利用生物信息手段探究黄鼠缺血耐受机制

Exploring the mechanisms of ischemic resistance in ground squirrels by bioinformatics tools

Group2

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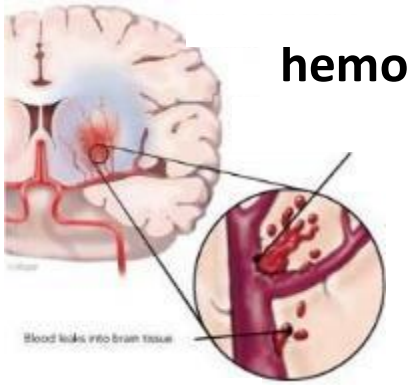
Stroke（World Stroke Day June 24th)

★Brain vascular disease has become a top cause leading to citizen death in China



hemorrhage

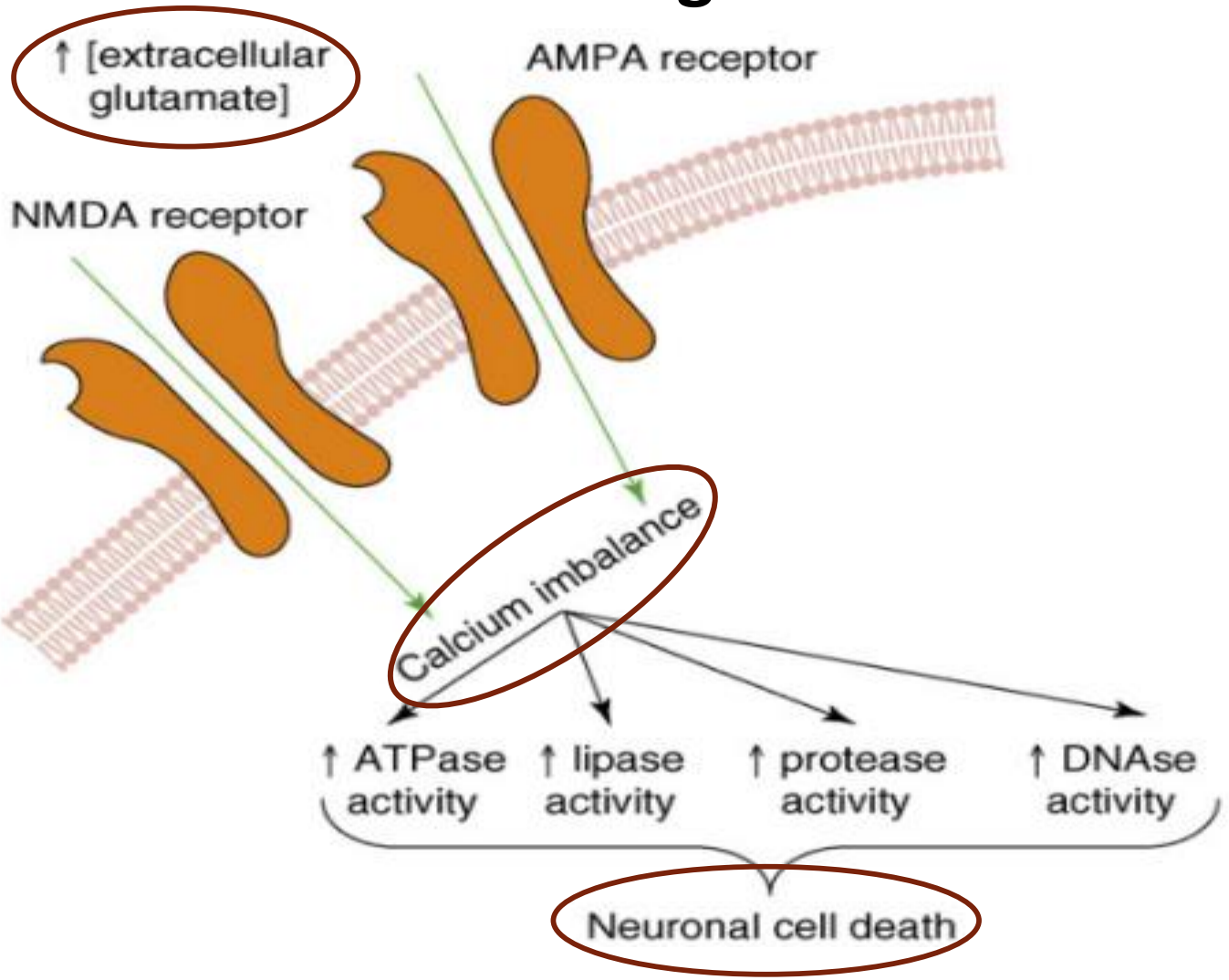
★classified into: ischemic stroke and hemorrhagic stroke

缺血型中风、出血型中风

★no effective treatment

Ischemic(>80%)

Glutamate acids accumulation and calcium overload during ischemia



Besancon *et al.*, *Trends Pharmacol Sci,* 2008

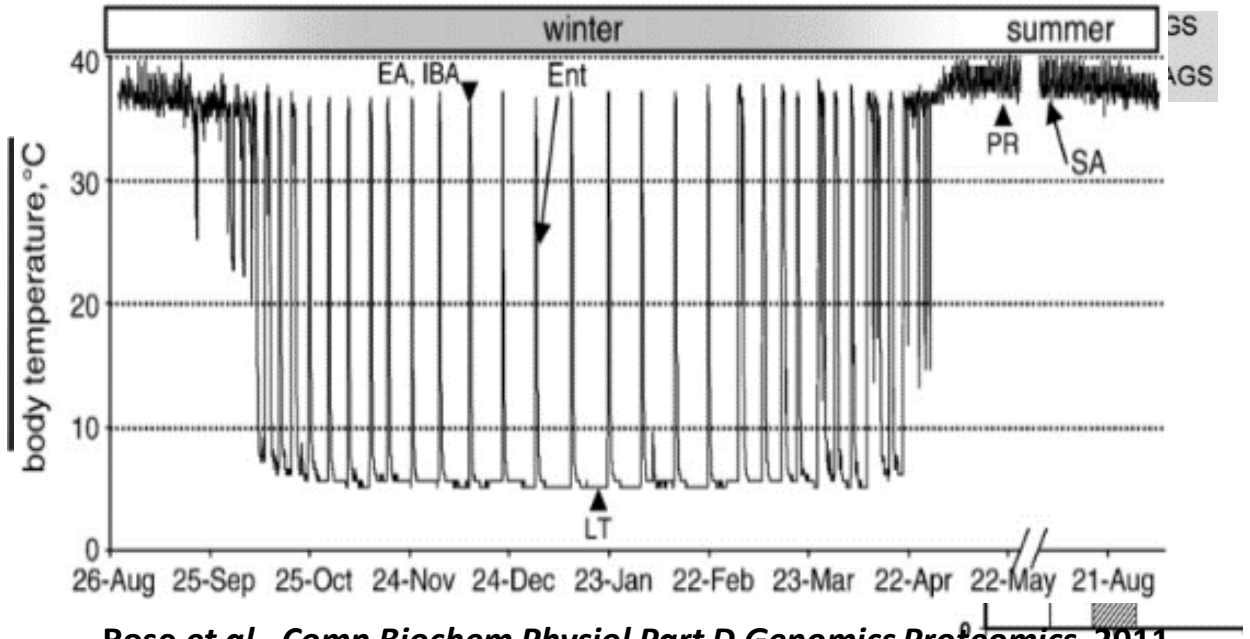
Hibernators（冬眠动物）

Hibernation is a state of inactivity and metabolic depression in endotherms during winter

