**C. Elegans as a model system in the development of the nervous system with respect to genes and behaviour**

Neurogenome and facts of C. elegans: It has 6 chromosomes with 19k genes on it and a total of 100Mbp (suggesting small genes or no abundance of redundant genes). It has 80 GPCRs, 1 CREB, 15 neuropeptides etc. Every C. elegans normally has exactly 959 cells of which 302 are neurons.

Ways to study C. elegans: Cell ablation with laser-microsurgery. Forward and reverse genetics. Optogenetics.  
Forward genetics: Use EMS to generate mutation ((frequence in worms: 1/3000 when 50 mM EMS used) and there are several strains like the bristol or hawaii strand.  
Reverse genetics: RNAi library present (bad uptake of dsRNA in neurons).  
Optogenetics: blue light => depolarization (activation of neuron); yellow light => hyperpolarization (inhibition of neuron) – maybe use viral vector for the expression of light-sensitive ion channels in neurons.

In cell ablation, we can remove cells or neurons discretely with laser-microsurgery and observe phenotype.

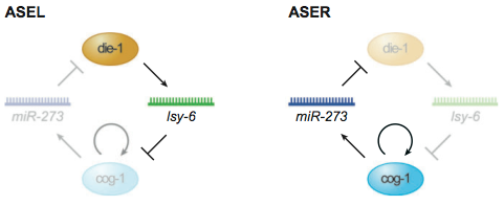
Asocial worms: Social behaviour in worms is defined by eating in groups vs eating alone. Only one gene determines behaviour (npr-1 gene): Cross asocial bristol N2 strand with social ABI strand. Only one locus determines phenotype – social behaviour is recessive.

Worms that do no learn: Learning is linked to glr-1 (AMPA type glutamate receptor). Normal worms can remember good conditions when exposed to a positive stimuli (food) with another such as temperature (normal temperature for worms is 20°C). Wildtype worms can associate favorable temperature with food, but learn mutants do not.

Worms that still do not learn: Exposing starving worms to an attractant like NaCl leads to an aversive response to NaCl in wildtypes if there is no food around. Learn mutants do not learn and will be attracted to another NaCl source again despite the absence of actual food.

Unc mutants: Quantification of locomotion – swimming tests for frequency of thrashing or reversal movement. Locomotion is basis for most behavioural assays. A-type motorneurons with AVA interneurons make backward movement. B-type motorneurons with AVB interneurons make forward movement.

Asymmetry in NS:



ASEL and ASER are both chemosensory neurons that express different genes and sense different ions (ASEL: Na+, ASER: K+)

Organization of sensory system: sensory neurons have amphids (heads) and phasmids (tails). They possess chemosensation, osmosensation, mechanosensation, thermosensation and light sensitivity.

Genes in chemosensation: arr-1, tax-6 (calcineurin), let-60, mek-2, osm-9.

**The formation of synapses**

Structure of a synpase: presynapse, postsynapse and the active zone (the space between the presynaptic and postsynaptic side which is coined by the fact that it consists of many different cytoskeletal matrix proteins that interact with the respective other site).

Presynaptic site (axon): It releases the vesicles. Vesicle formation occurs within the presynaptic cell. Depending on the type of the vesicle, it can either produce an excitatory (NMDA, AMPA) or inhibitory (GABA) reaction in the postynaptic cell. It differentiates into a presynapse, when it is exposed to neuroligin on another cell (can be any cell – not necessarily a neuron, as long as neuroligin is expressed (experimental evidence: neuroligin expression on HEK cell)). It also has synapsin (peripheral cytoplasmic protein), which is not essential, but has a modulatory role by making sure that SV are released at the membrane properly (it binds SV).

Active zone formation on the presynaptic side can occur without a postsynapse partner – positively charged lysine beads are enough to induce AZ formation: On the surface, we find neurexin, ephrin, synaptobravin (v-SNARE).

Postsynaptic side and PSD: Postsynaptic side assembly occurs only when there is contact to neurexin (neuroligin on the postsynaptic side interacts with neurexin on the presynaptic side – one can express neurexin on any given cell and postsynaptic assembly will occur). If contact is with neuroligin-1/3 then excitatory synapse will be formed, else if neuroligin-2 is contact partner then inhibitory synapse will be formed.

PSD-95 is a cytoplasmic scaffolding protein that binds to the inner part of the membrane. It is also a docking place for NMDAR and also neuroligin, but is present without. AMPAR has SAP-102 as the scaffolding protein.

