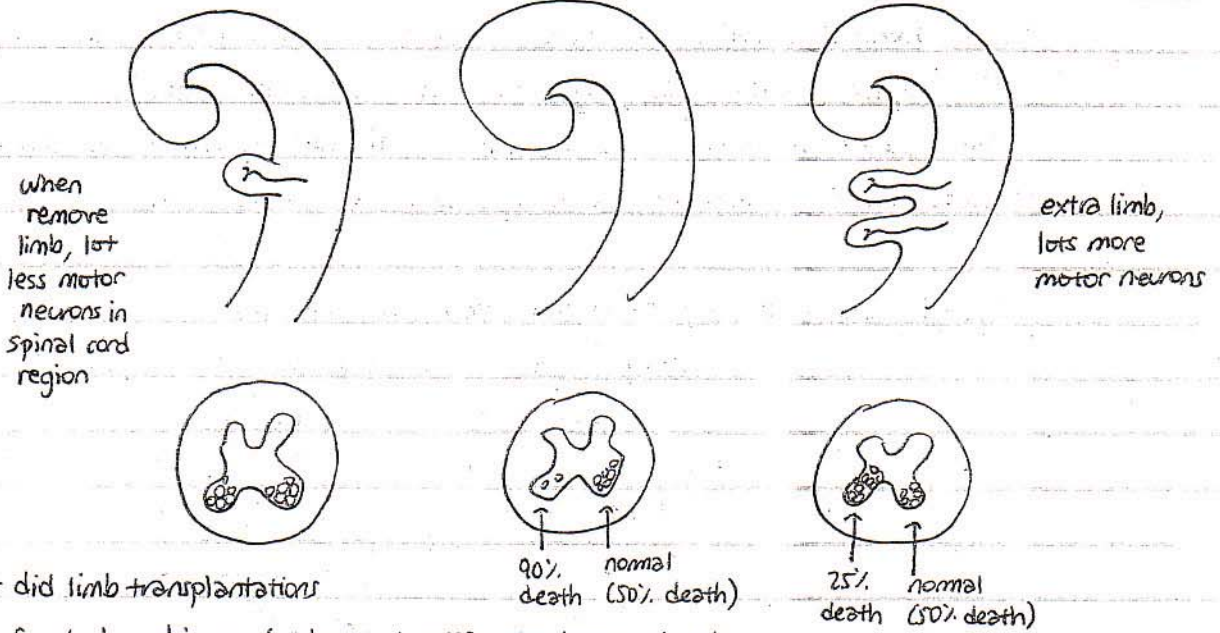


proneural genes - drive neuron cell fate (eg Achaete - scute)

neurogenic genes - limit neuronal cell fate (eg Notch - Delta)

growth factors: many neurons die in formation of neural system

- Victor Hamburger, Rita Montalcini (?), Stanley Cohen



- Victor did limb transplantations

- Rita found that this wasn't about cell proliferation but cell death

- purification of NGF (nerve growth factor): drives motor neuron cell survival

- started looking also in sarcoma cell lines, found similar effects: when digest w/ snake venom, very robust outgrowth of neurons, used snake venom to purify factor

- took submandibular glands of mice (like venom producing parts of snake), purified to single protein to sequence: lead to NGF

↳ whole family, includes BDNF

neurotrophin 3

neurotrophin 4/5

(look very similar to BMPs; lack gives less motor neurons)



bind trk receptors: signal to cell death machinery (turn off): this inhibits caspases through signaling pathway



if leave limb bud intact but paralyze muscles (nAChR antagonists), huge increase in number of neurons

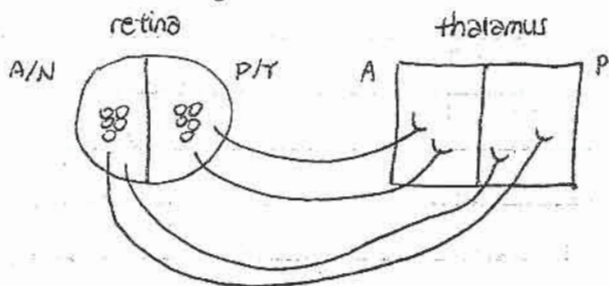
- as soon as muscle gets innervated, doesn't need to have more neurons innervating, turns off NGF (so too many neurons initially → proper wiring of brain)

axonal pathfinding:

