Ústav experimentální medicíny AV ČR, v.v.i.
EU Centre of Excellence

Krajská zdravotní, a.s.

NOVÉ POHLEDY NA BIOLOGII NERVOVÝCH BUNĚK (?)

- Definice problematiky
- Hlavní oblasti zájmu neurobiologie
- Zinek a CNS

evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

NERVOVÉ BUŇKY

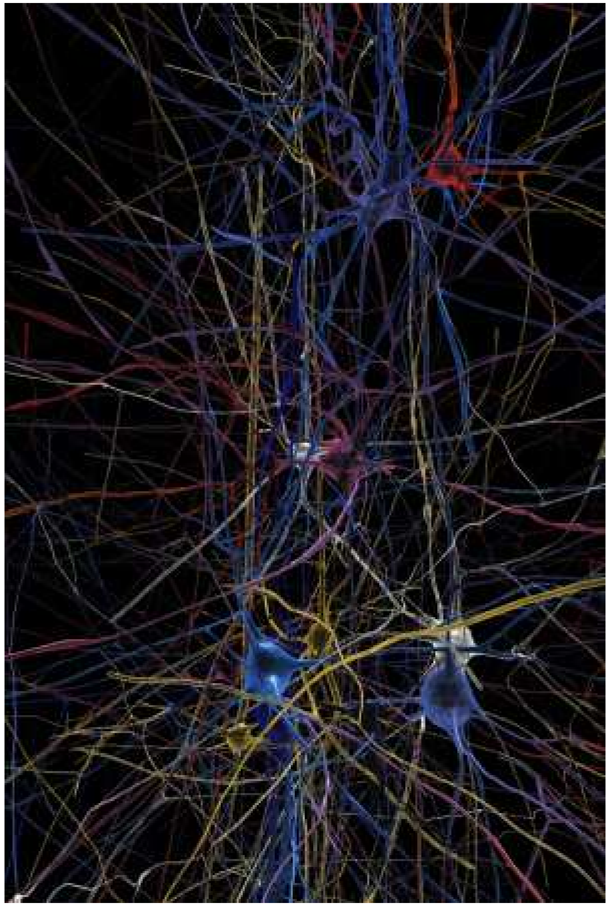
- Evoluční konzervovanost
- Superspecializace
- Elektrická excitabilita, synapse
- Regenerace a degenerace
- Plasticita
- Stabilní populace

PROČ NÁS ZAJÍMÁ BIOLOGIE NERVOVÉ BUŇKY?

1) Biologie

2) Mapování

3) Stárnutí a/Patologie



http://www.nature.com/news/big-neuroscience-billions-and-billions-maybe-to-unravel-mysteries-of-the-brain-1.12519

MAPOVÁNÍ

1) Topografie – konektomika

2) Topografie – funkční

Tomografie, Resonance, Světelná a elektronová mikroskopie

MAPOVÁNÍ

C. elegans	302
Mlž	20 000
Hmyz	>100 000
Člověk	>1 000 000 000

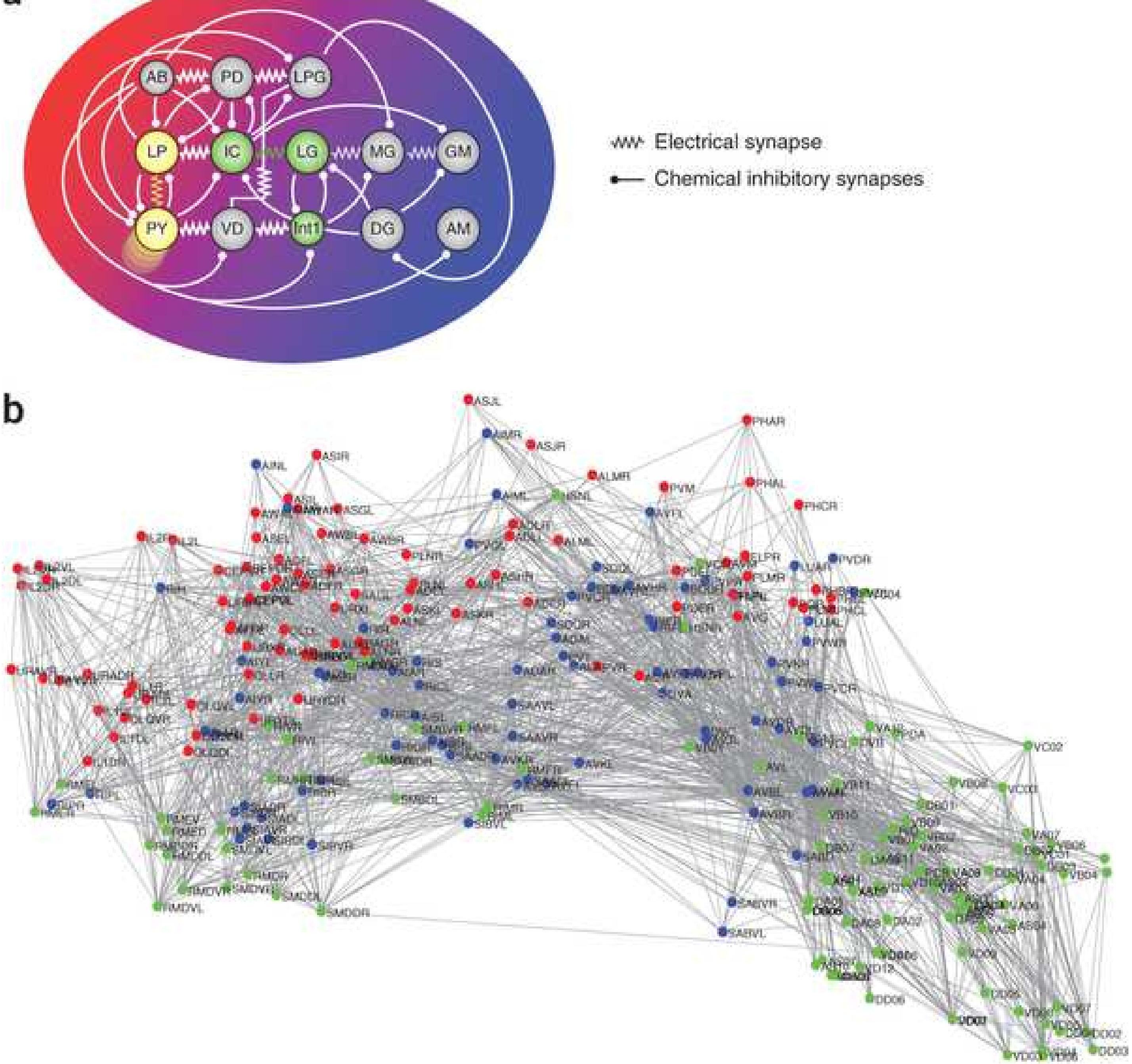
http://blog.neuinfo.org/wp-content/uploads/2011/06/celegans.jpg

