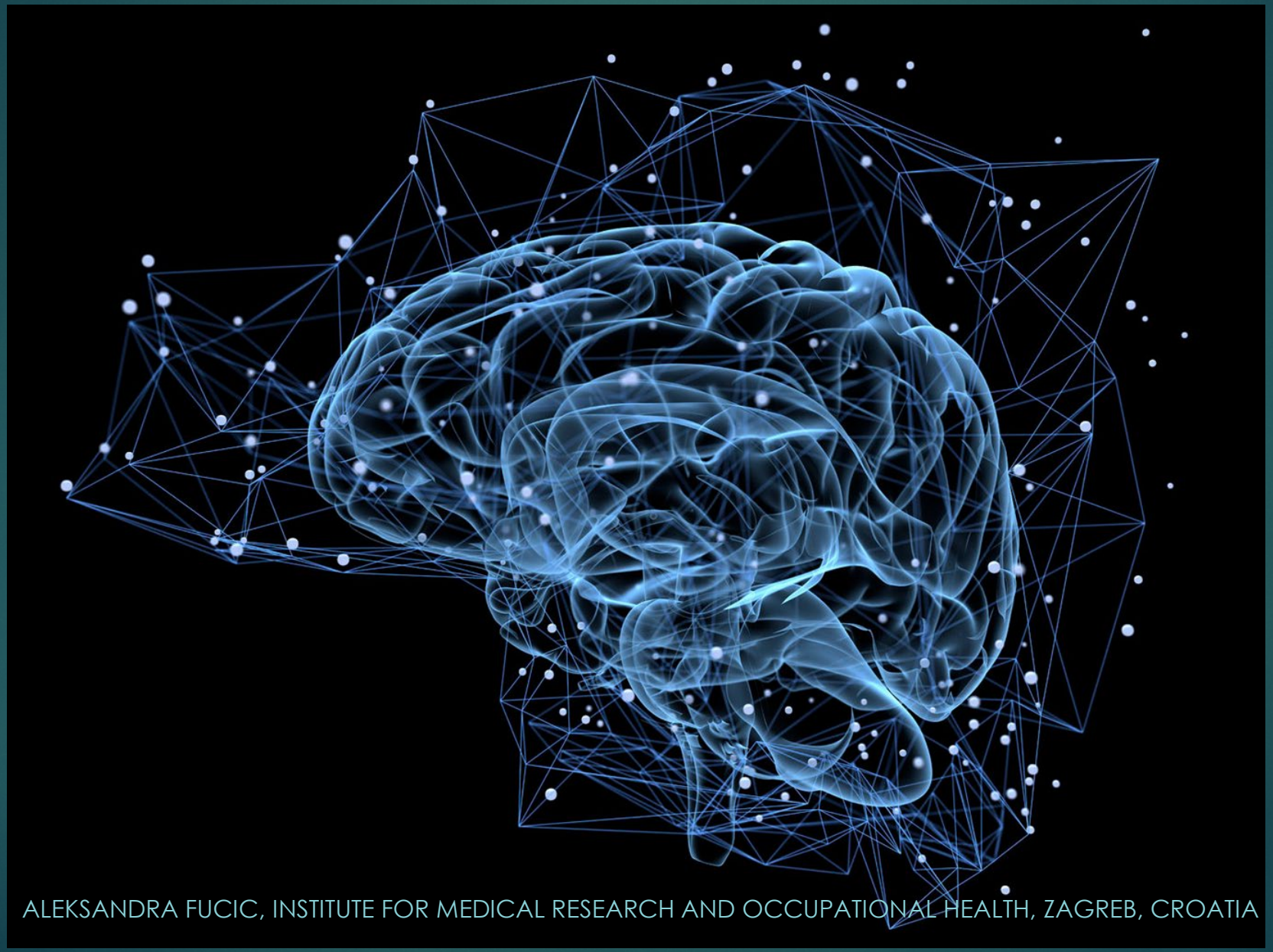
Development of the 4D program for simulation of human intrauterine frontal cortex development



ALEKSANDRA FUCIC, INSTITUTE FOR MEDICAL RESEARCH AND OCCUPATIONAL HEALTH, ZAGREB, CROATIA

BRAIN

Complex energy information system

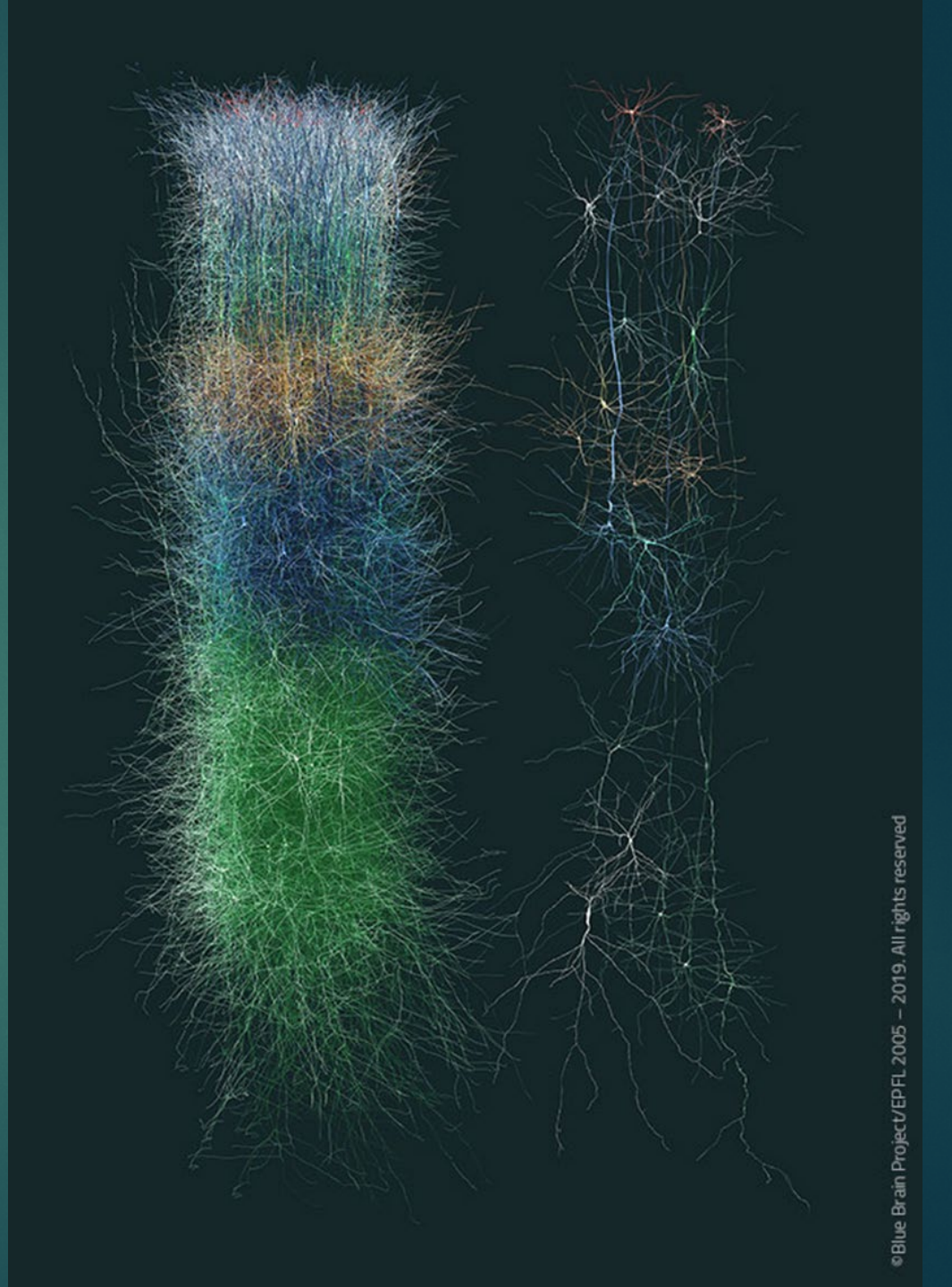
Neuroendocrine organ

- Neurotransmitters

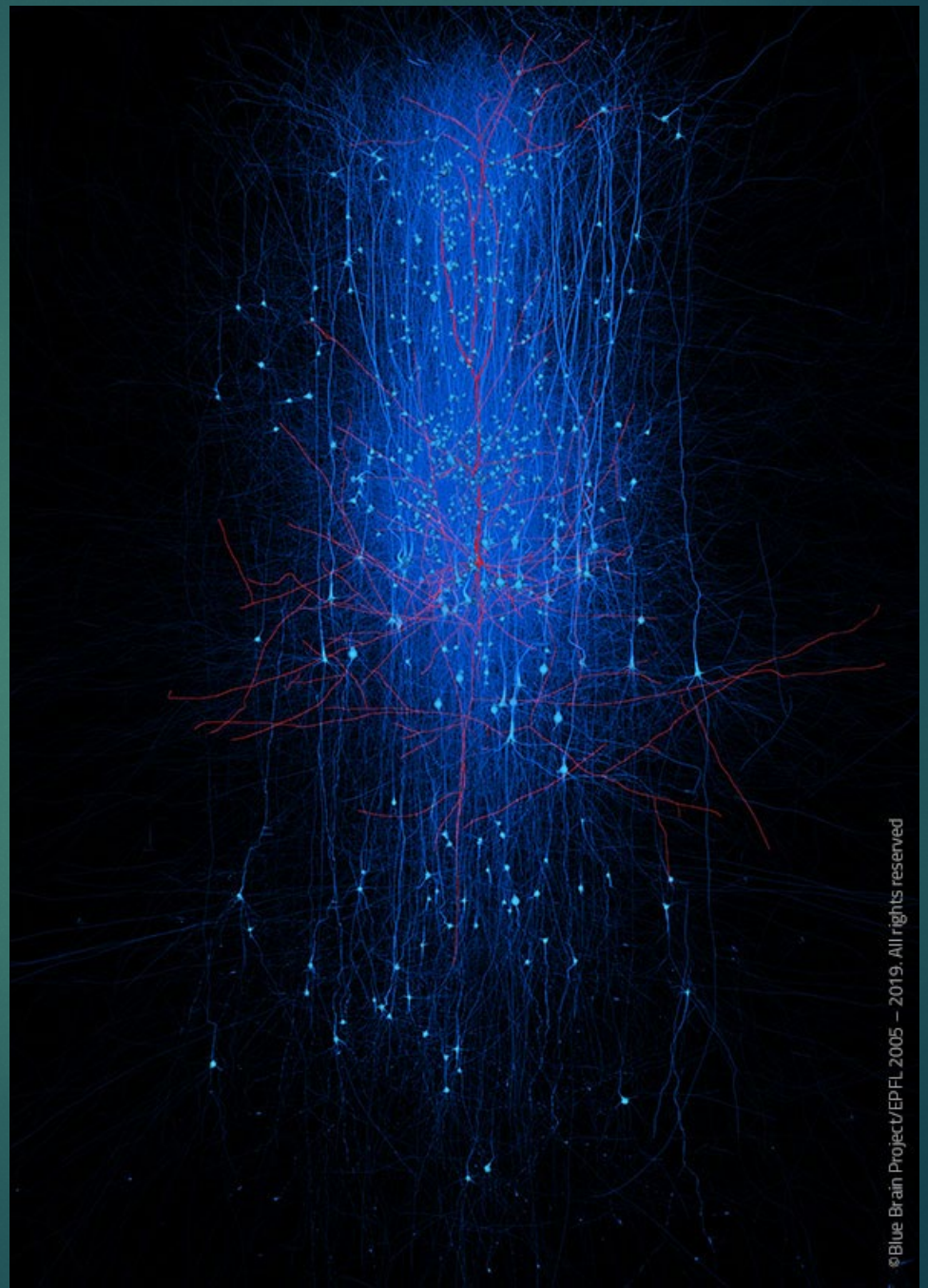
Neuromodulators:

- Neuropeptides
- Hormones

Diversity in cross talk and time of activity from msec to years



Neuropeptides facilitate activity of certain brain compartments- cross talk with estrogen

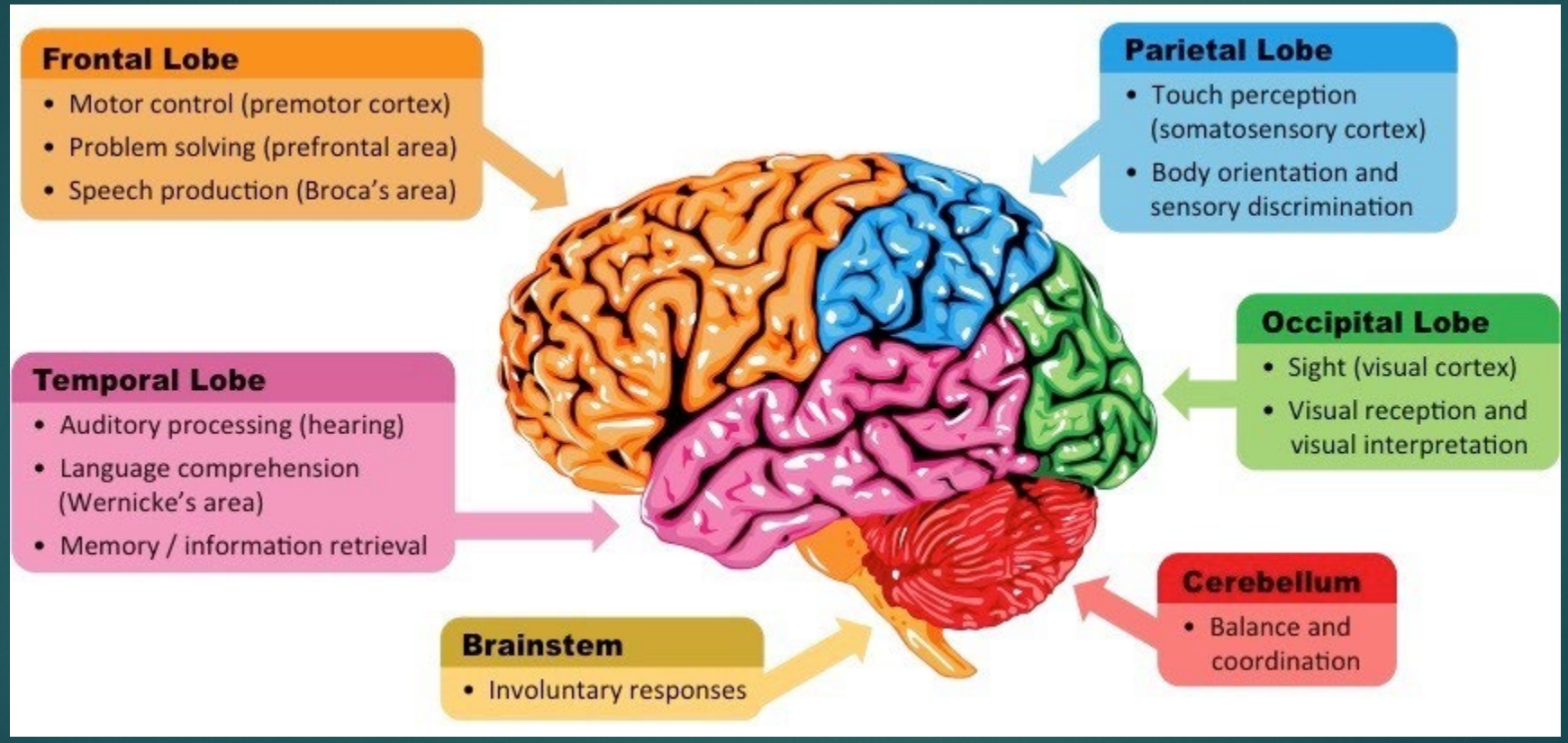


- Though we used to think of estrogen target tissues as mainly those with female reproductive functions, in the last decade we have discovered that every cell/tissue/organ system in the body responds to estrogens in some way

