Using Animal Models In Perinatal Brain Research: brain development + imaging

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Centre for the Developing Brain

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Learning Outcomes

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Brain Development:

- Use of rodents in research
- Brain development in Rodent and Human

Imaging:

- Purpose of histology and immunohistochemistry
- Labelling different cell types in the brain to assess injury and development

Animal models:

- Small and large animal models of brain injury
- Preterm + Term brain injury models

Preclinical Imaging:

- Preclinical imaging in rodents

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Translational Pipeline

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Inform about the human condition

Proof of Concept in animals

Proof of Concept in humans

Explore mechanisms, biomarkers, trials

Basic Research

Lab Discovery:
In Vitro + In Vivo Experiments

Pre-Clinical *(Toxicology etc)*

Clinical Trials - Early

Clinical Trials - Late

- Mechanisms
- Biomarkers
- Drug Trials + Tolerance

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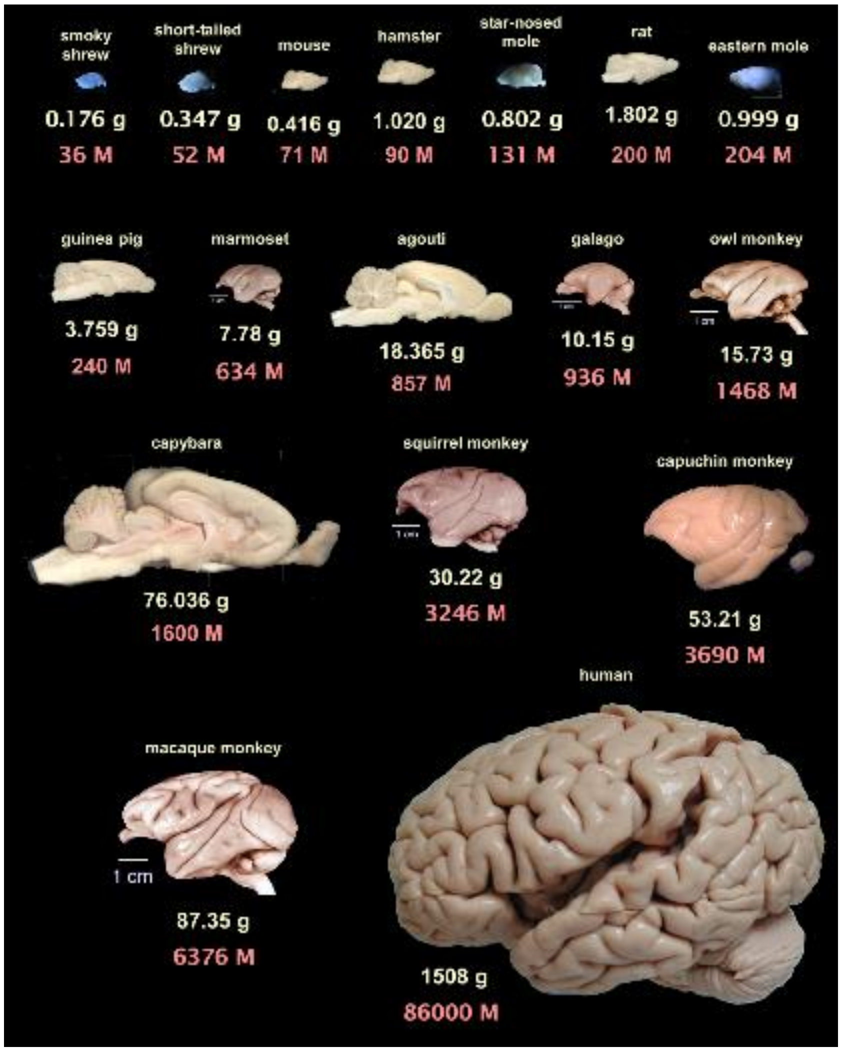
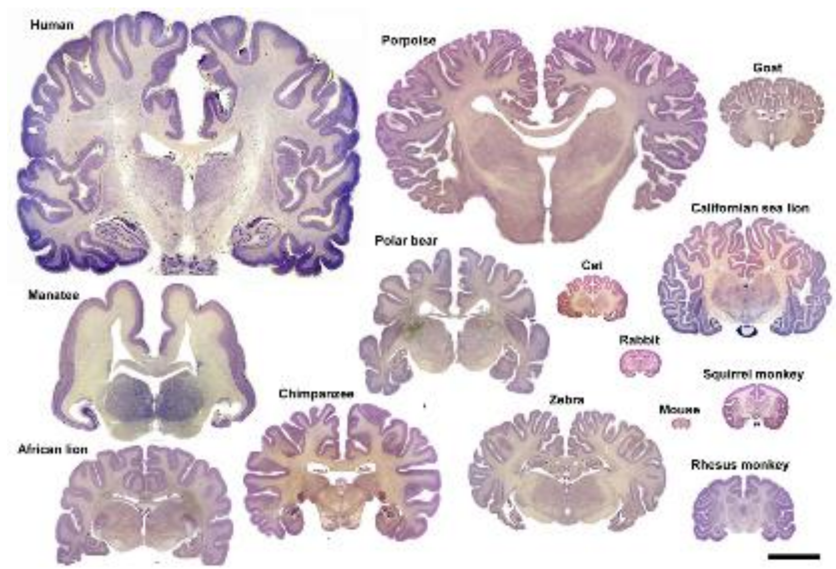


Herculano-Houzel (2009) Front. Hum. Neurosci.,
The human brain in numbers: a linearly scaled-up primate brain



DeFelipe (2011) Front. Neuroanat., The evolution of the brain, the human nature of cortical circuits, and intellectual creativity

Brain growth spurt of mammals

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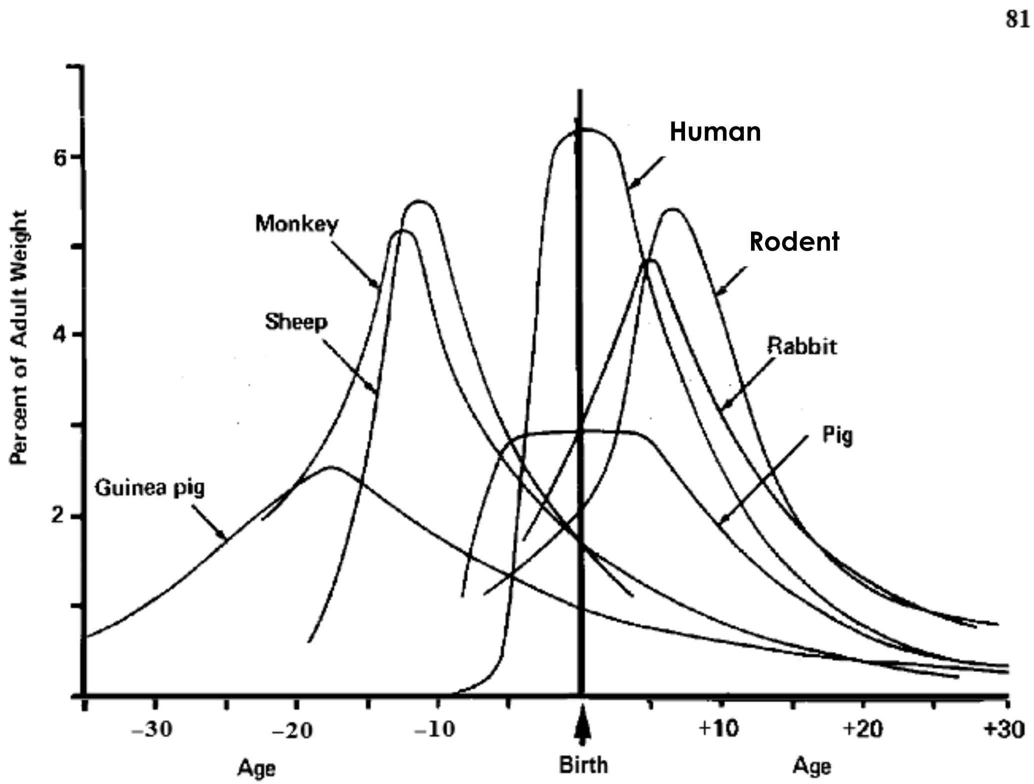

Fig. 1. The brain growth spurts of 7 mammalian species expressed as first-order velocity curves of the increase in weight with age. The units of time for each species are as follows: guinea pig [3]: days; rhesus monkey [1]: 4 days; sheep [9]: 5 days; pig [2]: weeks; man [5]: months; rabbit [8]: 2 days; rat [4]: days. Rates are expressed as weight gain as a percentage of adult weight for each unit of time.

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Dobbing and Sands 1979

Use of rodents in research

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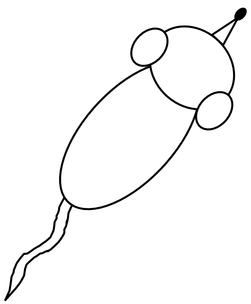

Pros	Cons
Large sample size	Altrical species: - brains develop post-birth
Large litter size - mice: 4 – 8 - rats: 4 - 12	Lissencephalic: - smooth brains, no sulci or gyri - white/grey matter ratio not comparable to humans
Mechanistic studies: - Time course	Difficult to monitor physiological parameters
Gene and protein manipulation (knock out and knock in)	Difficult to take blood samples
Test numerous experimental paradigms: - dose responses - pre/post-treatment	Small tissue samples
Assess long term outcome - behavioural measurements	Anesthetised during procedure
	Experimental procedures ex-*utero*

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Transgenic mice

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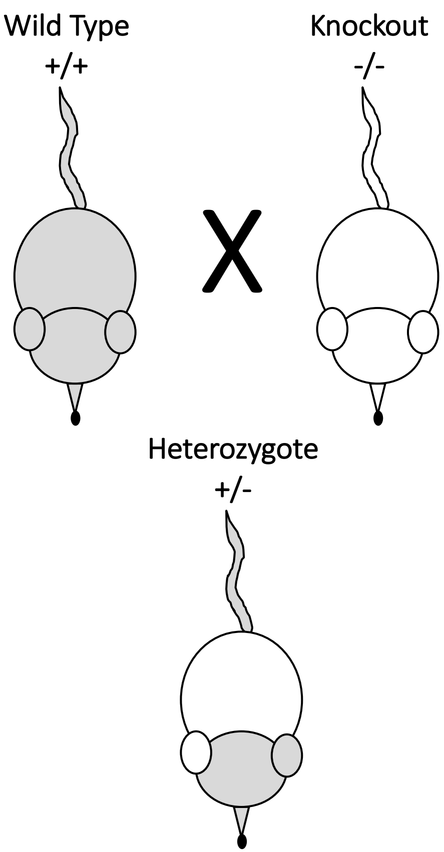
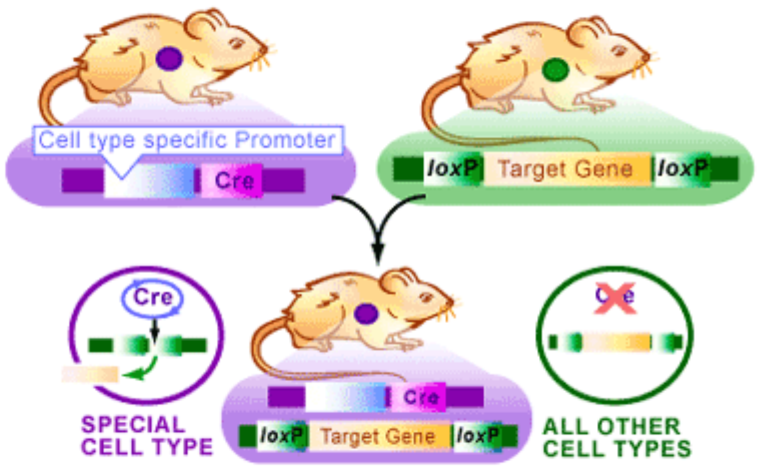
Traditional KO Mice



Specific gene of interest is absent from **all** cells in the body, from conception

Conditional KO Mice

Cre/Lox



https://www.scq.ubc.ca/targeting-your-dna-with-the-crelox-system-2/

Specific gene of interest (Lox) is absent from a specific cell of interest (Cre)

This KO in the cell may not happen from conception.

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Mouse models of Down Syndrome

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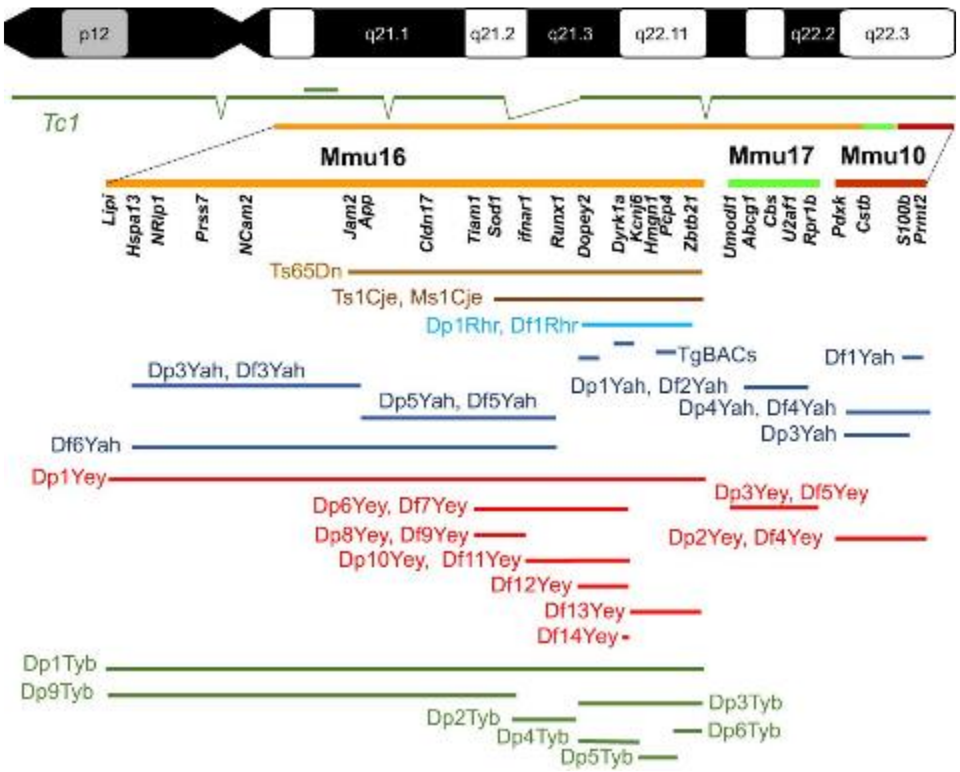
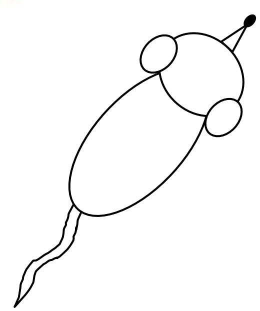


Contribution of genes

Assess brain growth + behaviour

Heart Defects



Trial therapies

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Brain development in the Rodent and Human

Human neurodevelopment

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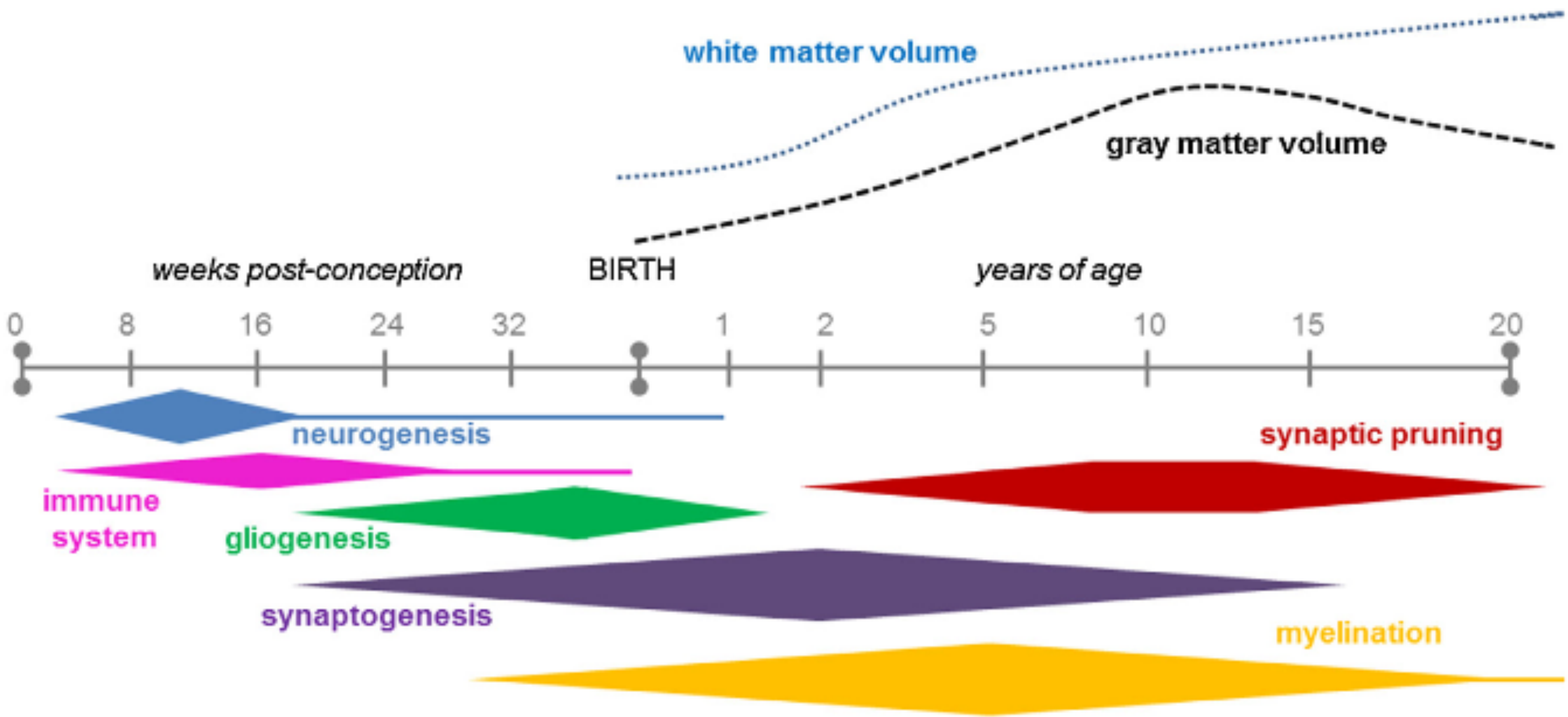
B.D. Semple et al. / Progress in Neurobiology 106–107 (2013) 1–16



