TAUB INSTITUTE
for research on
ALZHEIMER'S DISEASE
and the aging brain

Novel PDE5 Inhibitors as a Therapeutic Tool Against Alzheimer's Disease

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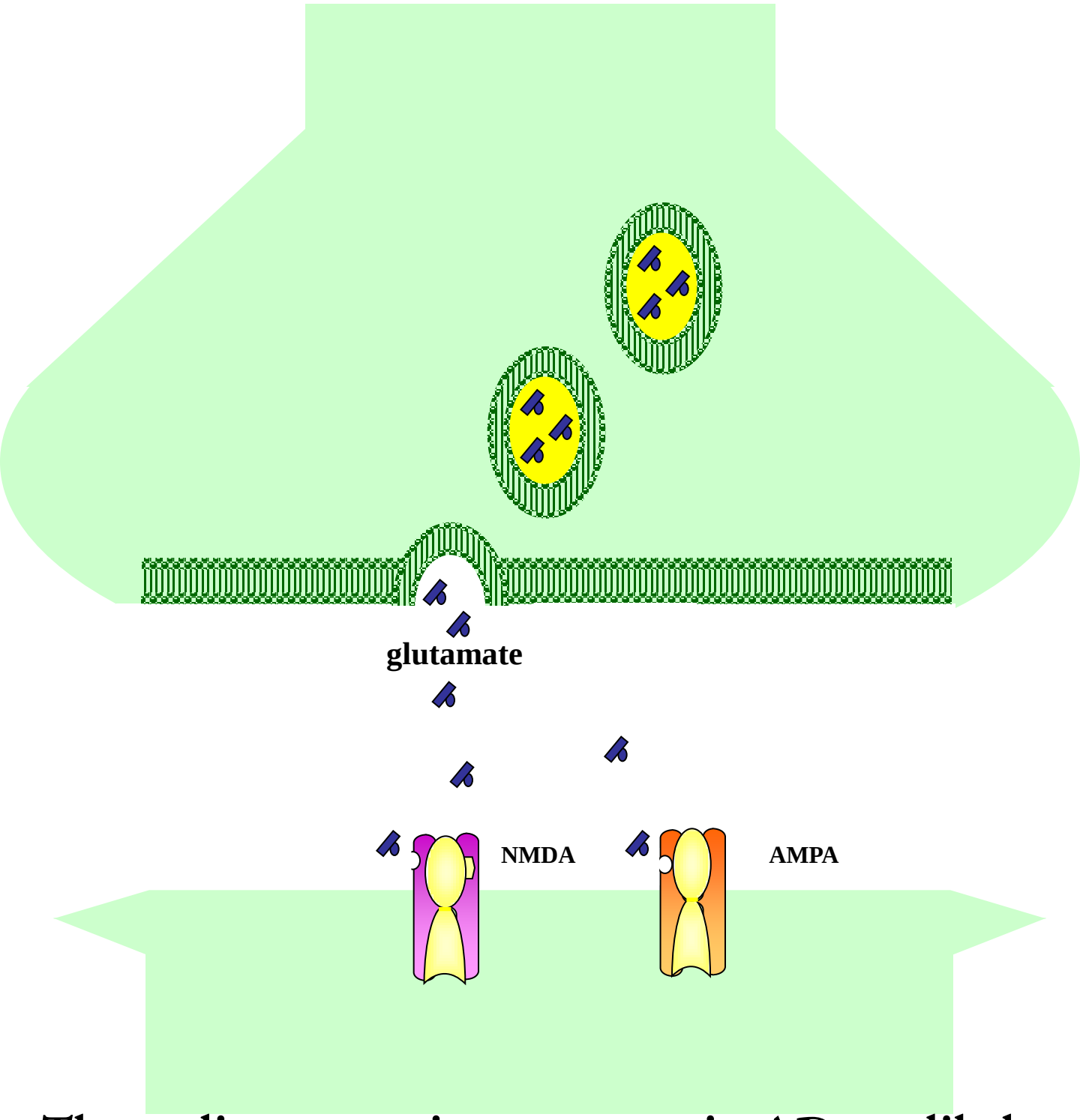
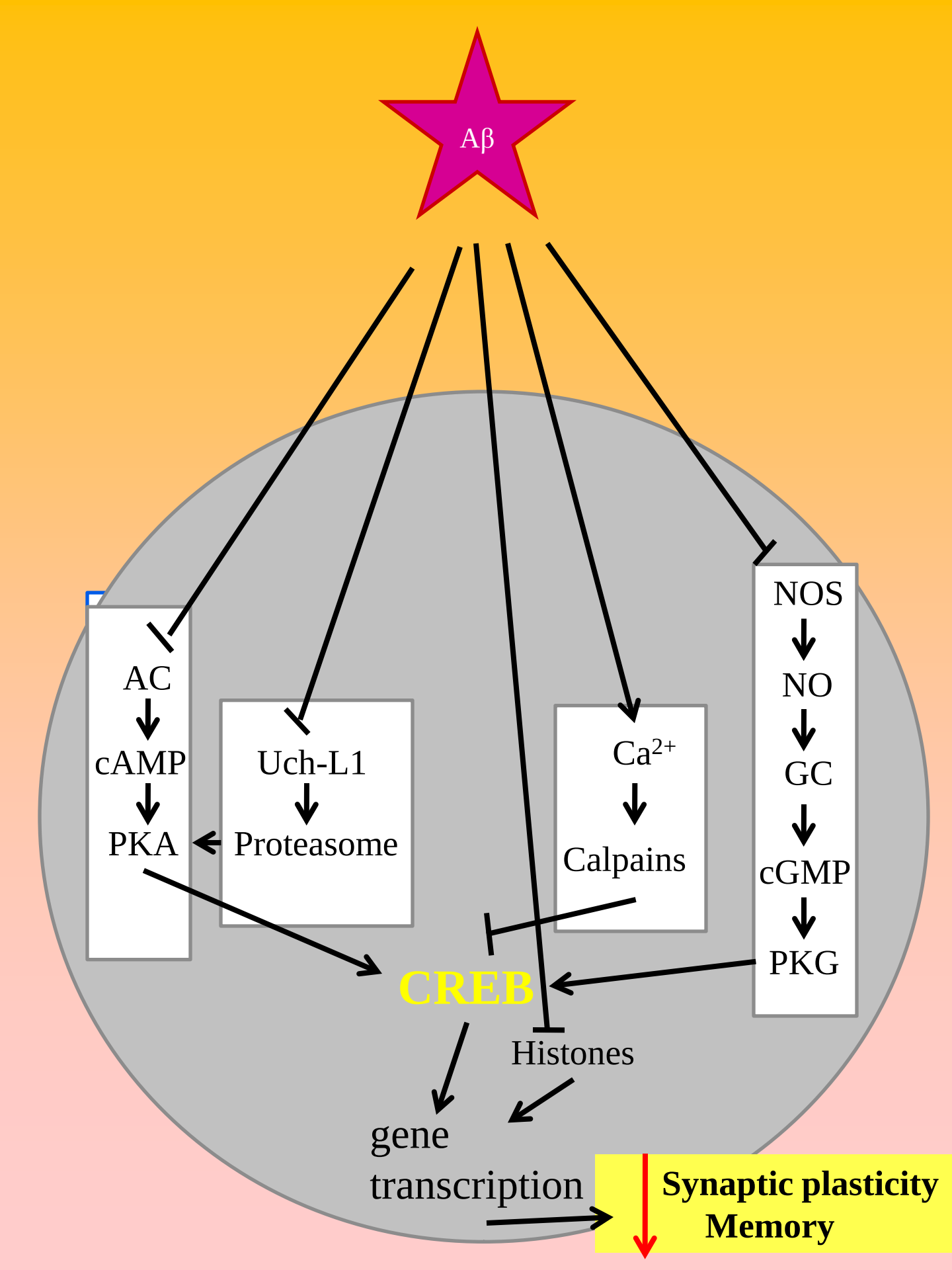
Synaptic alterations are highly correlated with the severity of clinical dementia