Hypothermia resistance

Ischemic reisistance

耐低温、耐缺血



Rose *et al.*, *Comp Biochem Physiol Part D Genomics Proteomics,* 2011

★ **Other(anti muscle disuse atrophy、anti-cancer *et al)***

Ma *et al.*, *J Neurosci Methods,* 2008

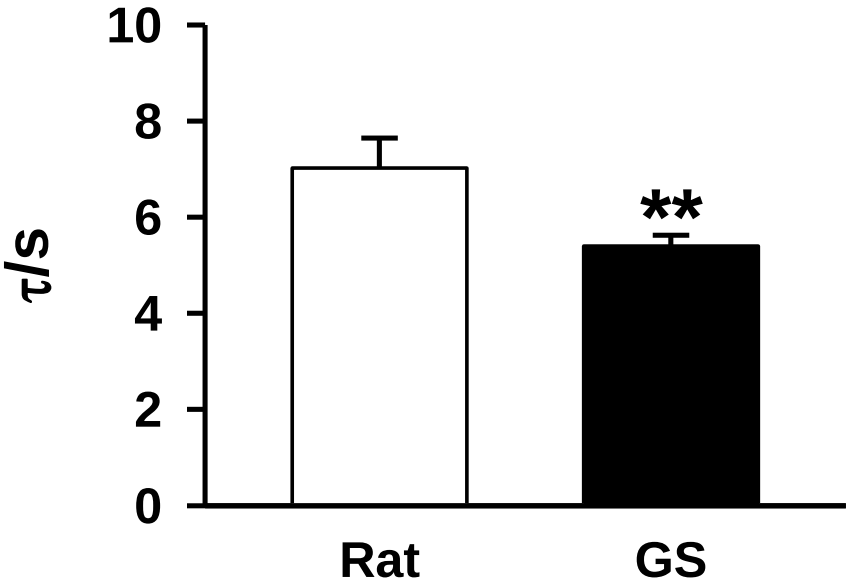
AGS: Arctic ground squirrel

Exploring neuronal ischemic resistance mechanisms in GS

Spermophilus dauricus

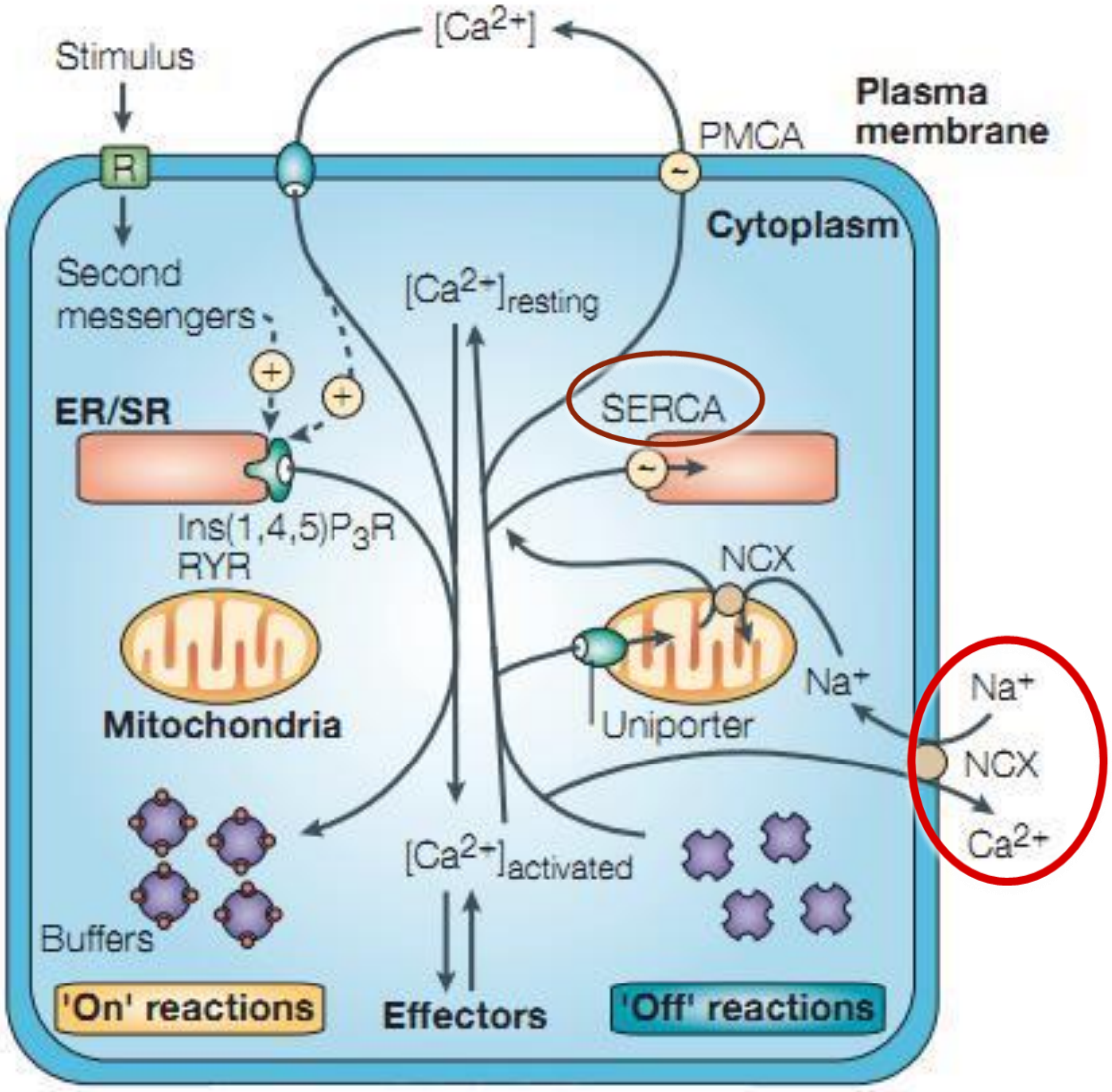
- **Whole animal level**
- **Tissue level**
- **Cellular level**

The velocity of calcium removal was faster in ground squirrel neurons than in rat neurons



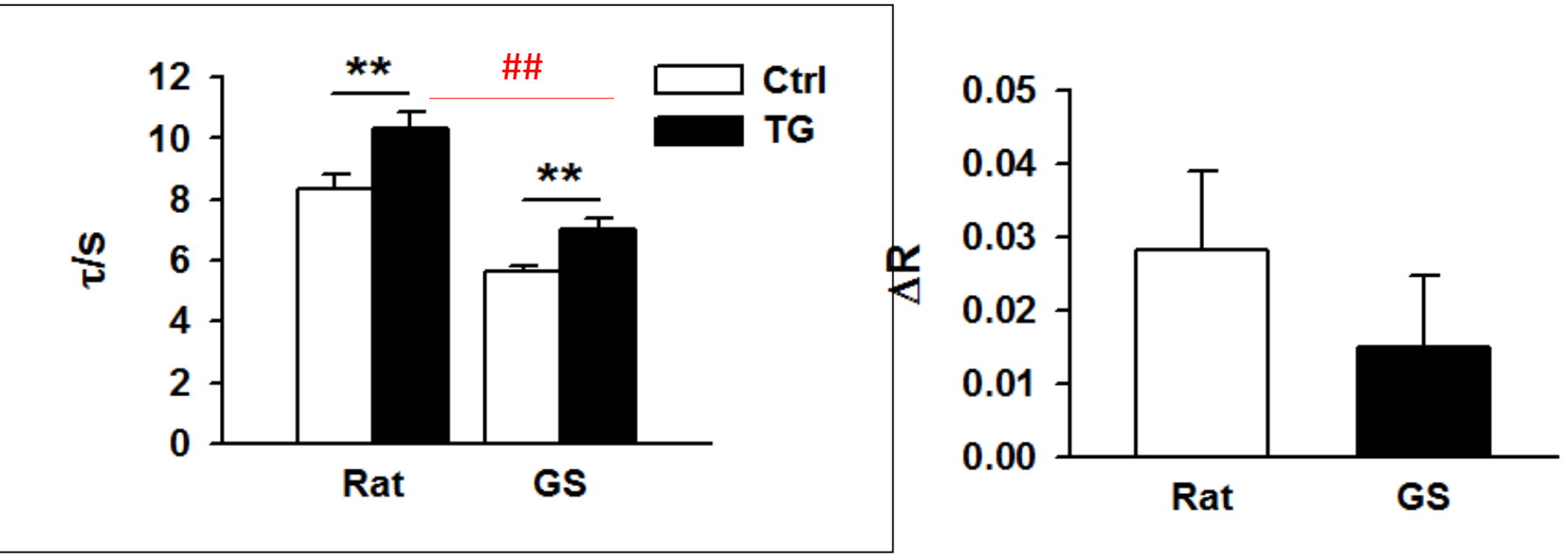
200 μM 谷氨酸处理3h后检测； n=42-62个神经元，4-5次独立实验。

Cellular Calcium homeostasis



Modified from Berridge *et al.*, N*at rev mol cell biol*, 2003

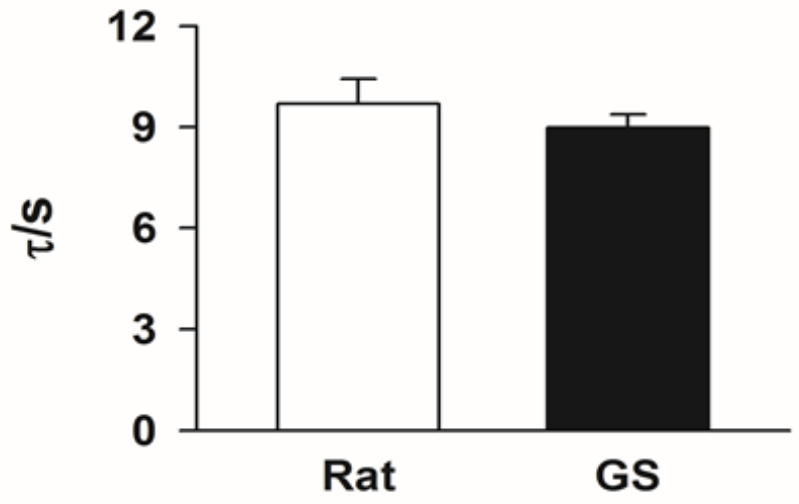
Calcium removal mediated by SERCA showed no difference in GS neurons than in rat neurons



TG: thapsigargin, SERCA（内质网钙泵）特异性抑制剂；R=1/τ； n=56-70个神经元，3次独立实验。

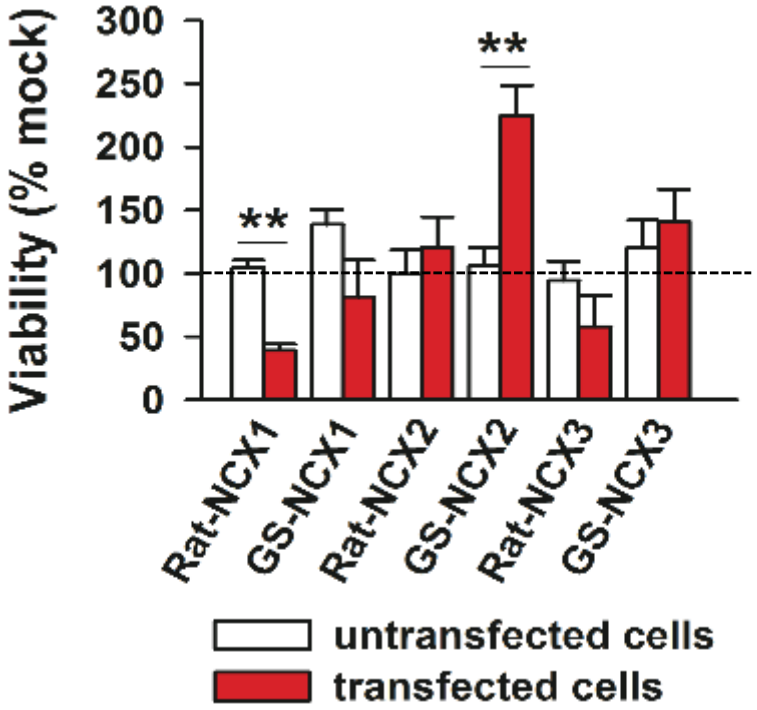
Calcium removal mediated by NCX was faster in ground squirrel neurons than in rat neurons