- Ramon y Cajal: identified growth cones, proposed idea that growth cones integrate information to find target
- R. G. Hamison: techniques for culturing neurons; saw living neurons migrating 1910
- Roger Sperry: chemoaffinity hypothesis (target secretes factor) 1940-1960
- Corey Goodman: labeled track hypothesis (Abs label specific tracts) 1980
- Mark Tessier-Leigne: genetic manipulation (w/ Corey Goodman)

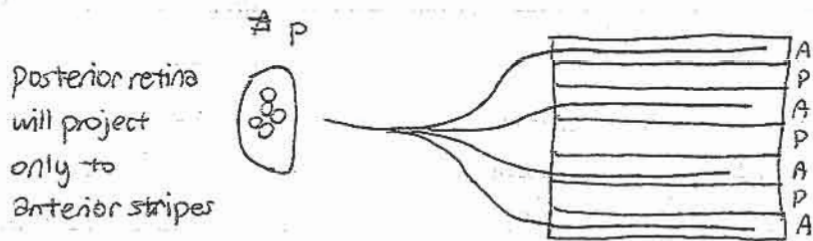
- Sperry's experiment: frog has retina → thalamus connections

very specific regions of retina connect w/ specific regions of thalamus



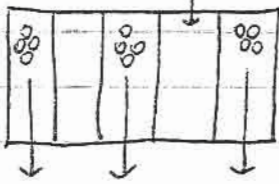
- can cut optic nerve of amphibians: will regrow (mammals won't): rotate eye 180°
- this frog will shoot 180° wrong way at flies
- R-A will always go to T-P, etc

- cut thalamus in A/P stripes, lay next to each other on collagen matrix



- if boil stripes, will kill factor that causes this? no, same pattern of migration
- if boil posterior stripes, axons go everywhere: make inhibitory factor

caudal cells expressing ephrin ligand (repels)



← expressing ephrins
(anterior/nasal axons sense + are repelled by these in thalamus)

- this happens in neural crest cells

- also in axon trafficking (stripe experiment)

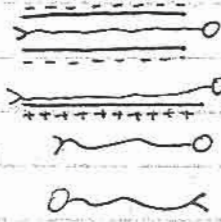
4 ways neuron can get to target: (cues)

1. inhibitory short-range

2. chemoattraction short-range

3. inhibitory long-range (eg ephrin stripes)

4. chemoattraction long-range

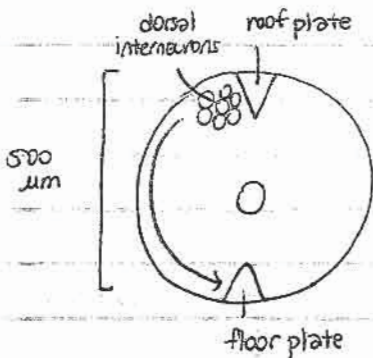


} must be type of cell adhesion molecule, usually

} must be secreted molecule:
50µm → 250µm

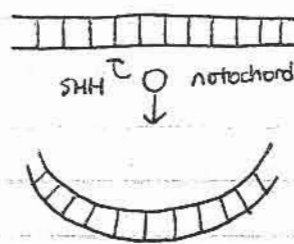
- advantage of negative cue: safety factor built in (if short-range positive cue & axon gets lost, you're screwed)

- all axonal migration events require influencing actin cytoskeleton



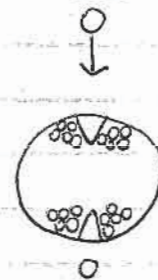
dorsal interneurons migrate through cord to get to floor plate
(could be neg. roof plate cues
pos. roof plate cues
floor
local neg./pos. cues)

- test w/ cell culture: take floor plate, new dorsal interneurons

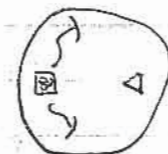


BMPs give dorsal fates, later repel those cells away: also, SHH (along w/ netrin) is attractant at floor plate

- redundancy in nervous system

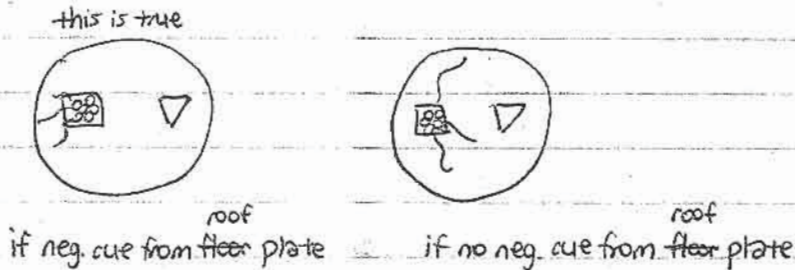


3
(if long-range pos. cue)



(if not long-range pos. cue)

same experiment w/ roof plate



so: positive attractant from floor plate, negative repellent from roof plate. ∴ isolate factors?