Also, one can simulate the creation of a spine on a dendrite by releasing caged glutamate (using photons to open the cage). Locally, this simulates a transmitter release from the presynaptic side, inducing spinogenesis in the postsynaptic side within seconds (neuroligin-1 necessary – leads to accumulation of PSD-95 in the postsynaptic cell). Contact with neurexin (presynaptic cell) is not necessary, but sufficient. In vivo, there are mobile presynaptic cells that move around and simply release glutamate to induce spinogenesis.

Active zone exocytosis can occur on orphan presynaptic cells with AZ: Such neurons are bathed in FM solution (a fluoroscence). The FM is integrated into the membrane of these neurons. Then, unload them by stimulating SV release: FM gives a signal that it occurred and it can be measured.

Homothillic and heterothillic proteins in the AZ: Presynaptic cell --- postsynaptic cell: heterothillic:

Ephrin --- EphB2, neurexin --- neuroligin-1/2/3, synaptobravin --- syntaxin and SNAP-25, neurotransmitters --- NMDAR/AMPAR/GABAR

Homothillic: sidekick, cadherins, protocadherins, NCAM, SynCAM, Nectin

It goes without saying that there are GABA SV for inhibitory synapses on the presynaptic cell and glutamate SV for excitatory synapses on the presynaptic cell. Also, frizzled is present on the postsynaptic cell, where Wnt can bind and activate beta-catherin that leads to transcriptional changes in the genome (maybe related to synaptic plasticity).

The SV do not diffuse randomly, but through nanocolumns on which they travel to the receptor ( => PALM; can also be used to count PSD-95 – PALM: express GFP on target (protein, genes, cells) and selectively turn them on and off through photoactivation. Record several pictures during this time and repeat process for the whole area. Output: lots of clusters, combine them, fit a gaussian, get high resolution image).

Genes involved: dsyd-1: KO results into small synapses. Liprin-alpha: KO results into a bit smaller and very slim synapses. Both genes are important for proper synapse formation.

Rab3-GTPase: Required in normal AZ distribution in both sides. No large defect if KO’ed.

Timing in synaptogenesis: Cell adhesion molecule interaction (neurexin with neuroligin), assembly of presynaptic side (SV formation and formation of proteinaceous cytomatrix), assembly of postsynaptic side (accumulation of PSD-95 and other scaffolding proteins, NMDAR and AMPAR or GABAR).

Maturation and the neuromuscular junction: The neuromuscular junction (in mice) is used to study maturation of synapses: General process: axon cone elongates, contact formation with the dendrite, formation of basal lamine (induction of formation of the synaptic cleft), multiple axons converge, myelination of all axons under one myelin sheath.

After birth, all muscles (and other target cells) have multiple innervations to make sure, every cell is innervated. Over the course of weeks, weakly activated axons are eliminated and strongly activated axons become established resulting into monosynaptic innervation.

In purkinje cells, it has been shown that Arc is essential for elimination: climbing fibers induce neural activity, opening special P/Q type Ca2+ channels in its vicinity, which activate Arc. Arc eliminates other climbing fibers further away from the activation site resulting into monosynaptic innervation in the end.

LTP in purkinje cells: LTP only occurs at large inputs from the climbing fiber (and simultaneous activation) which increases the conductance in AMPAR by protein modifications etc. Thus, only the strongest synapse will be the winner (due to elimination of other synapses through Ca2+/Arc postsynaptic signalling).

**How learning on a neurobiological level relates to sleeping and waking cycles**

It has been shown that sufficient sleep enhances learning when slow wave activity (=: SWA) is present during REM sleep. On a neurobiological level, existing synapses are optimized, which means that weights are newly distributed such that sum of weights is the same as in the previous morning (only a model). During sleep, synaptic synchronization is optimized: Some synaptic connections will be eliminated that had low weights anyway (removal of mistakes, noise, weak connections) while other connections are strengthened, also known as synaptic depression. EEG show spiking activity when SWA occur (they are ON) and no activity when SWA are OFF. The level of amplitude is correlated to the level of synchronicity.

During the day, new synapses are formed when learning or weights increase for existing synapses. More synapses mean more energy is required and space. During the night, energy expenditure and space are optimized. Wakefulness favors synaptic potentiation.

Synaptic homeostasis: Synaptic strength is normalized in a 24 hours cycle.

When depressed of SWA by accoustic intervention during sleep, no learning can occur on a neurobiological level. SWA will have a high amplitude where learning occurs.

EEG is used to gather data on electrical changes across cortical areas, EOG records eye movement, EMG records muscle movement.

