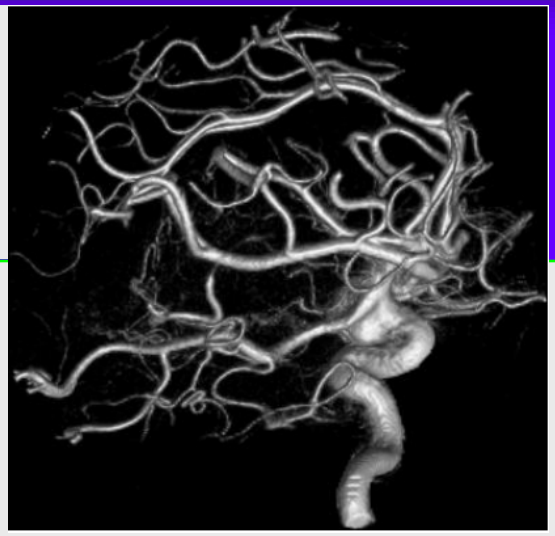


- RoboN is much shorter than full length receptor
- After IP (removing bound Robo/Slit complex) R+S (lysate) <<R+S(medium)-> More slit in medium.
- RN+S(medium) think band -> A lot of RoboN bound to Slit in medium, thus a good inhibitor.

Conclusion:

- Slit is a chemorepellent for neurons migrating from the SVZa.
- Slit acts as a diffusible molecule, the concentration gradient of which guides the direction of migrating neurons, not inhibiting their growth.
- Effective distance from the neurons is ~1 mm.

Discussion:



- previous findings that Slit is an axon repellent support the idea that there are some molecular guidance mechanisms in common between axon projection and neuronal migration.
- Whether slit guides the movement of cell body or axon depends on the responding cell. Is there another molecule involved in the two?
- Neural cell adhesion molecule (NCAM) might act locally to help SVZ neurons migrate.
- In case of unwanted cell migration, inserting Robo (to make the cell responsive to Slit) might offer a potential therapeutic application.

Questions:

- Knocking down slit in Septum result in symmetric migration pattern, does it mean that slit is the only protein in regulating neuronal migration?
- Is there an opposing O.B. gradient that fine tunes Slit gradient? Along the RMS, enough caudal septal Slit (effective in ~1mm) to guide the neurons the whole way?
- Is function of Slit 1 different from Slit 2?