

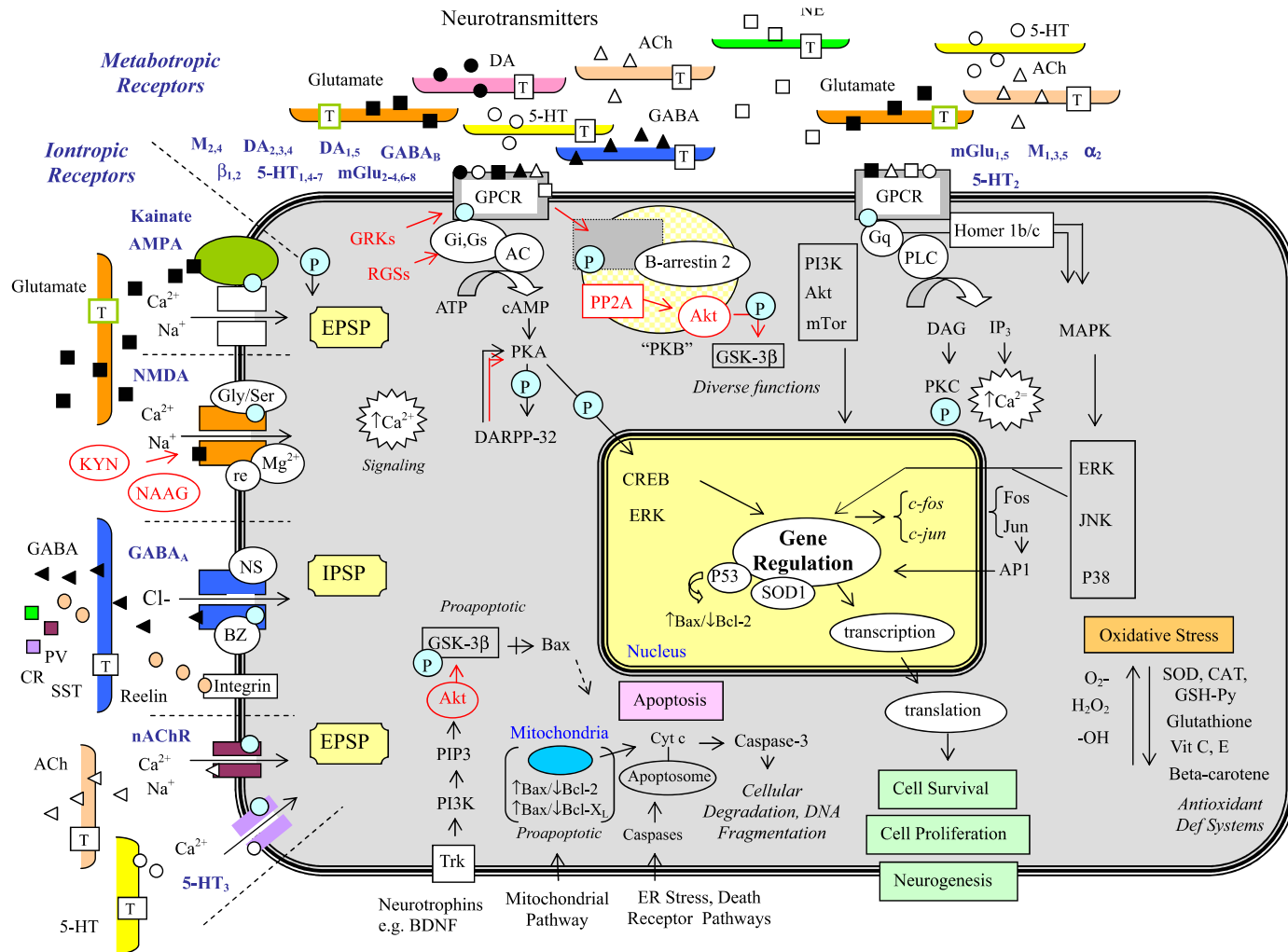
BUILDING AOPs FOR NEUROTOXICITY: PERSPECTIVE FROM AN ACADEMIC

Univ.-Prof. Dr. med. Ellen Fritsche

Mitglied der

The logo features the word 'Leibniz' in a large, black, cursive script. Below it, the words 'Leibniz-Gemeinschaft' are written in a smaller, blue, sans-serif font.

Molecular Targets in Brain Cells



Processes involved in Neurodevelopment

S.L. Andersen / Neuroscience and Biobehavioral Reviews 27 (2003) 3–18

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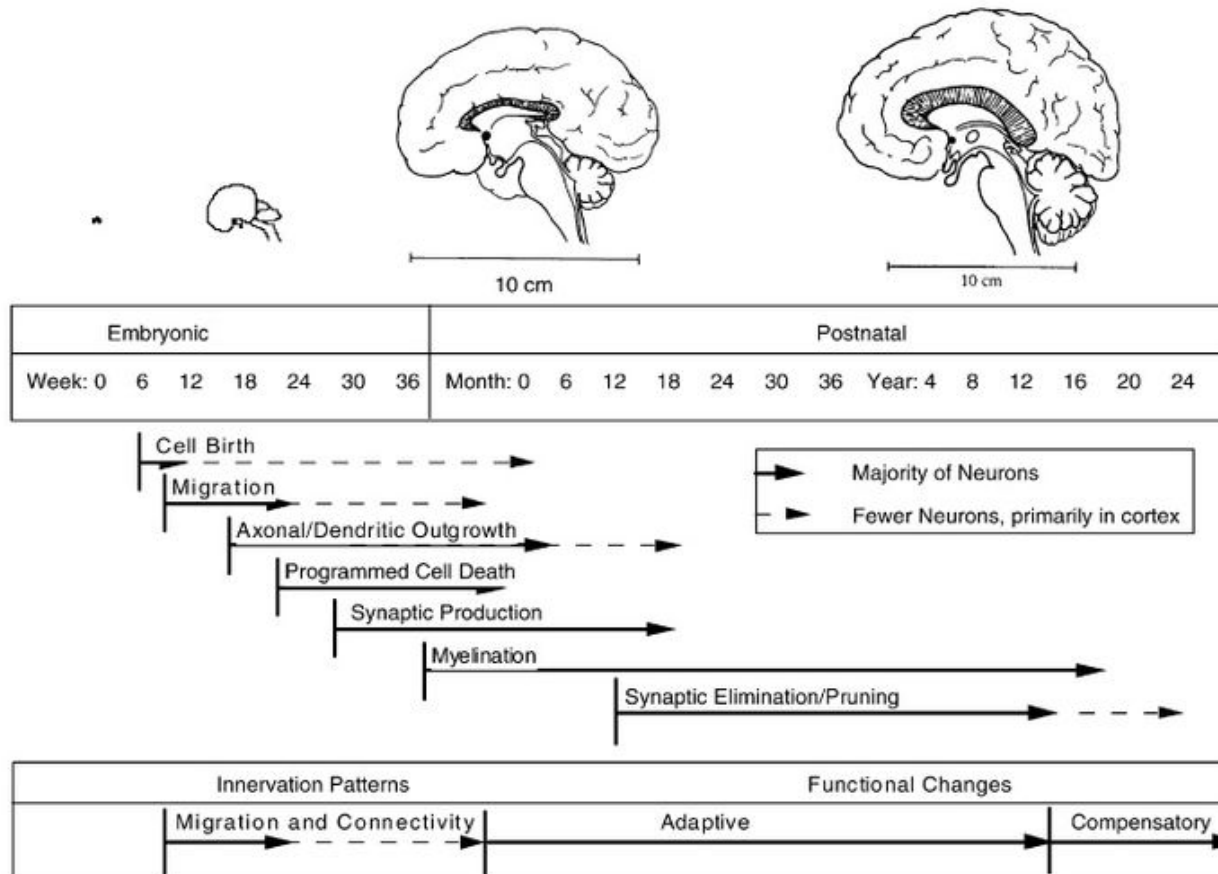


Fig. 1. The stages of brain development (top) and different windows of vulnerability (bottom). Developmental processes occur in phases, setting the stage for potential periods of vulnerability. Insults early in life (bottom) will be assimilated into innervation patterns, whereas a later pre-pubertal insult will cause functional changes that are more adaptive.

Identification of AOPs for neurotoxicity

**Critical Reviews
in Toxicology**

<http://informahealthcare.com/txc>
ISSN: 1040-8444 (print), 1547-6898 (electronic)

Crit Rev Toxicol, 2015; 45(1): 83–91
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informa
healthcare

REVIEW ARTICLE

Putative adverse outcome pathways relevant to neurotoxicity

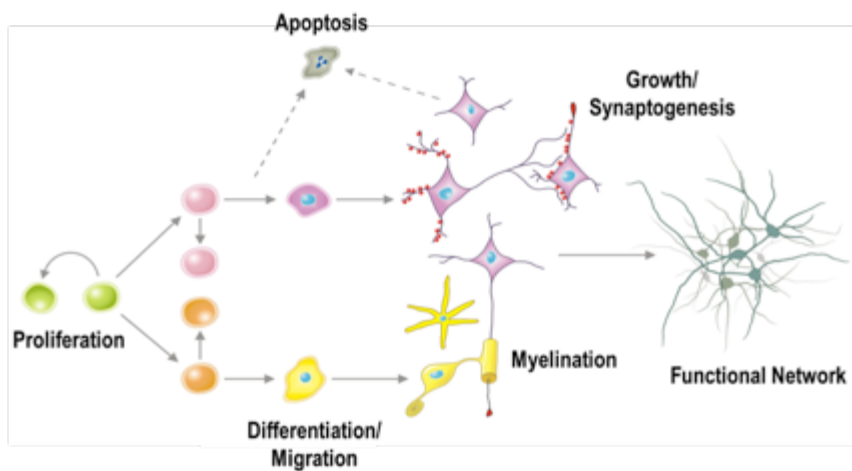
Anna Bal-Price¹, Kevin M. Crofton², Magdalini Sachana¹, Timothy J. Shafer², Mamta Behl³, Anna Forsby⁴, Alan Hargreaves⁵, Brigitte Landesmann¹, Pamela J. Lein⁶, Jochem Louisse¹, Florianne Monnet-Tschudi⁷, Alicia Paini¹, Alexandra Rblaki¹, André Schrattenholz⁸, Cristina Suñol⁹, Christoph van Thriel¹⁰, Maurice Whelan¹, and Ellen Fritsche¹¹

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Identification of AOPs for neurotoxicity

- I. **NMDAR blockage** during development induces impairment of cognitive and learning abilities
- II. **NMDA receptor over-activation** mediates neuronal cell death and reduction (or loss) of cognitive, sensory and motor function.
- III. **GABA_A receptor**-mediated neurotoxicity results in hyperexcitability and convulsions
- IV. Acute Neurotoxic Effects of Pyrethroids Mediated by Disruption of **Voltage-gated Sodium Channels**
- V. Binding of chemicals to membrane receptors, enzymes or cytoskeletal proteins results in **delayed neuropathy**
- VI. Impairment of learning and memory induced by binding of electrophilic chemicals to **SH(thiol)-group** of proteins during development
- VII. Inhibition of **mitochondrial respiration** or uncoupling of oxidative phosphorylation decreases or blocks ATP production resulting in neurodegeneration.
- VIII. Multiple molecular initiating events trigger **neuroinflammation** leading to neurodegeneration
- IX. The developmental neurotoxicity of non-dioxin-like PCBs: Sensitization of **ryanodine receptors** interferes with neurodevelopmental processes that determine neuronal connectivity
- X. ROS-dependent reduced adult neurogenesis due to decline of **neural progenitor cell** function: higher susceptibility during aging.