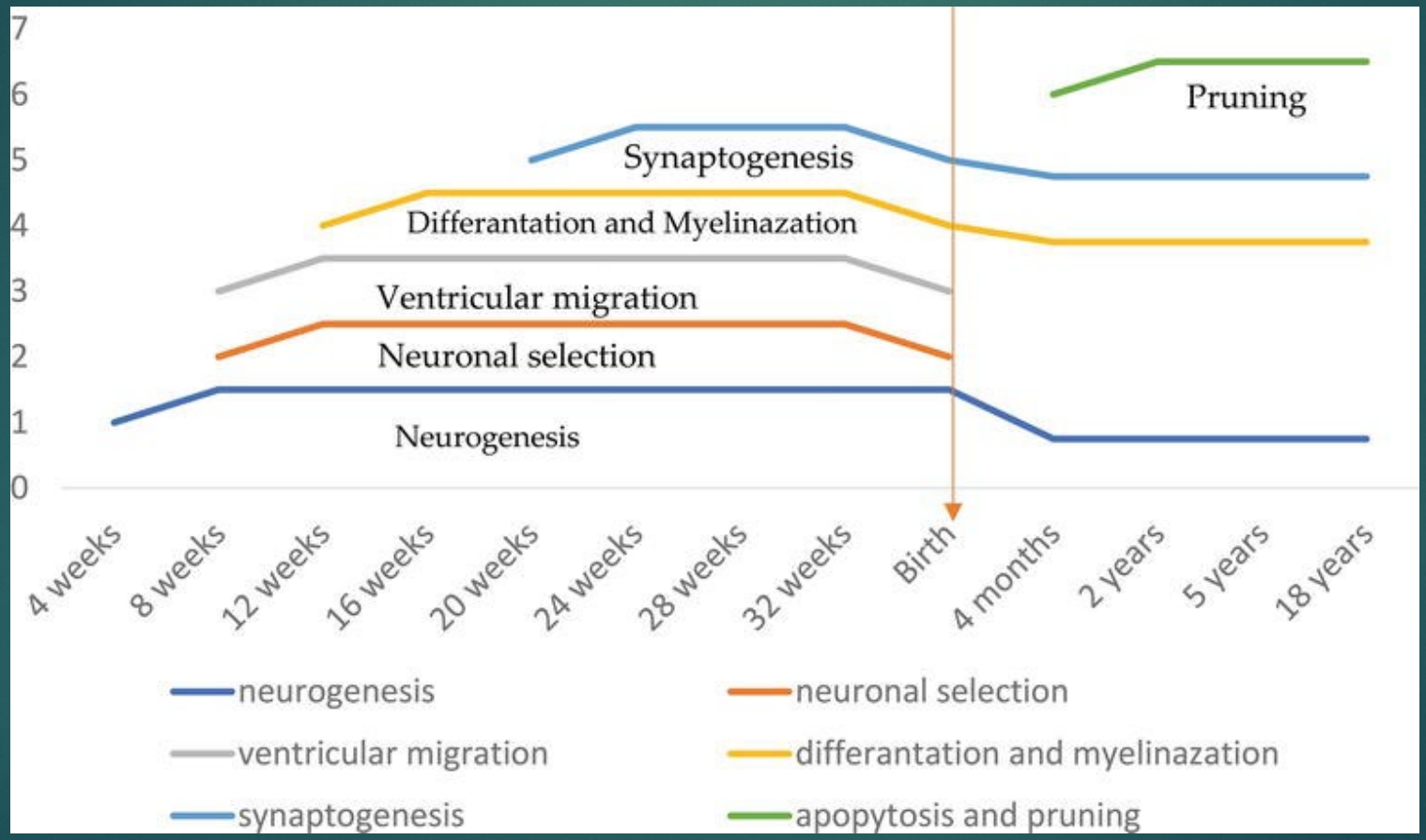
Estrogen

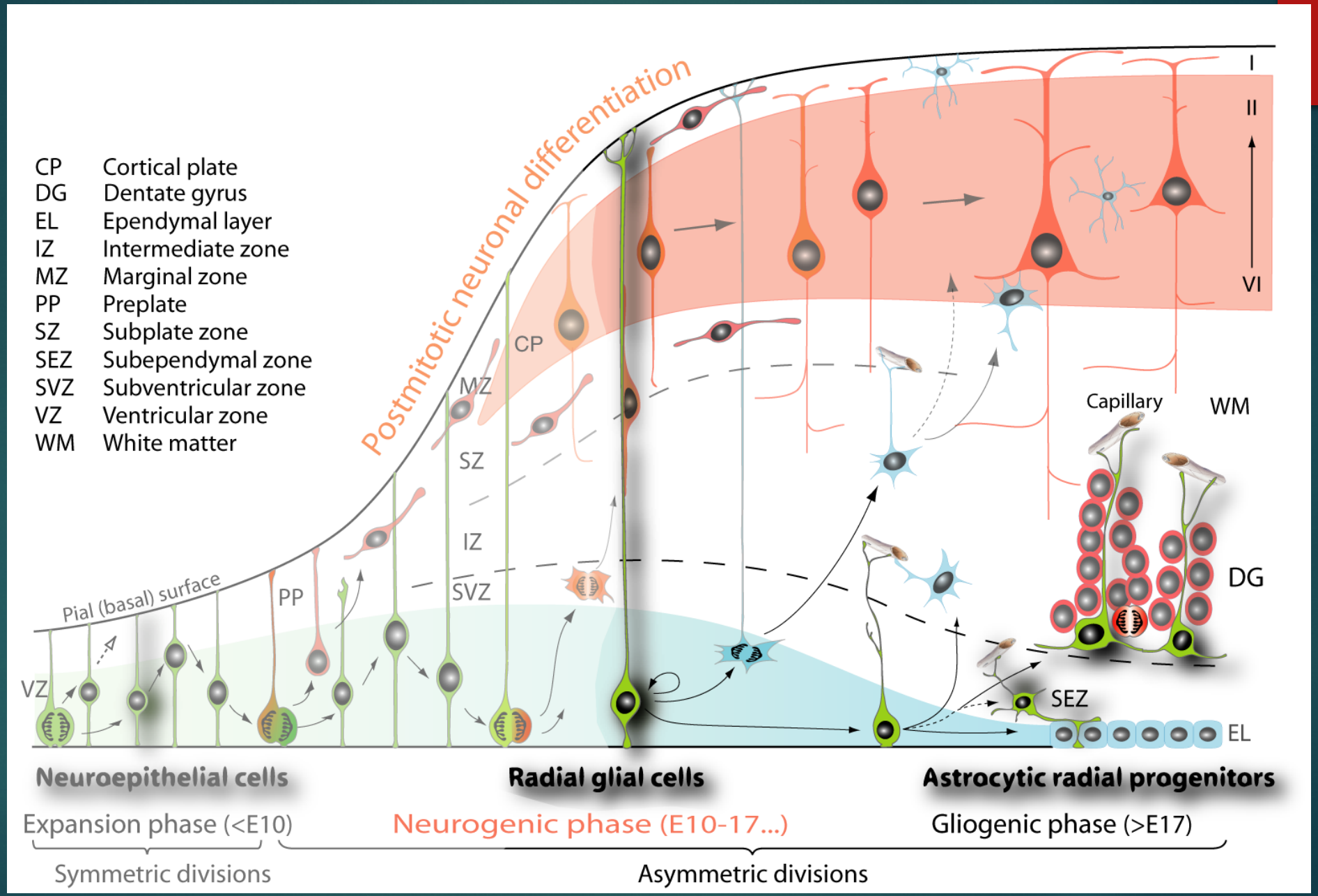
- Neuromodulator
- Hormone (intra-organ and on the level of organism)
- Paracrine
- Neurotransmitter

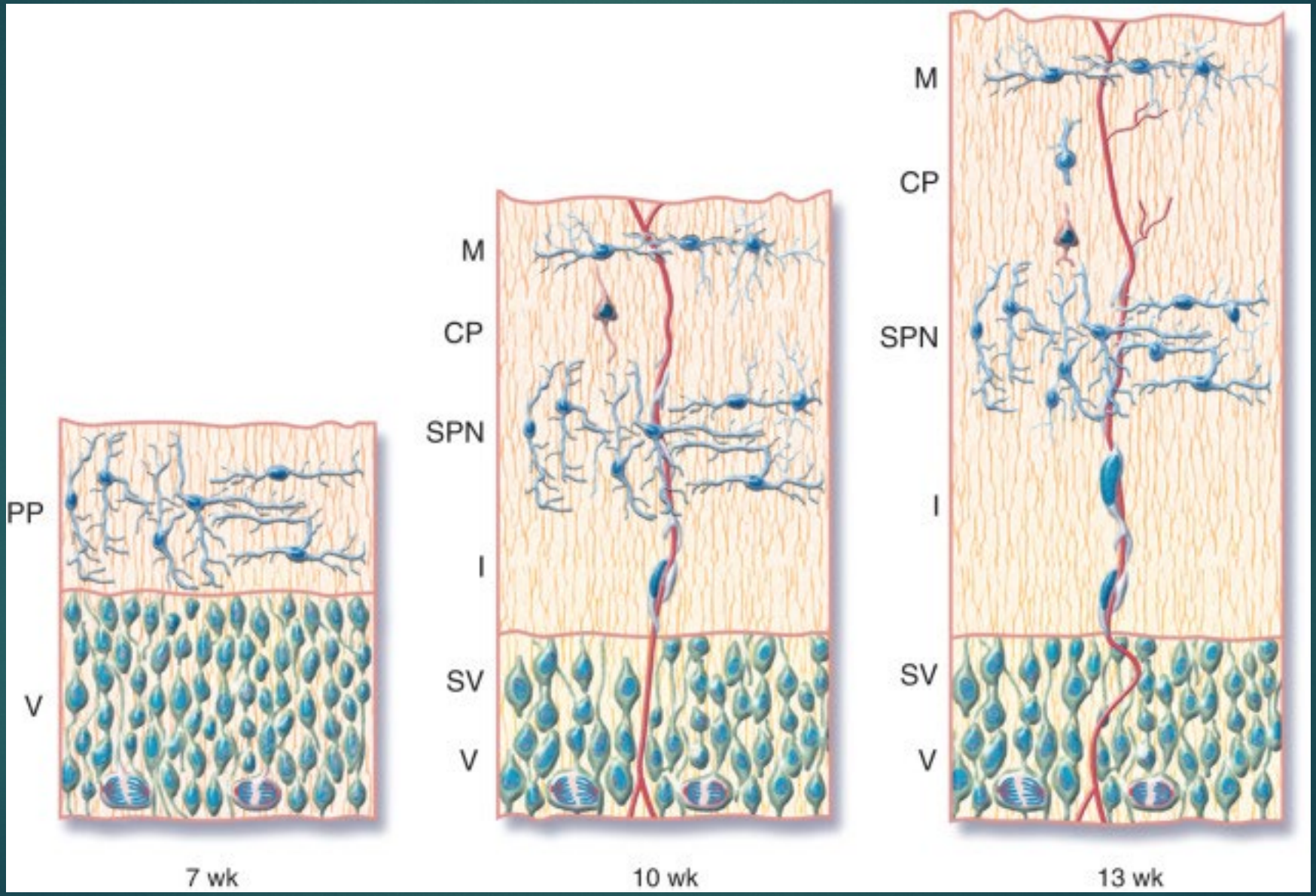
Frontal cortex



Human frontal cortex development




CP Cortical plate
DG Dentate gyrus
EL Ependymal layer
IZ Intermediate zone
MZ Marginal zone
PP Preplate
SZ Subplate zone
SEZ Subependymal zone
SVZ Subventricular zone
VZ Ventricular zone
WM White matter
Postmitotic neuronal differentiation
I
II
VI
CP
MZ
SZ
IZ
SVZ
PP
VZ
Pial (basal) surface
Capillary
WM
DG
SEZ
EL
Neuroepithelial cells
Radial glial cells
Astrocytic radial progenitors
Expansion phase (<E10)
Neurogenic phase (E10-17...)
Gliogenic phase (>E17)
Symmetric divisions
Asymmetric divisions


PP
V
7 wk
M
CP
SPN
I
SV
V
10 wk
M
CP
SPN
I
SV
V
13 wk

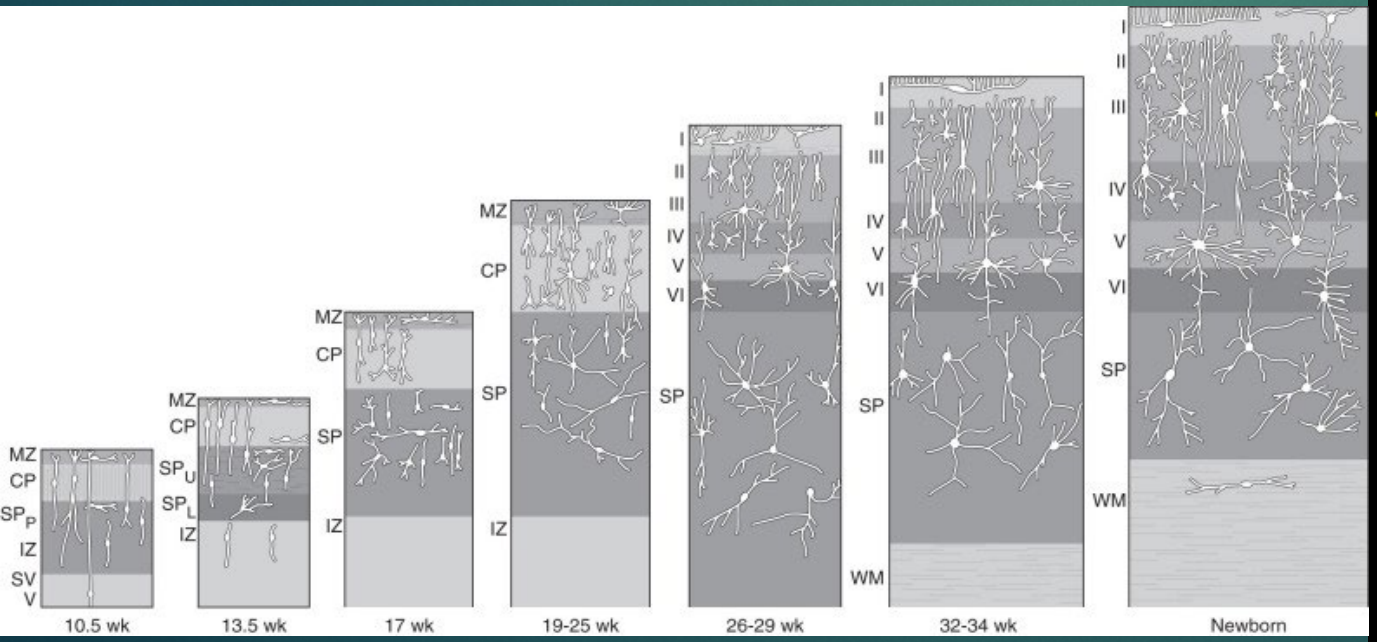
- https://www.youtube.com/watch?v=BJL_I783SBM

