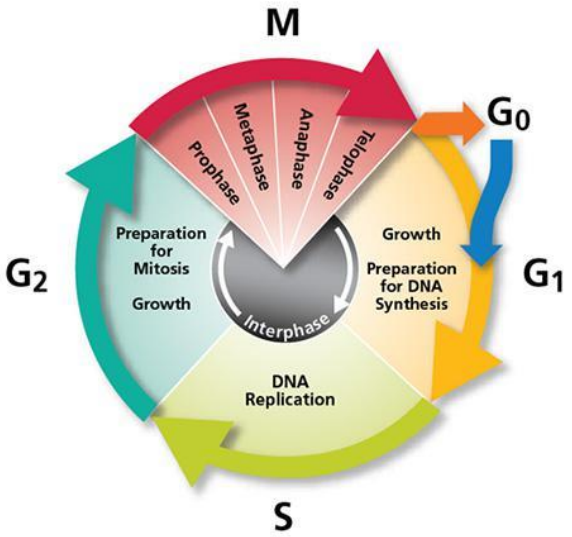
INTRODUCTION

DEFINITIONS

CGE	Caudal ganglionic eminence
CTX	Cortex
DRG	Dorsal root ganglion
EGL	External granular layer
ESPC	Excitatory post-synaptic current
HIP	Hippocampus
IGL	Internal granular layer
LGE	Lateral ganglionic eminence
MGE	Medial ganglionic eminence
ML	Molecular layer
NGF	Neural growth factor
NMJ	Neuromuscular junction
NT	Neurotransmitter
OB	Olfactory bulb
OSN	Olfactory sensory neuron
RGC	Retinal ganglion cell
SVZ	Subventricular zone
VZ	Ventricular zone



OVERALL

PROLIFERATION

- Differentiation ↔ Cell Migration
  - They can't be separated since differentiation depends on migration of the cell and vice versa
- Connectivity: Axonal pathfinding, synapse formation, circuit formation
- Maturation: Cell death, pruning

NEUROGENESIS

It starts with 3 parts in the beginning: **prosencephalon**, **mesencephalon** and **rhomboencephalon**. Each species has an individual way to develop them. In humans:

- Prosencephalon divides into two parts: diencephalon and telencephalon → cortex (covers **most** parts of the brain)
- Rhombencephalon divides into Metencephalon and Myencephalon

DEVELOPMENT

- Development means increase in size und increase in complexity, at **9 months** the brain reaches its **final shape**
- Size of **forebrain** increased during **evolution**
- Structures may look very different in adult organism (→ eye) but their development is very similar in invertebrates and vertebrates, sharing of molecular mechanisms, even if **morphology** differs

NEURAL CONNECTIVITY

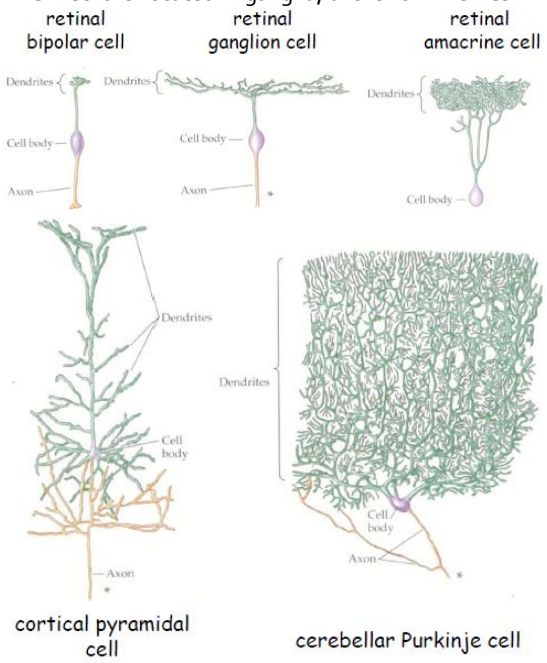
Basis of neural function with 14'000'000'000 neurons interconnected by fibers with total length of 500'000 km. Neurons extend **long processes** to connect to their targets. Axons use **guidepost cells** as intermediate targets, these consist of **attractive** and **repulsive** cues.

SYNAPSE FORMATION

- On average, every neuron is connected to 1000 neurons
- The neuromuscular junction is the best understood model for synapse formation
- Both in ZNS and PNS synapses are eliminated during development and maturation
  - Starts as poly-innervation and then is pruned to one axon per structure

COMPONENTS OF THE NERVOUS SYSTEM

CNS: neurons are arranged into nuclei or into layers, axons form tracts.
PNS: neurons located in ganglia, axons form nerves.



NEURON

Basic cellular element of nervous system

- Afferent neurons: mostly inhibitory
- Interneurons: participating in local aspects of a circuit
- Efferent neurons: excitatory

GLIAL CELLS

3 types in **mature central** nervous system

- Astrocyte: maintain chemical environment of neurons
- Oligodendrocyte: myelination (AP propagation) – Schwann cell
- Microglial cell: scavenger cells

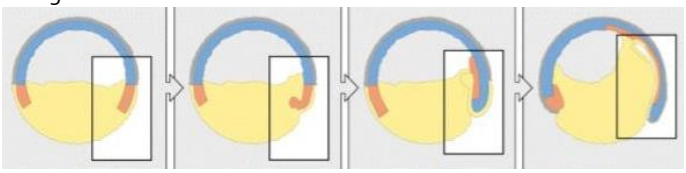
NEUROGENESIS

GASTRULATION

Blastula becomes a gastrula. Produces the three germ layers: ectoderm, mesoderm and endoderm. **Timing** is important, meaning not everything can be repeated at another stage. Cells change, environments change and **same factors** have completely **different effect** on cells at a different time.

- If you isolate cells from same part of blastula but at a different stage, they differentiate into different cell types.
- Cells moving through the blastopore lip

Involution at blastopore → migrate underneath → new environment → changes fate and becomes mesoderm



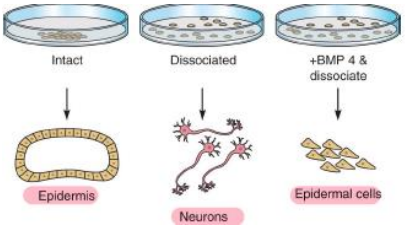
EXPERIMENT BY MANGOLD AND SPEMANN

Induction of **second** body axis after implantation of organizer node (**Henson's node**) and inserted into another blastula (with its own node). It resulted in a frog with two heads and two body axes, node has great inducing power.

- Cells are from recipient blastopore → therefore **organizer** and not an origin

ANIMAL CAP EXPERIMENT

Dissection of animal cap cells (with vacuole) is isolated and grown on a plate → blastula! It reveals neuronal development as **default** pathway. If cells are dissociated and the factor diffuses, neural cells grow. If *BMP4* is added, development into epidermis cells is possible. With cell-cell contact, epidermis is formed.



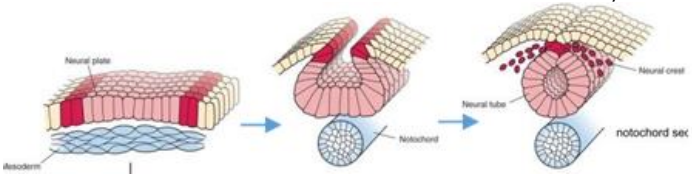
- BMPs lead to prospective epidermis ventrally
- BMP inhibitors lead to prospective neural tissue dorsally

→ Experiment **after** gastrulation you get neurons instead of epidermis with intact cap!

NEURULATION

Neural development proceeds along a **rostro-caudal** gradient, beginning **rostrally** as Hensen's node (organizer) migrates down during development, as embryo forms behind it → nervous tissue

Neurulation begins with building of the neural plate in **ectoderm**. Neural ectoderm then proliferates much stronger than the until both neural crests meet → folding due to faster cell division. It builds a furrow and finally becomes a closed structure: the **neural tube**. The area where the two crests met, is called the roof plate and the tube separates itself from the overlying ectoderm. The cells that fuse in the end are the **neural crest cells** which form the future nervous system.



NOTOCHORD

Underneath the neural ectoderm lies this **mesodermal** structure, which plays an important role in organizing neural ectoderm (**dorso-ventral axis**), by secreting important factors. The notochord is sufficient to induce the **floor plate**.

PROLIFERATION

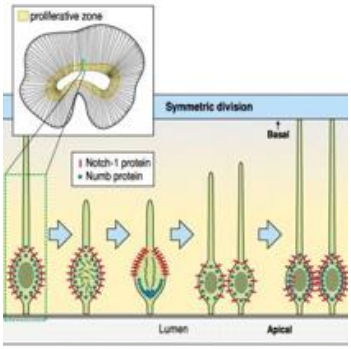
After neurulation cells divide **rapidly** and **asymmetrically**, developing many different **cell types**. After each division, the daughter cells differentiate and **G1** cell stage gets longer.

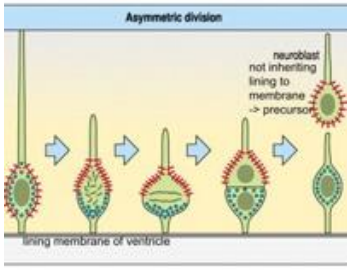
Cells proliferate in the **ventricular zone** of the **neural tube** and specialized areas of the nervous system:

- Brain: ventricular and subventricular [second proliferative zone, because one wouldn't be sufficient in humans] zone
- Spine: ventricular zone
- Cerebellum: ventricular zone and external germinal/granule cell layer

TIMING MATTERS DURING NEUROGENESIS

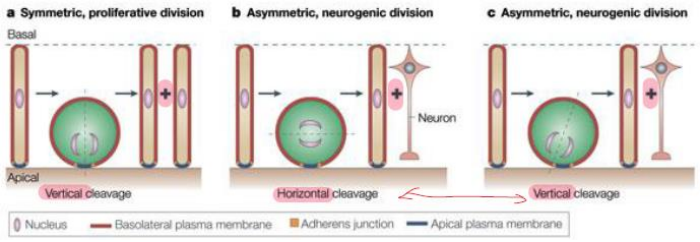
- **Expansion phase**: fastest way of making cells, when you've made enough you can make other cells (mostly symmetrical divisions)
- **Neurogenic phase**: towards the end again more symmetrical than asymmetrical division, because less stem cells needed





ASYMMETRIC CELL DIVISION

The cell with '**membrane attachment site**' (blue) stays a **stem cell**, the other one differentiates



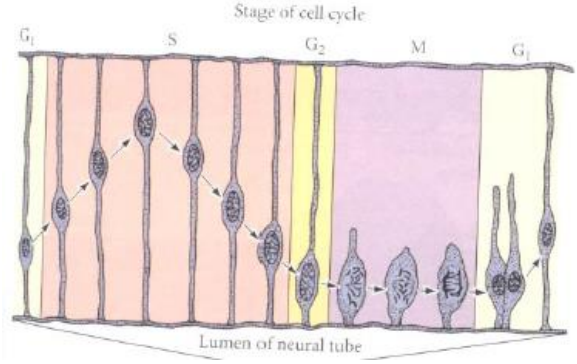
SNAREs decide on symmetric **vs** asymmetric division, it is essential **which type** of SNARE proteins are used

- Basal-**apical** combination results in a symmetric proliferative division
- Basal-**lateral** in an asymmetric, neurogenic division

→ Basel = v-SNARE, Apical and Lateral = t-SNARE

OSCILLATING MIGRATING BEHAVIOR

- Stem cells span the whole **lumen** of the **neural tube**
- For doubling the DNA, the **cell body** migrated **up**
- For the division phase, it **retracts the process** from **basal** surface
- One daughter remains **attached** to **apical** side

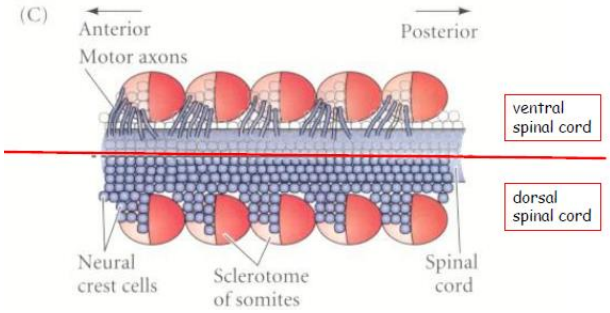


SEGMENTATION

- Sensory neurons are found in DRG along anterior-posterior axis

HOW IS SYMMETRY ACHIEVED

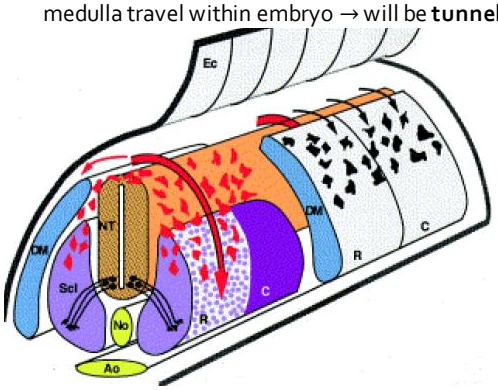
- Somites are essential for the segmentation of the PNS
  - Somite structures have anterior and posterior parts
  - Cells can **only migrate** through **anterior parts** → **Tunneling** cells into certain areas (higher density per volume)
- Gaps because of signal, causing inhibition of migration in certain areas



MIGRATION AND DIFFERENTIATION CANNOT BE SEPERATED IN NEURAL CREST DEVELOPMENT

Different **factors**, different **environments** → different **development** → influence on migration and differentiation

- Non-neural cells
  - Melanocytes [black dots in picture], *connective tissue and skeletal tissue* travel along ectoderm (future skin) and will not be tunneled → Melanocytes are **NOT** affected → therefore homogenous migration and pigmentation
- Neural crest cells
  - Migrate near aorta
  - Caudal part of the somite is inhibitory for neural crest cell migration
  - Sensory neurons, peripheral glia, autonomic neurons, adrenal medulla travel within embryo → will be **tunneled**



CELL CYCLE

- The overall length of the progenitor cell cycle increases during embryogenesis
  - **G1** phase is getting **longer** → factors can influence this phase
- Cells in **Go** resting phase can be influenced by factors from the environment to divide

CELL LENGTH HYPOTHESIS

- 2 cells that are in the same environment, are stochastically slightly different
- Factors from the environment have an influence on these cells
- Proteins
  - Increase in the difference of the cells (accumulation of proteins)
  - One is over threshold sooner than another → asymmetric cell division
  - When both are above threshold → symmetric division again

→ Time of protein accumulation deciding on division type!

CELL DIFFERENTIATION AND PATTERNING

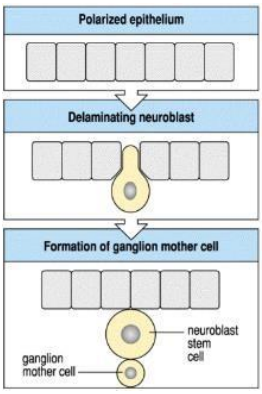
HOW TO MAKE NEURONS AND GLIAL CELLS

LATERAL INHIBITION

Defines the **number** of neuronal cells. All cells of **ectoderm** are **identical**, there are only slight differences on the molecular level. Some express a certain factor more than the other, this is achieved through the activation of the *Notch* signaling pathway, which changes the pattern of gene expression.

- *Delta/Notch* Feedback-Loop: *Delta* can bind to *Notch* receptor of **neighboring** cell, whose intracellular part will enter nucleus and **downregulate** *Delta* expression → the bigger one signal gets, the more it can downregulate the surrounding cells
  - Activation of *Notch* signaling prevents development of cells into neurons → **regulation** of **proportion** of nervous tissue

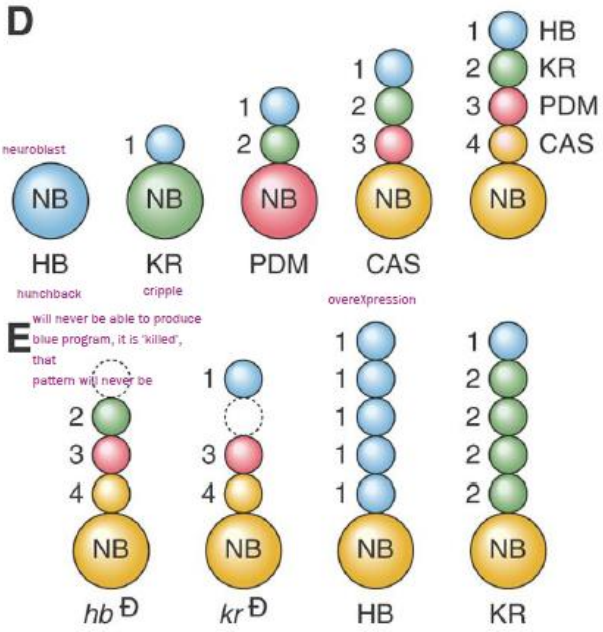
NEUROBLAST FORMATION



- Neuroblasts divide asymmetrically to give a neuroblast stem cell a ganglion mother cell
  - A cell from the **polarized epithelium** differentiates and then delaminates
- Asymmetric division:
  - Ganglion mother cell (will divide **exactly once** symmetrically)
  - Neuroblast stem cell (can go on with asymmetric cell divisions)

EARLY AND LATE-BORN NEUROBLASTS DIFFER

- They adopt new potentials; different programs get turned on by transcription factors → neuroblast will divide asymmetrically in the program it is **currently** in



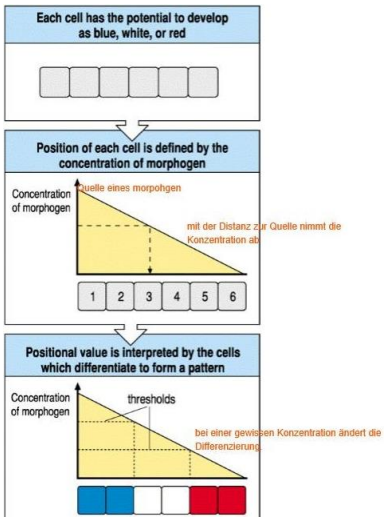
- Analogue: Progenitor cells in the retina give rise to different cell types depending on **time** → progenitor slightly changes over time → gives rise to different types of cells
- Oligodendrocytes and motoneurons are derived from the same precursor pool (**location** the same but **time** changed)

PATTERNING THE NERVOUS SYSTEM

The neural tube has an anterior and a posterior end. The cells that involute first will be at the brains end (rostral) → first step of body axis formation.

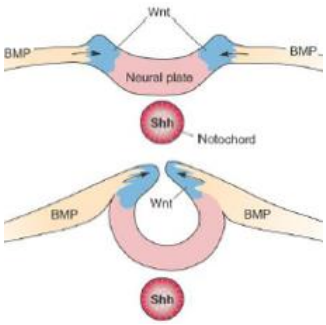
FRENCH FLAG MODEL

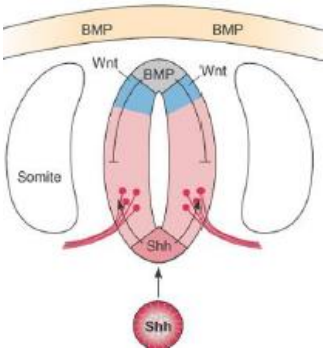
- Describes how cells become different from each other
- Not only morphogens decide differentiation but also HOX-genes



POLARIZED NEURAL TUBE - DORSOVENTRAL

BMP and Shh polarize the **dorso-ventral** axis of the neural tube. The **crest cells** which form the **roof plate** (ventral) produces ***BMP***. The **floor plate** (ventral) is induced by ***Shh***. These two factors act on all the cells lying between roof and floor plate.



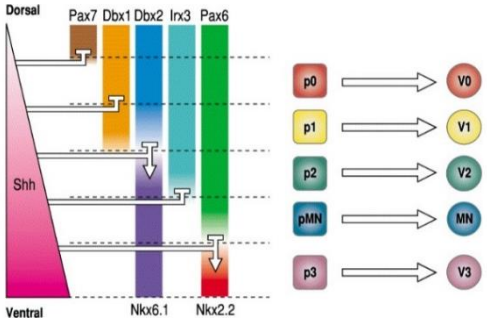


SHH

Shh ventralizes the spinal cord and the **concentration** of *Shh* determines **cell type** in the **ventral** spinal cord → highest concentration of *Shh* is found at ventral part of spinal cord.

- **Repression** of **class I** homeodomain (Hox) genes [Pax, Dbx, Irx]
- **Induction** of **class II** genes [Nkx]
- **Mutual repression** produces sharp boundaries
  - Overlapping gradients create a **cross repressive** system

Neurons along the DV axis of neural tube are characterized by specific patterns of transcription factor expression with continuous refinement of programs and cells.



THE NOTOCHORD -CHORDA DORSALIS

- If the notochord is removed the neural tube will **not** differentiate
- If you transplant a second notochord, a **second floor** plate develops → two ventral halves

PATTERNING OF ANTERIORPOSTERIOR – LONGITUDINAL AXIS

The brain contains two organizers that pattern the brain along the AV axis. This axis is organized by **Hox gene** expression patterns.

- **MHB** (Mid-Hindbrain-Boundary)
- **ZLI** (Zona Limitans Intrathalamica)

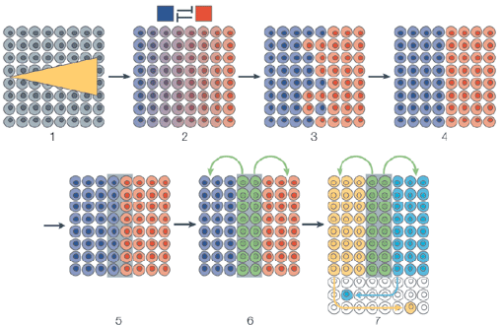
HOX GENE EXPRESSION PATTERNS

Hox genes are expressed in different segments of the nervous system, so we speak of **segmentation** instead of the whole length of the axis.

- Gradients not stable
- Differentiation guided by HOX genes: longitudinal axis organized by HOX gene expression patterns → Rhombomere [seven transient bulges, sites of differential cell proliferation (faster division at rhombomere boundaries), differential cell mobility (not easy to cross into adjacent one) and differential cell adhesion (preference to stick to cells of own rhombomere] identity is determined by HOX code
- Hox genes help cranial nerves find their destination

MODEL FOR BOUNDARY FORMATION

Cells try to be surrounded by the same cell types → clear boundaries. If one cell is different from neighbors and doesn't fit in, it will migrate to the right place. A new factor at a boundary can induce a new cell type.



SUMMARY

- Basic patterning mechanisms are conserved between vertebrates and invertebrates
- **Organizers secrete morphogens** which induce specific gene expression patterns in responsive cells
- Responsive cells differentiate to distinct cell types according to the morphogen concentration found at **their** location
- Boundaries are formed by a combination of mutual repression and cell sorting
- **Timing** is important in development

PAPER

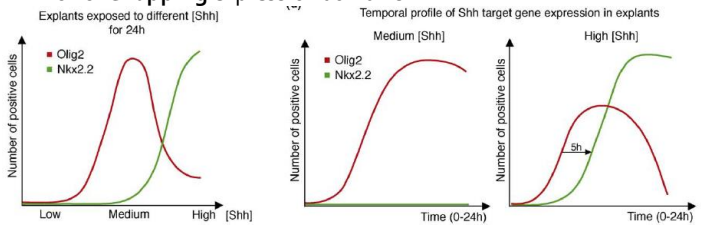
TEMPORAL DYNAMIC OF PATTERNING BY MORPHOGEN GRADIENTS – KUTEJOVA (2009)

Morphogens act as **graded positional cues** to control cell fate specification in developing tissues. They are signaling gradients regulating differential gene expression in a concentration-dependent manner. New observation that pattern formation is a **dynamic process** has raised questions about the influence of **time** on **morphogen activity**. This paper proposes that **spatiotemporal dynamics** of a cellular response to a morphogen gradient depends on a **combination of temporal alterations** to the gradient itself, the signal transduction and downstream interactions between target genes.

Cells **directly** respond to morphogen concentrations by activating different target genes at distinct **concentration thresholds**, which produces **spatially** organized domains of gene expression. This model says that this proportional gradient of **signaling activity** and **cellular response** depends **exclusively** on **signaling levels** → this view is widely accepted but **new complexity** is being added.