CB-DMB treatment



CB-DMB: NCXs（钠钙交换体）的特异性抑制剂； n=73-96个神经元，3次独立实验。

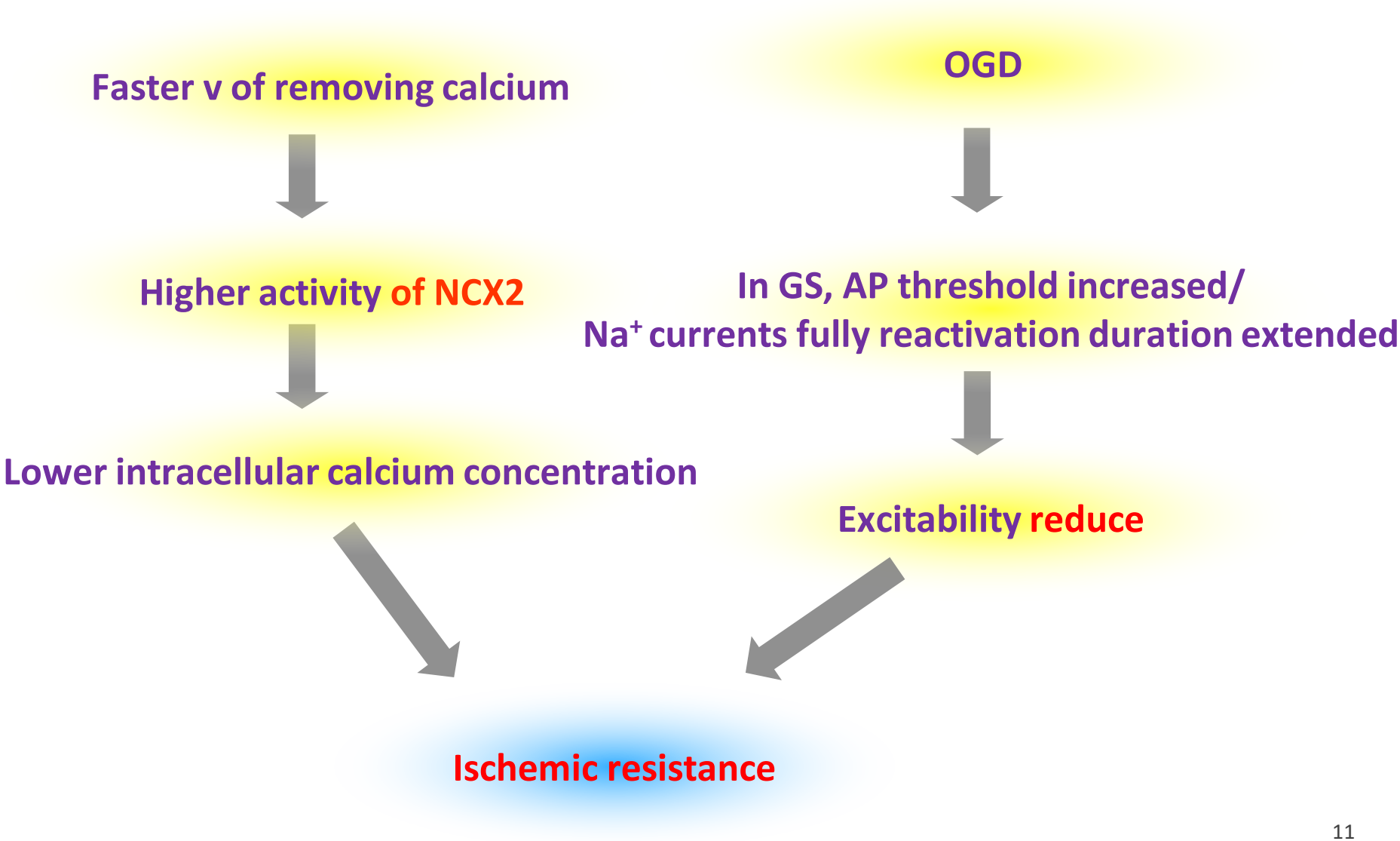
Expression of ground squirrel NCX2 in rat neurons increased neuron survival against glutamate toxicity



过表达黄鼠NCX2亚型的大鼠神经元对谷氨酸毒性有更高的耐受性

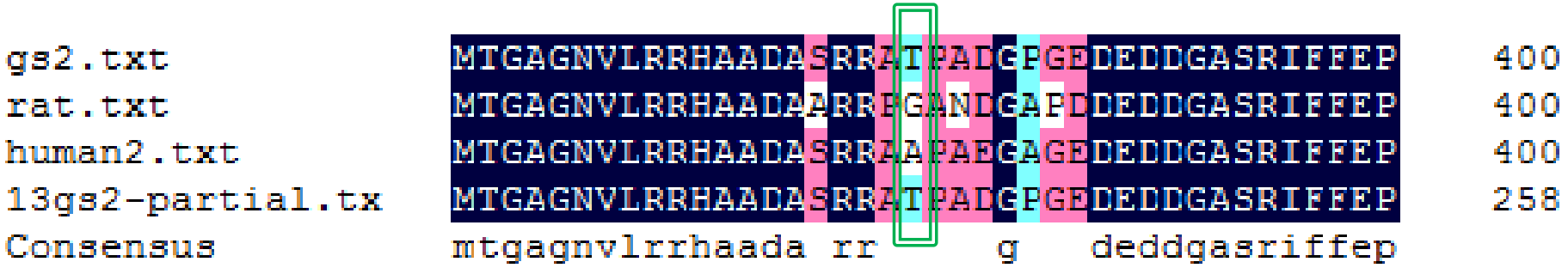
Zhao et al., PLoS one, 2014

Summary


Faster v of removing calcium
Higher activity of NCX2
Lower intracellular calcium concentration
OGD
In GS, AP threshold increased/
Na+ currents fully reactivation duration extended
Excitability reduce
Ischemic resistance

What is the mechanism?

NCX2 sequence alignment



DISPHOS 1.3

Disorder-Enhanced Phosphorylation Sites Predictor

Paste your FASTA formatted protein sequence

Or select a file: 选择文件 未选择任何文件

Default Predictor

Group Predictors

Group predictors are trained to minimize the number of total misclassified residues for a particular group (see Usage for detailed instructions).

Kingdoms and Viruses

Eukaryotes

Organism	Functional Category	
H. sapiens	regulation	cancer
M. musculus	cytoskeleton	membrane
R. norvegicus	ribosomal	inhibitors
C. elegans	transport	kinases
S. cerevisiae	degradation	biosynthesis
D. melanogaster	metabolism	GPCRs
A. thaliana		

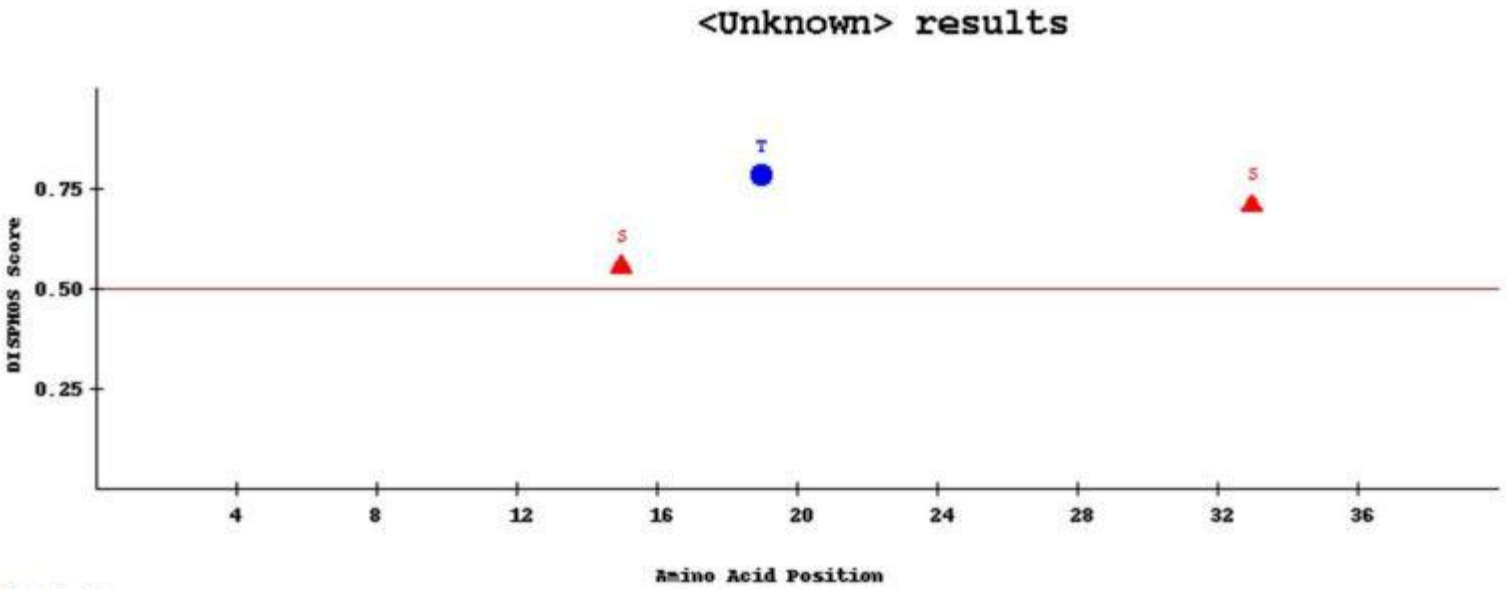
Archaea

Bacteria

Viruses

Predict 重置

DISPHOS



Statistics

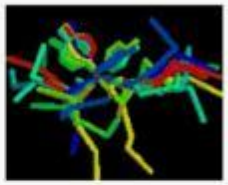
Number of phosphorylated serines: 2 out of 2 (100.000%)
Number of phosphorylated threonines: 1 out of 2 (50.000%)
Number of phosphorylated tyrosines: 0 out of 0 (0.000%)

Position	Residue	Score	Sequence	Yes/No
2	T	0.019	***MTGAGN	
16	S	0.558	AADASRRAT	YES
20	T	0.784	SRRATPADG	YES
34	S	0.713	DDGASRIFF	YES

*An asterisk indicates prediction by similarity to the training sequences

NETPHOS

NetPhos 3.1 Server



The **NetPhos 3.1** server predicts serine, threonine or tyrosine phosphorylation sites in eukaryotic proteins using ensembles of neural networks. Both generic and kinase specific predictions are performed. The **generic** predictions are identical to the predictions performed by NetPhos 2.0. The **kinase specific** predictions are identical to the predictions by NetPhosK 1.0. Predictions are made for the following 17 kinases:

ATM, **CKI**, **CKII**, **CaM-II**, **DNAPK**, **EGFR**, **GSK3**, **INSR**, **PKA**, **PKB**, **PKC**, **PKG**, **RSK**, **SRC**, **cdc2**, **cdk5** and **p38MAPK**.