Sleep patterns in different stages of human development:

Infants sleep in intervals throughout the day most of the time. The trend seems to be monophasic sleep as one ages. 1 year old sleeps triphasic (during night, before noon, after noon), 4 year old diphasic (night and afternoon). With 10 years of age, monophasic sleep seems to be prevalent. Also, amount of REM sleep decreases in the first year (infants have a lot of REM sleep).

Also, synaptic density has a U-shaped graph: many synapses are formed in early ages and are optimized (higher weights assigned to often stimulated synapses while weak synapses are eliminated), while in adults, there are more synapses eliminated than created. Children benefit more from sleep-related learning (memory task: before sleep, memorize a sequence of keys on the keyboard; next morning: check if still present and if even performance increased). White matter increases linearly though.

**Neurogenesis in the adult brain and its implications on learning and cognition**

Radial glia cells are the neural stem cells in the adult brain. They proliferate in the ventricular and subventricular zone. Their lineage can be traced by a number of methods: CreER-loxP system with confetti configuration, MML virus (does not go into cell nucleus, therefore stays in cytoplasm and is dependent on mitotic activity of the radial glia cell), BrdU labelling (thymidine analogue which can also be incorporated into DNA).

Social enrichment and exercise: Exercise enhances proliferation in neural stem cells in the hippocampus. It does not increase the survival of the new cells and the brain will assume about the same number of cells prior to proliferation after 4 weeks. Social enrichment does not increase proliferation but favors cell survival.

Ablation in neurogenesis: transgenic mice, locally ablating cells, radiation:

Transgenic mice: use suicide genes to kill cells, but cells die randomly.

Locally ablating cells: which genes are specific (to put viral vectors into)? Traumatic injection.

Radiation: Radiation has other unspecific effects, such as inflammation etc.

How to produce brave mice: Mice are given fluoxetin (anti-depressant) to boost bravery: When food is exposed on a lit area while it is surrounded by darkness, mice are normally very cautious about approaching the lit area due to exposure to predators. When starved and on fluoxetin, mice will go into the lit area. This effect can be reversed with radiation (destruction of fluoxetin).

Genetic influences on cognition (acetyl CoA + FASN => palmitate or malonyl CoA + NADPH => palmitate): Mutating FASN in R1812W leads to a defect in lipid metabolism for neural cell membranes due to protein misfolding (because of the BBB all lipids must be synthesized de novo). Such individuals will have higher amounts of fatty acids, but less neurogenesis, which can result into lipotoxicity. Mutating FASN in R1819W lets proliferation be unaffected compared to controls(?).

The dentate gyrus is known to act cognitively as a spatial pattern seperator. Mice with decreased neurogenesis in the dentate gyrus have impaired spatial seperation (-> Morris’ water maze).

Dynamic regulation of neurogenesis: There are many ways to impair neurons and neurogenesis: alcohol, drugs, ischemia, stress, depression, epilepsy and other mental disorderds, physical insults (martial arts etc.) and age.

Young adult born neurons: They are highly excitable: lower induction of threshold for LTP, increased amplitude of LTP, increased intrinsic excitability, picro-toxin independent LTP.

**The development and study of neural crest stem cells**

Stem cells: Totipotent stem cells can become any cell type in the whole organism. Those are normally only human ESCs which can be obtained from the blastula taking cells from the blastocyst. Pluripotent stem cells cannot become any cell type, but are restricted to its tissue or to a certain number of cells. NCSCs are pluripotent cells. Both types are capable of self-renewal and they can give rise to differentiable daughter cells.

Hierarchy: totipotent, pluripotent, multipotent, unipotent, progenitor cell.

How to trace neural crest cells: CreER-loxP system under a promoter specific for neural crest cells temporal as well as spatial control). For example, use lacZ gene to get blue cells (lacZ integrates in the membrane and colors the membrane respectively). This way, one can analyze the progeny of a single neural crest cell. First mouse has Cre under a NC-specific promoter and second mouse lacZ under ROSA26R. Crossing them leads to progeny that only expresses lacZ during Wnt signalling (temporal control, ROSA26 is constitutively active).

Neural crest cells give rise to neuronal and non-neuronal cells:

Neural cell types: Oligodendrocytes, Schwann cells, neurons, sensory nervous system (dorsal root ganglia), autonomic nervous system, enteric nervous system

Non-neural cell types: melanocytes, smooth muscle cells (outflow tract of heart), adrenal medulla (chromaffin cells), craniofacial bones and cartilage, odontoblasts (produce dentin in teeth)

The NCSC model system: A NCSC will divide into different cell types depending on signalling molecules and self-renew. NCSC + Wnt/beta-catenin => neurons, NCSC + BMP => melanocytes, NCSC + TGF-beta => mesenchymal cells. Apparently, Wnt/BMP lead to self-renewal.