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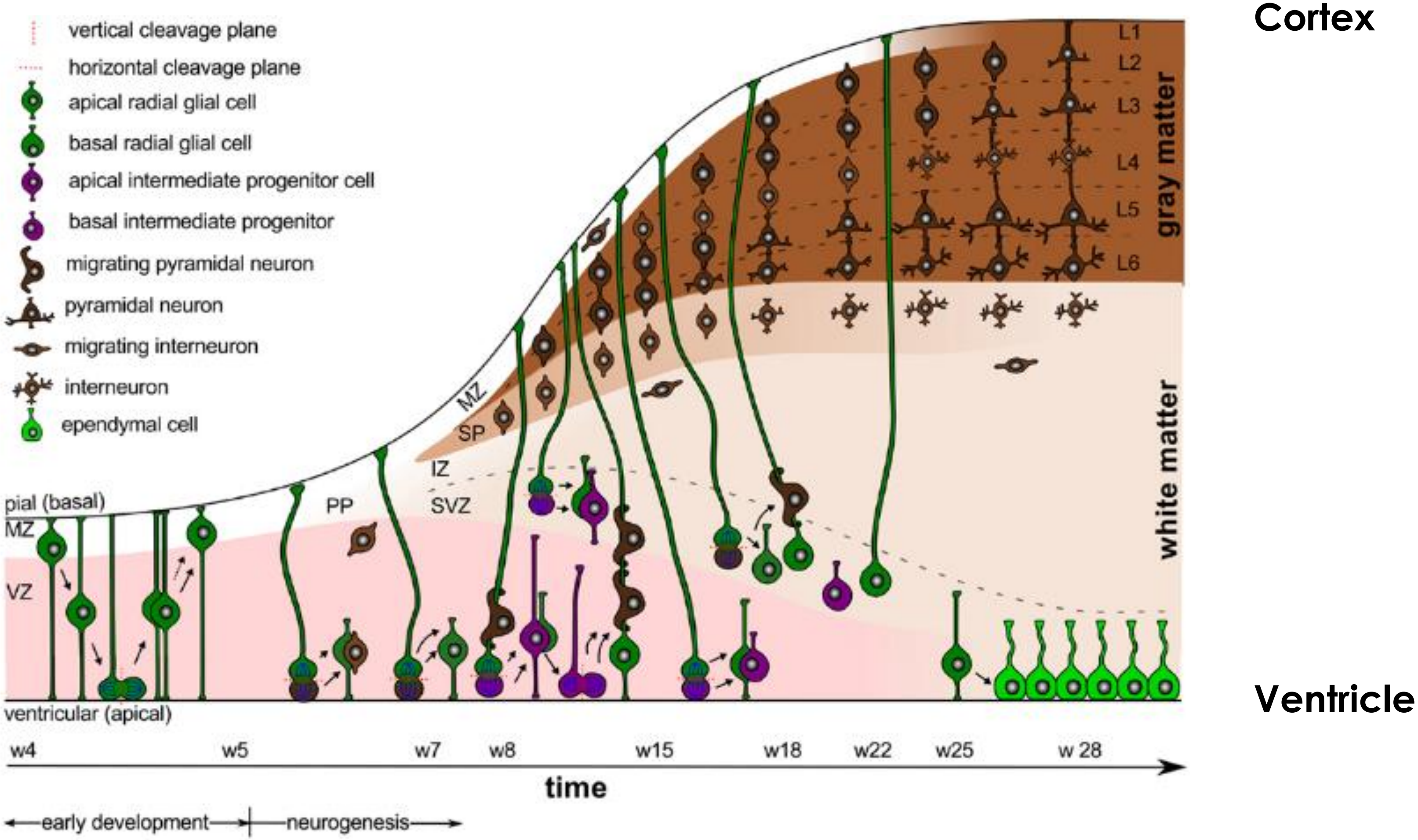
Human neurodevelopment

centre for the developing brain



Budday et al., 2015 Front. Cell. Neurosci., 08 July 2015 | https://doi.org/10.3389/fncel.2015.00257

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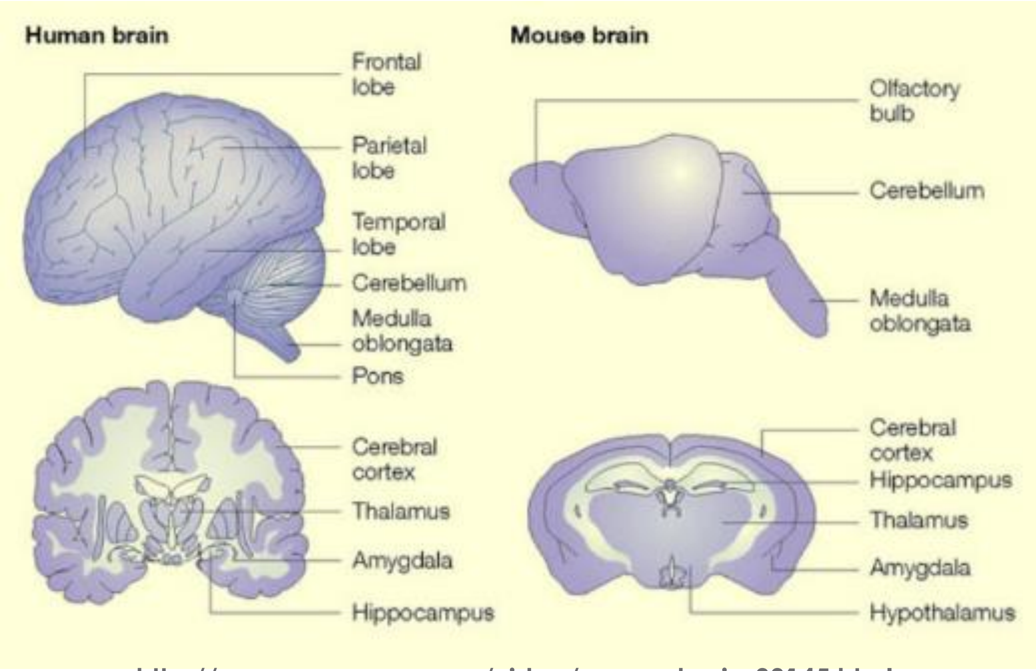
Human and mouse brain development



http://www.awwnews.com/video/mouse-brain-00145.html

Stagni et al.

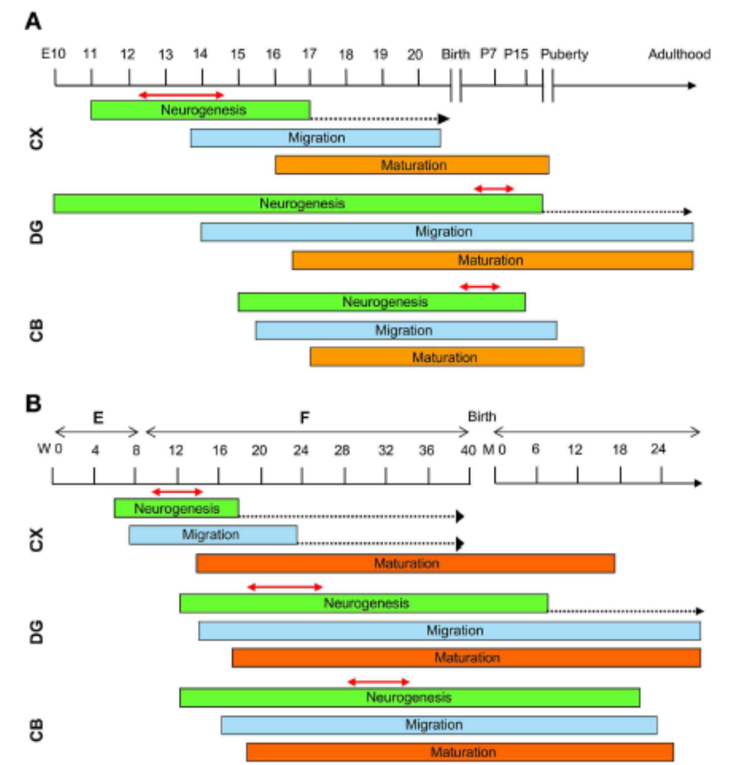


FIGURE 4 | Schematic representation of the timeline of brain development. Timeline of mouse **(A)** and human **(B)** brain development. See text for explanations. The dotted arrows indicate a reduction in the rate of neurogenesis. The double-headed red arrows delineate the period of maximum neurogenesis in the different brain regions of the mouse and human brain. Abbreviations: CB, cerebellum; CX, neocortex; DG, dentate gyrys; E, embryonic; F, fetal; M, month; P, post-natal; W, week.

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Glia - oligodendrocytes

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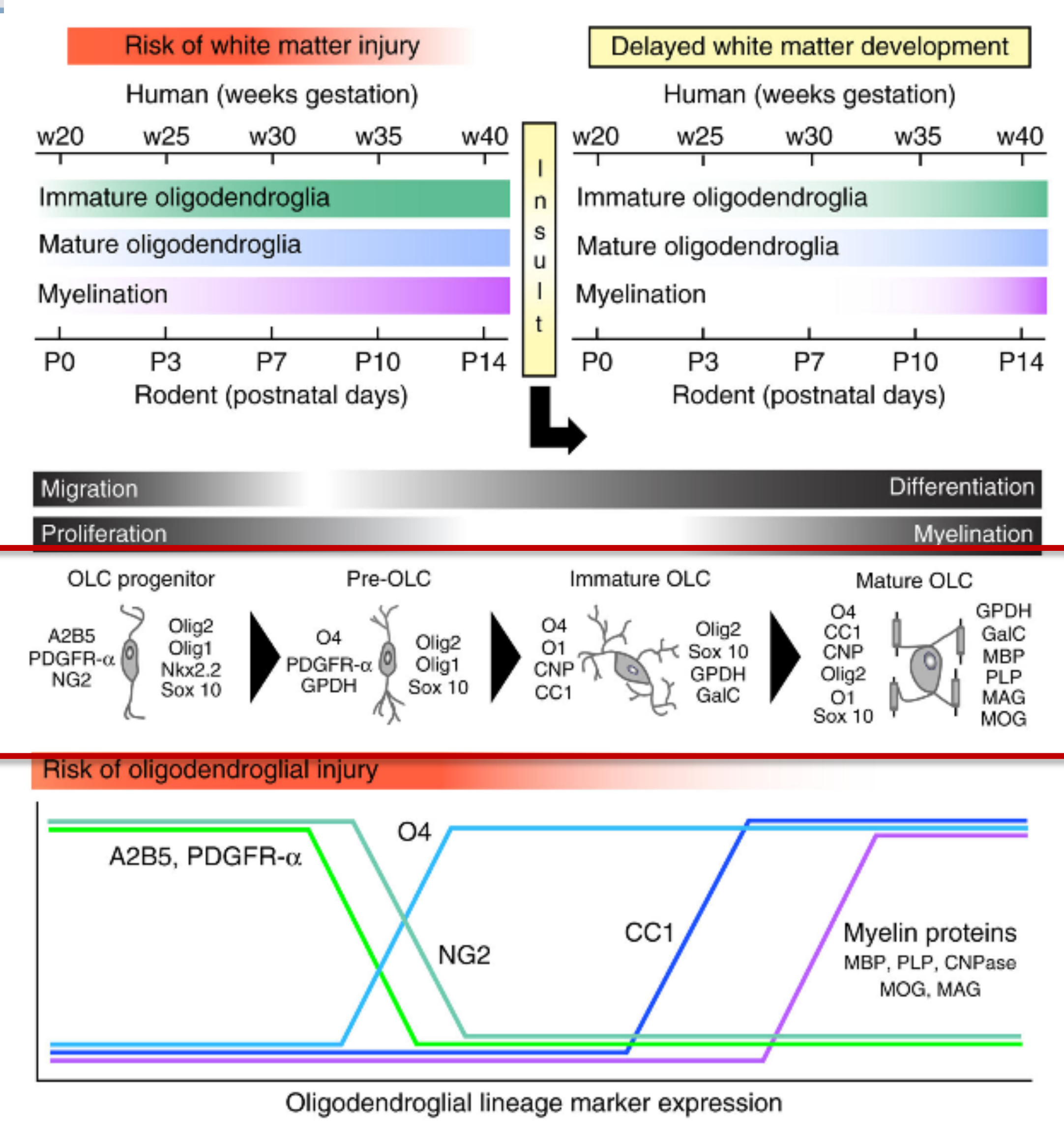

Label different lineages

Salmaso et al., 2014 Nat Neuro

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Adult brain

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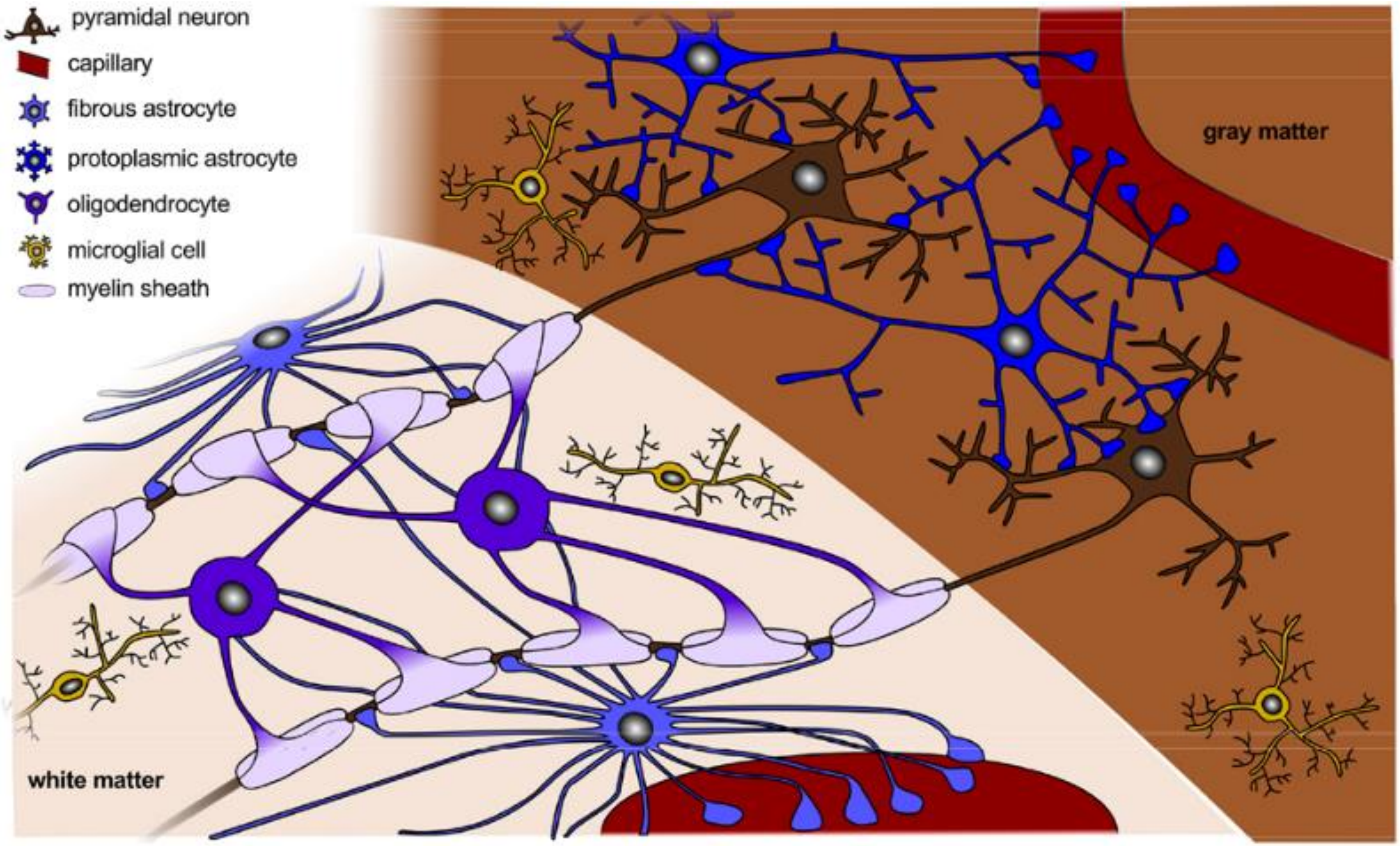

Budday et al., 2015 Front. Cell. Neurosci., 08 July 2015 | https://doi.org/10.3389/fncel.2015.00257

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Human and mouse brain development – cortex