See the version history of this server.

NOTE: the online service at http://www.cbs.dtu.dk/services/NetPhosK is currently off-line;
for the kinase specific predictions this service should be used instead.

Instructions | Output format | PhosphoBase

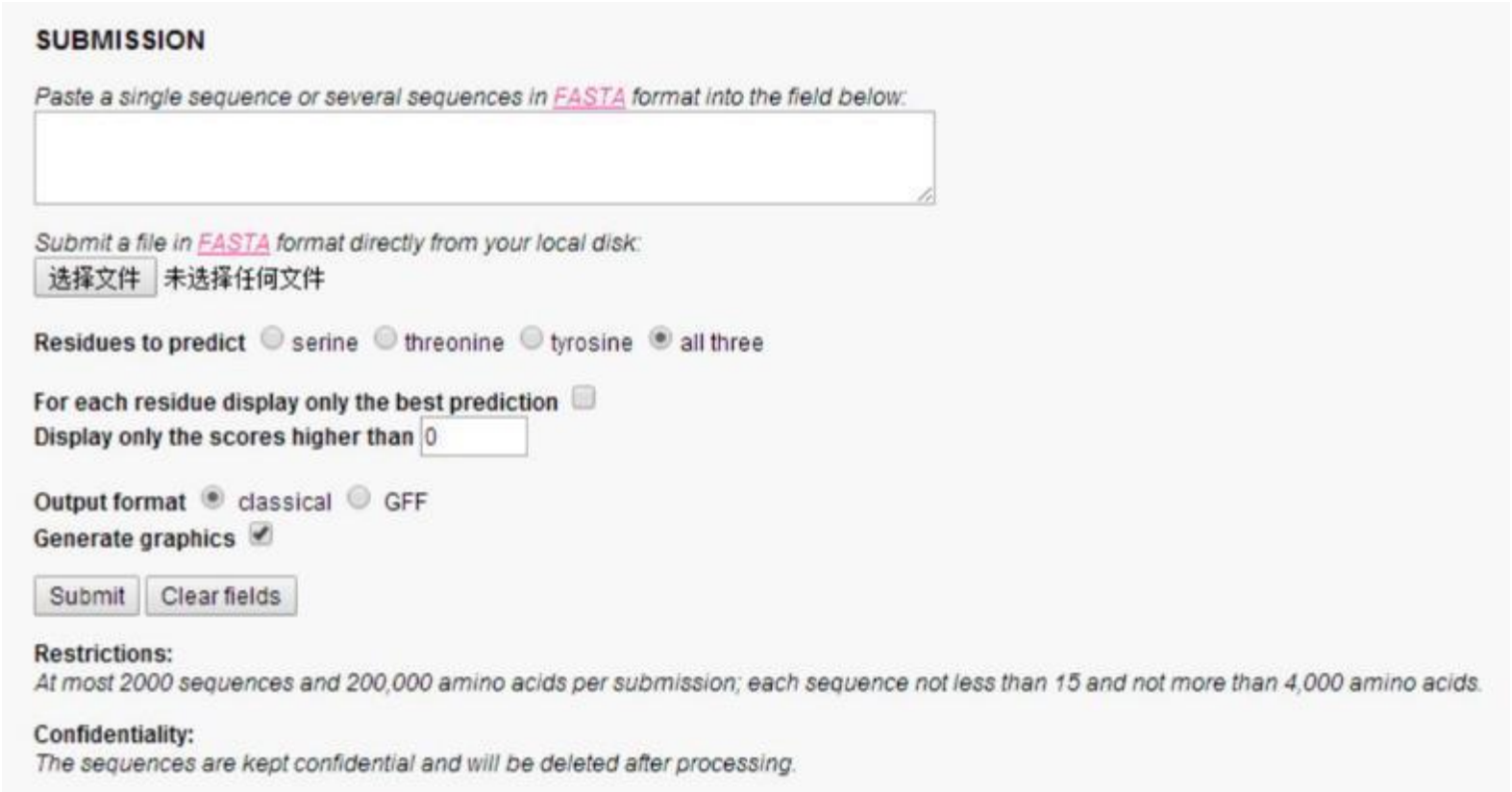
SUBMISSION

Paste a single sequence or several sequences in FASTA format into the field below:

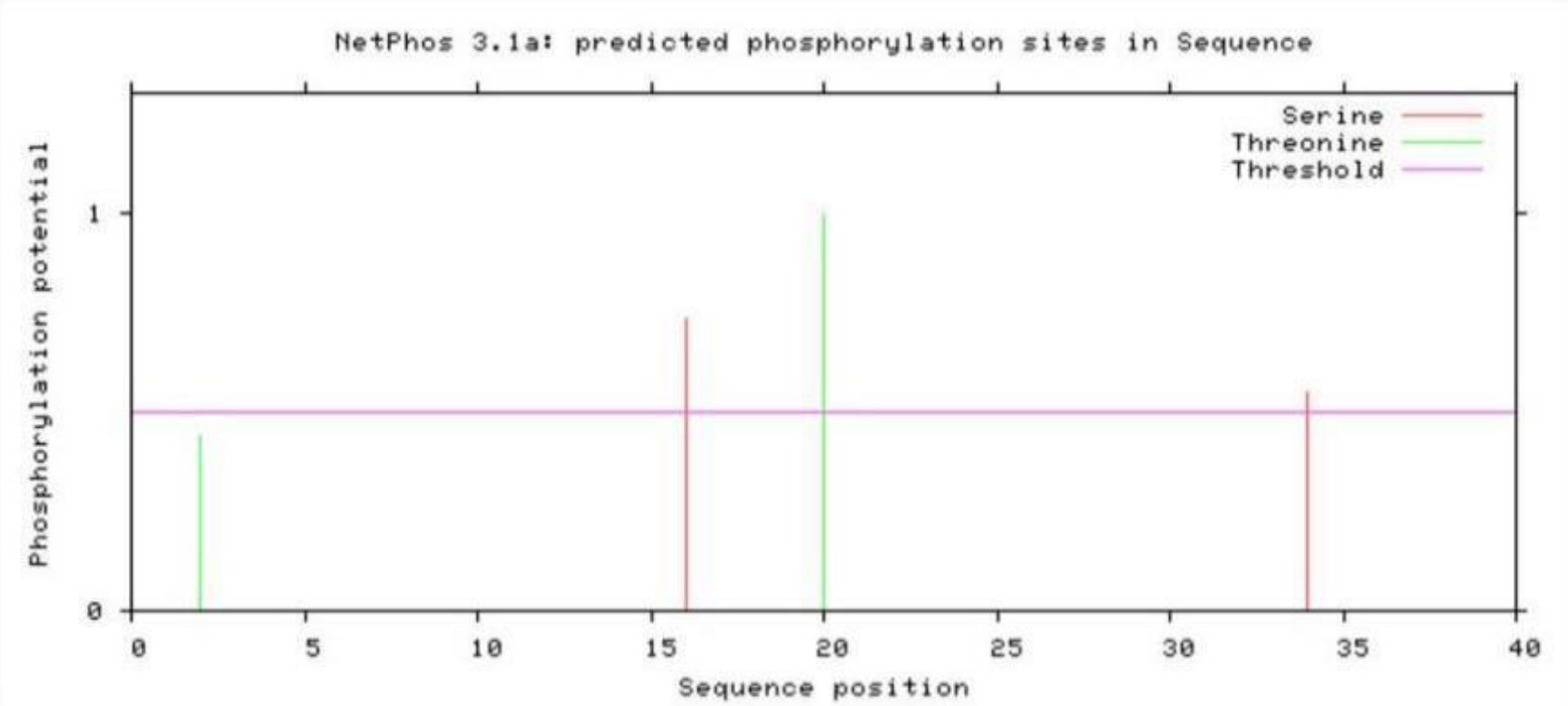
Submit a file in FASTA format directly from your local disk:

选择文件 未选择任何文件

NETPHOS


SUBMISSION
Paste a single sequence or several sequences in FASTA format into the field below:
Submit a file in FASTA format directly from your local disk:
选择文件
未选择任何文件
Residues to predict
serine
threonine
tyrosine
all three
For each residue display only the best prediction
Display only the scores higher than
0
Output format
classical
GFF
Generate graphics
Submit
Clear fields
Restrictions:
At most 2000 sequences and 200,000 amino acids per submission; each sequence not less than 15 and not more than 4,000 amino acids.
Confidentiality:
The sequences are kept confidential and will be deleted after processing.