Neural crest cell derived stem cells in the skin: Cells that are p75/Sox10 positive (neural crest stem cells) have been identified that are multipotent, giving rise to neural and non-neural cells.

**Neurogenesis and the principle of the occurrence of specific cells and their spatial separation**

The very early stages (gastrulation): The blastula results into the gastrula (the three germ layers ectoderm, mesoderm and endoderm are produced out of the blastocyst). There is a difference between removing the animal cap of the blastula and the animal cap of the gastrula: The former will differentiate into the epidermis while the latter differentiates into neurons. More specifically:

If cell culture (animal cap – ectoderm) is dissociate, then neurons, else if cell culture intact + BMP4, then epidermis, else if cell culture dissociate + BMP4, then epidermal cells.  
Pathway: Noggin/Chordin/Follistatin inhibit BMP4, ectoderm develops into neurons by default, else BMP4 induces epidermis formation when cell culture intact (cells are close together such that juxtacrine and paracrine signalling is effective).

Symmetric and asymmetric cell division: In mitosis of neural stem cells, there are specific SNAREs that decide cell fate: on the base of a cell, there is the apical membrane. Apical t-SNAREs can bind to the apical membrane, thus during mitosis, both daughter cells inherit apical membrane and proliferative cell division occurs. If during mitosis, the SNARE complex should be tilted to the side, such that basolateral membrane is inherited in one of the daughter cells by binding of lateral junction t-SNAREs, then only the daughter cell with the apical membrane will remain a stem cell while the other daughter cell will proceed to become a progenitor cell. We call this neurogenic division.

There are three areas, where cell proliferation occurs in the brain: the ventricular zone, the subventricular zone (cells from both zones migrate outwards) and the granule cell layer (outer most layer in the cerebellum – cells migrate inwards).

Morphogens across a gradient lead to different fates: Morphogens are proteins that are capable of inducing morphological changes within a cell. Across a gradient, cells will be exposed to different concentrations of a morphogen. Thus, some cell will reach a threshold (several thresholds may exist) that is enough to induce morphological changes (and therefore, cell fate) while other cells remain undifferentiated such that patterning in the nervous system can arise. In the french flag model, there are 3 possible states of undifferentiated cells depending on their exposure to the morphogen (low exposure: cell type 1, intermediate exposure: cell type 2, high exposure: cell type 3).

Lateral inhibition produces progenitor cells in tissues and leaves the other cells as stem cells: Since morphogen concentrations are never truly homogenous, there will be stochastic differences in discrete cells. Example: Delta/Notch signalling: Stochastically, one cell will have more delta ligand, which binds to notch on its neighbouring cells. In the notch cell, this leads to the suppression of hairless, therefore enchancer-of-split proteins is active inhibiting achaete-scute proteins. These cells remain stem cells. Also, these cells lose their delta ligand due to the inhibition of achaete-scute proteins. The first cell will lose its notch (gamma-secretase – a protease that removes notch) and achaete-scute proteins will be active, which also promotes bHLH proteins.

The cell cycle length hypothesis: External factors and inner concentrations of molecules can influence the time spent in G1 phase (cells start spending more time in G1 phase). At a certain threshhold, the cell will divide asymmetrically. Before and after, there will be symmetric division.

Another possibility for asymmetric cell division is the concentration of a specific molecule: Numb – at some point, one daughter cell will inherit all of the Numb and the other will not inherit anything and remain a stem cell (Numb is an inhibitor of notch signalling).

General mechanism: Asymmetric division of proteins in the ER lumen or in other locations.

The importance of having a notochord: The notochord is crucial for the development of motoneurons and the creation of a proper floor plate in the neural tube. The notochord is responsible for sonic hedgehog signalling (Shh signalling). The notochord is sufficient and necessary for floor plate induction (also several notochords produce several floor plates in the neural tube, depending where the Shh concentration is acting (most of the time, perpendicular to the notochord)). The roof plate of the neural tube signals Wnt and BMP4. The following relationship holds true: Shh promotoes Sfrp-1, Sfrp-1 inhibits Wnt resulting into inverted gradients for the signalling of Shh and Wnt.

Mutual repression: The process of sharp boundary formation – cell type 1 wants to have cell type 1 around (promotes other cells to become cell type 1) and therefore inhibits all other cell types in its proximity. Cell type 2 wants to have cell type 2 around (promotes other cells to become cell type 2) and therefore inihibits all other cell types in its proximity. That way, patterning generates sharp boundaries with no intermediate cell types.