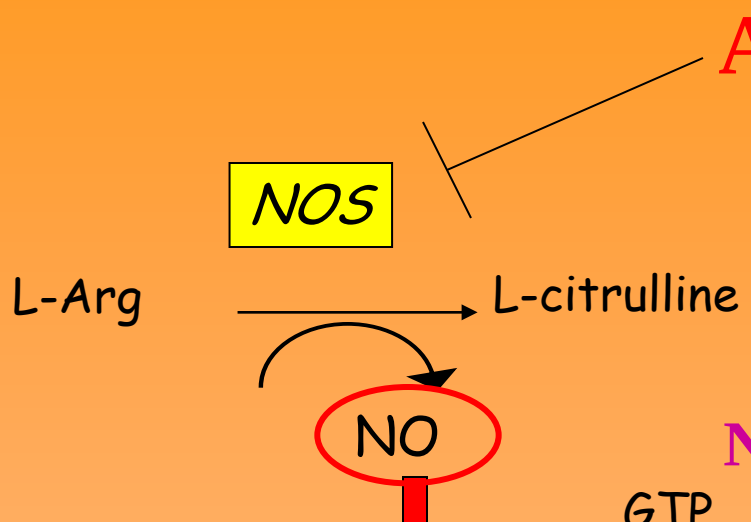
The earliest amnesic symptoms in AD are likely to be due to subtle changes in the way in which cell communicate at synaptic level

HYPOTHESIS

Can we prevent
β-amyloid-induced
impairment
of memory formation
using drugs acting
at the downsream level
of β-amyloid production?


Aβ
AC
cAMP
PKA
Uch-L1
Proteasome
Ca2+
Calpains
NOS
NO
GC
cGMP
PKG
CREB
Histones
gene
transcription
Synaptic plasticity
Memory

Aβ modulation of NO/cGMP/PKG/CREB pathway



NO-donors

GTP

The Journal of Neuroscience, July 20, 2005 • 25(29):6887– 6897 • **6887**

Neurobiology of Disease

Amyloid- Peptide Inhibits Activation of the Nitric Oxide/cGMP/cAMP-Responsive Element-Binding Protein Pathway during Hippocampal Synaptic Plasticity

analogs

PDE5 inhibitors

The Journal of Neuroscience, June 24, 2009 • 29(25):8075– 8086 • **8075**

Development/Plasticity/Repair

Phosphodiesterase 5 Inhibition Improves Synaptic Function, Memory, and Amyloid- Load in an Alzheimer's Disease Mouse