POINTS TO MORE COMPLEXITY

- Relative sizes and positions of target gene domains change during development
- Different morphogen target gene activation occurs at different times
  - ❖ *Olig2* expression is initiated **before** *Nkx2.2* → as *Olig2* expression rises, the more ventral cells activate *Nkx2.2*. The *Nkx2.2* expressing cells than **downregulate** *Olig2* expression → generating two **nonoverlapping** expression domains.



Examples suggest that target genes requiring **higher morphogen concentrations** are induced **later** than those responding to lower concentrations. → Time at which a target gene is detected gives us info to its concentration response and its final expression pattern.

TEMPORAL CHANGES IN MORPHOGEN GRADIENT

Morphogen concentrations change in tissues over time meaning the gradient is **not fixed**. This means threshold concentrations for a given target gene will occur at different positions in the tissue at different times. This will cause the pattern of gene expression to shift **with time** → **temporal evolution** of spatial pattern depends on changes of the gradient as **development proceeds**.

Concentration profile is determined by morphogens:

- Diffusion coefficient
- Degradation
- Production rate

After the onset of production, it needs time to achieve its **steady state**, until then the concentration at different positions will gradually increase.

- **Exponential** gradients reach their steady state quicker (*Bicoid*)

For tissues that are patterned **rapidly** (comparable to $\frac{1}{degradation\ rate}$), changes in expression patterns may be a consequence of target genes responding to the changing gradient as it **relaxes** to **steady state**.

- In other cases, transcriptional responses are established over a **longer** time that the **relaxation time** of the gradient (*Dpp*)

In these long-time scales of pattern formation, changes in the shape of the gradient will depend predominantly on **temporal changes** of the **kinetic parameters** that govern morphogen gradient **formation** and **maintenance** → diffusion, degradation and production rates.

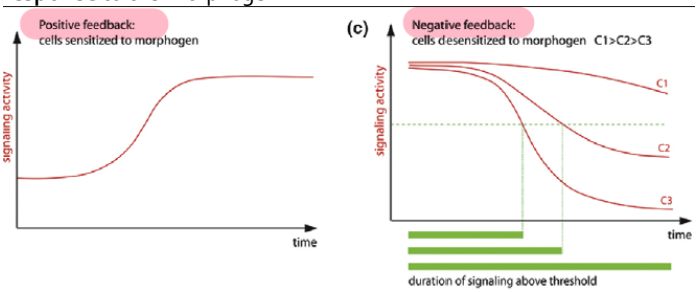
- ❖ The shape of the *Shh* gradient in the neural tube is dynamic, with **increasing amplitude** as development proceeds, this is most likely due to the increase in *Shh* producing cells. A contributing factor are *Shh* binding proteins such as *Ptc1* (Shh receptor) is expected to modulate effective diffusion and degradation rates of the morphogen, and the switch from *Olig2* to *Nkx2.2* expression → resulting in **temporal changes** in *Shh* **distribution**.

TEMPORAL CHANGES IN SIGNAL TRANSDUCTION

Signaling cascades involve binding and activation of target receptors on plasma membrane, activating downstream transcription factors, which enter nucleus and activate gene expression. The **time** it takes for this signal transduction will **influence** the spatial and temporal **profile** of signaling activity in responding cells

- If **fast** and **with linear** amplification, the levels of activated transcription factor **will be proportional** to morphogen concentration at a given position and time (*Dpp*)

The ongoing level of signal transduction can be altered by **cellular response** to the morphogen.



- ❖ Positive feedback can be seen in Drosophila *Cv-2* which modulates *BMP* signaling by **promoting** *BMP*-receptor interactions in *BMP*-dependent manner → **bi-stable** signaling activity profile.
  - ○ Enhancement of receptor-ligand binding due to previous signaling!
- ❖ In case of *Shh* the signal transduction pathway introduces **nonlinearity** in the cellular response, with the **morphogen concentration** determining the **duration** of **signaling**. There is a 'temporal adaptation' mechanism, where cells become progressively **less** sensitive to *Shh* exposure
  - ○ **High** *Shh* leads to **slow** decay (C1) and **lower** *Shh* leads to **faster** decrease in signaling activity (C3) → *Shh* triggers production of *Ptc1* which accumulates and progressively higher levels of *Shh* are required to sustain signaling activity levels → gradual **desensitization**

TEMPORAL CHANGES IN MORPHOGEN-REGULATED TRANSCRIPTIONAL NETWORK

A reason for **sequential** gene activation could be that the **induction** of the **later response genes** requires **prior changes** in the **transcriptional state** of responding cells, these changes themselves would depend on an earlier phase of **morphogen signaling** → sequential cell context.

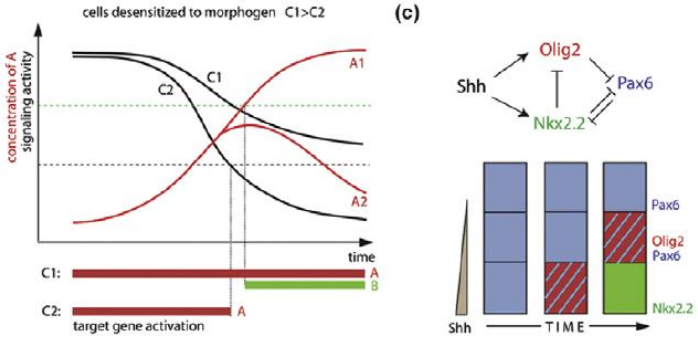
A mechanism able to produce this behavior is a **feed-forward loop**, which has been proven in the case of *Dpp*.

- ❖ *Dpp* **directly** induces expression of transcription factor *zen*. *Zen* then in **combination** with *Dpp* signaling activates a **second gene** *race* → *race* and *zen* depend on *Dpp*, but *race* expression is delayed due to requiring *zen* expression.

CONCLUSIONS

NO MORPHOGEN NECESSARY

Downstream networks can be complex, multifaceted and the structure of the network can lead to cases in which a gradient of morphogen is **not** required. The signal could act simply as an on/off switch which initiates patterning and subsequent **gene induction** relies on a **regulatory network**.



- B is only expressed when A reaches threshold with morphogen (black), if amount of C is decreased before A has been able to be produced → no production of B
- *Nkx2.2* accumulation requires **repression** of *Pax6*

INSUFFICIENT EXPLANATION

Transcriptional networks can explain delayed onset of activation of some morphogen target genes and can produce complex temporal patters, in many cases they are **not sufficient** to explain morphogen responses in cells.

- Maybe because transcriptional network never stabilizes across tissue due to continuing changes to morphogen gradient and tissue growth

SUMMARY

Spatiotemporal dynamics of cellular responses to morphogens depend on:

- Changes of morphogen gradient itself
- Dynamics of its signal transduction
- Downstream interactions between target genes

→ Combination of all three

It is challenging to determine which steps introduce nonlinearity and/or are rate-limiting and how these mechanisms achieve the accuracy and robustness that characterizes embryonic development.

FUTURE RESEARCH

Quantitative analysis of morphogen concentration, signaling activity and target gene activation in **real time**. Necessity for biosensors and tools to measure activity of transcription factors and other components of morphogen signaling pathways.

AXON GROWTH AND SURVIVAL

The complexity of axon tracts increases rapidly during early stages of development → already after 36 hours. Early tracts are used as scaffolds for the following tracts. An axon must **survive, grow, find and get to** target, **recognize** target and finally **connect to** target.

SURVIVAL

Trophic factors are essential for survival. They are helper molecules that allow a neuron to develop and maintain connections with its neighbors. These small proteins work through receptors on surface of nerve cells → Nerve growth factor (**NGF**) [decides survival or apoptosis].

Amount of neurotrophic factor available can predict how many axons/neurons will survive. The factors are produced in a limited amount, if axon receives enough it will survive → **reducing** the target **area** enhances cell death. The receptors are very factor-specific.

Cells that die by **apoptosis** can be **recognized** by specific features → DNA fragmentation can be labeled by **dUTP-biotin**. During apoptosis, there are **apoptotic bodies** (through process of pyknosis), which are phagocytized, and **cross-linking** of proteins. The whole process requires protein **synthesis** → active process.

- ❖ An experiment showed that when transcription is **blocked** there is **less** apoptosis than in a control organism.

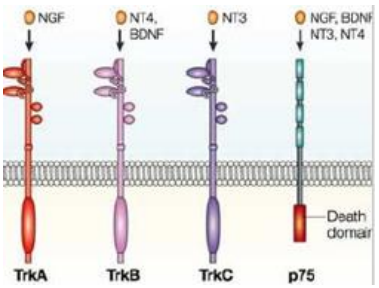
NEUROTROPHINS

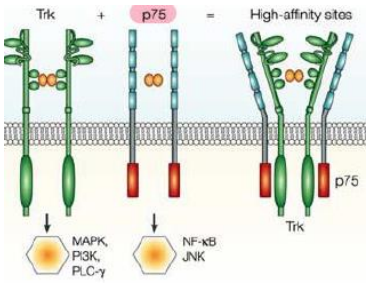
Two functions: **keeping neurons alive**, adjusting their number in tissue and an error mechanism to **eliminate wrong neurons**.

SUPPORT SURVIVAL OF **SENSORY** NEURON SUBTYPES

3 different tracts (A, B, C) that respond to the binding of different neurotrophic factors by releasing signals for the cell to stay alive

- P75 which is responsible for death process is **blocked** when **bound** to the tracts → creating **high affinity sites** for neurotrophins





TARGET-SPECIFIC RELEASE

Different factors are expressed in different tissues, which produce **different** neurotrophins. Neurotrophins make sure that 'wrong' neurons **die** when they are in the wrong place → **selective support** of neurons.

- The transmitter in **skin** is *NGF* (sensory)
- The transmitter in **muscle** is *NT3* (motor)

ADAPTING INNERVATION

Neurotrophins allow the adaptation of innervation to tissue size.

- There is less cell death in the DRGs of the limb buds → more neurons needed for extremities

NEUROTOPHIN SIGNALING

It requires endocytosis and retrograde transport

INTERNALIZATION

Mechanism unclear may be clathrin-dependent or independent, but it known that an **intact cytoskeleton** is needed.

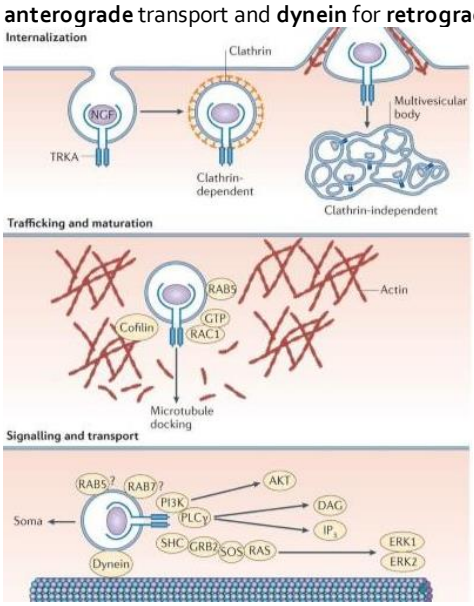
TRAFFICKING AND MATURATION

Actin guides complex to dock on microtubule. Actin is needed to allow uptake of information.

- *Cofilin* signaling leads to an 'actin turnover', as an actin-binding protein it regulates assembly and disassembly of filaments

SIGNALING AND TRANSPORT

Movement along microtubule with help of motor proteins. **Kinesin** for **anterograde** transport and **dynein** for **retrograde**.



SURVIVAL

Neurotrophins **prevent apoptosis** and **support synaptogenesis** and innervation. They are **not** the same thing as axon guidance factors!

GROWTH

Axons extend long processes to connect to targets. Mechanically **not** easy to **maintain** the structure because you need a **cytoskeleton** to maintain the overall structure and transport processes. Growth cones depend on a **dynamic cytoskeleton**.

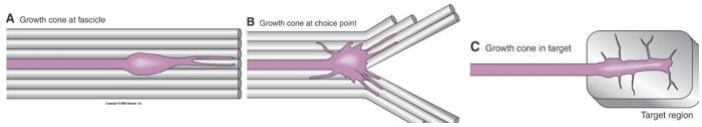
GROWTH CONE

Axons grow by adding new **microtubules** at **distal** end. They also grow by **stretching** during growth of body (still same axon as when a baby).

- Only this growth when whole organism is also growing, but during development the stretching is hardly enough, instead needs new material by adding element **within** the axon and not at the end.
- Mature circuit hardly grows so more stable than in development

SHAPE

The shape of the growth cone differs depending on the **environment** or **growth phase** of the axon.



FASCICLE
A bundle of structures, such as nerve or muscle fibers, pointed with little filopodia that follow direction of tract

CHOICE POINT
Larger and more complex growth cone with filopodia in all directions to explore environment (but always on tract surface)

IN TARGET
First overshoots target, then **retracts** and spreads within, deciding which contacts are most **efficient** and only those survive.

SPEED

The speed of growth cone depends on **location**. The speed **drops** when it gets to target region where the environment needs to be sampled and there are cells to connect to and places to grow synapses.

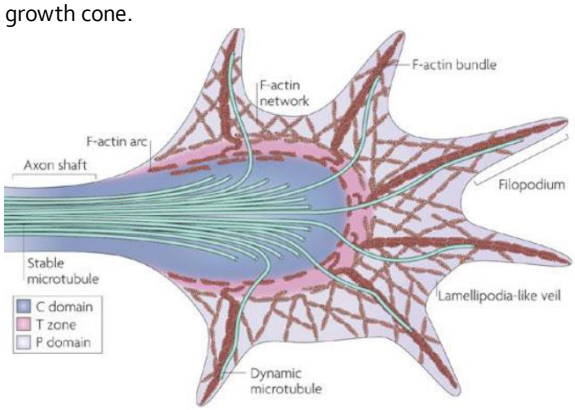
- Optic tracts: **fast**
- Tectum: **slow**

Non-permissive substrates **induce turns** of the growth cones at the **boundary** of the substrate. The growth cone wants to stay where it **can** grow, therefore initiates a turn away from area that is **less** permissive for growth.

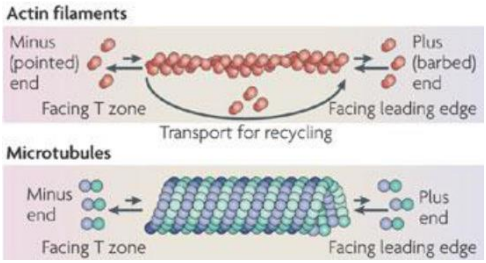
- The search for factors which help growth and which guide is difficult in research since they have similar functions and hard to separate.

DYNAMIC CYTOSKELETON

Growth cones depend on a **dynamic skeleton** with actin and microtubules, they meet in the peripheral zone and stabilize the growth cone.



Treadmilling cycle of **actin filaments** at steady state. Actin polymerize at **plus** end and depolymerize at **minus** end via **dephosphorylation** of ATP, staying a constant length, but individual subunits move around → cytoskeleton is in a **dynamic steady state**, using energy to maintain.

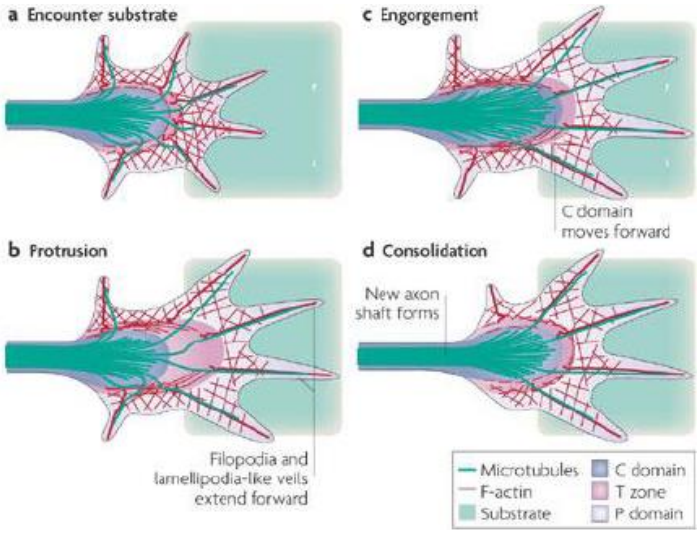


- **Ena/Vasp** are concentrated at **tips** of filopodia and promote **plus end elongation**
- Filopodia **growth** and **retrograde actin flow** are **inversely** correlated → if filopodia is growing, there is less backwards pull towards cell body and when filopodia are stable the backward flow is stronger

NAVIGATION

Every second axon dies at some point, way too many axons are built. This a good mechanism to adapt to size of target area, as well as a correction mechanism, helping connectivity in the brain. We know extremely little about how axons recognize their target cell – random?

When the growth cone reaches an appropriate substrate, it need a filopodial anchor to build tension. By growing there is a **pulling force** on the growth cone and if the tension gets **too high**, the growth cone **dislocates** → makes a turn.



- Adding of actin subunits at tip, leading to longer filopodia
- Secure attachment to substrate and building up of **tension**
- Traction force is achieved by transition zone (T zone), tension is released by pulling growth cone forward
- Actin filaments advance, dragging microtubules along

FORCE

Filopodia can exert force on existing = **other** axons, dislocating them.

TURNING

Filopodia are the ones that induce growth turning, e.g. to laminin.

STEERING OF GROWTH CONE

Information derived from interaction of surface receptors with guidance cues is transmitted to cytoskeleton.

- Profilin: add more actin subunits
- Cofilin: taking off more actin filaments

→ balance of profilin and cofilin induce turns

→ IN VITRO

Depolymerization of **actin filaments** induces turn in **other** direction.

- ❖ If you expose one side of growth cone to graded concentration of substance that depolymerizes actin on one side, there will a turn away from higher concentration, towards the with intact actin filaments → *cytochalasin*

Stabilization of **microtubules** induces a turn in that direction.

- ❖ Gradient of stabilizing substance, turn in that direction → *taxol*
- ❖ Gradient of destabilizing substance, turning away → *nocodazole*

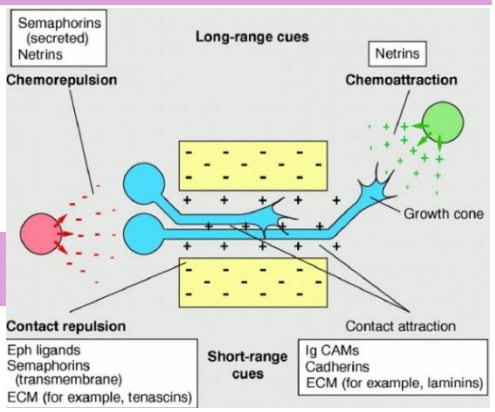
→ IN VIVO

Information derived from the interactions of **surface receptors** with **guidance cues** is transmitted to the cytoskeleton.

FOUR MECHANISMS COOPERATE TO GUIDE AXONS

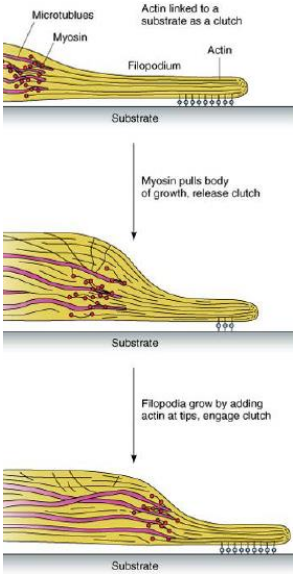
Long-range cues:
- Chemorepulsion
- Chemoattraction

Short-range cues:
- Contact repulsion
- Contact attraction



CLUTCH MECHANISM

Actin is linked to substrate (anchor) → **myosin** pulls body of growth, release clutch → Filopodia grow by adding actin at tips, engage clutch.



AXON BRANCHING

Important for neuronal connectivity. Less material needs to made, there is a rearrangement in cytoskeleton for new branches (assembling of actin on one side).

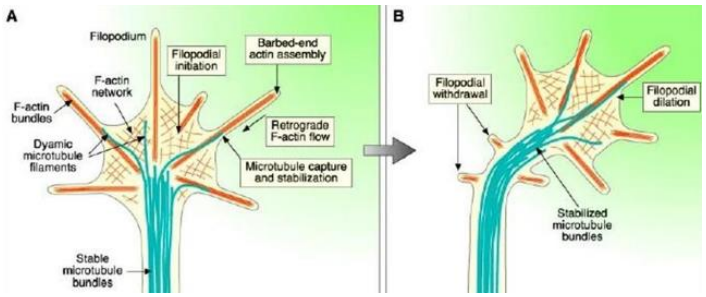
- 5 targets, multiple solutions
  - Single axon guidance along 5 targets
  - Multiple axons to each
  - Axon branching to targets
- Growth cone bifurbication
- Axon collateral branching

AXON COLLATERALS

They are formed by a well-orchestrated sequence of cytoskeletal changes. First protrusion of actin filaments, then invasion by microtubule and finally maturation.

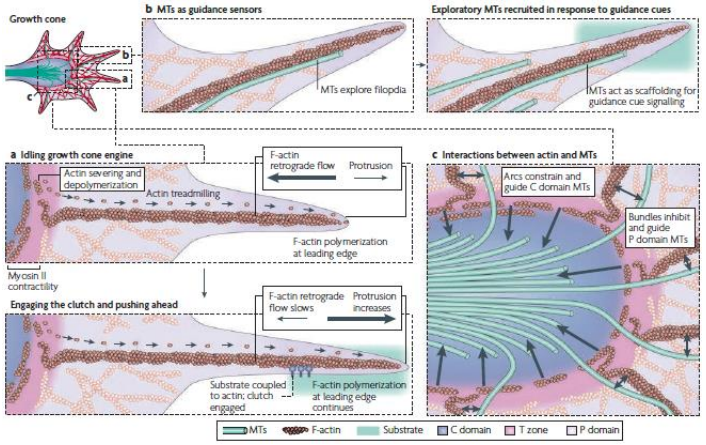
GENERAL

- Actin filaments are required for axon guidance
  - ❖ Cytochalasin-treated tectum creates lost axons
- Signal transduction pathway linking Rho GTPases to the cytoskeleton



PAPER

TRIP OF THE TIP: UNDERSTANDING THE GROWTH CONE MACHINERY – LOWRY (2009)



Growth cone functions as both 'vehicle' and 'navigator', maintaining movement and a motor to move forward, as well as a mechanism to provide traction. Its navigator capabilities consist of guiding the system with spatial bias and translating environmental signals into directional movement. The function and regulation of both its abilities provide new insights.

INTRODUCTION

For growth cones the road consists of **adhesive molecules**, either presented on a neighboring cell surface (**CAMs**) or assembled into a dense ECM (**laminin, fibronectin**). These molecules allow adherence but also activate **intracellular signaling pathways** used by growth cone machinery. The presence of **anti-adhesive** surface-bound (slits, ephrins) molecules provide 'guard rails' determining boundaries of path.

In addition, there are diffusible **chemotropic cues** representing road signs with steering instructions → morphogens, secreted transcription factors, neurotrophic factors and neurotransmitters.

The original theory was that some cues are always attractive or repulsive but it is now clear that the response is **not** due to intrinsic property of the cue but the **specific receptors** that are **engaged** in **growth cone** and the **internal signaling milieu**.

GROWTH CONE VEHICLE

Three stages of advance: **protrustion, engorgement** and **consolidation**.

ENGINE: F-ACTIN RETROGRADE FLOW

Continuous movement of F-actin **from** the leading edge **towards** center of the growth cone, allows the engine to idle and drive movement in response to directional cues. This **retrograde flow** is driven by **myosin II**, which seems to be tethered in the **t**ransition zone, and by the **push** from F-actin polymerization in the **p**eripheral domain

- Side note: actin not only engine, since axons that lack actin polymerization can **still** move forward

ENGAGING CLUTCH

Formation of a complex that acts as a clutch, mechanical coupling of receptors and F-actin flow, thus **preventing** retrograde flow and **driving** actin-based **forward protrusion**.

- Filopodia are dispensable for accurate growth cone guidance but **required** for normal growth cone **motility**

Increased levels of localized actin (F-actin bundles) assemble at site of adhesion and the **c**entral domain and F-actin **arcs** re-orientate from **C**-domain to adhesion site → C-domain moves **forward**.