Potential of retinal progenitor cells: following events happen in the subsequent order on a time axis:

G – ganglion cell, H – horizontal cell, A – amacrine cell, C – cones, B – bipolar cell, R – rods, M – Müller glia cell.

While Olig1 and Nrgn2: cell proliferation of neural progenitors. When changed to Olig1 and Nkx2.2: neural progenitor becomes glia progenitor cell (oligodendrocyte for example).

Hox gene clusters: Hox genes are important for segmentation and they are highly conserved: Body segmentation of drosophila and spinal chord segmentation in humans (longitudinal axis basically).

There are two further organizing boundaries for the anteroposterior axis: mid-hindbrain boundary ( =: MHB boundary) and the zona limitans intrathalamica (important for the development of the diencephalon through Shh signalling, boundary between the thalamus and the prethalamus).

**How an axon grows**

Axonal survival – the nerve growth factor and neurotrophins: Neurotrophins are the main signalling molecules that ensure axonal survival in target tissue (receptor mediated). Structure of such receptors on axonal surface: p75 + receptor corresponding to the right neurotrophin. p75 alone is enough to induce apoptosis. When bound to neurotrophic receptor structures, its activity is inhibited as long as signalling is active. If an axon grows in the wrong tissue, it will be eliminated, because it does not have the proper receptors for the specific neurotrophins present in the tissue. Thus correct innervation is linked to axonal survival (control mechanism).

Neurotrophins: NGF (trkA), NT4/BDNF (trkB), NT3 (trkC) (in muscle, also GDNF).

To inhibit apoptosis NGF and other neurotrophins have to be endocytosed (either through a clathrin coat or just through bulk endocytosis) and transported retrograde to activate a cascade (on microtubules with dynein) with Erk1/2 signalling in the end. Also, Erk1/2 signalling is involved in the production of postsynaptic axons.

Axon grow by elongating their finger-like tips structures also known as filopodia: Structure of filopodia: The rod part of the axon is made of microtubulues mostly. Those microtubules extend into the fingers of the filopodia. The fingers (and “palm”, called growth cone) are made of an F-actin network (actin basically). There are actin bundles and myosin on which the microtubules are pulled, so they can reach the tip of the filopodia fingers.

How to observe whether growth of extension occurred: Label microtubules with fluorescence, then expose to radiation (staining – fluorescence removed) so that a spot is marked. Before contact establishment: Spot stays at the same place, filopodia actually grows in length. When seeking, filopodias extend in all directions until an attractant is found. Then growth occurs in this direction and elimination of all other filopodia (their material is probably recycled for the growing filopodia). Molecular mechanisms of growth: the finger of the filopodia is constantly rebuild: the inner parts depolimerize (minus end) and the material is transported to the outer ends (finger tip) and polymerizes there (plus end). Microtubules grow the same way. Depolymerization does not consume energy, while polymerization consumes ATP. In shrinkage: rate of depolymerization > rate of polymerization (and energy expenditure sinks).

After contact establishment, there is no growth anymore, but stretching (extension). The marked spot becomes elongated across the space axis.

The clutch mechanism: Filopodia is connected to the substrate via integrins and actins, then microtubuli draw the nucleus towards the filopodia site with myosin, the back side retracts too and loses its connectivity to the substrate via secretases and filopodia grow by adding actin at tips.

Contact attractants: Ig CAMs, Cadherins, ECM (e.g. laminins), NrCAM, NgCAM (for fasciculation), Kirrel2 (fasciculation in sensory axons). Chemoattractants: netrin, Shh (precommissural axons), Wnt4 (postcommissural axons). Contact repulsion: Eph ligands, ECM (e.g. tensins), transmembranal semaphorins. Chemorepulsion: secreted semaphorins, netrins, BMP7 and draxin (precommissural axons), Shh (postcommissural axons).

Filopodia can also dislocate axons from their position or be attracted to them (pioneer axons).

Models for axon branching: How do axons branch? Most likely, they develop branches while the main axon is elongating. The new branches also inherit some of the actin network and microtubules. That way, a neuron can reach several target areas. Other thinkable solutions would be that a neuron simply grows several neurons from the soma being attracted to different target areas (and that an axon simply follows the attractants – the trajectory will be curvy then).

Process: distal elongation of main axon, protrusion – protruded area inherits actin filaments, filopodia formation, invasion by microtubules, final maturation of a new axon branch, elongation of the new axon branch.

**Proper axon guidance exemplified by commissural axons**

Axons find their target through an intricate interplay between short-range cues, intermediate targets and long range cues.