http://www.neptunfood.cz/home/?acc=info-slavka-jedla-mytilus-edulis

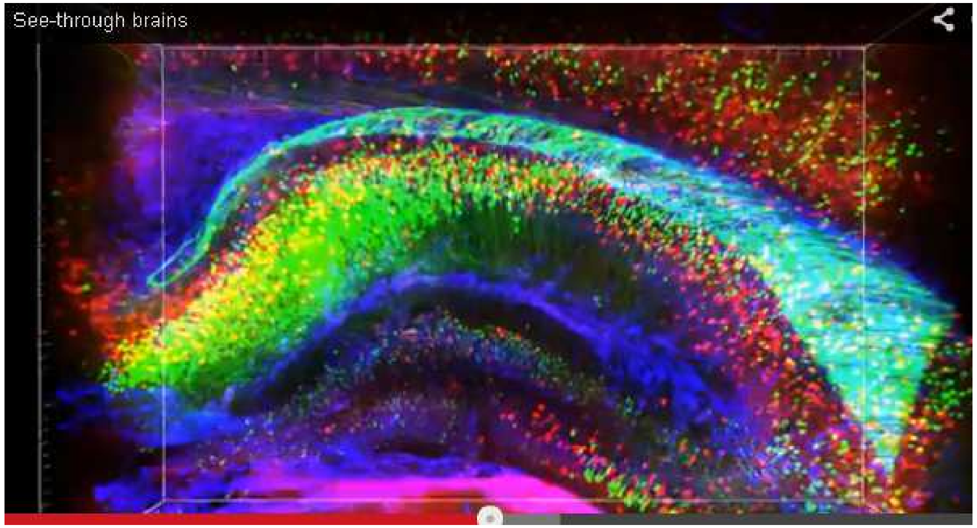
http://www.blesk.cz/tag/uhlobaron/1

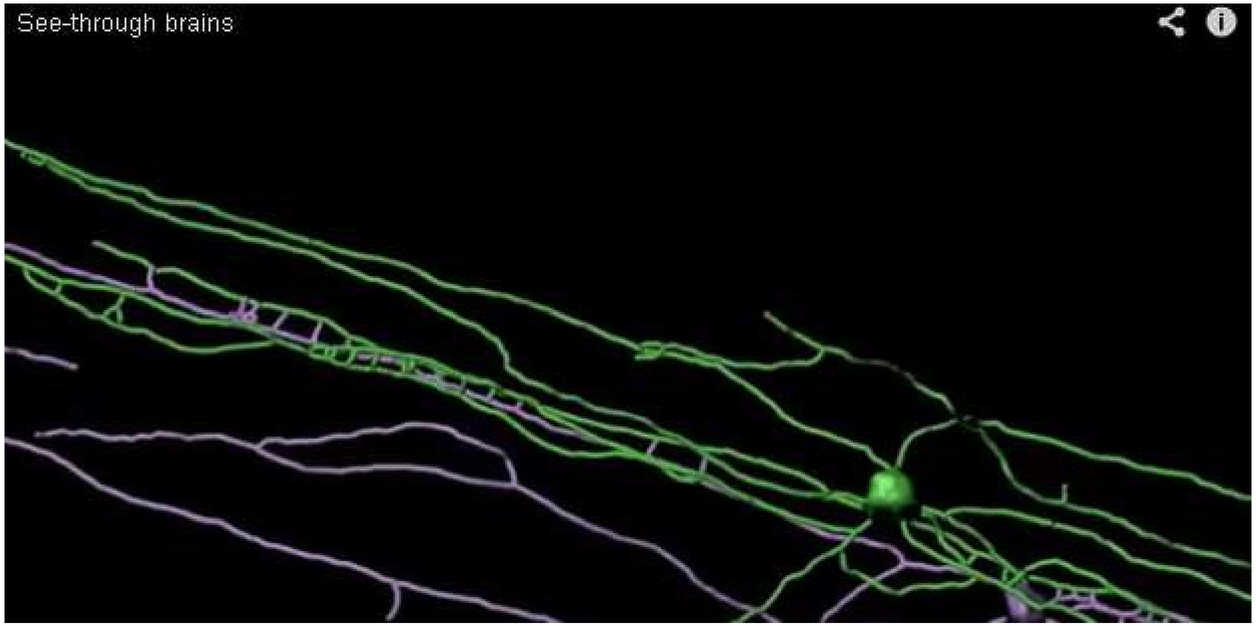
a

b


AB
PD
LPG
LP
IC
LG
MG
GM
PY
VD
Int1
DG
AM
Electrical synapse
Chemical inhibitory synapses