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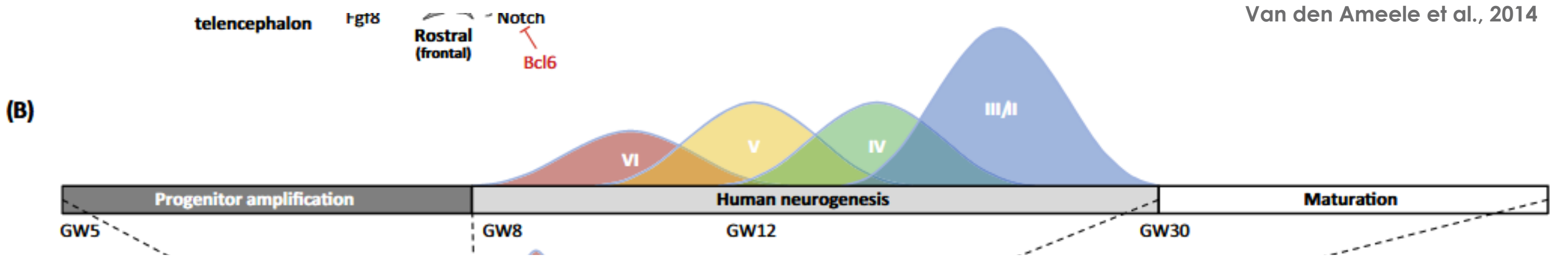
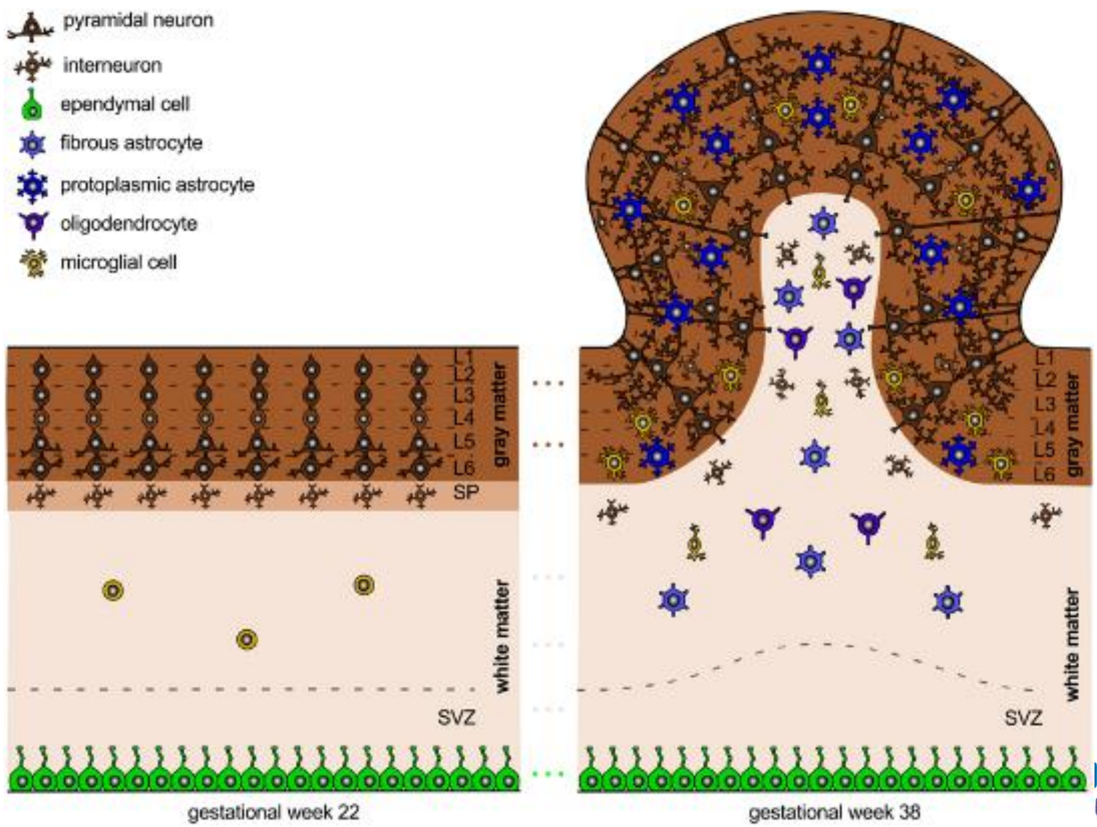
Van den Ameele et al., 2014





Budday et al., 2015 Front. Cell. Neurosci., 08 July 2015 | https://doi.org/10.3389/fncel.2015.00257

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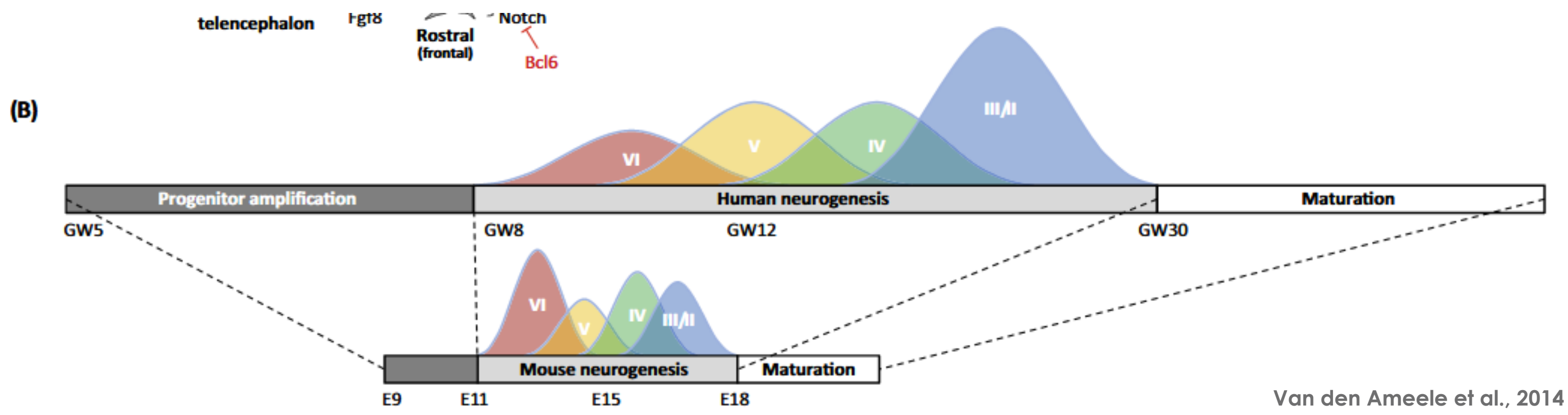
Human and mouse brain development – cortex

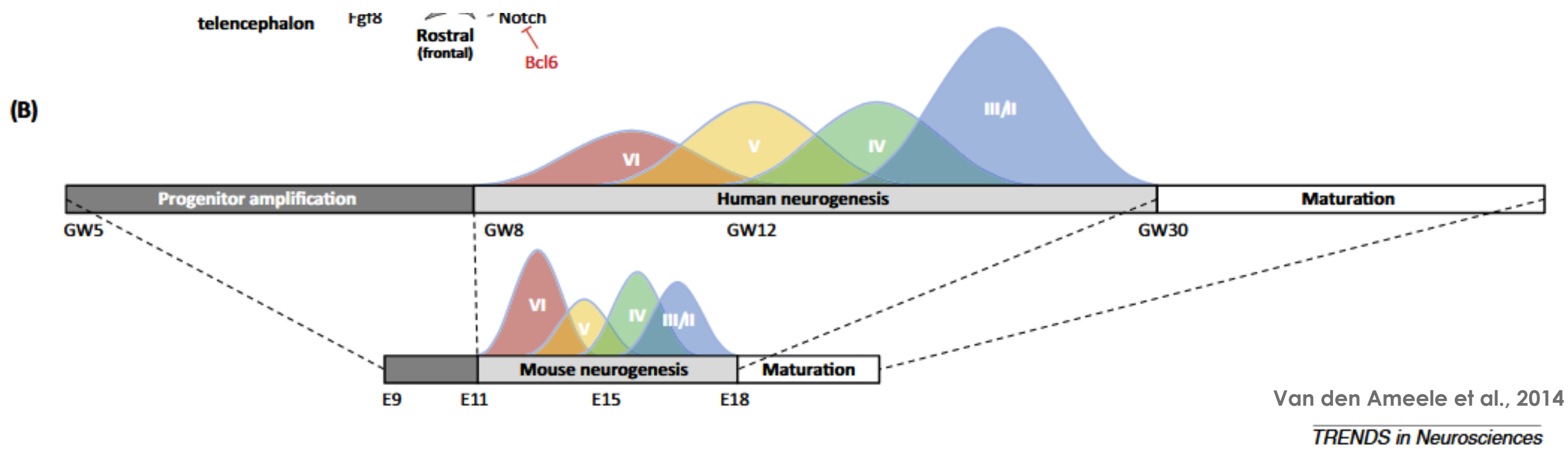


Van den Ameele et al., 2014

TRENDS in Neurosciences

Human and mouse brain development – cortex

centre for the developing brain

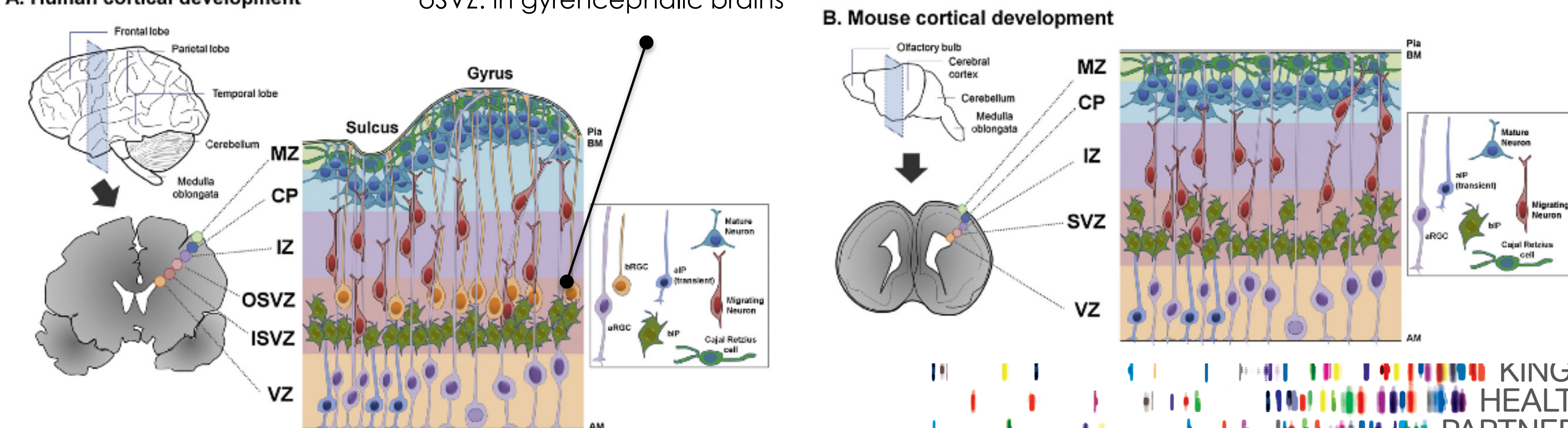


Van den Ameele et al., 2014

TRENDS in Neurosciences

D.M. Romero et al. / Seminars in Cell & Developmental Biology 76 (2018) 33–75

oSVZ: in gyrencephalic brains



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Histology + Immunohistochemistry: Visualising cells

Histology

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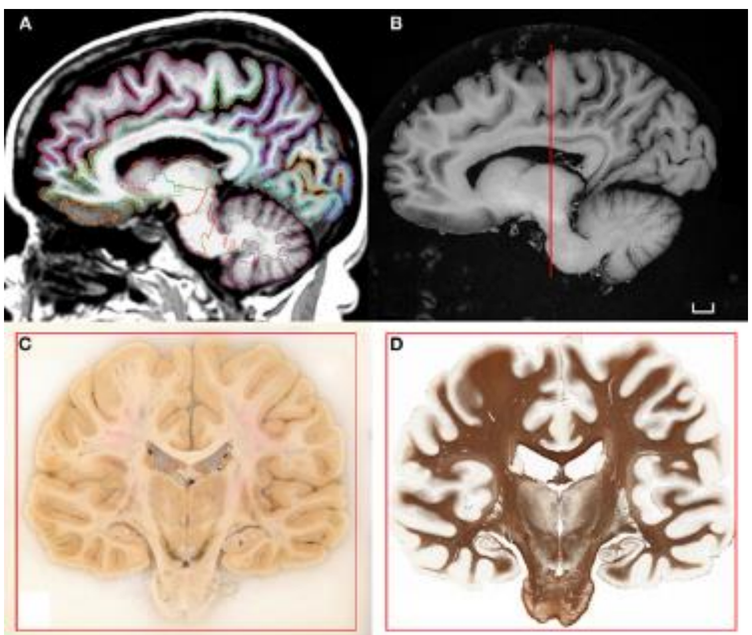
- Staining tissue sections to help visualise and/or identify
- Examining biopsy tissue to help diagnose a disease
- Visualised under a light microscope



Annese et al., 2012 Front Neurofrom

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H&E

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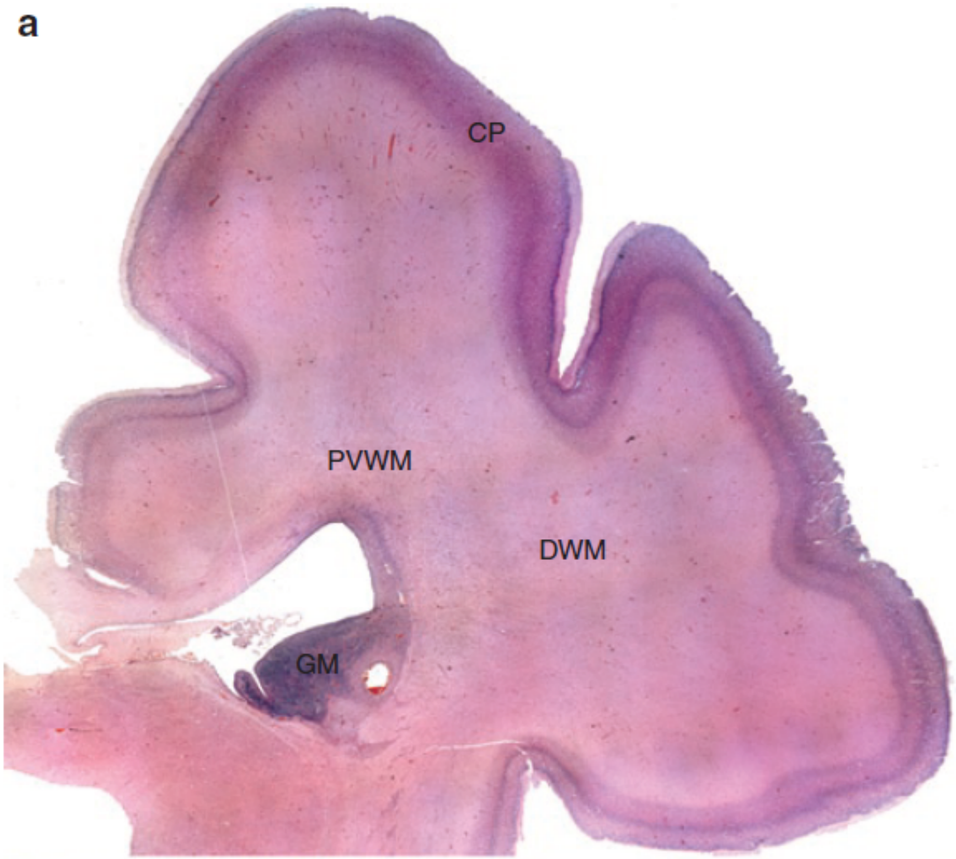

H&E in a coronal section at the level of the parietal lobe, 23+6/7 PMA Control brain

Supramaniam et al., (2013) PedRes

Most commonly used stain

Haemotoxylin basic dye

- Used to stain acid (basophilic) structures purplish blue
- Examples:
  - nucleus
  - DNA in nucleus
- RNA in ribosomes and RER

Eosin:

- Acidic dye, negatively charged
- Stains basic (eosinophilic) structures red/pink
- Examples
  - cytoplasm

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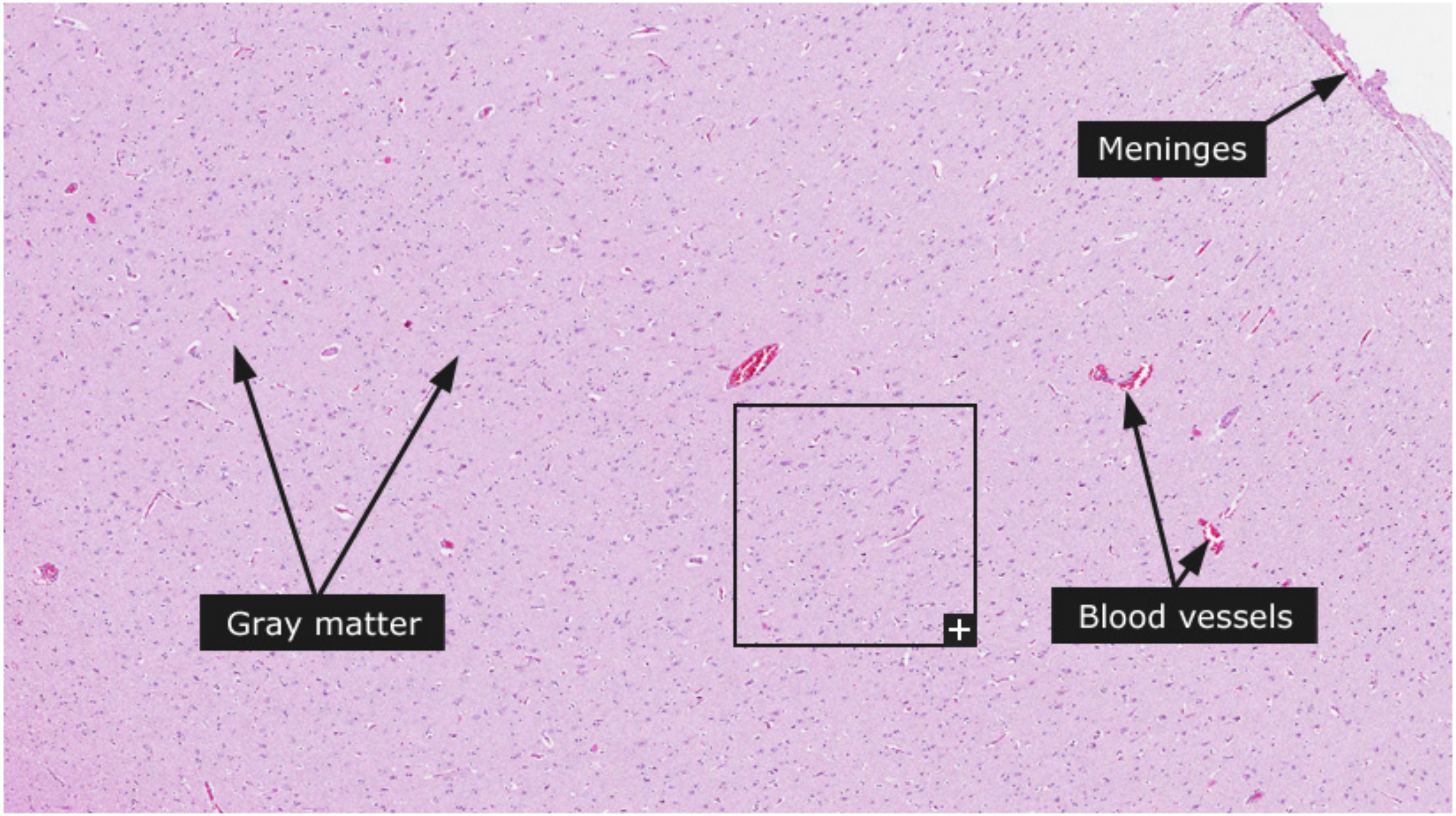
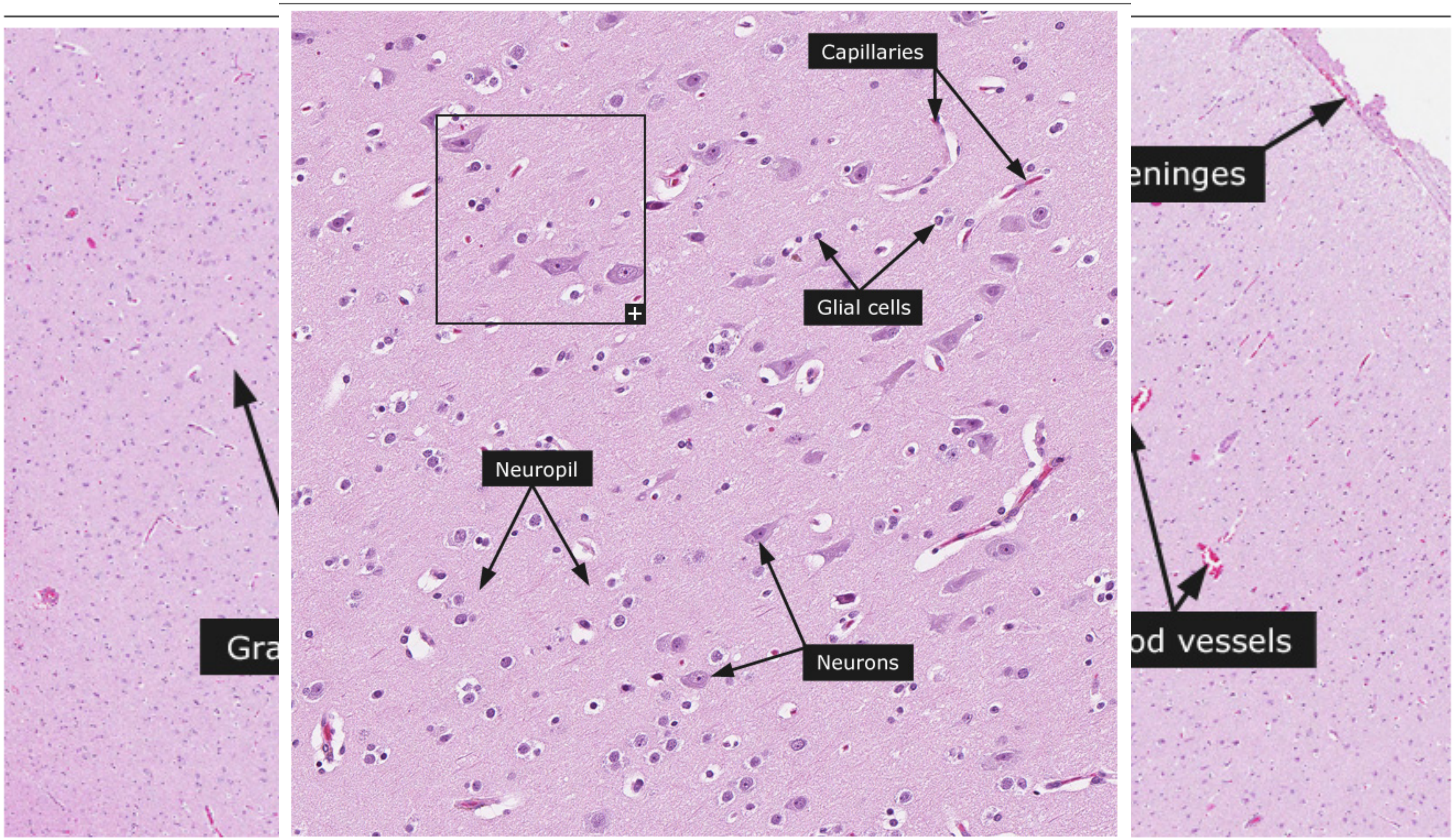
Cerebral cortex