The Neurosphere Assay



Fritsche et al. Environ Health Perspect 2005

Moors et al. Toxicol Appl Pharmacol 2007

Moors et al. Environ Health Perspect 2009

Moors et al. Genes & Immunity 2010

Tegenge et al. Cell. Mol. Life Sci. 2010

Schreiber et al. Environ Health Perspect 2010

Gassmann et al. Environ Health Perspect 2010

Verner et al. Toxicol in Vitro 2011

Fritsche et al. Methods Mol Biol 2011

Gassmann et al. Toxicol in Vitro 2012

Bal-Price et al. ALTEX 2012

Baumann et al. Curr. Protoc. Toxicol. 2014

Gassmann et al. Arch. Toxicol. 2014

Fritsche Methods Pharmacol. Toxicol. 2014

Alépée et al. ALTEX 2014

Baumann et al. Arch Toxicol 2016

Baumann et al. Methods Pharmacol Toxicol 2015

Barenys et al. Arch Toxicol 2017

Schmuck et al., Arch Toxicol 2017

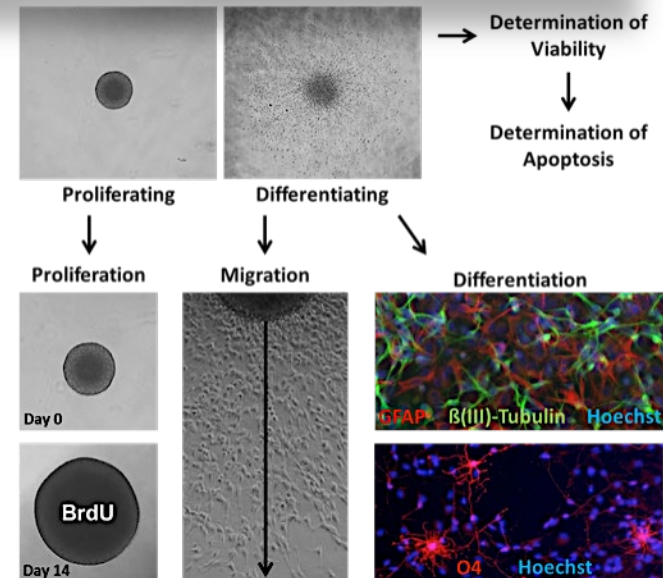
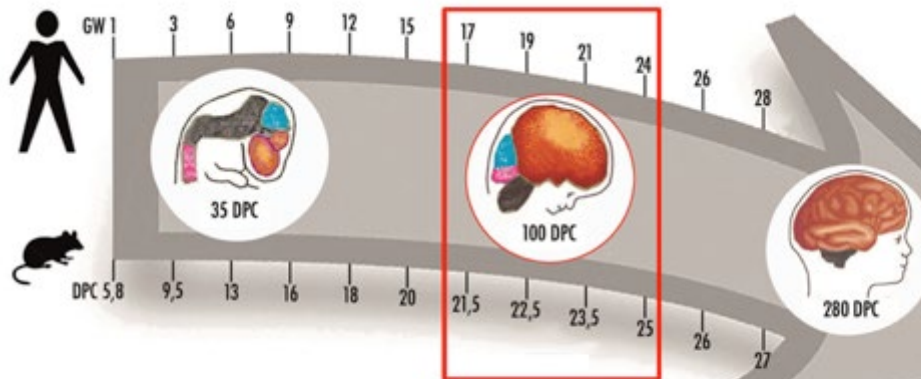
Dach et al., Sci Rep 2017

