Presentation 8 – Ilya Bezprozvanny

Neuronal cell culture model for the study of Gulf War illness

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GWI vs HD – clinical symptoms

Clinical symptoms of GWI:

chronic headaches

memory problems

difficulty concentrating

sleep disturbances

problem with balance

numbness and tingling in extremities

mood changes

The clinical symptoms of GWI are similar to symptoms of Huntington's disease (HD), a progressive neurodegenerative disorder

Huntington's disease (HD)

Gait abnormality in a patient with moderately advanced Huntington's disease (HD).

From M. Groves, J.-P. Vonsattel, P. Mazzoni, K. Marder, Huntington's Disease. *Sci. Aging Knowl. Environ.* **2003** (43), dn3 (2003).

GWI vs HD – brain studies

Brain imaging studies by Haley at al indicated that the functional state of basal ganlgia and brain stem neurons is abnormal in GWI patients

Huntington's disease (HD) results from dysfunction and degeneration of medium spiny striatal neurons in the basal ganglia

GWI vs HD – cause of pathology

Expression of mutant Huntingtin protein with expanded stretch of polyglutamines leads to neuronal dysfunction and death of striatal neurons in HD

HYPOTHESIS:

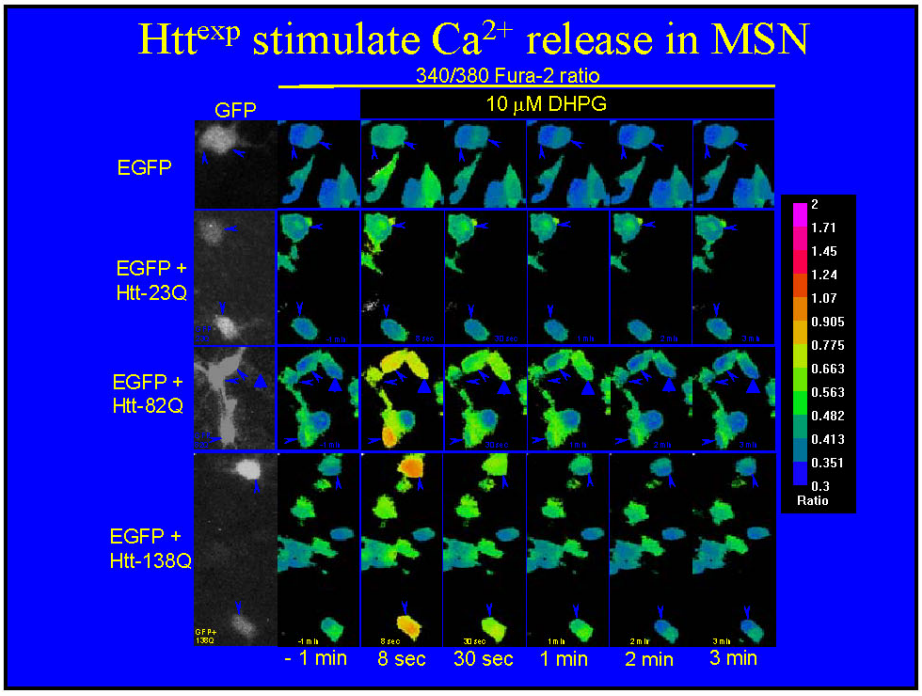
In GWI the neuronal dysfunction is likely to result from prolonged exposure of veterans to low levels of neurotoxic substances (pesticides, sarin nerve gas, DEET, pyridostigmine, depleted uranium) during Gulf War I.