Cerebral cortex

http://www.proteinatlas.org/learn/dictionary/normal/cerebral+cortex

Cerebral cortex



Cerebral cortex

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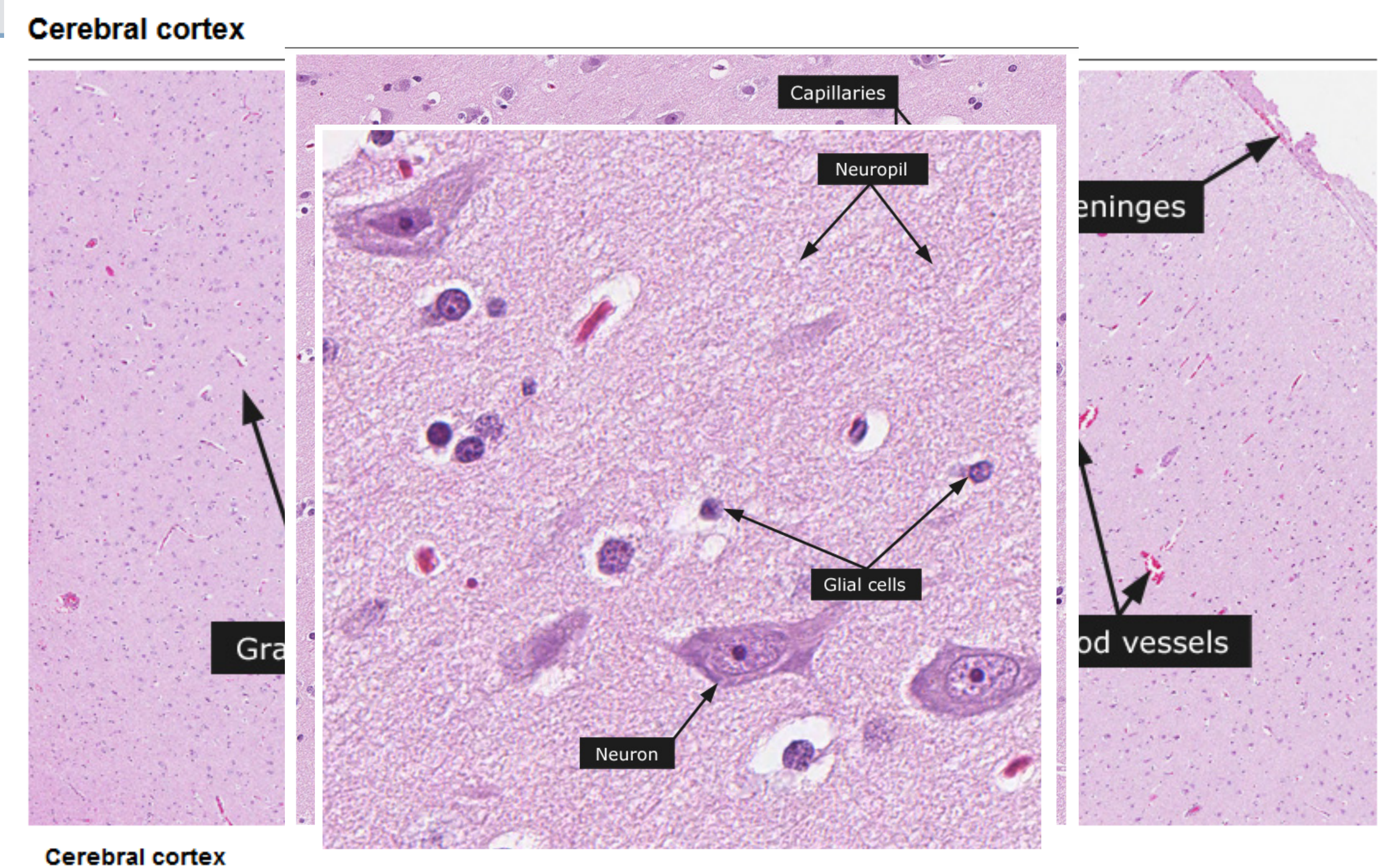
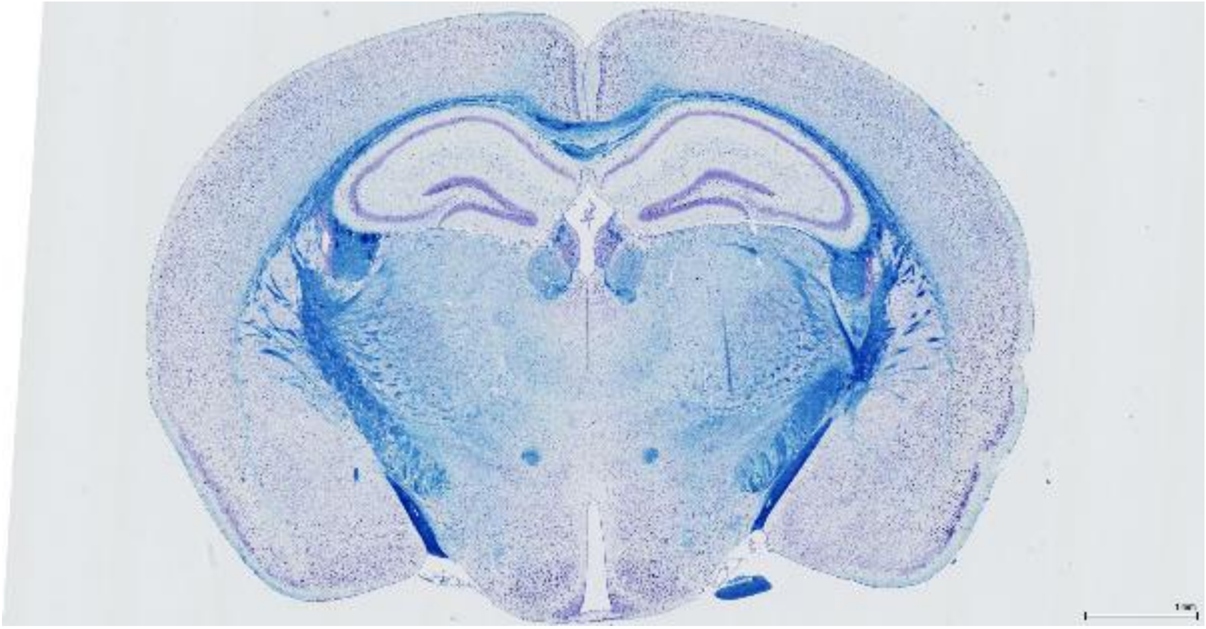


http://www.proteinatlas.org/learn/dictionary/normal/cerebral+cortex

Luxol Fast Blue

Commonly used to visualise myelin



Golgi Stain

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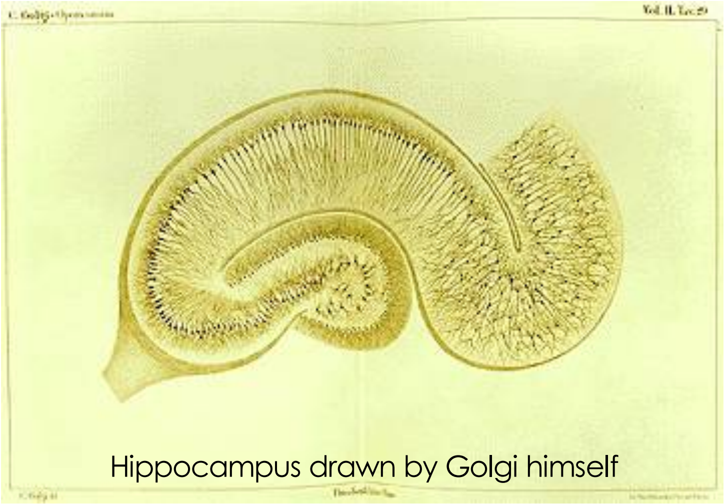
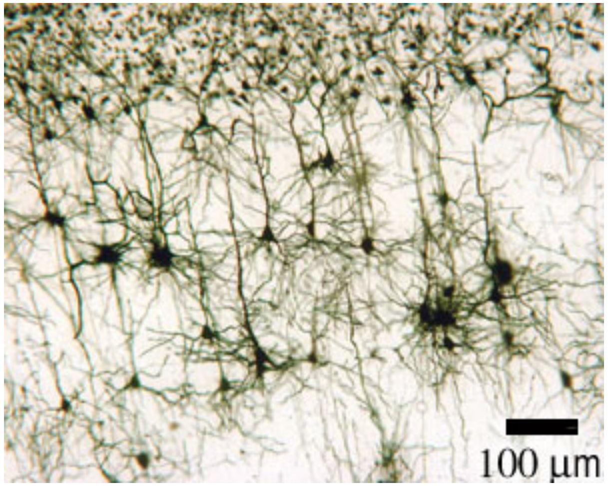
- Type of silvers stain used to visualise nervous tissue
- nervous tissue are densely packed and little information on their structures and interconnections can be obtained if all the cells are stained.

- Thin filamentary extensions of neurons, are too slender and transparent to be seen with normal staining techniques.



Hippocampus drawn by Golgi himself



http://www.histology.leeds.ac.uk/tissue_types/nerves/Nerve_neuron.php

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Immunohistochemistry

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- Technique used to **visualise** the **location** and **distribution** of specific antigens (proteins) through antigen-antibody interactions.
- Tissue is removed from patient/animal, frozen or chemically preserved
- Localization of cellular components while maintaining the original architecture of the surrounding tissue
- *Immunocytochemistry:* whole cells to stain, extracellular matrix removed
  - eg. Blood smears, swabs, tissue culture cell lines
- Label:
  - different cell types
  - Cell cycle: dying/proliferating cells
  - proteins of interest expressed by cells
- Single/multiple antigens

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Immunohistochemistry

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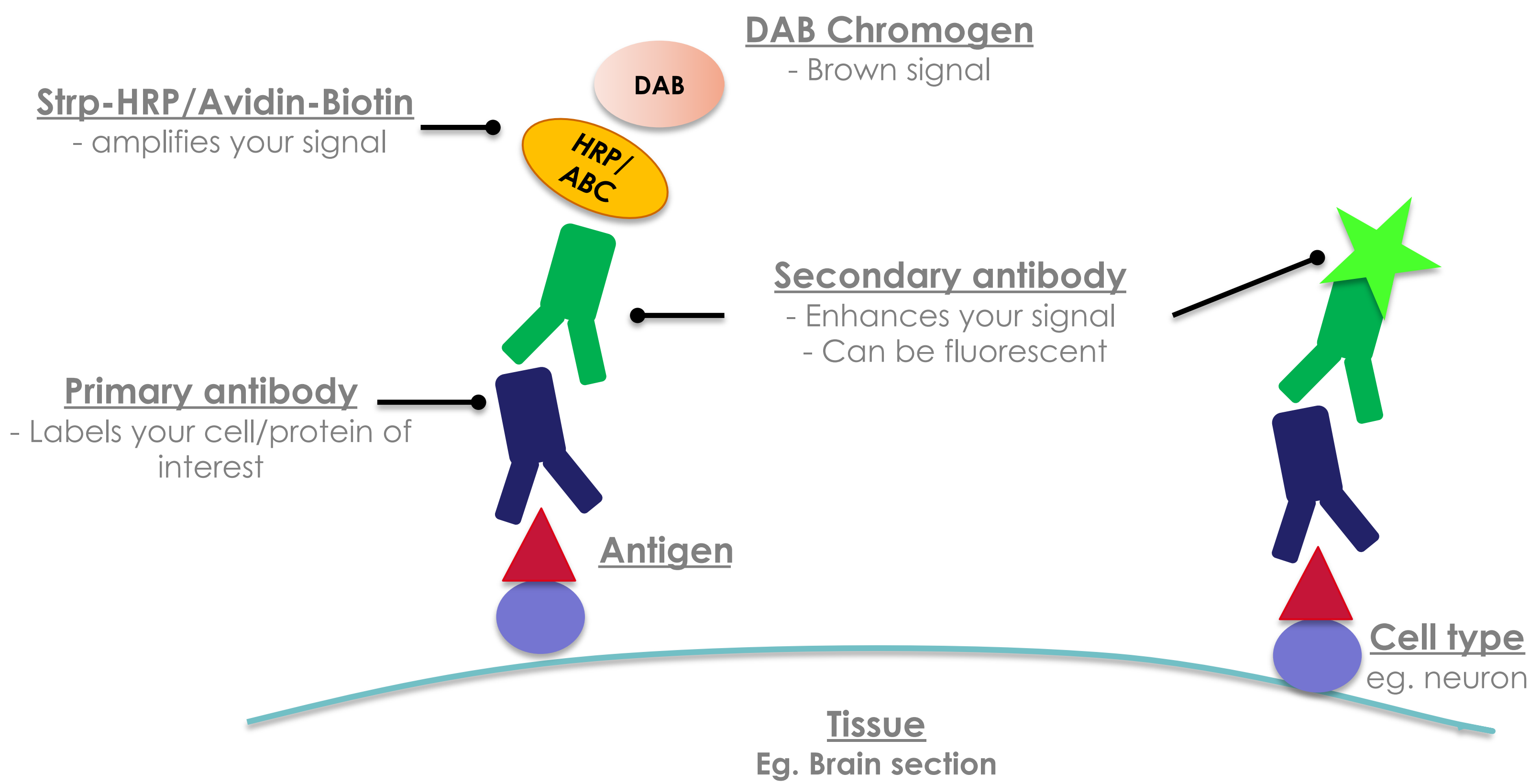
Peroxidase Labelling