(hard to do this b/c floor plate very small, hard to isolate factor)

Marc Tessier-Leigne found that axons also attracted to chick brains, used 20,000 chick brains to isolate attractant factors. (hoped was same one in spinal cord)

- isolated netrin: secreted protein

similarity to laminin (basal membrane components)

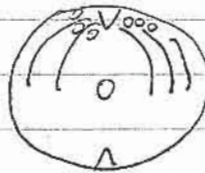
- tested by doing in situ, saw that floor plate making netrin

Knocked out, got axons going about halfway (driven away by roof plate), then stopped

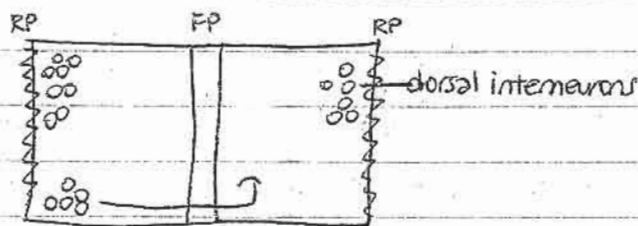
- DCC receptor on growth cones recognizes netrin (deleting this also gets axons halfway)

↳ plays role in colorectal cancer

(in mice?)



- open up spinal cord:

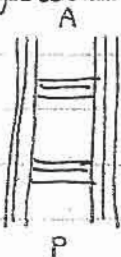


cross floor plate,
head upwards
towards brain

must turn
off netrin signal,
get another signal

maybe floor plate
also secreting something
negative

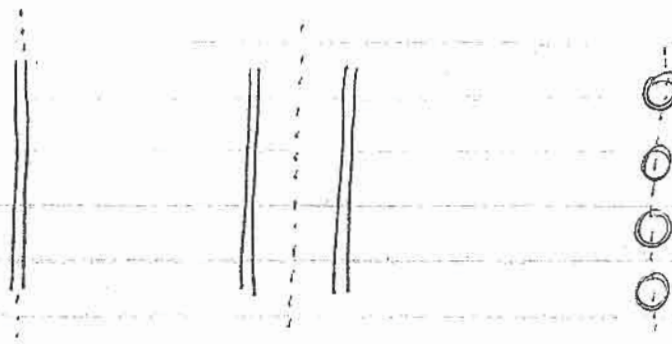
- Corey Goodman's lab dissected w/ genetics:



crossing of midline
of axonal tracts

but can't test in
cell culture (axons
pass through floor
plate, no longer
attracted, but not
repelled)

slit mutant:
mutants:



slit mutant
(or comm GOF
+++)

commissure

commissureless mutant
(or robo GOF)

roundabout mutant (robo)
(or comm GOF +)

slit: may be enhancement of signal, lack of negative signal?

large secreted molecule w/ many extracellular motifs, secreted at midline & gradient outwards (inhibitory): w/o, axons never want to leave midline

commissureless: may be constitutive slit signaling, or positive always off? protein in vesicular trafficking (endocytosis)
neg. signal always on until midline crossed

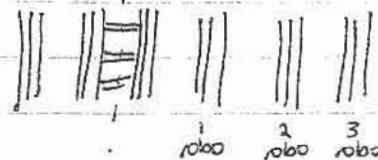
roundabout: receptors for slit
↳ on growth cones

- in floor plate, slit always on: robo binds & is repelled
- positive signal is netrin (bound by DCC)
- somehow can't get negative until after receive positive, must then switch pos. off
- robo expressed only after axon crosses midline, b/c comm inhibits robo by endocytosing & getting it degraded beforehand
- some signal at midline turns comm off, so slit/robo can repel
- robo binding slit lets it bind DCC, inactivating it: won't cross midline again

↳ robo

slit gradient

- axons follow more than one tract:



different tracts
express different
robo receptors:
of robo will
drive you farther
from midline;
robo code

netrin & slit - secreted molecules w/ EGF repeats

- diffuse across ECM

- robo + DCC have extracellular repeats; have IgG + fibronectin repeats, TM domains

- semaphorins also guidance molecules

- bind plexins

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