agonists

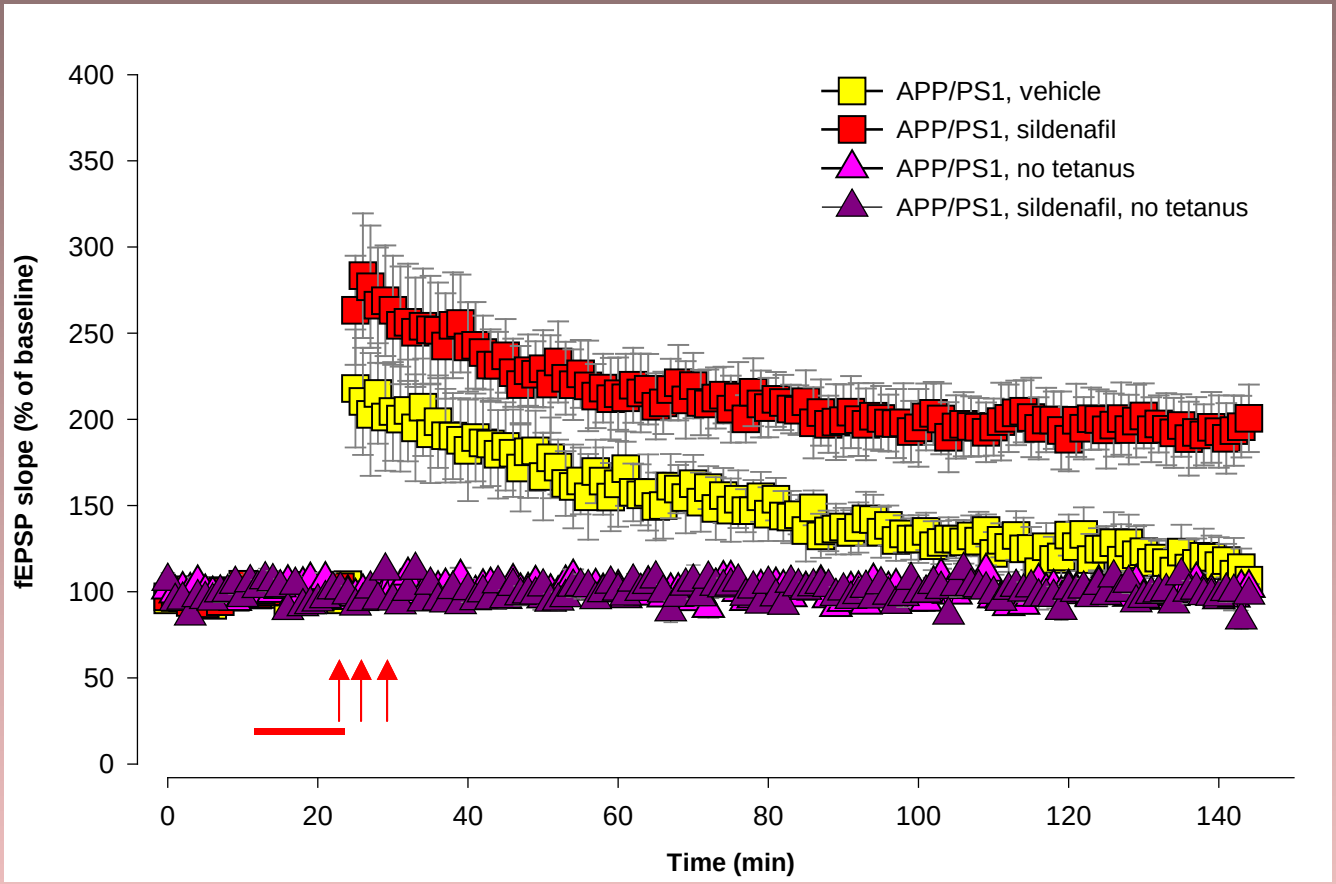
CREB

synaptic plasticity
memory

Puzzo et al, J. Neurosci. 2005

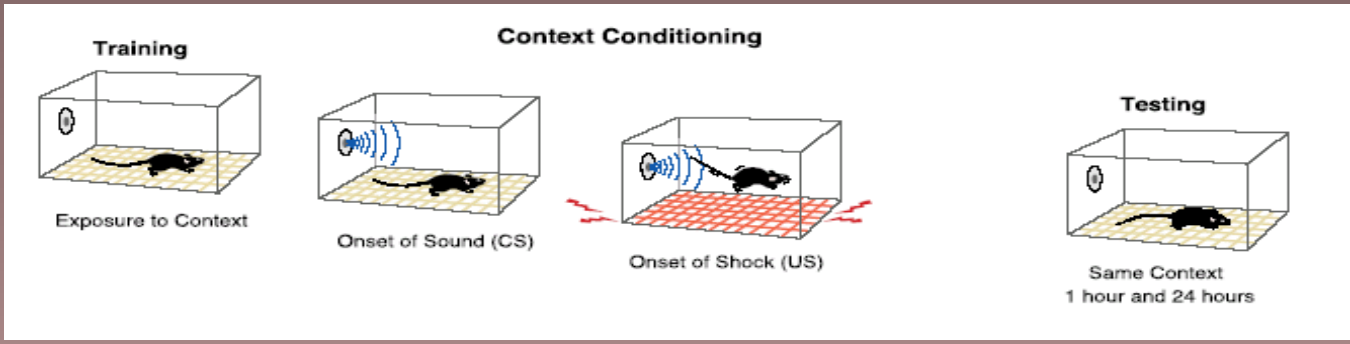
Can we improve synaptic and cognitive abnormalities at early stages of amyloid deposition?

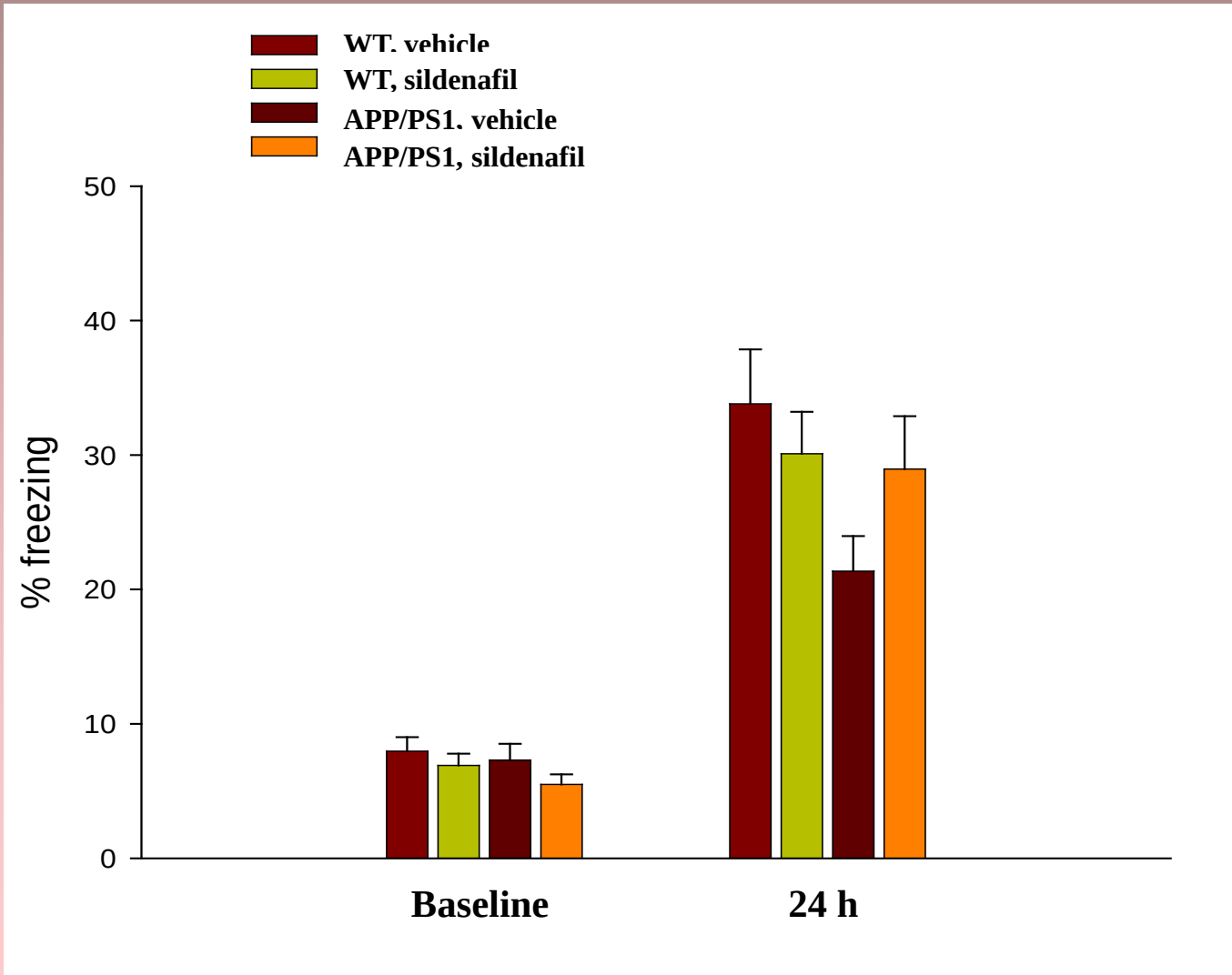
Recovery of LTP impairment in 3 month-old APP/PS1 mice by Sildenafil (Viagra)