Fluorescent Labelling



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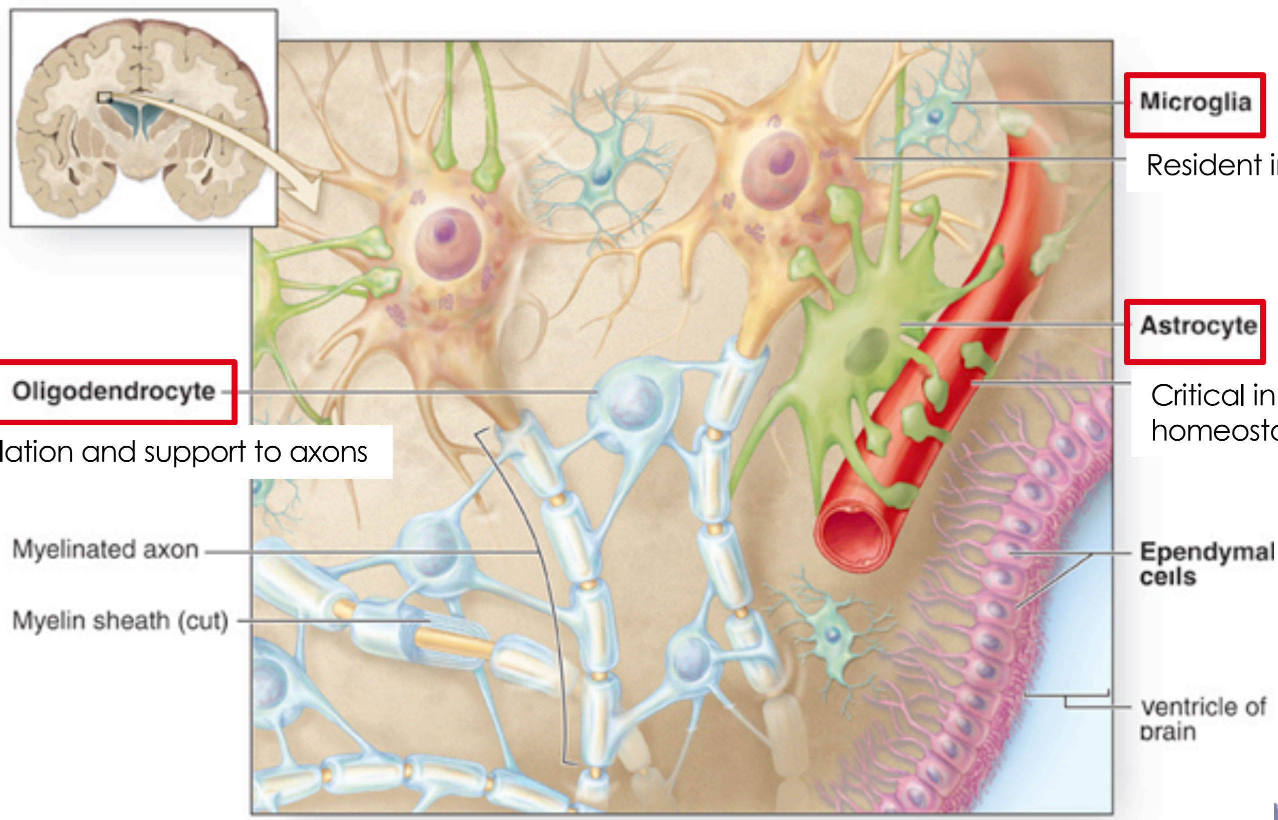
Glia

- Support neurons
- 10 – 50 x more
- React to injury
- Regulate metabolism

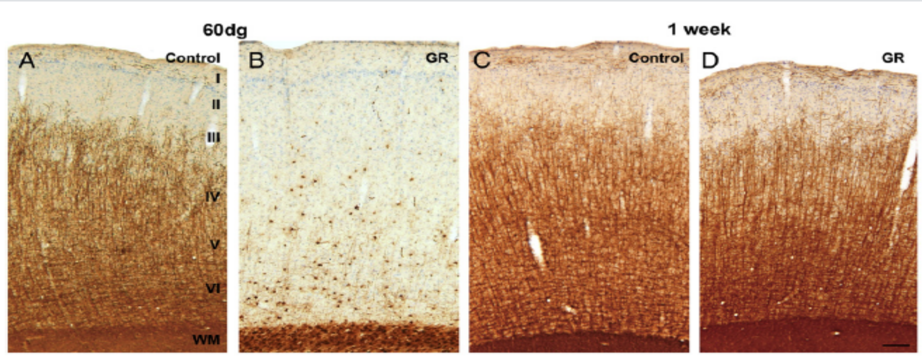


Glia

Oligodendrocytes

- Cell bodies
- Fibres
- Proteins label different maturation states

Tolcos et al., (2011) Exp Neurol



***Myelin Basic Protein**: myelinated fibres & cell bodies*

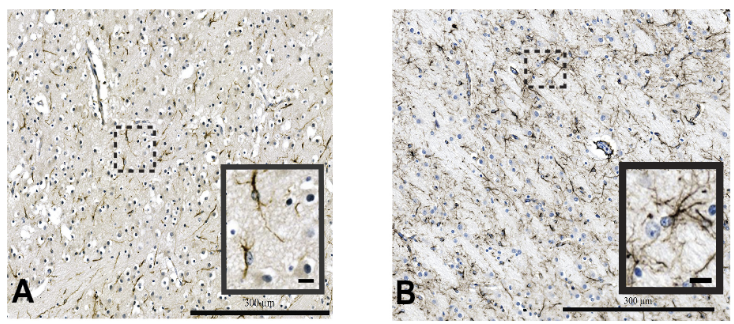
Astrocytes

- Cell bodies
- Morphology

Vontell et al., (2015) JNEN

Control

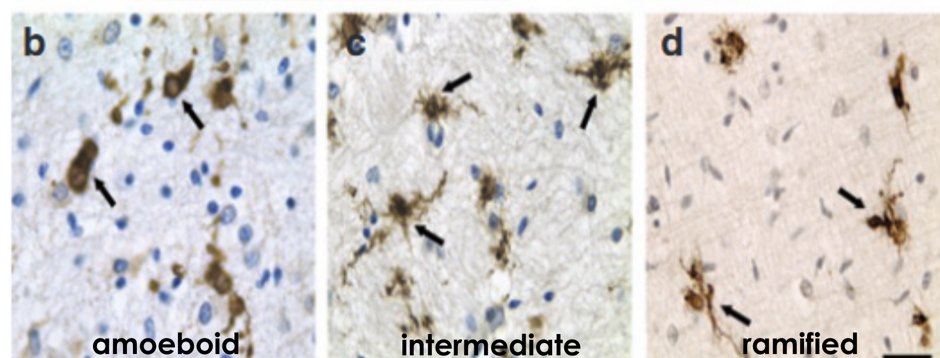
Preterm White Matter Injury



Fibrous & reactive morphology

Microglia

- Cells
- Morphology
- What they are producing



Supramaniam et al., (2013) PedRes

Neurons

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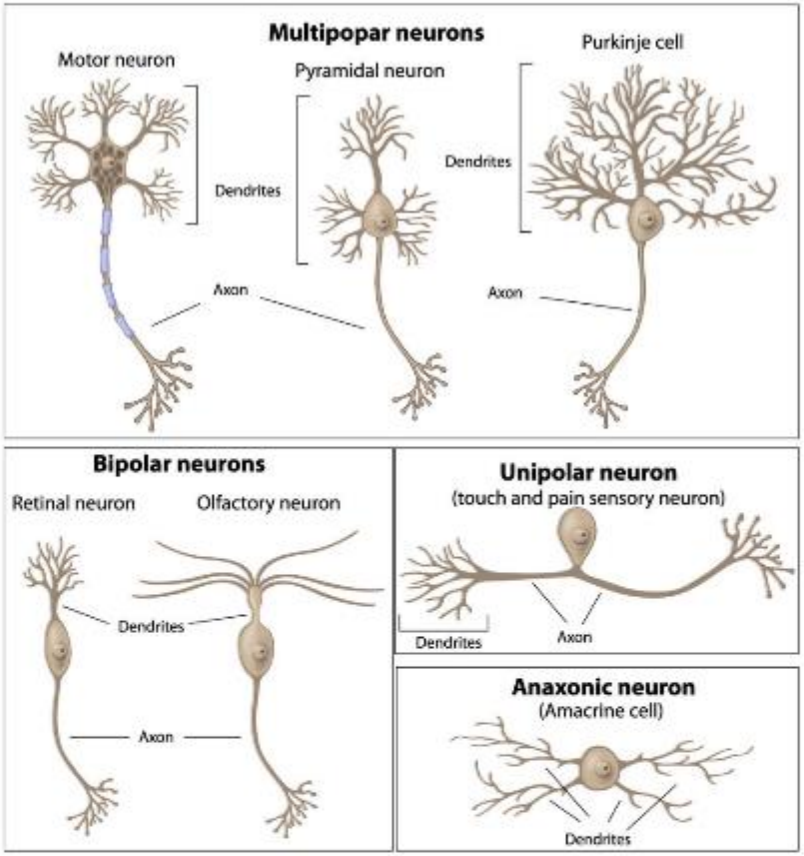
- More than 10^{10} neurons in the CNS
- 3 functions types:
  - Sensory (afferent; tissue → CNS)
  - Motor (efferent; CNS → tissue)
  - Interneuron (connects neurons within CNS)
- Different sizes & shapes:
  - **Unipolar:** dendrite and axon emerging from same process
  - **Bipolar**: axon & 1 dendrite are on opposite ends of the soma
  - **Multipolar:** 2 or more dendrites, separate from the axon
- **Label any part of the neuron**

Types of Neurons



http://www.interactive-biology.com/3247/the-neuron-external-structure-and-classification/

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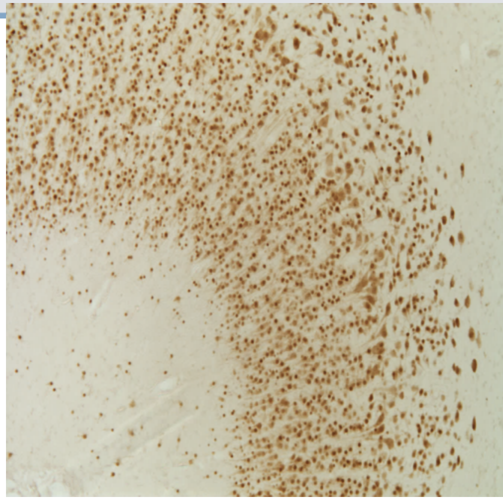
Neurons

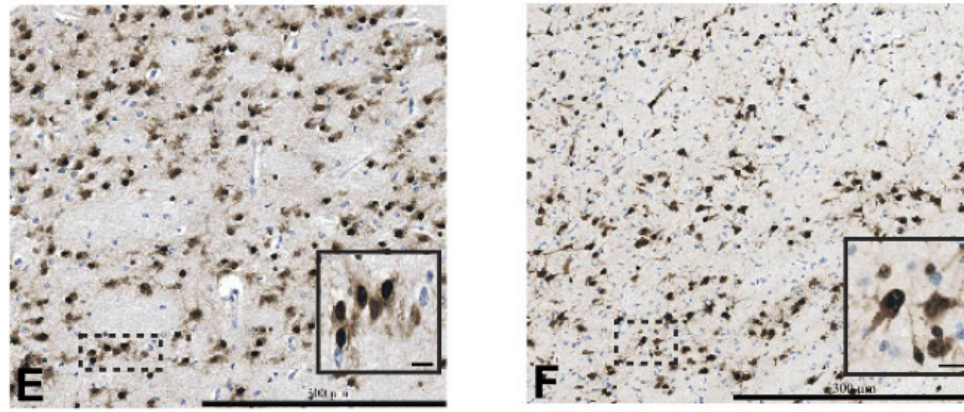
Neuronal nuclei (NeuN)



Preterm fetal sheep

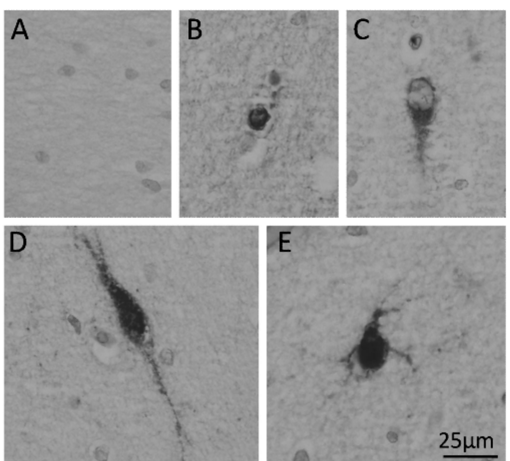
HuC/HUD

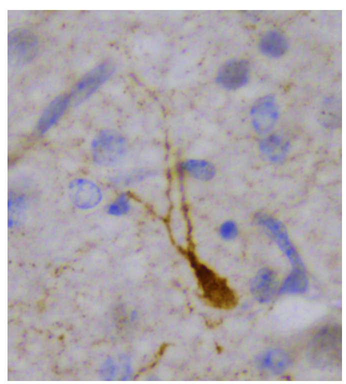
Control | **↓ Preterm White Matter Injury**



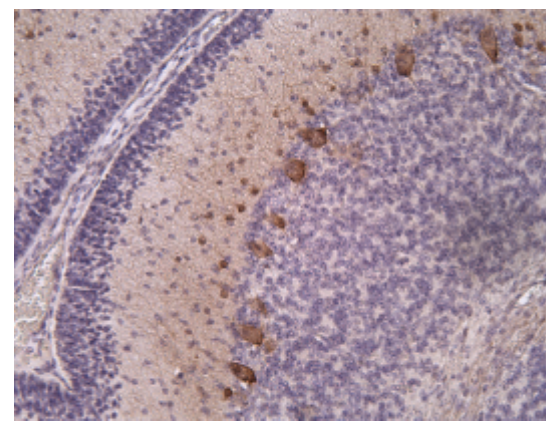
Preterm human brain
Vontell et al., 2015

Different **morphology** of GABAergic neurons in cerebral white matter





Somatostatin stained cell in the preterm fetal sheep brain
- Can assess neurite length



Parvalbumin stained in the preterm fetal sheep cerebellum

PARTNERS

Animal models of perinatal brain injury

Term Infants
Hypoxic-Ischemic Encephalopathy

Preterm Infants
Preterm birth
Inflammation and /or
Hypoxia-Ischemia

Perinatal Brain Injury

Perinatal Stroke

How can we study?

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Term Infants
Hypoxic-Ischemic Encephalopathy

Preterm Infants
Preterm birth
Inflammation and /or
Hypoxia-Ischemia

Perinatal Brain Injury

Perinatal Stroke

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Human neurodevelopment: Preterm

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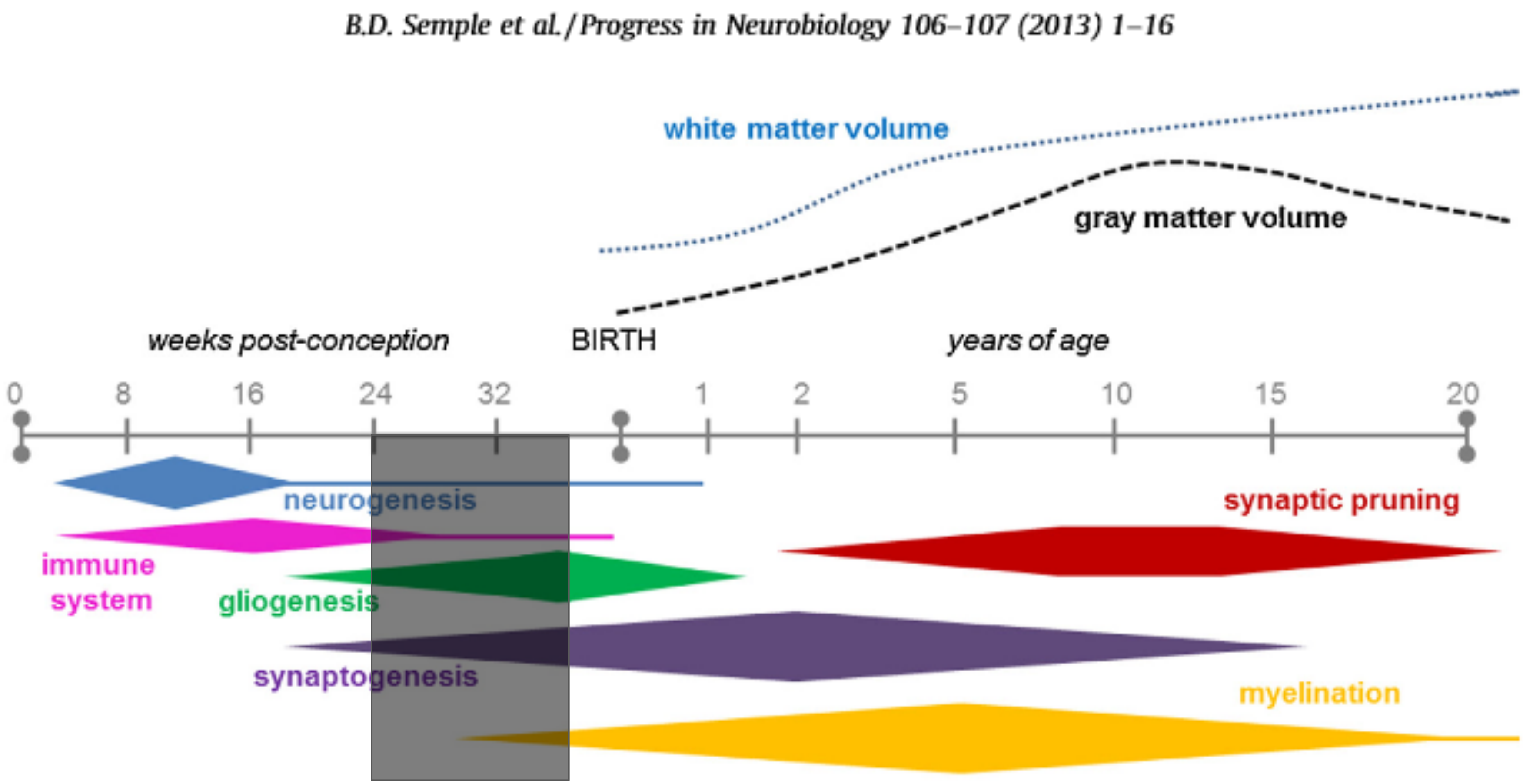

Preterm

Extremely Preterm: <28weeks

Very Preterm: 28-32 weeks

Mod to Late Preterm: 32 – <37 weeks

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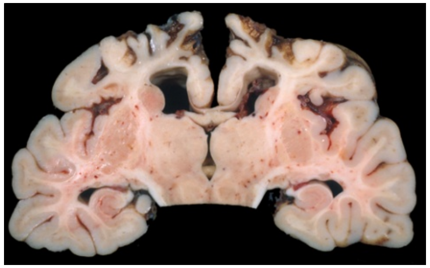
Preterm Brain Injury

Infection

Hypoxia-Ischemia

Preterm Birth
1/8 deliveries

Encephalopathy of prematurity
(23 – 36 weeks GA)



PVL
Necrosis of white matter

GM/IVH

Preterm Brain Injury

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Infection

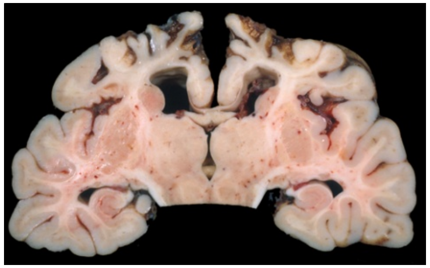
Hypoxia-Ischemia

Preterm Birth
1/8 deliveries

Encephalopathy of prematurity
(23 – 36 weeks GA)

Vulnerable cell type: Oligodendrocytes (precursors)
Injury: grey matter dys-maturation
Diffuse cortical white matter injury