MICROTUBULES AS PART OF VEHICLE

Major role in steering in two complementary ways:

- **Individual** P domain MTs act as **guidance sensors.** The introduction of adhesive cues leads to an **increase** in the number of exploratory MTs interacting with the adhesion site.
  - Carrying signals or by acting as a scaffold for localized recruitment of key signaling components for navigation (Rho GTPases)
- **Bulk** C domain MTs steer the **advance** of the growth cone
  - Move into area of new growth, as **consolidation** of new region of **axon shaft** forms behind them → fixing of axonal direction

MICROTUBULE INTERACTIONS WITH ACTIN

Actin has a role in determining **MT localization**, acting as a **barrier** to **premature** MT invasion, as a **guide** during **advance**. Perturbation of actin structures result in a redistribution of MTs and a **change** in the direction of growth.

- P-domain MTs and F-actin bundles:
  - Exploratory MTs follow trajectory of actin bundles, yet bundles are **not** required for advance. They **inhibit** MT penetration into P domain when MTs are **coupled** to F-actin bundle-specific **retrograde flow**, showing that MT-actin coupling and uncoupling would have an effect on MT dynamics.
- C-domain MTs and actin arcs
  - Disruption of actin arcs results in **failure** of MT **consolidation** during axon outgrowth, leading to an abnormally **broad** C-domain.

Pathfinding does not consist of just moving forward, it includes pauses, turns and retracts

GROWTH CONE NAVIGATOR

Navigation system needs to be able to translate multiple environmental directions and integrate separate signaling pathways to locally modulate dynamics of cytoskeletal machinery. There are numerous signal transduction molecules that convey guidance information: kinases, phosphatases and calcium ions, but most comprehensively Rho family of GTPases, which controls **cytoskeletal dynamics downstream** of nearly all guidance signaling receptors.

ACTIN VS MICROTUBULES

- Steady-state/treadmilling vs dynamic instability

AXON GUIDANCE

A cooperation of attractive and repulsive forces.

HISTORY

SPERRY'S CHEMOAFFINITY HYPOTHESIS

The specificity of axonal connections within a neural map is determined by molecular tags (address labels) on projecting axons and their target cells.

→ not a different signal for every axon but **concentration differences**.

- ❖ Experiments with frog eyes - Sperry concluded that each individual optic nerve and tectal neuron used some form of chemical marker which dictated their connectivity during development. Reasoning that when the eye had been rotated, each optic fiber and each tectal neuron possessed cytochemical labels that uniquely denoted their neuronal type and position and that optic fibers could utilize these labels to selectively navigate to their matching target cell, hence the visual motor impairment of top and bottom field → **target innervation is preserved**.
- Two perpendicular molecular gradients are necessary to identify each cell in a 2D target
- Bonhoeffer stripe assay supports Sperry's chemoaffinity hypothesis

WHAT ARE THE MOLECULAR TAGS?

LABELED-PATHWAY HYPOTHESIS

'Follow the one that knows how to get there'

- Follower axons can recognize **pioneer axons** and follow them
- If you take away the pioneers (with laser), followers will **not** find target area → they cannot understand guidance information
  - In lower vertebrates one of the followers will revert to a pioneer, look for target and then followers will connect to that pioneer, induces delay in system → completely true in invertebrates

SOLVING PROBLEM BY BREAKING INTO LITTLE STEPS

Navigation depends on **landmarks** and information about direction, molecular cues in the ECM or presented by cells in developing NS.

- Axons use **guidepost cells** as **intermediate targets**
- Neurons send axons straight out until they hit **choice point** after choice point which induce turns until finding target
- If you ablate one of the guideposts the axon will be lost

MOLECULAR BASIS OF AXON GUIDANCE

SUBSTRATE

Growth cones readily grow onto a **more attractive substratum**.

- Translocates onto **Schwann cell** and remains on its surface, because it is more attractive than the other surroundings → molecular structure is preferable

ADHESION

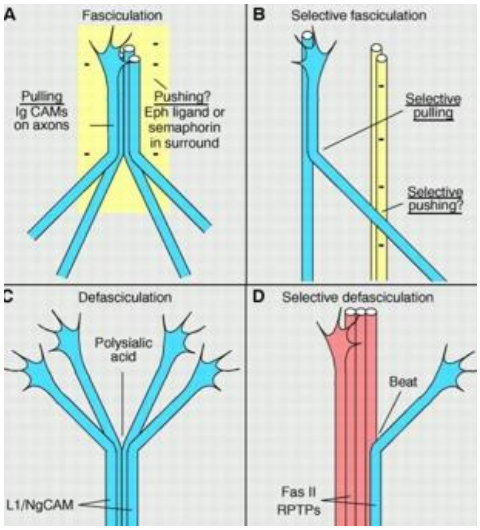
Axons select their pathway according to **adhesive strength**.

- ❖ Metal grids as support for tissue section, lined with Pd powder.
  - When Pd with petri – no preference for growth
  - When adding tissue cultures – slight preference for that
  - When **collagen** is added – strong preference for **non**-Pd path
- Test for **growth**
  - How long did axon get in window of time?
- Test for **adhesion**
  - Puff axon with constant rate of solution and measure time it takes dissociate → rate for adhesion between neuron and substrate

No correlation between strength of adhesion, growth rate or substrate preference, only actual adhesiveness deciding → plays a role by supporting neuron and lowering error rate.

- Laminin: highest growth rate
- L1: strongest adhesion

FASCICULATION



Axon-axon adhesions, split up at end again to innervate target.

SELECTIVE FASCICULATION

Sub-bundling of axons with common targets → selective pushing and pulling. Axons recognize each other by surface molecules.

DEFASCICULATION

Introduction of **polysialic acid** (PSA), which creates a choice point in bundle

SELECTIVE FASCICULATION

Beat acts as a de-adhesion molecule

CNS AND PNS SEPERATION

Growth cones from the PNS **collapse** upon contact with CNS axons. They then start to retreat and try again, but they cannot cross or bundle up with the axons → must be something acting repulsive on surface for PNS axons.

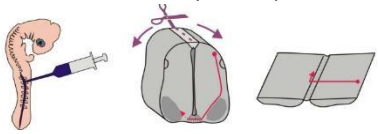
The molecule is **Semaphorin3A** and it repels NGF-dependent **sensory** axons. Growth itself is **not** inhibited but growth cones in that direction are collapsed → in vivo assumed to induce turn and not full collapse.

Extent of collapse is dependent on **concentration** of repellent.

COMMISSUAL NEURONS

During CNS development, the decision of a neuron to cross or not to cross the **midline** of the **neural tube** is critical. In vertebrates, this choice is mediated by the **floor plate** and enables the embryo to develop successful **left** and **right body halves** with respect to nervous tissue. Commissural neurons, located near the **dorsal** midline, send axons **ventrally** and across the floor plate and form a single commissure. Upon reaching the opposite side of the neural tube they project anteriorly or posteriorly within the tube. The trajectory of these axons toward the FP is guided in part by **netrins**.

- Axons grow towards floor plate, **cross it**, then grow towards brain in longitudinal axis → attracted to FP but once reached, repelled by it and move on in other direction.
- Open book explant →



NETRIN

Netrin is a **chemoattractant** for commissural axons and is highly expressed in cells of the floor plate (ventral). *Netrin* is necessary and **sufficient** for long-range guidance of commissural axons in a **dose-dependent** manner (gradient).

DRAXIN

Commissural axons are **repelled** by roof plate (dorsal) by expression of draxin, which has similar repellent effect like *BMP7*.

AXONIN 1 AND NRCAM

The interaction between **growth cone** *axonin-1* and **floor plate** *NrCAM* makes the axons **enter** the floor plate.

- *NrCAM* is binding partner for *axonin-1* in commissural axon guidance
- If **no** *NrCAM*: defasciculated, axons grow as individuals but still **find their way** → cross midline
- If **no** *axonin-1*: no crossing over, stay on **ipsilateral** side

→ Fasciculation **not** required for axonal pathfinding in higher vertebrates. Axons do **not** bundle up without *NgCAM*.

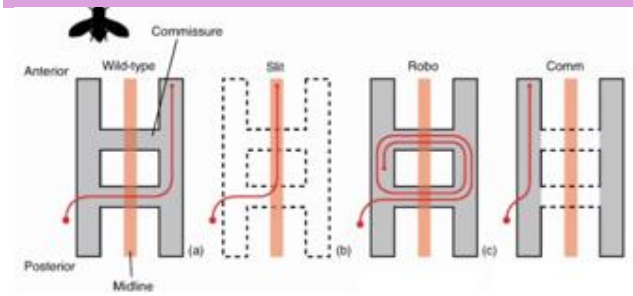
LEAVING FLOOR PLATE

Once contact to floor plate has been made, the axons **lose responsiveness** to *netrin.* Attractive effect is silenced after first contact, no matter the distance

The **balance** between positive and negative **signals** is shifted upon floor-plate contact and prevents the axons from recrossing.

- Axons can only read the positive signs **before** crossing because they lack receptors for negative signals which they start expressing once crossing.
- Trafficking is an important regulator of protein expression on growth cone surface

INVERTEBRATES



The midline glia in the ventral nerve cord is equivalent to the floor plate in vertebrates and there are also midline-derived guidance cues.

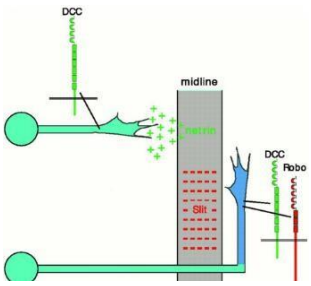
ROBO, SLIT AND COMM

- Main function of *Slit* is acting as a **midline repellent**, preventing the crossing of longitudinal axons across the midline of the CNS of most bilateral animal species
- When *Robo* gene is mutated the commissural neurons cross midline again and again
- With a *Comm* mutation the commissural neurons **fail** to cross midline

CHOICE POINTS

BEHAVIOR

Axons switch their behavior at choice points.



ATTRACTION TO MIDLINE

- **Netrin** is chemoattractant produced by **midline cells**
- **DCC** is receptor for *netrin* expressed in axons **once** they reach midline and are exposed to *netrin*

CROSSING AND MOVING FROM MIDLINE

- First: Upregulation of **Robo** expression → repulsion of **Slit**
- Then: **Loss** of netrin responsiveness, **despite continued** DCC expression → binding of DCC and Robo to each other?

HOW IS SWITCH IN BEHAVIOR POSSIBLE?

- Changes in transcription
- Changes in translation
- Changes in protein stability
- Changes in vesicle trafficking / membrane insertion

FLOOR PLATE EXIT

Commissural axons turn rostrally upon exit of floor plate due to gradients of **diffusible** and **non-diffusible** cues.

WNTS

*Wnt*s are an **attractant** for **post-commissural** axons and are expressed in a **decreasing** rostral to caudal gradient

SHH

Sonic hedgehog is a **repellent** and expressed the other way around, decreasing from floor plate to roof plate and they guide commissural axons along the **longitudinal** axis of the spinal cord. *Shh* has different functions during different stages in development

- $E0$: *Shh* acts as a morphogen
- *Shh* as **chemoattractant** for **pre**-crossing axons
- *Shh* as **repellent** cue for **post**-crossing axons

→ Commissural axons **change *Shh* receptor** at midline

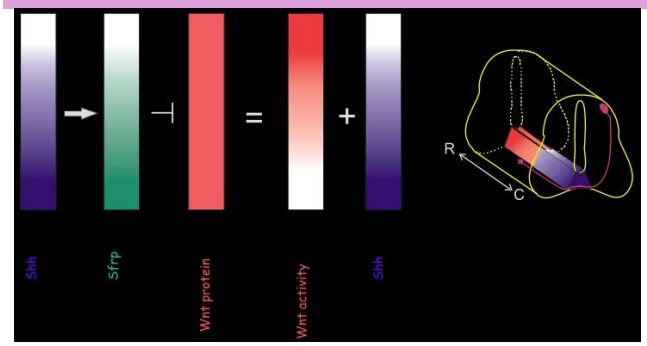
- *Glypican1* mediates the switch in commissural axons responsiveness to *Shh* at midline, **before**: they express *boc/ptc*, **after**: *Hhip*

→ *Wnt4* attracts and *Shh* repels **post**-commissural axons
→ *Shh* guides post-commissural axons directly and indirectly by regulating *Wnt* activity by influencing *Sfrp1* expression

SFRP1

This is the protein that forms the *Wnt* **gradient**. *Sfrp1* inhibits the *Wnt* protein and therefore where *Sfrp1* is higher, there is **less** *Wnt* activity (→ caudally)

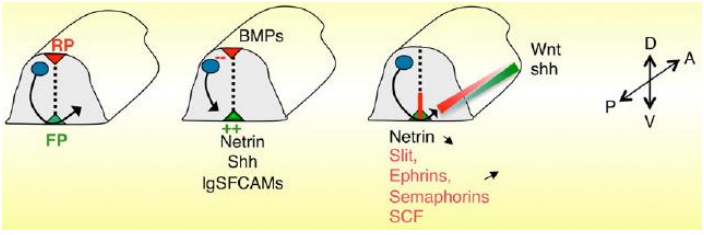
SUMMARY



PAPER

AXONAL COMMISSURES IN CNS: HOW TO CROSS THE MIDLINE? – NAWABI (2011)

The problem of pathfinding for neuronal projections navigating very long distances is solved by the subdivision of pathways into shorter sections through formation of intermediate targets (choice points). The puzzling aspect of this stepwise process is that axons must first be instructed to **enter** and later **exit** this intermediate target.



Multiple commissural axon tracts to establish reciprocal connections between both sides of CNS. In vertebrates, ventral glial cells of floor plate (FP) segregate between those forming ipsilateral and contralateral circuits. Studies revealed that the ipsilateral and commissural axons **respond** differently to **same set** of **midline-derived guidance cues**.

- Ipsilateral axons are prevented from crossing midline by repellents to which commissural axons only develop a sensitivity after crossing the midline

The level of expression of **cell surface receptors** will thus be the first **instrumental parameter** for setting the **sensitivity** of the growth cone to guidance cues.

- In fly, commissural axons maintained unresponsiveness to *Slit* repellent until crossing via **proteasomal degradation** of *Slit* **receptor** *Robo*.
- Silencing of *Netrin*-mediated **attraction after** midline crossing, attributed to the formation of a complex between *DCC* and *Robo*
- *Sema3B* is a midline **repellent** to which pre-crossing commissural neurons are unresponsive to, through processing of co-receptor *Plexin-A1*. Midline-derived cues lead to suppression of this processing, allowing *Plexin-A1* accumulation in commissural growth cones and sensitization to *Sema3B* follows

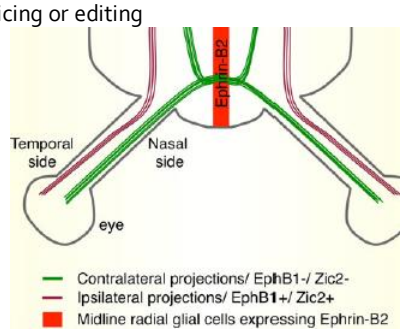
TRANSCRIPTIONAL CONTROL OF GUIDANCE RECEPTORS

Regulation of transcription is one the first ways to exert **spatio-temporal control** of **receptor expression**. Axon trajectory **defines** neuronal identity and must therefore be **encoded** by **transcription factors**. Influencing factors could be:

- mRNA traffic
- Stability
- mRNA modifications: alternative splicing or editing

IN THE VISUAL SYSTEM

Optic chiasm provides good example of linking transcription factors to guidance receptor expression in commissural systems. Visual information collected by each retina must be conveyed to specific centers located on **both** sides of the brain, to allow binocular vision and spatial positioning.



At optic chiasm (crossing of contralateral projections from nasal side), axons are exposed to variety of guidance cues from FP cells. *Ephrin-B2* ligand and *Eph-B1* receptor are found to play major role in **sorting** of ipsilateral and contralateral projections.

- In *EphB1*-deficient mice, the number of crossing fibers **increased** → **this B1** receptor helps repel from midline! (ectopic expression by targeted electroporation shows ipsilateral path)
  - *Zic* factors are involved in specification of **medial body axis** and in early acquisition of **neuronal identity**. *Zic2* was shown to induce and control *EphB1*expression → **ipsilateral** path

IN SPINAL AND VENTRAL CORD COMMISSURAL PROJECTIONS

Recently shown that *LIM* transcription factors control *Robo* expression. The study showed how transcriptional activity can control specific steps of axon **navigation**, such as midline crossing, suggesting that vast programs might be require to specify key steps.

IN DROSPHILA

Comm controls ipsilateral and contralateral choices, since it was found sufficient to **induce** contralateral projections. *Comm* is expressed **transiently** at **pre**-crossing stage and **downregulates** cell surface sorting of *Robo* receptor → preventing a response to *Slit* and allowing midline crossing. **After** crossing *Comm* is downregulated and *Robo* is expressed at cell surface.

Examples show that transcriptional regulations play crucial roles in controlling the spatio-temporal distribution of key receptors involved in commissural guidance across the midline and even after crossing.

POSTTRANSSCRIPTIONAL REGULATION OF MIDLINE CROSSING

REGULATION OF GUIDANCE RECEPTOR EXPRESSION BY ALTERNATIVE SPLICING OF MRNA

Alternative splicing of mRNA allows generation of several proteins with different functional properties from a unique locus and contributes to genesis of **specific synapses**.

Robo/Slit signaling 'pushes' axons out of FP and prevents midline re-crossing, isoforms of *Robo3* through differential splicing have shown to be expressed at different times:

- **Pre**-crossing: *Robo3.1* and *Robo1*, inhibition of early *Slit* response, overexpression results in multiple re-crossings → no sensitivity
- **Post**-crossing: *Robo3.2* and *Robo2*, help for *Slit*-mediated FP exit, overexpression resulted in no crossing → overly sensitive

Other guidance receptors use this as well for functional diversity?

TRANSLATIONAL AND POST-TRANSLATIONAL REGULATION OF MIDLINE CROSSING

Neo-synthesized receptors are sorted to **secretory pathway** to be inserted in PM. Studies are reporting a variety of translational and post-translational **regulations** taking place during **protein synthesis, maturation, trafficking** and **turn-over**.

REGULATION OF RECEPTOR SYNTHESIS

FRMP has been shown to regulate specific sets of mRNA encoding proteins required for synaptic development and function.

- *Msi1* controls *Robo3* expression. *Msi1* is downregulated by local FP signals → cue-dependent regulation of *Msi* expression may result in **decreased** downstream *Robo3i* levels.

Axons show to contain functional organelles similar to Golgi and RER, suggesting that membrane-associated and secreted proteins could be synthesized **in axons**. Most proteins are trafficked to axons and growth cones. Recent studied show that **repulsive** behavior is associated with **local** synthesis of signaling molecules, destabilizing the cytoskeleton. Local synthesis of cytoskeletal components occurs in response to **attractive** signals.

PROTEOLYTIC CLEAVAGE OF GUIDANCE RECEPTORS

Kuz is a metalloprotease and regulator of *Robo*. Metalloproteases have been shown to disrupt ligand-receptor interactions and allow **repulsive response**. *Kuz* is necessary for receptor activation and initiation of transduction, mediates cleavage before crossing.

Semaphorin signaling, which in addition to *Slit* has been shown to contribute to commissural axon guidance at midline. *Sema3B* responsiveness can be silenced by activity of an **endogenous proteolytic** *calpain1*. When reaching FP, exposure to local signals **suppress** this protease activity, resulting in *Plexin-A1* accumulation and sensitization to *Sema3B*.

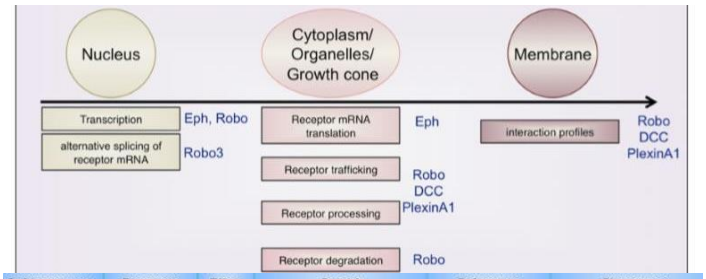
These examples show a mechanism of receptor processing in vertebrates which control temporal switch of commissural responsiveness to midline repellent and implicated *calpains* as regulators of axon guidance decisions and sensitivity to cues.

→ Crucial role of protease activity in setting receptor expression profiles at appropriate time and space during development of neuronal projections.

CONCLUSION

Midline crossing exemplifies navigation of axons at intermediate targets. The decision of crossing results from integration of successive and intricate regulations controlling the sub-cellular distribution of guidance receptors at growth cone surface. Key roles are attributed to mechanisms in series of guidance decisions:

- Protein synthesis
- Receptor trafficking
- Receptor processing

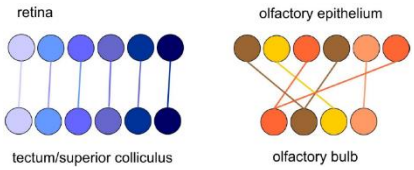


Guidance cues	Receptors	Effect	Role(s)	References	Phenotypes
BMP7 GDF7	BMPRI/ BMPRII	●	Repel commissural axons from the roof plate and drive their initial ventral trajectory	Butler & Dodd 2003	+/+ -/-
Netrin	DCC/Fra DSCAM Heparan Sulfate	● ● ●	Attracts commissural axons toward the FP	Serafini et al. 1996 Fazeli et al. 1997 Ly et al. 2008 Matsumoto et al. 2007	+/+ -/-
Shh	Boc Patch/Smo	●	Acts in synergy with Netrin to attract commissural axons to the FP	Charron et al. 2003 Okada et al. 2006	+/+ Net$^{-/-}$/Shh$^{-/-}$
NrCAM	Tag-1	●	Promotes the interactions between commissural growth cones and FP cells and allows FP entry	Stoeckli and Landmesser 1995 Stoeckli et al. 1997	+/+ -/-
Ephrin-B3	EphB1-EphB3	●	Prevents ipsilateral axons from midline crossing Prevents commissural axons from multiple re-crossing	Kullander and Klein 2002; Kadison et al. 2006 Jevince et al. 2006	+/+ -/-
Slits	Robo1,2,3	●	Prevent ipsilateral axons from midline crossing Prevent commissural axons from multiple midline re-crossing	Brose et al. 1999 Kidd et al. 1999	+/+ -/-
Sema3B	Nrp2/PlexA1	●	Expels commissural axons out of the FP Orients the rostro-caudal growth	Zou et al 2000 Nawabi et al 2010	+/+ -/-
SCF	Kit	●	Promotes post-crossing commissural axon growth to stimulate their exit of the FP	Gore et al. 2008	+/+ -/-
Wnt4	Frz3/LRP6	●	Controls the antero-posterior turning of post-crossing commissural axons	Lyuksyutova et al. 2003	+/+ -/-
Shh	HIP	●	Controls the antero-posterior turning of post-crossing commissural axons	Bourikas et al. 2005	+/+ -/-

NEURAL CIRCUITS

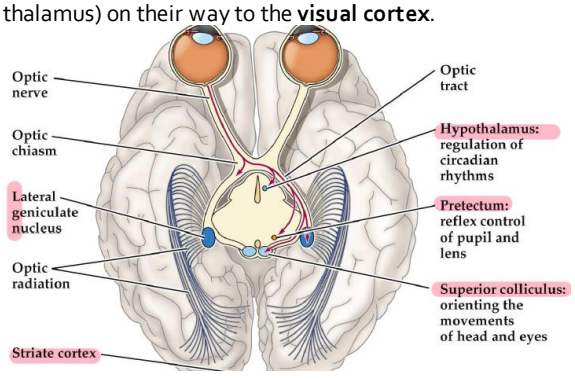
The **topographic** map of the **visual** system preserves **space information**, whereas the **olfactory** map preserves only **quality** of the **stimulus but** not **location**.

- Retina captures exact location in relationship to other locations
- Olfactory bulb had one neuron for one odorant



VISUAL SYSTEM

Projection from the retina are relayed in **lateral geniculate nucleus** (in thalamus) on their way to the **visual cortex**.

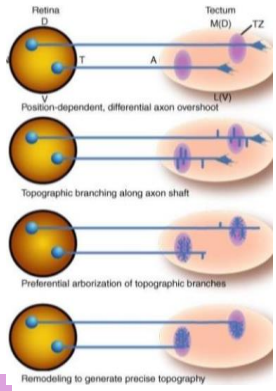


REVERSAL OF IMAGE

In the topographic map the **exact positional** information of stimuli is preserved. In the **retina,** an image is turned **upside down** but properly rearranged in **tectum**.