The labelled pathway hypothesis: New axons fasciculate with axons that are already at their target area and follow their lead. If such a pioneer axon were to disappear, the seeking axon will not know where to grow.

Axons may also make use of intermediate cells, called guidepost cells, that have contact attractants on their ECM (e.g. laminin or cadherins) for guidance. Adhesion does not correlate with growth rate though.

Mode of fasciculation: general fasciculation realized by a repulsive environment (semaphorin3a, ephrin) that fasciculates axons together. These have contact attractants on them (e.g. Ig CAMs). Selective fasciculation occurs when only one or few axons fasciculate with an established axon. General defasciculation is induced by the addition of polysialic acid and selective defasciculation can be induced with the addition of Beat.

Growth cones from the PNS collapse upon contact with CNS axons – CNS axons have contact repulsion. Also, it has been shown that DRG growth cones collapse in the presence of Sema3a.

The journey of a commissural axon: Commissural neurons are located at the roof plate and extend their axons called commissural axons down the floor plate, followed by rostral migration.

Initially, they are attracted by netrin-1 and Shh (DCC for netrin receptor on commissural axons and Boc/Ptc/Smo for Shh – Shh is initally attractive). The roof plate produces BMP7 and Draxin, which are chemo-repulsive. Also, commissural axons like to migrate as a fasciculated bunch as they possess NgCAM (KO of NgCAM is viable and commissural axons will find the floor plate nonetheless – NgCAM (fasciculation) reduces their ipsilateral migration). The outside of the floor plate has NrCAM and commissural axons have axonin-1. Both are essential for successful entry of the floor plate. In the middle of the floor plate, an additional receptor is added to the surface of the commissural axon: Glypican-1 mediates the change of receptors: attractive receptors leave and Hhip (:= Hedghehog interacting protein) is added which will recognize Shh as its binding partner and respond repulsively. From there on, Shh will be repulsive and netrin is not attractive anymore. Wnt4 is the main attractant and the postcommissural axon will migrate rostrally after it has left the floor plate due to the Shh/Wnt4 gradient (Shh promotes Sfrp1 which inhibits Wnt4 – high Shh around floor plate and low in roof plate, opposite for Wnt4).

In invertebrates such as Drosophila, the equivalent of the floor plate is the midline glia. Three genes are important: Slit, roundabout (robo) and commissureless (comm). Comm needed for crossing – it is like a bridge, slit is expressed in the midline glia, robo needed for no re-entry.

Robo1 (RabGDI needed for successful integration into the membrane). From there on, Shh will be repulsive and netrin is not attractive anymore. Wnt4 is the main attractant and the commissural axon will migrate rostrally after it has left the floor plate. Due to Robo1, there is no re-entry in the floor plate, since it is not attractive anymore.

KO phenotypes: Comm-delta: two straight lines, no crossing through the midline glia. Robo-delta: persistent re-entry of the midline glia (squares). If only midline present: Slit is enough to attract precommissural axons (double KO of comm and robo).

Levels of behaviour change at choice points: protein modifications (changes in protein stability), translational changes, changes in transcription, changes in vesicle trafficking/membrane insertion.

**The neural circuits of vision and olfactory sensation**

A discrete map: 1:1 map (each sensory neuron in the visual system has one target in the tectum) where spatial information has been preserved (allows orientation and 3D view).

Topographic map: Sensory neurons that are ventrally or dorsally located in the sense organ have their associated target neuron in the brain area located in the opposite way (mind that the picture on the retina is inverted, so it has to be reverted again), such that the spatial information is preserved. In case the sensory neuron connects to a wrong neuron that is located at the opposite side for instance (e.g. when sensory neuron is dorsal, but mistakenly finds target neuron in ventral area through wrong axonal guidance), then the organism’s perception will be inverted (example: eye and tectum – frog experiment). The formation’s underlying mechanism are ephrin concentration gradients and axon-axon competition (an already established axon will inhibit other axons from stealing its place, so they have to establish their position behind it).

The olfactory map: Several olfactory neurons in the olfactory epithelium target one glomerulus in the olfactory bulb if they have the same odor target. Spatial information not preserved and only quality of sensation (smell, odor). Olfactory map is established in a stepwise manner: first global targeting which is genetically programmed and then local sorting based on activity. Also timing and expression of repellants is crucial: involved chemorepellants: Robo2, Slit1, Sema3F, Nrp2.

Projections from the retina are relayed to the lateral geniculate nucleus when on their way to the visual cortex.

In the tectum, ephrins are expressed as gradients (anterior: low, posterior: high) and in the retina, the receptors are expressed as gradients (anterior: low, posterior: high).