Puzzo et al, J. Neurosci. 2009

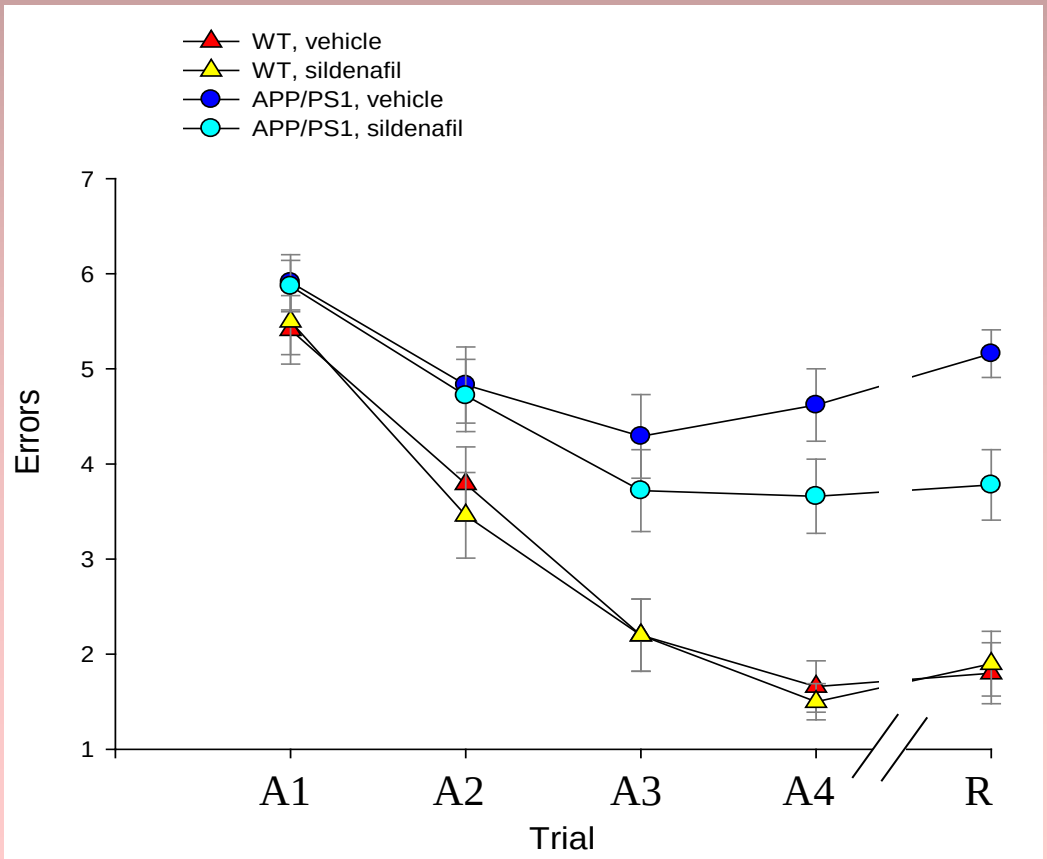
Sildenafil improves contextual conditioning in 3 month-old APP/PS1 mice





Puzzo et al, J. Neurosci. 2009

Sildenafil improves spatial working memory in 3 month-old APP/PS1 mice

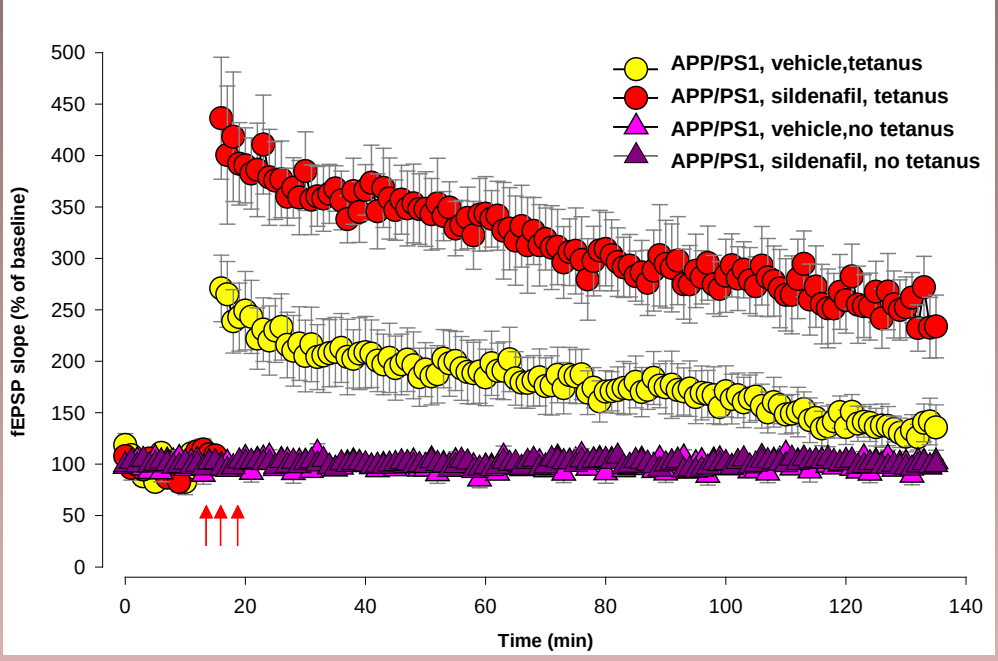


Puzzo et al, J. Neurosci. 2009

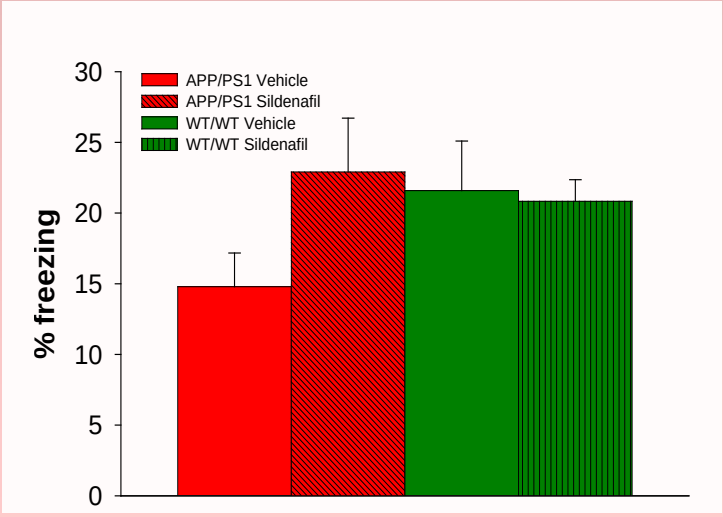
Can we improve synaptic and cognitive abnormalities when a substantial plaque load already exists?