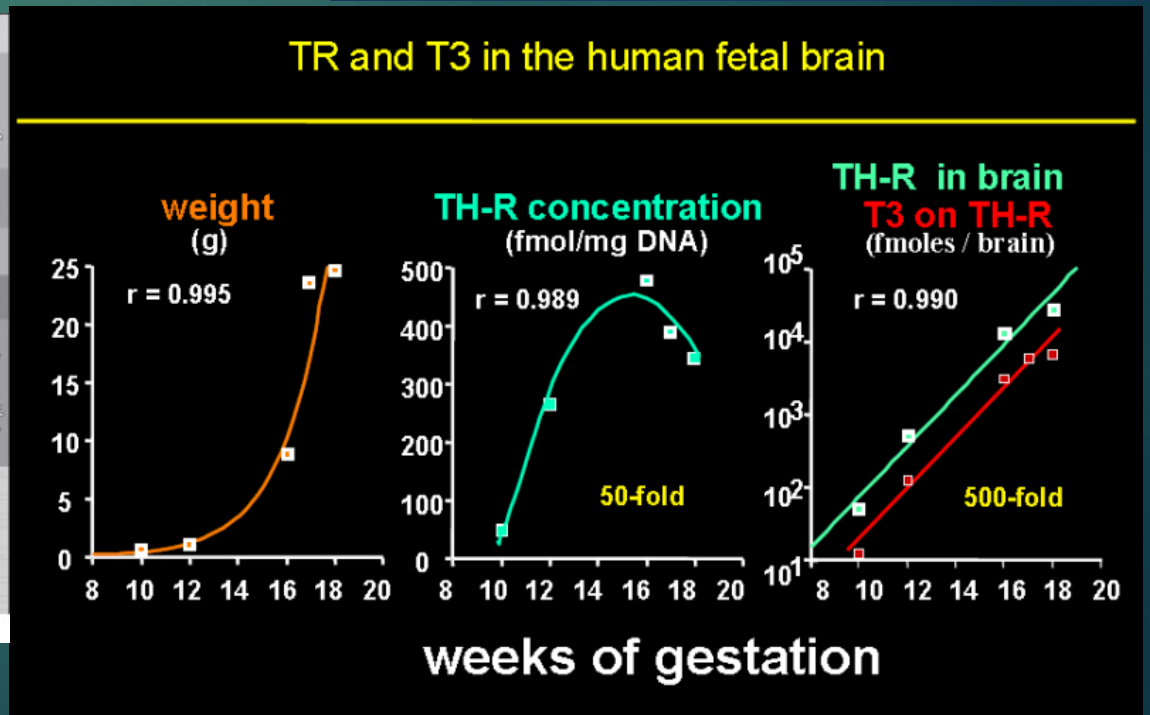
The most challenging period

- 16- 24 GW

MAJOR DEVELOPMENTAL EVENTS

Major development event	Peak occurrence
Dorsal induction	3rd – 4th wk prenatal
Ventral induction	5th – 6th wk prenatal
Neuronal proliferation and programmed cell death	2nd -4th mo prenatal
Neuronal migration	3rd – 5th mo prenatal
Neuronal differentiation and organization	
Synaptogenesis	6th mo – 3 yr
Initial pruning	3 – 5 yr
Secondary reorganization	Adolescence
Myelination	6th mo – 3 yr … 30 yr





During intrauterine development number of compounds from environment may cross placenta and disturb brain development

Disturbances on anatomical but most frequently on molecular level

Level of biological effects of environmental agents depend on

- Gestational age and corresponding metabolism of fetus
- Metabolism of placenta
- Metabolism of mother which changes during pregnancy duration

- Capacity of elimination of xenobiotics

- During fetal development, steroids (testosterone, estrogen) are responsible for the sexual differentiation of the brain.
- Estrogens are also able to exert effects in other brain areas of the fetus including the frontal cortex, where
- they modulate cognitive function and behaviors

Estrogen receptors

- Alpha
- Beta
- GPR30 (GPER1)

Large number of cells which bind estrogen are present during 16/17 GW in the cortical plate.

Expression of ER displays different spatial-temporal patterns during human cortical development (Gonzales et al., 2007)

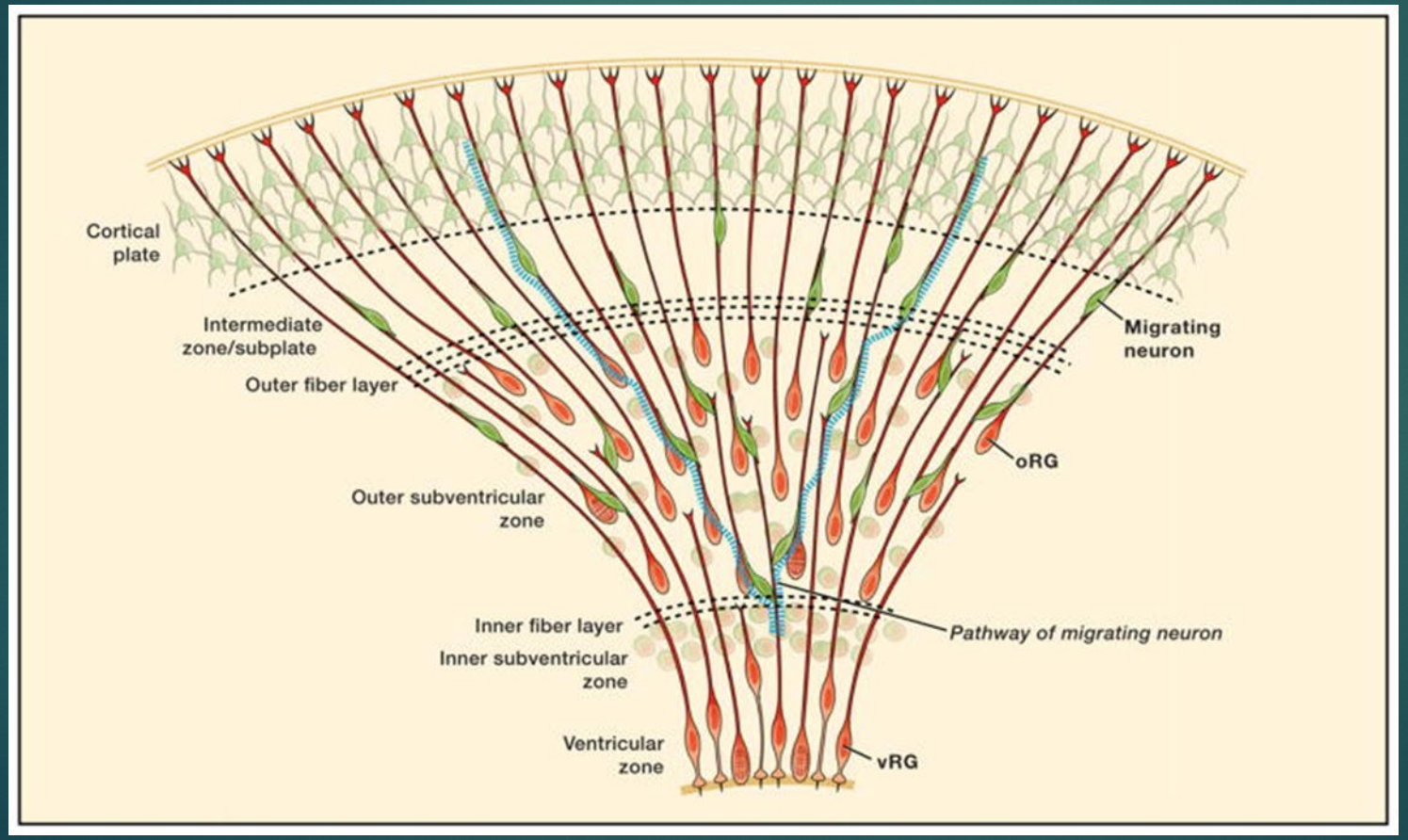
At early developmental stages (9 GW), virtually all cells have ERalpha, then frequency decreases during gestation but increases again after birth. By contrast, ERbeta staining is observed at 15 GW, it intensity gradually increases during cortical development to remain high in adulthood

TABLE 1. Semiquantitative Analysis of ERα and ERβ Cell Numbers in the Temporal Cortex[1]

	16–18 GW	20–25 GW	Newborn	Adult male	Adult female
ERα	76.25 ± 4.2(n=6)	38.40 ± 9.5 (n=6)	23.24 ± 4.33(n=2)	25.27± 4.34(n= 3)	30.12 ± 5.28(n=3)
ERβ	74.43 ± 9.5(n=4)	104.19 ± 17.58(n=6)	18.30 ± 4.24(n=2)	27.04 ± 4.64 (n= 3)	33.73 ± 4.92(n=3)

[1] All values are the mean of immunostained cell numbers present per unit area (100 × 200 μm) ± SEM in layers II–VI of the temporal cortex (medial temporal gyrus) at different prenatal stages and in the adult; n = number of subjects evaluated. ER, estrogen receptor; GW, gestational weeks.

Estrogen is a proliferative regulator and migratory stimulator.
Disruption to estrogenic signaling may result in an impaired migration and un-regulated proliferative cycle.
Cells may accumulate within the CP after being ejected too early from the cell cycle
Lui et al., 2011



- Cellular end points mediated by estrogen
- 1) apoptosis, preventing it in some regions but promoting it in others;
- 2) synaptogenesis, promotes in some regions and inhibits in others
- 3) Estradiol impacts immediate-early-gene expression

- Estradiol action is regionally specific and often involve neuronal/glial cross-talk

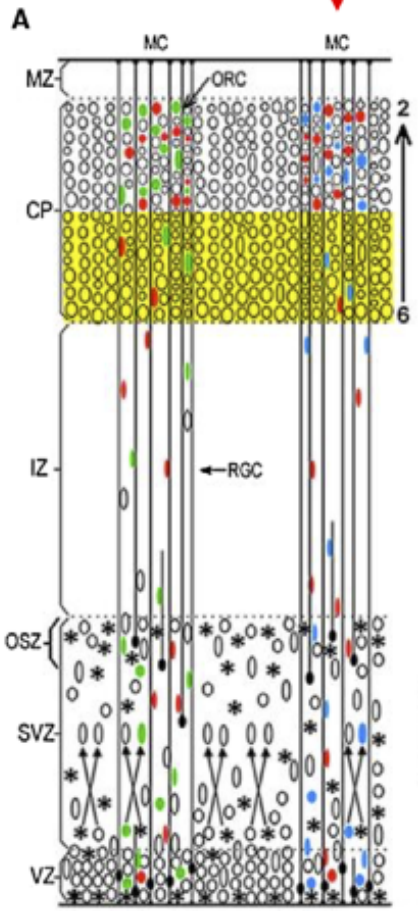
ERalpha precedes the expression of ERbeta in the proliferative zones, suggesting that ERalpha plays a role in early developmental processes such as cell proliferation in the VZ, whereas ERbeta might be more important for later events of corticogenesis

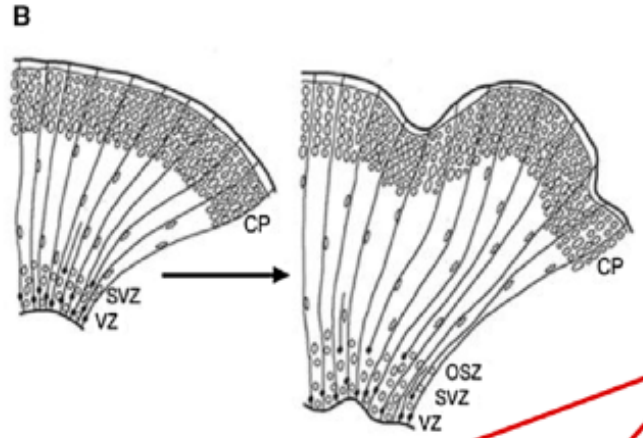
ERalpha is expressed in the Cajal-Retzius cells. These cells are important for correct positioning of cortical neurons because they are the main source of the extracellular matrix protein reelin, which is critically involved in the inside-out corticogenetic migration gradient