Hellwig et al. Tox in vitro 2018

Masjosthusmann et al. TAAP 2018

Ali et al. Brain Struct Funct 2019

Masjosthusmann et al. Chemosphere 2019

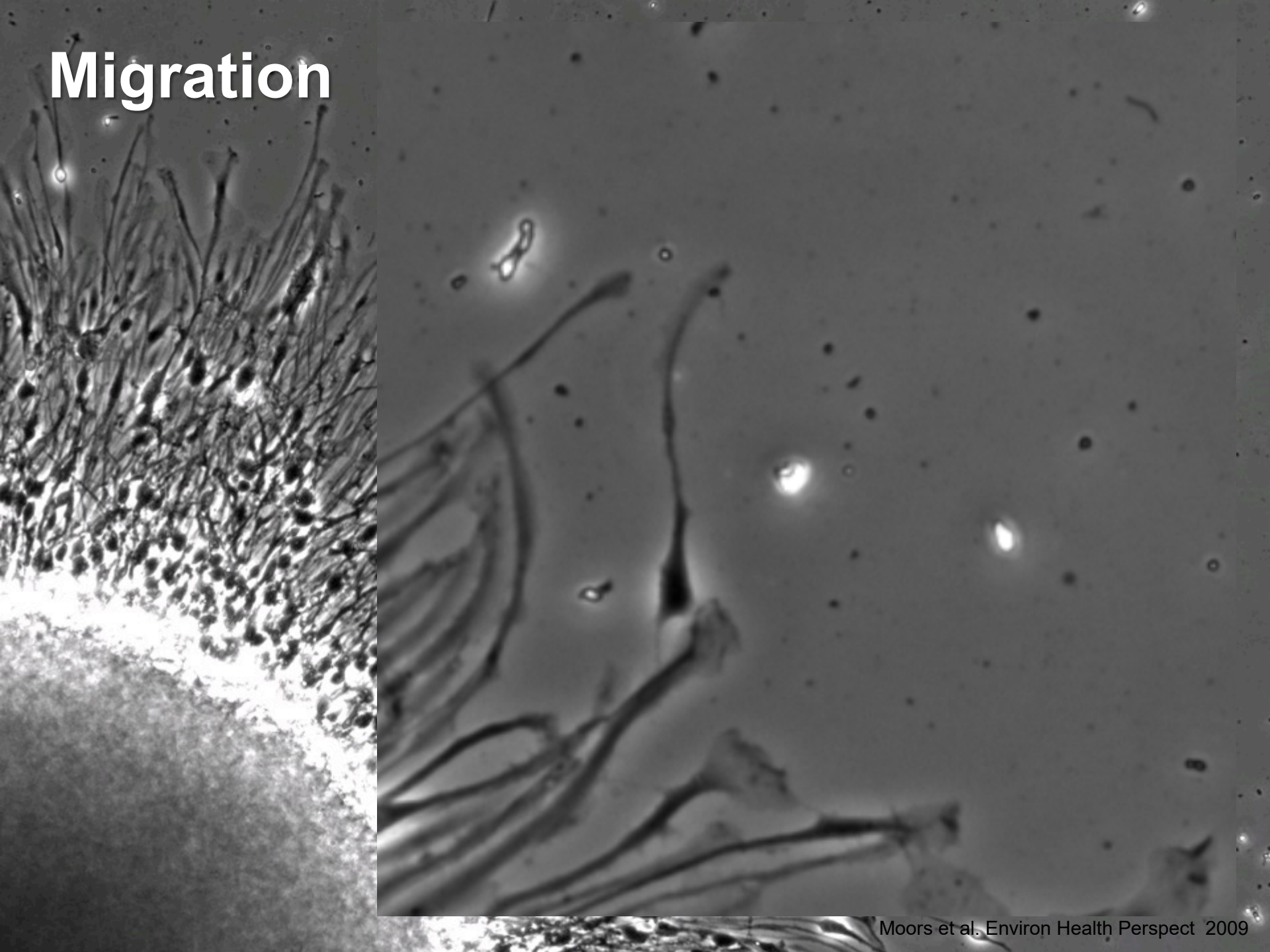


Leibniz

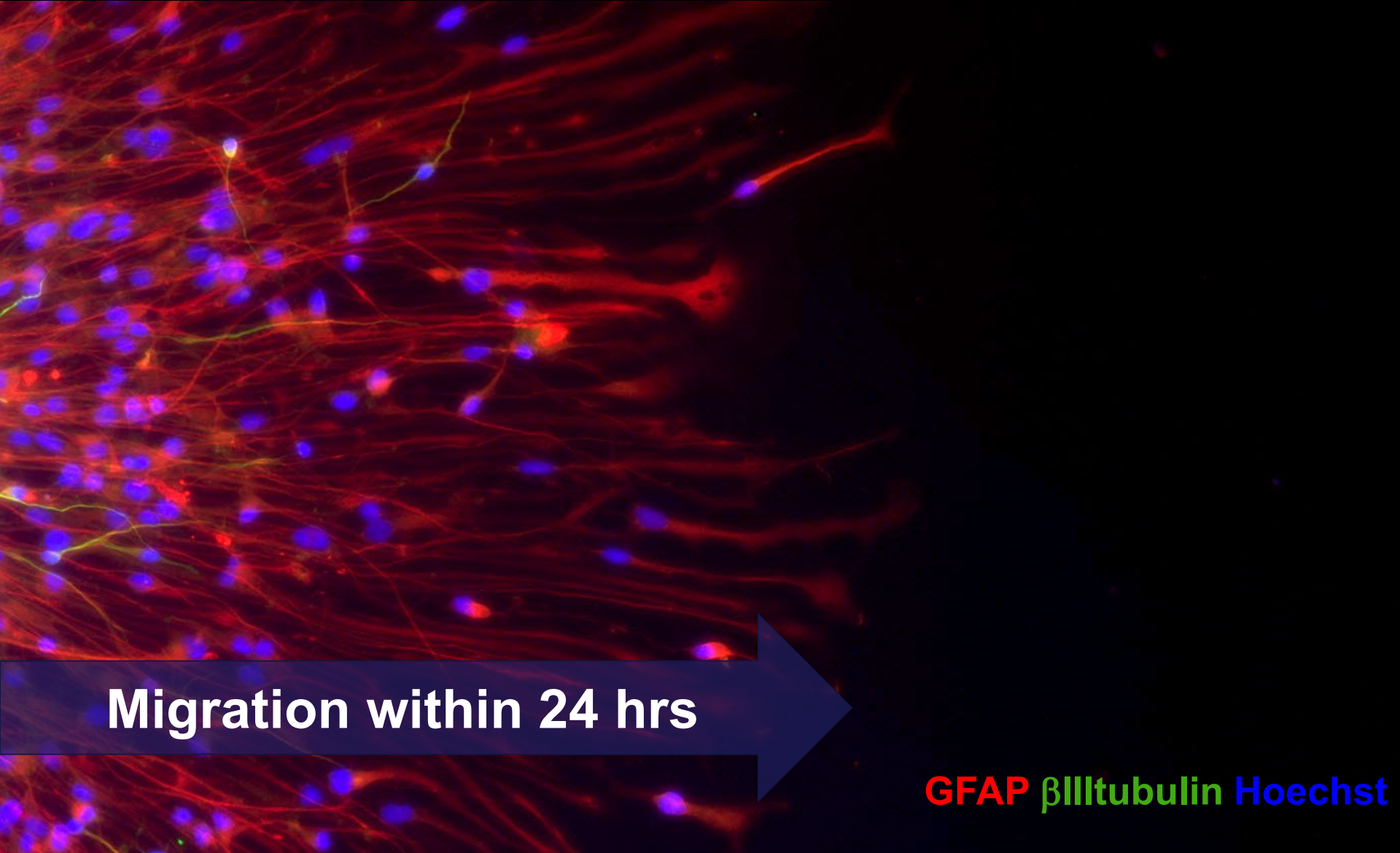
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Migration



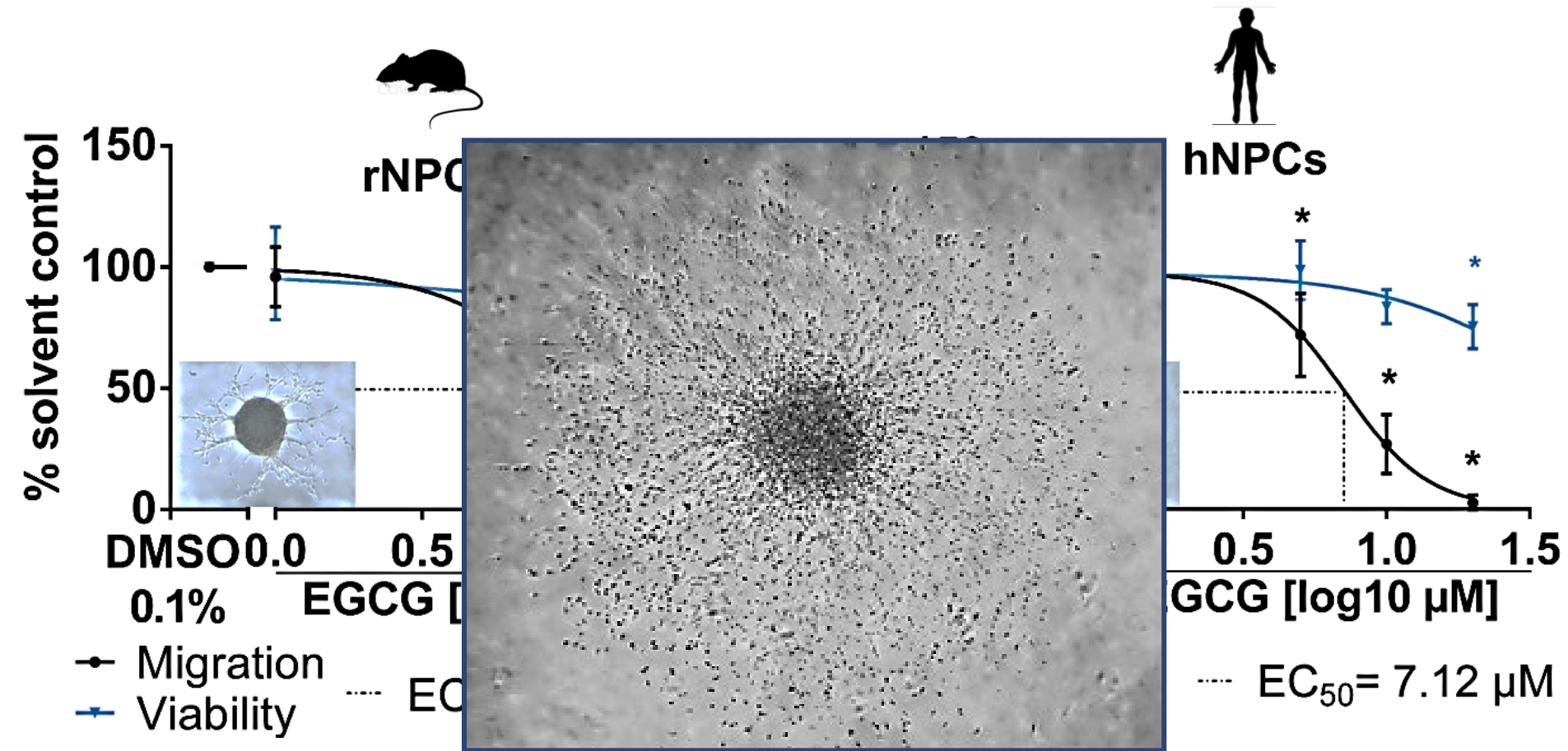
Radial Glia Migration/Differentiation



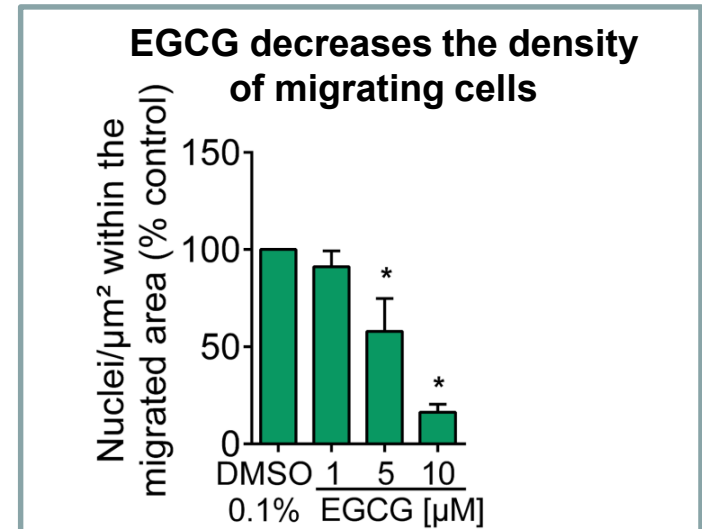
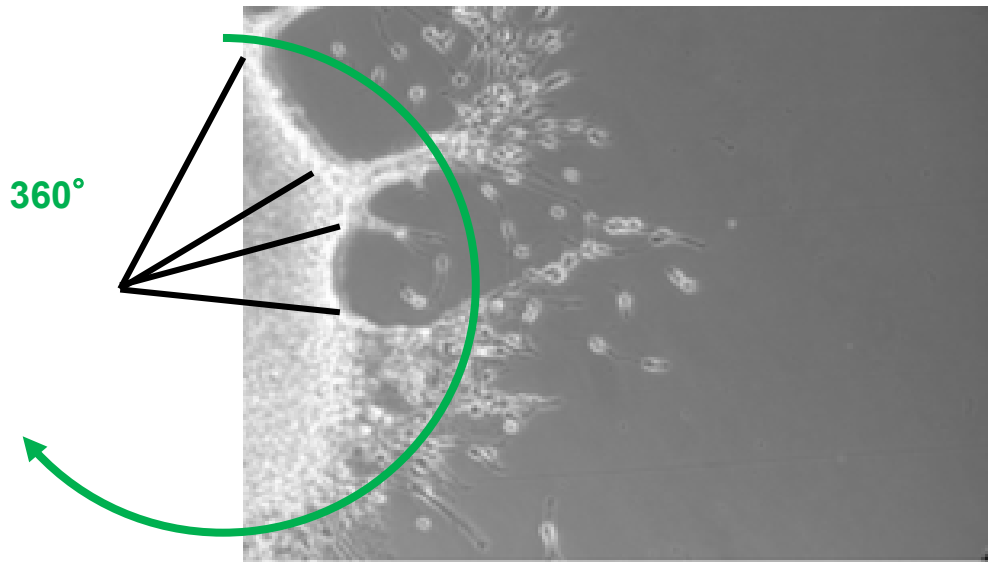
Migration within 24 hrs