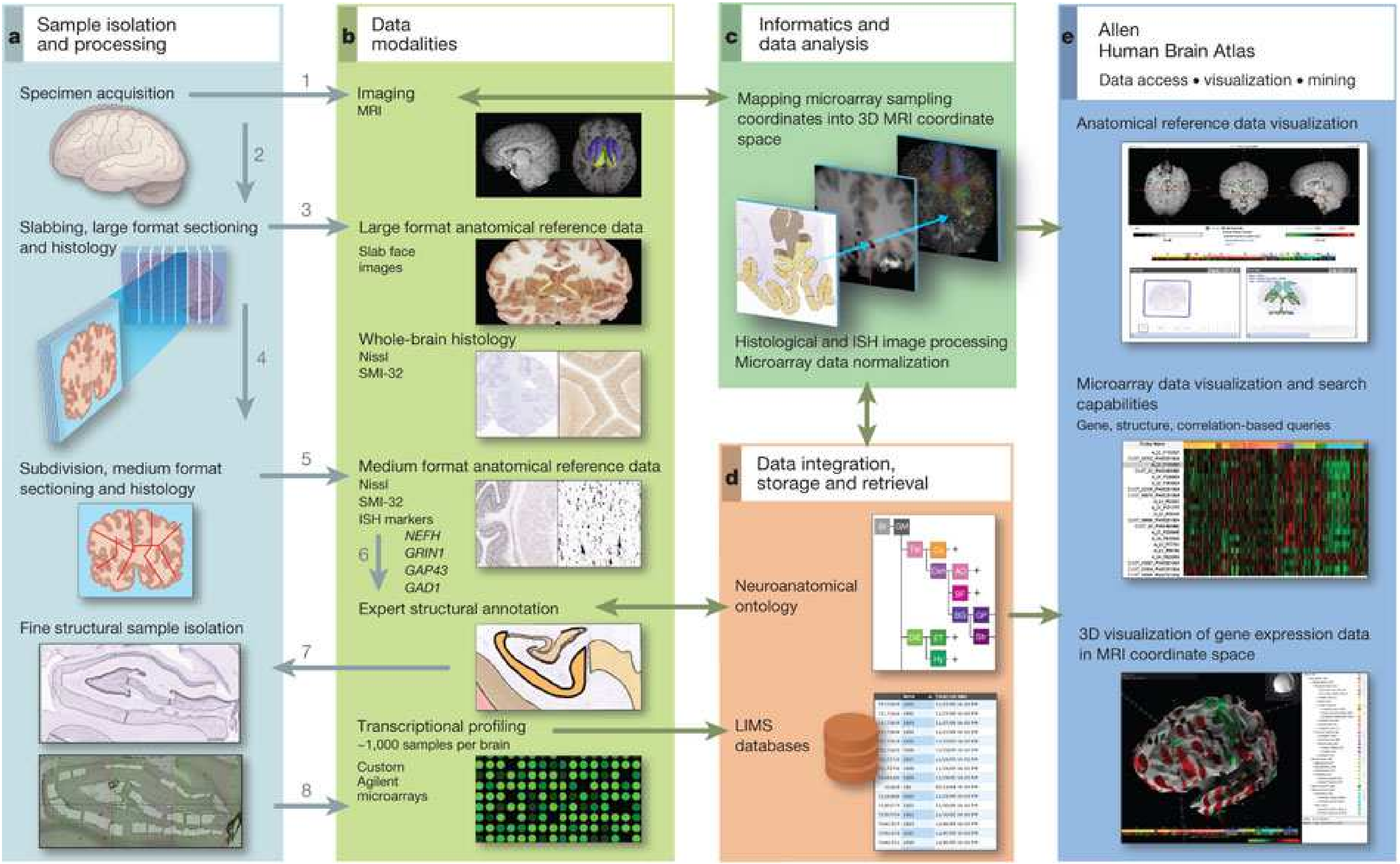
MAPOVÁNÍ I - CLARITY





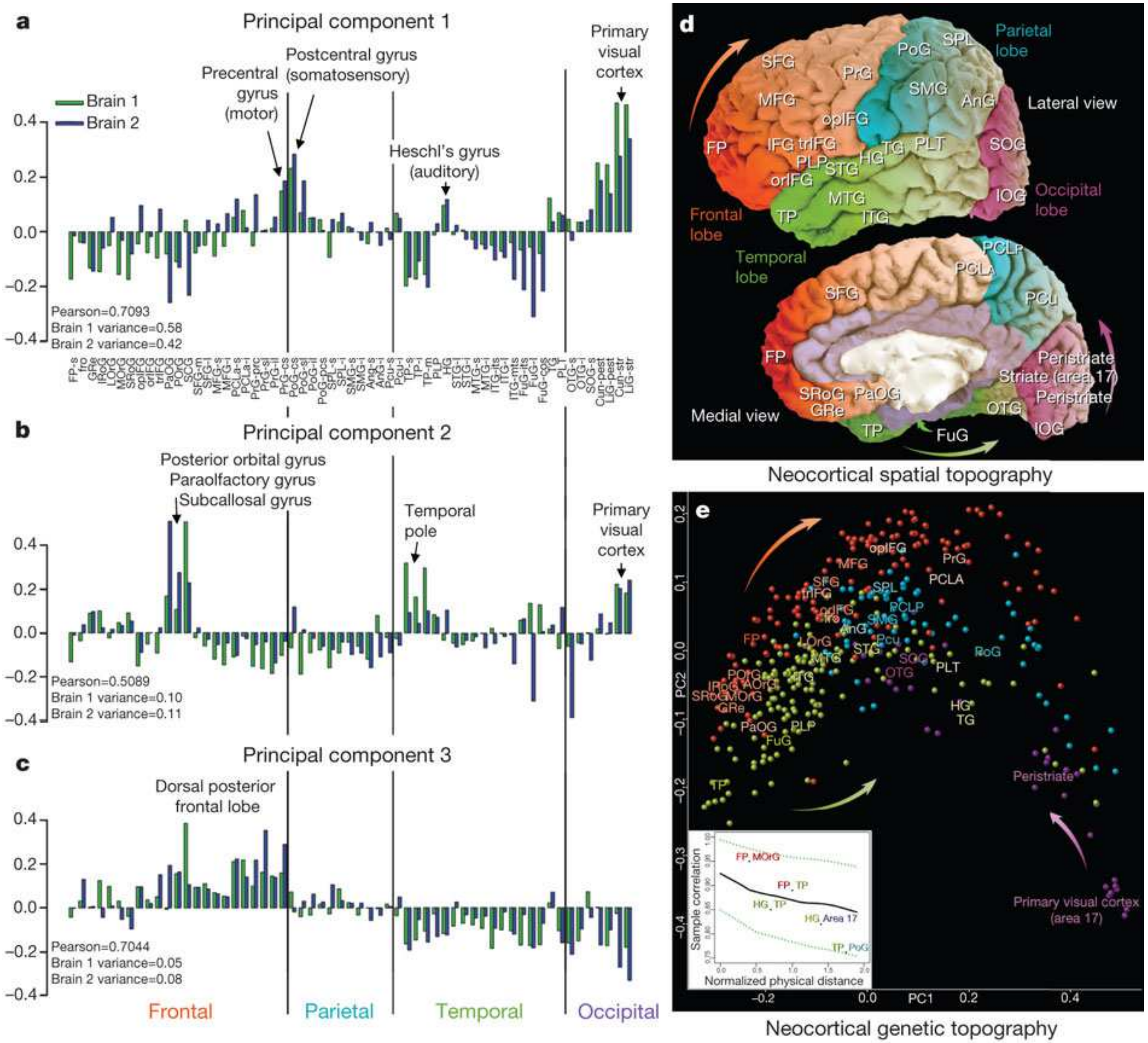
http://www.nature.com/news/see-through-brains-clarify-connections-1.12768