Gonzales et al., 2007

Functional columns are mixture of mini-columns
Torii et al., 2009

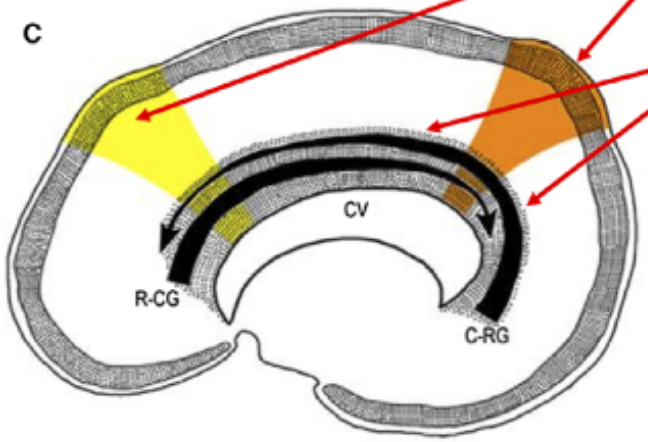
Reviewed in: Geschwind & Rakic, 2013
Bystron et al., 2008





Increase in cortical surface
Kuida et al., 1998
Chenn & Walsh, 2003
Kostovic et al., 2019
Rash et al., 2019

Introduction of new area,
e.g. Broca, Wernicke



Protomap of opposing molecular gradients
Cohen-Tannoudji et al., 1994
Mallamaci et al., 2000
Fukuchi-Shimogori & Grove, 2003
O'Leary & Bomgasser, 2006
Cholfin & Rubenstein, 2008
Miyoshi & Fishell, 2011
Elsen, Hodge,... Hevner, 2013

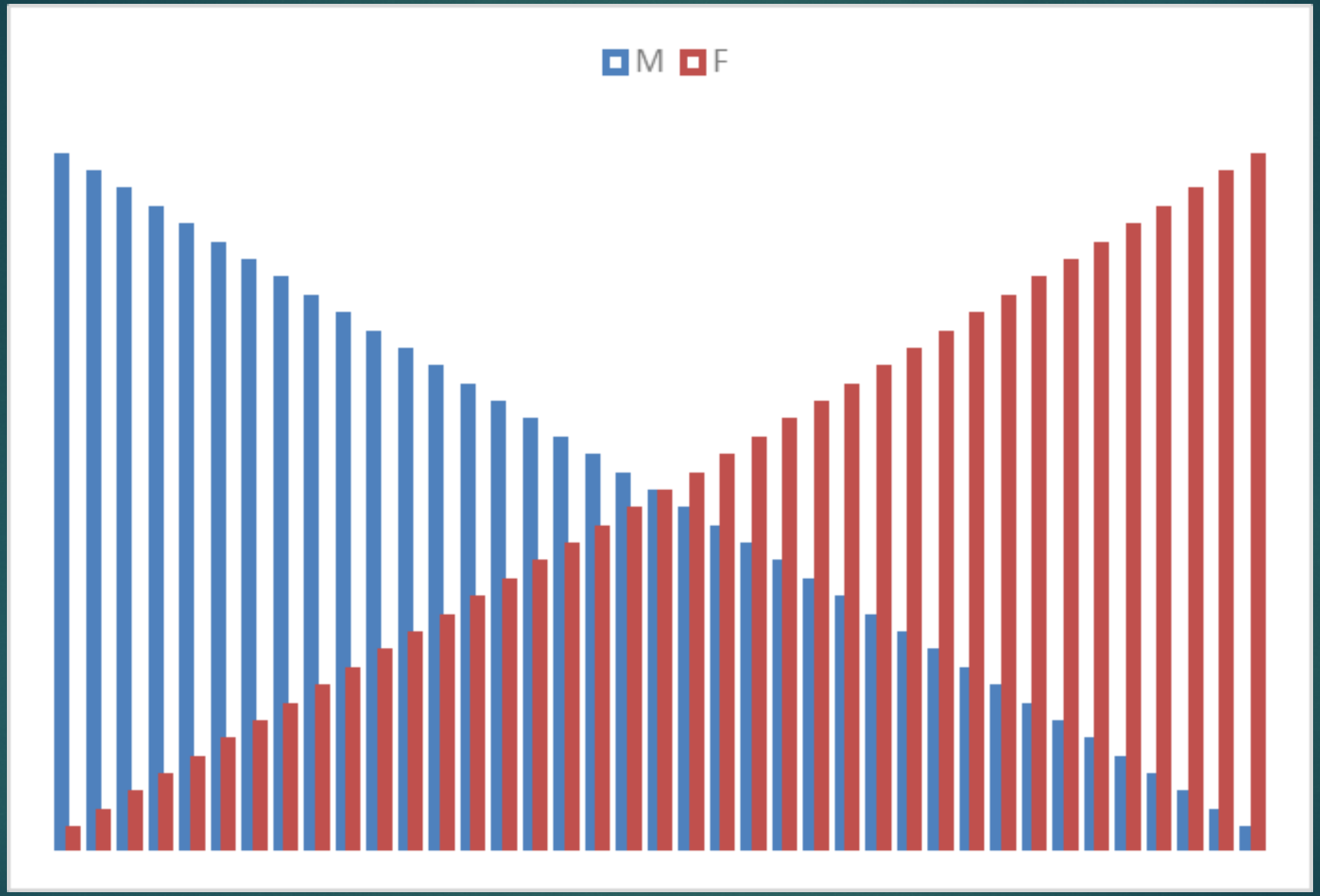
- This is why there is great interest for disturbances caused by agents which can be found in air, water and food which disturb estrogen and which we call

ENDOCRINE DISRUPTORS

Neural circuits strongly dependent on estrogen and thus to EDs

- Not only child neurobehavioural problems but life long risk of
- Alzhiemer disease
- Parkinson disease
- Epilepsy
- lower IQ
- Schizofrenia

- Associated with disturbance of estrogen/testosterone levels


M
F

- Our organism is making mistakes and couple with xenobiotic molecules which may be harmful

- adverse pathways that may be triggered by **lower dose** levels than conventional toxic effect

Estrogen-like activity

- Metals (aluminium, uranium, lead)
- PAH polycyclic aromatic hydrocar.
- dioxins
- Heterocyclic amines
- some antibiotics
- arsenic
- some pesticides

Components of plastics, additives, cosmetics

- Bisphenols
- Phthalates
- Parabens

Majority of environmental EDs are xenoestrogens

- In an animal model is has been shown that bisphenol disturbs levels of ERa, accelerate neuronal differentiation and migration.

- No data available on impact of ED on development of human frontal cortex
- Only epidemiology studies for some EDs
- No mechanistic insight

Smoking

- Transplacental exposure – mothers who smoke during pregnancy- fetus exposed to high levels of testosterone - mitogen

Alcohol

Increases estrogen

Phytoestrogens-

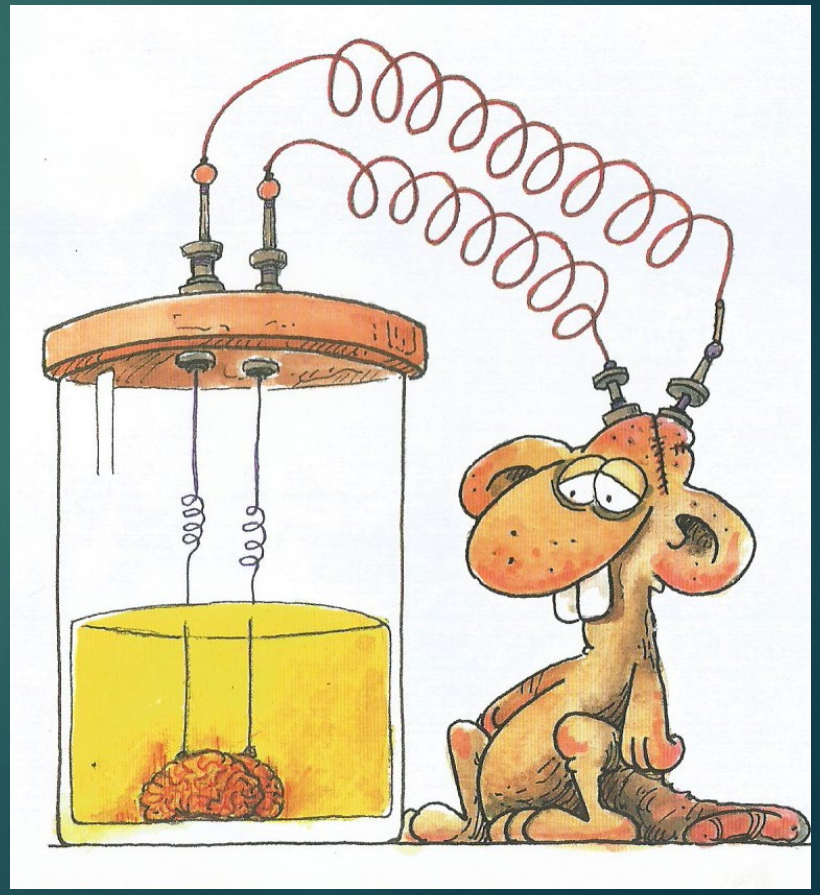
Aloe vera, soy food- slower elimination from fetus than from mother

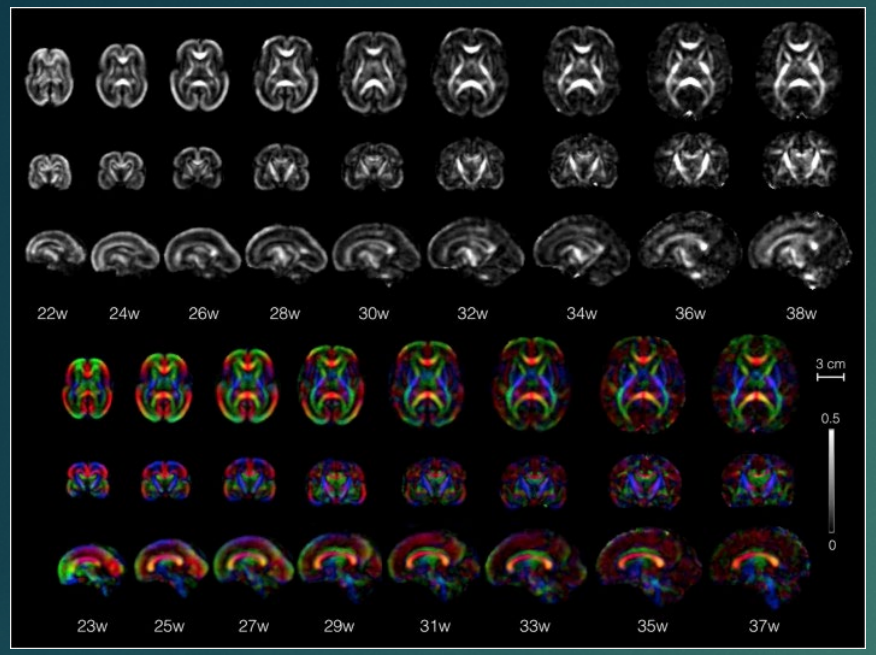
- Bisphenol A disrupts thyroid hormone function which is significant for brain development and
- Disrupt neocortical development by accelerating neuronal differentiation/migration (Nakamura et al., 2006)
- Paracetamol decreases testosterone and as shown on animal model males transplacental exposure causes disrupting of sexual neurobehavioral programming