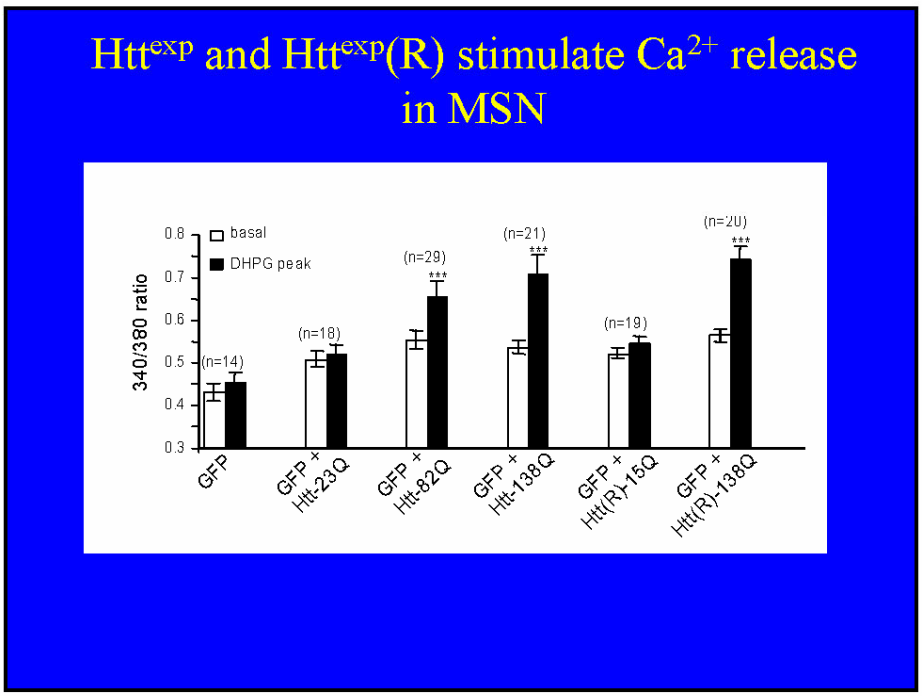
Neuron-2003 paper

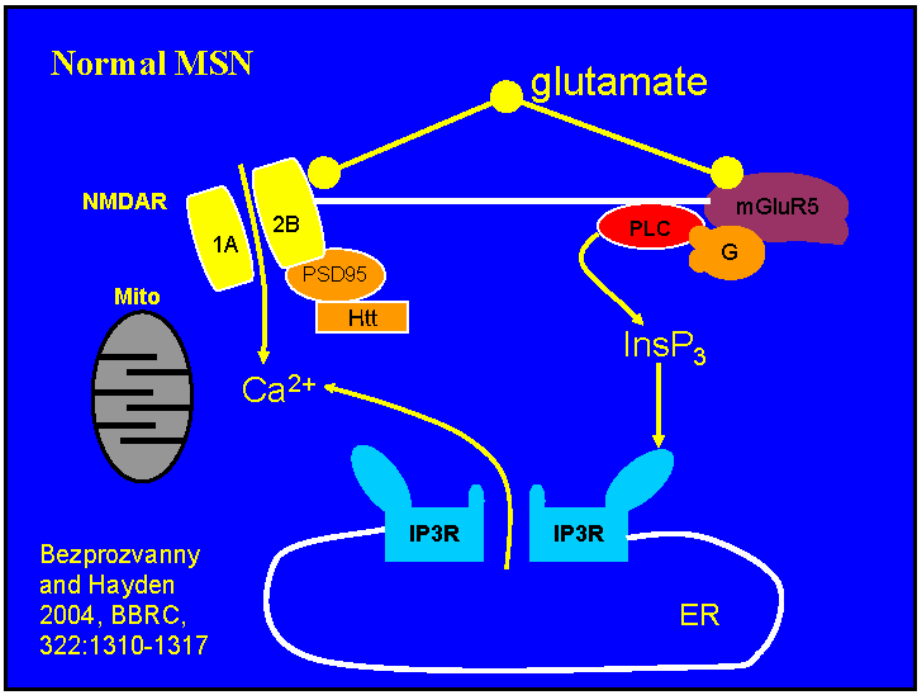
Neuron (2003), v 39, pp 227-239

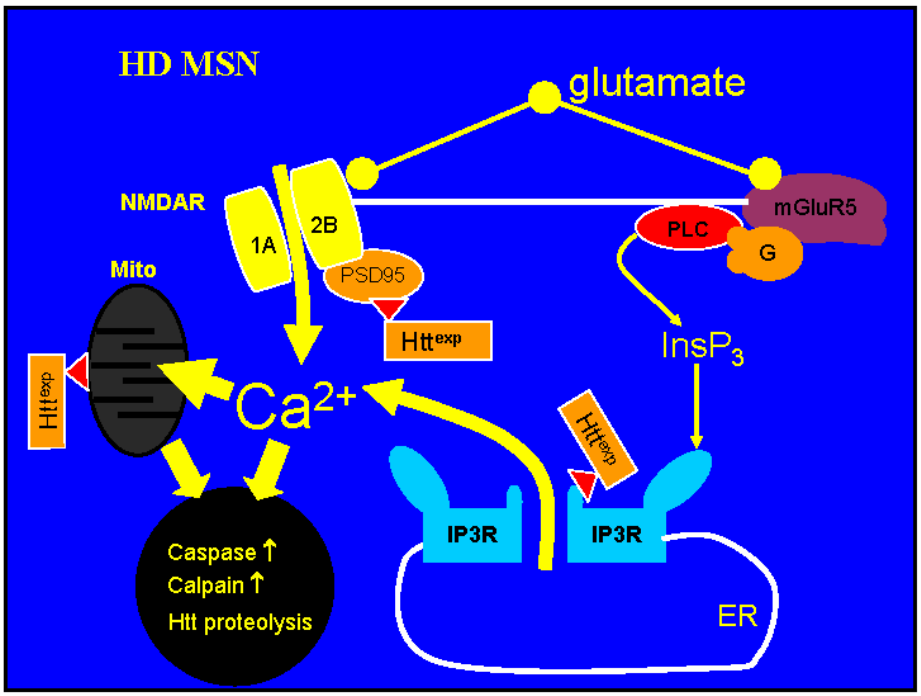
Huntingtin and Huntingtin-Associated Protein 1 Influence Neuronal Calcium Signaling Mediated by Inositol-(1,4,5) Triphosphate Receptor Type 1

Tie-Shan Tang, Huiping Tu, Edmond Y.W. Chan, Anton Maximov, Zhengnan Wang, Cheryl L. Wellington, Michael R. Hayden, and Ilya Bezprozvanny


Htt^exp stimulate Ca2+ release in MSN
340/380 Fura-2 ratio
10 μM DHPG
GFP
EGFP
EGFP + Htt-23Q
EGFP + Htt-82Q
EGFP + Htt-138Q
2
1.71
1.45
1.24
1.07
0.905
0.775
0.663
0.563
0.482
0.413
0.351
0.3
Ratio
- 1 min
8 sec
30 sec
1 min
2 min
3 min


Htt^exp and Htt^exp(R) stimulate Ca2+ release in MSN
basal
DHPG peak
340/380 ratio
0.8
0.7
0.6
0.5
0.4
0.3
(n=14)
(n=18)
(n=29)
(n=21)
(n=19)
(n=20)
***
GFP
GFP + Htt-23Q
GFP + Htt-82Q
GFP + Htt-138Q
GFP + Htt(R)-15Q
GFP + Htt(R)-138Q


Normal MSN
glutamate
NMDAR
1A
2B
PSD95
Htt
mGluR5
PLC
G
Mito
InsP3
Ca2+
IP3R
IP3R
ER
Bezprozvanny
and Hayden
2004, BBRC,
322:1310-1317


HD MSN
glutamate
NMDAR
1A
2B
PSD95
Httexp
mGluR5
PLC
G
Mito
InsP3
Ca2+
Caspase ↑
Calpain ↑
Htt proteolysis
IP3R
IP3R
ER