GFAP β III tubulin Hoechst

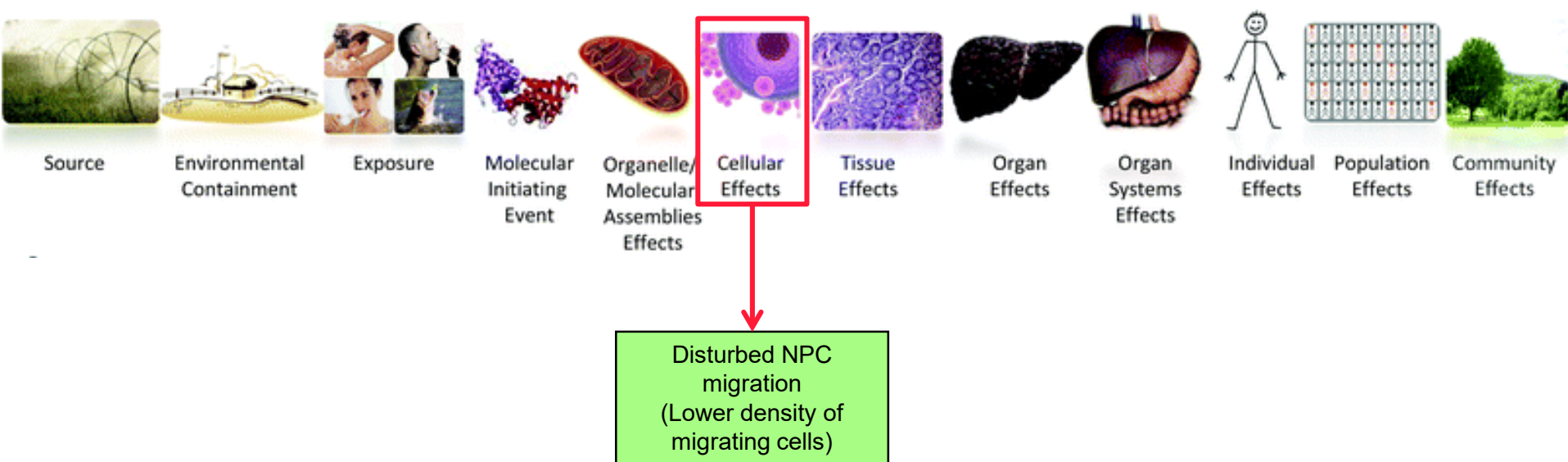
EGCG disturbs migration of rat and human NPCs *in vitro*



EGCG lowers cellular density of human NPCs *in vitro*



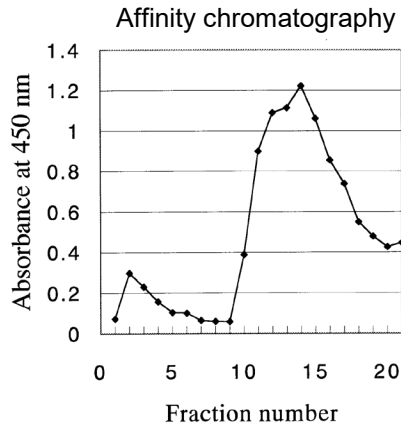
Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



from
Ankley et al., 2009

EGCG disturbs migration by interaction with laminin

Suzuki and Isemura, 2001



EGCG-Sepharose 4B column
+ laminin solution → collection 1 mL fractions

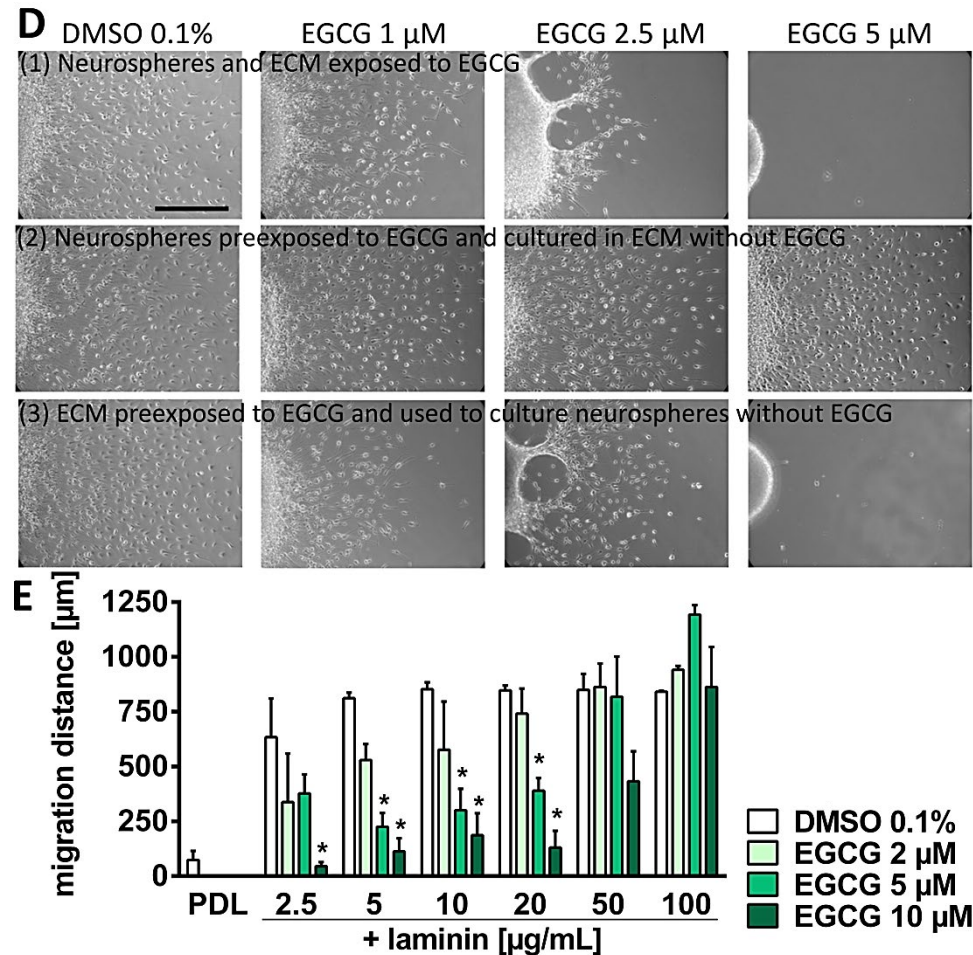
Fractions 0-10:

Laminin retained in the column

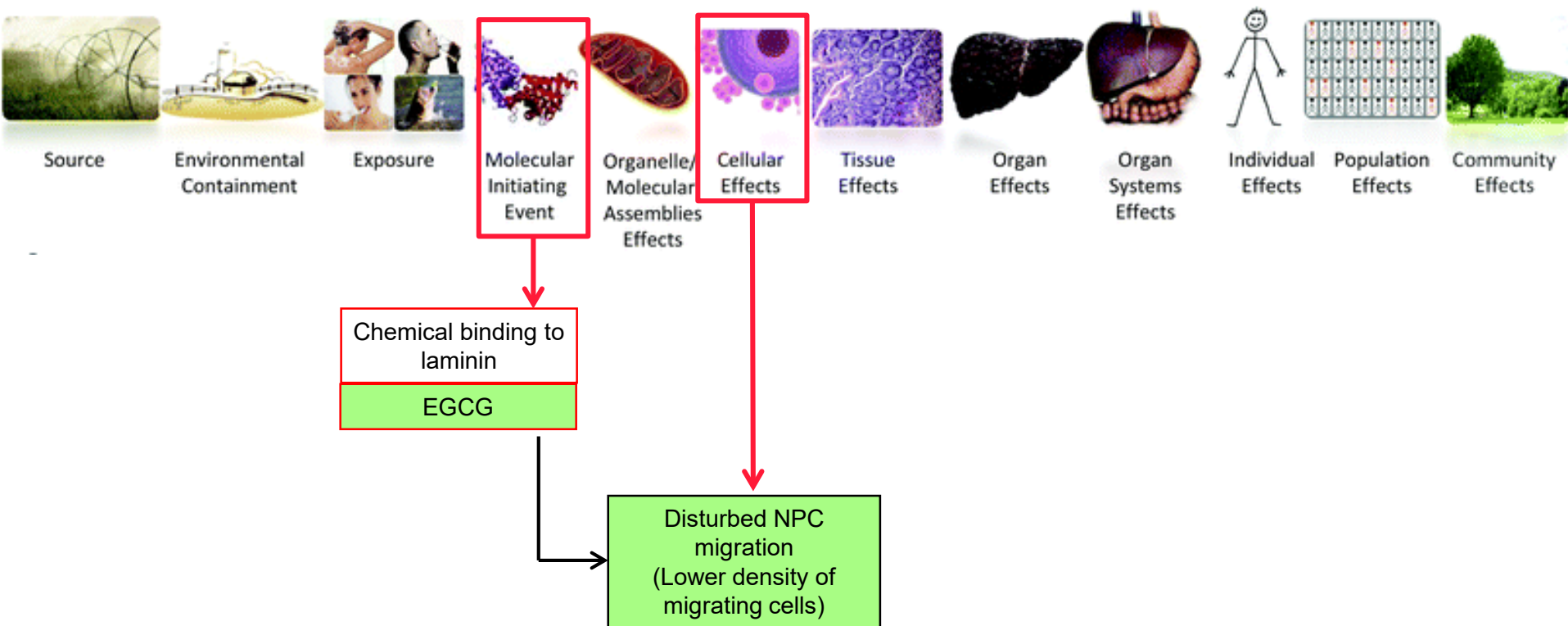
Fractions 10-20:

Laminin was eluted with a buffer
containing 4 M urea and 1 M NaCl

...