MAPOVÁNÍ II - TRANSKRIPTOM



MJ Hawrylycz et al. Nature **489**, 391-399 (2012) doi:10.1038/nature11405

MAPOVÁNÍ II - TRANSKRIPTOM



http://www.brain-map.org/

MJ Hawrylycz et al. Nature **489**, 391-399 (2012) doi:10.1038/nature11405

MAPOVÁNÍ III

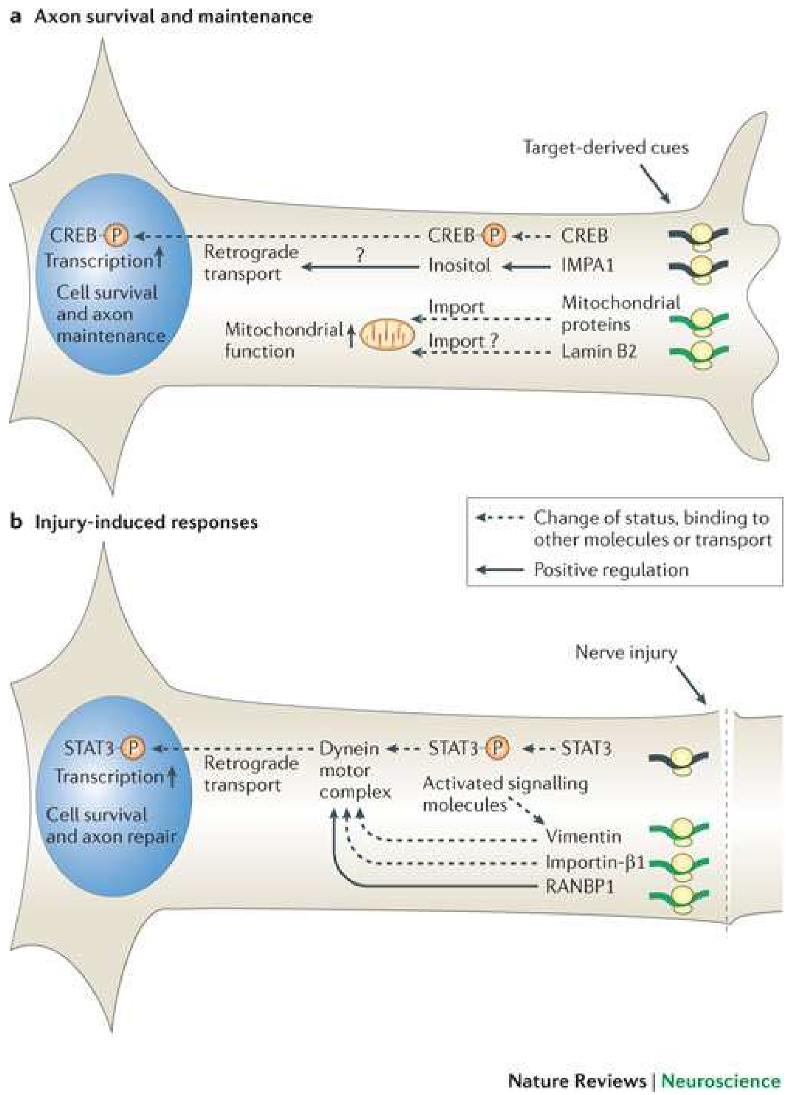
Human Brain Project

http://www.nature.com/news/whole-human-brain-mapped-in-3d-1.13245

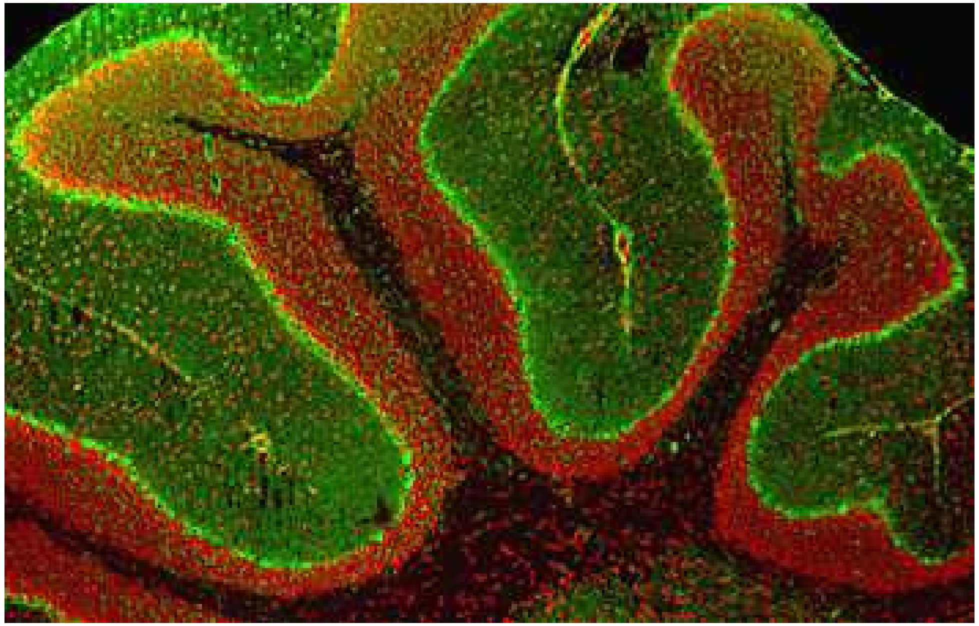
BIOLOGIE NERVOVÝCH BUNĚK

- Axonální mRNA lokalizace
- Neurální proteom – dendritický a synaptický
- Kontext ECM - proteoglykany chondroitin sulfátu (CSPGs)
- Planární buněčná polarita (PCP)
- Dendritické výběžky a rozvětvení dendritů
- Nekódující RNA
- Železo, vápník......

