POKROKY V NEUROVĚDÁCH 2019

Iontové kanály excitabilních buněk I.

Ionotropní glutamátové receptory

Prof. Ladislav Vyklický

13:00 – 13:40 (35+5)

Genetika ionotropních glutamátových receptorů ve vztahu k neurodevelopmentálním a neuropsychiatrickým onemocněním

Dr. Aleš Balík

13:40 – 14:20 (35+5)

Synaptická plasticita: Mechanismy LTP a LTD v hipokampu

Dr. Miloslav Kořínek

14:20 - 15:00 (35+5)

Excitotoxicita

Dr. Tereza Smejkalová

15:00 - 15:40 (35+5)

Napěťově aktivované iontové kanály a kanálopatie

Dr. Jan Krůšek

15:40 - 16:20 (35+5)

Ionotropní glutamátové receptory

POKROKY V NEUROVĚDÁCH 2019

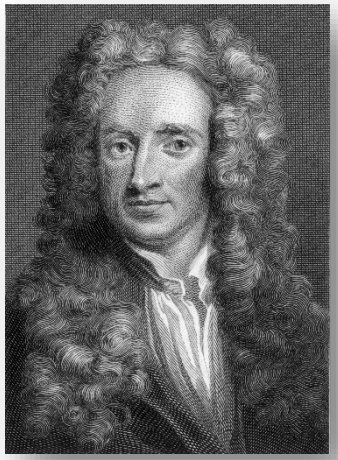
Prof. MUDr. Ladislav Vyklický DrSc

Oddělení buněčné neurofyziologie
Fyziologický ústav AVČR
Vídeňská 1083

Ladislav.Vyklicky@fgu.cas.cz

Newtonův gravitační zákon

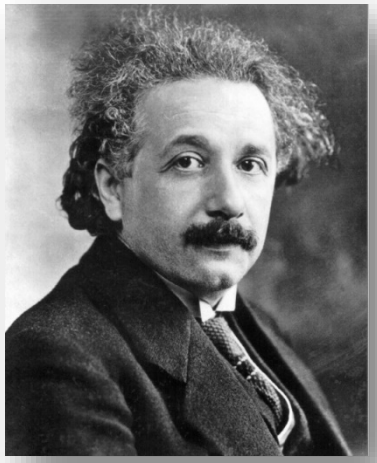
$$F_g = G\frac{m_1 m_2}{r^2}$$



Isaac Newton 1643 –1727

Ekvivalence energie a hmotnosti

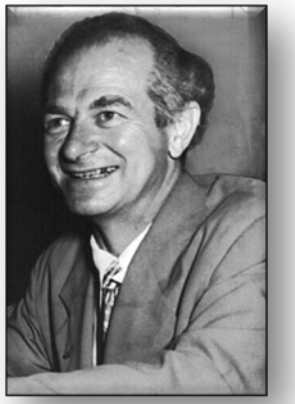
$E = mc^2$



Albert Einstein 1879 – 1955
1921 - Nobelova cena za fyziku

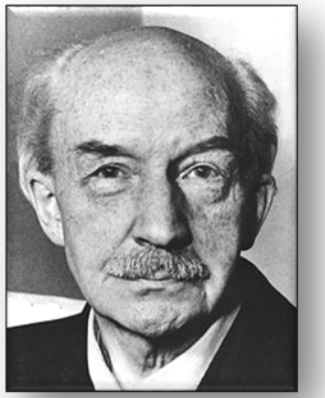
Kvantověmechanický model atomu

Louis Victor Pierre Raymond vévoda de Broglie 1892 – 1987
1929 – Nobelova cena za fyziku - objev vlnově korpuskulárního dualismu částic



Linus Carl Pauling 1901 –1994
1954 - Nobelova cena za výzkum podstaty chemické vazby

Max Born 1882–1970

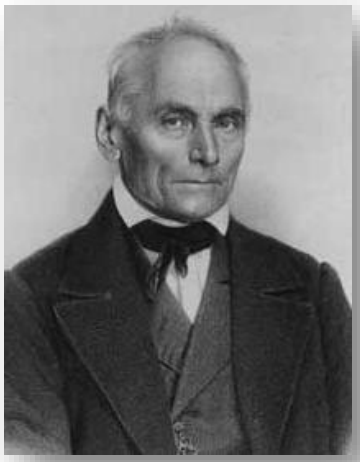


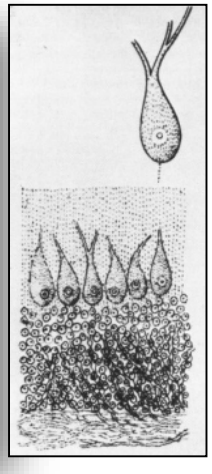
Walther Bothe 1882 – 1970

1954 - Nobelova cena za kvantovou mechaniku

Historické poznámky

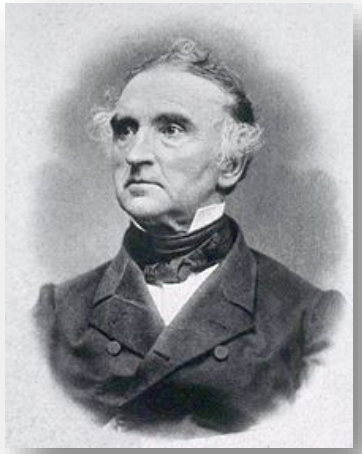
-220





Jan E. Purkyně (1787-1869)

-152



Glutamate was discovered and identified in 1866

Heinrich Ritthausen (1826 - 1912)

-118

… ***umami*** - is one of the five basic tastes along with sweet, bitter, sour and salty.

Kikunae Ikeda (1864 – 1936)

-100

1921 **Otto Loewi** (1873-1961)

1936 - Nobelova cena za fyziologii a medicínu

-66

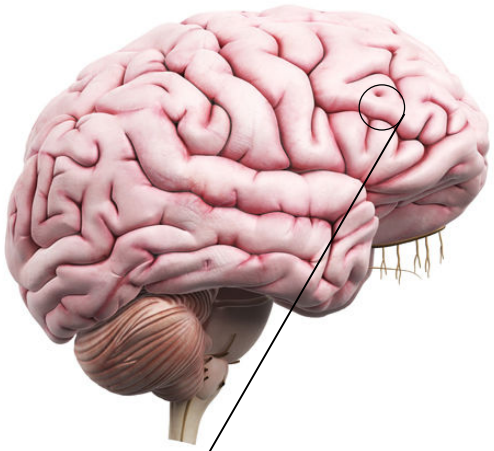
Keio Journal of Medicine
Vol. 3, No. 4, December, 1954

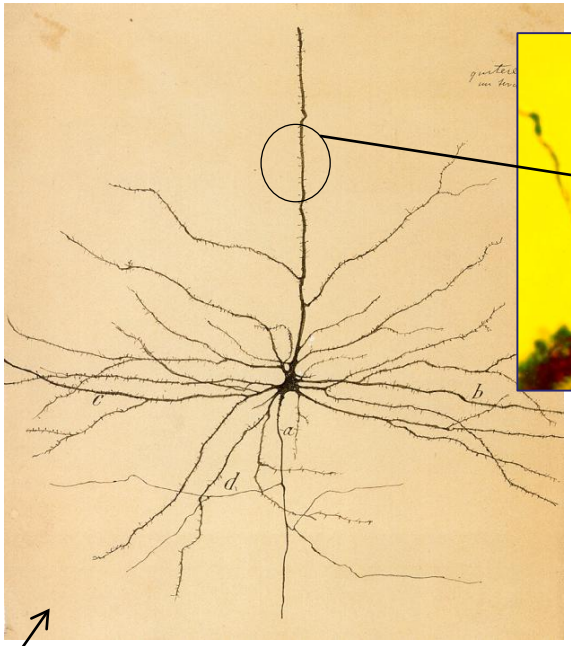
EFFECTS OF SODIUM GLUTAMATE ON THE NERVOUS SYSTEM

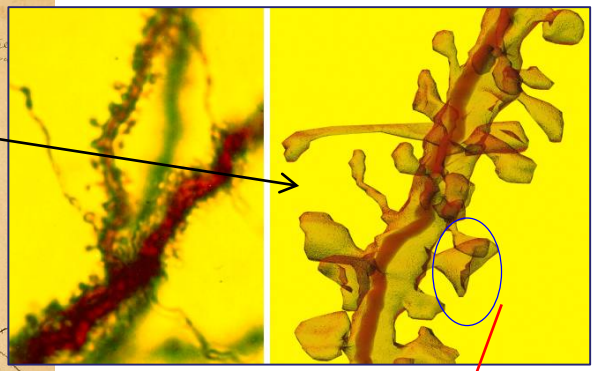
TAKASHI HAYASHI
Department of physiology, School of Medicine, Keio University

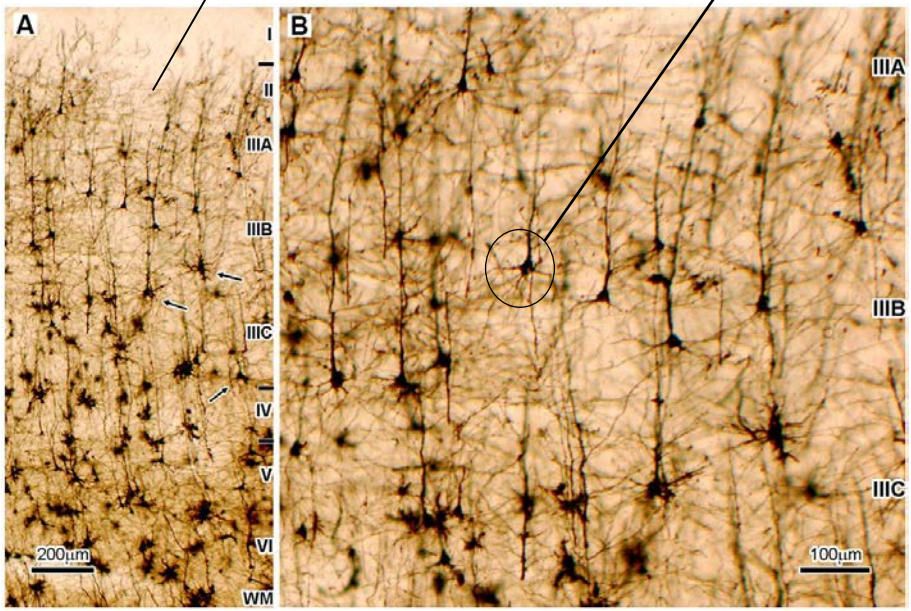
(Received on December 25th, 1954)

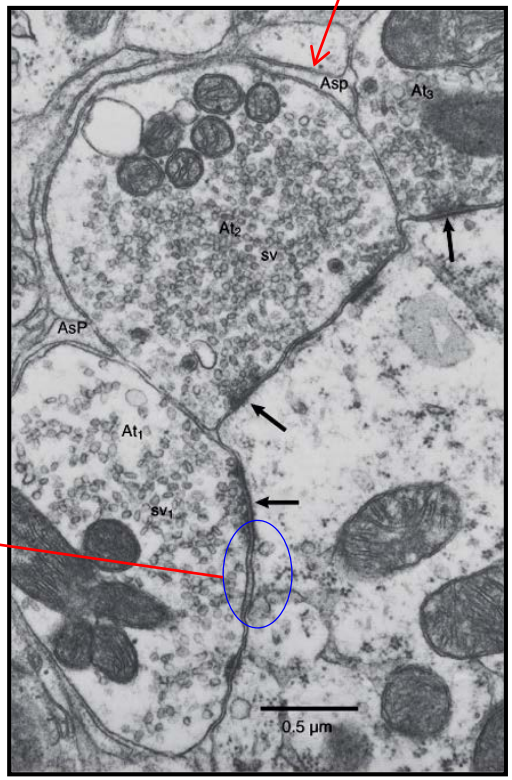
Takashi Hayashi 1897-1969




a
b
c
d




A
I
II
IIIA
IIIB
IIIC
IV
V
VI
WM
200µm
B
IIIA
IIIB
IIIC
100µm

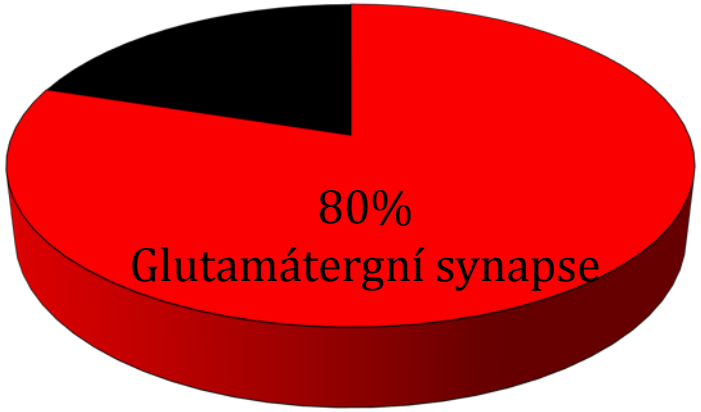

Asp
At3
At2
sv
AsP
At1
sv1
0.5 µm

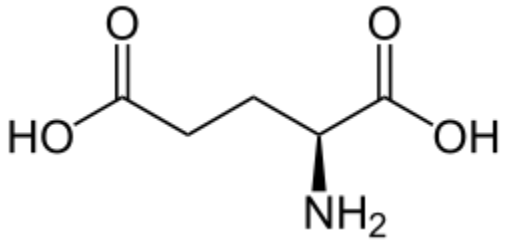
Synapse

Lidský mozek obsahuje *50,000,000,000 neuronů* ($5 . 10^{+10}$).