- Dorsal in retina → ventral in tectum
- Ventral in retina → dorsal in tectum
- Nasal in retina → anterior in tectum
- Temporal in retina → posterior in tectum

NEURAL CIRCUIT FORMATION IN VISUAL SYSTEM

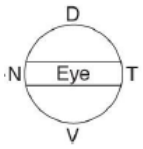


- **Position-dependent** with **differential axon** overshoot. **Axon guidance molecules** are needed to bring axons close to its TZ.
- When TZ is reached, there is **topographic branching** along axon shaft within TZ. Axons explore with **filopodial extrusions** from growth cone, resulting in **interactions** with several synapses.
- **Preferential arborization** of topographic branches
- **Remodeling** to generate precise topography → **pruning** and **retraction**

FORMING A TOPOGRAPHIC MAP

Neurons '**know**' where they belong spatially and what their destination is in relation to other neurons in the map. **Relative relationship** of neurons decides the pattern → competition shows the way.

- ❖ If you take away part of tectum, neurons reorganize and absolute position of neurons change, but relative relationship remains. Branching in tectum is **smaller**
- ❖ Take away part of retina and relative relationship remains, **larger** connections and branching in tectum



→ RGC axons read the **relative** repulsive strength of tectum and hone in on anterior-posterior position that is compatible with their active *EphA* expression level.

BONHOEFFER STRIPE ASSAY

Alternating stripes (anterior/posterior) of tectum on a dish with a strip of retina on top (nasal – temporal)

- Nasal axons grow on **every** substrate → able to grow into posterior
- Temporal axons can **only** grow on anterior stripes → avoid posterior, recognition of different types of stripes

This shows there is a **gradient of guidance molecules** in the tectum telling the axons where to go.

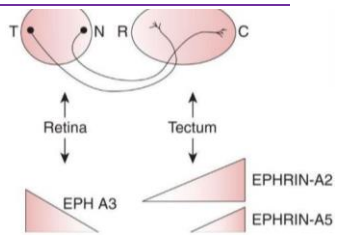
WITH HEAT

If membrane was heated the temporal axons **also** grew on both membranes → loss of repulsiveness because protein was degraded (Ephrin) → **repulsive cues**

EPH-RECEPTORS AND EPHRINS

Gradients of Ephrins and Eph-receptors control **retinal axon targeting**.

- Rostral = anterior
- Cortical/caudal = posterior



Temporal axons are repelled by **high concentrations** of **Ephrins**.

INTERATION OF LIGAND AND RECEPTOR

Eph receptors and Ephrins do **not** interact specifically → there are **more receptors** than ligands.

EPHA RECEPTOR

- Axons from **temporal retina** express **high level** of *EphA* receptors → grow until **anterior tectum** (low Ephrin concentration), they are repelled by high concentration of Ephrin in posterior tectum → **high** sensitivity
- Axons from **nasal retina** express **low level** of *EphA* receptors → grow until **posterior tectum** (no bothered by high Ephrin concentration) → **low** sensitivity

COMPETITION

Axon-axon competition contributes to topographic map by staying in relationship to neighboring axons to preserve image.

- Competition between **growth cones** in **tectum**
- Presence of ***Eph* receptors** on **growth cones** decide who moves on
- Required for fine tuning
- Type of axonal interaction
- The ones that go the farthest have **least amount** of *Eph* receptors on growth cones

OLFACTORY SYSTEM

Spatial information is **not** included in olfactory map. Axons from olfactory sensory neurons responding to **same odor** target the **same glomerulus** → one neuron – one receptor rule

STEPWISE ESTABLISHMENT OF MAP

- DM: dorsomedial
- VL: ventrolateral

GLOBAL TARGETING

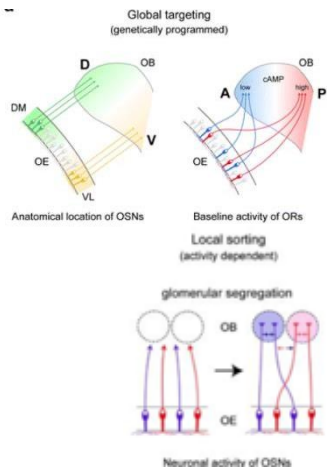
Comes first, genetically programmed.

- Anatomical location of OSNs
- Baseline activity of odorant receptors

LOCAL SORTING

Comes second, activity dependent.

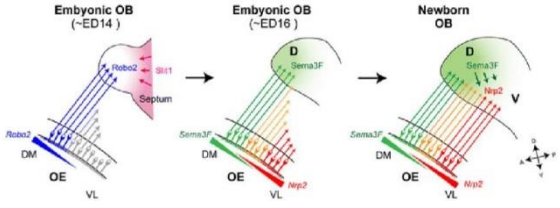
- Glomerular segregation
- Neuronal activity of OSNs



TIMING AND EXPRESSION

The timing and expression of repellants is crucial for axonal targeting. Involved molecules:

- *Robo2*
- *Sema3F*
- *Nrp2*



Olfactory epithelium begins to be developed from **dorsomedial** side, the first ones expressing **higher** amounts of *Robo2* and then **high amounts** of *Sema3F*, the newer axons start expressing *Nrp2* with the **highest** expression on the **ventrolateral** edge.

This sequential projection helps to **maintain topographic order** during the process of axonal projection. *Sema3F* secreted by the DM-zone axons in the OB **prevents** the late-arriving *Nrp2* axons from invading the dorsal region of the OB → Countergradient

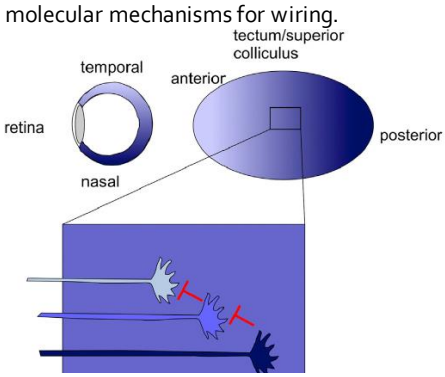
PAPER

WIRING MECHANISM FOR OLFACTION AND VISION, NOT COMPLETELY DIFFERENT AT ALL - STOECKLI (2014)

Gradients of repulsive *EphrinA*s **in the target** were thought to repel temporal RGC axons expressing high levels of *EphA* receptors, now it can be shown that *EphrinA* expressed on **nasal** axons (OF RETINA) contributes to repulsion of temporal axons.

Every target cell in tectum carries a specific **address label** for **incoming** RGC axons, when they express an appropriate combination of receptors for these labels they are guided to their target cells, resulting in the **topographic** map of the visual system that maintains **spatial information** of sensory input.

The olfactory system is a **discrete** map using **different** underlying molecular mechanisms for wiring.



EPH RECEPTORS AND EPHRINS

- *EphrinA*s and *EphA* receptors responsible for **rostrocaudal** mapping
- *EphrinB*s and *EphB* receptors for **lateral-medial** mapping of RGC axons in tectum

*EphrinA*s expressed in anterior < posterior gradient in tectum and their receptors in nasal < temporal gradient in retina.

AXON-AXON

Question remaining was why innervate tectum **at all**? Hypothesis that axon-axon interactions contribute to **topographic** map formation → **repellent**. This would require *EphrinA* expression **on** RGC axons to repel other RGC axons expressing *EphA* **receptors** → **trans**-interaction (cis- is on own cell), thus competing locally for target cells in tectum. This discovery brings visual system **closer** to olfactory system.

VISUAL VS. OLFACTORY

Axons innervating different areas of olfactory bulb did not intermingle due to expression of secreted **repulsive** signal or its receptor. A contribution of axon-axon interactions to innervation is not specific to olfactory system but also found in muscle for innervation of sensory and motor axons. This shows that the two systems are not so far apart as assumed until now. However a difference still to be named is that axon-axon interaction are important **before** contact with target in **olfactory system** and for **global** pattering, whereas in visual system in to **locally** sort out axonal topography.

CELL MIGRATION

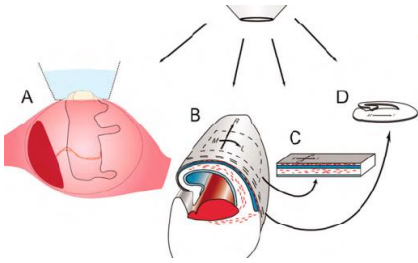
10^{11} neurons are in adult brain and highest rate of proliferation is 250'000 per minute.

TECHNIQUES TO LOOK AT MIGRATION

TIME-LAPSE VIDEO MICROSCOPY

REQUIREMENTS

- Efficient labeling of cells with **fluorescence markers** (vital dyes)
  - Labelling through viral infection, transgenic animals, membrane-permeant reactive tracer, electroporation [electrical field is applied to cells to increase the permeability of the cell membrane] or biolistic gene (gene gun)
- Detection with **confocal** or **two-photon** microscopy



IMAGING

- Live in uteri
- In situ [am Ort] in whole brains
- Tangential cortical explants
- Organotypic coronal slices

CONFOCAL MICROSCOPY

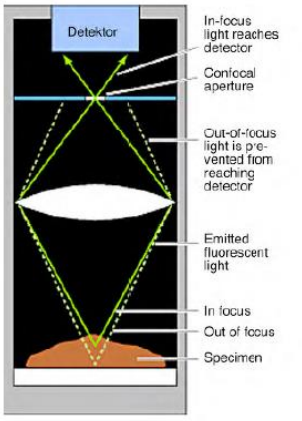
Focus on point in tissue, tissue emits light back, the emitted light is focused in very precise plane.

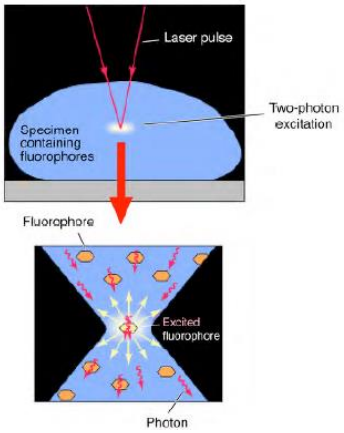
- UV-light source (laser), one photon excites fluorescence
- Restricted areas in sample can be focused, broader area
- Cannot go far into tissue, quickly stopped

TWO-PHOTON MICROSCOPY

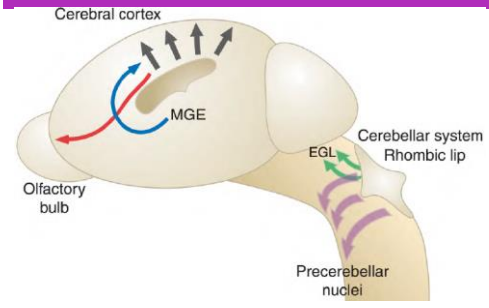
Focus on certain area, only focus will be emitted light which is only emitted when hit by two photons at the same time → happens only in focus plane.

- Infra-red light, two photons excite fluorescence
- In vivo use, penetrates very far





MIGRATION PATHWAYS IN CENTRAL NERVOUS SYSTEM



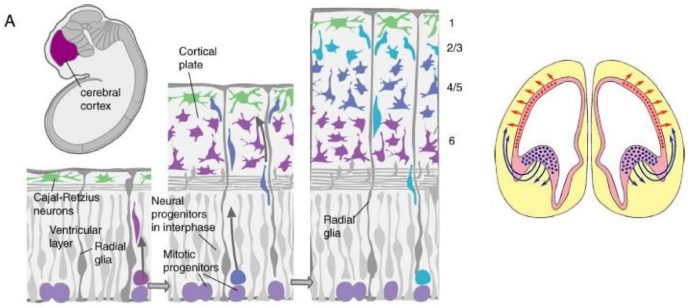
DEVELOPMENT OF THE CEREBRAL CORTEX

MIGRATION

RADIAL MIGRATION

Formation of **excitatory pyramidal** neurons in **cortex**

- Radial glia can **give rise** to **neuronal precursors** → Stem cells
- Radial glia for guidance to outer surface of neural tube
- Radial migration is **essential** for **cortical** development
  - Asymmetric division
  - Precursor migrates along glial process until reaching → Cajal-Retzius: **STOP-Signal,** to let go of radial glia fiber at top layer
  - Later born precursors will migrate through the previous built layer because the **environment changes**
  - → **'Inside-out'** growth of cortical layers
  - Different layers have different targets and identities
- Reelin

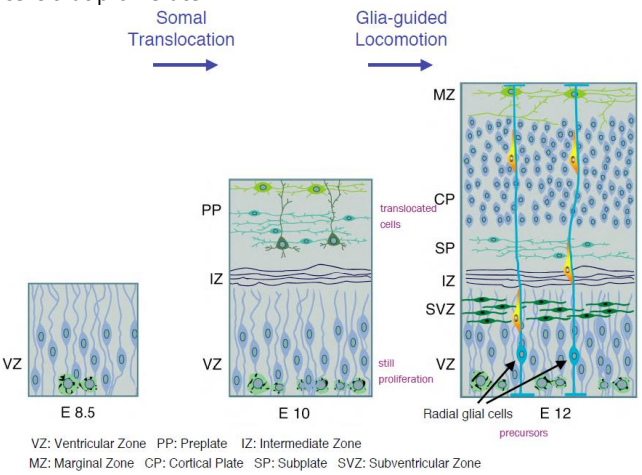


SOMAL TRANSLOCATION

Neurons lose contact to ventricular site and migrate through **intermediate** zone. They extend their processes up and soma are pulled up after → early in development

GLIA-GUIDED LOCOMOTION

Radial glia cells have long processes that extend up to the surface. Neurons use their processes to migrate outwards (away from ventricular zone) → up to 2 cm migration. Radial glia are **precursor** cells that proliferate in VZ.



TANGENTIAL MIGRATION

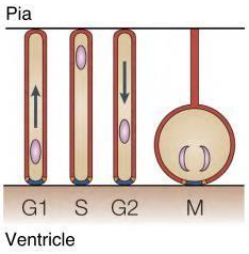
Immigration of **inhibitory** interneurons into cortex and olfactory bulb

- Migrate **parallel** to surface → become **interneurons**

EARLY NEUROGENESIS IN CEREBRAL CORTEX

NEUROEPITHELIUM

One cell thick, pseudostratified (nuclei at different levels / stages of cell cycle process).

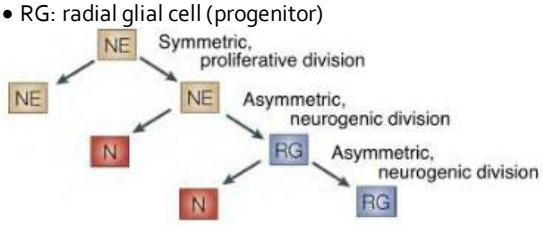


INTERKINETIC NUCLEUR MIGRATION

→ **not** cell migration but nuclei

CELL TYPES

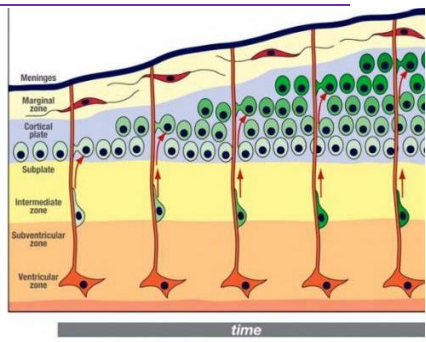
- NE: neuroepithelial
- N: neuronal cell
- RG: radial glial cell (progenitor)



INSIDE OUT DEVELOPMENT OF CORTICAL PLATE

Neurons always go **past** the **last formed** layer; the **youngest** cells are on **top**.

Injecting of **thymidine** in pregnant mouse during different stages of embryogenesis allows labeling and tracing (of mitotic cell), which shows that neurons 'born' later are farther out.



ADULT ORGANISM

There is also non-radial migration as well as perpendicular / lateral migration in the ventricular zone → tangential movement.

There are I (top) – VI (bottom) cortical layers, WM (white matter, axonal fibers and no cell bodies) and SVZ.

VENTRICLE DIRECTED MIGRATION

Interneurons originate from ventral forebrain (ganglionic eminence), then migrate **tangentially** through SVZ to find ventricle and then change direction by migrating radially to target region in cortex.

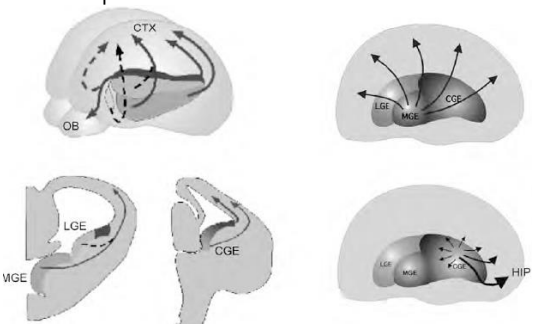
It is speculated that combinations of chemoattractant and chemorepellent molecules are involved in this ventricle-directed migration and that interneurons may seek the cortical ventricular zone to receive layer information.

TANGENTIAL MIGRATION

Final positioning of tangentially migrating neuronal precursors can be tricky, they have different point of origin and usually end up farther from ventricle (except those originating in marginal zone (MZ).

PATHWAYS

Coronal (towards ears) and sagittal (along midline) are the pathways available. This happens relatively early in development but there are still some precursor cells in adults.



SUMMARY OF MIGRATION OF NEURONAL PRECURSOR CELLS

CEREBRAL CORTEX

RADIAL MIGRATION
Formation of **excitatory pyramidal** neurons (glutaminergic) in the cortex, with at least two major migration modes:

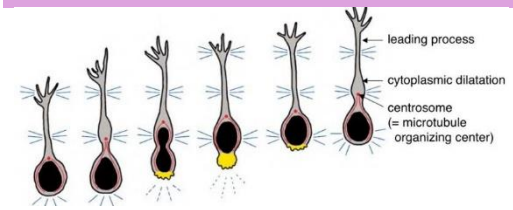
- Somal translocation (early in development)
- Glial-guided locomotion along radial glia → up to 2 cm migration

TANGENTIAL MIGRATION
Immigration of **inhibitory inter**neurons (GABAergic) into the cortex and olfactory bulb (especially in rodents)

CEREBELLUM

- **Radial** migration of **Purkinje** precursor cells
- Tangential migration of **cerebellar granule cell precursors** and precursors of **precerebellar nuclei**

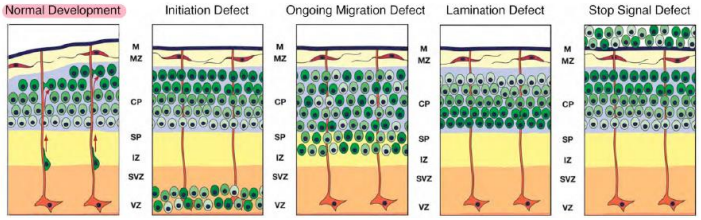
MODEL OF SALTATORY NEURONAL MIGRATION



- Leading process (growth cone) adhered to environment by **selective adhesion** → integrin receptors
- Dynamic reorganization of the cytoskeleton
  - **Centrosome** which encages nucleus gets pulled forward into budding cytoplasmic dilation
  - The material behind retracts immediately with this movement

→ **Discontinuous** movement

CORTICAL MIGRATION DEFECTS



INITIATION DEFECT

HUMAN DISORDERS

PERIVENTRICULAR HETEROTOPIA
Additional grey matter close to ventricular zone and ventricles are larger. Cells accumulate in the VZ, don't move up along the glia.

MUTATED GENES

- *Filamin A* (FLNA): actin-binding protein: x-linked mutation (males don't survive, women have **epilepsy**)
- *Arfgef2*: Vesicle trafficking

ONGOING MIGRATION DEFECT

Organization in the cortical plate is severely disturbed → no properly segregated layers and growth into lower layers, no folding.
→ Severe epilepsy, early death, retardation

HUMAN DISORDERS

LISSENCEPHALY/SUBCORTICAL BAND HETEROTOPIA
Also known as double cortex (two layers of grey matter with one thin layer of white matter in between.

MUTATED GENES

- *Dcx*: microtubule stabilization, process outgrowth, nuclear translocation
- *Lis1*: has effects on actin

LAMINATION DEFECT

Outside-in organization instead of inside-out, cells cannot find their destination so they stay on course until they can't move forward.
→ Cerebellum almost completely missing, ataxia, epilepsy, retardation

HUMAN DISORDERS

LISSENCEPHALY/CEREBELLAR HYPOPLASIA
Only rudiment of cerebellum → severe ataxia

MUTATED GENES

- *Reelin* (RELN): expressed by cajal cells in marginal zone (layer I)

STOP SIGNAL DEFECT

Neurons pass the marginal zone and over-migrate into the subarachnoid space. Disease can only be seen in autopsy
→ Ocular anomalies, hydrocephalus, only a couple months survival

HUMAN DISORDERS

COBBLESTONE LISSENCEPHALY
Nodular brain surface.

MUTATED GENES

- *POMT1*
- *POMGnT1*
- *Fukutin*

FUNCTIONAL NETWORKS OF NEURONAL MIGRATION FACTORS

Mutations leading to defects in the cortex development affect mostly genes involved in the **assembly, stability** and **dynamics** of the microtubule **cytoskeleton** → **monogenetic** diseases.

NEURAL CREST CELL MIGRATION

NEURAL CREST CELLS AND THEIR DERVIATIVES

CRANIAL

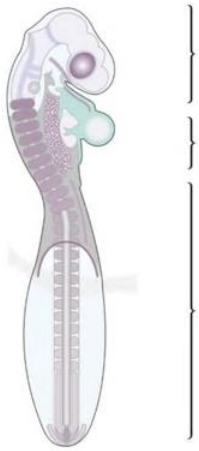
- Bone and cartilage
- Connective tissues (teeth, eyes, ears)
- Sensory neurons
- Glial cells
- Melanocytes

VAGAL

- Enteric neurons
- Smooth muscle
- Cardiac tissues
- Sensory neurons
- Glial cells
- Melanocytes

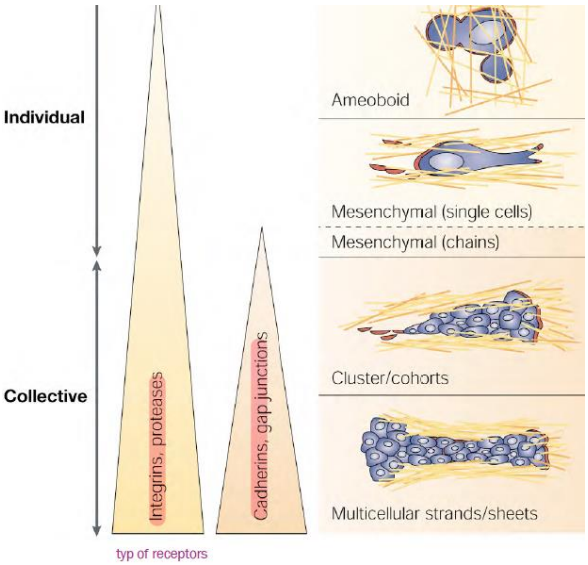
TRUNK

- Autonomic neurons
- Chromaffin cells (adrenal medulla)
- Sensory neurons
- Glial cells
- Melanocytes

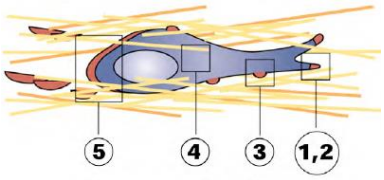


MODES OF CELLULAR MIGRATION THROUGH ECM

Cells migrate as mesenchymal (single or chain) cells in cluster/cohorts or in multicellular strands/sheets.



- Pseudopod protrusion at leading edge (farthest from nucleus): integrins bind to ECM
- Formation of focal contact: actin filaments are formed and bind to integrin intracellularly
- Focalized proteolysis of surroundings
- Actomyosin **contraction**
- Detachment of trailing edge → recycling of integrins, sliding movement



NEURAL CREST CELL MIGRATION IN EMBRYONIC TRUNK

VENTRAL MIGRATION PATH

- Dorsal root ganglia
- Sympathetic ganglia
- Medulla of adrenal gland

DORSO-LATERAL MIGRATION PATH

- Melanocytes (pigment cells of skin)

INHIBITORS OF CREST CELL MIGRATION

Somites are partitioned into a rostral and caudal part. There is **no** migration in **caudal** area.