Severe: periventricular leukomalacia
Germinal matrix/intraventricular haemorrhage



PVL
Necrosis of white matter

GM/IVH

Outcome: dependant on severity
CP, visual dysfunction, hearing impairments, cognitive & intellectual disabilities

Treatment: None

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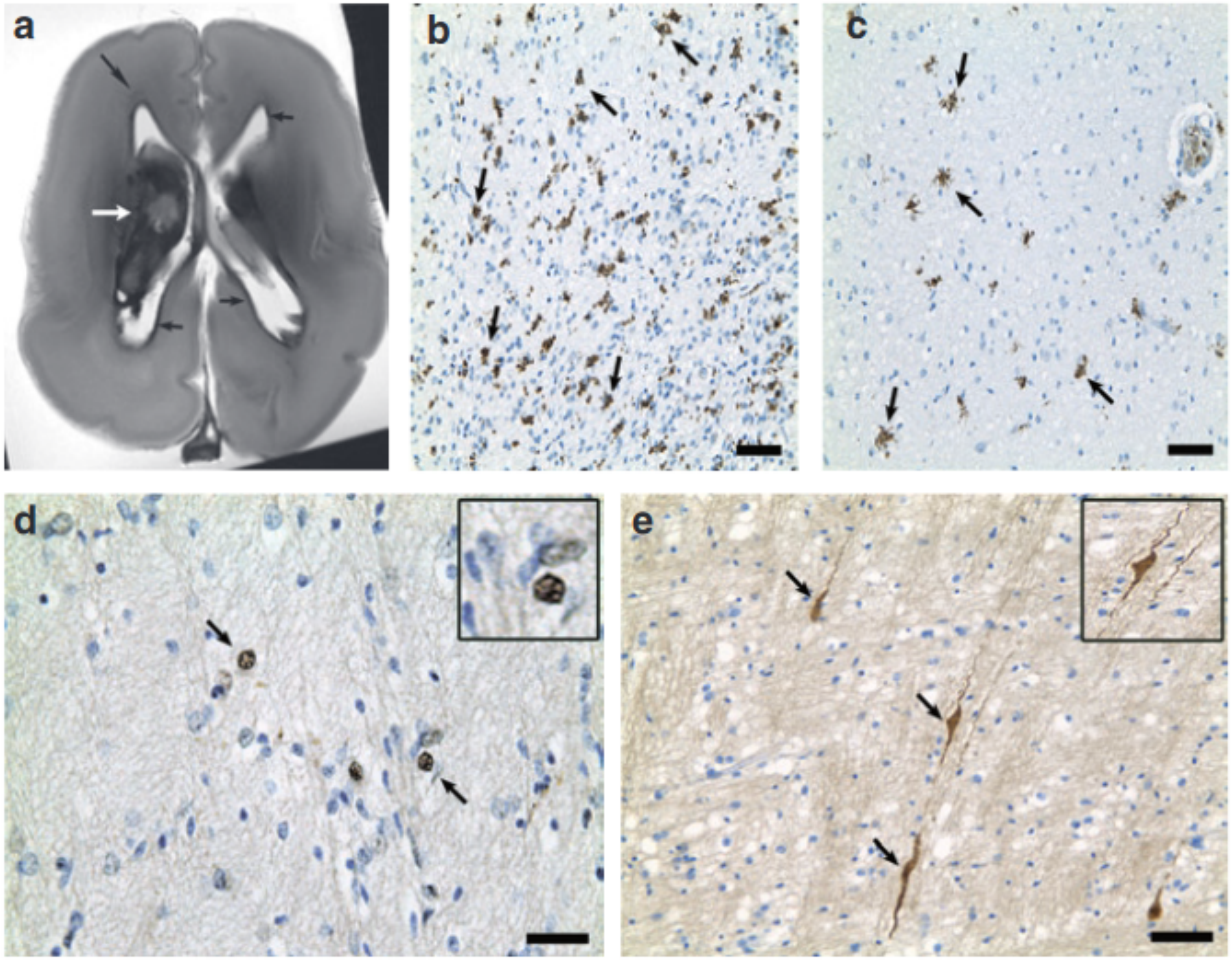
Cell death in preterm GM/IVH ($28^{+6/7}$ PMA)



Cell apoptosis
Caspase-3 +ve cells in Periventricular White Matter

Axonal Injury
Amyloid precursor protein +ve cells in Periventricular White Matter

How to model preterm brain injury?

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Clinical presentation

Sub-clinical infections

E.Coli

Staph E.

Chorioamnionitis
- preterm birth
- brain injury

Inflammatory responses or non-specific inflammation
- Hypoxia-ischemia
- Preterm birth
- Ventilation

What type of infection/inflammatory response?

Gram negative bacteria: LPS

Staph E, Staph A

Viral: Poly I:C

Moderate Systemic Inflammation: IL-1β

When?

Prenatal

Post natal

Type?

Acute
Repeated
Chronic

Species?

Mouse/Rat
Rabbit
Sheep

What are we trying to understand?

Response of microglia
Resident phagocytic/immune cells
Activation: beneficial and detrimental

Response of oligodendrocytes
Cell death?
Dysmaturation?

Effect of infection on:
Preterm ventilation
Prior to hypoxia-ischemia

Brain Injury

Term Infants
Hypoxic-Ischemic Encephalopathy

Preterm Infants
Preterm birth
Inflammation and /or
Hypoxia-Ischemia

Perinatal Brain Injury

Perinatal Stroke

Human neurodevelopment: Term

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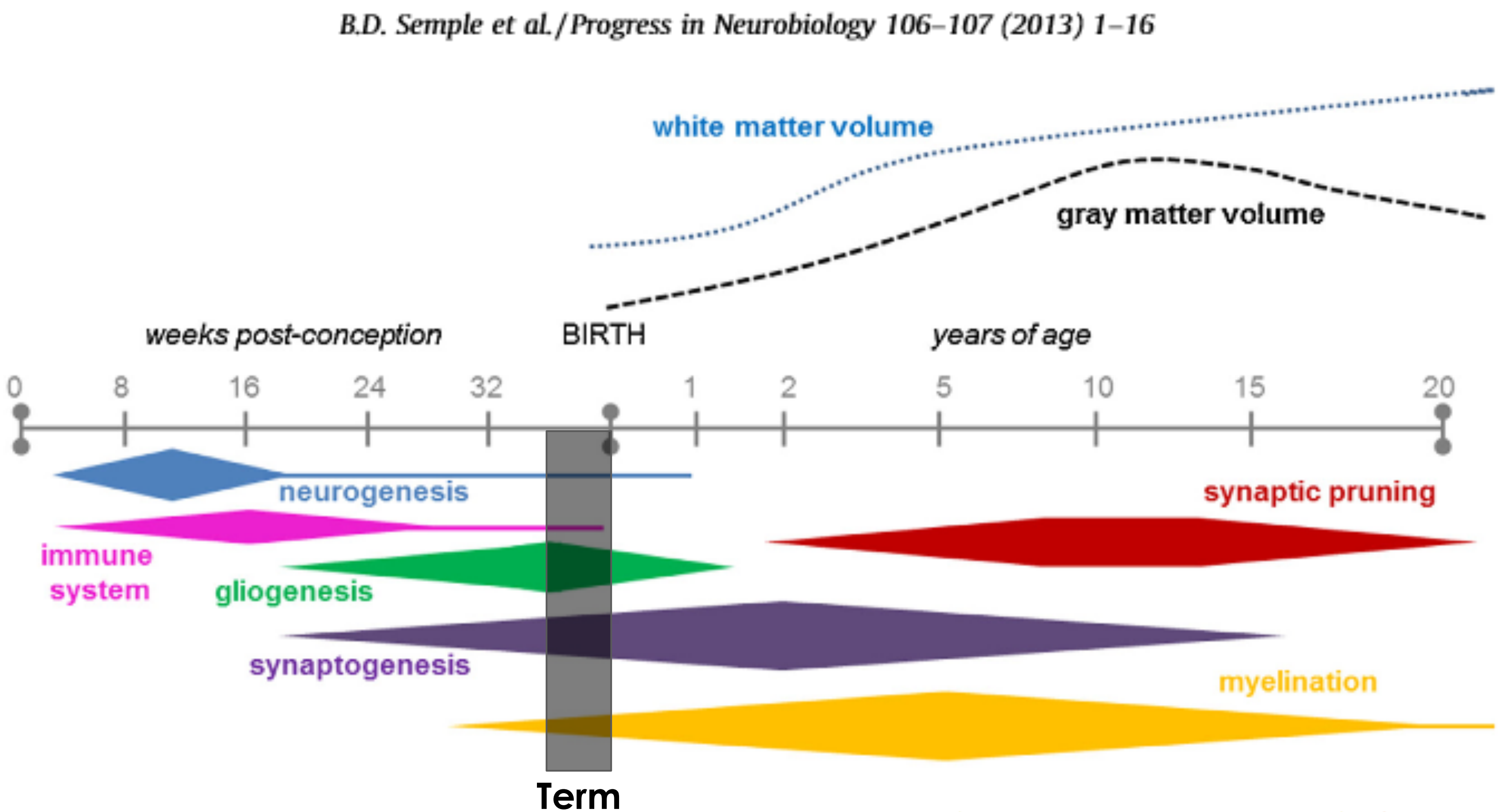

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Table 1
Summary of key developmental processes across comparable ages in humans and rodents.

Human	Rodent	Developmental milestones
23–32 wk gestation (pre-term infant)	pnd 1–3	Oligodendrocyte maturation state changes—pre-dominance of mitotically active pre-OLs[a]. Immune system development. Establishment of the blood-brain barrier.
36–40 wk gestation (term infant)	pnd 7–10	Peak brain growth spurt. Peak in gliogenesis. Increasing axonal and dendritic density. Oligodendrocyte maturation state changes–switch to a pre-dominance of immature OLs. Consolidation of the immune system.

Semple et al., 2013

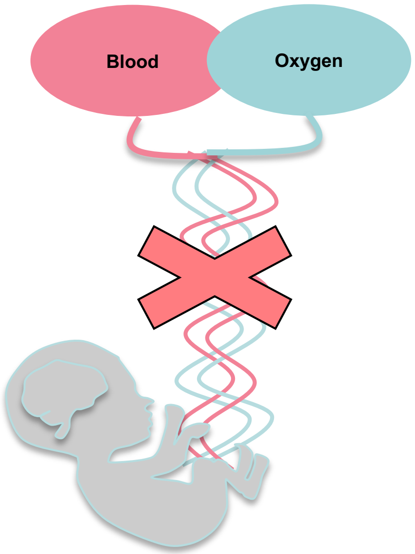
Perinatal Brain Injury

Birth Asphyxia

- Impaired blood flow to the fetus
- Premature rupture of membranes
- Problems with umbilical cord during labour
- High/Low maternal blood pressure

Infection

Hypoxic-Ischemic Encephalopathy
(>36 weeks gestational age)
Incidence: 1/3000



Perinatal Brain Injury

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Birth Asphyxia

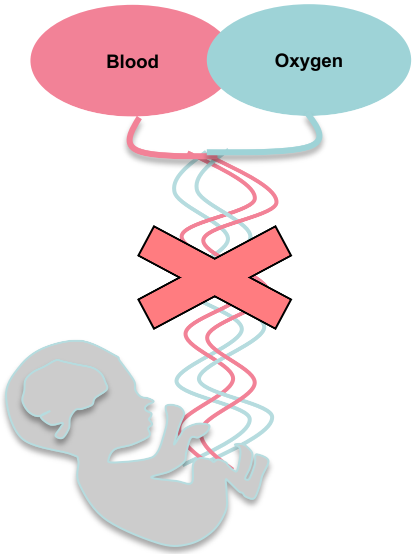
- Impaired blood flow to the fetus
- Premature rupture of membranes
- Problems with umbilical cord during labour
- High/Low maternal blood pressure

Infection

Hypoxic-Ischemic Encephalopathy

(>36 weeks gestational age)

Incidence: 1/3000



Vulnerable cell type: Neurons
Injury: Cortical
Diffuse white matter injury

Outcome: dependant on severity
CP, seizures, cognitive and intellectual disabilites

Treatment: Therapeutic Hypothermia
Need to treat 1 in 8/9 babies
Must be initiated within 6h

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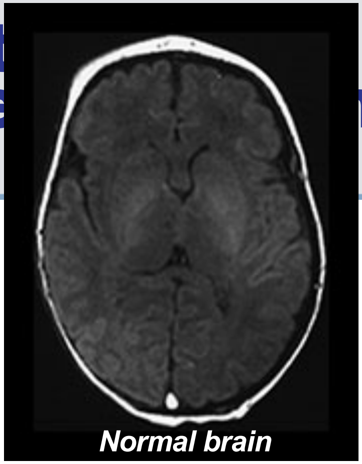
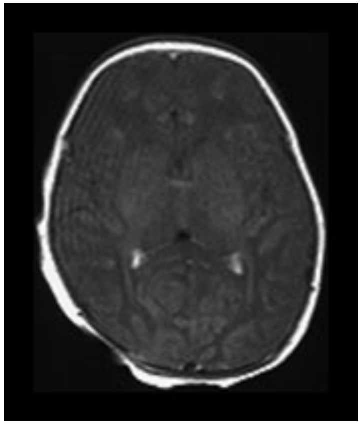
Term brain injury


Normal brain

- Hypoxic-Ischemic Encephalopathy
  - Brain injury due to asphyxia
  - Mature neonates (>37 wks) distressed prior to delivery
  - Severe acute asphyxia: associated with lesions in the basal ganglia, thalami, brain stem, hippocampus
  - Chronic, possibly repetitive insult: abnormalities within the cortex and white matter

- Early MRI findings (up to 1 wk following birth)
  - Brain swelling
  - Brainstem lesions
  - Loss of grey/white matter differentiation
  - Abnormal signal intensities in caudate, thalamus, internal capsule


Brain swelling

Additional information: http://www.mrineonatalbrain.com

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The Hypoxic-Ischemic Brain Injury Model: Rice-Vannucci

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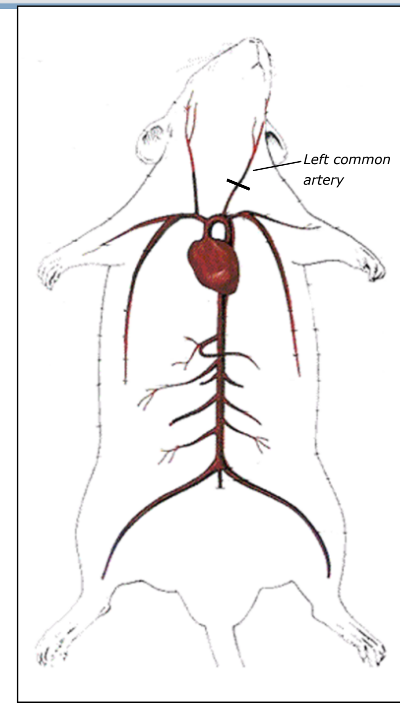
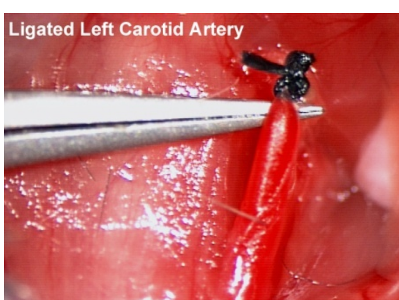


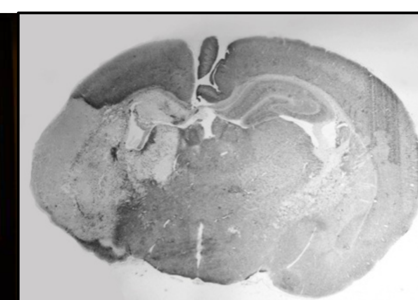
Mice

- PND 9
- Permanent ligation of the left common carotid artery
- Chamber with 10% O_2 at 36˚C
- Hypoxia: 50 or 60 minutes

Rat

- PND 7
- Permanent ligation of the left common carotid artery
- Chamber with 7.8% O_2 at 36˚C
- Hypoxia: 60 minutes





(Rice et al., (1981) Ann Neurol; Hedtjärn et al., (2001) J Neurosci)

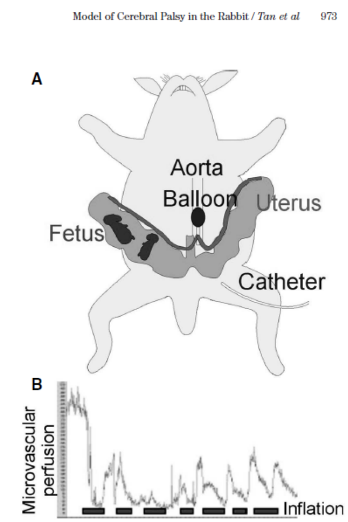
Rabbit model of acute placental insufficiency

- Gestational Length: ~32 days (NZ White Rabbit)
- Perinatal brain developers

- E22 (preterm ~70%) or E29 (term ~90%) Kits (embryonic day)
  - Uterine ischemia (through femoral artery catheter)
  - MRI analysis

    ↕
  - CP phenotype

    ↕
  - Histology
    - WMI: internal capsule
    - Microgliosis: BG and Thalamus



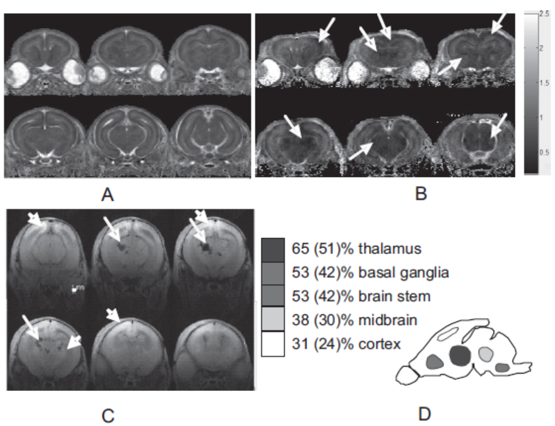
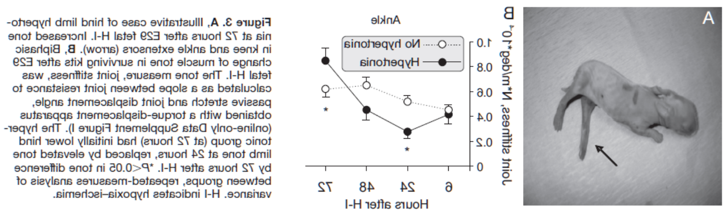


Figure 4. Diffusion weighted images 24 hours after H-I in E29 brain on showing no injury in **A** and severe injury in **B**, in areas of cortex, basal ganglia, thalamus, midbrain, brain stem (arrows indicates areas with abnormally low ADC less 0.7 μm^2/ms). Color map bar units are μm^2/ms. **C**, T2-weighted images 72 hours after H-I in E29 brain (n=19 kits) show ischemic regions (arrowheads, bright image) and hemorrhagic lesions (long arrows, black image) in thalamus and cortex. **D**, The frequency of injury is depicted as a proportion of all kits with any injury on MRI and a proportion of all studied kits after H-I in parenthesis.