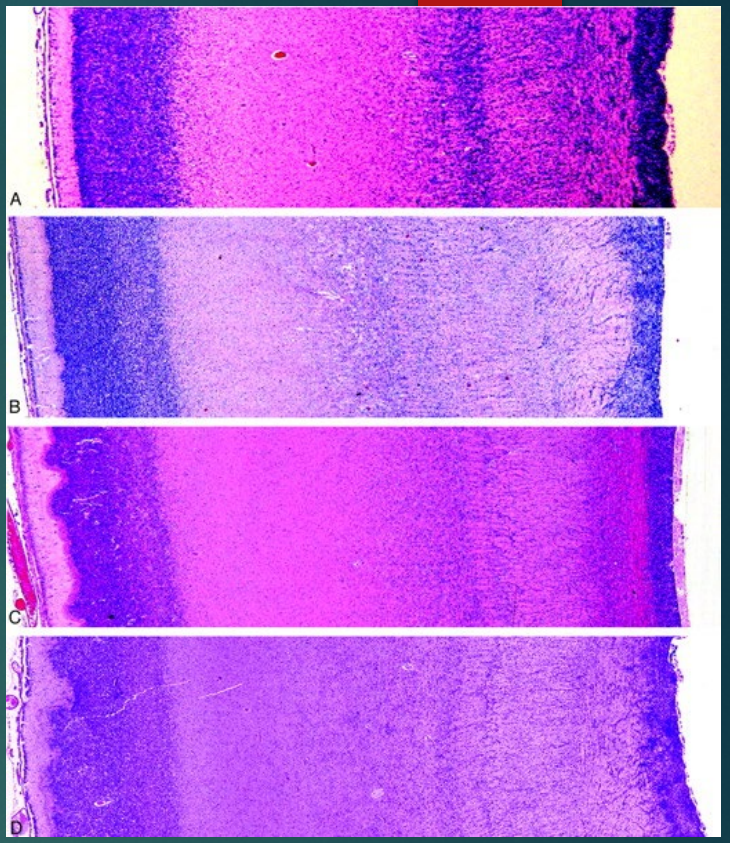
Preciados et al., Int. J. Mol. Sci.2016,17, 2086

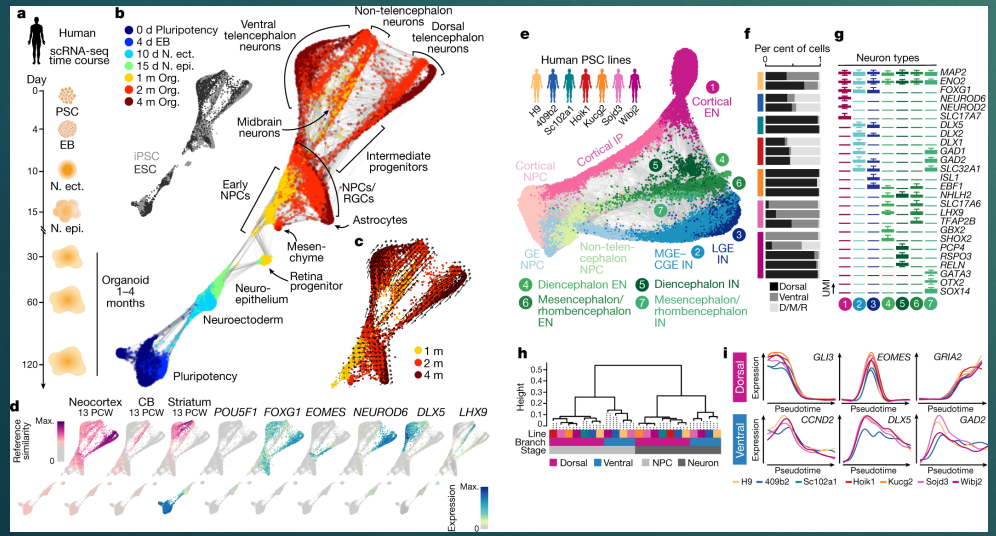
- Prenatal BPA exposure produce more aggressive and hyperactive behavior in offspring when compared to mothers with lower BPA levels
- prenatal urinary BPA concentrations in the mother and child were associated with anxiety, depression, and hyperactivity
- an association between BPA exposure and autistic spectrum disorders
- In autistic children, plasma levels of BPA and phthalates were significantly higher compared to controls
- Gender and ethnic difference due to different levels of sex hormones
- **Afro-american people have higher estradiol levels than white people.**
- **Mexican-Americans had higher testosterone than white people but similar estradiol concentrations**

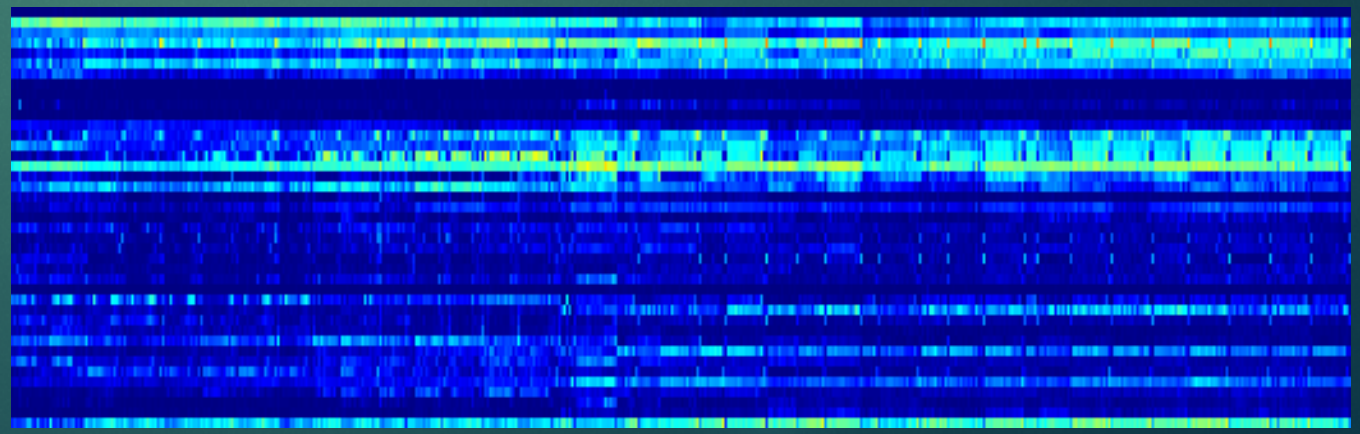
- Rodent studies questionable
- different levels of receptors, sex hormones and different structure and metabolic activity of placenta – still better than petri dish




22w
24w
26w
28w
30w
32w
34w
36w
38w
3 cm
0.5
0
23w
25w
27w
29w
31w
33w
35w
37w


A
B
C
D


a
Human
scRNA-seq
time course
Day
PSC
EB
N. ect.
N. epi.
Organoid
1–4
months
b
0 d Pluripotency
4 d EB
10 d N. ect.
15 d N. epi.
1 m Org.
2 m Org.
4 m Org.
iPSC
ESC
Ventral telencephalon neurons
Non-telencephalon neurons
Dorsal telencephalon neurons
Midbrain neurons
Intermediate progenitors
Early NPCs
NPCs/ RGCs
Astrocytes
Mesen-chyme
Retina progenitor
Neuro-epithelium
Neuroectoderm
Pluripotency
c
1 m
2 m
4 m
d
Neocortex 13 PCW
CB 13 PCW
Striatum 13 PCW
POU5F1
FOXG1
EOMES
NEUROD6
DLX5
LHX9
Reference similarity
Max.
0
Expression
e
Human PSC lines
H9
409b2
Sc102a1
Hoik1
Kucg2
Sojd3
Wibj2
Cortical EN
Cortical IP
Cortical NPC
GE NPC
Non-telen-cephalon NPC
MGE– CGE IN
LGE IN
4 Diencephalon EN
5 Diencephalon IN
6 Mesencephalon/ rhombencephalon EN
7 Mesencephalon/ rhombencephalon IN
f
Per cent of cells
Dorsal
Ventral
D/M/R
g
Neuron types
MAP2
ENO2
FOXG1
NEUROD6
NEUROD2
SLC17A7
DLX5
DLX2
DLX1
GAD1
GAD2
SLC32A1
ISL1
EBF1
NHLH2
SLC17A6
LHX9
TFAP2B
GBX2
SHOX2
PCP4
RSPO3
RELN
GATA3
OTX2
SOX14
UMI
h
Height
Line
Branch
Stage
Dorsal
Ventral
NPC
Neuron
i
Dorsal
Ventral
Expression
GLI3
EOMES
GRIA2
CCND2
DLX5
GAD2
Pseudotime
H9
409b2
Sc102a1
Hoik1
Kucg2
Sojd3
Wibj2