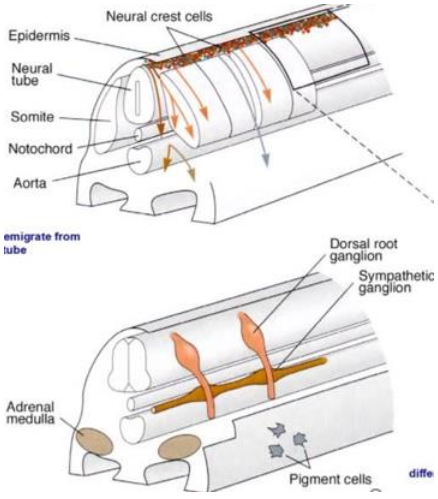
We also find **transient** inhibition between the ectoderm **and** dermomyotome.

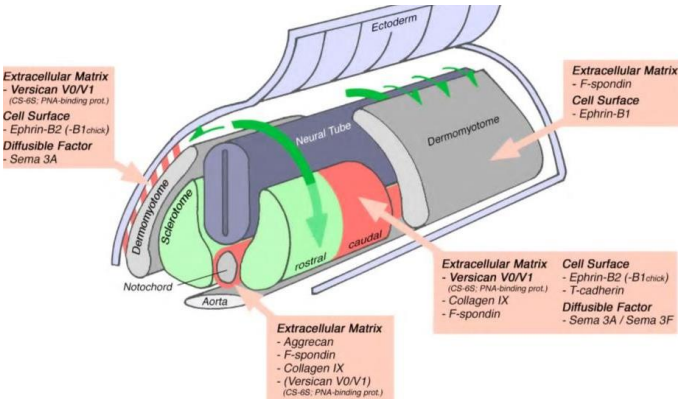
There is **no** migration **around** the **notochord**.

Factors in ECM, cell surface or diffused factor:

- *Sema 3A*
- *Collagen IX*
- *Ephrin-B1/2*
- *F-spondin*

→ *Fibronectin* **promotes** neural crest cell migration





SUMMARY

Neural crest cells emigrate from the **dorsal** neural **tube** shortly after its **closure**. Some subpopulations migrate large distances giving rise to a wide variety of **neural** and **non-neural** tissues. In PNS, **sensory, sympathetic, enteric** neurons and **Schwann** cells originate from the neural crest. **Cell surface** and **ECM molecules** of **surrounding tissues** guide neural crest cells to their targets.

PAPER

STRATEGIES FOR ANALYZING NEURONAL PROGENITOR DEVELOPMENT AND NEURONAL MIGRATION IN THE DEVELOPING CEREBRAL CORTEX – HIGGINBOTHAM (2010)

TIME LAPSE ANALYSIS OF ADHERENT NEURAL PROGENITOR CLONES

In vitro assays using isolated, single cortical progenitors from different embryonic ages are used to selectively study and manipulate RGP proliferation, differentiation and cell fate in a defined environment.

Dissociated cortical progenitors are plated on an adherent substrate at clonal density, where cell-cell contacts are minimized & the only extracellular cues are produced from the clones themselves or are exogenously added. Over several days in vitro, single progenitors generate other progenitors, distinct neuronal subtypes and glia

Time-lapse analysis: the effects of extrinsic factors or cell-cell contacts on progenitor division can be examined by adding diffusible cues to the medium or culturing progenitors at higher density

- Advantage:
  - it permits complete registering of individual cell division and behaviour and a clear analysis of intrinsic mechanisms at work in choosing cell fate
- Disadvantage:
  - dissociated progenitors neither encounter the permissive microenvironments found in vivo nor do they maintain critical features such as polarity and orientation they would have in the intact brain
  - Symmetric and asymmetric division in vivo rely on appropriate cell-cell contacts that are absent in isolated cultures → cortical slice assay helps with that

SYNAPSE FORMATION I

Seems to be convergent synapse evolution, synapses and neural systems likely evolved more than once, some organisms have synaptic genes but no synapses.

HOW TO BUILD A SYNAPSE?

- Presynaptic assembly
- Postsynaptic assembly
- Synaptic transmission

KEY REQUIREMENTS

- Flexibility and reliability
- Speed
- Repetitive use

SYNAPTIC MODULES

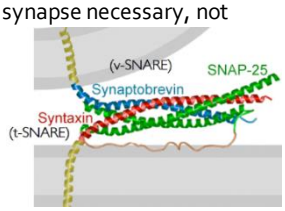
- Presynaptic
  - Vesicle fusion
  - Active zone
- Postsynaptic density (PSD)

→ Lots of independents, but all come together in the end to form one functional synapse.

VESICLE FUSION: SNARES

Vesicle fusion is enabled by SNAREs, which are soluble protein receptors. The process can just happen, no synapse necessary, not even Ca2+. Crucial proteins:

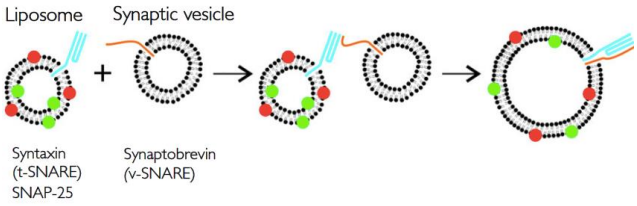
- Synaptic vesicle (*Synaptobrevin)* incorporated in vesicle membrane: v-SNARE
- *Syntaxin* and *SNAP-25* are located in target membrane: t-SNARE



FRET

Förster/**F**luorescence **R**esonance **E**nergy **T**ransfer. If two molecules are close, energy is transferred to neighboring light-sensitive molecule:

- **Decrease** in **donor** fluorescence and **increase** in **acceptor** fl.
- Can be used to probe **intermolecular distance** by looking at **transfer efficiency**



- FRET gets lower → dequenching due to fusion

EXPERIMENT

Vesicle fusion can be reconstituted in vitro, in the absence of active zone components or Ca2+. However, vesicle fusion at synapses can be accelerated by an increase in presynaptic free calcium concentration, without there is a lower rate.

KEY REQUIREMENT: SPEED

Rapid AP-triggered vesicle fusion requires a **short** distance (> 100 nm) between Ca2+ channels and synaptic vesicles → due to **low** affinity of vesicular protein that triggers fusion (*synaptotagmin-1/2*).

ACTIVE ZONE

- Platform for rapid fusion of synaptic vesicles after Ca2+ influx
- AZ-membrane is decorated by a proteinaceous cytomatrix (set of specialized proteins)

ACTIVE ZONE ASSEMBLY

CONTACT WITH POSTSYNAPTIC PARTNER REQUIRED?

Presynaptic differentiation can be **induced** by expression of a single **postsynaptic cell adhesion protein** (**Neuroligin**) on **non-neuronal** cells

Active zone **formation** can be induced after **contact** with **polylysine**-coated beads.

➢ Active zone assembly does **not** require a **postsynaptic** partner

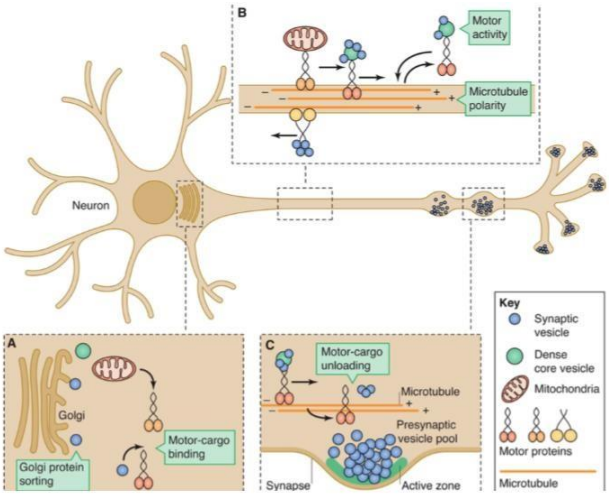
ARE ORPHAN AZS FUNCTIONAL?

Probe exocytosis using FM destaining → fluorescence signal if dye molecule is **in membrane**.

- Orphan synapses **can** undergo **exocytosis**
- Vesicle clusters are **mobile** and can undergo fusion
- **Self-assembly** of active zone → **functional synapses** can form without postsynaptic partner!

DELIVERY AS PREASSEMBLED UNITS OR SINGLE COMPONENTS?

- Most AZ components and synaptic vesicle proteins are delivered in **preassembled multi-vesicle** transport aggregates (‘**Digital**’) → chunks which are then localized
- Microtubule polarity (neg. → pos.) and specific MT-associated proteins specifically target synaptic components to synapses in axons **or** dendrites



MOLECULES INVOLVED IN AZ ASSEMBLY

- *Brp*: active zone component

GENES THAT REGULATE AZ ASSEMBLY AND SYNAPTIC FUNCTION

- Synapse defective-I (*dsysd-I*) is required for normal synaptic vesicle **targeting** in the PNS and CNS in drosophila and CNS in mice
- Liprin-*α* (*dliprin- α*)
- Small GTPase *Rab3* is required for normal AZ **distribution** and pre/post **matching**
  - Breaks in continuum and no ‘clusters’ of cells to form synapses, but **discontinuous AZ clustering**
  - *Rab3* mutant do **not** have a strong defect concerning **synaptic transmission** → normal function (Ca2+ channels are dislocated)

PRESYNAPTIC DEVELOPMENT ACROSS SPECIES

What seems to be predominantly necessary are **calcium channels**, **Neurexin** and a Leukocyte antigen-related receptor (**LAR**) on the presynapse → the **drosophila NMJ** can be used to uncover genes that are involved in AZ assembly.

Downstream of CAMs some proteins have proven to be important with guiding transport of further components to AZs (*SYD-2/Liprin- α)*.

POSTSYNAPTIC DENSITY (PSD)

Proteins that accumulate at postsynaptic side of synapse.

ASSEMBLY

IS CONTACT WITH PRESYNAPTIC PARTNER REQUIRED?

Neurexin expression in **non-neuronal** cells clusters **glutamate**- and **GABA** postsynaptic **scaffolding proteins** in dendrites → **Neurexin** can form a postsynaptic site → mimics presynaptic area.

Neuroligin expression in **non-neuronal** cells clusters glutamate and GABA **synaptic vesicles** → Neuroligin isoforms are involved in determining the **sign** of a **synapse**.

- Neuroligin-1/-3 → **excitatory** synapse
  - **Glutamate** receptors
  - *PSD-95* is scaffolding protein
- Neuroligin-2 → **inhibitory** synapse
  - **GABA** receptors
  - *Gephyrin* scaffolding protein

DELIVERY AS PREASSEMBLED UNITS OR SINGLE COMPONENTS

Non-synaptic clusters of postsynaptic scaffolding proteins → not near a pre-synapse. There is a **preassembled delivery** of **preformed** scaffold proteins.

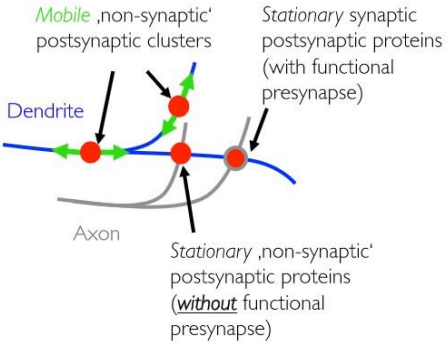
The exact shipping mechanism is unknown, there is evidence for specific and inclusive vesicles.

- AMPAR and NMDAR are shipped in **separate** vesicles
- PSD 95 and SAP 90 are recruited to synapses from **cytoplasmic pools**.

IN ADDITION

Contact per se is **not** sufficient to drive synapse formation and functional synapses are **stationary**.

Mostly, sites opposed to stationary non-synaptic scaffold clusters are **readily transformed** to **active presynaptic** terminals.



ACTIVITY DEPENDENCE

GLUTAMATE UNCAGING

2-Photon glutamate uncaging can be used to mimic NT release at individual synapses.

❖ Release a photon close to a dendrite where there is **no** evidence of a postsynaptic spine → trying to induce postsynaptic structure without actual pre-synapse.

Glutamate uncaging can be used to '**grow**' a postsynaptic structure (spine [spine is **not** equal to post-synapse but a pre-requisite]) within **seconds**.

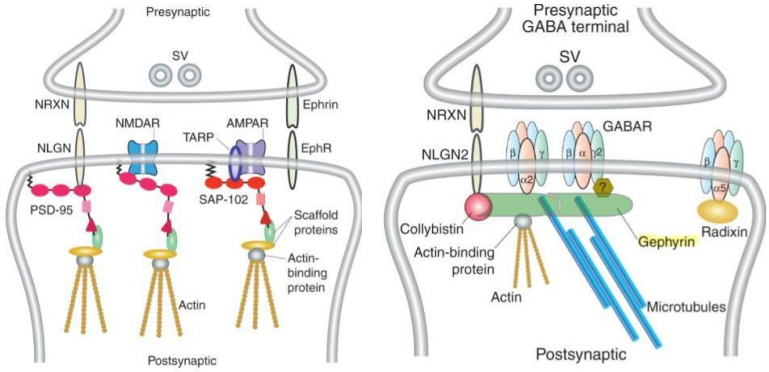
ACTIVITY DEPENDENT SPINOGENESIS

Glutamate uncaging induces spine growth and accumulation of PSD-95 (PSD protein in mature synapses) → **plasticity!** Only possible with presence of **Neuroligin-1** → regulator of activity-dependent spinogenesis.

POSTSYNAPTIC AND TRANSSYNAPTIC MOLECULES

EXCITATORY AND INHIBITORY SYNAPSES (PSD)

They are characterized by specific pre- and postsynaptic proteins.

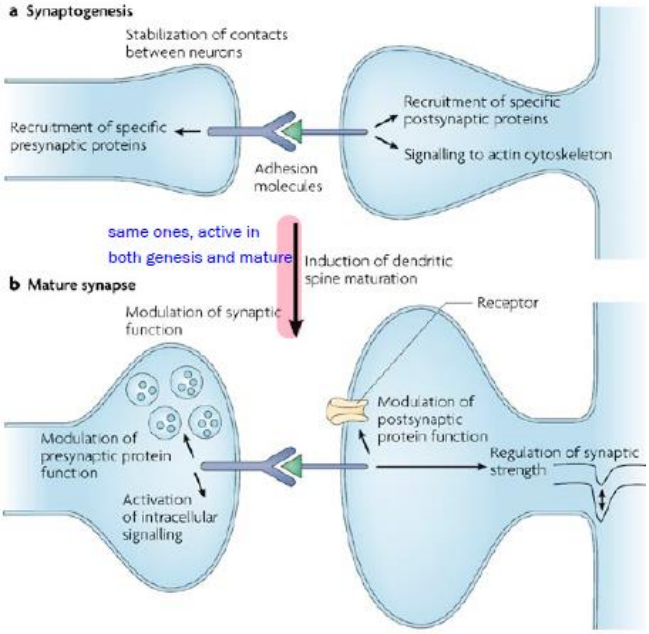


TRANSSYNAPTIC MOLECULES

Homophilic and **hetero**philic interaction between **cell adhesion** molecules at synapses. All transsynaptic molecules have a different function. Cell adhesion molecules have been linked to autism-spectrum disorders.

- Homophilic: expressed at both sides (***SynCAM***, *Sidekick*, *Cadherins*)
- Heterophilic (*NL/NRX*, *EphB/ephrinB*)

Transsynaptic cell adhesion molecules play specific roles in synapse **formation** (→ recruitment of specific pre-/postsynaptic proteins, signaling to actin cytoskeleton [post]) and in synaptic **plasticity** at **mature** synapses (→ modulation of pre-/postsynaptic protein function, activation of intracellular signaling [pre]).



- Cell adhesion molecules have been linked to ASD

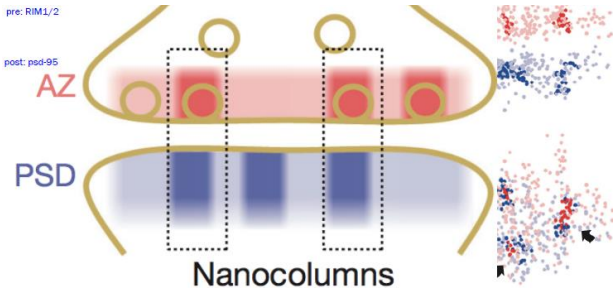
ALIGNMENT OF SYNAPTIC MODULES

ANALYSIS

PHOTOACTIVATED LOCALIZATION MICROSCOPY (PALM)

'Super-resolution' light microscopy approach based on stochastic [use of statistics] activation of fluorophores to increase spatial resolution and can be further increased using Gaussian fit function.

- Can be used to count PSD-95 molecules
- Reveals aligned 'nanoclusters' of presynaptic and postsynaptic molecules → 'nanocolumns'



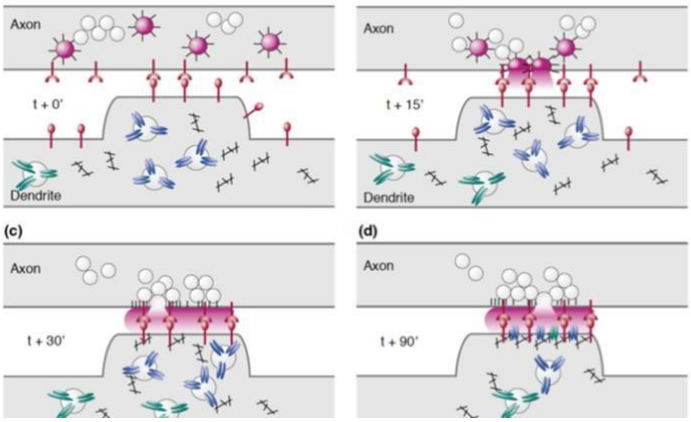
TIMING OF SYNAPTOGENESIS

Cell adhesion molecule **interaction** → **pre**-synaptic **assembly** → **post**-synaptic **assembly**.

CNS GLUTAMERGIC SYNAPSE

Multistep process initiated at, or shortly after initial axo-dendritic contact.

- Activation of 'classical' CAMs such as *cadherins*, or specific pairs of CAMs such as *neuroligin-neurexin*.
- Pleomorphic vesicular clouds become clustered pre-and post-synaptically at sites of cell-cell contact.
- The electron-dense core of the 80 nm vesicles suggests that these might also deliver synaptogenic factors that could help drive postsynaptic differentiation.
- Differentiation of the postsynaptic cell appears to occur by the sequential, in situ, recruitment of PSD **scaffolding molecules** followed by **glutamate receptors** and PSD **signaling molecules**.



MATURATION

DIFFERENCES BETWEEN SYNAPSES

SYNAPTIC TRANSMISSION STRENGTH

Degree of postsynaptic voltage/current change in response to AP stimulation. Differences in NT **release probability** affects synaptic strength, these differences depend on **number** of synapses per **dendritic branch** → **low number of synapses** per branch means **high release probability**.

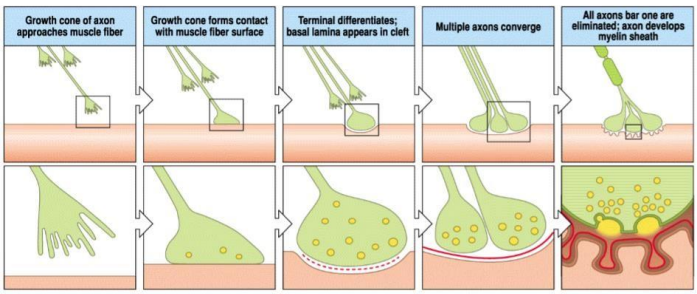
- Can be measured with FM destaining → when **rapid** there is **high** release probability

ELIMINATION AND COMPETITION

PRUNING

Many synapses are eliminated during maturation. Refinement of topography, convergence and postsynaptic compartment.

IN VIVO MOUSE NMJ IS USED TO STUDY SYNAPSE MATURATION
More than one axon go to postsynaptic clusters at the beginning of development, but they disappear over time → percentage of multiple innervated NMJ goes **down**.

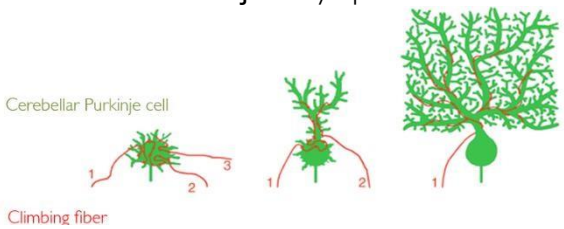


Synapse elimination results in **monosynaptic** innervation (in NMJ one **motoneuron** innervates one muscle fiber)

- Intermingled fibers become **segregated** and a **withdrawal** occurs, by the 'losing' axon
- Patterns of **motoneuron activity** modulate synapse elimination at NMJ

COMPETITION

All synapses but **one** are eliminated during development of **climbing fiber – cerebellar Purkinje cell** synapses.



Activity-dependent synapse **elimination** of cerebellar climbing fiber – Purkinje cell synapses requires PQ-type [voltage-gated] **Ca2+ channels** and *Arc*.

- Ca2+ activated *Arc* which eliminates unnecessary connections, without *Arc* no elimination → mutant

PLASTICITY

- Only the '**largest**' [ESPC amplitude] climbing fiber input becomes stronger → the strongest is also the most plastic
- **Synchronous** activation of **climbing fiber** and **Purkinje cell** is often used to **induce long-term increases** in synaptic strength (LTP)
  - LTP is induced through an **increase** in **conductance** of **AMPA**-type glutamate receptors → ESPC amplitude **increases** after CF-spike pairing = strongest, others are weak
  - Winner synapse undergoes LTP (strengthening) and eliminates the other synapses through **postsynaptic** Ca2+/Arc signaling

SUMMARY

HOW TO BUILD A SYNAPSE

- **Preformed** complexes of pre- and postsynaptic proteins
- **Specific trans-synaptic** signaling
- **Assembly** of pre-synapse leads to **assembly** of post-synapse
- Maturation (often) involves **synapse elimination**, which is **activity dependent**
- Changes in synaptic **strength** (synaptic **plasticity**) of mature synapses involve molecular mechanisms that were already used during **synaptogenesis**

PAPER

ASSEMBLING THE PRESYNAPTIC ACTIVE ZONE – OWALD (2009)

During nervous system development, synaptic circuitry must be defined by forming synaptic connections with high spatio-temporal precision. Synapse formation seems to proceed properly in the absence of neurotransmission, neuronal activity can trigger changes in the molecular composition and functional status of synapses.

SYNAPTIC MODULES

At the presynaptic site, there is the active zone (AZ):

- The AZ provides the platform for rapid fusion of neurotransmitter-filled synaptic vesicles (SVs) after calcium influx
- It is decorated with a proteinaceous cytomatrix (CAZ): a set of specialized proteins, variable morphologies at different synapse types, which are critical for effective organization of the associated SV exo-/endo-cycle machinery At the postsynaptic site

Postsynaptic density (PSD):

- Accumulation of neurotransmitter receptors
- Stability and dynamic regulation of neurotransmitter receptor population

In between of the pre- and post-synapse, there is the synaptic cleft:

- characterized by the presence of trans-synaptic pairs of cell adhesion molecules

MODULARITY OF SYNAPSE ASSEMBLY

Are the synaptic modules independent units of assembly, or does their formation require the presence of a synaptic site assembling the other modules in parallel?

- Vesicle fusion can be reconstituted in vitro, in the absence of cytomatrix scaffolds and even of calcium
- In immature neurons, mobile moving clusters of SVs have been observed exchanging with the neuronal plasma membrane in the absence of postsynaptic differentiation
- Presynaptic AZs can form in the complete absence of postsynaptic partner cells (no postsynaptic specialization) → Drosphila
- Formation of AZs (in cultured neurons) can be induced by the presentation of a single postsynaptic cell adhesion protein (Neuroligin) expressed on non-neuronal cells
- Postsynaptic differentiation is inducible by Neurexinin young hippocampal neurons

So, vesicle release machinery, AZ matrix and to some degree the postsynaptic specialization can display intrinsic assembly propensities, forming 'in isolation'. Under physiological conditions, it is expected, that synaptic modules communicate to fine tune the synapse assembly process.

A DIGITAL NATURE OF AZ ASSEMBLY AND STRUCTURE?

Are preassembled units of AZ proteins shipped to prospective AZs, or are AZs assemble de novo from diffuse pools of the relevant proteins?