AXONÁLNÍ mRNA LOKALIZACE

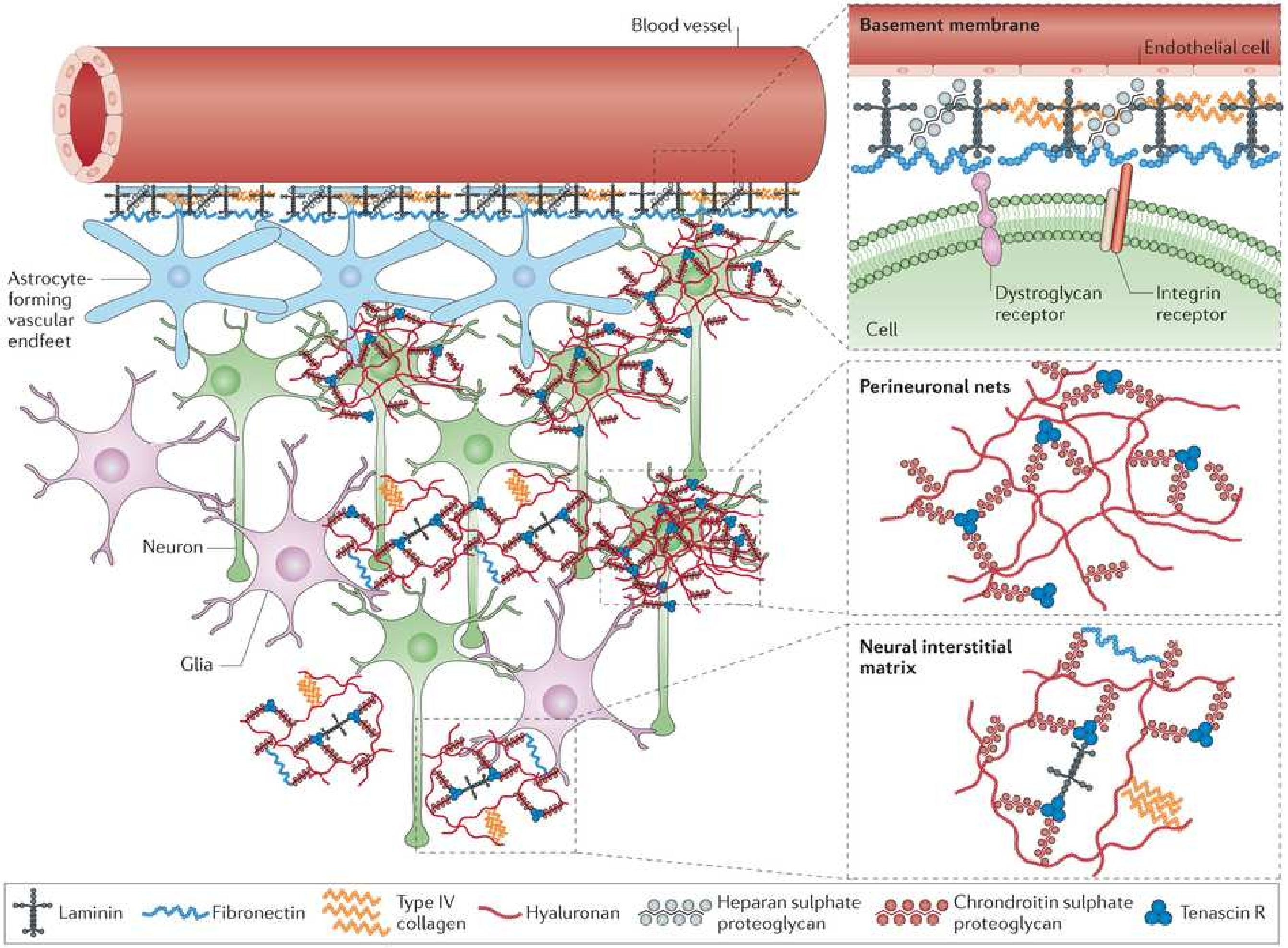


NEURONÁLNÍ PROTEOM



A neuron from the brain in which DNA has been stained red and the Dasm1 protein, which controls mammalian dendrite development, has been stained green. Dasm1 is abundantly expressed in the dendrites of neurons, but not in the axons. Image: Cover, PNAS, September 7, 2004. Copyright 2004, National Academy of Sciences, U.S.A

ECM A CNS



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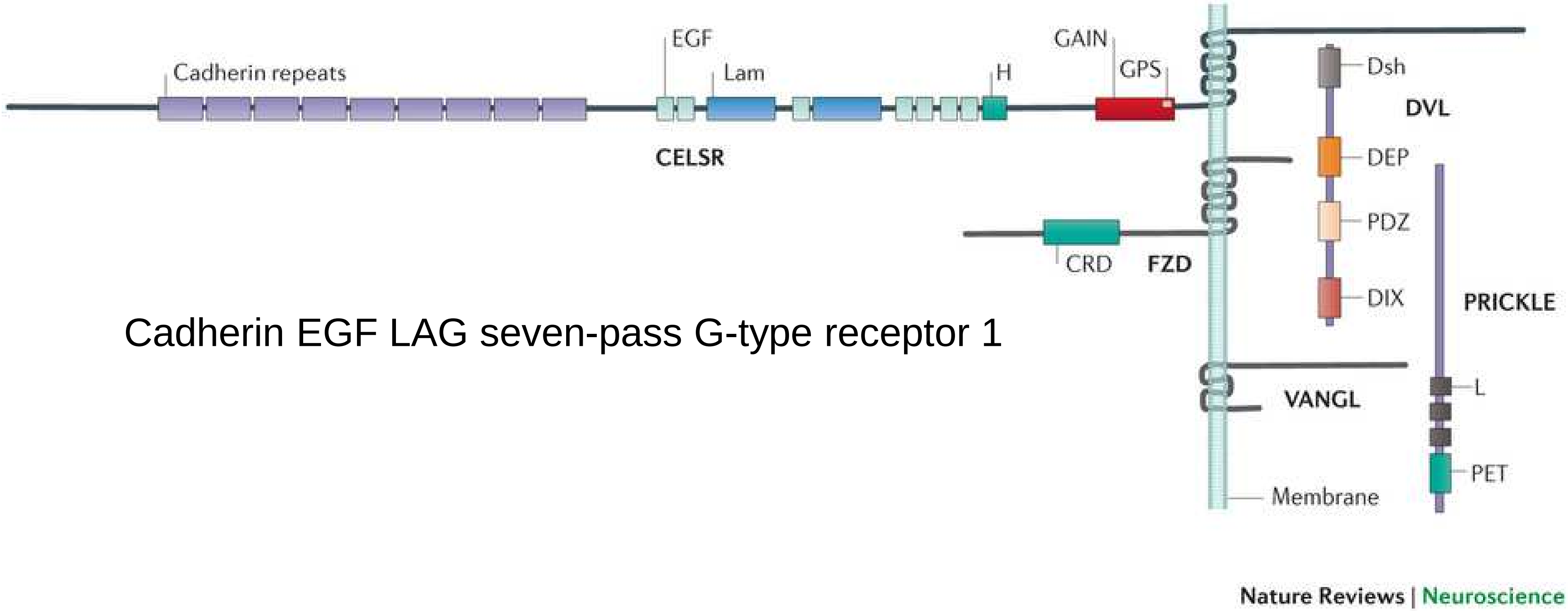
PLANÁRNÍ BUNĚČNÁ POLARITA