Map organization is controlled by ephrin gradients: Tectum: Ephrin2A high at caudal and low at rostral, same for Ephrin5A, but occurs later (caudally – in the middle of rostral-caudal line).

In eye, Ephrin3A is high in temporal retina (away from nose) and low in nasal retina (side close to the nose). Eph5A high in nasal retina and lower in temporal retina. Also, nasal retina connects to caudal tectum and temporal retina connects to rostral tectum.

Bonhoeffer stripe assay: Cut anterior (rostral) and posterior (caudal) membrane from the tectum. Place them nicely in stripes with anterior alternating with posterior (A-P-A-P…). On one end, place nasal retina membrane and then temporal retina membrane. Result: temporal retina axons avoid high ephrin concentration and grow only in the anterior stripes, while nasal retina axons have no specific preference.

**The mechanisms of cell migration in the developing brain and neural crest stem cells migration**

There are four modes of migration which neural cells undertake during corticogenesis: Somal translocation, Glia guided migration (both are radial migratory types), tangential migration (switches to radial migration at some point in migration) and ventricle directed migration of interneurons. The cortex develops inside-out (so new neural cells are above the old cells).

Radial migration mechanism involves reorganization of the cytoskeleton and nucleokinesis: Elongation of the exploring site of the cell for intermediate targets and attrative and repulsive cues by polymerization of the actin filament and microtubules at the tip, nucleokinesis (the cell nucleus moves towards the exploring end a bit by means of microtubules and their motor proteins) and retraction of the rear side. This is true for both somal translocation and glia guided migration.

Somal translocation as the dominant modus operandi in early corticogenesis: Neuronal cells from the ventricular zone (=: VZ) at E8.5 migrate to the preplate (=: PP) at day E10.

Glia guided migration: Neuronal cells, especially pyramidal cells, from the VZ migrate along the axons of glia cells (also located at VZ) to the cortical plate (=: CP) (after E10, around E12 effectively).

Tangential migration: Neuronal cells (inhibitory interneurons) from the ganglionic eminence migrate tangentially until they reach the VZ and then migrate radially to the CP. They migrate from the ventral telencephalon to the dorsal telencephalon (marginal zone) and then radially to the intermediate zone, then to the CP.

Ventricle mediated migration: Interneurons from the ganglionic eminence migrate to the VZ first, before after some time they migrate towards the pial surface (CP simply put).

Migration in the cerebellum: Granule precursor cells migrate tangentially (tangential to the pial surface) and then migrate non-pial surface directed radially (they basically migrate closer towards the VZ, but will stay in the CP obviously). Purkinje precursor cells migrate radially. This forms the external granule layer (outer external granule layer + inner granule layer), then molecluar layer, then purkinje cells layer and lastly the internal granule layer (outside-in).

Precursor cells of the olfactory bulb migrate tangentially from the (telencephalic) SVZ via the rostral migration stream to the olfactory bulb (with radial migration in the end).

Cortical migration defects and their associated diseases:

Initiation defect: Gene: FLNA and Arfgef2: Periventriuclar heterotopia.  
Stop defect: POMT1, POMGnT1, Fukutin: Cobblestone lissencephaly.  
Lamination defect: RELN: Lissencephaly, Cerebellar hypoplasia.  
Ongoing migration defect: Dcx, Lis1, etc.: Lissencephaly, Subcortical band heterotopia.

The derivatives of neural crest cells: Cranial: bone and cartilage, sensory neurons, melanocytes, connective tissue such as teeth and eyes, glial cells.  
Vagal: glial cells, sensory neurons, melanocytes, heart tubes, cardiac tissue, smooth muscle cells, enteric neurons.  
Trunk: glial cells, sensory neurons, melanocytes, autonomous nervous system neurons, chromaffin cells (adrenal medulla).

Modes of migration through ECM: Amoeboid, mesenchymal (single cells or in chains), as clusters/cohorts, multicellular strands/sheets.

The process of migration through the ECM: Pseudoprotrusion at the leading edge, formation of focal contact, focalized proteolysis, actomyosin contraction, detachment of the trailing edge.

**Mental disorders – Schizophrenia, ASD and MR**

In neurodevelopment, axon guidance, synapse formation and synaptic plasticity are affected in mental diseases.

Schizophrenia: Positive symptoms: hallucinations, illusions, thought disorder. Negative symptoms: anhedonia, social retreat/asociality, alogia (poverty of speech). Cognitive symptoms: working memory dysfunction, dysfunction in executive tasks, attention deficit.