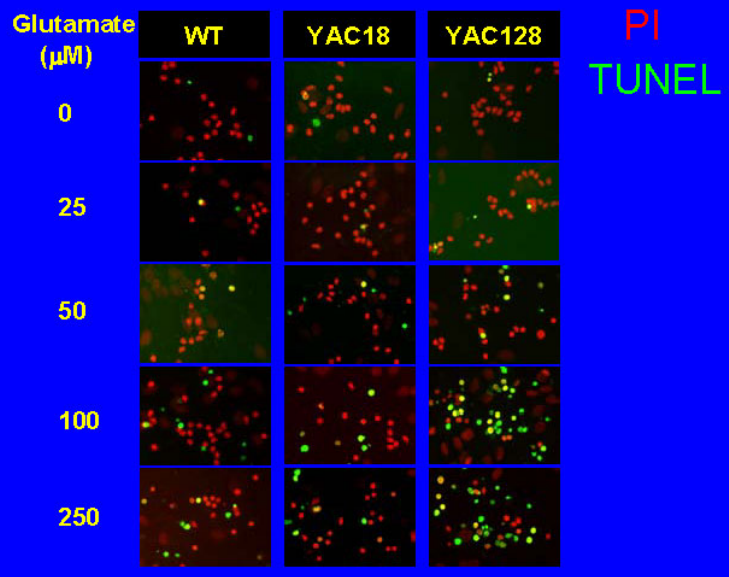
PNAS-2005 paper

Proceedings of the National Academy of Sciences (2005), v 102, pp 2602-2607

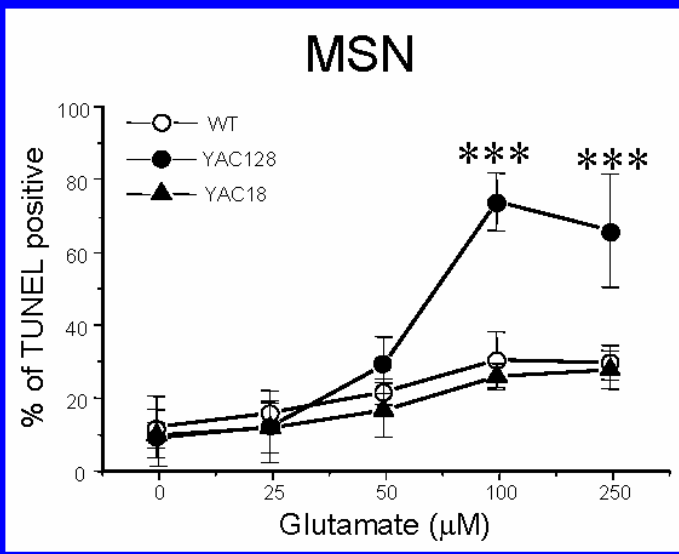
Disturbed Ca^{2+} signaling and apoptosis of medium spiny neurons in Huntington's disease.

Tie-Shan Tang, Elizabeth Slow, Vitalie Lupu, Irina G. Stavrovskaya, Mutsuyuki Sugimori, Rodolfo Llinás, Bruce S. Kristal, Michael R. Hayden and Ilya Bezprozvanny

In vitro HD model (MSN)



Glutamate-induced MSN apoptosis



Neuroscience Letters (2006), v 407, pp 219-223

Evaluation of clinically-relevant glutamate pathway inhibitors in in vitro model of Huntington's disease

Jun Wu, Tie-Shan Tang, and Ilya Bezprozvanny

Table 1
Effects of drugs on glutamate-induced apoptosis in WT and YAC128 MSN

Drug treatment	WT (% TUNEL-positive)		YAC128 (% TUNEL-positive)	
	0 μM glutamate	250 μM glutamate	0 μM glutamate	250 μM glutamate
Group 1 (1 μM)				
Control	10.53 ± 2.62	39.78 ± 4.11	10.20 ± 1.20	76.86 ± 2.46
Folic acid	9.18 ± 0.99	45.74 ± 2.74	9.80 ± 1.95	79.18 ± 2.87
Gabapentin	12.20 ± 1.37	38.65 ± 2.76	11.78 ± 1.79	81.04 ± 4.21
Lamotrigine	7.81 ± 1.52	30.39 ± 4.22	9.14 ± 1.00	67.44 ± 5.29
Memantine	8.15 ± 0.72	35.91 ± 3.78	9.90 ± 1.54	70.47 ± 2.39
Group 2 (1 μM)				
Control	20.52 ± 2.39	45.84 ± 2.19	24.30 ± 2.91	64.13 ± 4.02
Riluzole	18.59 ± 1.85	46.04 ± 5.97	26.30 ± 4.55	65.45 ± 3.50
Group 3 (10 μM)				
Control	14.88 ± 1.02	36.22 ± 2.75	18.59 ± 2.41	**70.60 ± 3.55**
Folic acid	23.26 ± 4.28	58.18 ± 1.84*	23.08 ± 2.21	78.09 ± 4.02
Gabapentin	20.57 ± 1.05	55.90 ± 4.21*	22.57 ± 1.93	64.33 ± 5.31
Lamotrigine	19.26 ± 2.14	35.73 ± 5.43	20.20 ± 1.86	61.59 ± 5.41
Memantine	21.66 ± 2.57	32.96 ± 4.37	17.97 ± 2.70	**37.83 ± 3.56***
Riluzole	18.15 ± 2.94	41.85 ± 4.18	21.86 ± 1.94	**52.51 ± 4.99***