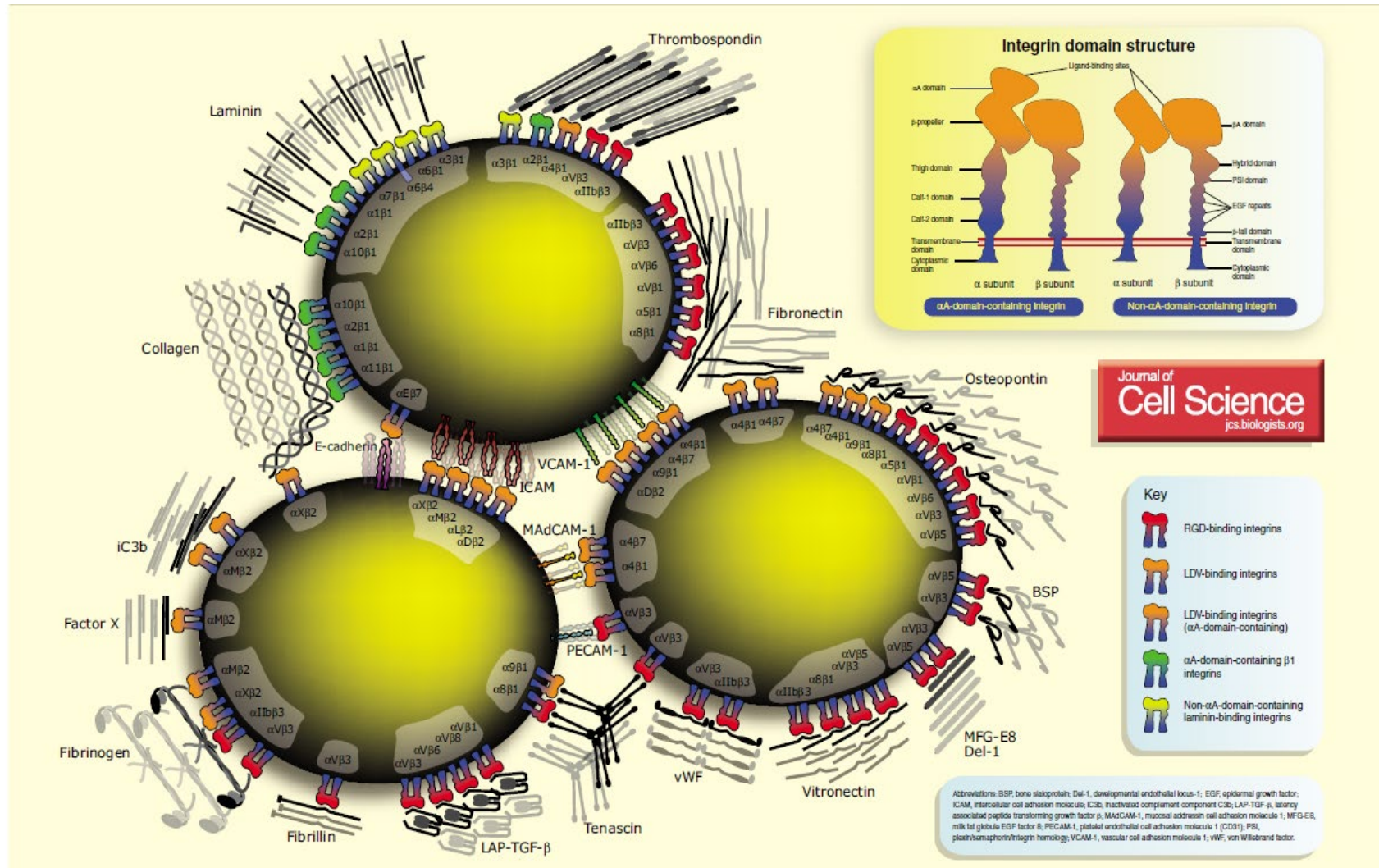
Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



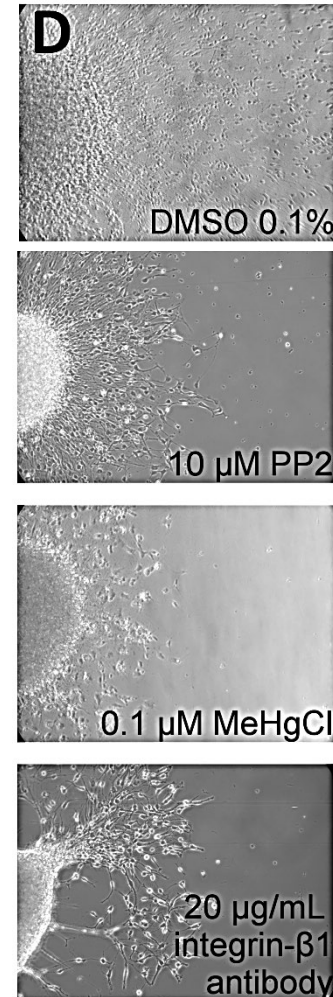
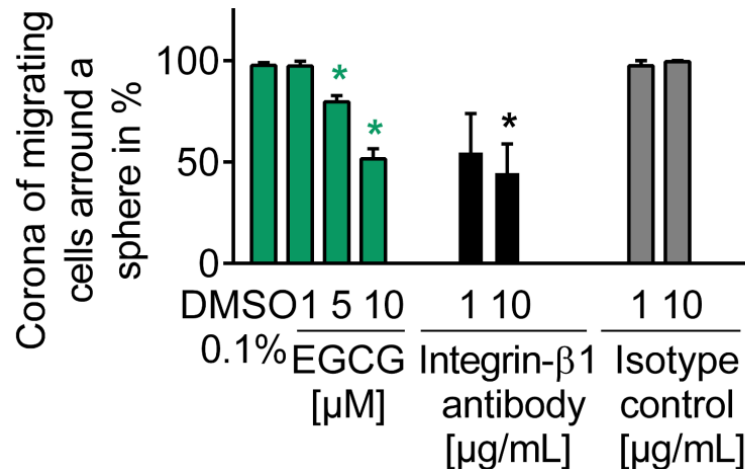
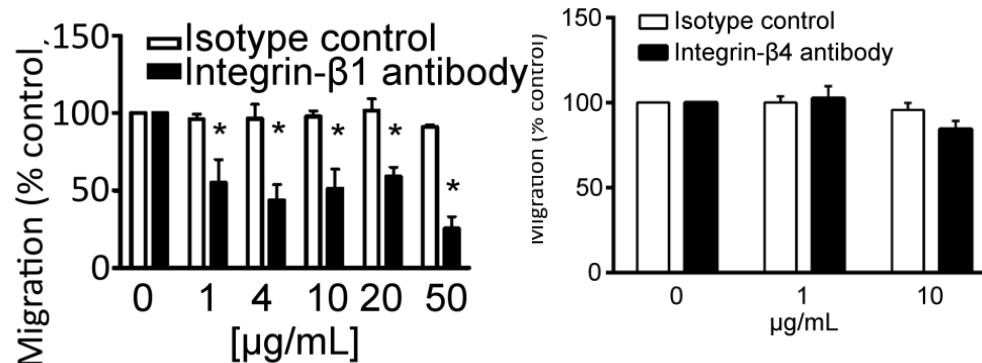
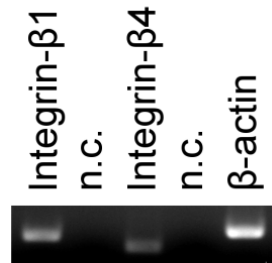
from
Ankley et al., 2009