- Presynaptic proteins have been suggested to be transported in specialized transport vesicles (80nm dense) → Piccolo/Bassoon transport vesicles (PTV) suggested to carry a comprehensive set of AZ materials, providing unitary building blocks of AZs.
  - more complicated: both PTV and SVs seem to be transported in a preassembled multi-vesicle transport aggregate, with the potential to form rapidly functional presynaptic sites
- At least at some specialized synapses not all players seem preassembled but rather arrive in a sequential fashion

PROVIDING THE BACK BONE

Mammalian CNS boutons persist for long periods and apparently do not eventually equilibrate and become identical in size, composition, or function.

At drosophila NMJ synapses, two different glutamate receptor complexes are co-expressed within individual PSD (*GluRIIA* or *GluRIIB*). *GluIIA* is a good candidate to confer persistence to postsynaptic assemblies in Drosophila → incorporates irreversibly and correlated with PSD growth

Presynaptic tenacity might be based on tenacity of CAZ, constructed of a static backbone (bassoon) and mobile machinery. This could allow clustering of less static synaptic proteins in a dynamic equilibrium.

SYNAPSE ASSEMBLY IN VIVO: SEQUENCE AND TIMING?

A rapid assembly leading to mature synapses in one to two hours or less, *in vivo* synapse assembly has recently been suggested to protract over many hours.

- The adult rodent neocortex showed that spine growth precedes the growth of synapses *in vivo*, newly formed spines became functional within a day after **LTP** induction
- Synapses form within hours after **spontaneous** spine formation

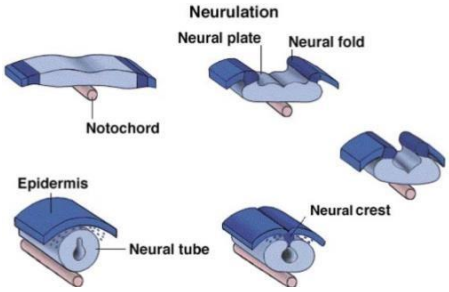
DEVELOPMENT OF NEURAL CREST CELLS

STEM CELL PROPERTIES

Finely tuned balance between SC **maintenance**, **proliferation** and **differentiation** → **generation** of specialized cell types at correct **location**, **time** and in appropriate **numbers**. The neural crest is a **good model system** is study SC biology. Neural crest cells generate most of PNS, skin pigment cells, smooth muscle in outflow tract of heart, craniofacial bone and cartilage, glial cells, adrenal medulla, ...

GENERATION OF NEURAL CREST CELLS

This happens during neurulation. Neural crest cells are **highly migratory** cell population in embryo, emerging at **dorsal** part of **closing** neural tube during neurulation → change from epithelial to **mesenchymal** structure (Epithelial-to-mesenchymal transition (**EMT**)).

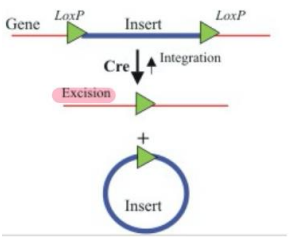


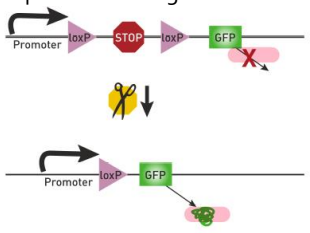
CRE/LOXP SYSTEM

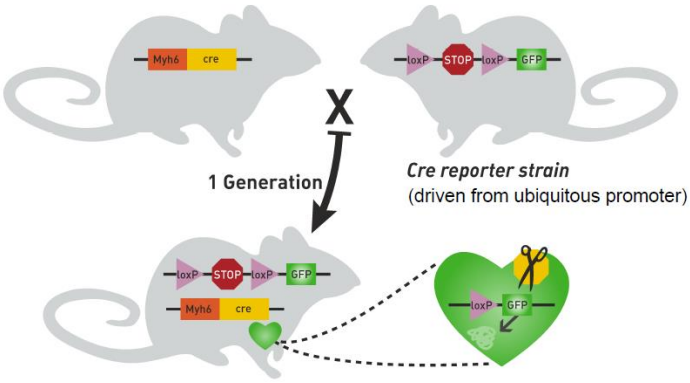
In vivo cell fate mapping using **Cre-recombinase-mediated** recombination in mice. It enables the **targeted removal** of DNA sequences.

Cre is a recombinase enzyme, which catalyzes cleavage and regeneration. The **recognition site** is called loxP, which must be before and after sequence of interest, the sequence meant to be **removed**. Cre recognizes loxP and **binds it**, by doing so it **cuts** the relevant sequence between the two loxP sites and **rejoins** them.

- To mark not only SCs but also their daughter cells you have to do recombination, then markers are also expressed in daughter cells



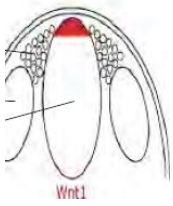




- **GFP** fluorescence **confirms** Cre activity in expected tissues

EXPRESSION OF WNT1

Specifically, in the **dorsal** neural tube and in **premigratory** neural crest population as **all axial** levels.



MAPPING CELL DERIVATIVES

You take two identically staged chicks in ovo, one with a black coat and one with white. Then you transplant one little part of the black embryo to the white embryo in the same stage. You might then have a white coated young chicken with some black spots. This way you can map the whole body and find out which parts derive from which cells in the embryo.

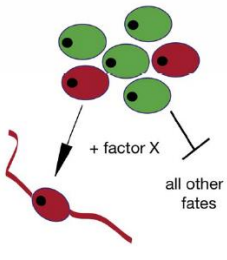
FATE VS. POTENTIAL

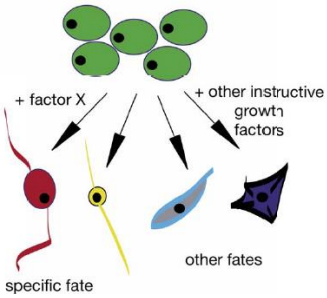
In vivo, NCCs differentiate based on their **axial** position. If you implant a transplant at another location than it originates from → it will adapt to the **new** location and differentiate into cells necessary at **that new location**.
→ Fates of neural crest cells are influenced by new **environmental signals** and **axial positions**.

DEVELOPMENT OF MULTIPLE CELL TYPES

SELECTIVE EFFECT
Selective effect of a factor on a **heterogeneous** population of **lineage-restricted** cells → selective elimination or proliferation.

INSTRUCTIVE EFFECT
Instructive effect of a factor on a **homogenous** population of **multi-potent** stem cells → fate specification at expense of all other possible fates.





→ How to distinguish?

NEURAL CREST CULTURE SYSTEM

Clonal analysis in vitro reveals multipotency and self-renewal capacity through neural crest explants that are then cultivated.

- *P75*: low affinity **neurotrophin** receptor (surface molecule allowing prospective identification and direct isolation)
- *Sox10*: a high mobility group (HMG) transcription factor involved in regulation of embryonic development and determining cell fate.

In vitro SCs **seem** similar to **ES cells**

IDENTIFICATION OF CUES REGULATING NCSC DEVELOPMENT

Instructive [because it becomes something at the expense of something else] growth factors regulate early emigrating NCCs, they differentiate into:

- +Wnt1/BMP2 → Neural crest stem cell
- +Wnt1 → Sensory neuron
- +BMP2 → neurons of autonomous nervous system
- +TGFβ → smooth muscles, cell cycle exit

IN VIVO RELEVANCE OF CUES

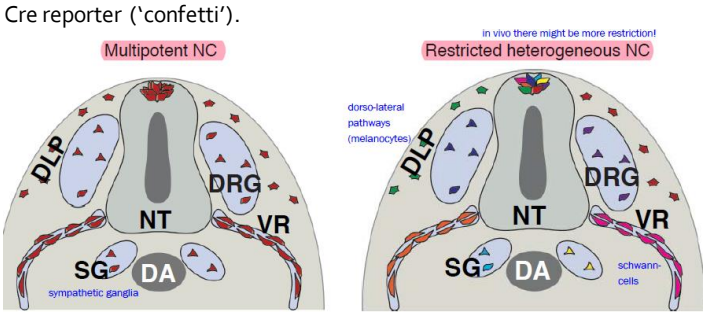
Combination of in vitro and in vivo approaches reveal relevance of TGFβ, Wnt, BMP and other signaling pathways for neural crest stem cell renewal and fate decisions.

APPROACH
Best is to find a way to **delete downstream segment** like a receptor to find out which gene can block TGFβ signaling.

TGFβ MUTANT
Conditional mutant [mutation that has wild-type (or less severe) phenotype under certain "permissive" environmental conditions and a mutant phenotype under certain "restrictive" condition] that prevents normal smooth muscle, bone and cartilage formation.

IN VIVO RELEVANCE OF MULTIPOTENCY

APPROACH
Clonal analysis of pre-migratory and migratory NCCs using multicolor Cre reporter ('confetti').



There will be different color combination depending on how Cre is combined (random stochastic manner), resulting in different colors of the cells.

COLOR FREQUENCIES
For very rare color combinations: low probability that a cohort of equally colored cells in a given area derive from several distinct mother cells expressing the same color combination

QUANTITATIVE ANALYSIS OF CLONES
In vivo tracing of unicolored clones in multiple derivatives → cell is **multipotent**.
There are both kinds of cells:

- **Fate-restricted founder** cells → **only** in certain areas (DRG, SG, VR)
- **Multipotent founder** cells → in multiple areas (DRG/SG/VR/...)

STATISTICAL EVALUATION

- In mice, vast majority of NCCs appear to be **multipotent** at the stage analyzed, with very **few** clones contributing to **single** derivatives
- Intriguingly, multipotency appears to be **maintained** in **migratory** NCCs → evidence for stem cells **in vivo**
- Neural crest-derived cells with **stem cell features** also persist in **adult structures**
  - prospectively identified *p75/Sox10*-**positive** neural crest derived cells in **adult skin** display **self-renewal capacity** and are **multipotent** → cornea, bone marrow, heart, gut, DRG, trunk skin in hair follicles

ADULT NEURAL CREST CELLS

Aged concept of Cajal was: 'everything may die, nothing may be regenerated', which has now been proven to be inaccurate.

New neurons are generated throughout life in distinct regions of the mammalian brain. This process, called **adult neurogenesis,** has been implicated in physiological brain function, and failing or altered neurogenesis has been associated with numerous neuropsychiatric diseases → important player in brain **homeostasis** and **disease**.

The mammalian brain retains the capacity to generate new neurons throughout life. Theses adult neural stem/progenitor cells (NSPCs) reside in two main locations in **adult** brain:

- Subventricular zone (SVZ)
- Hippocampal dentate gyrus (DG)

GETTING FROM A FERTILIZED EGG + PLURIPOTENT CELLS TO HIGHLY SPECIALIZED NERVOUS SYSTEM

- After gastrulation → neurulation
- Principle idea → generate **tissue polarity**
- Going from simple to highly diverse and complex

HOW TO VISUALIZE DIVIDING CELLS & THEIR PROGENY?

TOOLS

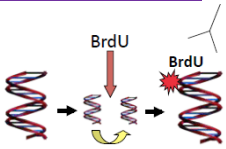
ENDOGENOUS MARKERS

pH3, Ki67, mitotic figures (replicating DNA) → are expressed at different developmental stages.

- use **antibodies** to identify the markers → visualization of stem cells

THYMIDINE ANALOGUES (BRDU)

BrdU is integrated into DNA of dividing cells, which can later be recognized and visualized by antibodies. Their advantage is **lineage tracing** to follow maturation process of NSPCs.



- Visualize newly formed **nuclei**

ANTIBODY

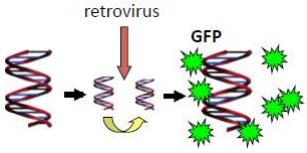
NeuN (neuronal nuclear antigen) that is a common biomarker for neurons. When combining BrdU and NeuN techniques it can be used to detect **newly born** cells that **differentiate** into **neurons** → evidence for lifelong neurogenesis.

FUSION PLASMIDS

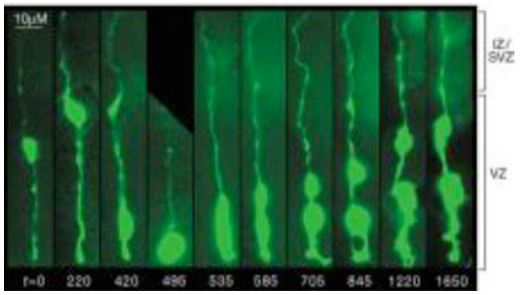
Tubulin, histones, etc...

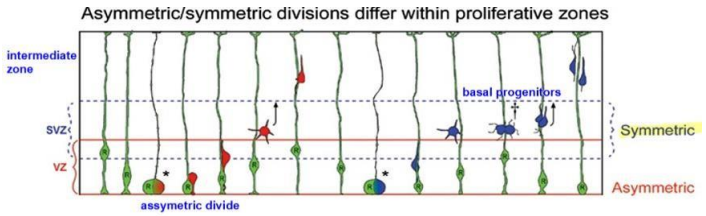
RETROVIRUSES + PROGENY

Dividing cell includes the retrovirus during cell division, since the nucleic membrane is **open**.



- Moloney mammalian leukemia (**MML**) viruses
- Can watch radial glia divide and generate neuronal progeny → division at the ventricular side (bottom)
- **Visualize cell morphologies**

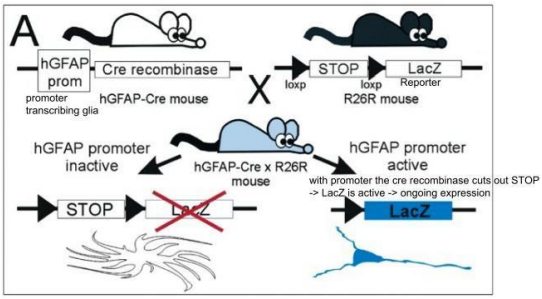




There are not only radial glial cells in VZ but also SVZ. Daughter cells migrate along radial glial cells, while glial cells stay rooted in VZ. A human brain has many more neurons than a mouse brain with a larger cortex surface meaning there is not enough space to put all the radial glia cells in the VZ → radial glia in **outer SVZ** in humans

TRANSGENIC LINEAGE TRACING

LoxP gets stopped if there is **no** Cre recombinase If LoxP gets stopped, no LacZ → with a promotor there is expression of LacZ.



C14

Radioactive carbons are found to be high in brain allowing birth dating of individual neurons. This showed that many neurons in hippocampus were born during adulthood → 80% of DG is replaced during lifetime.

ONGOING NEUROGENESIS IN MAMMALIAN BRAIN

In the DG newborn cells differentiate into excitatory granule cells (principal DG neurons). NSPCs in the SVZ generate restricted neural progenitor cells that migrate through a glial cell scaffold via the rostral migratory stream (RMS) towards the olfactory bulb, where newborn olfactory neurons mature and functionally integrate.

- Mice with enriched environment learn faster to find the platform → learning increases brain size
- Chronical stress and depression leads to degeneration of the hippocampus (chronic antidepressant treatment in rats increases neurogenesis in the hippocampus!)
- Mice with decreased neurogenesis are impaired at spatial pattern separation

LINEAGE PROGRESSION AND MOLECULAR REGULATION OF ADULT NEUROGENESIS

From the largely quiescent NSPCs, type-1 cells (**DG**), B-cells (**SVZ**) or radial glia-like cells (**DG & SVZ**), to a fully integrated and functional neuron, NSPCs must pass through several development steps:

- NSPC population is activated to generate proliferating, non – radial transit amplifying cells (**TAPs)** → type-2 cells (DG) and C-cells (SVZ)
- Type-2 cells and C-cells give rise to **immature** neurons (A- cells in SVZ**)**, which progress through neuronal differentiation
- Within the DG, immature neurons migrate up into the **granule cell layer**, projecting out a large dendritic arbor into the adjacent molecular layer and axons that innervate target cells

WHAT CONTROLS NSPC ACTIVITY?

Neurogenesis is controlled by **niche-derived** as well as **intrinsic** mechanisms, which together ensure the **appropriate levels** of

proliferation of NSPCs, as well as the correct differentiation, migration and integration of newborn cells.

- Transcriptional regulators
- Epigenetic mechanisms
- Niche-derived morphogens, neurotransmitters, growth factors and cytokines: GABA, glutamate, BDNF, EGF, Wnt, Shh, BMP, IL6, TNFα

SYSTEMIC REGULATION OF ADULT NEUROGENESIS

The number of neurons born in the adult brain is dynamically regulated by several **extrinsic environmental** factors:

Positive regulators: physical activity, environmental enrichment, olfactory or hippocampus-dependent learning, which enhance NSPC proliferation and/or survival of new neurons

Negative regulators: stress, certain forms of inflammation, alcohol abuse and age

Additional regulators: olfactory enrichment and/or deprivation

FUNCTIONAL SIGNIFICANCE OF ADULT NEUROGENESIS

Newborn neurons contribute to olfactory- and hippocampus-dependent learning and memory. Increased levels of neurogenesis correlate with **improved performance** in hippocampus-dependent learning and memory tasks (Morris water maze).

Studies that use transgenetic- and virus-based strategies to deplete or enhance neurogenesis, have identified a role for hippocampal neurogenesis in **spatial** and **object recognition memory**, **fear conditioning**, **synaptic plasticity** and **pattern separation** (= process of transforming similar representations or experiences into distinct and non-overlapping neural representations).

Failing or altered neurogenesis has been associated with several neuropsychiatric diseases, including **major depression** and **epilepsy** (neurons frequently migrate ectopically and show aberrant synaptic integration). A reduction in the number of neurons generated as well as reduced ectopic integration may contribute to hippocampus-dependent behavioral deficits. Hippocampal neurogenesis has been also linked to emotional control.

→ The same environmental factor may affect neurogenesis in one region but not another

CONTRIBUTION OF NSPCS FOR BRAIN REPAIR

The idea is to boost NSPC proliferation and/or newborn cell migration towards lesioned tissue either following acute injury, such as stroke, or during chronic neurodegeneration (for example Parkinson's disease). This may be achieved by through **chemokine-directed migration**.

Alternative sources of NSPCs include cells generated in vivo by directed differentiation of NSPCs in glial cells, or by reprogramming of somatic cells.

This may hold therapeutic potential for the treatment of diseases of CNS and may be useful to repair not only neuronal cell loss but also glial dysfunction (for example chronic demyelinating disease MS).

SLEEP AND DEVELOPMENT

HOW DO WE MEASURE SLEEP

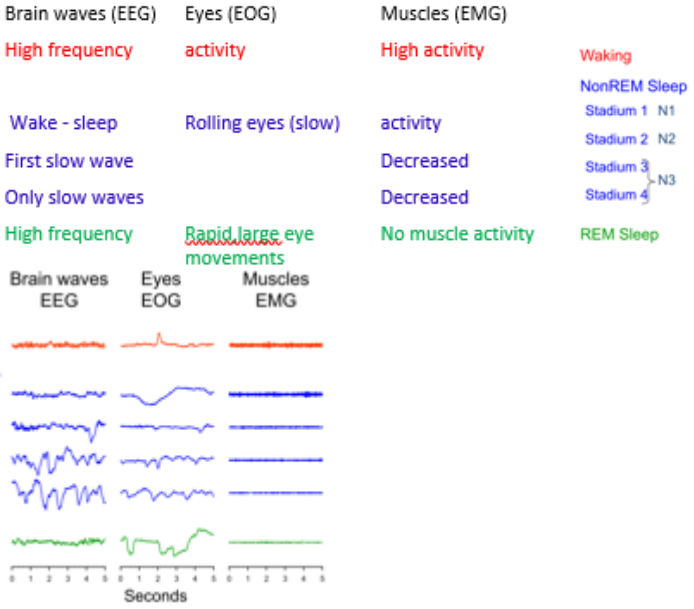
ELECTROENCEPHALOGRAPHY (EEG)

EEG measures **potential differences** in large cortical networks

RAPID EYE-MOVEMENT (REM) SLEEP

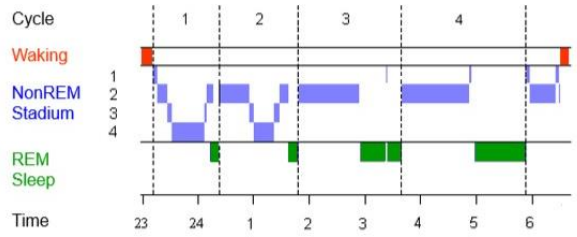
Discovered first in humans (earlier associated with dreaming), later in cats. Started golden years of sleep research.

SLEEP STAGES (VIGILANCE STATES)



- N1: if you wake them up, about 50% claim they were still awake
- N2,3: absence of eye movement – electrodes for eye movement sometimes also register brain waves, which is why line isn't zero
- REM: EEG shows fast frequency and low amplitude, as in wakefulness, complete absence of muscle activity, except eyes

SLEEP ARCHITECTURE



Alternation of non-REM & REM sleep during the night, about 4-5 sleep circles. Over the night:

- Deep non-REM sleep decreases towards end of night
- REM sleep duration increases (brief REM sleep periods in beginning)
- Number of slow waves & sleep depth decreases across sleep period

SLOW WAVE ACTIVITY (SWA) REFLECTS SLEEP HOMEOSTASIS

SLEEP HOMEOSTASIS

Balancing sleep pressure over 24hrs, it increases whenever you are awake, SWA correlates with it.

- Normal: SWA high at beginning of night
- Sleep deprived: more SWA
- Daytime nap: less SWA

→ Sleep is a **regulated** process
→ **EEG slow waves** reflect sleep homeostasis

NEURONAL LEVEL

Need animal model → vigilance states are the same in rodents

- Also in rodents SWA reflects sleep homeostasis
- Can study these aspects in animal models

SLEEP SLOW OSCILLATIONS

On the neuronal level EEG slow waves are reflected by an alternation between **ON** (spiking activity, neurons fire briefly at high rate) and **OFF** (no activity, neocortical neurons rest) periods

- **Early sleep**: all units are synchronized → large amplitudes of slow waves
- **Late sleep**: not synchronized anymore → more shallow sleep

→ Cause of changes in synchronization: Long term potentiation (LTP). The higher the synaptic strength, the better the synchronization.

SYNAPTIC STRENGTH AND SLOW WAVES

Computer model mimicking sleep (Quantification by measuring slope)

- **High synaptic strength**: Synchronized alternation → high amplitude waves
- **Low synaptic strength**: more shallow slow waves

→ On neuronal level: slow waves are reflected by ON & OFF periods

→ Level of synchronization is determining the size of the slow waves

DEVELOPMENT OF SLEEP

FROM POLY-TO MONOPHASIC SLEEP

In the first year of life a sleep pattern is established, since in beginning rest-activity pattern is very irregular. The older a baby gets the number of sleep phases decreases but duration of one phase increases.

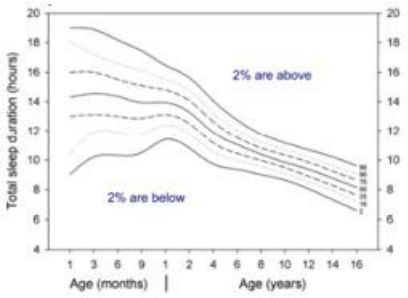
- Most adults develop a monophasic sleep pattern (not true for all societies)

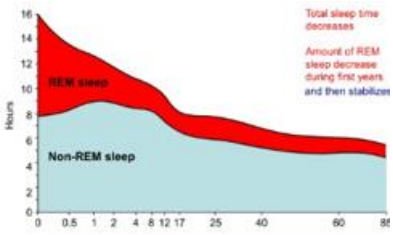
CHANGES IN SLEEP DURATION

Age-dependent **inter-individual** differences in sleep duration. Very individual in infants: some sleep 9-10hrs, others 19hrs. while getting older the total sleep time decreases as well as amount of REM sleep. SWA increases slightly with a peak around 5-10 years old.

SLEEP STAGES DURING DEVELOPMENT

- Total sleep time decreases
- Amount of REM sleep decreased during first years (then stabilizes)
- Inter-individual differences decrease in adulthood





SIMILAR TRAJECTORIES

Synapse density and energy consumption are in a very close relationship, low in the beginning, peaking at around 6 years old, then decreases again. Lots of synapses need lots of energy, efficiency is improved with pruning (optimization).