Prolonged Beneficial Effect by Sildenafil on Synaptic Dysfunction and Memory Loss

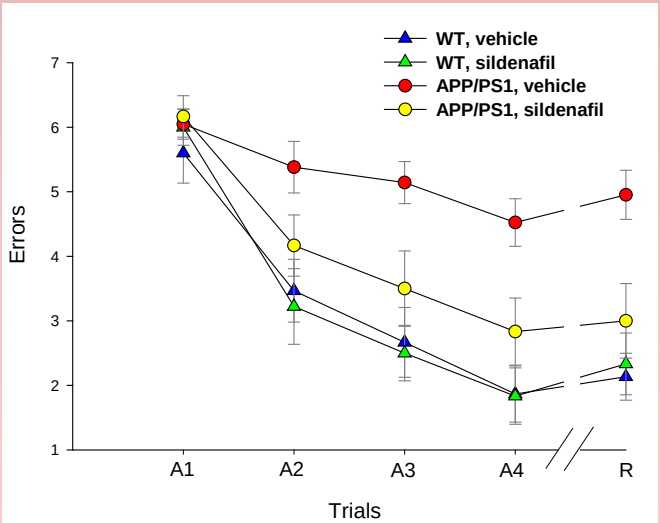
LTP



Fear Cond

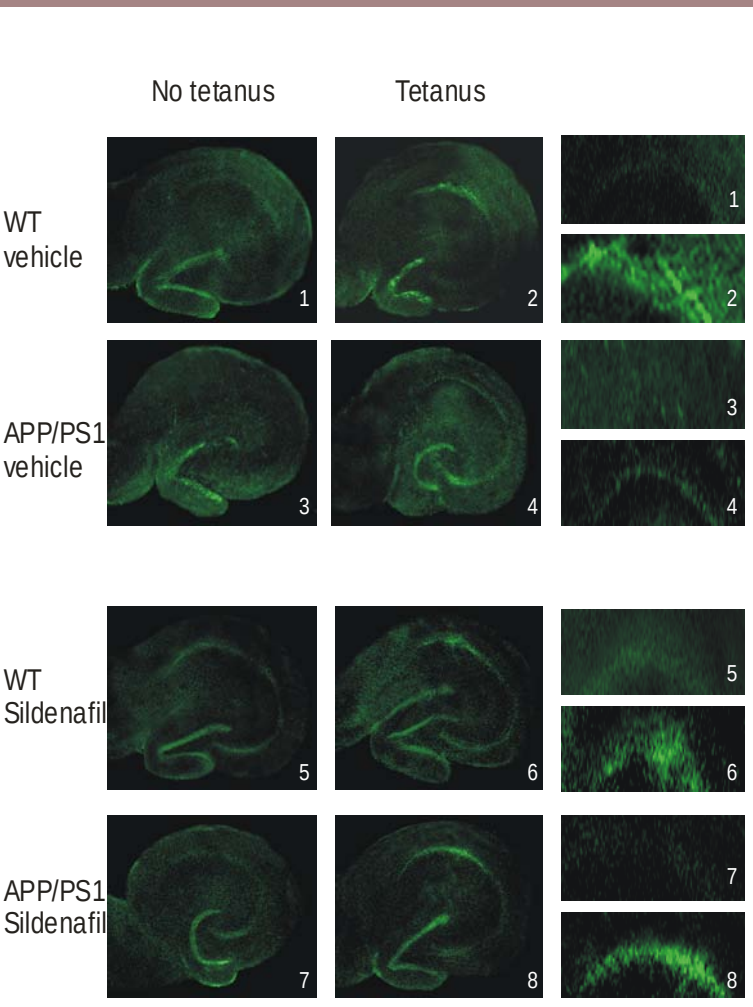


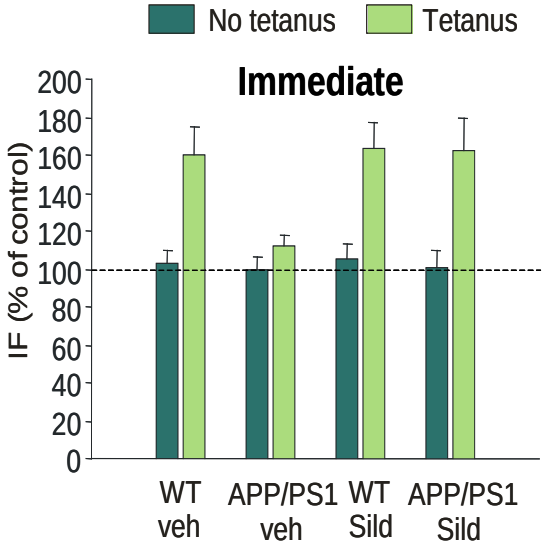
RAWM

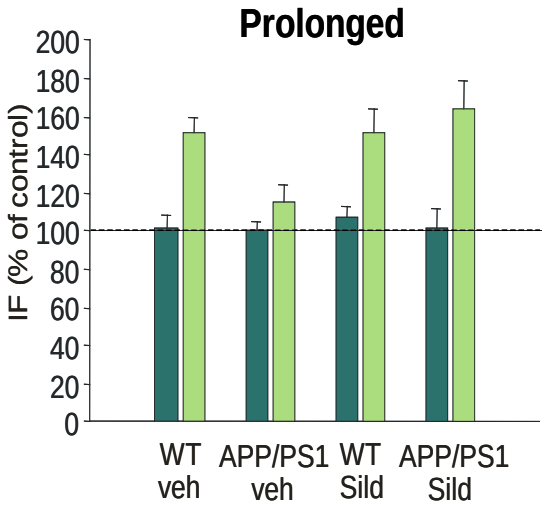


Puzzo et al, J. Neurosci. 2009

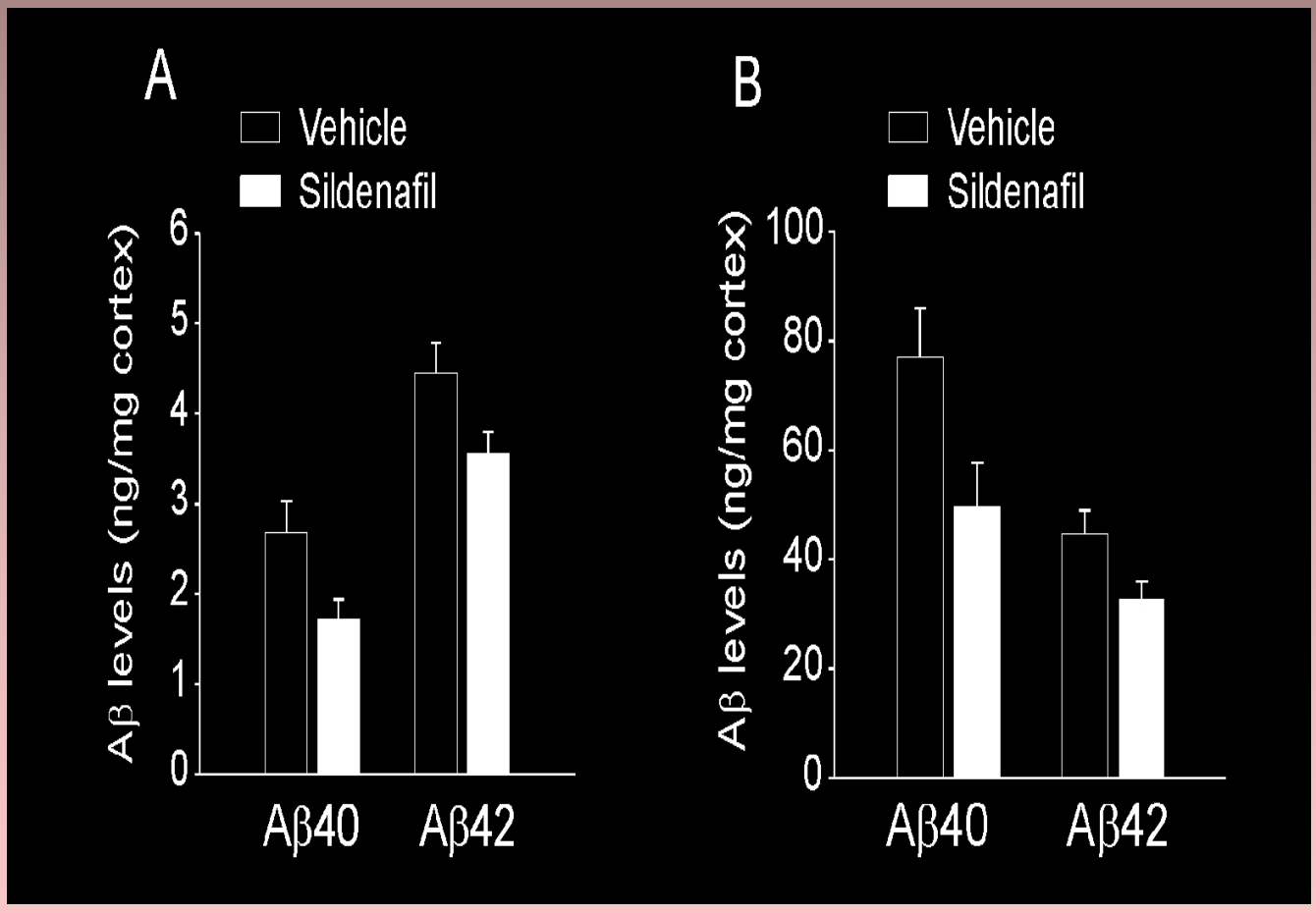
Sildenafil re-establishes normal levels of CREB phosphorylation in hippocampal slices from APP/PS1 mice