Cadherin EGF LAG seven-pass G-type receptor 1

http://www.nature.com/nrn/journal/v14/n8/fig_tab/nrn3525_F1.html

NEKÓDUJÍCÍ RNA

Class	Description	Refs
Antisense lncRNAs	lncRNAs derived from antisense transcription, present at ~70% of mammalian genomic loci and implicated in the regulation of sense protein-coding genes	3
Antisense termini-associated short RNAs	Small ncRNAs with 5′ poly-U tails originating from 3′ termini of genes in antisense orientation, suggesting transcription by RNA-dependent RNA polymerase	45
Centromere repeat-associated small interacting RNAs	Small ncRNAs (34–42 nucleotides) derived from centromeric repeats with putative roles in local epigenetic modifications and heterochromatin formation	47
Endogenous small interfering RNAs	Small DICER-dependent ncRNAs (21–26 nucleotides) associated with Argonaute proteins (AGO2) and involved in post-transcriptional and epigenetic silencing of protein-coding genes and transposons	33
Enhancer RNAs	lncRNAs transcribed from enhancer domains of, and expressed coordinately with, activity-dependent neuronal genes	109
Enhancer-like long ncRNAs	lncRNAs exhibiting enhancer activity, particularly for genes regulating development and differentiation	21
Large intergenic RNAs	lncRNAs derived from intergenic regions that act as guides for recruiting PRC2, CoREST and other chromatin-modifying complexes	17,18
lncRNAs	Large family of ncRNAs (>200 nucleotides) with diverse functional roles	24
MicroRNA-offset RNAs	Small ncRNAs (~20 nucleotides) produced from microRNA precursors and exhibiting independent expression relative to associated microRNAs, implying distinct functional roles	46
MicroRNAs	Small DICER-dependent ncRNAs (20–23 nucleotides) associated with Argonaute proteins (AGO1–4) and involved in post-transcriptional silencing of protein-coding genes and ncRNAs	28,29
Mitochondrial ncRNAs	Small ncRNAs and lncRNAs generated from both strands of the mitochondrial genome, regulated by nuclear-encoded mitochondrial proteins and expressed in cell type- and tissue-specific patterns	23,51
PIWI-interacting RNAs	Small ncRNAs (26–30 nucleotides) associated with the PIWI subclass of Argonaute proteins and involved in silencing of mRNAs and transposons	34
Promoter-associated long RNAs	lncRNAs transcribed from promoter domains of protein-coding genes, particularly cell cycle modulators, and capable of recruiting regulatory factors	20,150
Promoter-associated small RNAs	Small ncRNAs (20–200 nucleotides) possessing 5′ ends that coincide with the transcription start sites of protein-coding genes and ncRNAs	43
Small nucleolar RNAs	Small ncRNAs derived from intronic regions with roles in promoting RNA modifications, including pseudouridylation (H/ACA snoRNAs) and methylation (C/D snoRNAs), as well as pre-mRNA processing	36
Small RNAs derived from small nucleolar RNAs	Small ncRNAs derived from 3′ ends of H/ACA snoRNAs (20–24 nucleotides) and 5′ ends of C/D snoRNAs (17–19 nucleotides and >27 nucleotides) with microRNA-like functions	41
Small nuclear factor 90-associated RNAs	Small ncRNAs (117 nucleotides) associated with the nuclear factor 90 RBP and exhibiting accelerated evolution and expansion in hominids and region-specific expression in human brain	151
Splice-site RNAs	Small ncRNAs (17–18 nucleotides) derived from splice sites of highly transcribed genes, and expressed in developmental stage- and region-specific patterns	46
Telomere small RNAs	Small DICER-independent telomere-specific ncRNAs (~24 nucleotides)	48
Telomere repeat-containing RNAs	lncRNAs transcribed from telomeric repeats and dynamically regulated during the cell cycle with roles in heterochromatin formation and telomere functioning	22
Termini-associated short RNAs	Small ncRNAs originating from 3′ termini of protein-coding genes and ncRNAs	24
Transcription initiation RNAs	Small ncRNAs (18 nucleotides) originating downstream of transcription start sites of protein-coding genes, implicated in modulating CTCF localization and nucleosome density	44,52
Transcription start site associated RNAs	Small ncRNAs (20–90 nucleotides) originating from the -250 to +50 position relative to the transcription start site of protein-coding genes	152
tRNA-derived RNA fragments	Small ncRNAs (19–40 nucleotides) derived from cleavage of mature tRNAs constitutively by DICER and in response to stress by angiogenin (in humans), promoting stress granule assembly and inhibiting mRNA translation	49
3′-untranslated region-associated ncRNAs	lncRNAs derived from 3′-untranslated regions of protein-coding transcripts and exhibiting independent developmental stage- and tissue-specific expression profiles	19
Vault RNAs	Small ncRNAs (88–100 nucleotides) integral to the vault RNP complex with putative roles in multidrug resistance, apoptosis resistance and innate immunity	153,154
Y RNAs	Small ncRNAs (~100 nucleotides) required for DNA replication, cleaved into microRNAs and implicated in Ro RBP localization and function	155,156