- Number of neurons stay the same
- Pruning during puberty (synapses are removed again)

CHANGES IN CORTICAL EXCITABILITY

More synapses lead to **increased** network synchronization and **larger** slow waves.

- Responsiveness to stimulation in newborn is quite small
- Increased cortical excitability in pre-pubertal children
- Reduced responsiveness of the system in adolescents → refining process during adolescence (more synapses are eliminated than newly formed)

MEASURE STRUCTURAL CHANGES DUE TO PRUNING

- Two photon imaging in mice: quantify the spines over time
  - more spines are eliminated than newly formed during pruning period
- Structural magnetic resonance imaging: MRI cortical thickness (quantify volume of grey matter)
  - Grey matter becomes thinner (decrease in volume)
  - Cortex of pre-pubescent girl is thinner than that of an adult, peak cortical thickness between 7-13 years old depending on location

LOCAL MATURATION

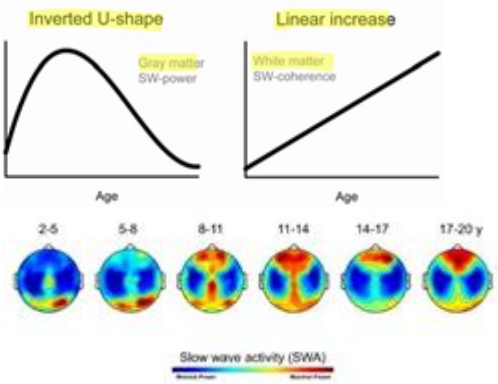
Not all areas are maturing at the same time. Cortical grey matter maturation starts in the back and ends in the front → **back to front**. Sleep slow waves mirror cortical maturation.

AGE DEPENDENT SWA TOPOGRAPHY

SWA maturation **from back to front,** correlates with performance of tasks getting better when that part of cortex is matured → parallel **anatomical and behavioral** maturation.

→ Gray matter maturation parallels changes in SWA

→ White matter shows a linear increase across age (up to 34yrs), parallel to SW-coherence



- Youngest children have highest activity in dorsal part of the brain and the older they get this activation translocates to the front.

FUNCTIONAL RELATIONSHIP (CONNECTIVITY – BEHAVIOR)

BACK BRAIN REGIONS

- synapse density peak is early
- fast skill development during first year (max at 4yrs)
- functions from visual cortex

FRONTAL BRAIN REGIONS

- later peak in synapse density
- skill development takes longer (max at 30yrs)
- higher cognitive functions
- → predominance of SWA shits from back to front
- → Parallels anatomical & behavioral maturation
  - How could this be explained?

SYNAPTIC HOMEOSTASIS HYPOTHESIS

Function of sleep is to maintain synaptic strength over time. Synaptic homeostasis may play a role during development.

DAY (WAKEFULNESS)

When you are awake, you learn things and overall synaptic strength is increases over the day. But at some point, the membrane is saturated and more cannot be added. All this costs energy and needs a counter activity. If you have a stronger, denser network, the SWA is increased.

- Learning induces synaptic potentiation
  - more synaptic strength
  - increased energy expenditure → saturation
  - Slow wave increase → sleep

NIGHT (SLEEP)

Slow waves are responsible for synaptic downscaling (renormalizing synaptic strength) and brings overall synaptic strength down→ loss of weak connections, only strongest synapses survive → sum will be the same as before but **relationship changes**, important selected over non-important *things*

  - energy expenditure decreases

- more potential to learn new things
- slow wave decrease → process comes to halt

SYNAPTIC STRENGTH IS BALANCED ACROSS 24HRS

→ synaptic homeostasis

Reducing synchronization, reduces slow waves → self-regulation.

- Wakefulness favors **synaptic potentiation / synapse formation** (more spines are formed than lost)
- Sleep favors **synaptic depression / synapse elimination** (more spines are lost than formed)

RELATED TO PERFORMANCE? → LOCAL INCREASE IN SWA

Local areas show increased SWA after learning during sleep. The more SWA in this area the better performance the next morning, confirming that **non-REM sleep** is important for **learning**.

→ if SWA is suppressed the subject could not increase the performance

→ Slow waves seem to be related to sleep-dependent performance changes

RELATIONSHIP TO MARKERS OF MATURATION - CHILDREN

- Experience-dependent increase in SWA is larger in children
- Children seem to benefit more from sleep

CONTINUOUS SPIKE-WAVE EPILEPSY IN NON-REM SLEEP

Children with epileptic spike waves during sleep lose cognitive abilities again over night:

- disturbing SW pattern eliminates renormalization of synaptic strength
- Saturation stays → cannot learn new things or loses abilities again

FROM GENES TO BEHAVIOR – CANEORHABDITIS ELEGANS

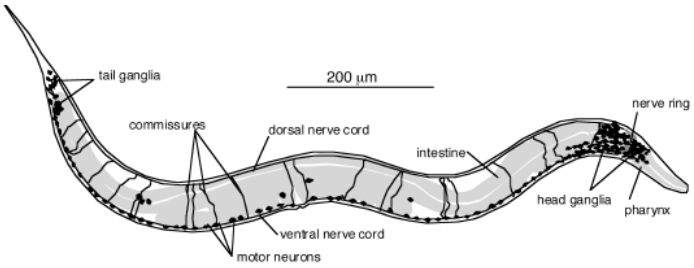
C. ELEGANS AS A MODEL FOR NEUROBIOLOGY

Compared to mouse it is much simpler and therefore function of nervous system is observable and influence on behavior can then be reverse analyzed.

ANATOMY

Animal is made of 959 cells; germ cells are not included because there is constant proliferation (stem cells) but somal = 959. Embryogenesis is 800 minutes long. It is known when which cell will be made and what is will become.

- Invariant lineage
- 131 die at a predetermined stage → apoptosis (not incl. 959)
- Different parts of lineage contribute to nervous system
- Extensive **cell migrations** during embryogenesis



ANATOMY OF THE NERVOUS SYSTEM

They can smell, perceive environment, move (motorneurons), also more complicated functions: such as behave and adapt to environment, **change** of behavior, simple forms of memory (for a few hours, maybe a day) → 302 neurons. Each neuron has a name and a specific place in

- Anterior **nerve ring** ('brain'), **dorsal** [lots of axons but low cell body count] and **ventral** [most cell bodies] nerve cords, **sensory** amphids [principal olfactosensory organs of nematodes] in the head and **phasmids** [unicellular sensilia in the lateral tail region] in the tail.
- Three classes of neurons: **motor-**, **inter-** and **sensory** neurons
- **Invariant** neuronal network and **invariant** connections, completely described through EM reconstruction → www.wormatlas.org

THE WORM 'NEURO' GENOME

Approximately 19'000 genes on 6 chromosomes. Genes with functions in nervous system:

- Channels: voltage -gated K+, **no** Na+, ligand-gated ion channels
  - How do they make APs without Na+ channels? Neurons are much shorter so no need for **propagation** of signal
- Neurotransmitters (same as us), synaptic function
- 80 known GPCR, 700 'orphans'
- G proteins
- 1 CREB: transcription factor, important in memory
- Kinases [P from ATP] and phosphatases

Much less cells than vertebrate but genetical complexity is **high**, almost comparable to vertebrate.

METHODS IN WORM NEUROBIOLOGY

STUDYING FUNCTION OF NEURON

Electrophysiology (not best idea), knock-out mutants (only one function can be found out at a time, removal of neurons with laser surgery (one after another to define function).

CELL ABLATIONS

This is used to identify function, nucleus is boiled through laser-microsurgery. Then testing is done to see effect of removing individual neurons on development, behavior, etc.

- Identification of all chemosensory neurons by ablation

FORWARD AND REVERSE GENETICS

FORWARD

Mutagenesis by EMS (produces **random** mutations in genetic material, typically produces only **point** mutations) and then screen for animals with altered behavior → desired phenotype.

REVERSE

Knock-outs by small deletions, CRISPR allows point mutations.

TRANSGENIC WORMS

GFP LABELLING

This allows labelling of specific neurons with GFP, markers which are the basis for genetic screens to identify mutants.

OPTOGENETIC MANIPULATION

- Depolarization → activation (Na+)
  - Blue light
- Hyperpolarization → inhibition (Cl-)
  - Green/yellow light

INPUT: SENSORY SYSTEM

They cannot see, hear or speak **unlike** mouse. No dedicated eye structure but can still **react** to light through light sensitive neurons (→ known because they tend to avoid light, stay below soil).

Very good taste and smell, recognition of and attraction to NaCl and eats any type of bacteria.

SENSORY NEURONS

They are organized in amphids and phasmids. Most have been identified, 5 chemosensory neurons (AWA, AWB, AWC, ASH and ADL).

- Chemosensation: taste (gustatory) and odor (olfactory sense)
- Mechanosensation: response to touch/vibration, most along full body axis
- Themosensation: sensitive to heat and cold, comfortable at 20°C
- Osmosensation
- Light sensitivity

OUTPUT: BEHAVIORAL ASSAYS

LOCOMOTION

They are capable of **forward**- and **backward** movement, which can be **quantified** through **swimming tests, frequency** of thrashing or **reversal** of movement. Many 'Unc' (uncoordinated movement) mutants from genetic screens. Modulation by external stimuli → mechanical stimulus, odorants, amount of food. Locomotion is **basis** for most **behavioral assays**.

- Smell stimulus: movement towards or away?

NEURONAL NETWORKS

There is **well-defined** connectivity and a **simple** structure, only 1-3 interneurons between sensory and motor neurons.

- **A-type** motor neurons with AVA interneurons → **backward** movement (after touch on nose)
- **B-type** motor neurons with AVB interneurons → **forward** movement

The circuits can be manipulated. Mechanosensory circuit has **one** layer of interneurons, thermotaxis and chemosensory circuits have **three** layers of interneurons.

QUESTIONS

Presentation of a stimulus and analysis of output (locomotion → taxis or aversion?).

- Adaptation and plasticity? → after 20th time reaction is muted
- Signal integration?
- Environment? → how do environmental changes affect behavior?
- Development changed anatomy? Do genetic changes cause change in anatomy or just function?

Analysis of pair stimulation, which is prioritized?

GENETIC ANALYSIS OF BEHAVIOR

QUANTITATIVE BEHAVIORAL ASSAYS

Specific assays, e.g. for chemosensation are the basis for **isolation of mutants** in **forward** genetic screens, it is important to have a well-defined genetic background. Combinations of multiple stimuli allow for discrimination. → Search for genes controlling **behavior** but **not** development. Animals behaving differently than most are marked.

- Odortaxis assay
- Associative conditioning
  - Conditioned and unconditioned stimuli → 2 attractants
- Chemotactic choice assay
  - Must cross copper border to get to attractant
- Thermotaxis assay
  - Cold block in the middle and hot border, creating temperature gradient → most form circle where 20°C

→ Chemotaxis index: I = Worms at attractant divided by total worms on plate

- **Attraction**: $0 \ll I \leq I$ → food
- **Repulsion**: $-I \leq I < 0$ → copper
- **Neutral**: $I = 0$ → ethanol

ADVANTAGES

With mice, only small numbers can partake in behavioral studies and because of *C. elegans* small size more animals per study possible and real statistical data can be collected.

EXAMPLES

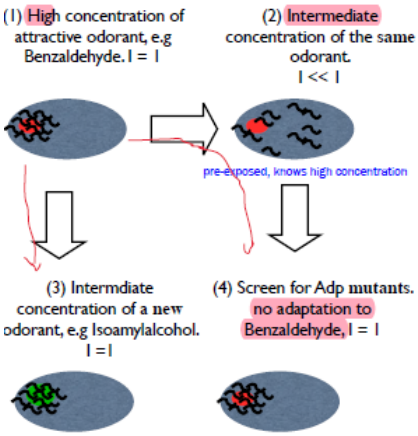
GENERATING ASYMMETRY IN THE NERVOUS SYSTEM

ASEL and ASER chemosensory neurons are **bilateral symmetric**, they respond to different stimuli and express different receptors → either one or the other is active. **Double negative feedback loop** controls neuronal asymmetry by generating and maintaining. Generation of mutants can make both active at the same time allowing cross-talk.

ADAPTATION TO ODORANTS (ADP MUTANTS)

There are 3 neurons for **reception** of volatile attractants. One neuron expresses **many different receptors** → odor discrimination occurs within a **single cell**.

- High concentrations cause adaptation without affecting odor discrimination
- Screen for *Adp* mutants showing **no** adaptation to stimulus



Genes implicated in olfactory adaptation:

- Ca2+ signaling
- Receptor desensitization: *arrestin*
- Ras GTPase

ADAPTATION TO MECHANOSENSATION

Nose touch induces reverse locomotion. Reversal **distance** is **lowered** and **response ratio** amplitude is **decreased**.

ASSOCIATIVE BEHAVIOR DURING THERMOTAXIS

Combining positive stimuli with specific environmental conditions (higher or lower than 20°C), results in a **modulation of behavior**, animals 'remember' favorable conditions.

Lrn mutants show **no** associative behavior, their genomes get tested. Very difficult molecular analysis (mapping) but several candidate genes been tested showing an *Lrn* phenotype.

- Glr-1: encodes a glutamate receptor

ASSOCIATIVE CHEMOTAXIS

Remembering negative experience such as starvation. Exposure of wild-type animals to an attractant in **absence** of food causes **aversive** response. Learning mutant show **no** aversion of attractant after food starvation, they retain **chemotaxis** to attractant (NaCl).

EXPERIENCE

- Motor neuron decides in which direction the head moves
- Controlled by three interneurons which express glutamate receptor, if knock out of glutamate receptor, is there same behavioral change?
- Input from gustatory neurons

GLR-1 GENE

- Encodes AMPA [ionotropic (ligand-gated ion channels) transmembrane receptor for glutamate that mediates fast synaptic transmission in the CNS] type glutamate receptor
- Introduce a Glr-1::GFP reporter to observe localization at synapses in **live** animals
  - Formation of clusters which become **smaller** in **trained** worms, but clusters **do not shrink** in Lrn mutants? → **same** number of clusters

→ in vivo model for synaptic plasticity

SOCIAL AND ASOCIAL WORMS

Wild-type strain N2 (Bristol) is **asocial**, so when food is abundant, N2 feeds solitary. About half of all 30 natural isolates of *C. elegans* are social (AB1). Crossing AB1 with N2: a single **locus** determines **feeding behavior**. N2/AB1 hybrid is asocial → social feeding is **recessive**.

NPR-1 GENE

Gene that controls feeding behavior. Nrp-1 expressed in AQR, PQR and URX (3 sensory neurons) that are in contact with internal body fluid.

- N2: 215V → asocial
- AB1: 215F → social
- N2/AB1: 215V/215F → asocial
- AB1 + nrp-1 transgene with 215V allele → asocial feeding

SENSING OXYGEN AND FOOD

1: O2 sensory neurons → tend to avoid high oxygen (head and tail)

- Depolarization of membrane and inflow of calcium from ER calcium channels and outside of cell
- Sustained cytosolic calcium elevation leads to neurotransmitter/neuropeptide release at end of sensory neuron

2: Interneurons → long-term increase in forward locomotion

3: Command interneurons → short-term reversals

- From head: some direct, some connected with interneurons
- From tail: direct

DEVELOPMENTAL DISEASES

Dendritic spines are usually aberrant, but no pattern visible, it affects neuroplasticity but that isn't the only reason. The general common factor is that there are mutated genes affecting neuro-skeleton, outgrowth and axon guidance.

Genes associated with neurodevelopmental diseases affect **more** than one step in neural **circuit formation** → **not** monogenetic diseases

PREVALENCE

• Schizophrenia	1.3 %
• Major depression	5.3 %
• Autism Spectrum Disorders	0.1-0.2 %
• Panic disorders	1.6%
• OCD	2.4 %
• Bipolar disorder	1.1 %
• Posttraumatic stress disorder	3.6 %
• Anorexia nervosa	0.1 %

SCHIZOPHRENIA

Described by Kraeplin and Bleuler in early 1900's. Kraeplin described it as dementia while Bleurer recognized a split personality with cognitive defects not being the main symptom.

DEFINITION

Brain disorder characterized by abnormal mental function and resulting disturbed behavior.

COGNITIVE SYMPTOMS

- Impaired attention
- Impaired specific forms of short-term and long-term working memory and learning
- Impaired executive function
- Deficits in perception

In addition, many patients have concomitant [naturally following] mood symptoms including depression and anxiety

POSITIVE SYMPTOMS (PSYCHOSIS)

- Hallucinations
- Delusions
- Thought disorder: paranoia

NEGATIVE SYMPTOMS

- Asociality
- Alogia: 'poverty of speech'
- Anhedonia: inability to feel pleasure in normally pleasurable activities

VULNERABILITY

Clearly related to **genetic** factors based on evidence from family, twin and adoption studies.

LIFETIME RISK

- 1% general population
- 50% in monozygotic twins
- ~17% in dizygotic twins
- Second-degree relatives (uncles, nephews, grandchildren, half siblings) are between 2-6%
- First-degree (children, siblings, parents) are between 6-17%

BUT genetic factors alone **cannot** explain occurrence of schizophrenia → there must be **environmental** factors.

ENVIRONMENT

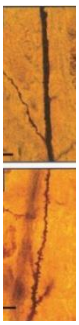
Identified risk factors:

- **Viral infection** during fetal or infant development
- Exposure to **toxic**, **traumatic** or **autoimmune insults**
- Poor **maternal nutrition**
- **Problems** during **gestation** (during labor/birth)

PHYSICAL CHANGES IN BRAIN

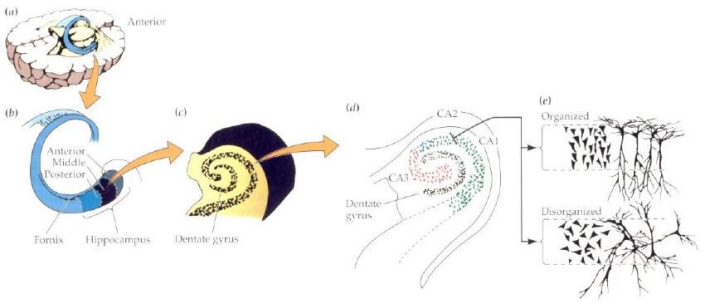
These factors not very useful for diagnosis but after death it can be determined if something was wrong.

- **Enlarged ventricles** when compared to sibling (MRI, must be compared to close relative since measurements are very individual, varying with **gender** and **age**) → indication of shrinkage of brain tissue
  - Male controls vs SCZ have greater differences than female counterparts
- **Reduced** size of **hippocampus** and **amygdala**
- Changes in **fine structure** and **function** of **cortical connections**, dopamine and glutamate transmission → only determinable after death
- **Spine density** on apical dendrites of **pyramidal cells** is **reduced** in schizophrenic patients.



CELL POLARITY DISTURBANCES

Cell polarity can be disturbed in brain, leading to disorganized pyramidal cells in dentate gyrus of hippocampus. The position of cell body, axons and **dendrites** are affected.



DOPAMINE HYPOTHESIS

Antipsychotic drugs that act on **D2 receptors** are effective in **some** patients. This is consistent with the hypothesis that **positive symptoms** of schizophrenia are due to an **excess** of **DA signaling** in the **striatal** [subcortical part of the forebrain and a critical component of the reward system] and/or **mesolimbic** [ventral tegmental area (VTA) and nucleus accumbens] areas of the brain → in need of inhibition.

However, **negative symptoms** are thought to be due to **deficits** in **DA signaling** in the **prefrontal cortex** probably mediated **D1** receptors.

The problem of treating these issues is difficulty in making receptor-specific drugs (D1/D2). Both get affected by blockers, so while positive symptoms might get reduced, negative symptoms are increased.

NMDAR HYPOFUNCTION HYPOTHESIS

NMDAR: glutamate receptor and ion channel protein found in nerve cells. When activated it allows positively charged ions to flow through the cell membrane. The NMDA receptor is very important for controlling synaptic plasticity and memory function.

In addition to the dopamine system, there is a lot of evidence for a contribution of the **glutamate system**. Morphological changes are seen in SCZ patients' brains, which show evidence that NMDA agonists/antagonists affect/cause symptoms in healthy subjects.

Once again, a hindrance in treating this aspect is the difficulty of making drugs with an affinity to NMDA receptors, blockage **not** possible → deadly.

FUNCTIONAL CHANGES IN BRAIN

Schizophrenia is a disease that does **not** have a biomarker to determine if disease is present, only certain diagnosis possible **post mortem**.

FRONTAL CORTEX

Reduced activity and restricted to much smaller area in frontal cortex in patients compares to unaffected **twin** sibling → also a symptom in other illnesses.

EYE TRACKING

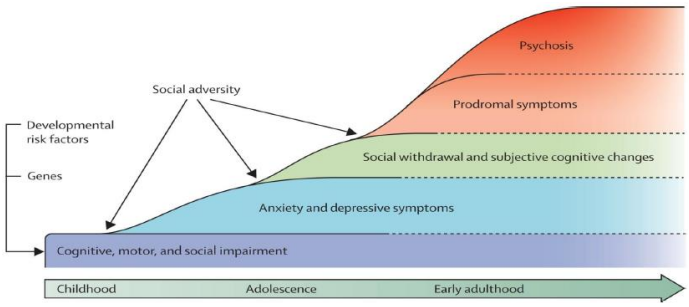
Neurophysiological characteristic difference between schizophrenics and their non-affected twin sibling, is the ability to follow movements of a pendulum smoothly → also symptom of other disorders.

NEURODEVELOPMENTAL BACKGROUND

Maturational processes are disturbed and cause manifestations supporting neurodevelopmental background of schizophrenia.

- **Apoptosis**: either too much or too little
- **Synaptic pruning**: too much in some areas and too little in others. This process only really begins in puberty, young adulthood, 12-14. Selection process and removal of not properly functioning synapses → reasoning why SCZ occurs in **adolescents**
- **Myelination**: lower levels in schizophrenic patients

TRAJECTORY OF ILLNESS



Schizophrenic patients often reveal neurological deficits during childhood and adolescence. However, they are not abnormal enough to be recognized and diagnosed, especially since similar neurological deficits are usually evident in non-schizophrenic relatives.

- Impaired cognitive skills
- Attention deficits
- Irritability
- Delayed gross motor development

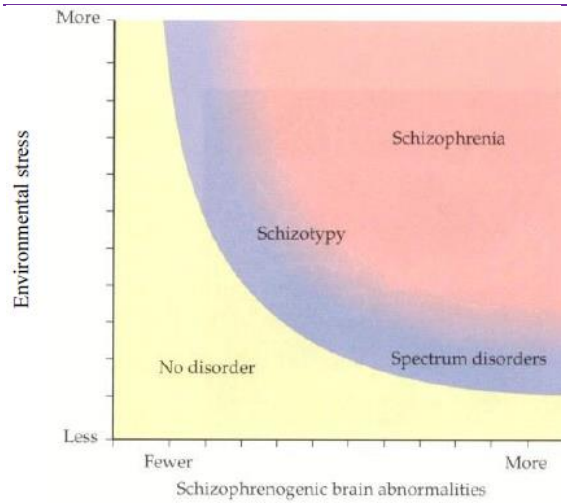
These are **indicators, not** diagnostic criteria.

During adolescence symptoms, such as anxiety and depression are manifested. There is a withdrawal from society which worsens depression. At this point it becomes obvious that something is wrong a diagnosis is made, the earlier symptoms are followed by prodromal [a prodrome refers to the early symptoms and signs of an illness that precede the characteristic manifestations of the acute, fully developed illness] symptoms and psychosis in early adulthood.

DISEASE

Etiology [cause] of schizophrenia involves multiple hits: **genes** conferring vulnerability and **environmental insults**. The difference to other neurological diseases is that manifestation is **not** immediate but only during second or third decade of life.

MIRSKY AND DUNCAN MODEL



Schizophrenia emerges when combination of **stress** and **brain abnormalities** exceeds a **threshold value**.

- Red area: clear to outsiders that something is wrong with patient
- Blue area: outsiders might recognize aberrant behavior but nothing major

PROGNOSIS

Schizophrenia is not just **one** disease but a combination, which are not the same for all patients. Due to this difference in causes, different medications help different patients. If there no drug that helps, there is most likely no recovery.

About **25%** of patients recover **completely** and show **no** obvious signs of having had disease.