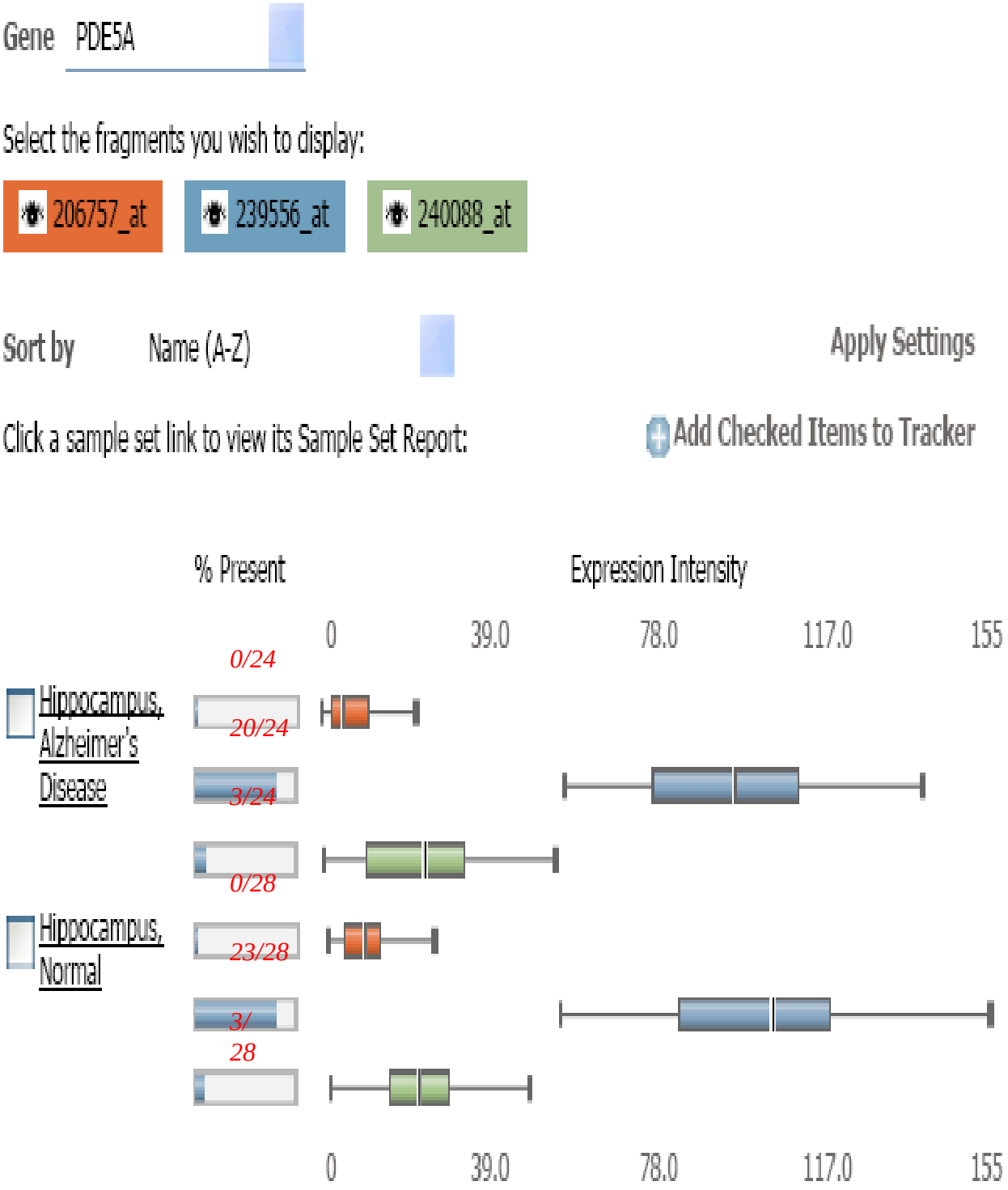


Sildenafil decreases Aβ levels in APP/PS1 mice