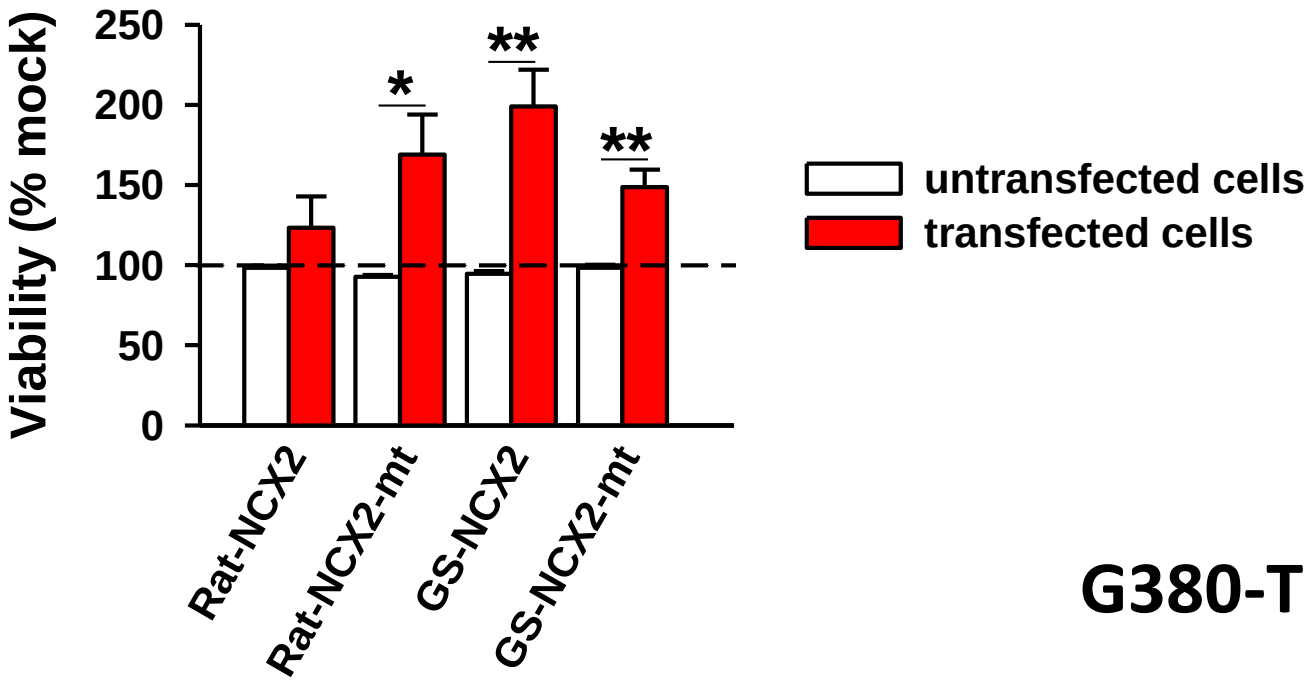
NETPHOS


NetPhos 3.1a: predicted phosphorylation sites in Sequence
Serine
Threonine
Threshold
Phosphorylation potential
1
0
0
5
10
15
20
25
30
35
40
Sequence position

NETPHOS

```
#
# Sequence                20 T    SRRATPADG    0.989    unsp         YES
# Sequence                20 T    SRRATPADG    0.583    RSK          YES
# Sequence                20 T    SRRATPADG    0.531    cdk5         YES
# Sequence                20 T    SRRATPADG    0.501    GSK3         YES
# Sequence                20 T    SRRATPADG    0.470    p38MAPK       .
# Sequence                20 T    SRRATPADG    0.466    PKA           .
# Sequence                20 T    SRRATPADG    0.436    CaM-II        .
# Sequence                20 T    SRRATPADG    0.418    PKG           .
# Sequence                20 T    SRRATPADG    0.370    CKI           .
# Sequence                20 T    SRRATPADG    0.359    DNAPK         .
# Sequence                20 T    SRRATPADG    0.291    cdc2          .
# Sequence                20 T    SRRATPADG    0.260    ATM           .
# Sequence                20 T    SRRATPADG    0.224    CKII          .
# Sequence                20 T    SRRATPADG    0.214    PKB           .
# Sequence                20 T    SRRATPADG    0.094    PKC           .
#
```

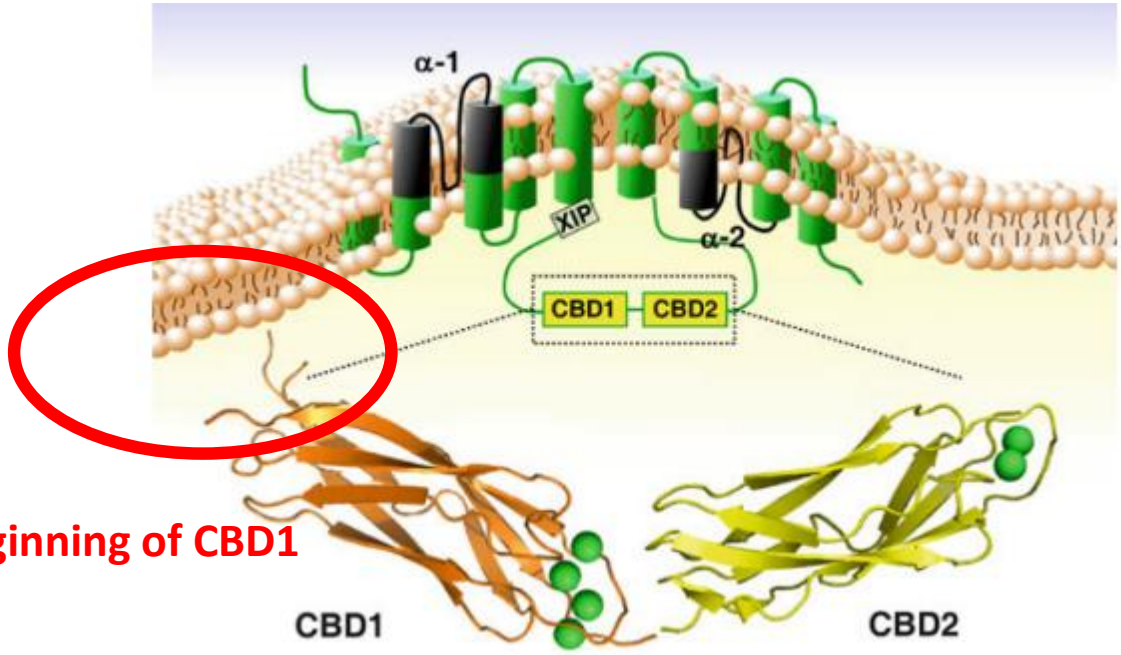
Expression of mutant NCX2 in cultured rat neurons increased neuron survival against glutamate toxicity



过表达点突变的NCX2的大鼠表现与黄鼠相似的毒性耐受性。

Rat-NCX2 Gly380Thr Rat-NCX2-mt
GS-NCX2 Thr380Gly GS-NCX2-mt

The structure of NCX



near the beginning of CBD1

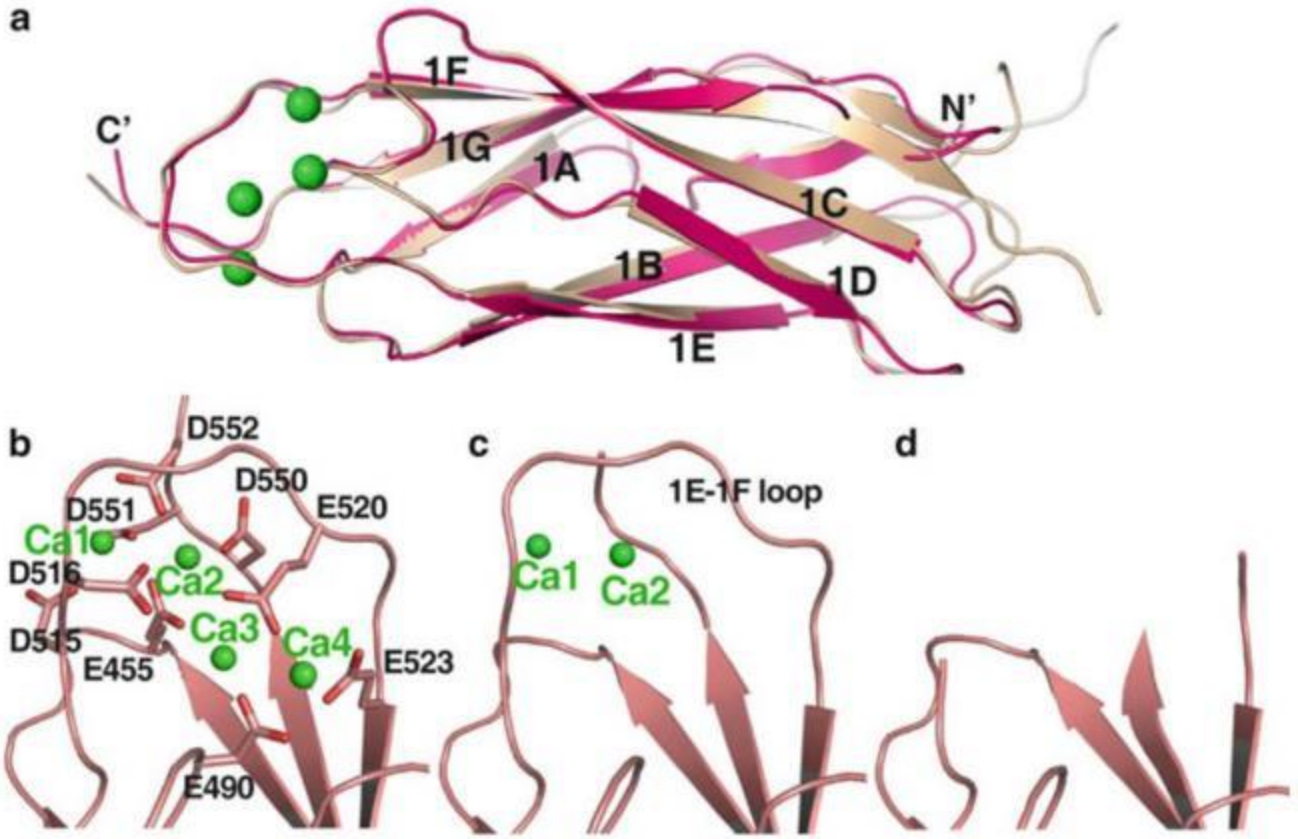
Annunziato et al., *Springer*, 2013

NCX2 sequence alignment

```
gs2.txt          MTGAGNVLRRHAADASRRATPADGPGEDEDDGASRIFFEP   400
rat.txt          MTGAGNVLRRHAADAARRPGANDGAPDDEDDGASRIFFEP   400
human2.txt       MTGAGNVLRRHAADASRRAAPAEGAGEDEDDGASRIFFEP   400
13gs2-partial.tx MTGAGNVLRRHAADASRRATPADGPGEDEDDGASRIFFEP   258
Consensus        mtgagnvlrrhaada rr   g   deddgasriffep
```