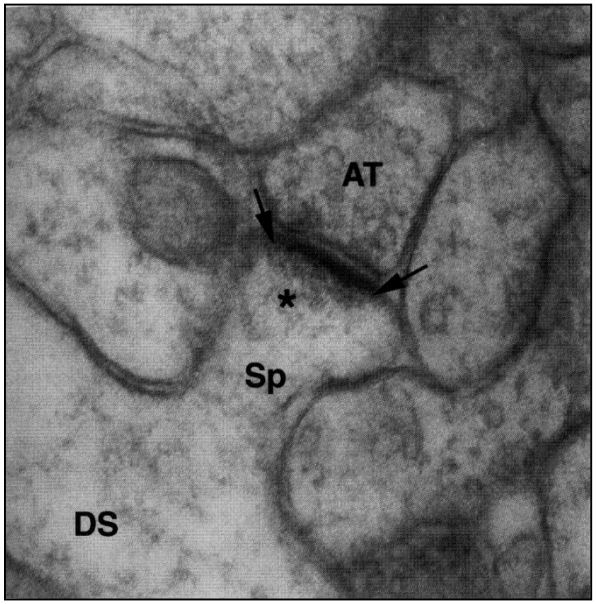
Na neuronu může být až *200,000 synapí*

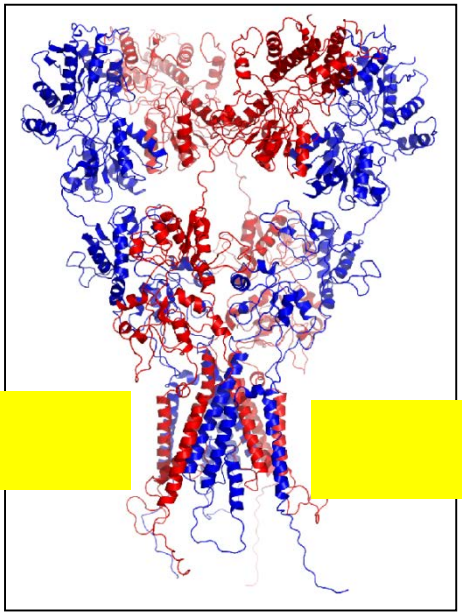
10^{+13} synaptických kontaktů v mozku člověka





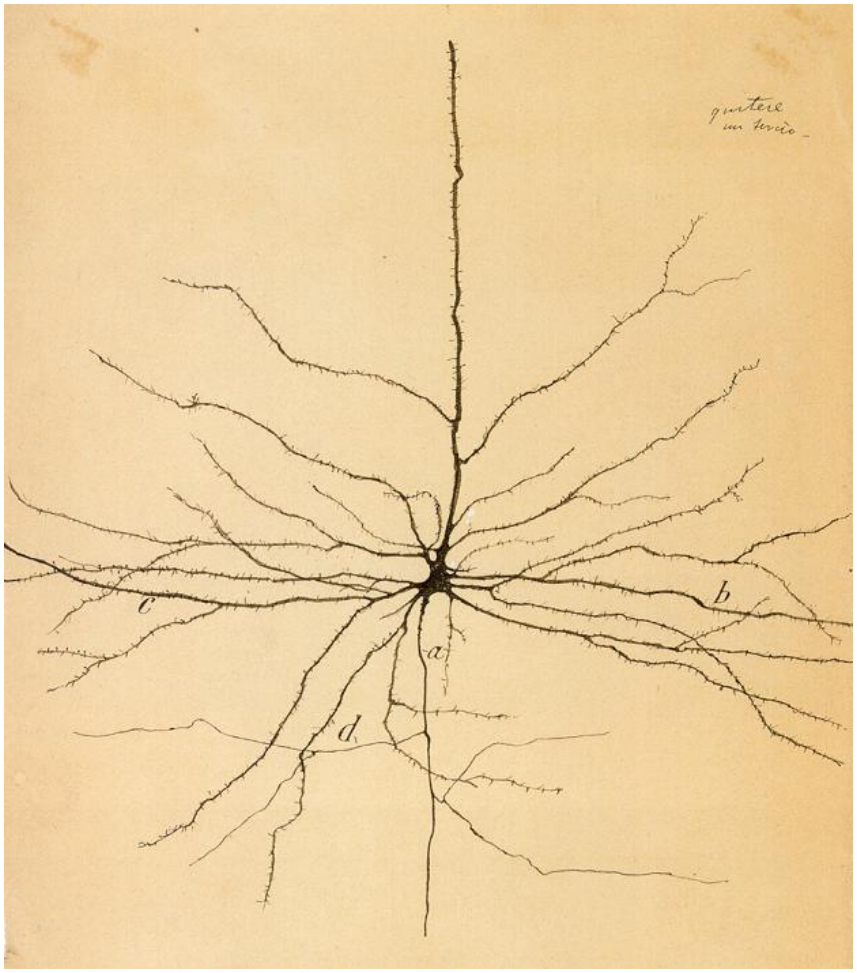
Kyselina glutamová



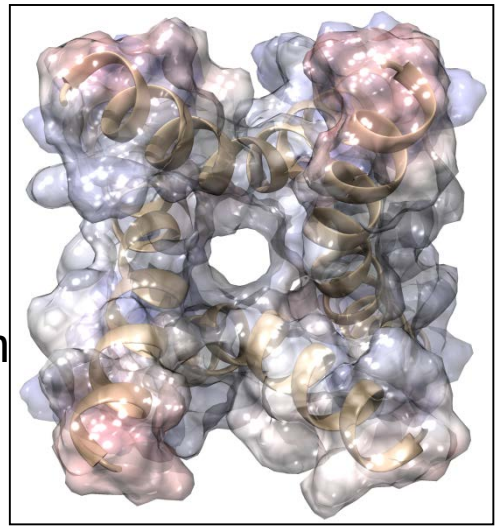


180 Å

18 nm
(10^{-9} m)

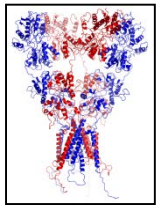


6 Å
1.0×10^{-10} m



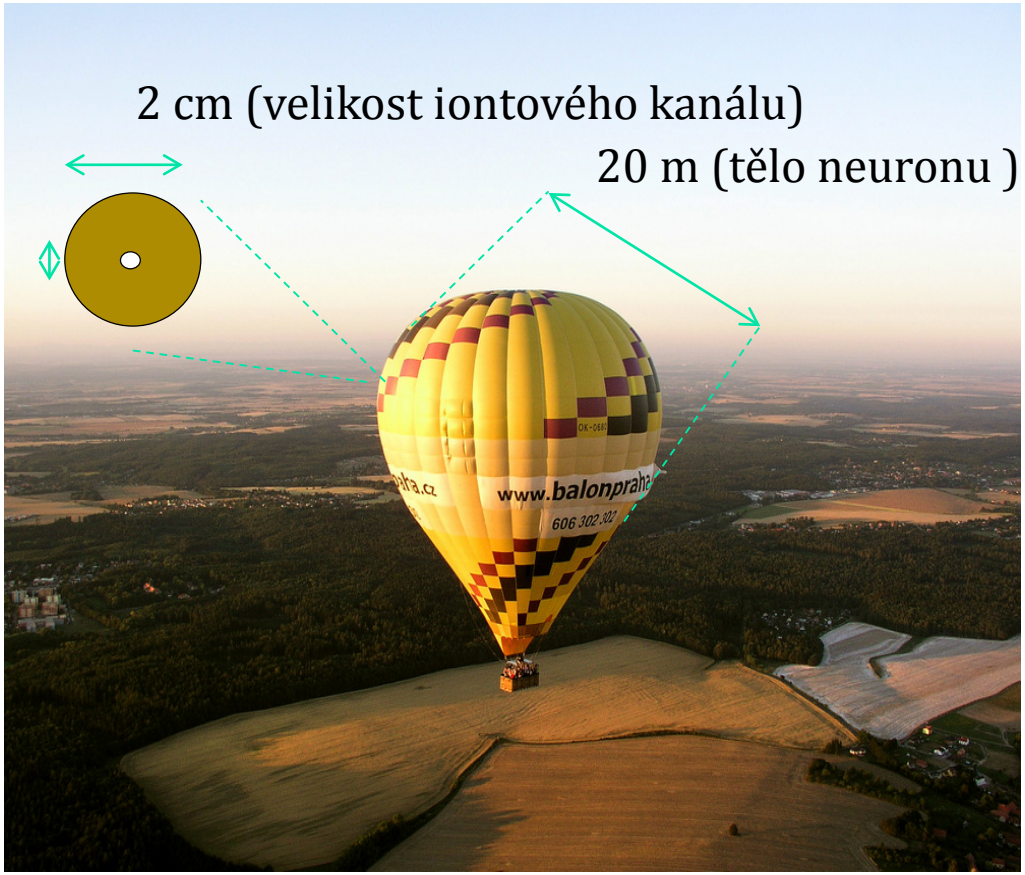
18 µm
(10^{-6} m)

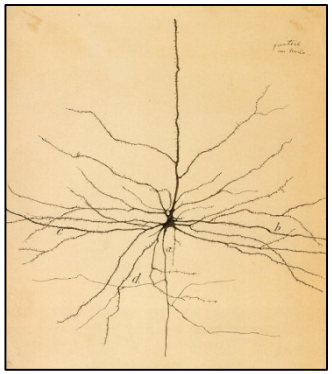
Pór = 0.6 mm
vlas



18 nm
(10^{-9} m)

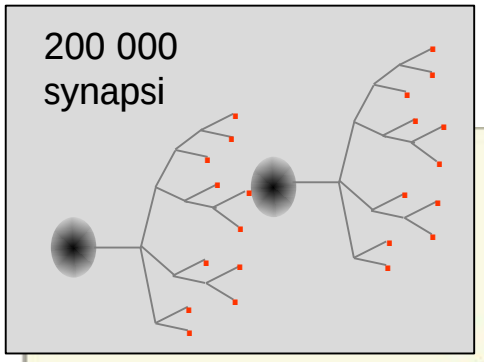
6 Å
1.0×10^{-10} m

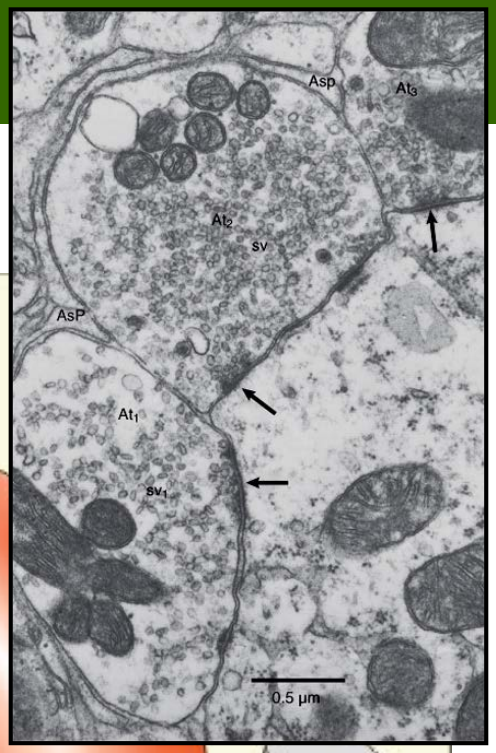




18 µm
(10^{-6} m)