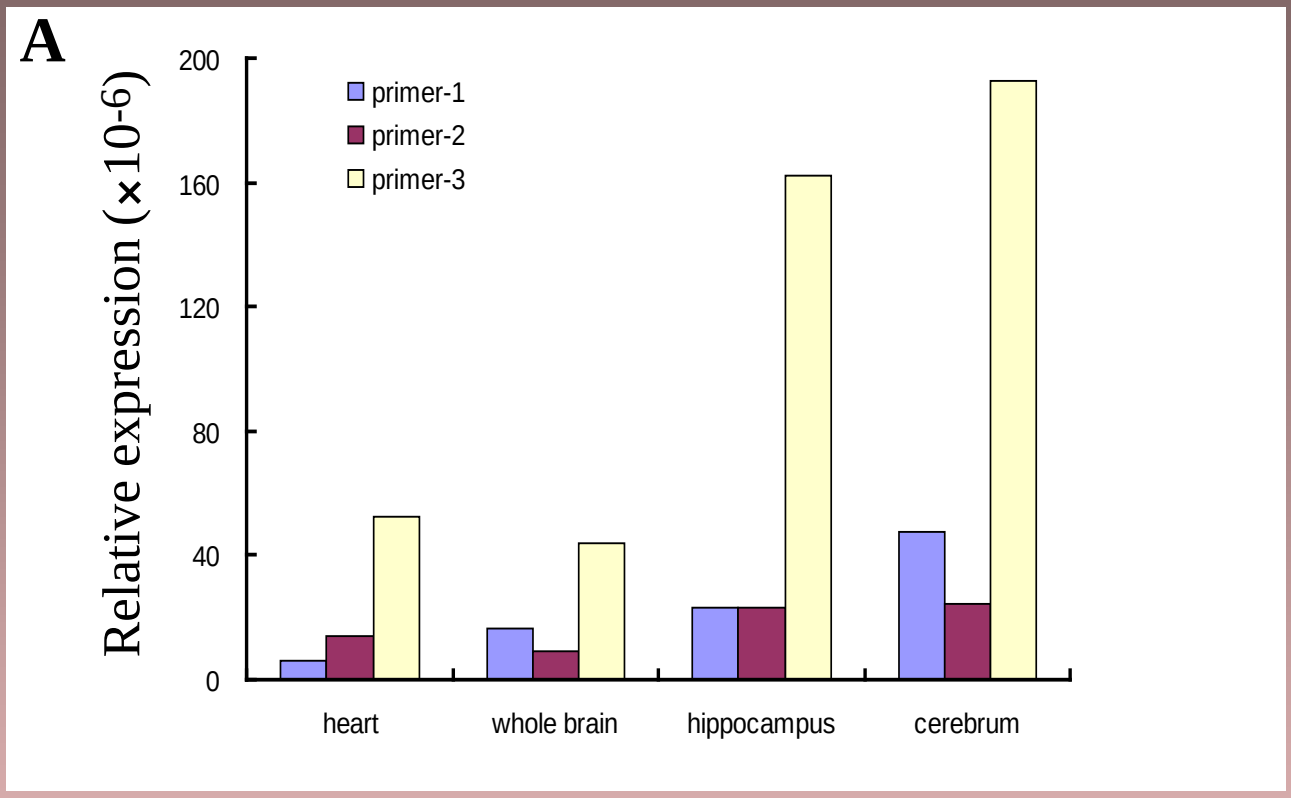


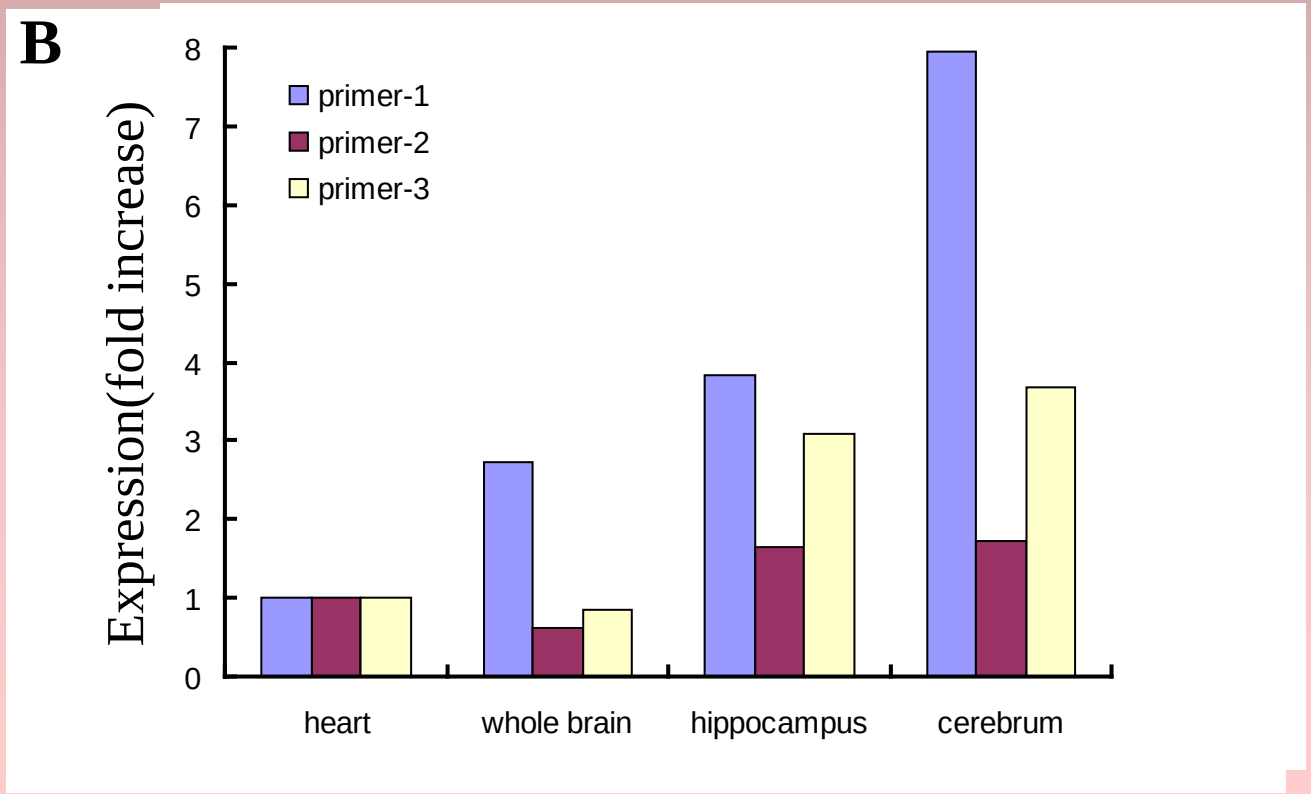
Is there any PDE5 in human hippocampus?