Tan et al., 2005 J Child Neuro; Drobyshevsky et al., 2012 Stroke

SUMMARY

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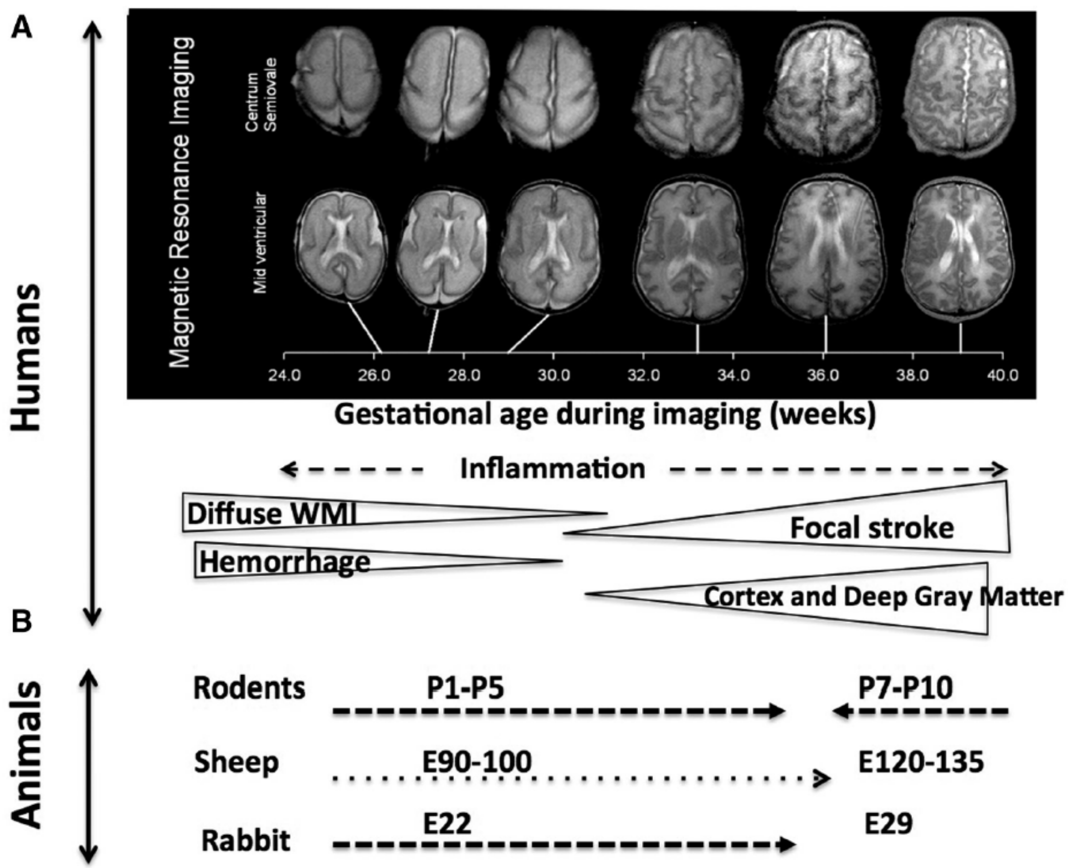

Figure. Age-dependent preterm and at-term brain injury patterns. **A**, Magnetic resonance imaging–delineated brain maturation during 24 to 40 weeks of gestation (reprinted from Kapellou et al.[2] Copyright ©2006, The Authors [see: http://creativecommons.org/licenses/by/4.0/]) and the predominant patterns of injury in humans. **B**, Appropriate age ranges in individual species to mimic ischemia-related preterm and at-term brain pathophysiology.

Mallard & Vexler 2015 Stroke

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Pre-Clinical Imaging

Why do pre-clinical MRI?

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Courtesy of T. Roberts

1. Reproduce diseases in a controlled, reproducible manner
   - Surgically / Genetically

2. MRI is non-invasive (i.e: unlike histology), which facilitates longitudinal studies

3. Drug and biomarker development
   - A biomarker is*: "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention, including therapeutic interventions"

4. MRI sequence/technique development

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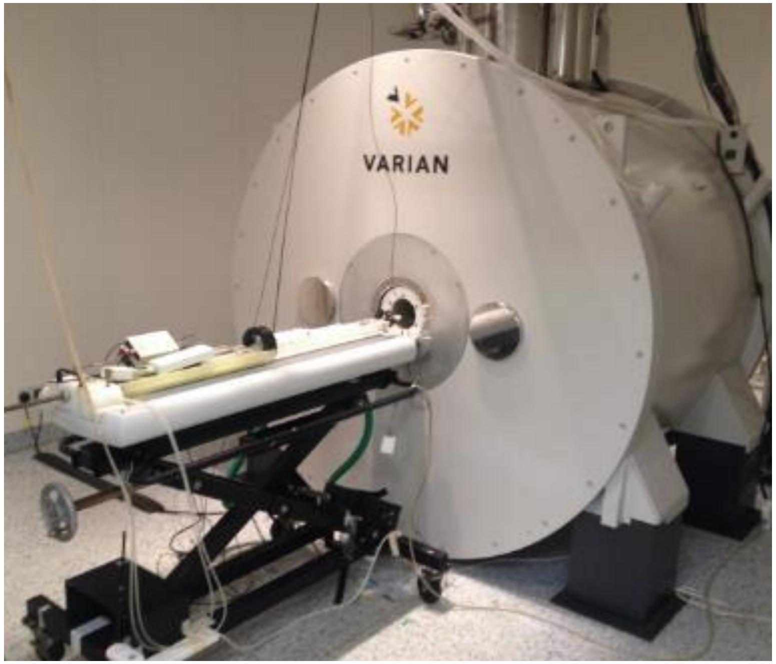
*Nature Reviews Clinical Oncology 14, 169–186 (2017)

Pre-clinical MRI scanners

Courtesy of T. Roberts

- 9.4T MRI scanner



- 1.0T 'Benchtop' Scanner

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Pre-clinical MRI scanners

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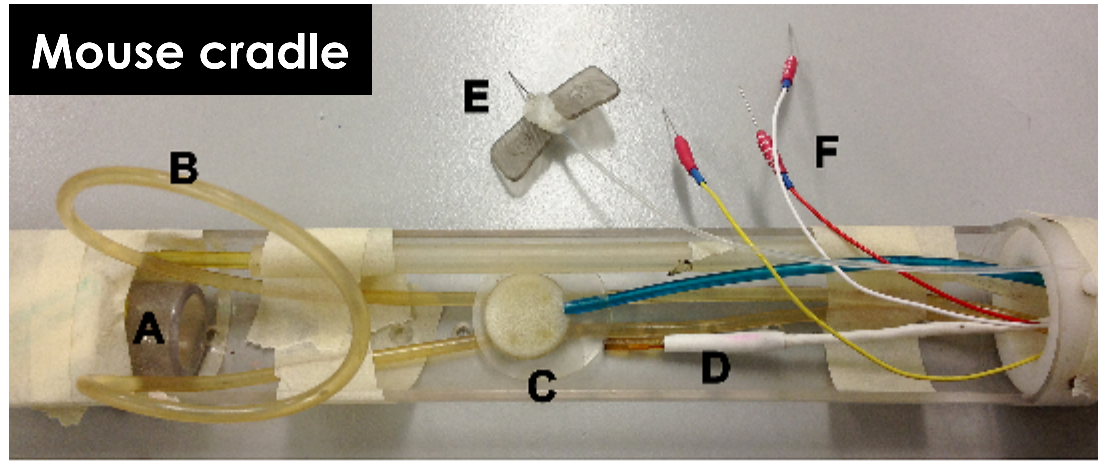
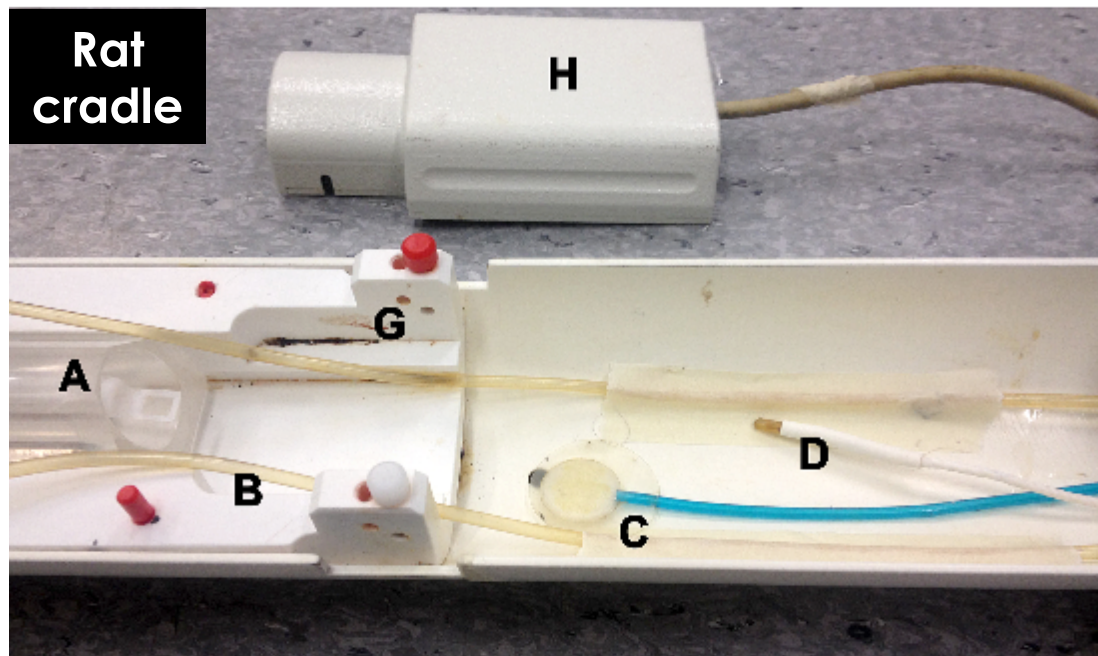
Courtesy of T. Roberts

Animal imaging cradles

A. Anaesthetic nose cone
B. Water heating tubes
C. Respiration pad
D. Temperature probe
E. Contrast agent infusion line
F. ECG electrodes
G. Ear bars for head stability
H. Multi-channel head coil





Pre-clinical MRI scanners

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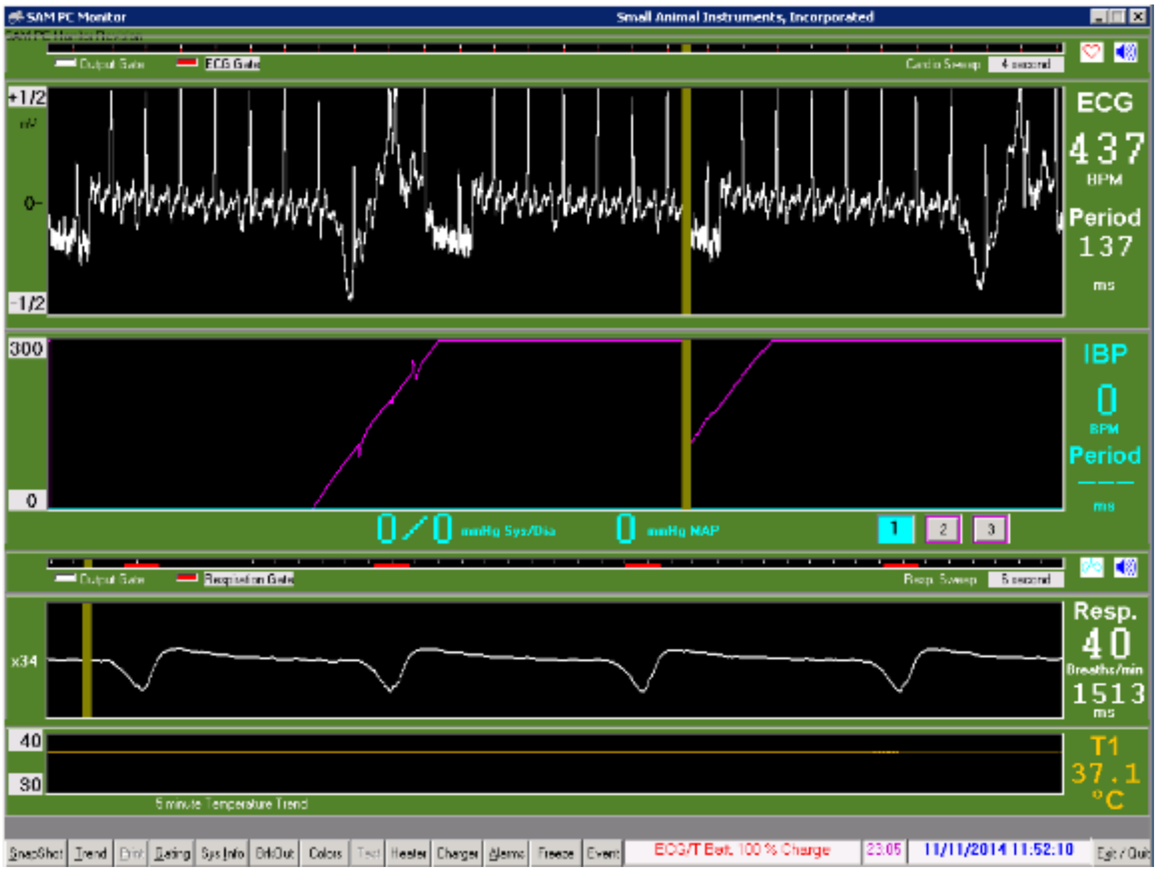
Courtesy of T. Roberts

Animal monitoring software



- ECG:
  - Mice = ~400-600bpm
  - Rats = ~330-450bpm
  - Adult humans = ~ 50-100bpm
  - Adult fetal = ~ 100-150bpm
- Blood pressure (not usually required)
- Respiration
- Temperature

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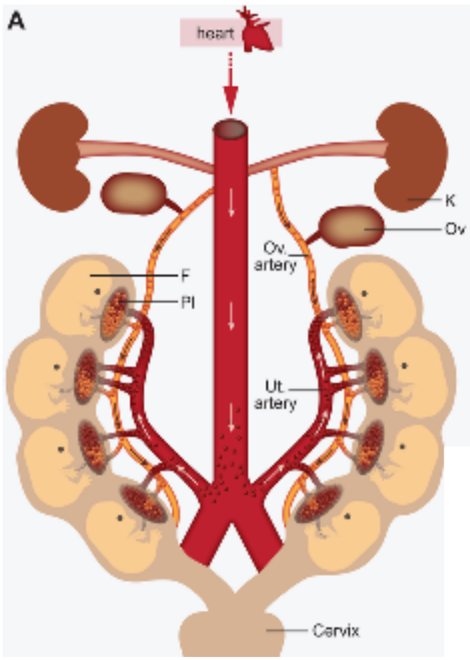
Practical Considerations

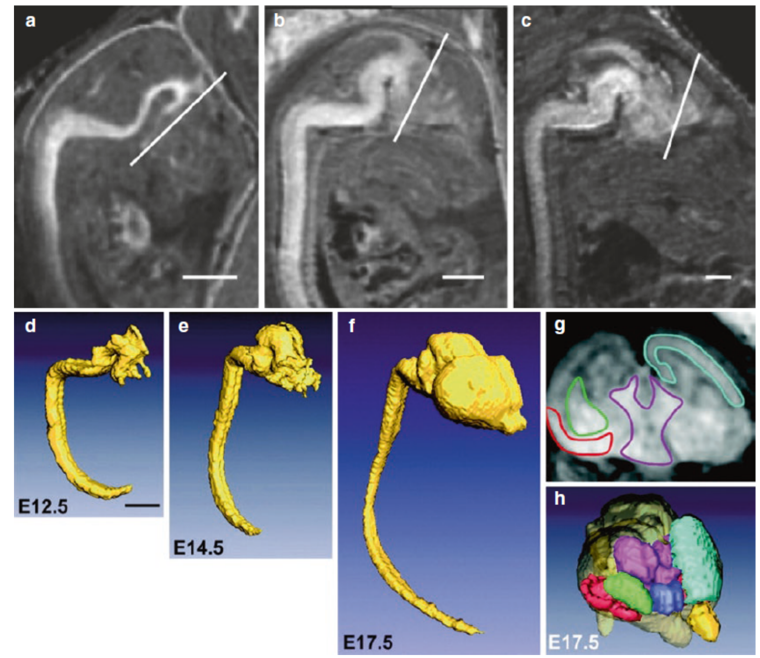
- In utero vs. postnatal?
- Living or post-mortem?
  - Living: need anaesthetic
  - Time? How long will the scan take?