Excitační synapse



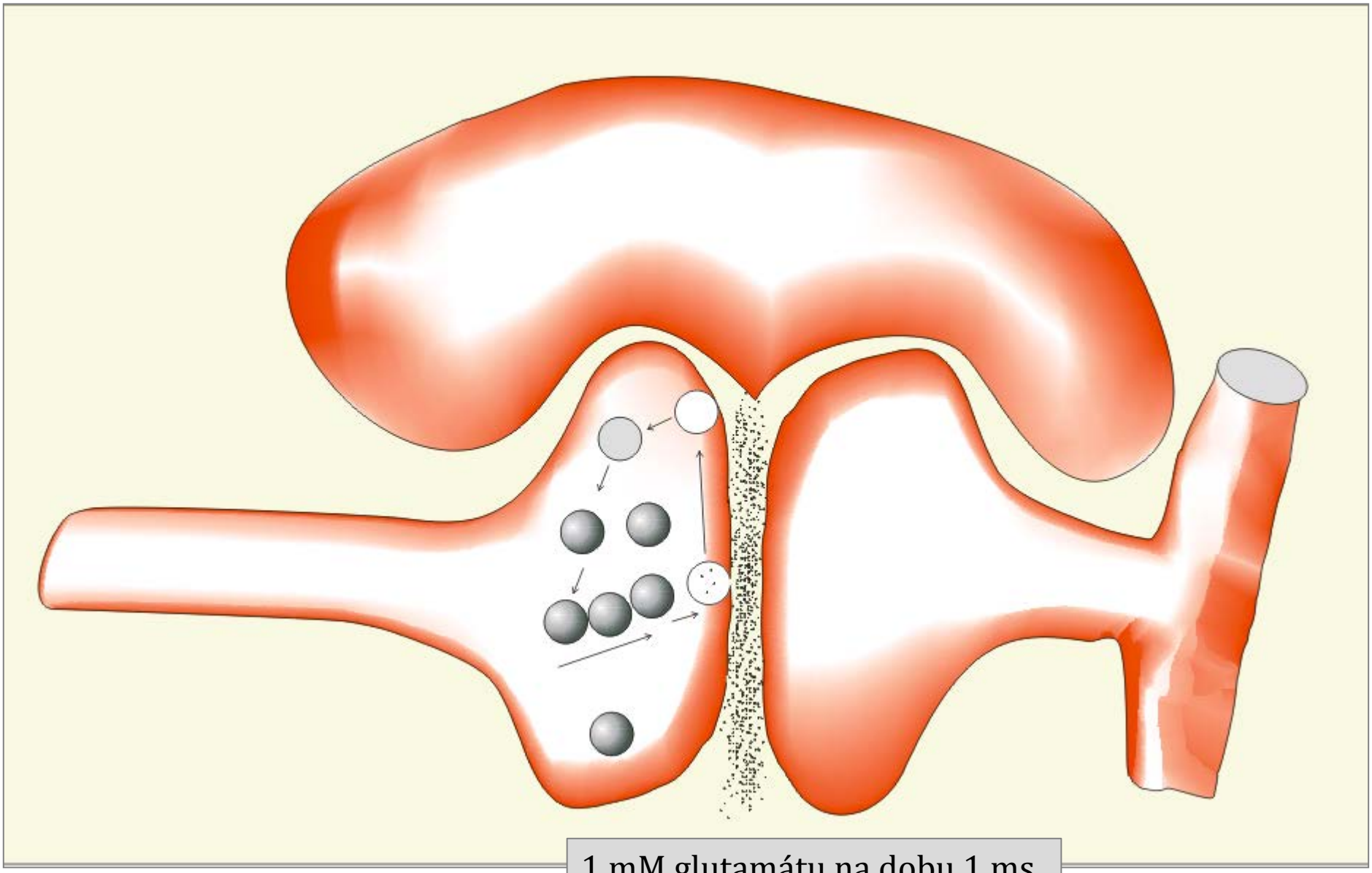


Glie

Presynaptický neuron

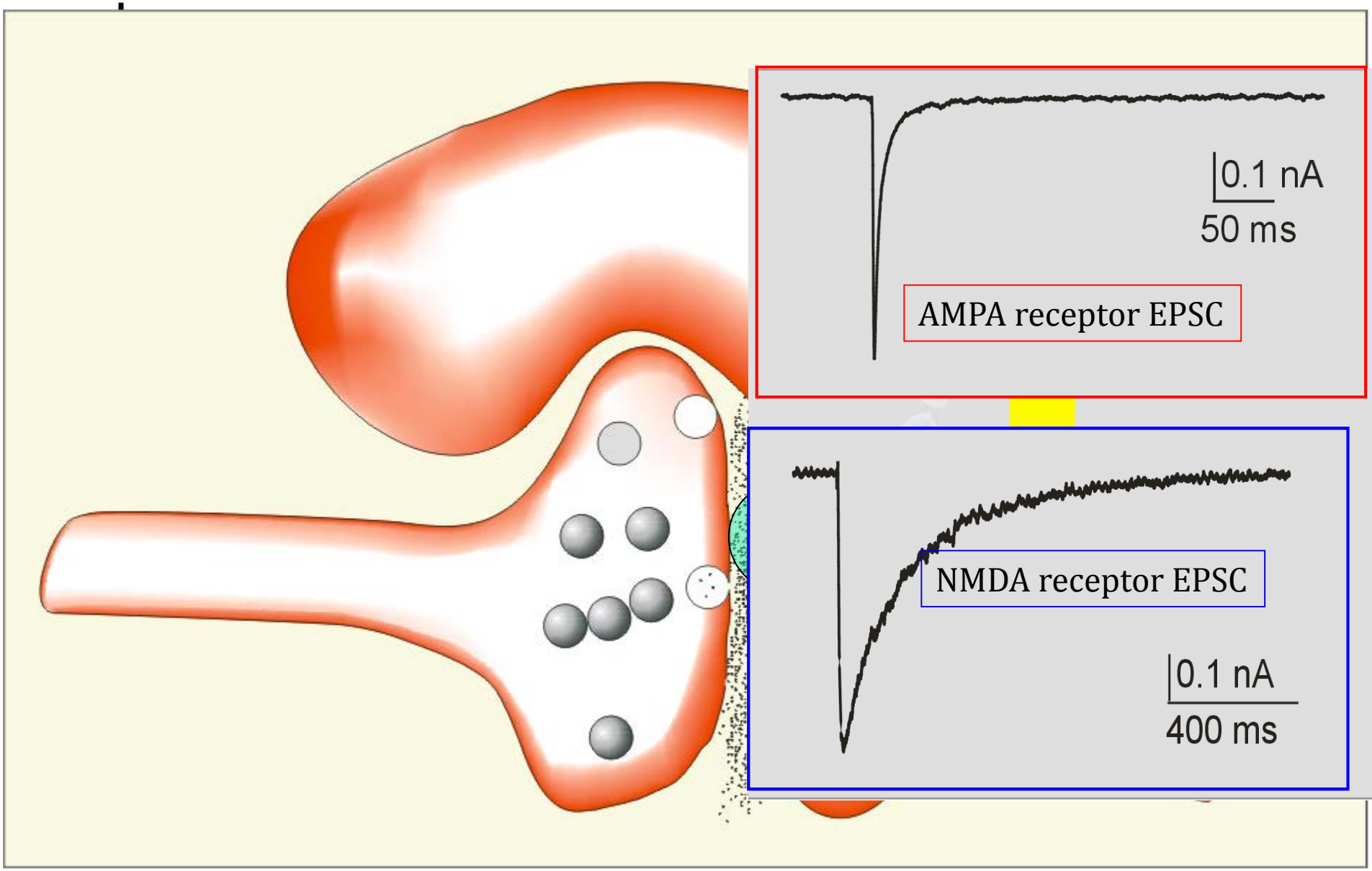
Postsynaptický neuron

Uvolňování glutamátu z presynaptické terminály



1 mM glutamátu na dobu 1 ms

Excitační postsynaptické proudy



Podjednotkové složení ionotropních glutamátových receptorů

Ionotropní glutamátové receptory

Podjednotka	Gen	Chromozóm
AMPA *(α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate)*		
GluA1	GRIA1	5q33
GluA2	GRIA2	4q32-33
GluA3	GRIA3	Xq25-26
GluA4	GRIA4	11q22-23
Kainát		
GluK1	GRIK1	21q21.1-22.1
GluK2	GRIK2	6q16.3-q21
GluK3	GRIK3	1p34-p33
GluK4	GRIK4	11q22.3
GluK5	GRIK5	19q13.2
NMDA *(N-methyl-D-aspartát)*		
GluN1	GRIN1	9q34.3
GluN2A	GRIN2A	16p13.2
GluN2B	GRIN2B	12p12
GluN2C	GRIN2C	17q24-q25
GluN2D	GRIN2D	19q13.1qter
GluN3A	GRIN3A	9q31.1
GluN3B	GRIN3B	19p13.3

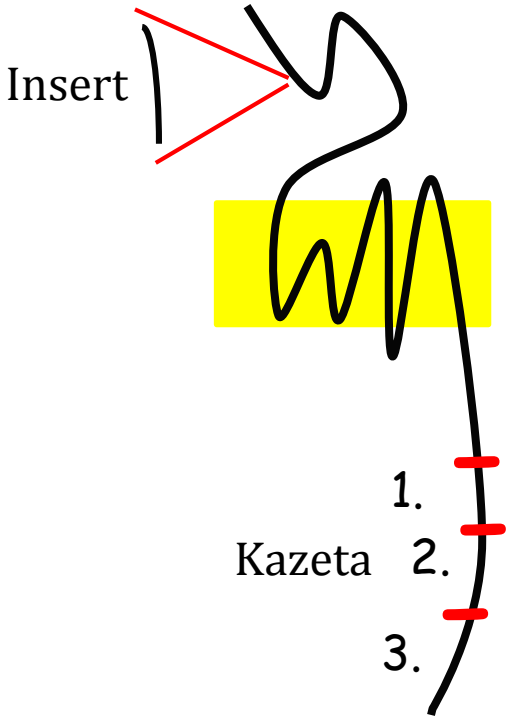
```
ORIGIN
        1 mkpraeccsp kfwlvlavla vsgsrarsqk sppsigiavi lvgtsdevai kdahekddfh
       61 hlsvvprvel vamnetdpks iitricdlms drkiqgvvfa ddtdqeaiaq ildfisaqtl
      121 tpilgihggs smimadkdes smffqfgpsi eqqasvmlni meeydwyifs ivttyfpgyq
      181 dfvnkirsti ensfvgwele evllldmsld dgdskiqnql kklqspiill yctkeeatyi
      241 fevansvglt gygytwivps lvagdtdtvp aefptglisv sydewdyglp arvrdgiaii
      301 ttaasdmlse hsfipepkss cynthekriy qsnmlnryli nvtfegrnls fsedgyqmhp
      361 klviillnke rkwervgkwk dkslqmkyyv wprmcpetee qeddhlsivt leeapfvive
      421 svdplsgtcm rntvpcqkri vtenktdeep gyikkcckgf cidilkkisk svkftydlyl
      481 vtngkhgkki ngtwngmige vvmkraymav gsltineers evvdfsvpfi etgisvmvsr
      541 sngtvspsaf lepfsadvwv mmfvmllivs avavfvfeyf spvgynrcla dgrepggpsf
      601 tigkaiwllw glvfnnsvpv qnpkgttski mvsvwaffav iflasytanl aafmiqeeyv
      661 dqvsglsdkk fqrpndfspp frfgtvpngs ternirnnya emhaymgkfn qrgvddalls
      721 lktgkldafi ydaavlnyma grdegcklvt igsgkvfast gygiaiqkds gwkrqvdlai
      781 lqlfgdgeme elealwltgi chneknevms sqldidnmag vfymlgaama lslitficeh
      841 lfywqfrhcf mgvcsgkpgm vfsisrgiys cihgvaieer qsvmnsptat mnnthsnilr
      901 llrtaknman lsgvngspqs aldfirress vydisehrrs fthsdcksyn nppceenlfs
      961 dyisevertf gnlqlkdsnv yqdhyhhhhr phsigsassi dglydcdnpp fttqsrsisk
     1021 kpldiglpss khsqlsdlyg kfsfksdrys ghddlirsdv sdisthtvty gniegnaakr
     1081 rkqqykdslk krpasaksrr efdeielayr rrpprspdhk ryfrdkeglr dfyldqfrtk
     1141 ensphwehvd ltdiykersd dfkrdsvsgg gpctnrshik hgtgdkhgvv sgvpapwekn
     1201 ltnvewedrs ggnfcrscps klhnysttvt gqnsgrqaci rceackkagn lydisednsl
     1261 qeldqpaapv avtsnasttk ypqsptnska qkknrnklrr qhsydtfvdl qkeeaalapr
     1321 svslkdkgrf mdgspyahmf emsagestfa nnkssvptag hhhhnnpggg ymlskslypd
     1381 rvtqnpfipt fgddqcllhg sksyffrqpt vagaskarpd fralvtnkpv vsalhgavpa
     1441 rfqkdicign qsnpcvpnnk nprafngssn ghvyeklssi esdv
```

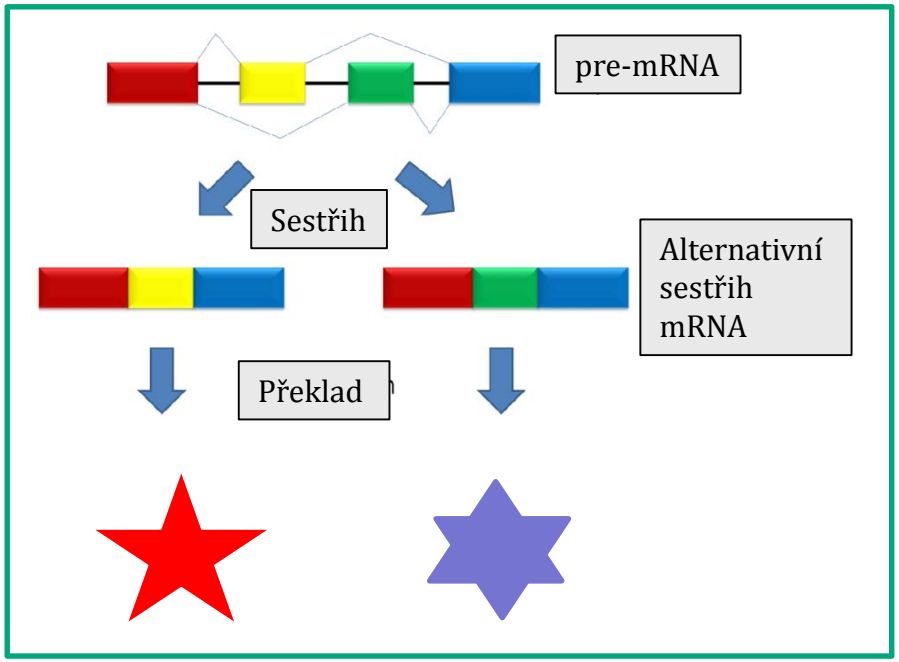
Alternativní sestřih

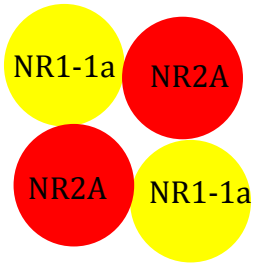
GluN 1-1a
GluN 1-1b
GluN 1-2a
GluN 1-2b
GluN 1-3a
GluN 1-3b
GluN 1-4a
GluN 1-4b