More than half of the remainder substantially improve but nonetheless show some **residual signs**, such as occasional memory or sleep problems, not feeling 'right' or intolerance of tension and stress.

About 75% of those that do improve do so within the **first 3 years of diagnosis**.

MENTAL RETARDATION

GENERAL

- Significantly sub-average intellectual functioning
- Significant limitations in adaptive functioning in at least **two** of these skill areas:
  - Communication
  - Self-care
  - Living independently
  - Social and interpersonal skills
  - Work
  - Leisure
  - Health and safety
- Onset **before** age 18, if not that it is something different, then usually called → early onset dementia
- Syndromic (all patients share some symptoms like morphology of face and eyes, heart problems, etc.... → down syndrome, phenotype goes hand in hand with disease) and non-syndromic (not recognizable in face or body, no grouping as cohort possible) forms

PREVALENCE

- **Male to female** ratio for moderate to severe MR (IQ < 50) is **1.4** and **1.9** for mild MR (IQ 50-70).
- **~0.4%** of general population are mentally retarded

X-linked mental retardation (**XLMR**) accounts for **10-16%** of all severely retarded patients and **20-25%** of all levels of MR. XLMR is **6-8 times** more prevalent than expected when compared to **~3%** of **gene contribution** by X chromosome.

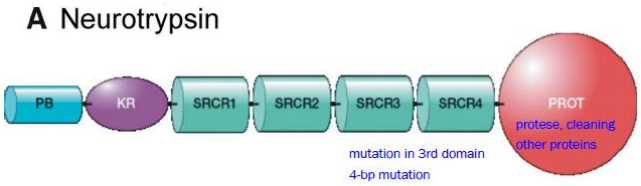
- **~40%** of 885 **protein-coding genes** identified on X chromosome are expressed in **brain**
- **2/3** of XLMR are **non-syndromic** (NS)
- **~100** genes associated with XLME have been described so far

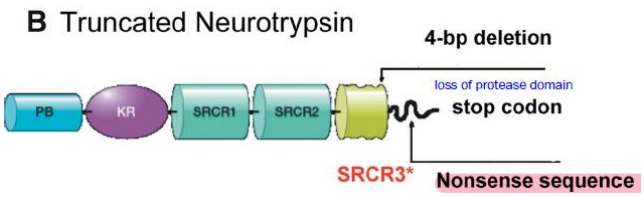
NS-**autosomal** MR (both genders in family are affected, so **not** x-linked) is much more difficult to study, but the identification of **Neurotrypsin** in 2002 led to a frameshift.

PROTEINS INVOLVED

NEUROTRYPSIN

A deletion induces a **premature stop codon** resulting in a **truncated** version of Neurotrypsin (NT).

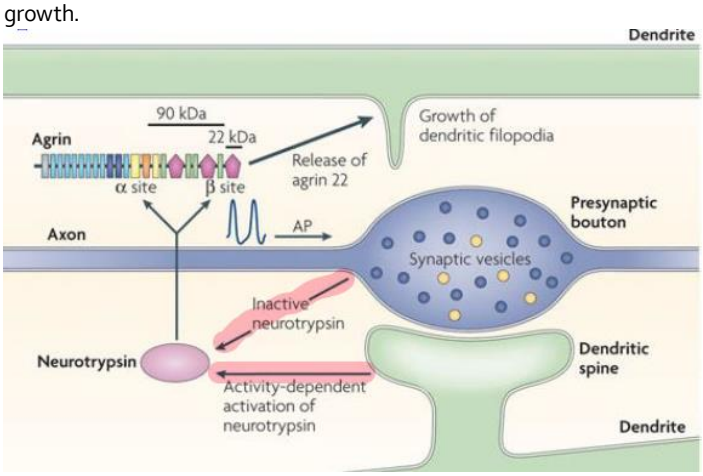




Neurotrypsin release depends on **synaptic activity** → more activity, more release and is involved in **synaptic plasticity**.

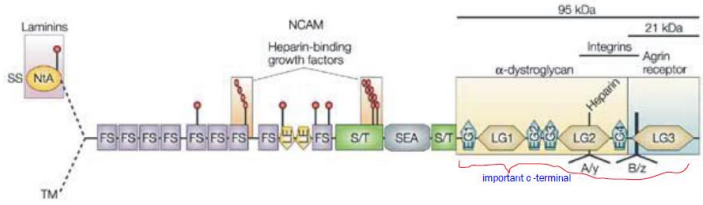
COINCIDENCE DETECTOR

NT is released from synapses (presynaptic bouton) in an inactive form and then **activated** by synaptic activity (postsynaptic bouton – dendritic spine) → pre-and post-synapse **must** be active in the same moment for NT to be released **and** activated. These events allow for cleavage of Agrin to agrin22, which then induces dendritic filopodial growth.



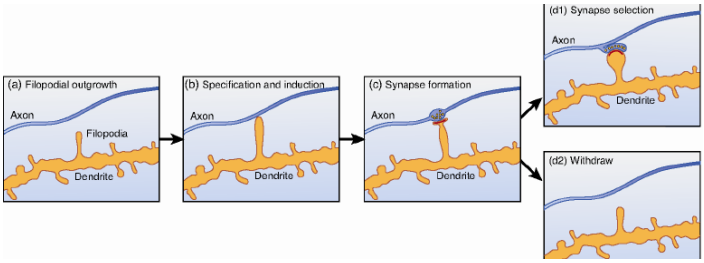
AGRIN

A motor neuron derived organized of NMJ and involved in CNS synaptogenesis. Agrin **cleavage** depends on presence of **Neurotrypsin** and is stimulated by **synaptic activity**.



Healthy is when synaptic activity releases Neurotrypsin, which then cleaves agrin at two sites, resulting in *agrin22*, which is needed for filopidia formation to allow synaptogenesis.

DENDRITIC FILOPODIA



If there is less synaptic plasticity and less likelihood of synapse formation, which is important for learning and memory there is a problem.

LTP

In Neurotrypsin knockout mice LTP is **intact** but LTP-associated formation of filopodia is **abolished**. This means synapse **strengthening** is possible but **formation** of new ones very difficult. Filopodia formation can be **induced** through injection of **agrin22**.

CAUSES OF MR

GENES INVOLVED IN MR

- Neurite outgrowth → regulation of actin cytoskeleton
- Axon guidance
- Synapse formation and plasticity
- Neurotransmitter release

OTHER CAUSES

MR can be caused by exposure to drugs during pregnancy, about 40% of children born to **alcoholic** mothers show a distinctive profile of **anatomical, physiological** and **behavioral** impairments known as Fetal Alcohol Syndrome (FAS) or Fetal Alcohol Spectrum Disorders (FASD).

FAS

Brain anatomy can be affected at macroscopic level in children with FAS, such as a **reduced corpus callosum**, changes in **nasal area** and **cerebellum**.

SYMPTOMS

- low birth weight
- small head circumference
- organ dysfunction
- facial abnormalities (including smaller eye openings, flattened cheekbones, and indistinct philtrum)
- epilepsy
- poor coordination/fine motor skills
- poor socialization skills (such as difficulty building and maintaining friendships)
- lack of imagination or curiosity
- learning difficulties, including poor memory, inability to understand concepts such as time and money, poor language comprehension, poor problem-solving skills
- behavioral problems (including hyperactivity, inability to concentrate, stubbornness, impulsiveness, and anxiety)

AUTISM

Autism is a **neurodevelopmental** disorder that is defined by **deficits** in social interaction, impaired communication and by unusual **restricted and repetitive** behaviors.

Autism begins in **infancy**, **before** three years of age. Diagnosis is preceded by observations of abnormal behavior relating to interactions or communication.

- Young children often do **not** interact with peers or parents, **no** sharing of happiness → in their own world
- Repetitive behaviors develop in preschool years
- Signs of **sensory overload**, avoidance of novel stimuli

DIAGNOSTIC CRITERIA

SOCIAL INTERACTION

Qualitative impairment in social interactions, as manifested by at least **two** of the following:

- Marked impairment in use of multiple **nonverbal** behaviors (eye-eye)
- Failure to develop **peer** relationships
- Lack of spontaneous seeking to **share** enjoyment with others
- Lack of social or emotional **reciprocity**

COMMUNICATION

Qualitative impairments of communication, as manifested by at least **one** of the following:

- Delay in, or total lack of, development of **spoken language**
- Marked impairment in initiating or sustaining a **conversation**
- **Stereotyped** and **repetitive** use of language
- Lack of varied, spontaneous make-believe or **imitative play**

BEHAVIOR

Restricted, repetitive and stereotyped patterns of behavior, as manifested by at least **one** of the following:

- Preoccupation with one or more stereotyped or restricted **patterns of interest**
- Adherence to **nonfunctional** routines or rituals
- Stereotyped and **repetitive** motor mannerisms
- Persistent preoccupation with **parts** of objects

SIGNS IN CHILDREN

- Avoid eye contact with parents when held
- Push away from close contact
- Severely impaired language acquisition
- Automatic acts (incessant rocking)
- May or may not be mentally retarded
- Up to 1/3 with ASD report **epilepsy**

CONCEPTUALISATION OF SPECTRUM: ASD

Common theme of **qualitative** deficits in **social behavior** and **communication**.

- Childhood disintegrative disorder (Rett syndrome)
- Asperger's Disorder
- Pervasive Developmental Disorder – Not otherwise specified (PDD-NOS)

PREVALENCE

- Sibling recurrence risk is approximately **4.5%**
- Population incidence 3-6/1000 (for full spectrum of autistic disorders)
- Concordance rate for **monozygotic** twins is **60%** for classical autism, up to **92%** for full spectrum → autism is **most heritable** psychiatric disorder
- Concordance rate for dizygotic twins: ~3-10%
- Autism is 4-5 times **more** prevalent among **males**

COMMON TO ALL ASD

Disturbance of normal social behavior, ranging from **subtle** abnormalities in social reciprocity, particularly with peers, to much more **obvious** difficulties in use of eye contact, facial expression and social motivation. **Interpretation** of **facial expressions** markedly impaired.

GENES

Genome-wide linkage studies with large patient cohorts have provided large data sets, showing many genes are linked to **synaptogenesis and axon guidance**. It is **not** a monogenetic disease, many different genes involved. Autism as an '**under-connectivity**' syndrome → change in **functionality**, **long-distance** relationships in brain are affected.

ENVIRONMENTAL FACTORS

Many are suggested but association not unequivocally proven for any factor.

- Thalidomide [immunomodulatory drug for certain cancers] use
- Certain viral infections: rubella, influenza, cytomegalovirus
- Maternal anticonvulsants → epilepsy sufferers

PROBLEMS TO BE SOLVED

- Definition of brain regions that are most severely affected
- Types of alterations (structural vs. neurochemical)
- Biochemical tools for diagnosis → so far none
- Overcome problems with diagnosis due to heterogeneity

MOST PREVELANT ABNORMALITIES

- **Reduced** corpus callosum, some areas affected more others (2,4), complete or partial agenesis [failure of an organ to develop during embryonic growth and development due to the absence of primordial tissue]
- **Changes** in cerebellar structure, loss of cells in **deep cerebellar nuclei** in some studies
- **Loss** of **Purkinje cells** is commonly found in studies

ANATOMICAL/MORPHOLOGICAL FINDINGS

- Changes in **synapse formation** and **elimination**
- Cells more **densely** packed and **smaller**
- **Reduced** complexity of **dendritic arbors**

→ All this with a grain of salt since only ~30 brains have been looked at!! These finding are no more than anecdotal as of now.

DISTURBANCE OF MIRROR NEURON SYSTEM

MNS helps understand intentions → setting or clearing the table

PAPER

WHAT DOES THE DEVELOPING BRAIN TELL US ABOUT NEURAL DISEASES – STOECKLI (2012)

The cost of brain disorders is larger than all other disease areas combined and carries 1/3 of the total burden of disease. Yet drug development in this area has been stagnant, mostly due to a lack of drug targets. There needs to be a shift from translational to **basic** research to meet the need for drugs and therapies in the future.

INTRODUCTION

- Currently available drugs can delay disease onset or alleviate symptoms
- Molecular and cellular underlying mechanisms of SCZ are not understood → etiology and pathogenesis are not understood
- Many neurodevelopmental disease have malfunctions with neural circuits but the underlying causes in SCZ seem to be quite different
- Today's medication helps with reducing positive symptoms but not improving cognitive defects and comes with severe side effects

DEFECTIVE SYNAPTOGENESIS

Dominant hypothesis has been that **excessive dopaminergic transmission** in forebrain was key factor in SCZ **pathogenesis**. This was based effectiveness of D2 receptor **antagonists**.

More recently, an **imbalance** between **inhibition** and **excitation** in **neural circuits** was postulated as **basis** of SCZ, the main evidence showing a link between **GABAergic interneuron dysfunction** and cognitive impairment. It was also found that **communication** between different brain areas was **more affected** that the function of individual brain areas → defective neural circuits.

- Deficits so far attributed to **late** stages of development, **pruning** of excessive synapses, and **aberrant** synaptic plasticity → genes involved in **synaptic structure** and **function**

NEW RESEARCH

Overrepresentation of genes known to affect neural circuit formation, pathways and processes were axon guidance, integrin signaling, *ephrin* receptor signaling and *Shh* signaling → **early** stages of development. Even earlier seemingly affected pathways: *Notch, Wnt, semaphorin* and *neuropilin*.

- *DISC1*: scaffold protein, required for neuronal **migration**
  - Neurite outgrowth
  - Axon guidance defects
- *NRG1* and *ERBB4*: control number of GABAergic interneurons, their migration from MGE to destination in cortex, fails without genes
  - *ERBB4 mutants* show a decrease in synaptic contacts between interneurons and pyramidal neurons

→ Findings consistent with imbalance between excitation and inhibition

ASDS RESULT FROM ABERRANT NEURAL CIRCUIT FORMATION

Importance of excitation and inhibition balance for normal cortical function is **undisputed** and SCZ is not the only disease where this balance is perturbed: ASDs are also associated, well studied for Rett syndrome.

RETT SYNDROME

Monogenetic form of autism which is an **x-linked** developmental disease. Females develop normally for first 6-18 months, then loss of milestones and gaining of autistic traits. Shift in changes of balance between excitation and inhibition **before** onset of detectable symptoms in mice. Brains are smaller in size and weight and cells are smaller and more densely packed.

- *MECP2*: represses expression of many different target genes
  - Duplication of this gene found in patients with mental retardation, seizures, respiratory problem, …
  - Mice with deficiency **in GABAergic neurons** show **reduced** level of GABA and therefore **higher** excitability of **cortical neurons**
  - Mice with **global** deficiency show **lower** cortical excitability

Developmental deficits caused by absence of *Mecp2* was **reversible**, giving hope that even structural deficits can be overcome, to some degree, at the **functional** level.

ASDS ARE HIGHLY HERITABLE BUT POOR UNDERSTANDING OF GENETICS

Most forms **not** monogenetic, diagnosed in +/150 – 1/200 live births, with core features:

- Problems with social interaction and communication
- Stereotypic, repetitive behaviors
- Language deficits
- Mental retardation
- Motor disturbances
- Epilepsy

Different than for SCZ, involvement of factors in **early** stages of development in pathogenesis of ASD widely accepted, these processes are: cell migration, axon pathfinding, synapse formation → **initial wiring** of the brain. **Late** developmental events such as pruning **might** also be involved. One can also observe **transient macrocephaly**, which is a postnatal event. **Post mortem** finding show:

- Aberrant positions of **Purkinje** cells
- **Decreased** connectivity of the two hemispheres, **concluded** from **smaller** corpus callosum

JOUBERT SYNDROME

Aberrant axon guidance during development is widely accepted as contributor to disease in some ASD patients, but particularly for this. Imaging can detect differing axon tracts, therefore very severe.

- Autosomal recessive disorder
- Hypotonia, ataxia, abnormal breathing patterns, mental retardation and autism
- Aberrant axonal connectivity and underdevelopment of cerebellar vermis [midline fissure] and peduncles [white matter connecting to brainstem]

Common denominator in developmental process is the **cilium**, which are signaling centers of cells during development, linked to **Wnt** and **Shh** signaling during **patterning** and **differentiation**.

GENES LINKED

- ***NLgN3/4***: involved in synapse **formation**
  - Genes **encoding** for *NLGN3/4* and *Neurexin 1/3* were identified as ASD susceptibility genes
  - Trans-synaptic adhesion molecules which organize inhibitory and excitatory synapses → create **balance**
- Contactin-associated protein-like 2 (***CNTNAP2***): encodes protein similar to neurexin
  - Binds to *Cntn2* at nodes of Ranvier → interaction essential for proper molecular organization
  - In mouse CNS cell migration and number of GABAergic interneurons were affected in **absence** of *CNTNAP2* and reflected autistic traits.

AXON GUIDANCE MOLECULES AS CANDIDATE DISEASE GENES

Applicable to neurodevelopmental and neurodegenerative disorders.

- ***L1CAM***, ***Robo1***, *semaphorins*… these have been found in GWASs for ASD, SCZ and MR
- **Contactin family *CNTN1-6***: involvement linked to ASD, intellectual disability, SCZ and Alzheimer's disease
  - Involved in cerebellar development as axon guidance molecules and in synapse formation
  - Direct interaction with *L1CAM* and *NrCAM*

WNT SIGNALING

Involvement of *Wnt* signaling in axon guidance (cell polarity, MT dynamics), synapse **formation** and (synaptic plasticity) has been clearly demonstrated, as well as in differentiation and patterning as a morphogen.

- *Wnt*s, receptors, *frizzled*s or intracellular components of signal transduction cascade have been linked to many neurodevelopmental diseases
  - Possible link between *DISC1* and *Wnt* signaling

CALL FOR MORE BASIC RESEARCH

Diagnoses of all neural diseases are based on symptoms that are variable and extremely difficult to specify qualitatively and quantitively. In addition, brain function, cannot be segregated and one malfunction in one brain will trigger responses in other areas and symptoms will therefore be a **summary of neural circuit function**. Reduced efficiency can come from:

- Decreased number of cells
- Aberrant cell migration
- Mislocalization of neurons
- Aberrant axonal connectivity due to:
  - Guidance defects
  - Axonal degeneration
  - Altered synapse formation
  - Reduced synaptic efficiency and plasticity

→ These steps are **not independent** from each other.

AXON GUIDANCE

Fewer neurons means fewer axons and fewer synapses, mislocalized neurons can undergo apoptosis, failing to reach target areas. Axon guidance depends on tightly regulated set of guidance cues and receptors, provided by **intermediate targets** and other **axons** → precise **temporal** control is key of correct navigation.

→ Focus should be on **candidate genes** and **linkage analyses** and try to **assign** them to different processes in brain **development** and **function**!

PAPER

SCHIZOPHRENIA: AN INTEGRATED SOCIODEVELOPMENTAL-COGNITIVE MODEL – HOWES (2013)

The **dopamine hypothesis** attempts to explain **pathogenic mechanisms**, the **neurodevelopmental hypothesis** attempts to describe the **origins**. The **cognitive model**, a new alternative has gained popularity in the past 10 years, however **without** integrating the other existing models.

DOPAMINE DYSFUNCTION

- **Pre**synaptic dysregulation as major locus suggested by **molecular imagine** due to increased dopamine **synthesis** capacity, **release** and **baseline** synaptic **concentrations**
  - Potential as diagnostic test since this phenomenon is not observed in people with other common psychiatric disorders, yet specific to those with **psychotic symptoms** (positive)
  - Greater release, greater induction of psychotic symptoms and vice versa
  - Dysfunction is **dynamic**, increasing with worsening of disorder

NEURODEVELOPMENTAL HYPOTHESIS

based on three main lines of evidence:

- Pre- and perinatal hazards
  - Low birthweight
  - C-section
  - Hypoxia
- Developmental deviance (neuromotor, physical)
- Imaging studies of structural brain defects present at onset of symptoms (but **no** findings post mortem)

Dopamine dysfunction regarded as manifestation of subcortical **hyper** function and considered secondary to interaction between **primary cortical lesion** (dorsolateral prefrontal cortex) and **normal maturational processes**. Dopaminergic changes **persist** into **adulthood**

SOCIAL RISK FACTORS

- Being an immigrant and readily identifiable as a minority
- Growing up in a city: greater brain response to stress
- Childhood adversity: loss of a parent or abuse
- High cortisol concentrations
- Social isolation, can be recovered if dominant position in social hierarchy after isolation
- Social instability and social defeat

Stress **increases** striatal dopamine **release** in human beings, most likely related to **severity** of social stressor. People with schizophrenia and those at risk show an **enhanced** dopaminergic response to psychosocial stress.

SENSITIVITY AND SENSITATION OF DOPAMINE SYSTEM

C-section and hypoxia only affect dopaminergic but not serotonergic system. Isolation rearing is associated with increased dopamine release but reduced serotonin release to challenges.

SENSITISATION

Dopaminergic system shows sensitization [marked **amplification** in a response after repeated stimulation] to several drugs and stressors. In addition, previous exposure to one challenge leads to an increased response to a different challenge → **cross-sensitization**.

OVERALL

Environmental insults can affect several neurotransmitter systems, but dopaminergic system seems to be particularly sensitive and with the ability of cross-sensitization, insults can have additive or even multiplicative effects.

GENES, NEURODEVELOPMENT AND DOPAMINE SYSTEM

- Variance in schizophrenia is considered **genetic**
- Presynaptic dopamine functions have **low** heritability and **high** contribution from environmental factors
- **Gene-environment interactions** must be considered in GWAS
- Copy number variants

COGNITIVE THEORIES AND LINK TO DOPAMINERGIC DYSFUNCTION

Hypotheses so far can explain biology of psychosis, but not much understanding of symptoms. These models suggest:

- Exposure to social adversities lead to threatening world views
- Stress results in anomalies of conscious experience triggering a search for an explanation
- Biased cognitive schema result in erroneous judgement that experiences are externally driven and uncontrollable → paranoid delusions

Environmental adversity dysregulates dopamine system and from biased cognitive schema, the net result is **additional stress** and further dopamine dysregulation → hardwiring and habit development. Vicious cycle:

- Stress increases dopamine dysregulation

- Leading to more stress
- Further dopamine release
- Hardwires psychotic interpretation

This is a dynamic model in that degree of dopaminergic dysfunction fluctuates in response to **psychological response** to **abnormal dopamine signaling** → can account for relapses and remissions which dopamine hypothesis could not.

INTEGRATED SOCIODEVELOPMENTAL MODEL

This model combines dopamine, neurodevelopmental and socio-developmental hypotheses with cognitive theories.

- Developmental deviance due to
  - Variant genes
  - Hazards to brain
  - Social adversity in childhood → biases cognitive schema
- Subsequent stress results in dysregulated dopamine release
  - Leading to aberrant assignment of salience
- Interpretation within context of biased cognitive schema contributes to further stress

Endurance of dopamine dysfunction could account for **negative symptoms** between episodes and regarding cognitive function. The model explains the overlap both in **risk factors** and **brain abnormalities**.

STRENGTHS AND LIMITATIONS

- Support of link between developmental risk factors and altered dopamine function
  - Link between environmental risk factors and dopamine dysfunction is less well established
- Glutamatergic **hypo**function could contribute to dopaminergic dysfunction, still needs to be tested
- Estrogens could also be involved
- Stimulant use and increased risk of schizophrenia due to drugs inducing dopamine sensitization
- More evidence needed to account for persisting negative symptoms

IMPLICATIONS AND FUTURE DIRECTIONS

- Research into developmental trajectory of dopamine function beginning earlier
- Examining interactive effects of social risk factors
- Interaction between genes affecting dopamine system and environmental risk factors
- Interactions between neurodevelopmental and later social effects on dopamine system
  - Dynamic change and effect of stressors
- Cognitive schema in transition from experiencing aberrant salience to development of psychosis

This model suggests the life events and associated cognitions play a key part and that by **altering** cognitive schema and reducing stress, **psychological therapies** and **social interventions** can interrupt the vicious cycle that dysregulates dopamine → avoidance of hardwiring, the earlier the better.

→ Treatment needs to address **psychological, socio-developmental** and **biological** factors.

GENERAL

- We have about 60 random mutation which our parents do **not** have
- Neurodevelopmental diseases have problems with neural circuit **formation** and neurodegenerative diseases with **maintenance**