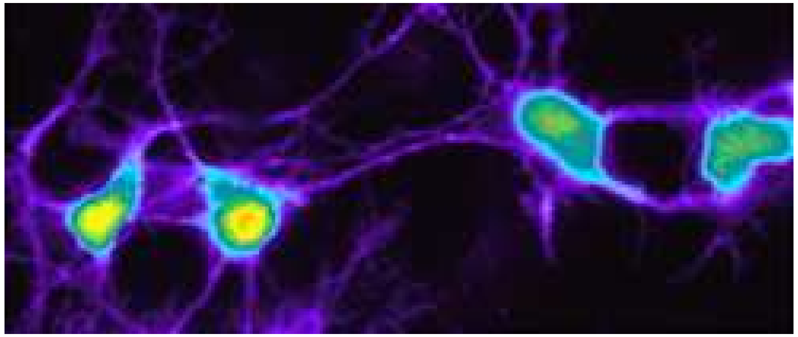
CoREST, repressor element 1-silencing transcription factor corepressor 1; CTCF, CCCTC-binding factor; lncRNA, long non-coding RNA; ncRNA, non-coding RNA; PRC2, Polycomb repressive complex 2; RBP, RNA-binding protein.

ZINEK – POHLEDEM VĚDY

- Nutriční specialista - fyziolog
- Biochemik
- Ekolog a toxikolog
- Genetik
- Neurovědec (neurobiolog)

ZINEK – BIOLOGICKÉ FUNKCE

- Buněčná integrita - cytoprotekce
- Proliferace
- DNA syntéza a genová exprese
- Enzymatická katalýza
- Signalizace
- Reparace a regenerace
- Buněčná smrt



http://www-dsv.cea.fr/en/institutes/institute-of-life-sciences-research-and-technologies-irtsv/laboratories/chemistry-and-biology-of-metals-laboratory-lcbm/biology-of-metals/transmembrane-transport-and-storage-of-zinc-in-neurons.-roles-of-trpc6-channels

ZINEK – INTRACELULÁRNÍ SIGNALIZACE

- Specifické senzory kovů, transportéry (DTC, ZnT) a vazné proteiny
- Influxní a effluxní mechanizmy
- Labilní (volná) a vázaná forma

VÁZANÁ

- Proteiny obsahující zinek (metallothioneiny)
- Proteiny regulované zinkem (tubulin)
- Protein-zinkové komplexy (zinek-inzulin)

VOLNÁ

- Zinkozomy

ZINEK – KONCENTRACE

- Intracelulární celková – nM až mM
- Intracelulární volná – pM až nM
- Nejvyšší koncentrace – prostata
  - – pankreas
  - – CNS

ZINEK – NEURONY V CNS

1) Intracelulární vázaný (80%)

2) Intracelulární volný (?)