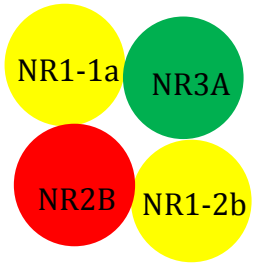
GluN 2A
GluN 2B
GluN 2C
GluN 2D

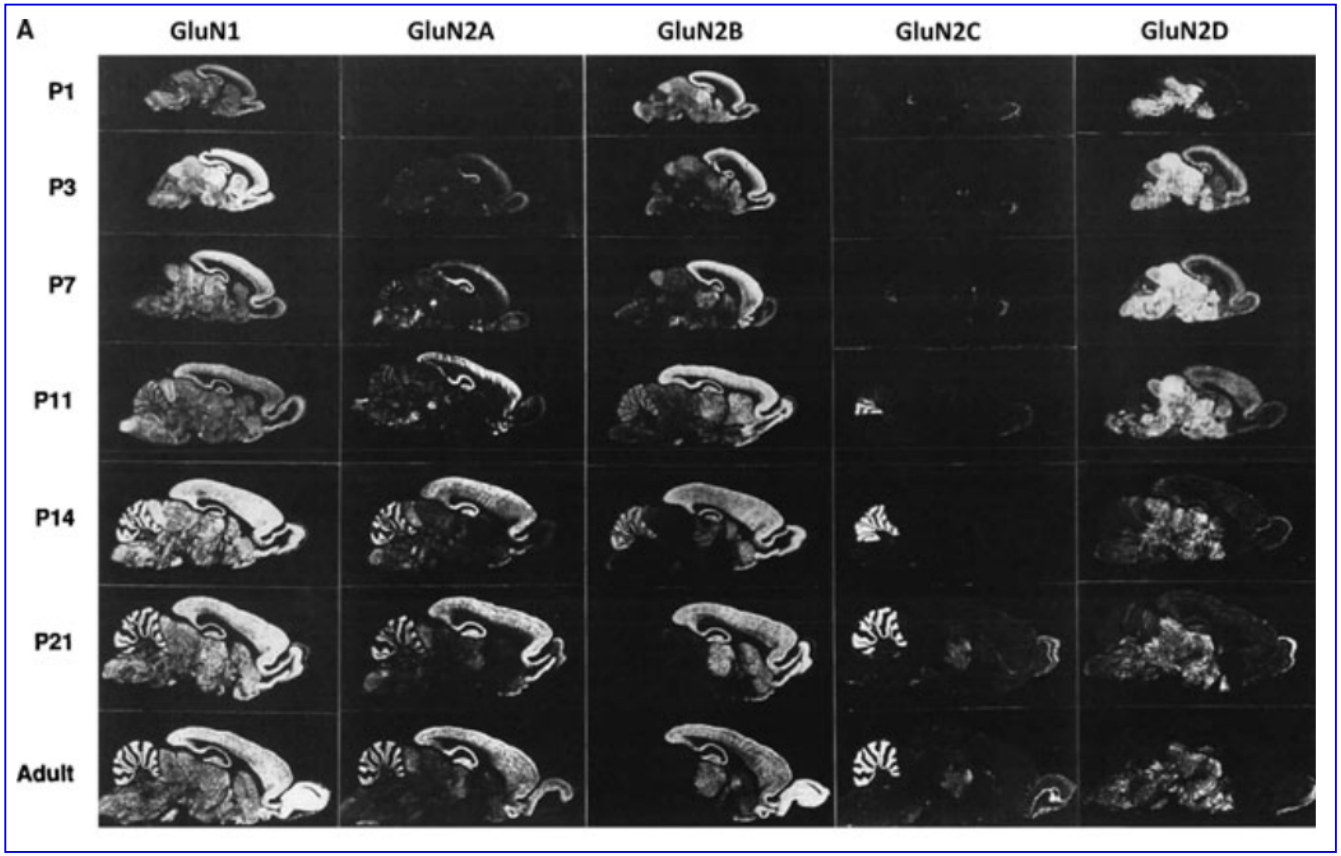
GluN 3A
GluN 3B



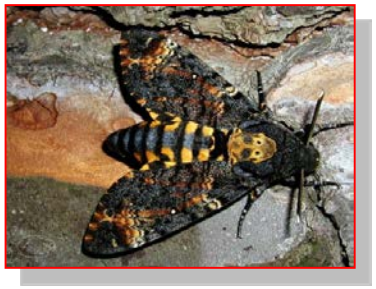


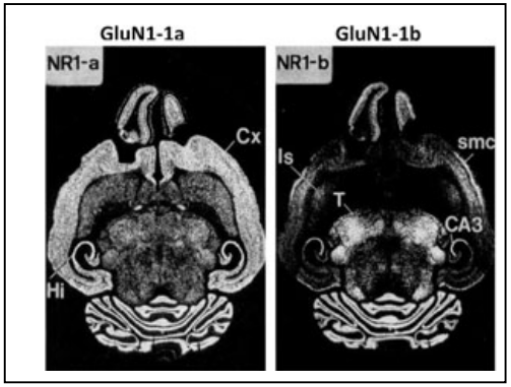


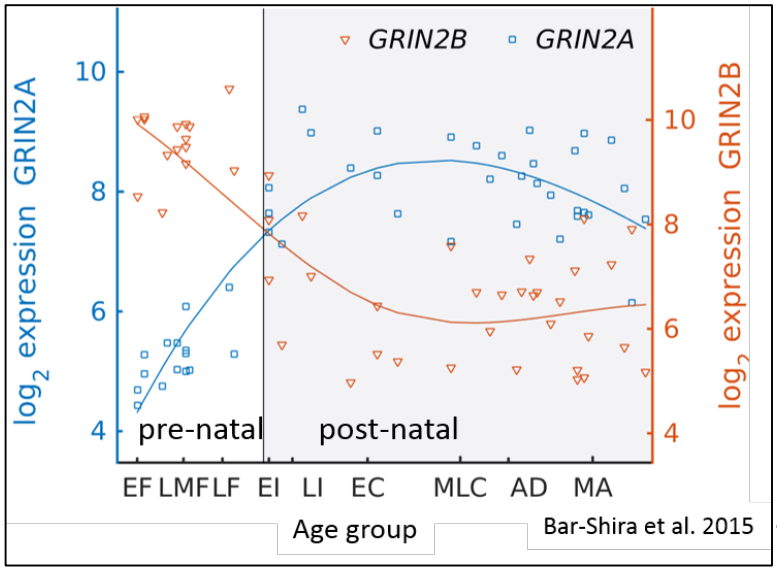




Hollmann, O'Shea-Greenfield, Rogers, Heinemann (1989) Cloning by functional expression of a member of the glutamate receptor family. *Nature* **342**:643-648.



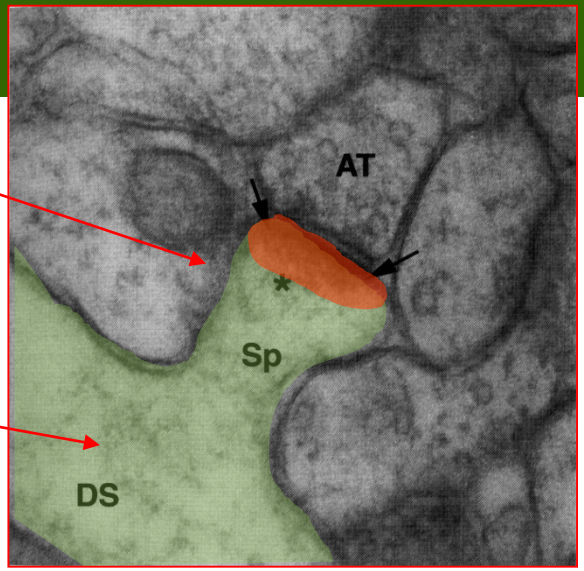


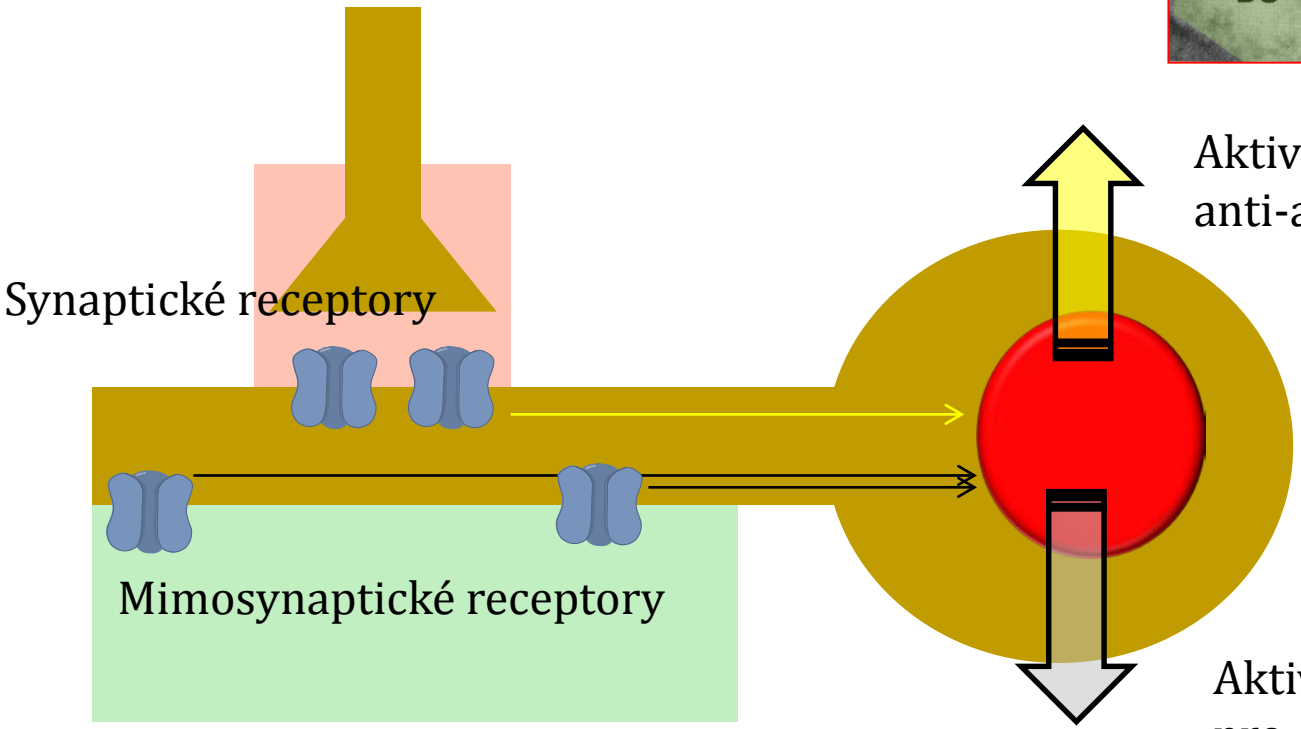


.. to be or not to be ....