EGCG and structurally related catechins inhibit hNPCs' adhesion to laminin

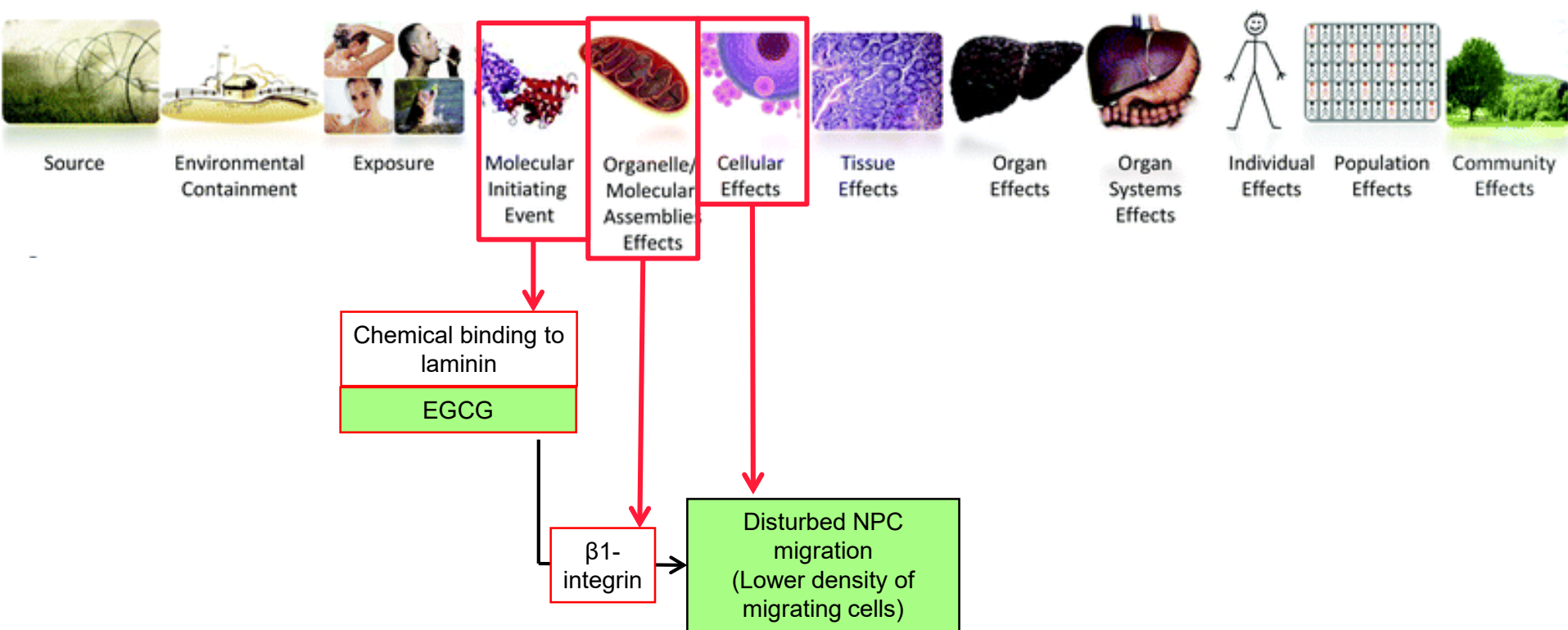
Adhesome



EGCG prevents laminin binding to integrin- β 1

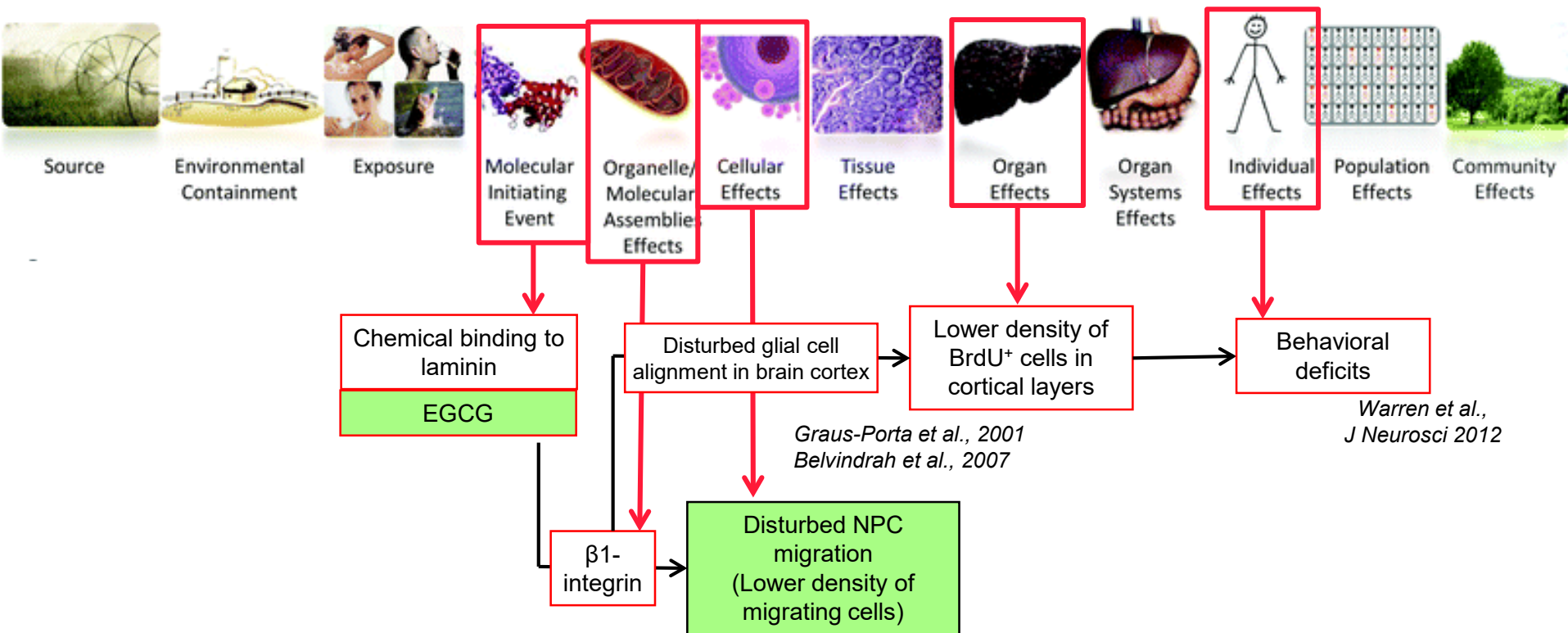


Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



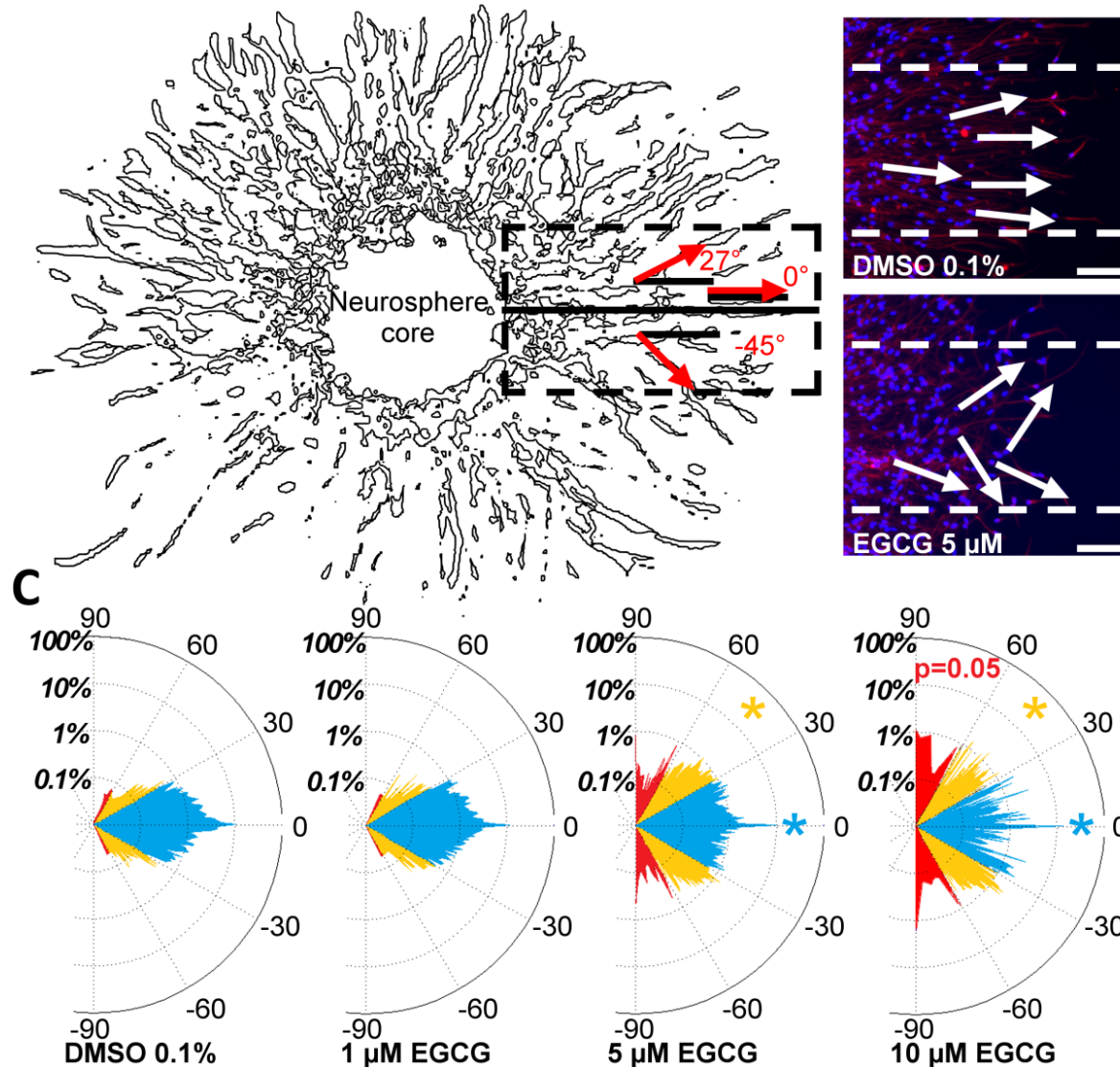
from
Ankley et al., 2009

Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



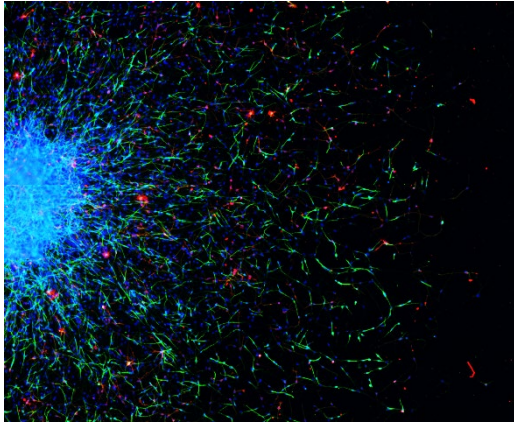
from
Ankley et al., 2009

EGCG disturbs alignment of radial glia cells

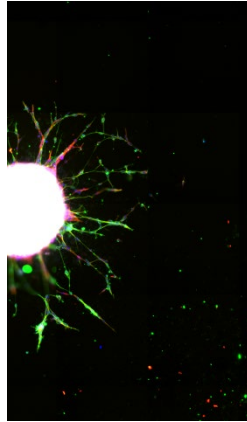


EGCG causes secondary neuronal loss

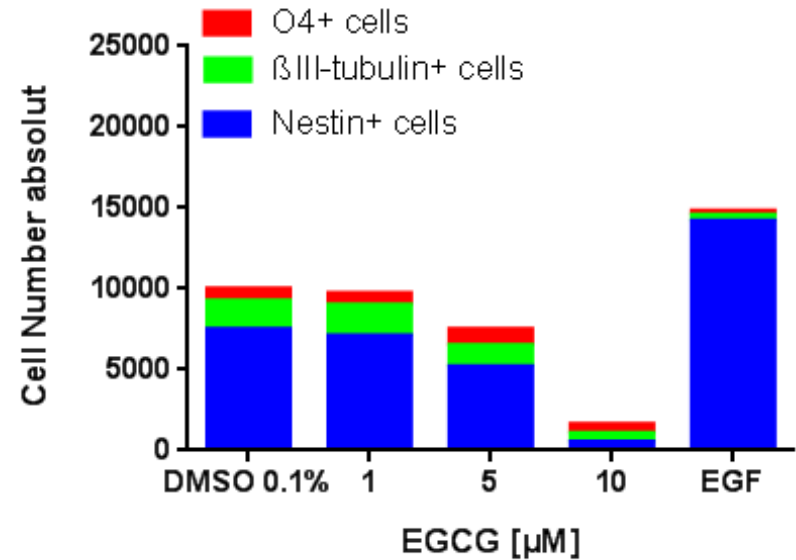
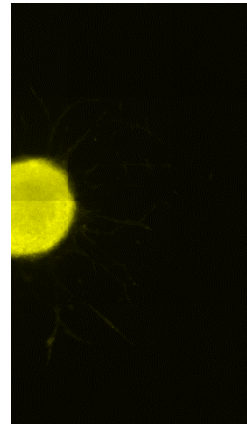
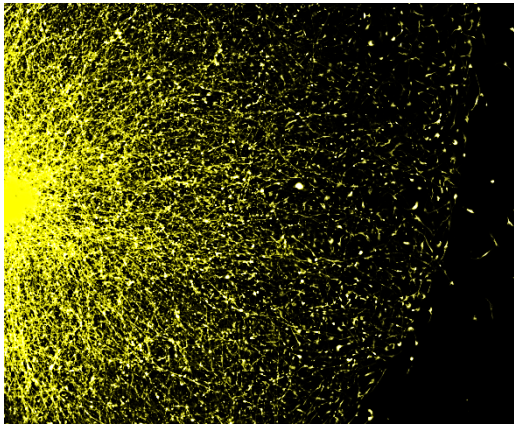
Control