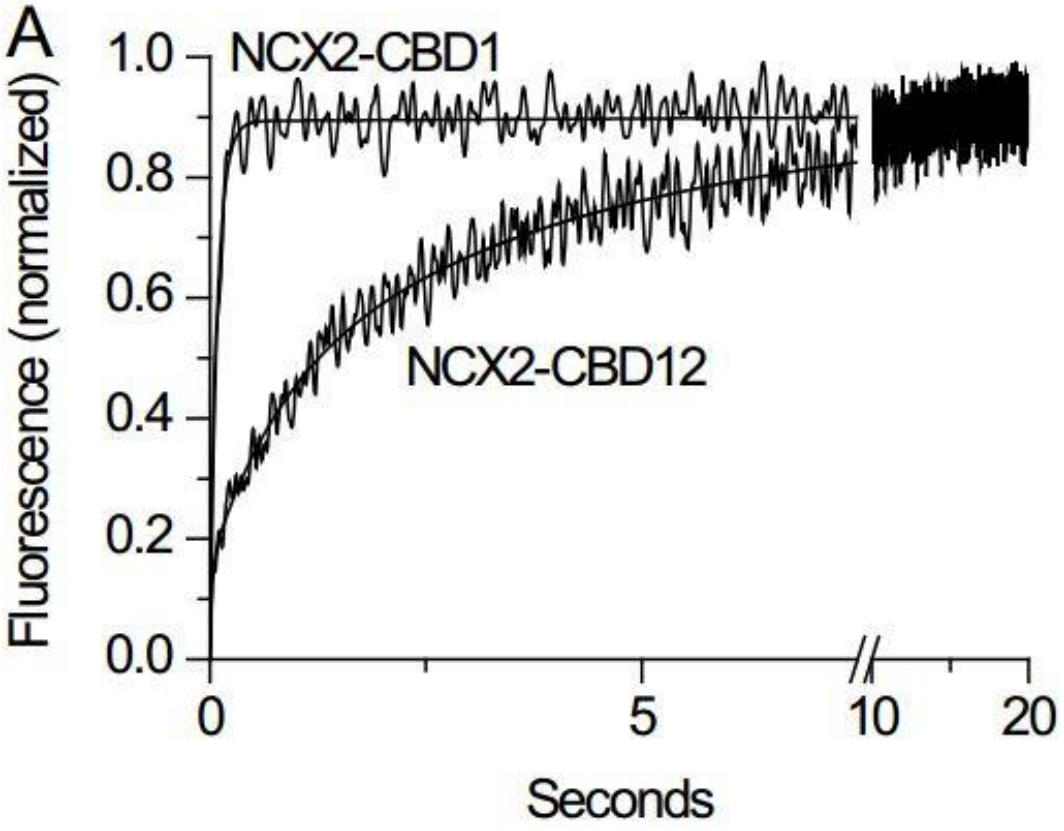
Predicted phosphorylation sites，DISPHOS： 0.725； Netphos 2.0： 0.989

The structure of CBD1



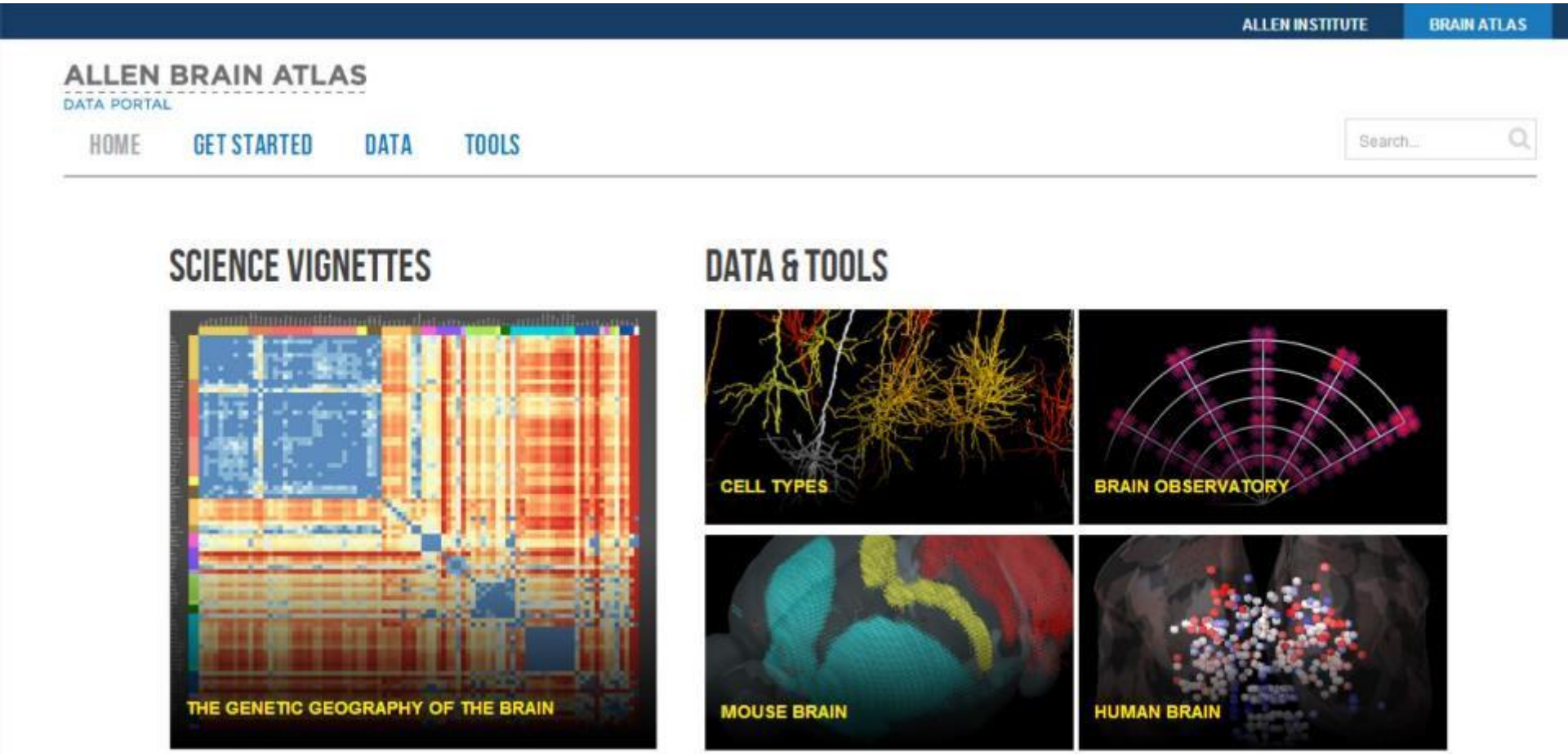
Zheng, L., Wu, M., & Tong, S., *Springer*, 2013

The structure and function of CBD1



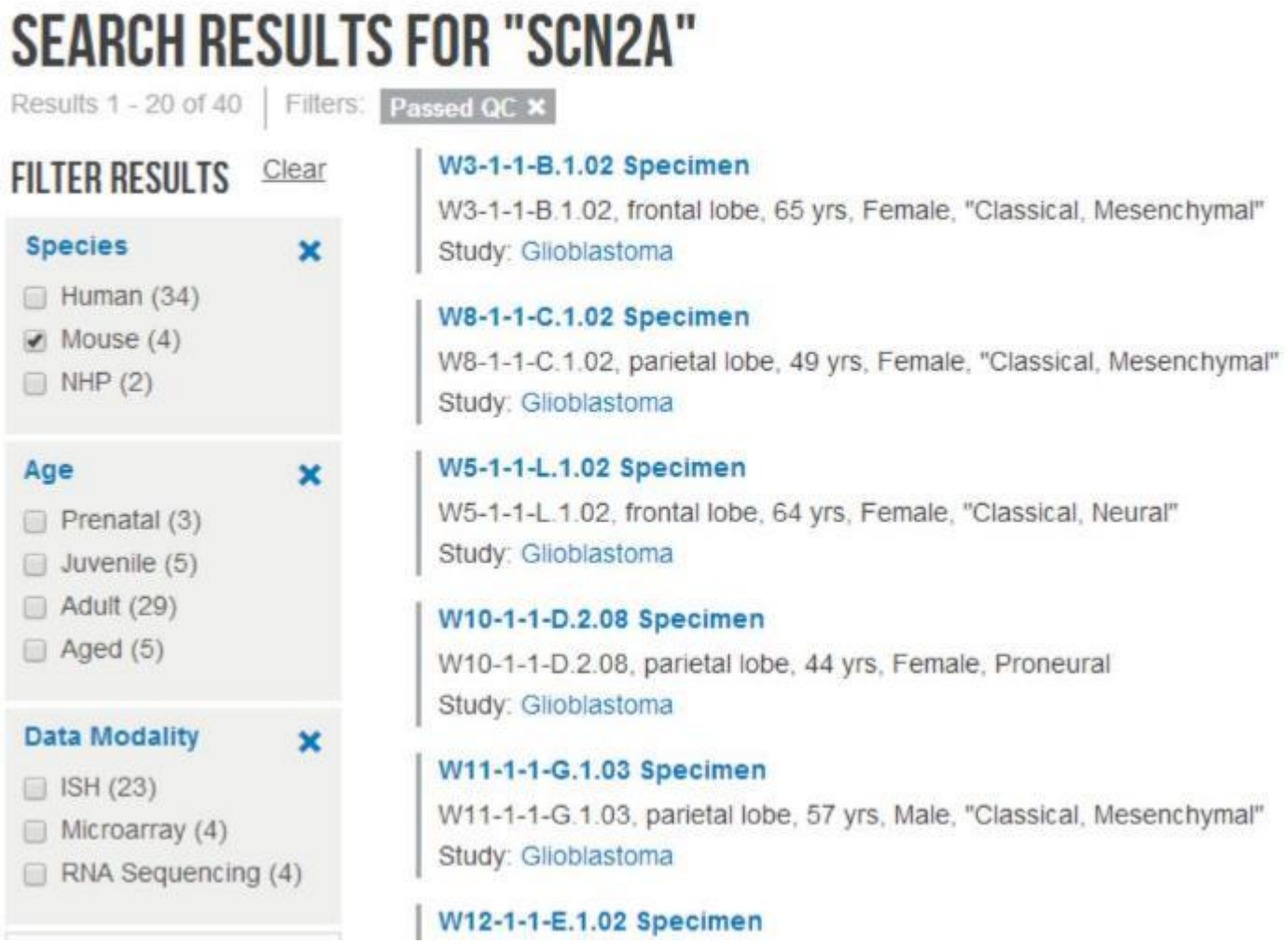
CBD1有着很快的钙解离效率，可以快速排钙。 **Tal, Inbal, *et al.*, *Cell calcium*, 2016**