Synaptické receptory

Mimosynaptické receptory



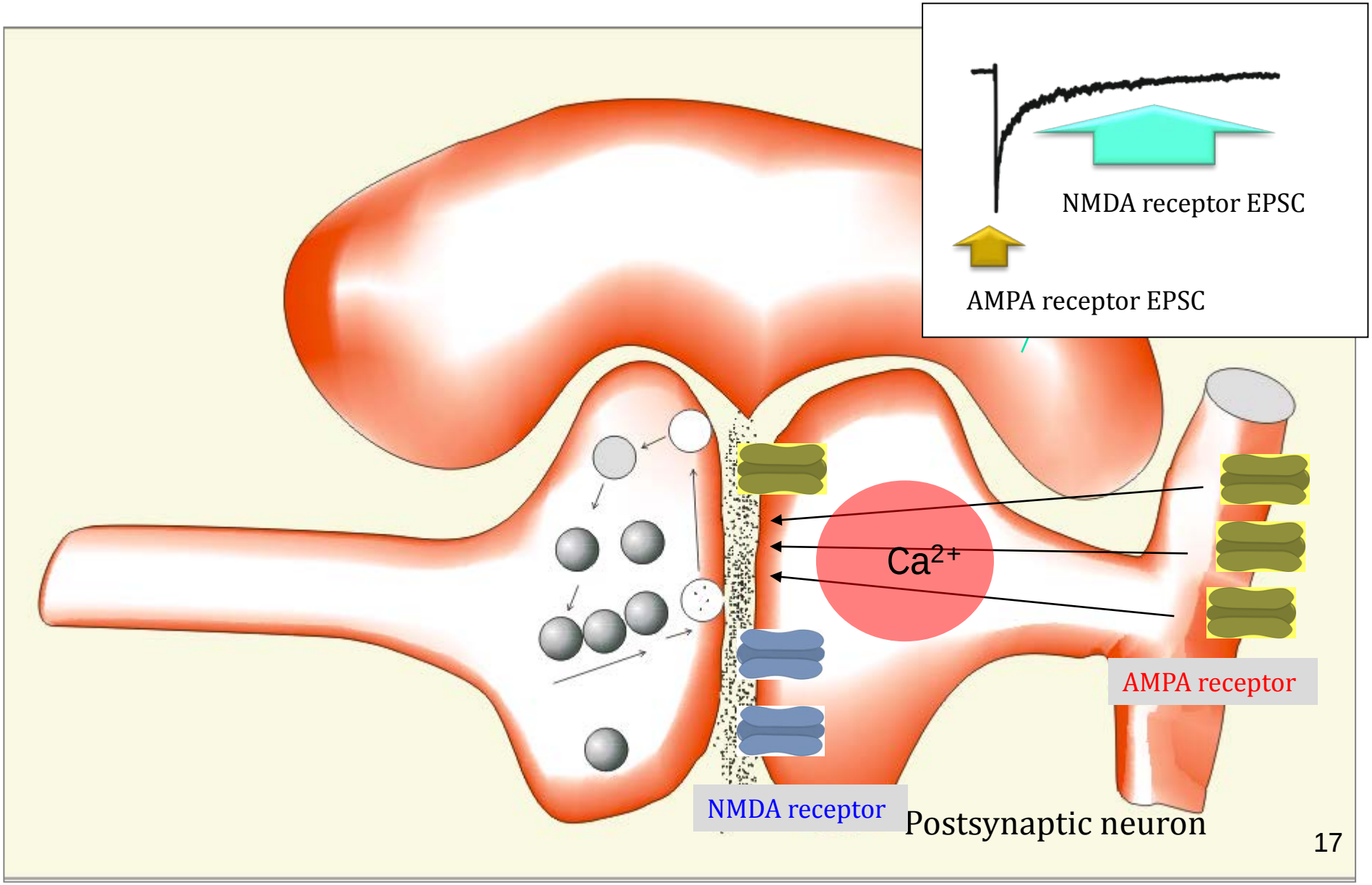


Aktivace
anti-apoptotických genů

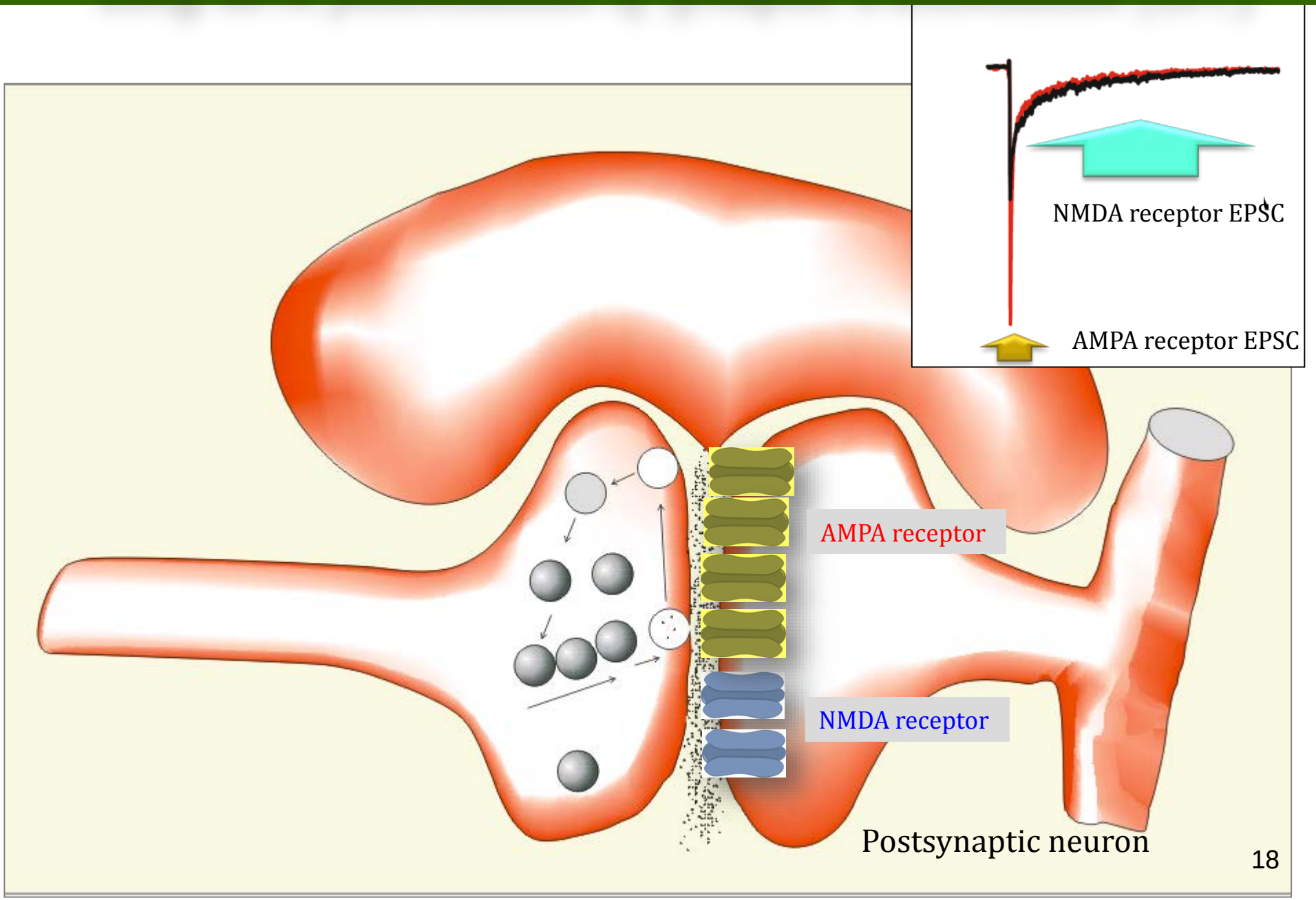
Aktivace
pro-apoptotických genů

Pamatujete si tento obrázek?

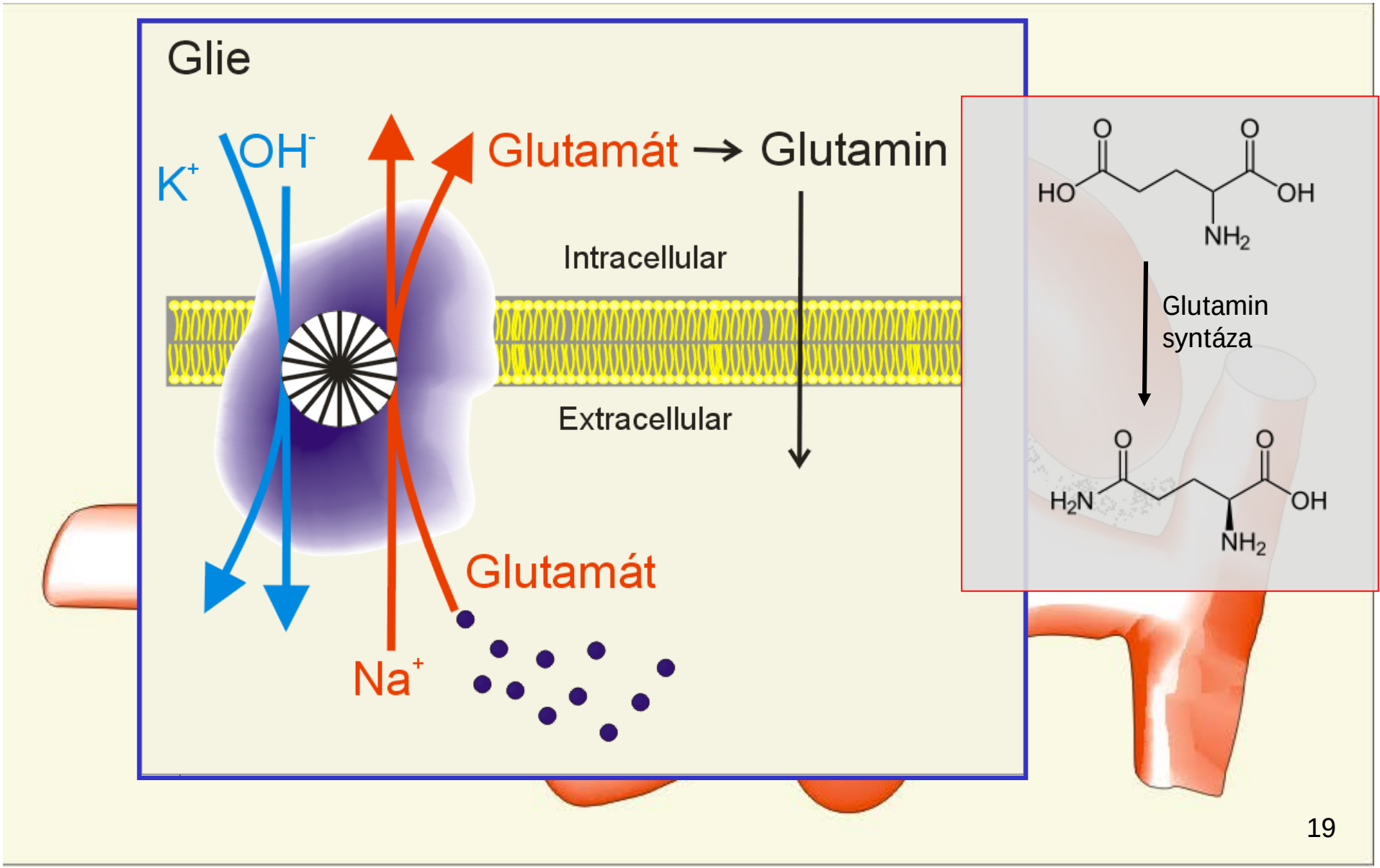
Long term potentiation of synaptic transmission (LTP)



Long term potentiation of synaptic transmission (LTP)



Odstranění glutamátu

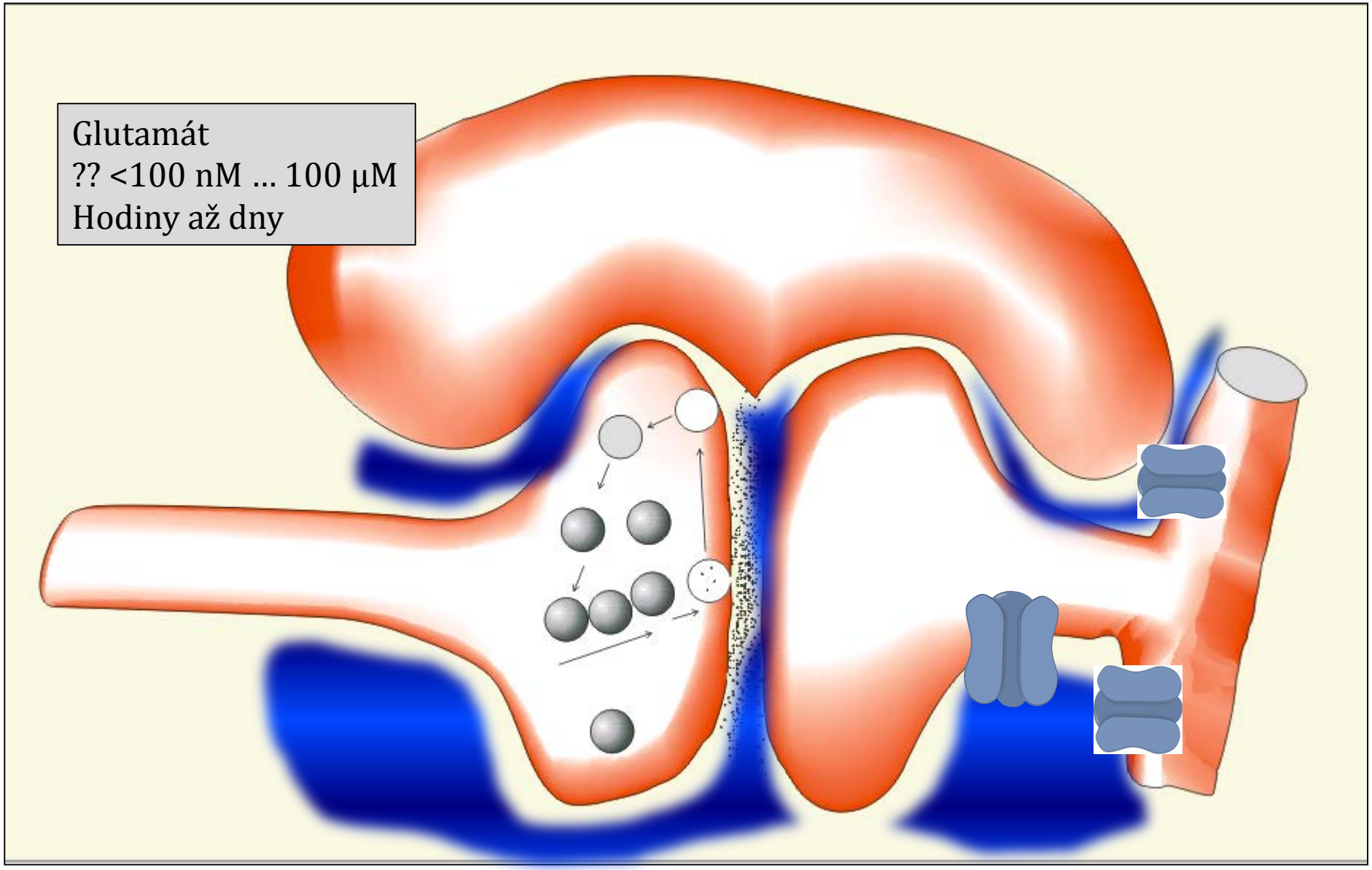


Pamatujete si jméno tohoto vědce?

Pamatujete si jméno tohoto vědce?

Takashi Hayashi

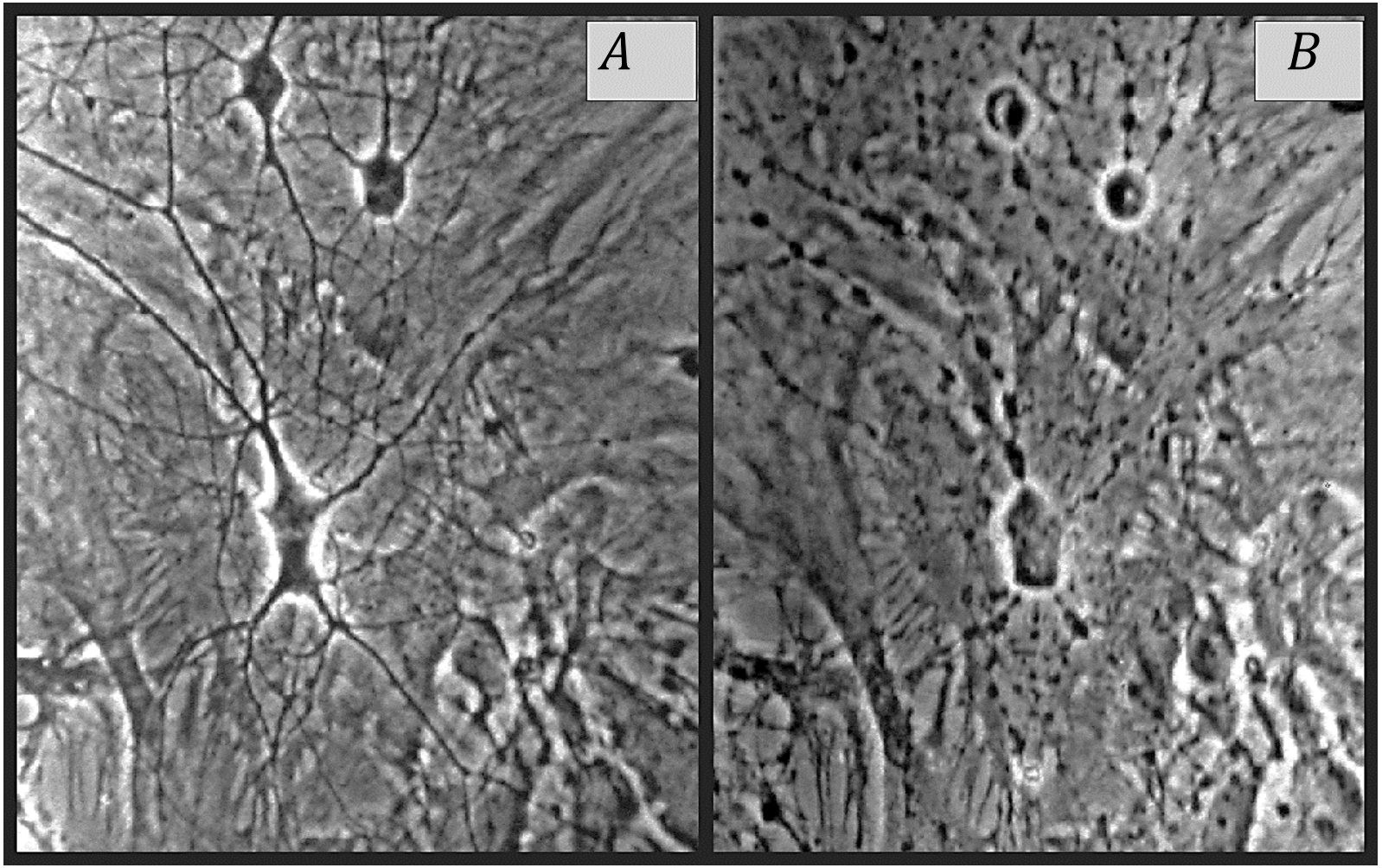
Excitotoxicita



Excitotoxicity

Control

Glutamate 30 min



"Glutamátergní" teorie vzniku:

Výrazná neurodegenerace

- Alzheimerova choroba
- Důsledky mozkové příhody
- Tramatické poškození mozku
- Parkinsonova choroba
- Tardivní dyskinezie
- Huntingtonova choroba
- Amyotrofická laterální skleroza
- Olivopontocerebellar degenerace
- AIDS
- Alergická encefalomyelitida