NMDAR hypothesis – glutamate system: morphological changes to NMDAR leading to dysfunctional activity.

DA signalling hypothesis: Positive and negative symptoms are correlated to dopamine signalling. Positive symptoms act on D\_2 receptors (excess) and negative symptoms act on D\_1 receptors (deficit). Medication is not always easy, because the receptors are quite similar resulting into off traget effects.

There is clear genetic evidence for the development of schizophrenia (concordance: 50%, in dizygotic twins: 17%), but environmental factors also need to be taken into account: viral infections during fetal development, poor maternal nutritioning (during famine), toxic/traumatic/autoimmune insults, problems during gestation or labor/birth.

Evolution of symptoms: cognitive/motor/social impairment, anxiety and depressive symptoms, social withdrawal and subjective cognitive changes, prodromal symptoms, psychosis. Prodormal symptoms and psychosis normally start in early adulthood.

Schizophrenic brains: There are several changes from normal patients: smaller size in amygdala and hippocampus, enlarged ventricles, not nicely defined cell polarity. Frontal cortex in the schizophrenic sibling is reduced when compared to healthy sibling. Also, synaptic fine tuning was malfunctioning throughout development resulting into too many and too few synapses (depends on brain area). Therefore, synaptic connections exist that are not normally supposed to be present while others are missing (failed apoptosis) and issues with myelination occur.

Model by Durcan and Mirsky: brain anomalities vs. environmental stress: threshold curve is similar to 1/x => many abnormalities, but few/no environmental stressors lead to healthy people and few abnormalities but many environmental stressors can lead to schizophrenia etc.

Autistic Spectrum Disorder (=: ASD): Concordance is 60%, in dizygotic only 3-10%. 2/3 of disease genes are X-chromosome linked of the 885 genes.

Signs for autism: Communication: no language or late development, usage of stereotypic phrases and repetetive, problems with sustaining a conversation or developing a conversation (especially initiation), lack of varied, spontaneous make-believe play or imitative play.

Social impairments: avoids eye-to-eye contact, lack of sharing happiness and enjoyment spontaneously with others, does not develop peer-to-peer relationships (friends), lack of emotional or social reciprocity.

Behavioural: stereotype or repetitive motor mannerism, persistent preoccupation with one object, adherence to non-functional routines or rituals, preoccupation with one or more restricted or stereotyped patterns of interest.

Around 1/3 of ASD children also report epilepsy.

Other similar disorders: They all have qualitative deficits in communication and social behaviour in common.

Childhood disintegrative disorder: first two years of development normal, then regress (caused by a mutation on chr X – lethal for boys, girls can live).

Asperger’s syndrome: Similar to ASD, but normal speech, individuals are very intelligent and highly functional.

PDD-NOS – pervasive developmental disorder-not otherwise specified: Group where all undefined patients belong to.

Environmental stessors are drugs against epilepsy (anti-convulants), certain viral infections (rubella, influenza, cytomegalovirus), thalidomide use.

The ASD brain: Very small or no CC, reduced prefrontal cortex, smaller cerebellum, general changes to overall brain structure, loss of Purkinje cells, changes in synapse formation and elimination, reduced dendritic arbor complexity, cells are more densely packed and they are smaller.

Additionally, the have a dysfunction in the mirror neuron system (=: MNS): MNS activates neurons in the temporal lobe when an observer is exposed to an activity by another individual. The MNS simulates the expierence ⬄ activates the same/similar neurons that the individual in action has activated. The MNS is involved in associative and higher order functions of the brain, such as understanding intention, reading non-verbal signs (face expression etc.).

Mental retardation:

Non-syndromic autosomal forms are hard to recognize by mere eye. Syndromic forms are often recognized as down syndrome. Many forms are related to mutations on X chromosomes (40% of 885 protein coding genes in the brain are on the X chr).

In mentally retarded patients, LTP is still possible for existing synapses, but new synapse formation is not possible (no new formation of dendritic filopodia which are precursors to synapses). This is caused by a mutation in neurotrypsin (non-syndromic gene, not X-linked), which cleaves agrin (needed for synapse formation). Agrin is a NMJ gene that is also used and modified by neurotrypsin in the CNS. The cleaved form is 21-22kD (it’s simply the last domain of the entire gene). The truncated, non-functional disease lacks the active part (C-terminal).

Neurotrypsin release from SV is stimulated by synaptic activity (on the dendritic side) by an unknown molecule.

Fetal alcohol syndrome (alcoholic mothers during pregnancy): small changes to facial features, basically no CC left, brain anatomy changes to some extent in general.