* $p < 0.05$ compared with control experiment.

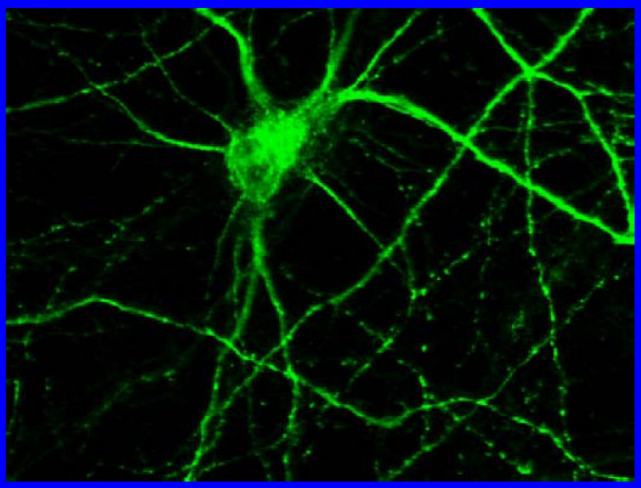
In vitro GWI model

1. Establish primary cultures of mouse neurons from brain regions implicated in GWI by brain imaging studies (substantia nigra, caudate putamen, hippocampus, thalamus, cortex and amygdala).

2. Expose cultured neurons to "GWI agents" (pesticides, sarin nerve gas, DEET, pyridostigmine, depleted uranium) and assay for neuronal dysfunction and death as in our HD studies.

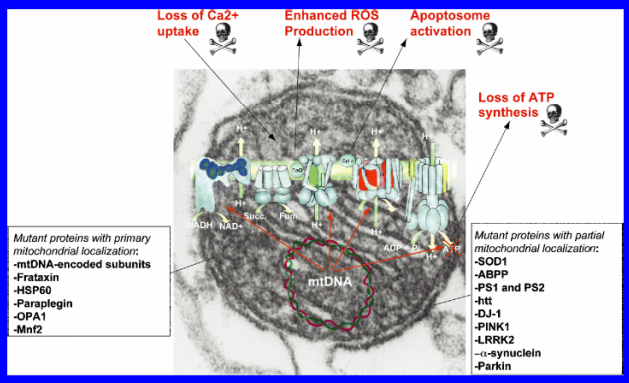
3. Test if NMDA receptor antagonist memantine can protect cultured neurons from exposure to "GWI agents".

Mouse cortical neurons in culture

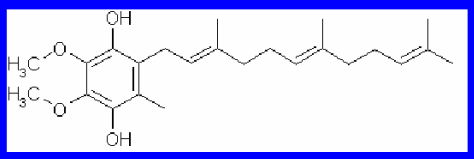


GWI as mitochondrial disorder

Hypothesis: "GWI agents" damage neurons by damaging their mitochondria



Coenzyme Q10 as treatment of GWI



Co-Q10

Co-Q10 is an essential factor in the mitochondrial electron transport chain and important antioxidant in mitochondria.

Co-Q10 has been shown to be beneficial for treatment of mitochondrial and neurodegenerative disorders, including HD

We plan to test the ability of co-Q10 to restore the function of neurons exposed to "GWI agents" in cell culture and whole animal models of GWI.