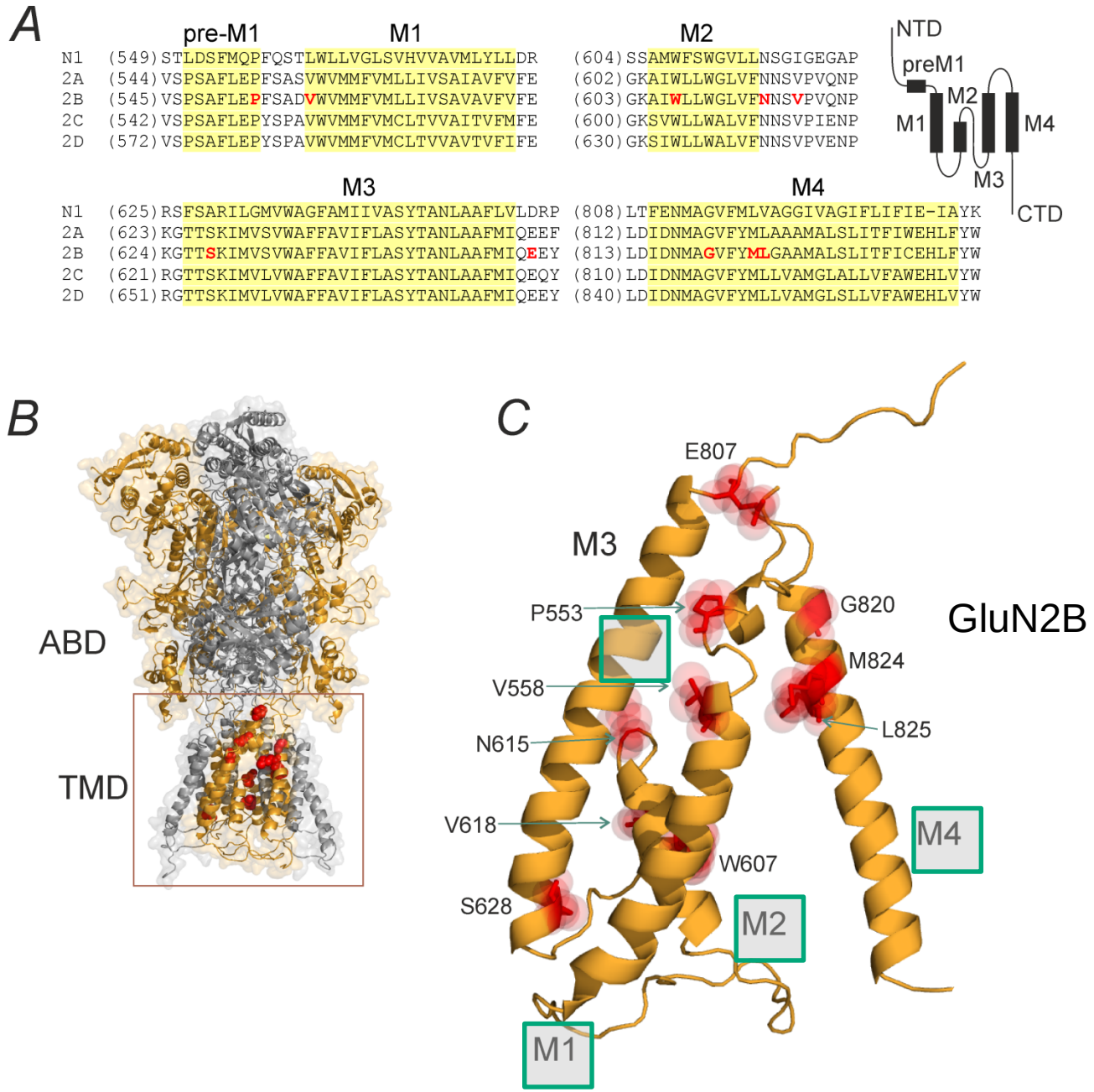
Další

- Epilepsie
- Anxieta
- Deprese
- Schizofrenie
- Chronická bolest
- Léková závislost

Human diseases and mutations in GRIN genes

GluN2B mut.	Genotype	Phenotype	Age of onset	Source
P553L	c.1658C	ID, hypotonia	Early postnatal	(de Ligt et al., 2012)
V558I	c.1672G	ID	-	(Hamdan et al., 2014) (Lelieveld et al., 2016)
W607C	c.1821G	ID, DD, dysmorphic features	-	(Yavarna et al., 2015)
N615I	c.1844A	WS, ID	7 weeks	(Lemke et al., 2014)
V618G	c.1853T	ID, WS, Epi-encephalopathy	4 months	(Lemke et al., 2014)
S628F	c.1883C	ID, DD, Epi-encephalopathy	-	(Platzer et al., 2017)
E657G	c.1970A	ID, DD	-	(Platzer et al., 2017)
G820E	c.2459G	ID, microcephaly	Early postnatal	(Hamdan et al., 2014)
G820A	c.2459G	ID, DD, DMD, ES, ASD	months	(Platzer et al., 2017)
M824R	c.2471T	ID, DD, microcephaly, Rett-like picture, Epi activity on EEG	months	(Zhu et al., 2015)
L825V	c.2473T>G	ASD	-	(Awadalla et al., 2010) (Swanger et al., 2016)

ID - intellectual disability; DD - developmental delay; WS - West symdrome; Epi – epilepsy and/or seizures, infantile spasms; ASD - Autism Spectrum Disorder; DMD -Dyskinetic movement disorder; ES - epileptic spasms; GVL - generalised cerebral volume loss


A
pre-M1
M1
M2
N1 (549)STLDSFMQPFQSTLWLLVGLSVHVVAVMLYLLDR (604)SSAMWFSWGVLLNSGIGEGAP
2A (544)VSPSAFLEPFSASVWVMMFVMLLIVSAIAVFVFE (602)GKAIWLLWGLVFNNSVPVQNP
2B (545)VSPSAFLEPFSADVWVMMFVMLLIVSAVAVFVFE (603)GKAIWLLWGLVFNNSVPVQNP
2C (542)VSPSAFLEPYSPAVWVMMFVMCLTVVAITVFMFE (600)GKSVWLLWALVFNNSVPIENP
2D (572)VSPSAFLEPYSPAVWVMMFVMCLTVVAVTVFIFE (630)GKSIWLLWALVFNNSVPVENP
M3
M4
N1 (625)RSFSARILGMVWAGFAMIIVASYTANLAAFLVLDRP (808)LTFENMAGVFMLVAGGIVAGIFLIFIE-IAYK
2A (623)KGTTSKIMVSVWAFFAVIFLASYTANLAAFMIQEEF (812)LDIDNMAGVFYMLAAAMALSLITFIWEHLFYW
2B (624)KGTTSKIMVSVWAFFAVIFLASYTANLAAFMIQEEY (813)LDIDNMAGVFYMLGAAMALSLITFICEHLFYW
2C (621)RGTTSKIMVLVWAFFAVIFLASYTANLAAFMIQEQY (810)LDIDNMAGVFYMLLVAMGLALLVFAWEHLVYW
2D (651)RGTTSKIMVLVWAFFAVIFLASYTANLAAFMIQEEY (840)LDIDNMAGVFYMLLVAMGLSLLVFAWEHLVYW
NTD
preM1
M1
M2
M3
M4
CTD
B
ABD
TMD
C
E807
M3
P553
G820
M824
V558
L825
N615
V618
M4
W607
S628
M2
M1
GluN2B

Intellectual disability

Estimates of the prevalence of ID range widely, from **9 to 40 per 1,000 (1-4%)**

Intellectual disability (ID), also known as **general learning disability**, and **mental retardation** (MR), is a generalized neurodevelopmental disorder characterized by significantly impaired intellectual and adaptive functioning. It is defined by an IQ score under 70 in addition to deficits in two or more adaptive behaviors that affect everyday, general living.

- ❑ **Mild ID (IQ 50–69)**
- ❑ **Moderate ID (IQ 35–49)**
- ❑ **Severe or profound ID**

Some of the early signs can include:

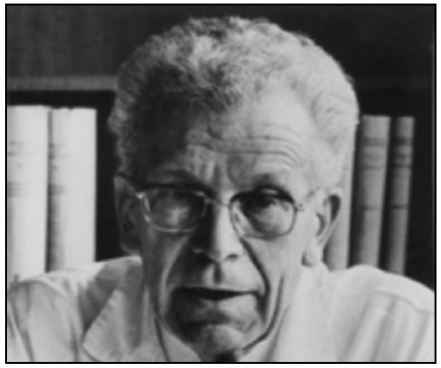
- Delays in reaching or failure to achieve milestones in **motor skills development** (sitting, crawling, walking)
- **Slowness learning to talk** or continued difficulties with speech and language skills after starting to talk
- Difficulty with **self-help and self-care** skills (e.g., getting dressed, washing, and feeding themselves)
- Poor planning or **problem solving abilities**
- **Behavioral and social problems**
- **Failure to grow intellectually** or continued infant-like behavior
- Problems keeping up in school
- **Failure to adapt or adjust to new situations**
- **Difficulty understanding and following social rules**

Autism

Most recent reviews tend to estimate a prevalence of **1–2 per 1,000 for autism** and **6 per 1,000 for ASD**

Autism is a **developmental disorder** characterized by troubles with **social interaction** and **communication**, and by **restricted and repetitive behavior**. Parents usually notice signs in the first **two or three years of their child's** life. These signs often develop gradually, though some children with autism reach their developmental milestones at a normal pace and then worsen.

Paul Eugen Bleuler (1857 – 1939) Swiss psychiatrist - he started using the term around 1911

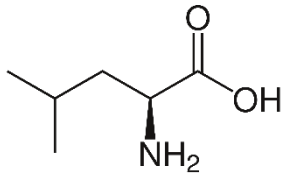


Hans Asperger (1906-1980) Austrian pediatrician, medical theorist, and medical professor - … his work on "autistic psychopathy". .. 400 children with autistic psychopathy.

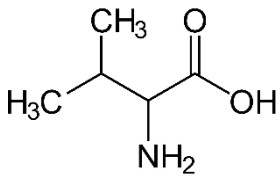
Leo Kanner (1894 – 1981) Austrian-American psychiatrist - Autistic disturbances of affective contact. Nerv Child ***1943***; 2:217–50.

...kde jsou geny, tam jsou problémy ...

Family	Subunit	Gene	Chromosome
AMPA *(α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate)*			
	GluA1	GRIA1	5q33
	GluA2	GRIA2	4q32-33
	GluA3	GRIA3	Xq25-26
	GluA4	GRIA4	11q22-23
Kainate			
	GluK1	GRIK1	21q21.1-22.1
	GluK2	GRIK2	6q16.3-q21
	GluK3	GRIK3	1p34-p33
	GluK4	GRIK4	11q22.3
	GluK5	GRIK5	19q13.2
NMDA *(N-methyl-D-aspartate)*			
	GluN1	GRIN1	9q34.3
	GluN2A	GRIN2A	16p13.2
	GluN2B	GRIN2B	12p12
	GluN2C	GRIN2C	17q24-q25
	GluN2D	GRIN2D	19q13.1qter
	GluN3A	GRIN3A	9q31.1
	GluN3B	GRIN3B	19p13.3



Leucin



Valin

GluN2B (L825V)

vfymlgaama

```
ORIGIN
        1 mkpraeccsp kfwlvlavla vsgsrarsqk sppsigiavi lvgtsdevai kdahekddfh
       61 hlsvvprvel vamnetdpks iitricdlms drkiqgvvfa ddtdqeaiaq ildfisaqtl
      121 tpilgihggs smimadkdes smffqfgpsi eqqasvmlni meeydwyifs ivttyfpgyq
      181 dfvnkirsti ensfvgwele evllldmsld dgdskiqnql kklqspiill yctkeeatyi
      241 fevansvglt gygytwivps lvagdtdtvp aefptglisv sydewdyglp arvrdgiaii
      301 ttaasdmlse hsfipepkss cynthekriy qsnmlnryli nvtfegrnls fsedgyqmhp
      361 klviillnke rkwervgkwk dkslqmkyyv wprmcpetee qeddhlsivt leeapfvive
      421 svdplsgtcm rntvpcqkri vtenktdeep gyikkcckgf cidilkkisk svkftydlyl
      481 vtngkhgkki ngtwngmige vvmkraymav gsltineers evvdfsvpfi etgisvmvsr
      541 sngtvspsaf lepfsadvwv mmfvmllivs avavfvfeyf spvgynrcla dgrepggpsf
      601 tigkaiwllw glvfnnsvpv qnpkgttski mvsvwaffav iflasytanl aafmiqeeyv
      661 dqvsglsdkk fqrpndfspp frfgtvpngs ternirnnya emhaymgkfn qrgvddalls
      721 lktgkldafi ydaavlnyma grdegcklvt igsgkvfast gygiaiqkds gwkrqvdlai
      781 lqlfgdgeme elealwltgi chneknevms sqldidnmag vfymlgaama lslitficeh
      841 lfywqfrhcf mgvcsgkpgm vfsisrgiys cihgvaieer qsvmnsptat mnnthsnilr
      901 llrtaknman lsgvngspqs aldfirress vydisehrrs fthsdcksyn nppceenlfs
      961 dyisevertf gnlqlkdsnv yqdhyhhhhr phsigsassi dglydcdnpp fttqsrsisk
     1021 kpldiglpss khsqlsdlyg kfsfksdrys ghddlirsdv sdisthtvty gniegnaakr
     1081 rkqqykdslk krpasaksrr efdeielayr rrpprspdhk ryfrdkeglr dfyldqfrtk
     1141 ensphwehvd ltdiykersd dfkrdsvsgg gpctnrshik hgtgdkhgvv sgvpapwekn
     1201 ltnvewedrs ggnfcrscps klhnysttvt gqnsgrqaci rceackkagn lydisednsl
     1261 qeldqpaapv avtsnasttk ypqsptnska qkknrnklrr qhsydtfvdl qkeeaalapr
     1321 svslkdkgrf mdgspyahmf emsagestfa nnkssvptag hhhhnnpggg ymlskslypd
     1381 rvtqnpfipt fgddqcllhg sksyffrqpt vagaskarpd fralvtnkpv vsalhgavpa
     1441 rfqkdicign qsnpcvpnnk nprafngssn ghvyeklssi esdv
```