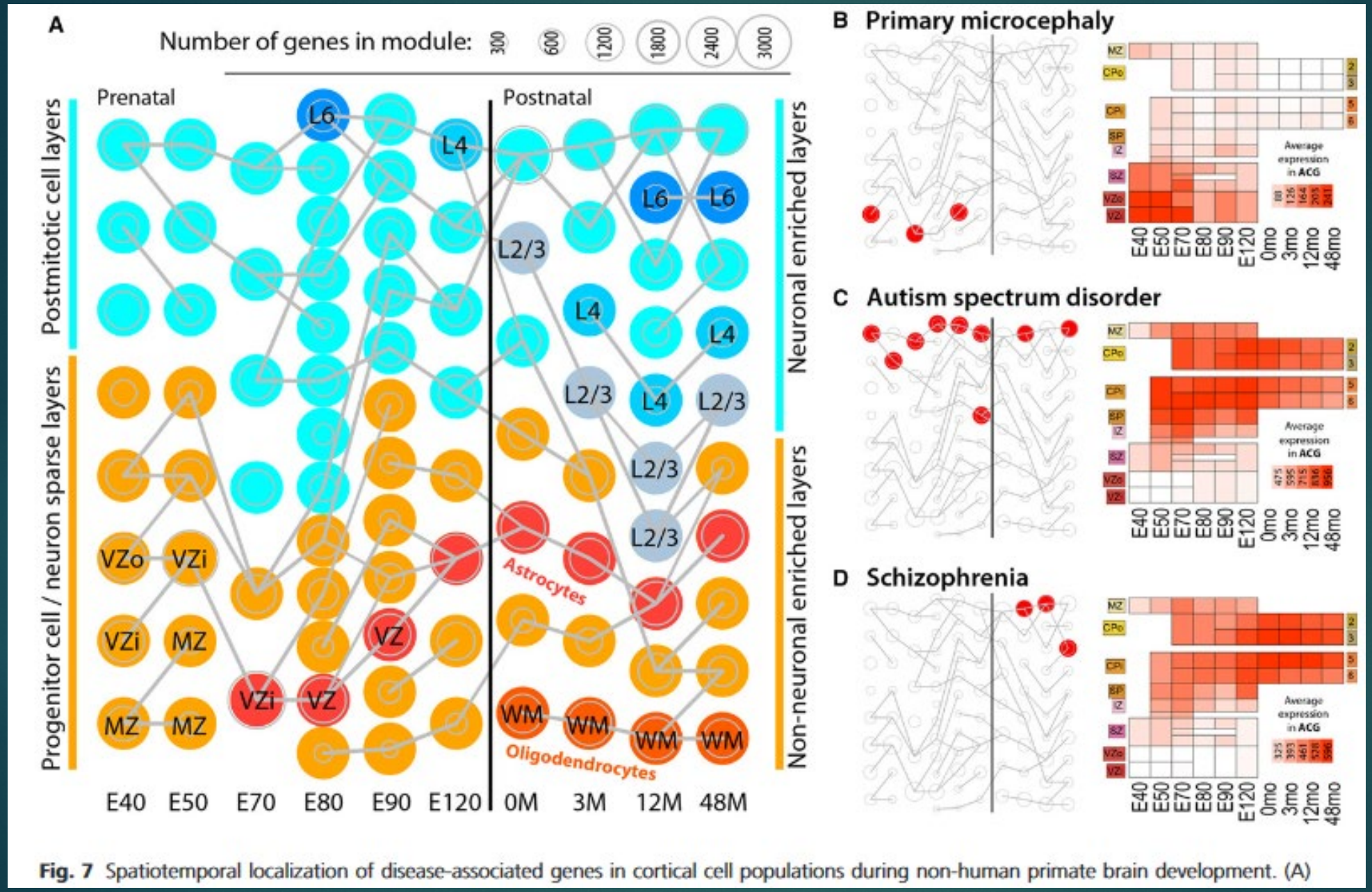
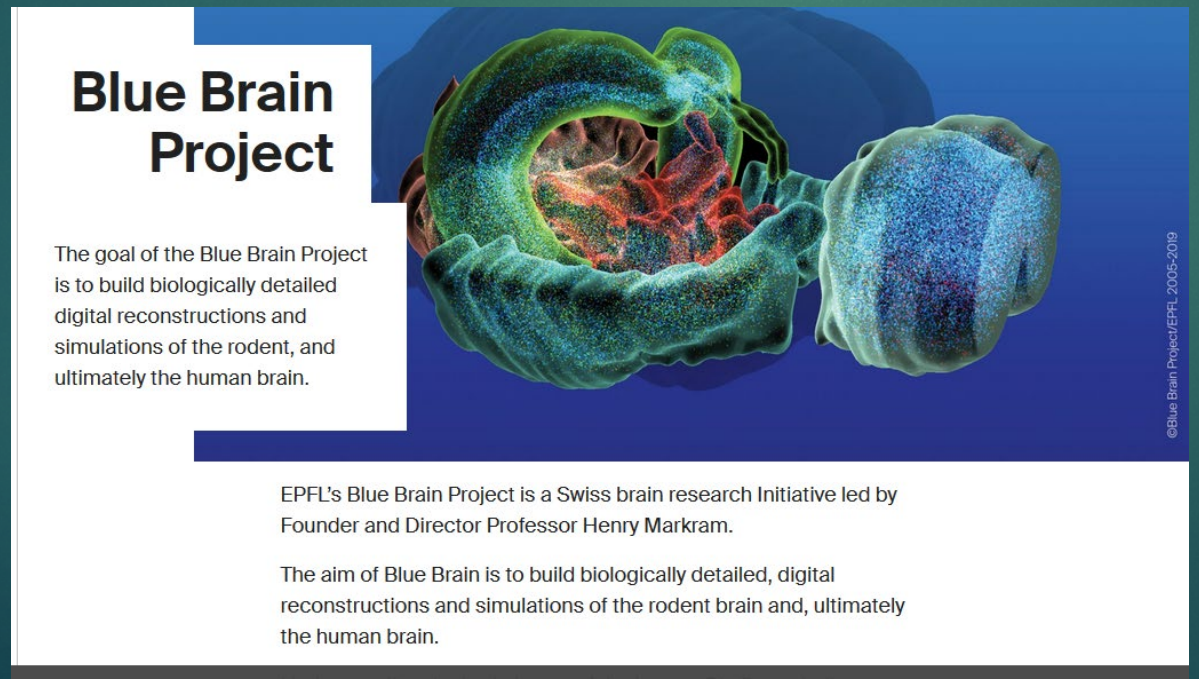


Fig. 7 Spatiotemporal localization of disease-associated genes in cortical cell populations during non-human primate brain development. (A)

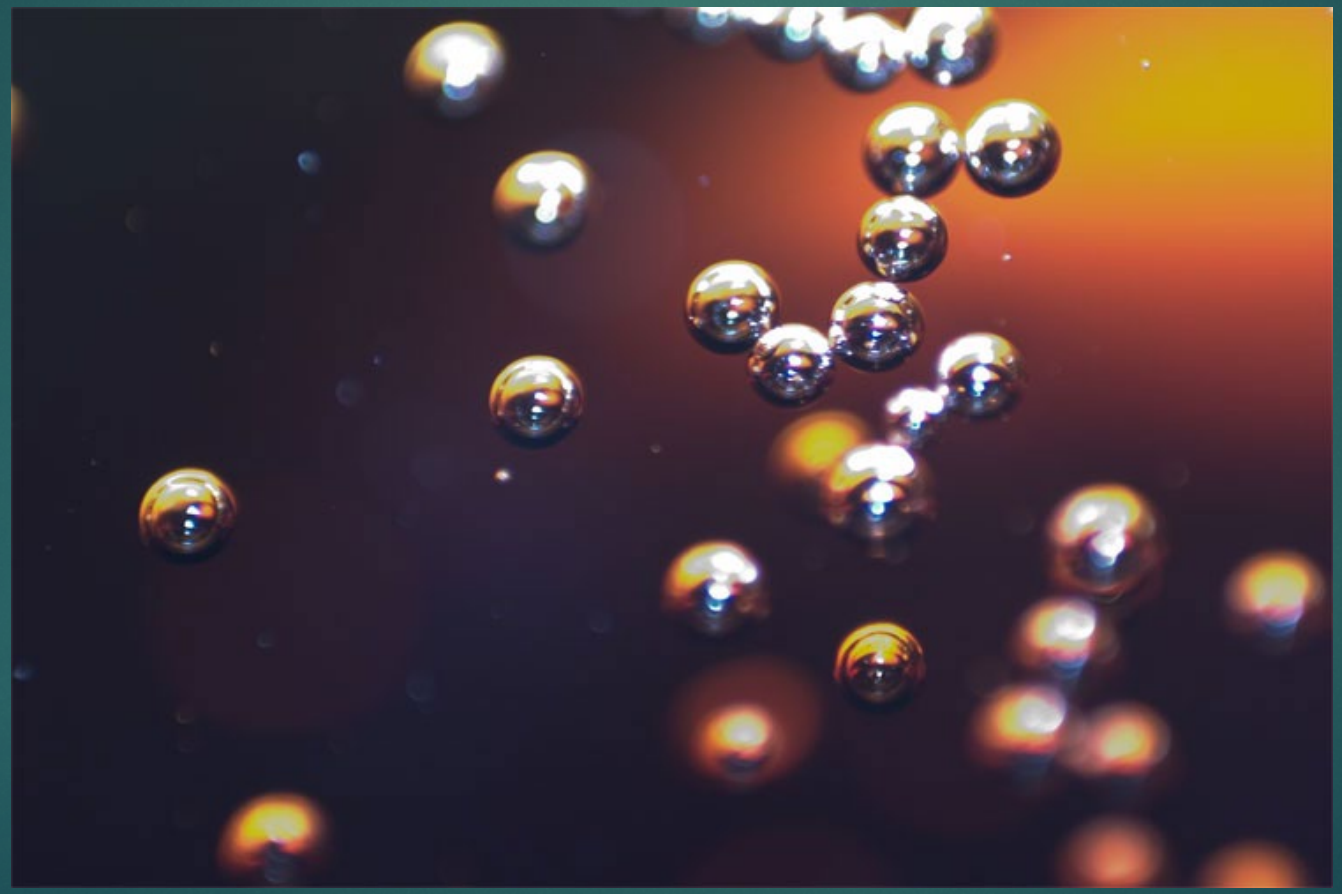
What they discovered is that the brain is full of multi-dimensional geometrical structures operating in as many as 11 dimensions.

Human brains are estimated to have a staggering 86 billion neurons, with multiple connections from each cell webbing in every possible direction, forming the vast cellular network that somehow makes us capable of thought and consciousness. The progression of activity through the brain resembles a multi-dimensional sandcastle that materialises out of the sand and then disintegrates.

École Polytechnique Fédérale de Lausanne, Geneva, Switzerland



Migration of cells through cortical layers- fluid mechanics
Migration of bubbles through the gel

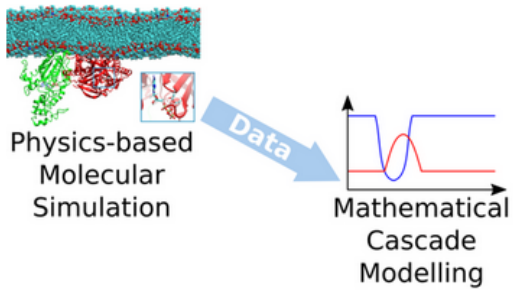


Constraining Kinetic Models of Signalling Cascades using Molecular Simulation

Publications

- Neil J. Bruce, Daniele Narzi, Daniel Trpevski, Siri Camee van Keulen, Anu G. Nair, Ursula Roethlisberger, Rebecca C. Wade, Paolo Carloni and Jeanette Hellgren Kotaleski, Regulation of adenylyl cyclase 5 in striatal neurons confers the ability to detect coincident neuromodulatory signals, (2019), BioRxiv.

The processes that determine how a neuron responds to external chemical signals are key to understanding processes such as synaptic plasticity. Accurately modelling these processes requires simulating phenomena that occur on a wide range of spatial and temporal scales, requiring multiscale modelling approaches.



What makes modelling of molecular signalling cascades special?