ALLEN BRAIN ALTAS



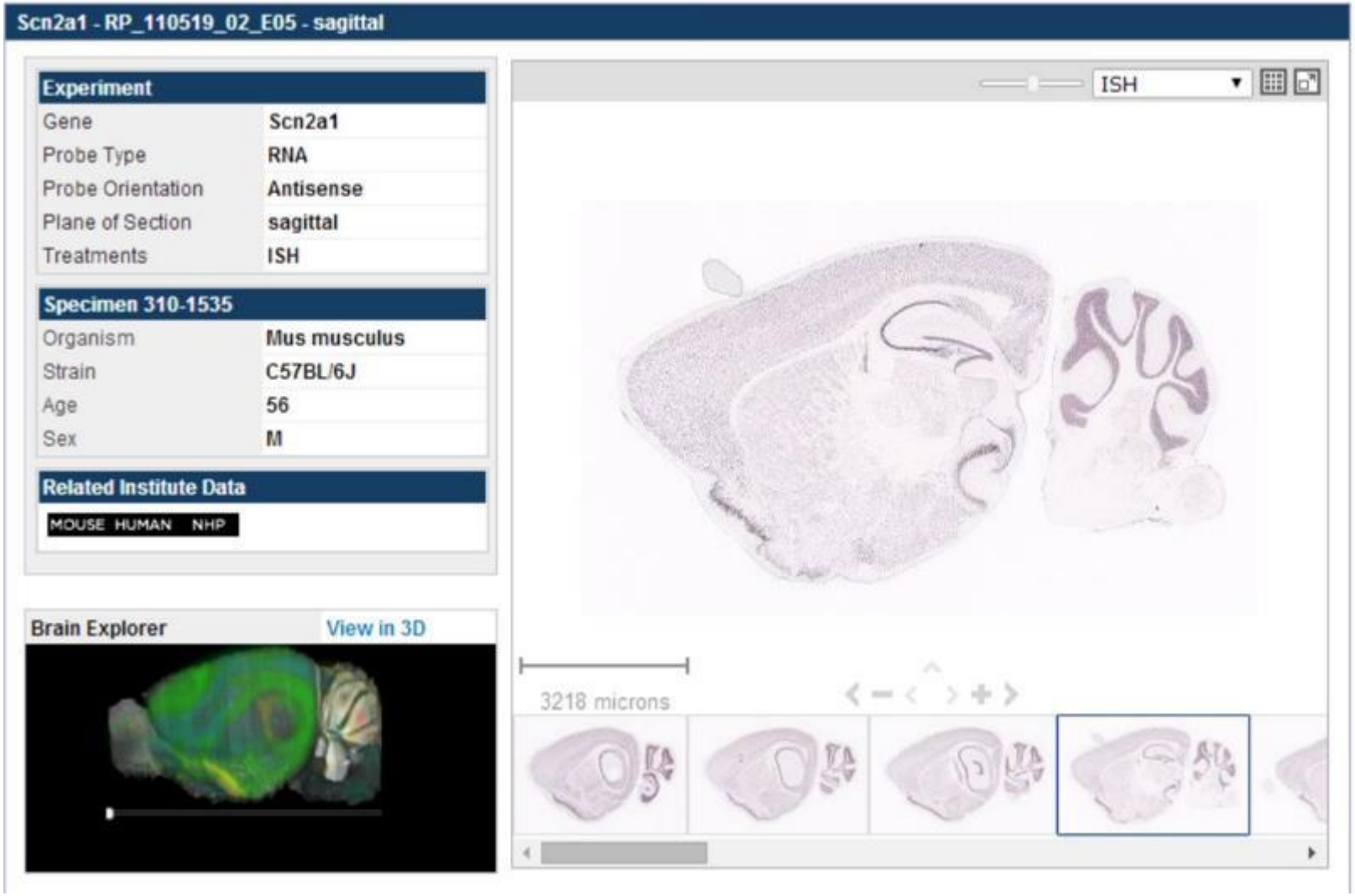
脑内基因表达数据库

ALLEN BRAIN ALTAS



ALLEN BRAIN ALTAS

ALLEN BRAIN ALTAS



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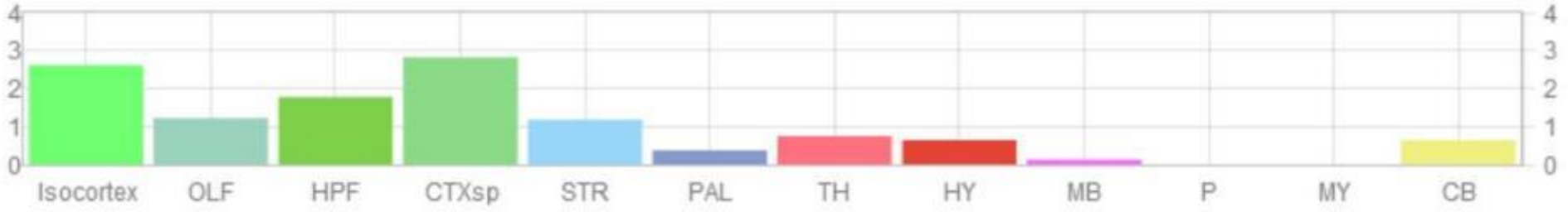


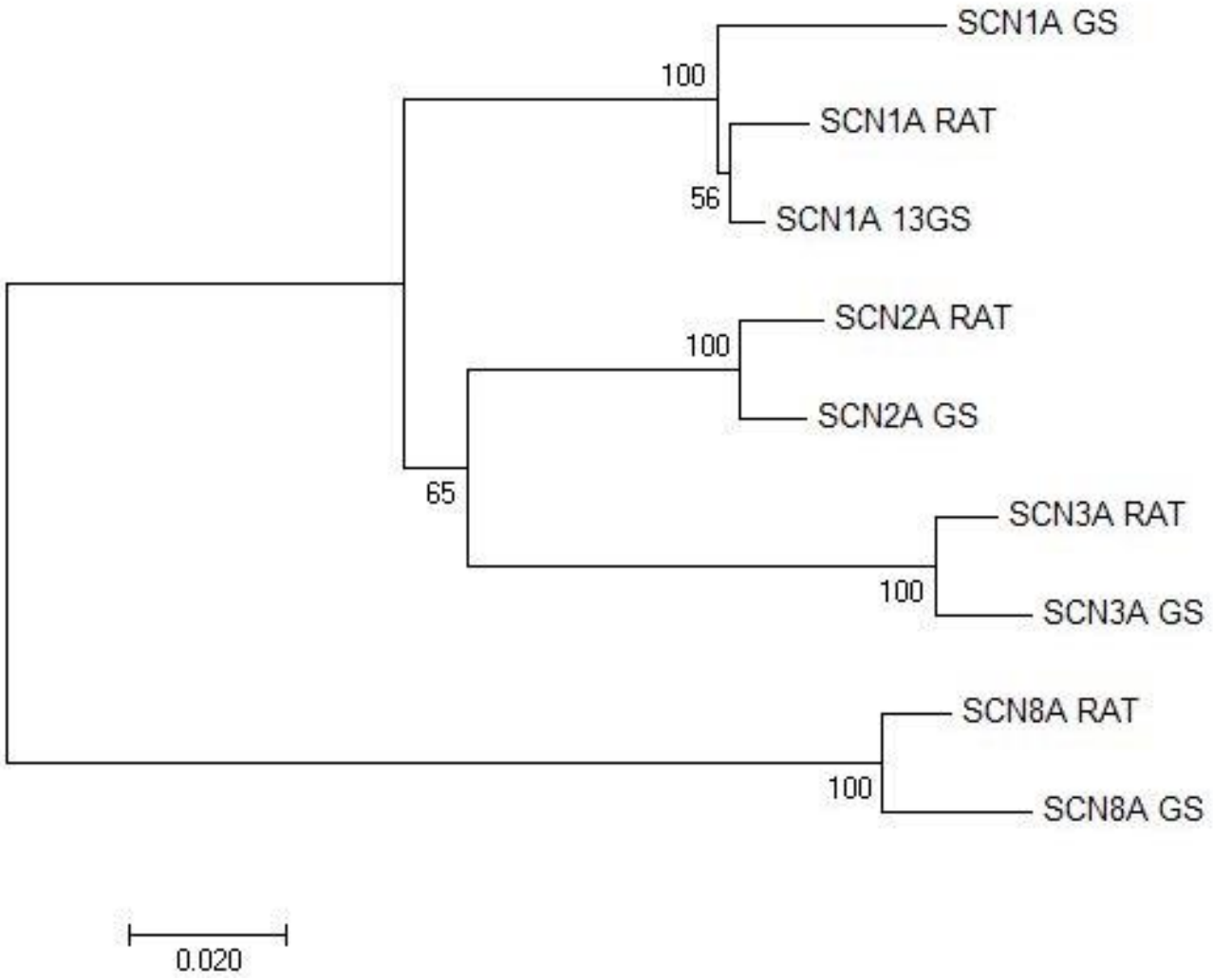
Probe RP_110519_02_E05			
Type	RNA	Orientation	Antisense
NCBI Accession	TC1499159.1	Forward Primer	CAGAGAAGACTGACGTGACACC
GI	-1006	Reverse primer	ATTGCTCTCCTCCACTCTTCAG

Sequence:

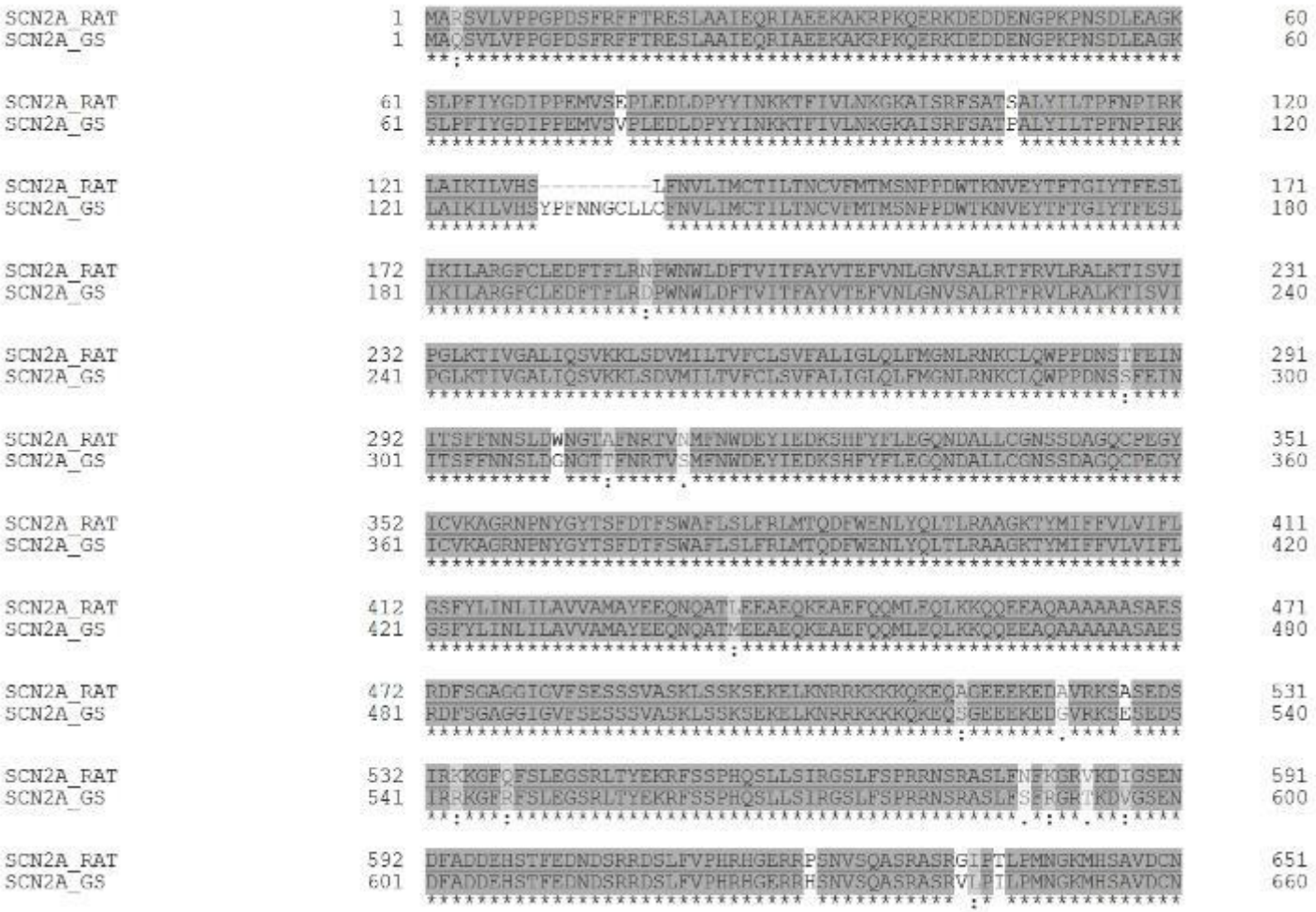
CAGAGAAGACTGACGTGACACCCTCCACCACTTCGCCACCTTCCTACGATAGCGTGACCAAACCGGAGAAGGAAAAATTTGAAAAGGACAAATCAG
AAAAAGAGGACAAGGGGAAAGATATCAGGGAAAGTAAAAAGTAAAAAAATAAAAAATTAATGAAAATTAAAAATTAAAAAAAAAAGAATATTCCATTTTGTGA
TCAACTGTTTACAGCCTGTGTTGGTGATGTATCTGCGACTCCCTCAGGAGGTCTATGCCAAACTGACTGTTTCTATAAGTGTATACTTAAGGTCAGT
GCCTATAACCAGACAGGACCTCGGGTCAGCAAAGTGGGACTCGCCAAACCAGACAAAGAGCACCGACAGGAGGCTTCTGTTTTCGCAACCAGCT

Nav1.1, Nav1.2, Nav1.3,Nav1.6 express in brains.

The sodium channel proteins show similar Characteristic between GS and rat



Sequence alignment of Nav1.2 between GS and rat



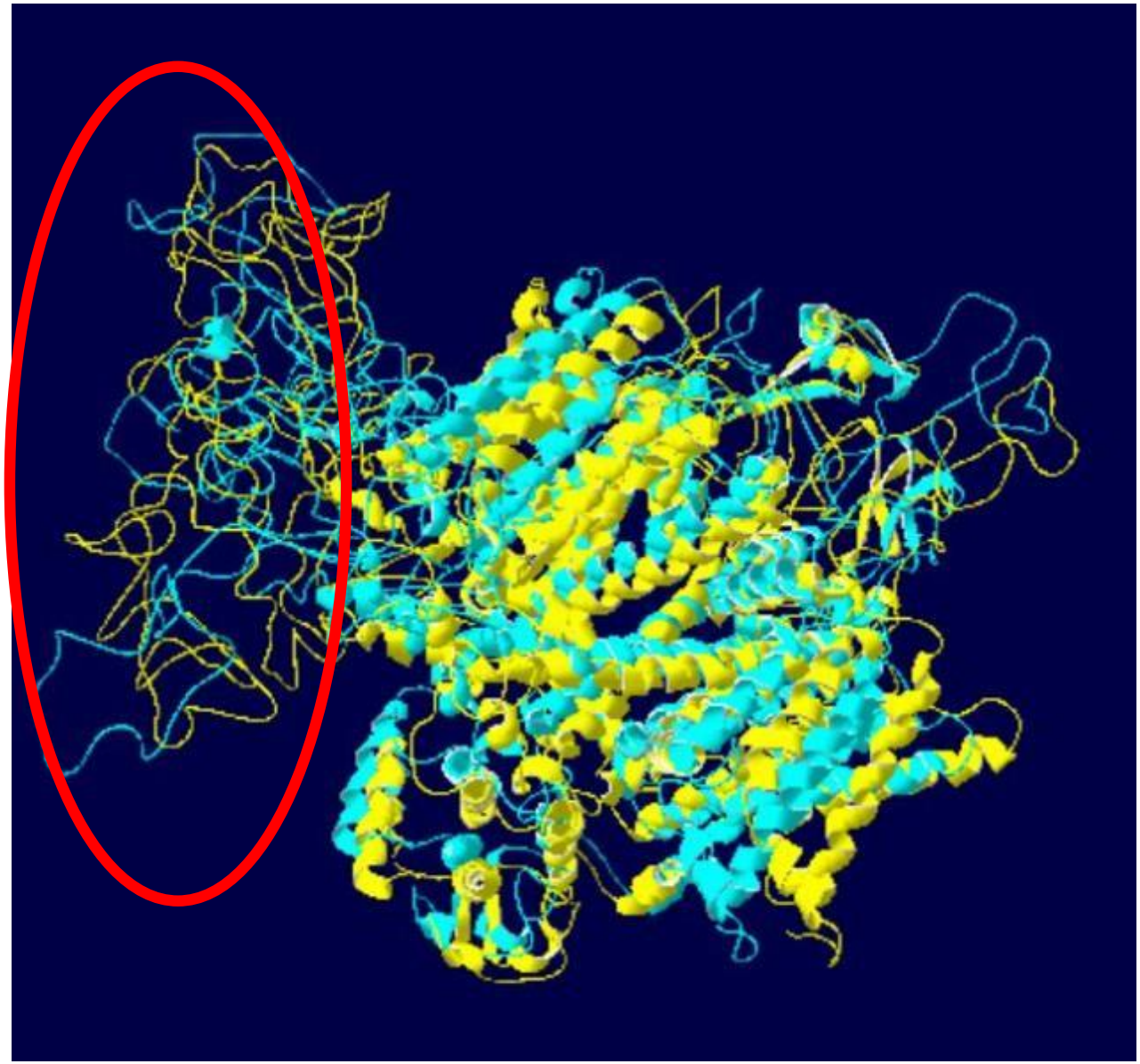
S106P
T646I
S709N
S927A
T1971I

Sequence alignment of Nav1.2 between GS and rat

T1971I may be a possible phosphorylation site

Sequence	1971	S	PSTTSPPSY	0.987	unsp	YES
Sequence	1971	S	PSTTSPPSY	0.637	cdk5	YES
Sequence	1971	S	PSTTSPPSY	0.542	p38MAPK	YES
Sequence	1971	S	PSTTSPPSY	0.501	GSK3	YES
Sequence	1971	S	PSTTSPPSY	0.472	CaM-II	.
Sequence	1971	S	PSTTSPPSY	0.458	CKI	.
Sequence	1971	S	PSTTSPPSY	0.453	cdc2	.
Sequence	1971	S	PSTTSPPSY	0.367	DNAPK	.
Sequence	1971	S	PSTTSPPSY	0.281	ATM	.
Sequence	1971	S	PSTTSPPSY	0.281	RSK	.
Sequence	1971	S	PSTTSPPSY	0.265	PKG	.
Sequence	1971	S	PSTTSPPSY	0.253	CKII	.
Sequence	1971	S	PSTTSPPSY	0.161	PKA	.
Sequence	1971	S	PSTTSPPSY	0.113	PKB	.
Sequence	1971	S	PSTTSPPSY	0.095	PKC	.

Swiss-model



Summary

* We used DISPHOS and Netphos to predicted phosphorylation sites in NCX2.
* The expression of GS NCX2 or mutated Rat NCX2(G380T) can increase its resistance to glutamate acid injury.
* We compared the sequence of all the Nav proteins and predict the T1971I in Nav 1.2 may be a possible phosphorylation site.
* We Homology modeled all these protein and found the different structure are mainly localized in the S2-S5 region.

Acknowledgement

Prof. Luo,Jing-chu
Prof. Chai Zhen
Prof. Wang,Shi-qiang
Wei Jun
Su Zi-jun
My group members
All the ABC classmates

It’s a wonderful semester that I’ll never forget.

Thanks for listening !