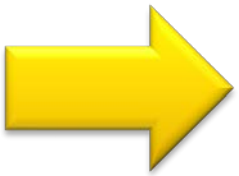
Surface expression

Functional properties:

i. agonist affinity

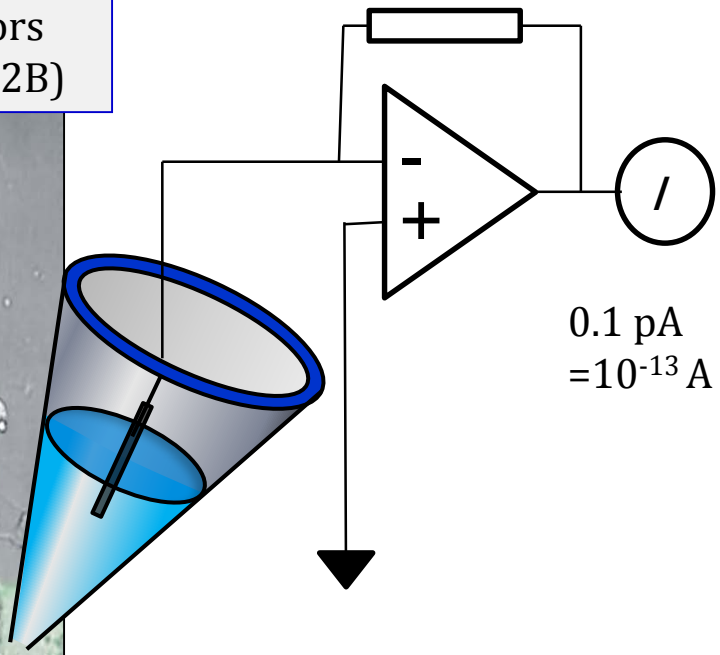
ii. receptor desensitization

iii. probability of opening

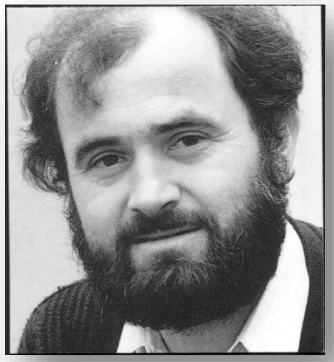


„Patch-clamp“ technika

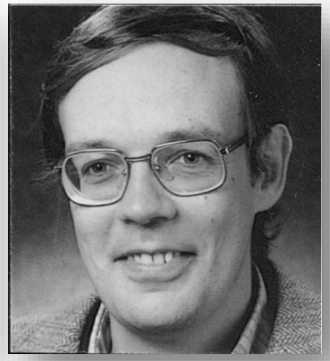
Recombinant receptors
HEK293 (GluN1/GluN2B)