Kinetic modelling of biochemical signalling networks allows us to investigate how various chemical pathways interact to control cellular processes. By simplifying the interactions between the molecules in these pathways to a set of parameterised mathematical equations that describe the changes in concentrations of various chemical species due to the chemical reactions occurring in neurons, we can build models that describe how neurons react over timescales of seconds or minutes. Often, the data needed to build these models are sparse, and the parameters in the models must be fitted in order to reproduce observed phenotypic data.

International Journal of
Molecular Sciences

MDPI

Article

Exploiting Gene Expression Profiles for the Automated Prediction of Connectivity between Brain Regions

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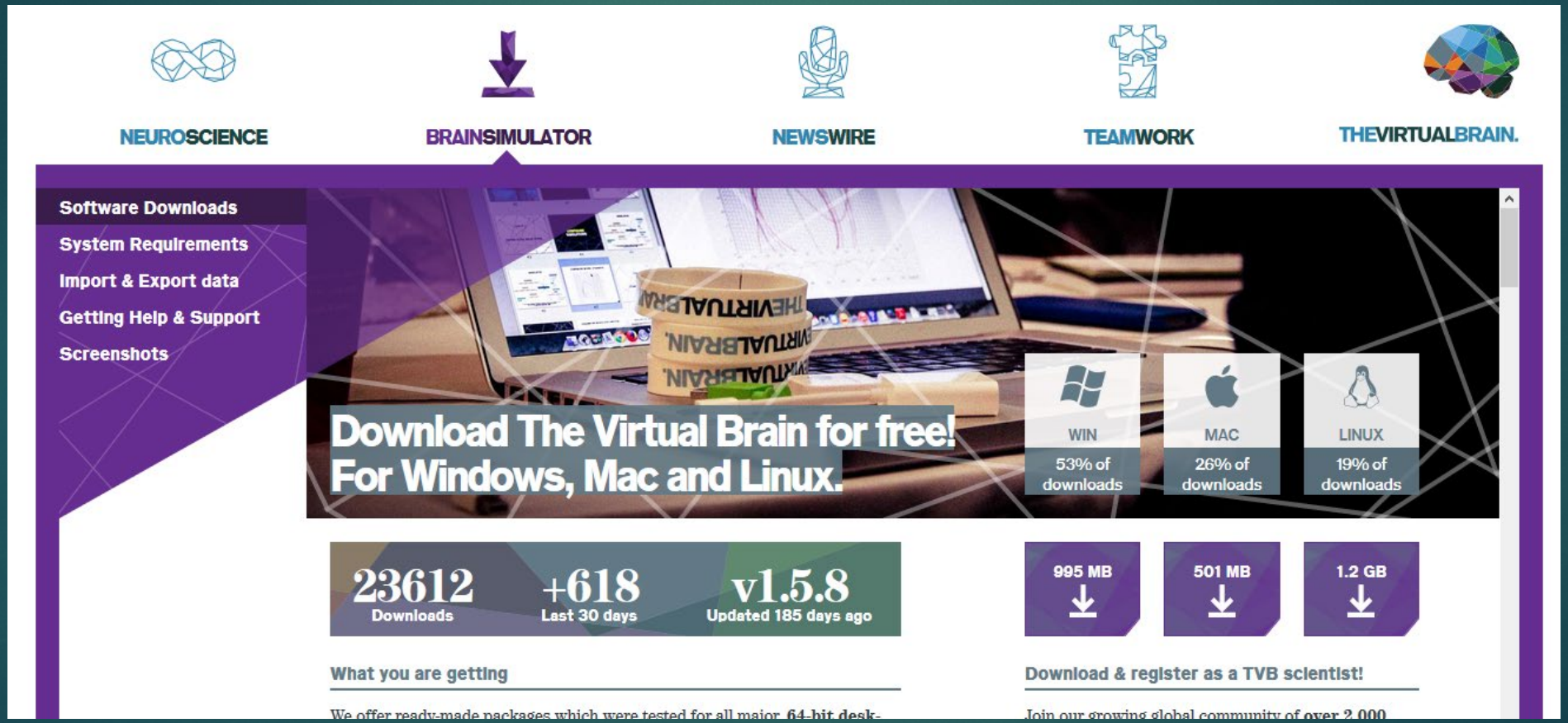
Received: 7 February 2019; Accepted: 20 April 2019; Published: 25 April 2019

check for updates

Abstract: The brain comprises a complex system of neurons interconnected by an intricate network of anatomical links. While recent studies demonstrated the correlation between anatomical connectivity

Brain simulator



underlying stroke, tumour, simulate the whole brain mouse dynamics, and perform multiscale modeling of resting state fMRI and EEG activity in humans.

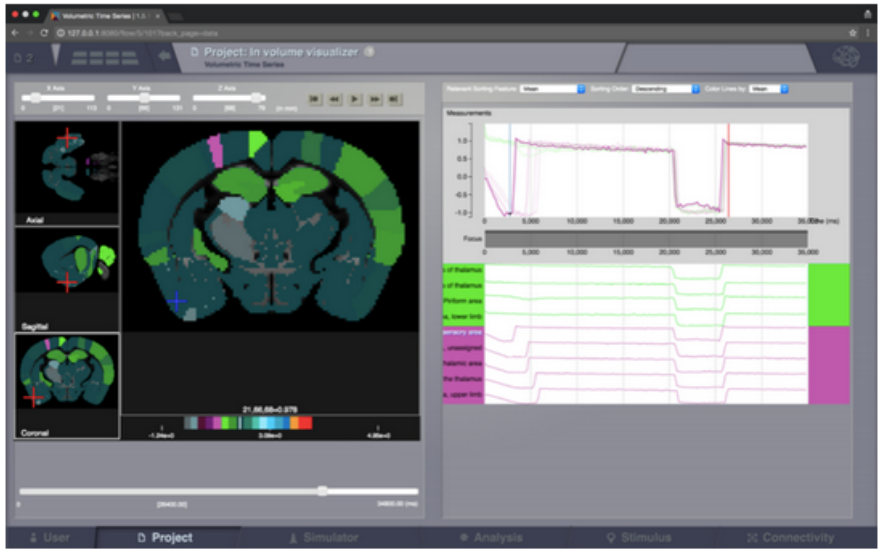
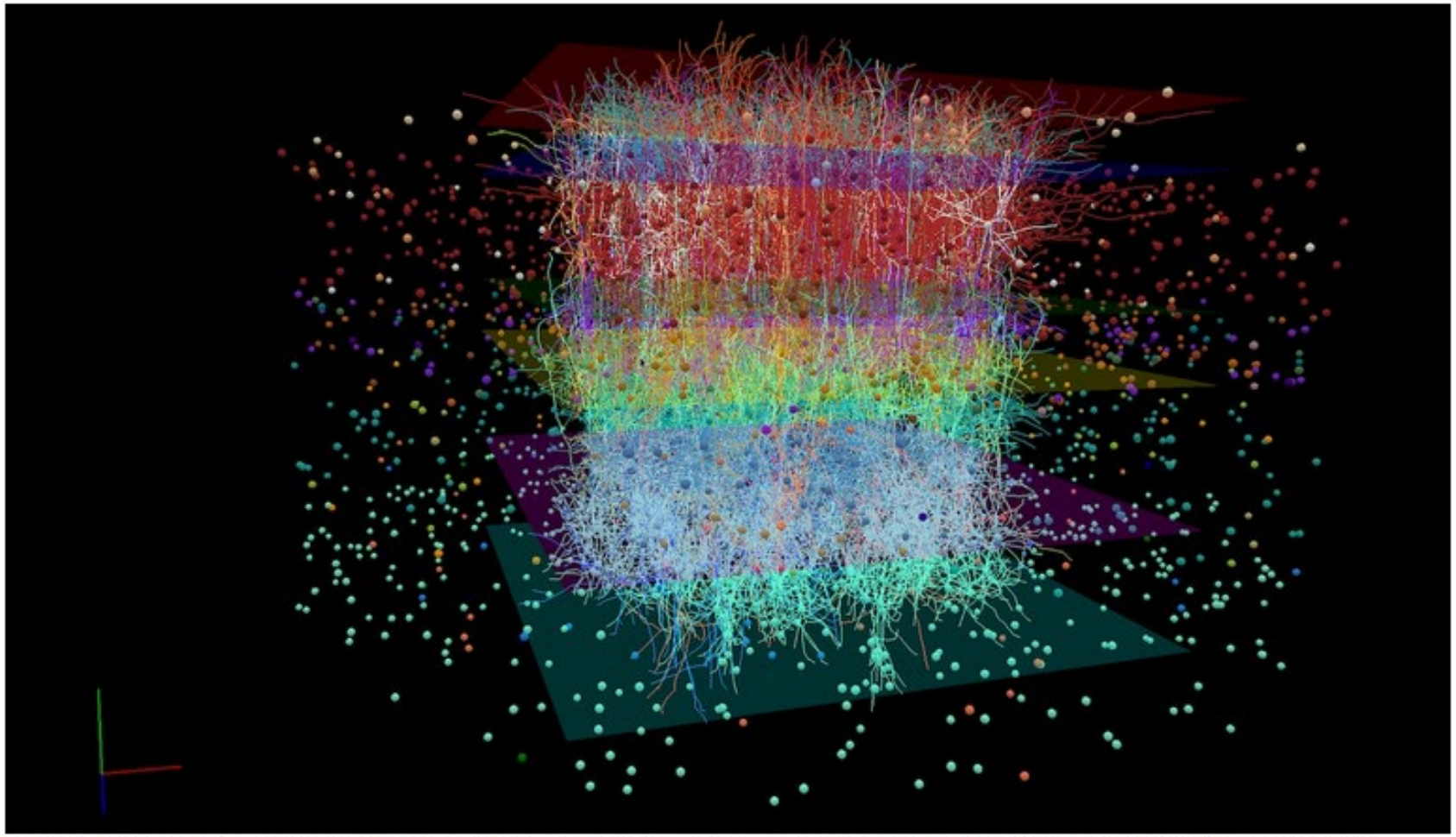


Figure 2 An example for application of TVB: simulated epileptic seizure in the mouse brain.

Image imported from https://www.thevirtualbrain.org/tvb/zwei/brainsimulator-screens

Air Traffic Management



A visualization of a large-scale model of a mouse visual cortex brain circuit, built at the Allen Institute and containing 230,000 neuron building-block models. Image courtesy of Sergey Gratiy, Ph.D.

Conclusion

Kanthaka program

- Simulation of intrauterine human brain development which include time
- The most dynamic period of human frontal cortex development is between 16 and 24 GW
- Inventory of available data should be performed
- Mathematical tools exist
- Kanthaka would be crucial in simulation of deviations in human brain development after action of one or mixture of chemicals
- The priority for application of such stress system program would be endocrine disruptors


sukh sokhi photography

Investigation

- Principle of pattern formation in plasticity process
- Sustainible chain of common/define cross roads which may be changed by deviation due to exposure to xenobiotics

Questions?