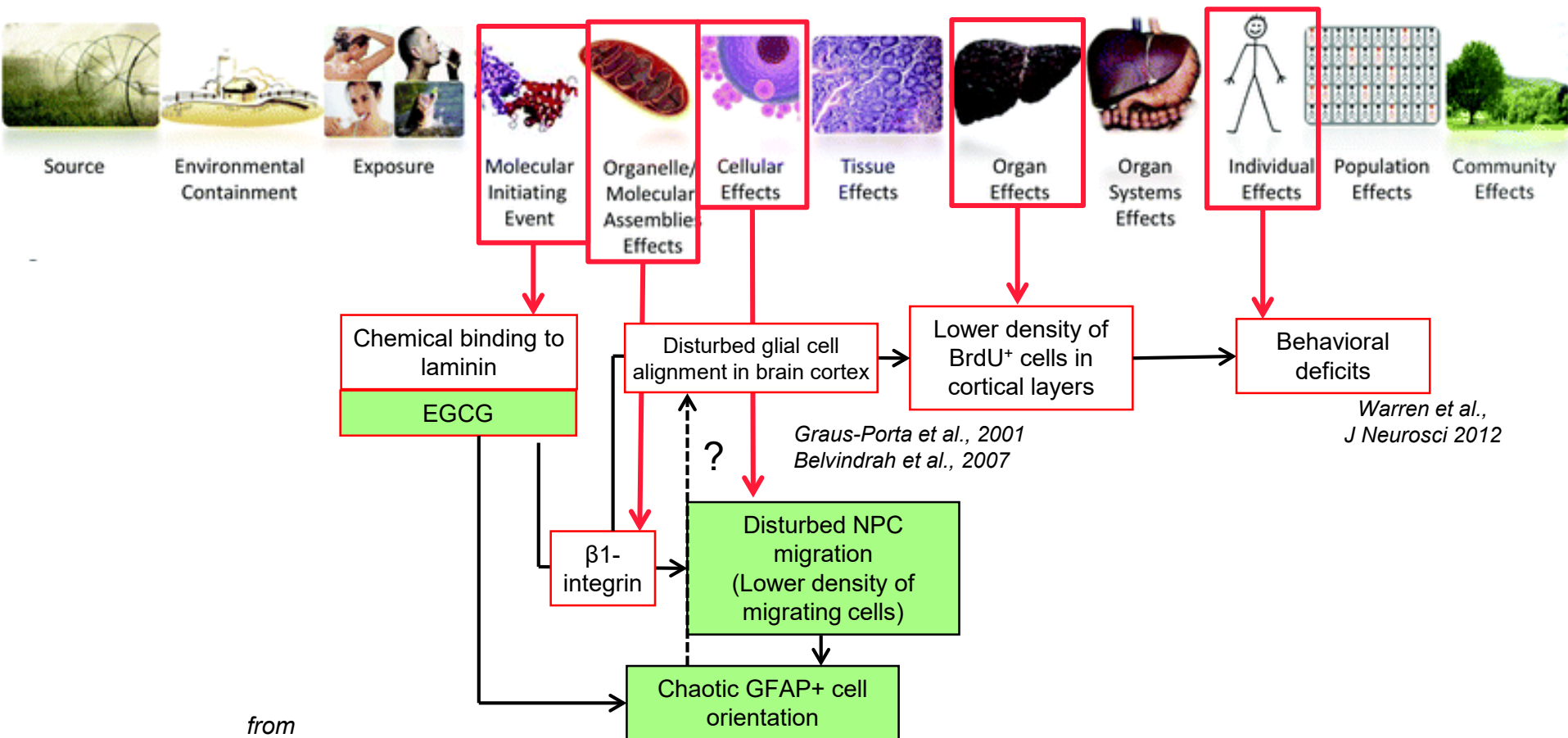
EGCG 10 μ M



β -III tubulin, O4, nestin



Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



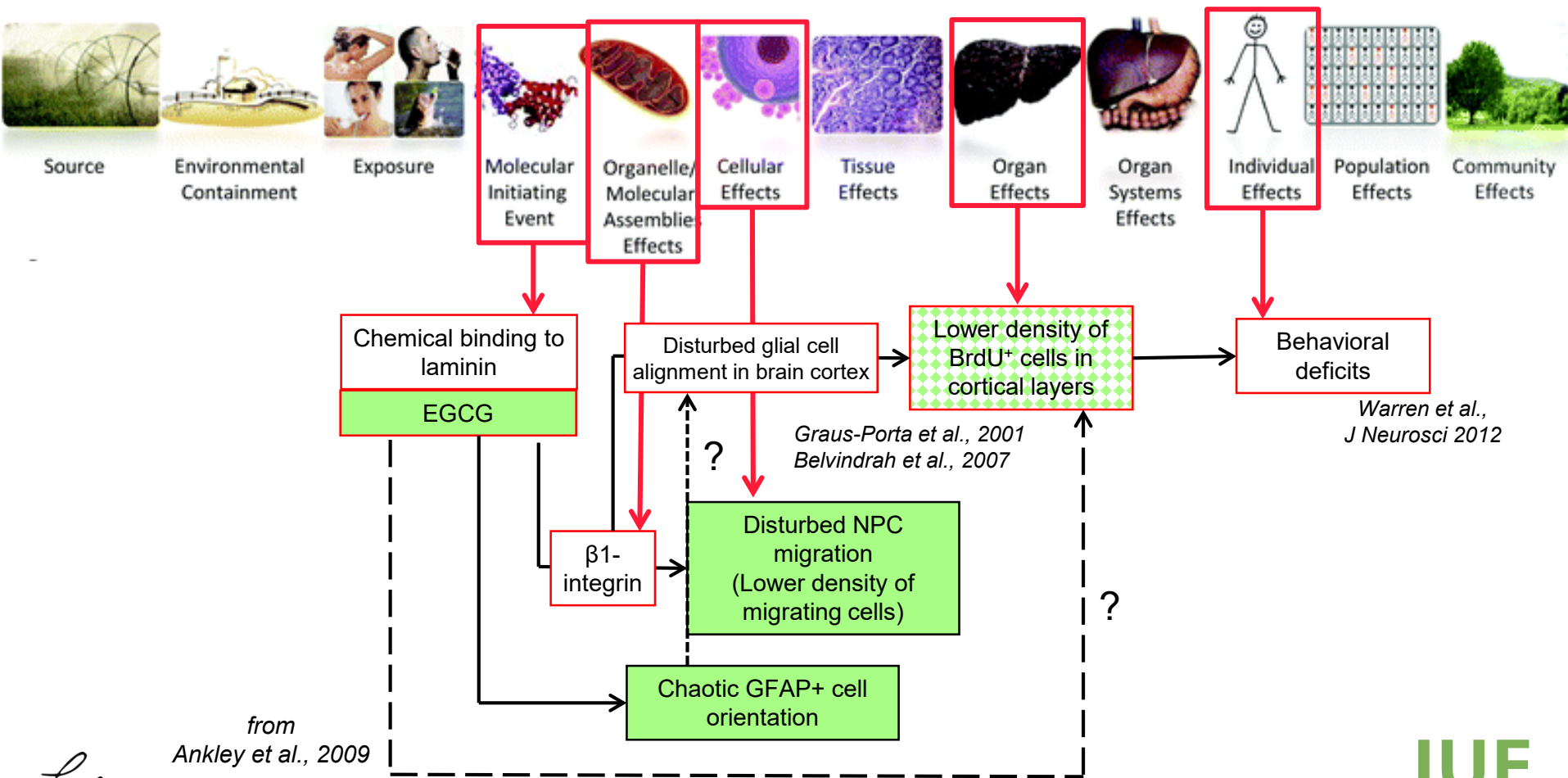
from
Ankley et al., 2009

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Bal-Price et al. Neurotoxicology 2016

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Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



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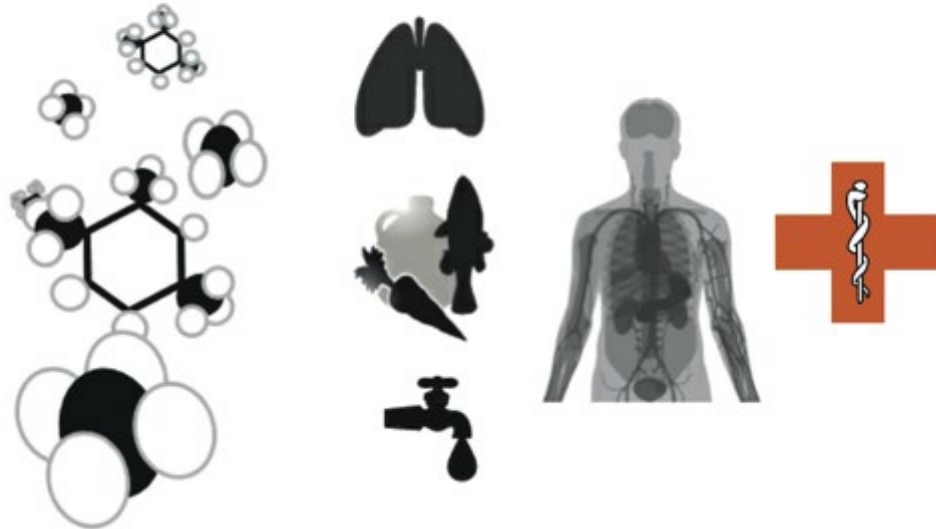
Bal-Price et al. Neurotoxicology 2016

Exposure

Source ➤ Emissions ➤ Concentration ➤ Exposure ➤ Dose ➤ Health effects



www.pinterest.com



Modified after Shonkoff et al. 2014

Putative, qualitative ➔ quantitative AOP

Pharmacokinetic assessment of EGCG food supplement intake

Pharmacokinetics



EGCG oral supplements available via the Internet:

$$\begin{array}{ccc} \text{Concentration} & & \text{Dose} \\ 0.1 \text{ g EGCG/mL} & \times \text{ (two spoons)} & = 3 \text{ g} \end{array}$$

Ullmann et al., 2003
Shanafelt et al., 2009
Chu et al., 2007
Suganuma et al., 1998
Zini et al., 2006

From EGCG **pharmacokinetics in humans**:

Dose	C_{\max} plasma
1.6 and 2 g EGCG/d	7.4 and 8.7 μM

Rev. in Barenys et al. 2015

Considering **pregnant women**, extrapolating from:

Dose	C_{\max} maternal plasma
3 g EGCG/d	11.9 to 22.2 μM

From EGCG **pharmacokinetics in rats**:

C_{\max} maternal plasma	C_{\max} fetal brain
0.6 μM	0.0761 μM

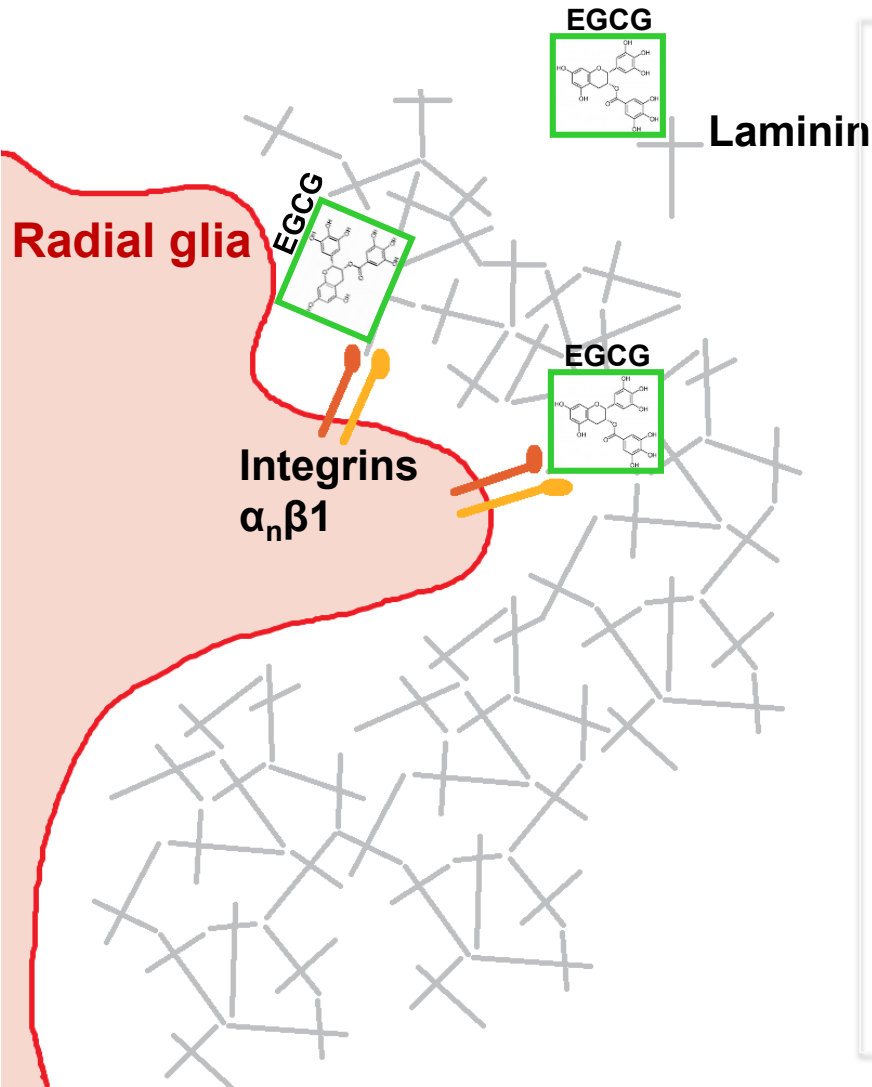
Assuming similar pharmacokinetics in pregnant rats and humans, which is supported by comparable EGCG kinetics in non-pregnant individuals

Dose	C_{\max} maternal plasma	C_{\max} fetal brain
3 g EGCG/d	11.9 to 22.2 μM	1 to 3 μM

LOAEC hNPCs

- migration	5 μM
- adhesion	1 μM
- GFAP ⁺ process orientation	5 μM
- cell density	5 μM

Summary: Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction



- High concentration EGCG disturbs NPC migration/adhesion
- MoA: EGCG binds laminin and thereby prevents laminin-integrin binding
- Disturbed adhesion causes altered radial glia alignment
- In vivo, altered integrin function causes structural and functional neurodevelopmental defects
- Pharmacokinetic considerations call for caution of high dose food supplement intake during pregnancy

CHM –天麻 (TM) Non Toxic CHM

- Tian Ma, *Gastrodia elata* Blume
- Tall Gastrodia Tuber (TGT)



CHM –雷公藤 (LGT)

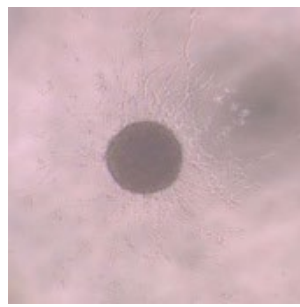
Strong Toxic CHM

- Lei Gong Teng, *Radix Et Rhizoma Tripterygii Wilfordii*
- Common Threewingnut Root (CTR)

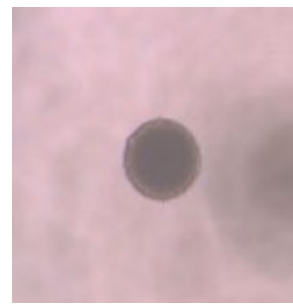




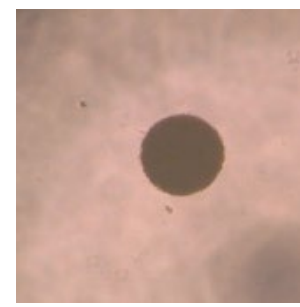
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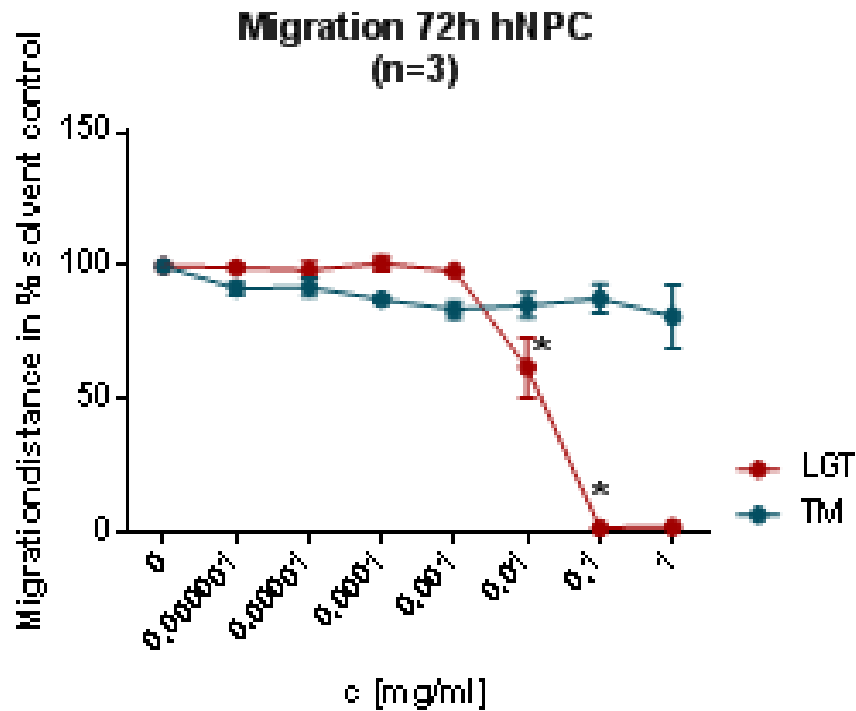
0,01mg/ml
LGT



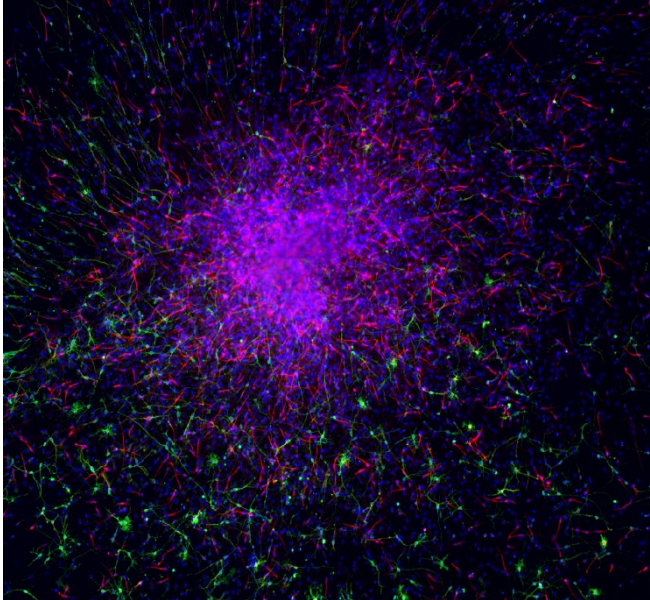
0,1 mg/ml
LGT



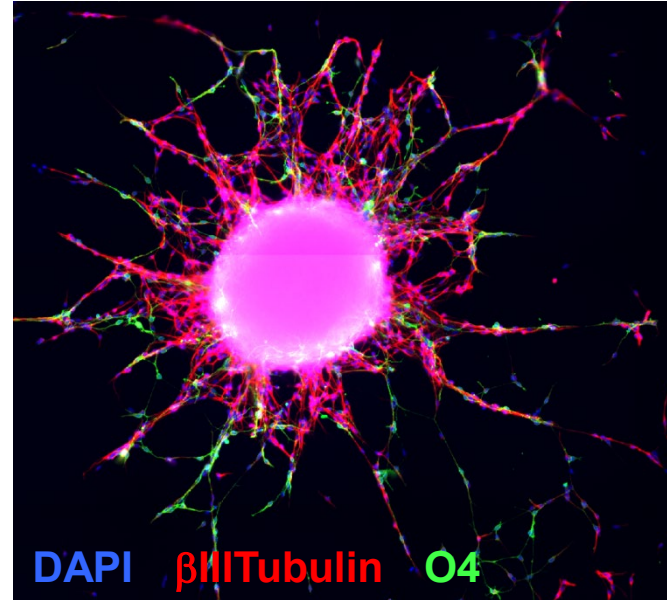
1 mg/ml
LGT



Neurosphere exposure towards LGT



control



0,01 mg/mL LGT

Acknowledgements

Dr. Julia Tigges

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Anna Wellenberg

Britta Kühne

Barbara Petzuch

Anastasia Geldaris



VIP – Validation of
innovative products



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für Bildung
und Forschung



Ministerium für Innovation,
Wissenschaft und Forschung
des Landes Nordrhein-Westfalen



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Thank you for your attention!