0.1 pA
$=10^{-13}$ A



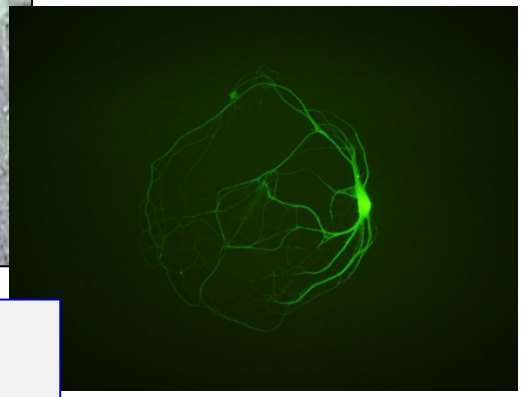
Erwin Neher



Bert Sakmann

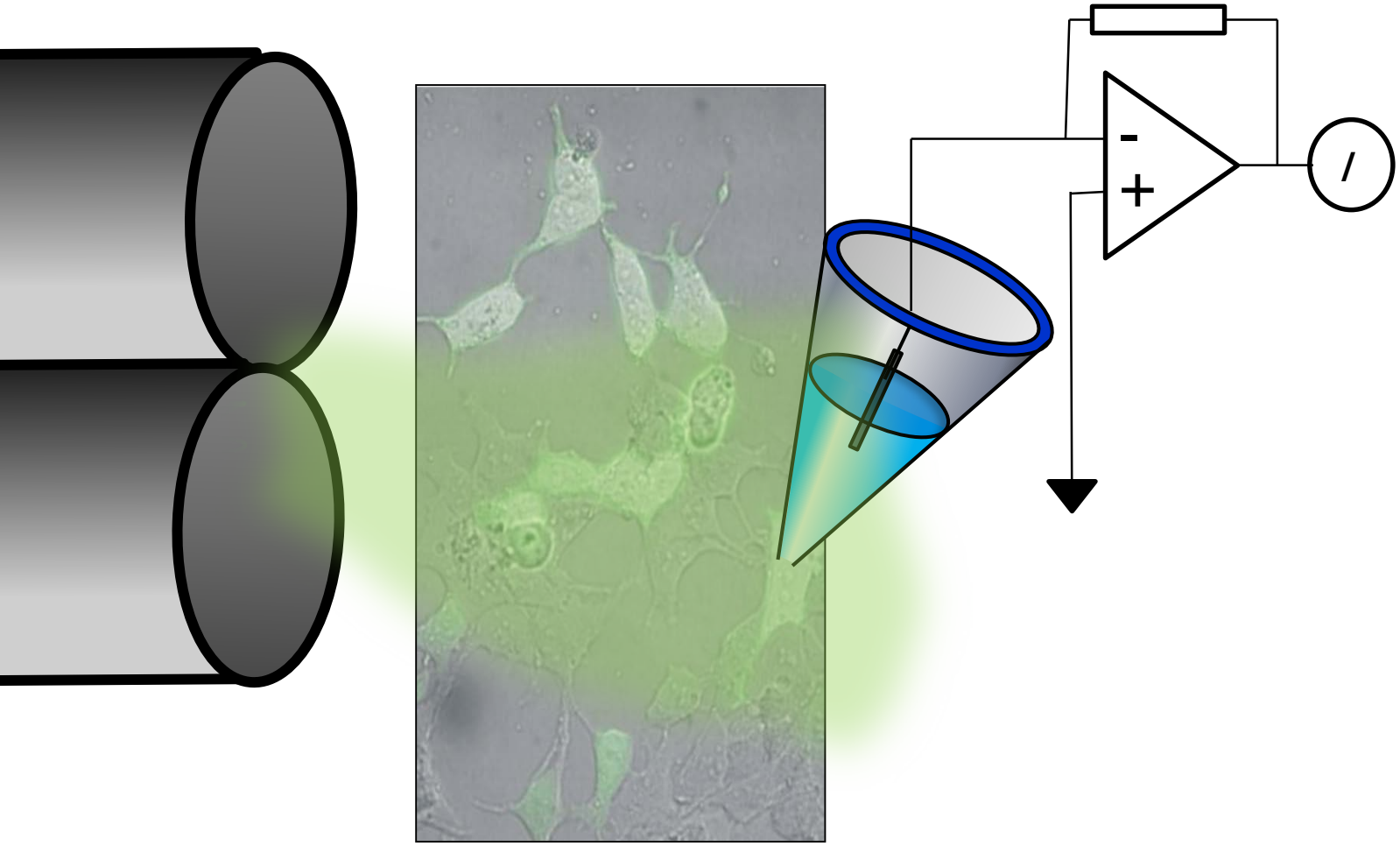
NP... Medicine 1991

Patch-clamp amplifier

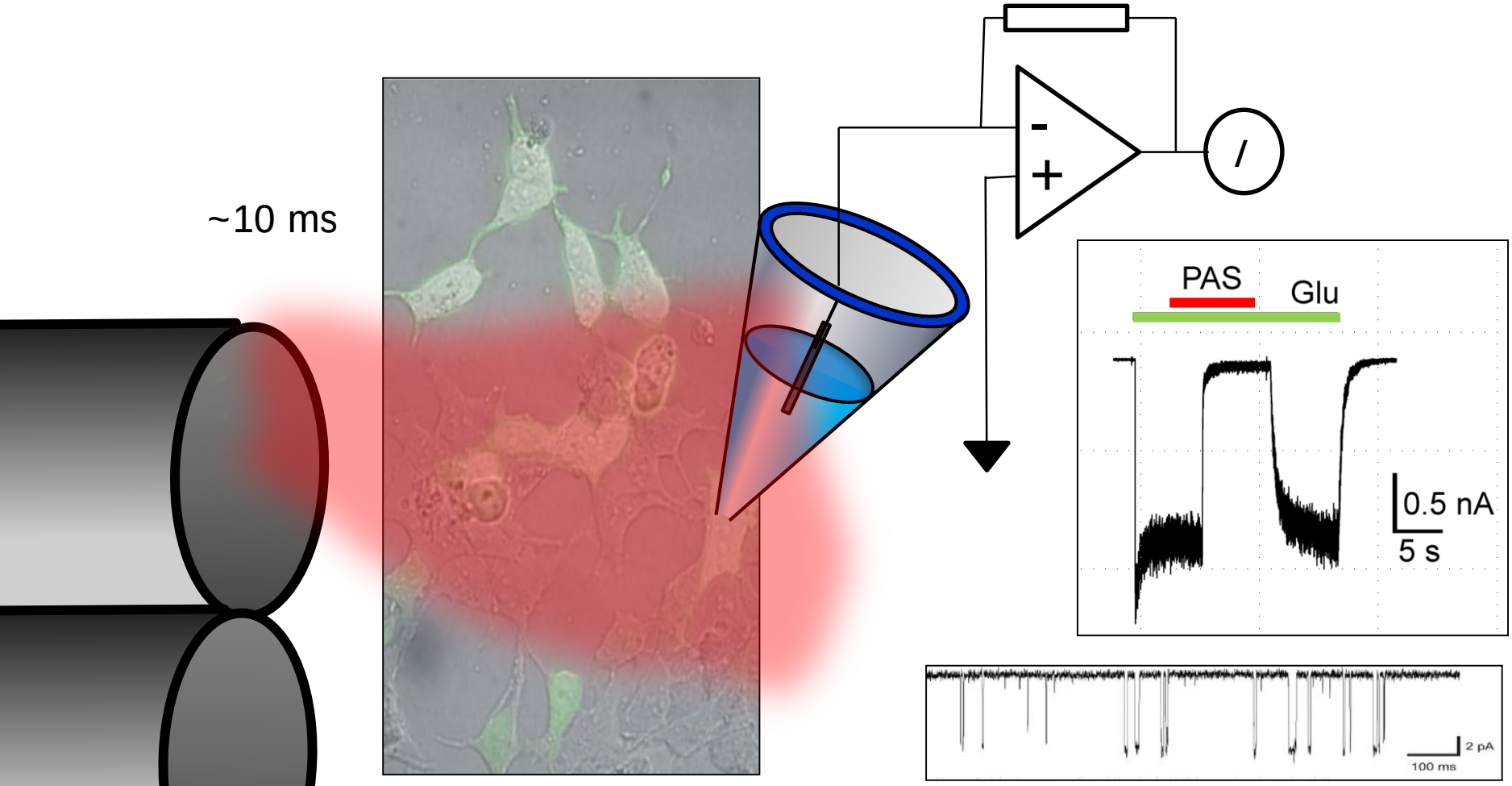


Hippocampal
cultures

„Patch-clamp“ a rychlá aplikace látek



„Patch-clamp“ a rychlá aplikace látek

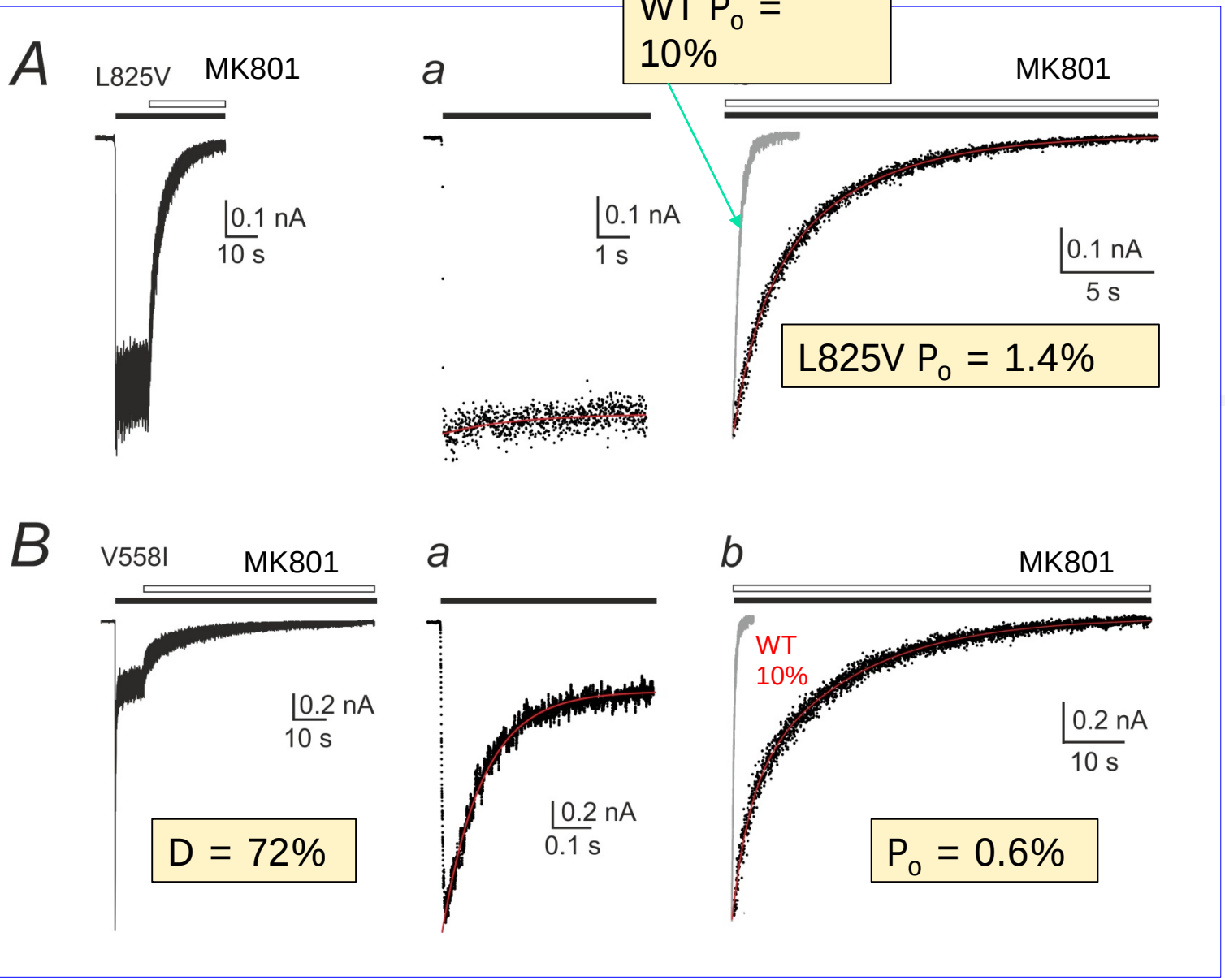


Pravděpodobnost otevření a desensitizace

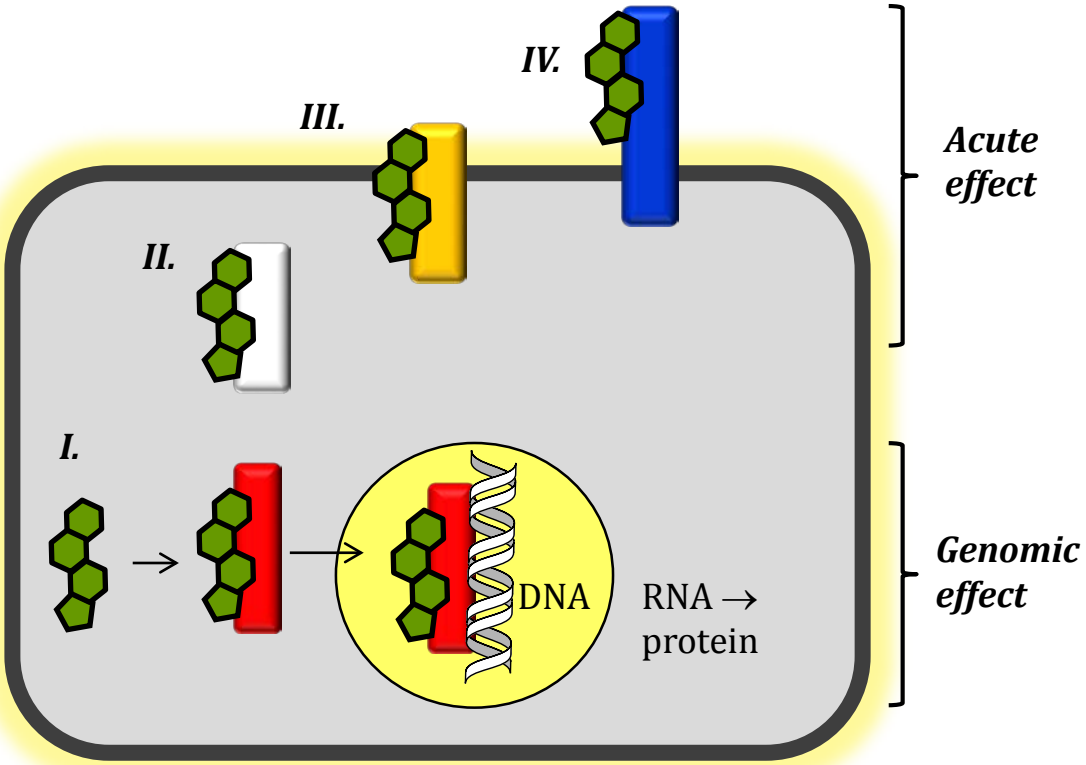
$R + A \rightleftarrows RA + A \rightleftarrows RAA \rightleftarrows O$

$k_r \uparrow\downarrow k_d$

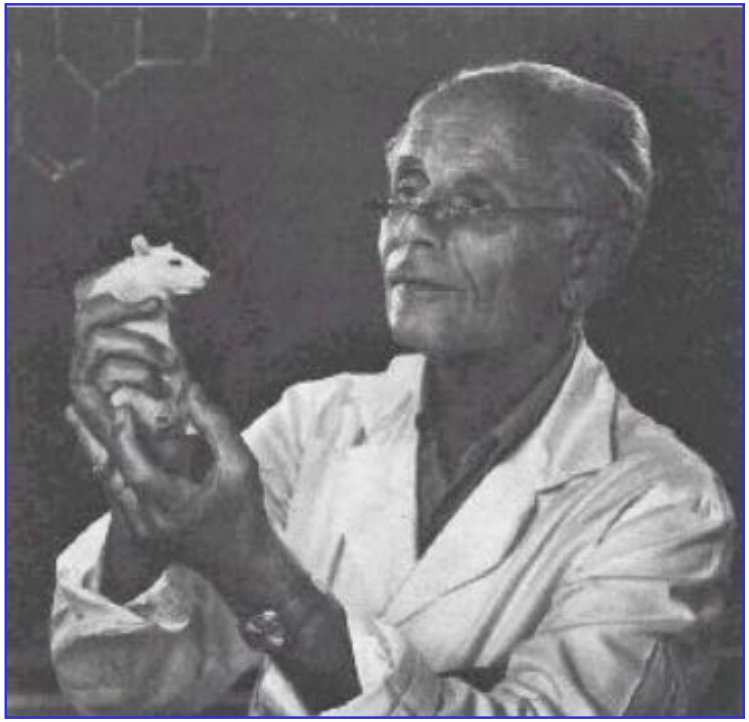
D



Acute „non-genomic“ action of steroids



Anesthetic Effect of Steroid Hormones,
Proc. Soc. Exptl. Biol. Med. **46**, 116-21 (1941)



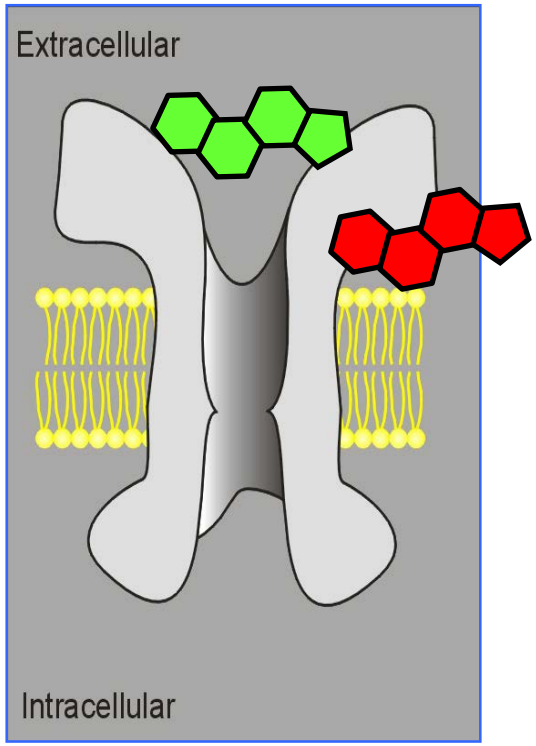
H. H. B. Selye (1907 - 1982)

The Austrian-Hungarian endocrinologist was born in Vienna and graduated at Prague's German Faculty of Medicine. ... is known as "the father of stress„.

Steroids and NMDA receptors

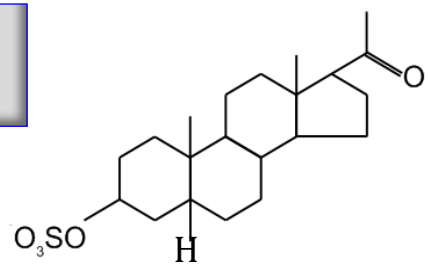
Neurosteroids:

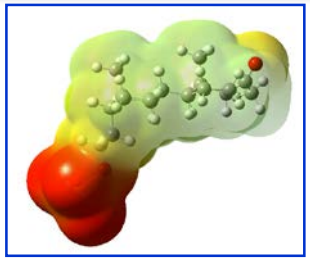
- ❑ are synthesized *de novo* in the CNS
- ❑ can reach a high local concentrations
- ❑ have a direct non-genomic effect



Pregnanolon sulfate (PAS) 20-oxo-5β-pregnan-3α-yl sulfate

1.

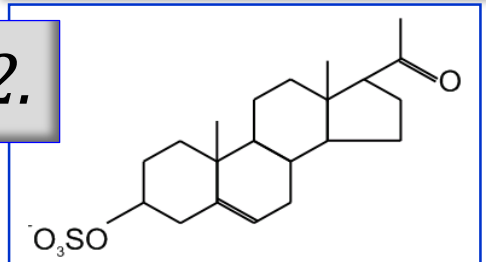


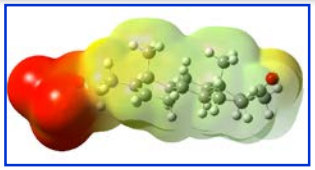


Inhibition

Pregnenolon sulfate (PES) 20-oxopregn-5-en-3β-yl sulfate

2.

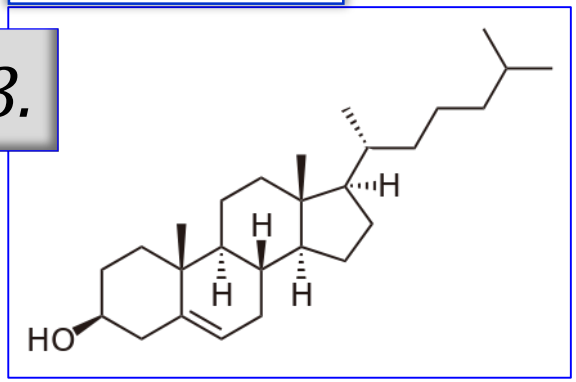


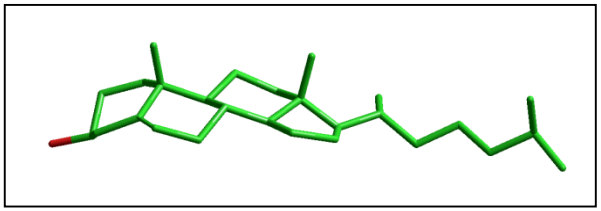


Potentiation

Cholesterol

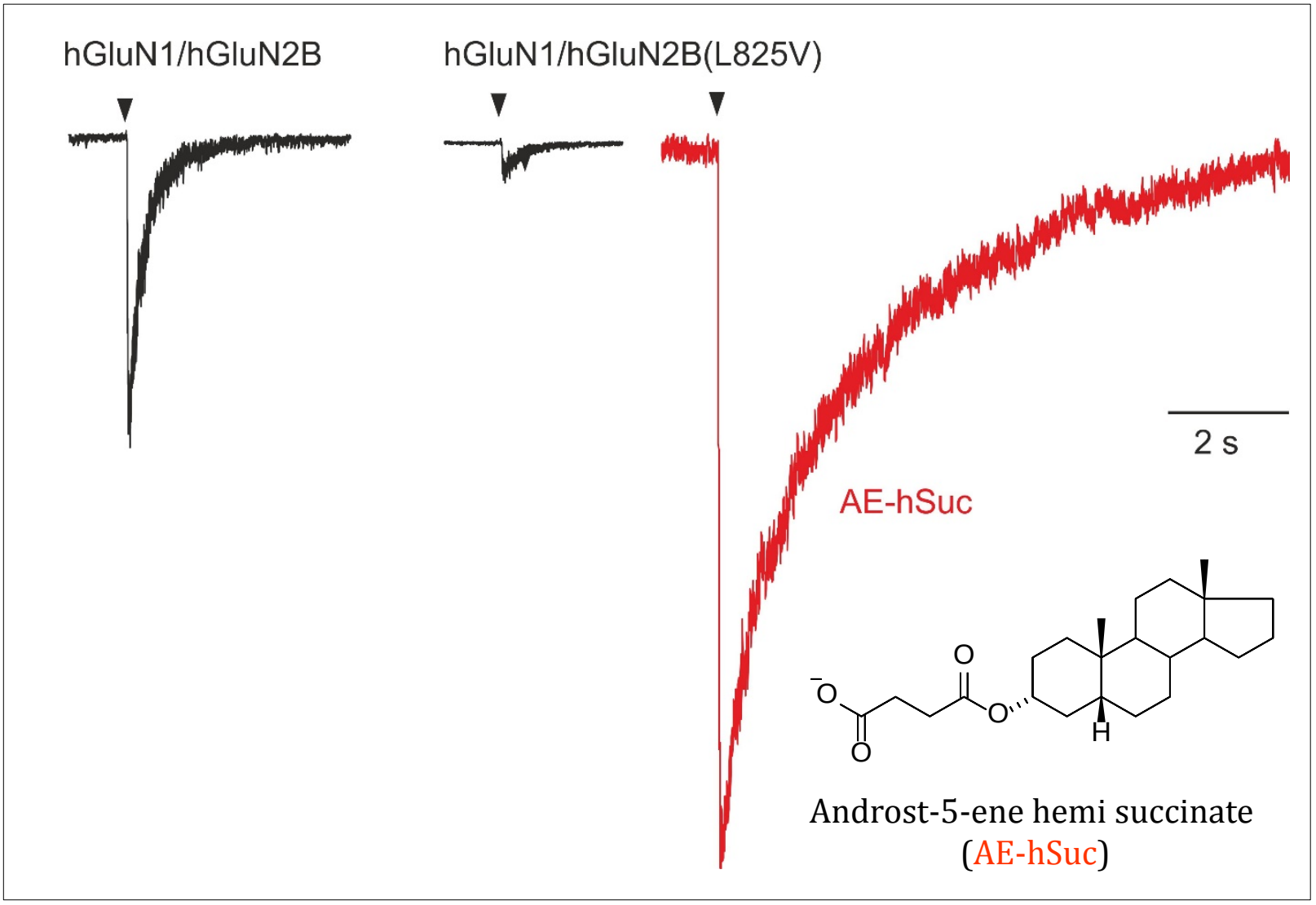
3.





Dependency

Steroids can compensate NMDA receptor malfunction



Androst-5-ene hemi succinate
(AE-hSuc)

Věda je velké dobrodružství

Děkuji za pozornost