3) Synaptický (vezikule) (10%) – 300 μM

GLUZINERGICKÉ NEURONY

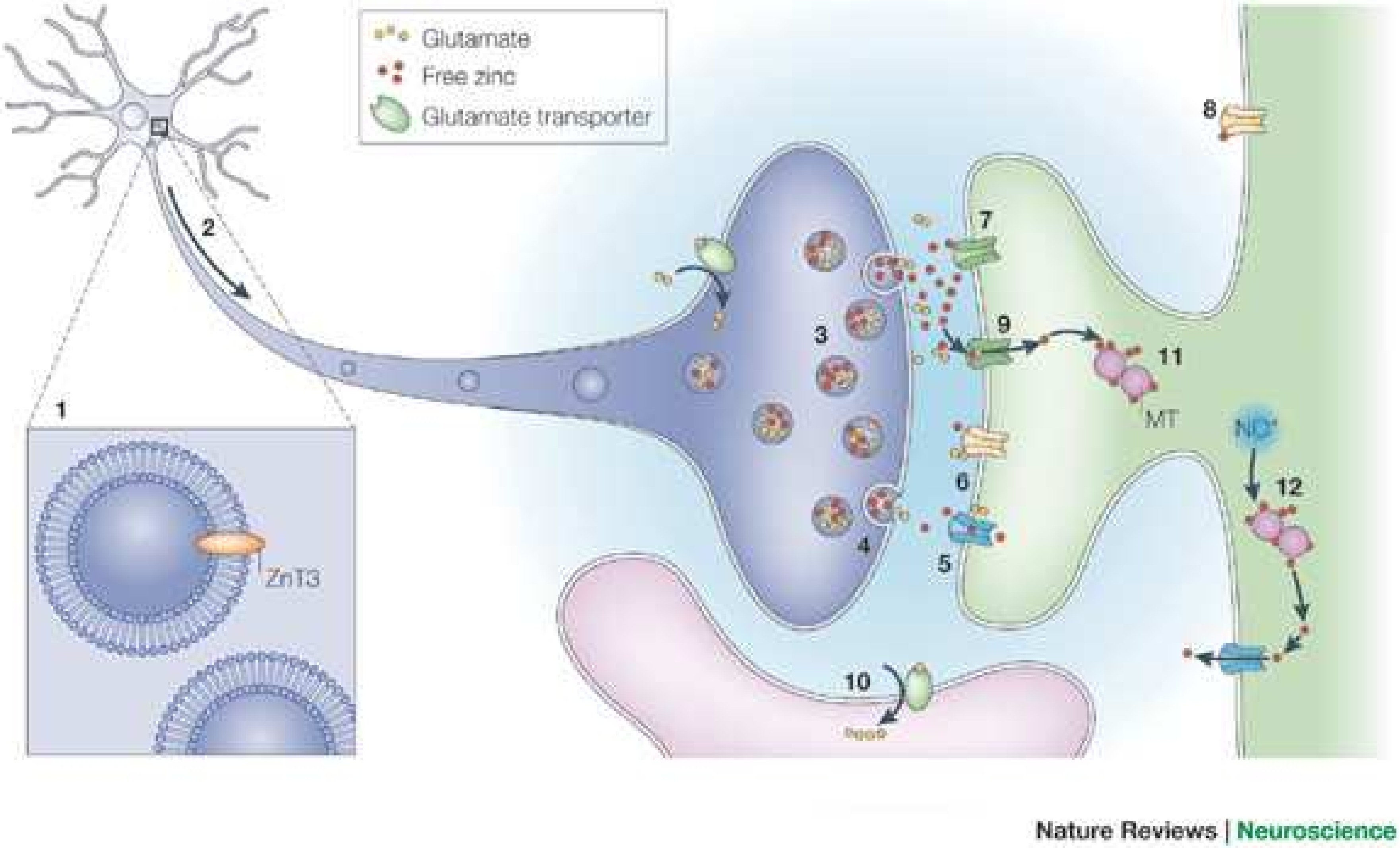


Table 1 | **Zinc-sensitive targets in the central nervous system**

Specific protein	Main effect of zinc	References
Glutamate receptors		
AMPA	Up- and downregulates	254
NMDA	Tonic downregulation Phasic effect disputed	99,255
Metabotropic	Downregulates	256
Other receptors		
$GABA_A$	Mostly downregulates	17,257
$GABA_B$	Mimics, downregulates	258
Glycine	Upregulates	125
Sigma 2	Mimics	259,260
Acetylcholine	Up- and downregulates	124
Adenosine	Up- and downregulates	121
Serotonin	Downregulates	261
Dopamine	Up- and downregulates	117,118
Catecholamine	Up- and downregulates	120
Melanocortin	Upregulates	262
Zinc receptor	Mobilizes intracellular Ca^{2+}	134
Proton receptor	Zinc–proton synergy	89
Opioid	Downregulates	263
Channels		
Ca^{2+}	Blocks, inhibits	264
K^+	Up- and downregulates	265
Na^+	Mixed	102,266
Cl^-	Facilitates	267
Ca^{2+}-amyloid	Blocks	268
Transporters		
Glutamate	Decreases uptake	135,269
Dopamine	Decreases uptake	136,270

AMPA, α-amino-3-hydroxy-5-methyl-4 isoxazole propionic acid; $GABA_A$/$GABA_B$, γ-aminobutyric acid type A/B; NMDA, *N*-methyl-D-aspartate.

NEUROBIOLOGIE ZINKU

- Iont vs molekula

- Chemické chování

ZINEK A MOZEK

- Inhibice excitačních a inhibičních receptorů

- Normální mozek – redukce excitability (endogenní antikonvulzant)

- Aktivní role při synaptické plasticitě (učení a paměť)

ZINEK, MOZEK A TOXICITA

- Eukaryotní buňky umírají – 100 nM Zn
- 1988 (potkaní model)
- ZnT3 knock-out myši
  - CA3 oblast hipokampu
  - CA1 a thalamické neurony
- Oxid dusnatý a MT3

ZINEK A NEURODEGENERATIVNÍ STAVY

- Alzheimerova choroba

  - β amyloid

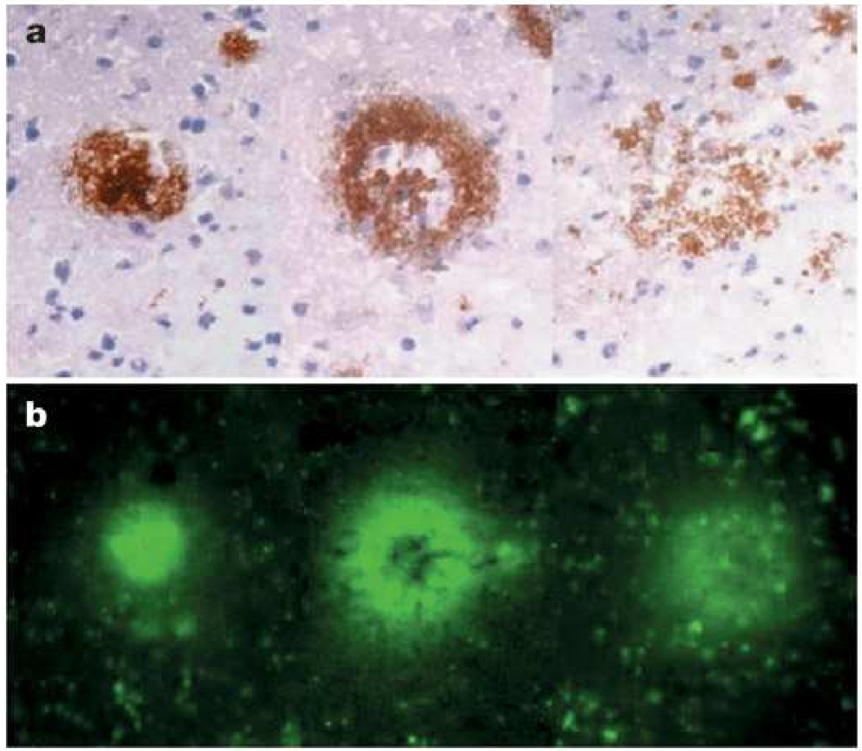


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Table 2 | **Metal levels in patients with Alzheimer's disease and healthy individuals**

Location	Zinc μg g^{-1} (μM)*	Copper μg g^{-1} (μM)*	Iron μg g^{-1} (μM)*
Plaque rim	67 (1024)‡	23 (357)‡	52 (938)‡
Plaque core	87 (1327)‡	30 (474)	53 (951)‡
Total senile plaque	69 (1055)‡	25 (393)‡	53 (940)‡
Alzheimer's neuropil	51 (786)§	19 (304)	39 (695)
Control neuropil	23 (346)	4 (69)	19 (338)

*Numbers in brackets represent molar concentrations, which were converted with the assumption of a sample density equivalent to 1 g cm^{-3}; ‡$p<0.05$ (plaque values compared with neuropils from patients with Alzheimer's disease); §$p<0.05$ (neuropils from patients with Alzheimer's disease compared with neuropils from control individuals). Adapted from REF. 195.

ZINEK JAKO TERAPEUTICKÝ CÍL

- Inhibice NOS (excitotoxické poškození)
- Pufrace volného zinku (clioquinol - AD)
- Pyruvát

NEUROBIOLOGICKÉ PERSPEKTIVY

- Hloubková topografie NS
- Definice signálů vnějšího prostředí
- Interaktom