In utero pre-clinical MRI

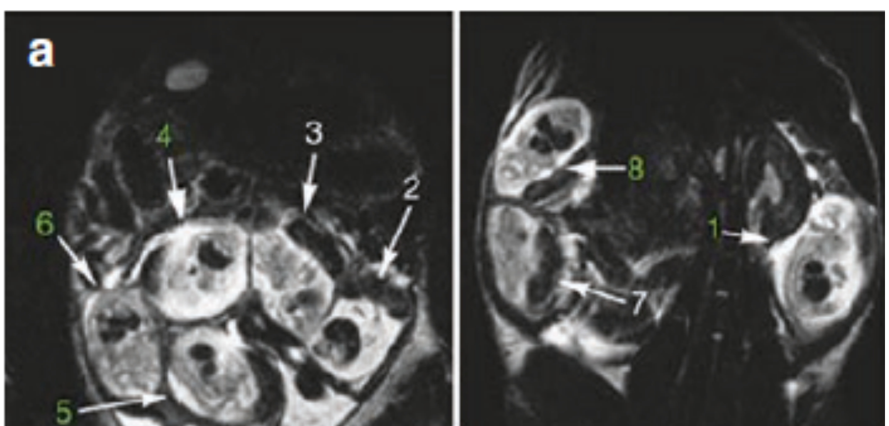
Courtesy of T. Roberts



Manganese contrast agent



Magn Reson Med. 2008;59(6):1320–8



Nat Med. 2007;13(4):498–503

- *In utero* imaging is very challenging:
  - Motion
  - Limited resolution/contrast

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Ex vivo pre-clinical MRI

Courtesy of T. Roberts

- Decide on animal model
- Decide embryo gestational age
  - Mouse pregnancy ~18-21 days

- Extract embryos and fixate (with PFA etc.)
- Place in sample holder

- Run MRI overnight (~12 hours) or at weekend (48 hours+)
  - Can achieve 50µm/pixel resolution (>1mm in humans)

Uses of Pre-Clinical Imaging

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- Injury progression
- Term brain injury in mice, at P9
- 11.7T Bruker;
- *in vivo* imaging, anesthetised, 12min
- Volumetric

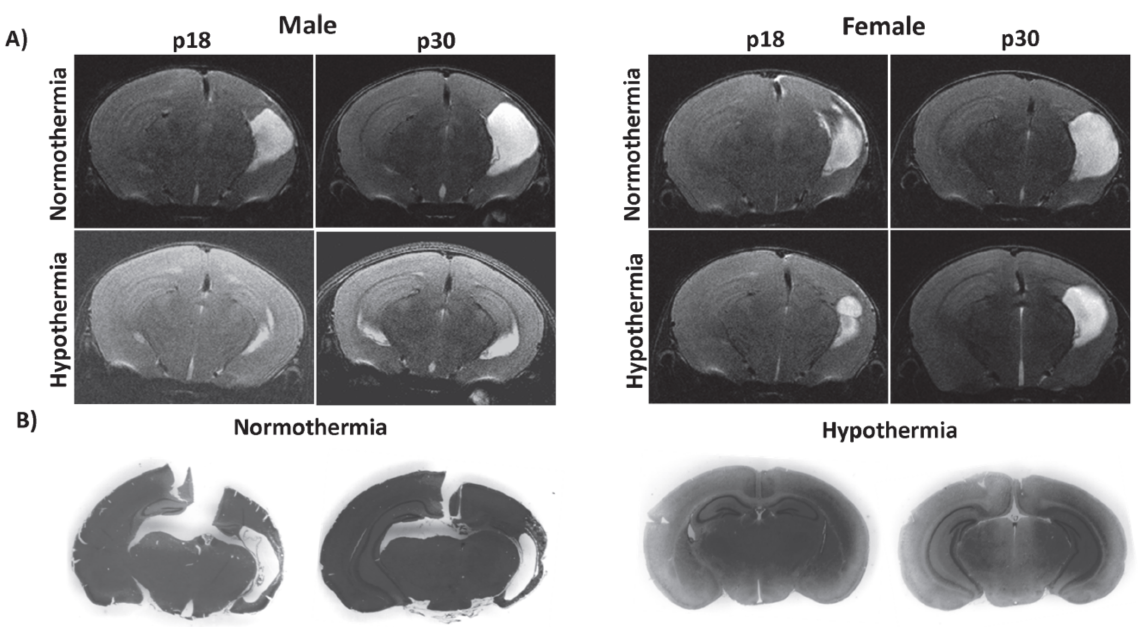

Fig 5. Representative MRI and gross histopathology at p18 and p30. A) Representative T2—weighted MR imaging animals at each time point, treatment, and sex. Images at p18 and p30 are from the same animal. The trend of mild to moderate, variable injury and neuroprotection particularly in females is demonstrated. **B)** Low power views H&E stained anterior and posterior sections from p30 male mice.

doi:10.1371/journal.pone.0118889.g005

Burnsed Et al., 2015

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NeuroImage 54 (2011) 80–89

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

ELSEVIER

An MRI-based atlas and database of the developing mouse brain

Nelson Chuang [a], Susumu Mori [a,e], Akira Yamamoto [a], Hangyi Jiang [a], Xin Ye [b], Xin Xu [a], Linda J. Richards [f,g], Jeremy Nathans [b,d], Michael I. Miller [c,h], Arthur W. Toga [i], Richard L. Sidman [j], Jiangyang Zhang [a,*]

- 11.7T Bruker;
- *Ex-vivo* imaging

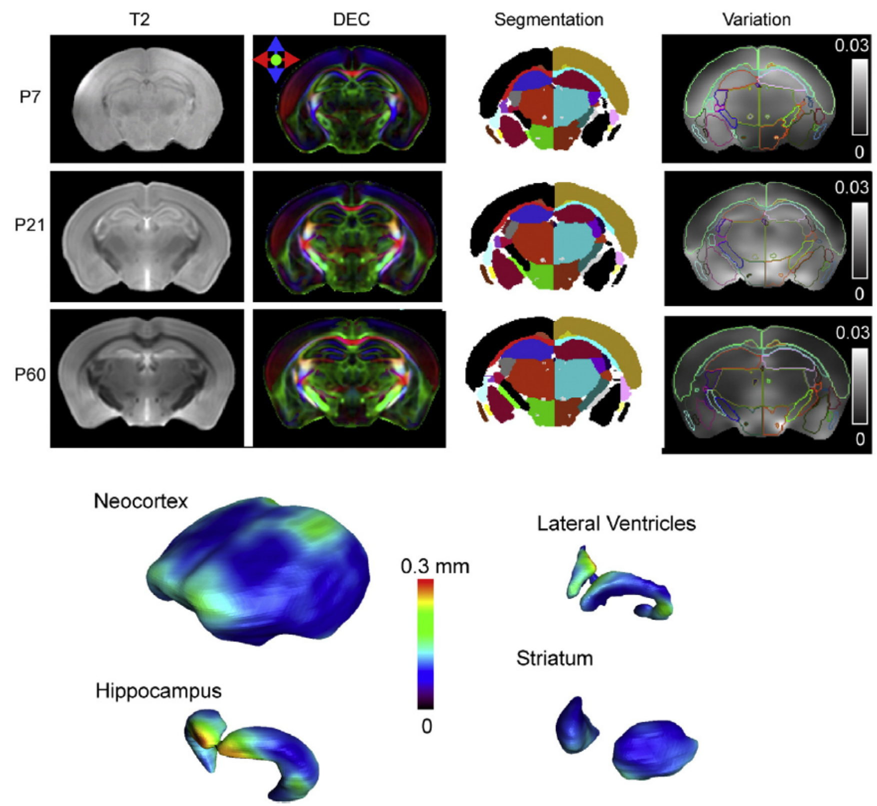

Fig. 6. Top: Population average T_2, directional encoded colormap (DEC) images of the P7, P21, and P60 mouse brain and maps of segmentation and maps of anatomical variation overlaid with outlines of structural segmentations. The unit in the variation maps is mm^2. Bottom: anatomical variations in the P60 mouse brain rendered on the surfaces of the neocortex, hippocampus, lateral ventricles, and striatum.

NeuroImage 125 (2016) 144–152

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

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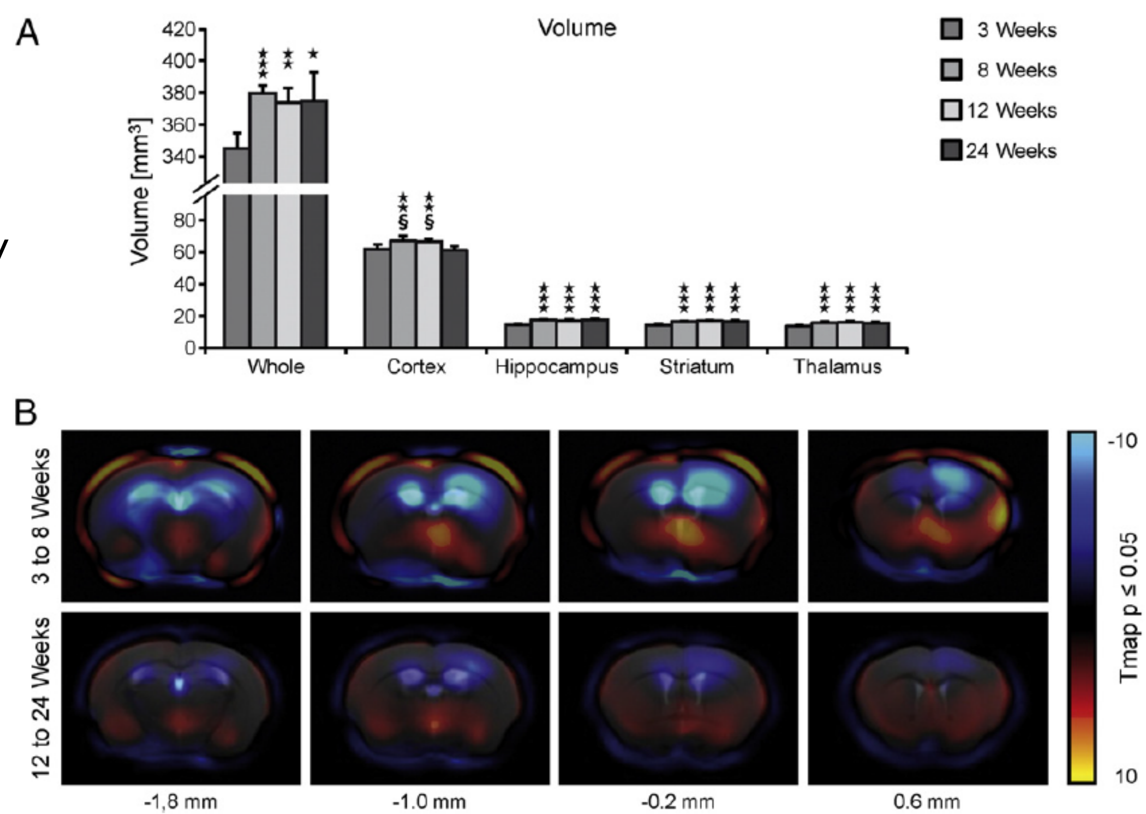
Morphological maturation of the mouse brain: An in vivo MRI and histology investigation

Luam Hammelrath [a], Siniša Škokić [a,b], Artem Khmelinskii [c,d], Andreas Hess [e], Noortje van der Knaap [a,f], Marius Staring [c], Boudewijn P.F. Lelieveldt [c,g], Dirk Wiedermann [a], Mathias Hoehn [a,c,d,*]

- In Vivo Imaging, 9.4T Bruker; [3, 8, 12, 24 weeks]
- Culled mice → histology
- Assessing cortex and white matter

L. Hammelrath et al. / NeuroImage 125 (2016) 144–152



MRI + Histology

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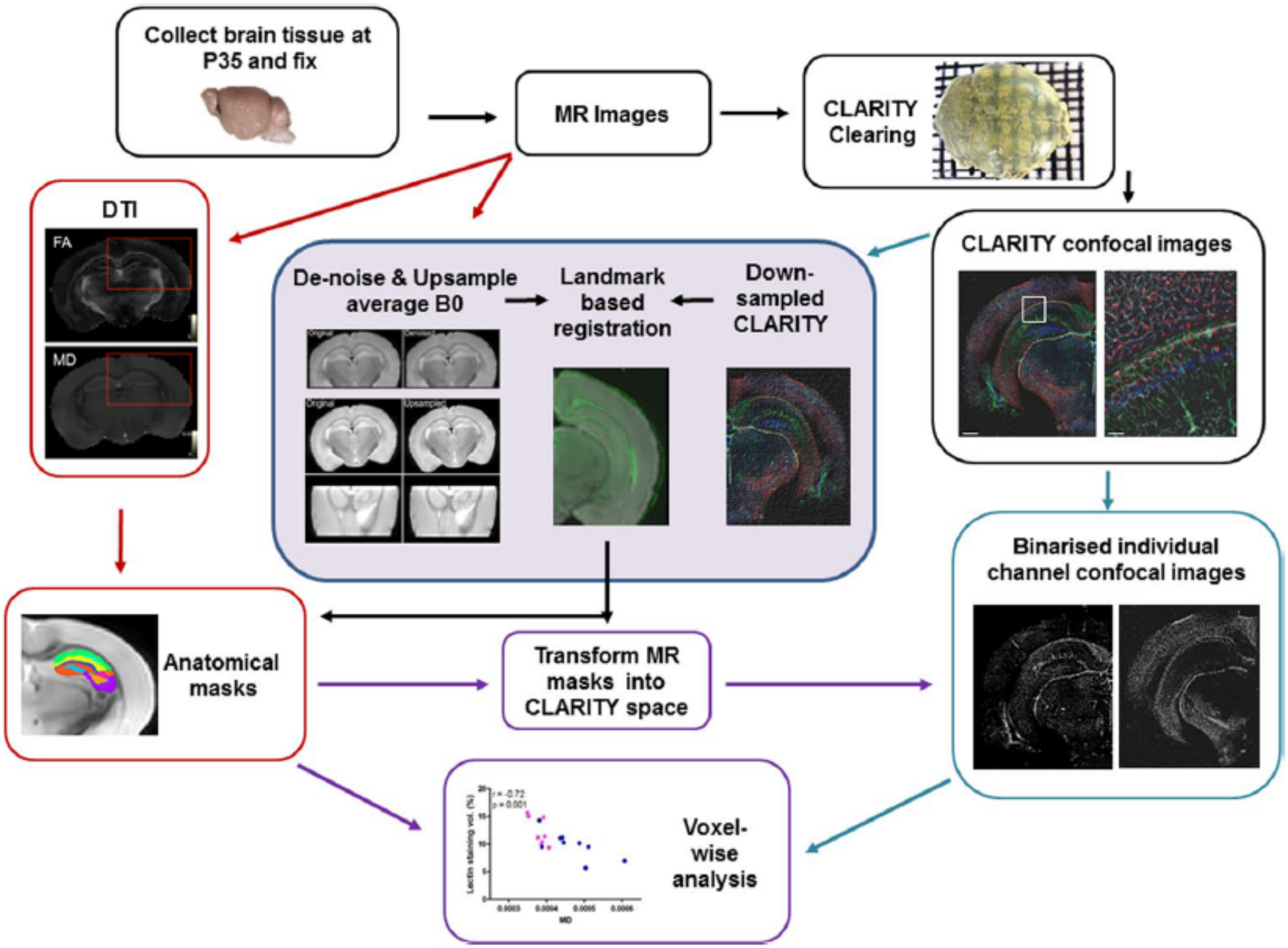

Stolp et al., 2018

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High Resolution Episcopic Microscopy (HREM)

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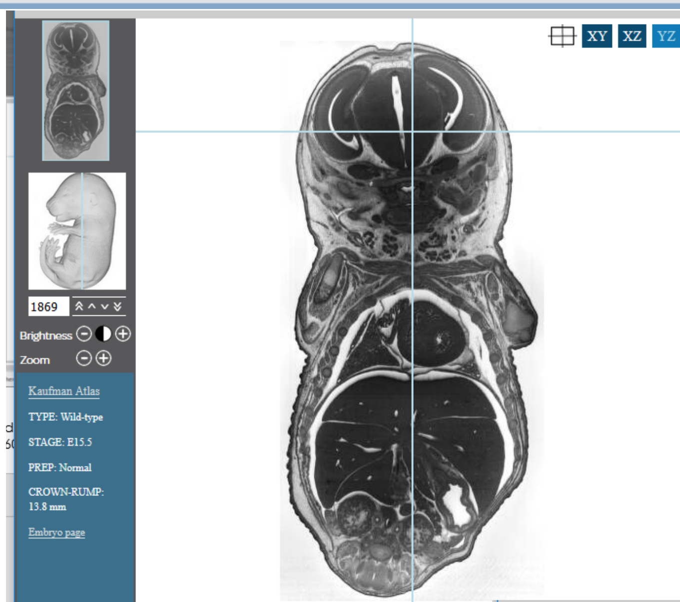

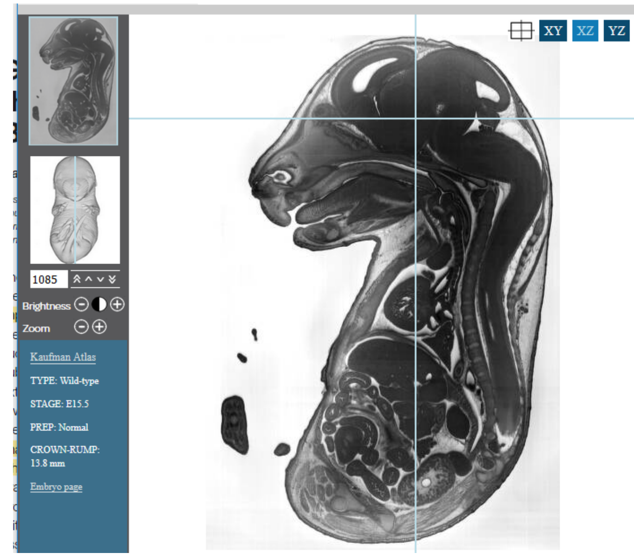

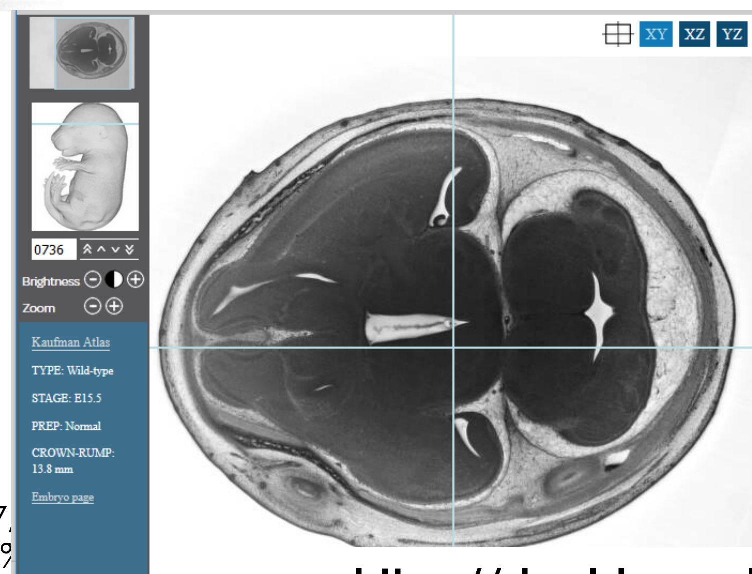

https://dmdd.org.uk/stacks/DMDD1957/ 957%3B1581%3B1%3B1.1%3B0.5%
3B0.5%3B0.16082698585418934%3Bfalse%

https://dmdd.org.uk/movies/DMDD1957/xz

Questions?