Database of human brain Gene Logic's ASCENTA System



Expression levels of PDE5 mRNA in heart, whole brain, hippocampus and cerebrum of humans (Quantitative RT-PCR)





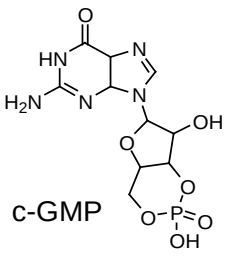
Agents increasing cGMP levels by enhancing CREB phosphorylation produce a beneficial effect on synaptic transmission and cognition

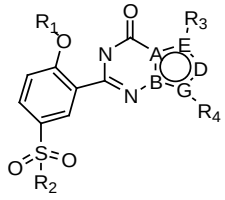
PDE Superfamily

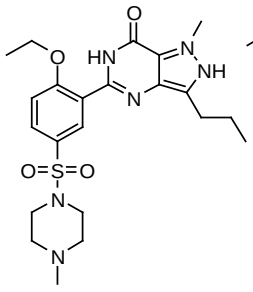
PDE isoenzyme	*No. of isoforms*	*Substrate*	*Km* (μM) *cAMP*	*Km* (μM) *GMP*	*Tissue expression*	*Specific inhibitors*
1	8	Ca^{2+}/calmodulin-stimulated	1–30	3	Heart, brain, lung, smooth muscle	KS-505a
2		cGMP-stimulated	50	50	Adrenal gland, heart, lung, liver, platelets	EHNA (MEP-1)
3	4	cGMP-inhibited, cAMP-selective	0.2	0.3	Heart, lung, liver, platelets, adipose tissue, inflammatory cells	Cilostamide Enoxamone Milrinone Siguazodan
4	20	cAMP-specific	4		Sertoli cells, kidney, brain, liver, lung, inflammatory cells	Rolipram, Roflumilast Cilomilast
5	3	cGMP-specific	150	1	Lung, platelets, vascular smooth muscle	Sildenafil, Zaprinast
6		cGMP-specific		60	Photoreceptor	Dipyridamole
7	3	cAMP-specific, high-affinity	0.2		Skeletal muscle, heart, kidney, brain, pancreas, T lymphocytes	BRL-50481
8		cAMP-selective,	0.06		Testes, eye, liver, skeletal muscle, heart, kidney, ovary, brain, T lymphocytes	none
9	4	cGMP-specific,		0.17	Kidney, liver, lung, brain	BAY 73-6691
10	2	cGMP-sensitive, cAMP-selective	0.05	3.0	Testes, brain	none
11	4	cGMP-sensitive, dual specificity	0.7	0.6	Skeletal muscle, prostate, kidney, liver, pituitary and salivary glands, testes	none

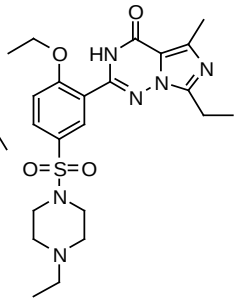
Our aim is to move the PDE5 inhibitory project forward to the stage where it not only provides new biological insights but also, when appropriate, can serve as the basis for future development of new therapeutic strategies

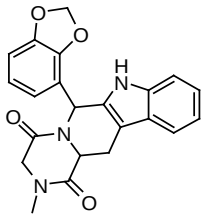
Currently used PDE5 inhibitors











	BBB Perm.	IC50 against PDE5	Half life	Select. ratio for PDE1	Select. ratio for PDE6
SILDENAFIL	+	6 nM	3-4 hrs	180	**12**
VARDENAFIL	**?**	0.17 nM	4-5 hrs	>1000	**3.5**
TADALAFIL	**-**	5 nM	17-18 hrs	>1000	1000

None of the commercially available PDE5 inhibitors possesses the selectivity required for chronic administration to an elderly population with comorbid conditions

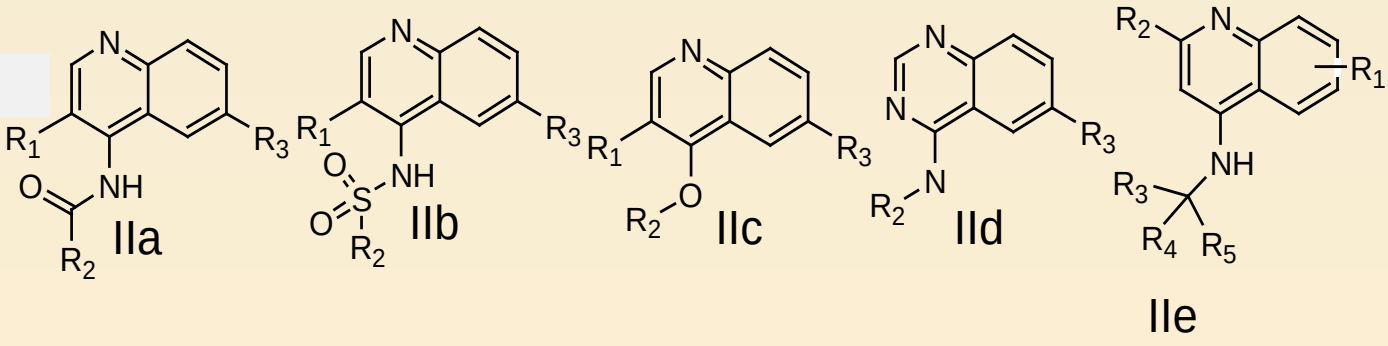
We have launched a computer-aided med/chem program to identify molecules that counteract synaptic and memory dysfunction by inhibiting PDE5

Our goals are to obtain novel drugs with

- high specificity and potency
- good PK, bioavailability and CNS penetration
- safety
- novel compositions of matter with an unobstructed intellectual property path to development

Given that many PDE5 inhibitors have been developed in the past decades, we avoided wasting resources to develop an entirely new scaffold with high potency and excellent selectivity:

we identified quinoline derivatives as the top candidates for the design and synthesis of novel PDE5 inhibitors to be optimized against AD



IC_{50} against PDE5 = 0.05 nM
select. ratios > 7800 vs PDE1-4
160 vs PDE6)

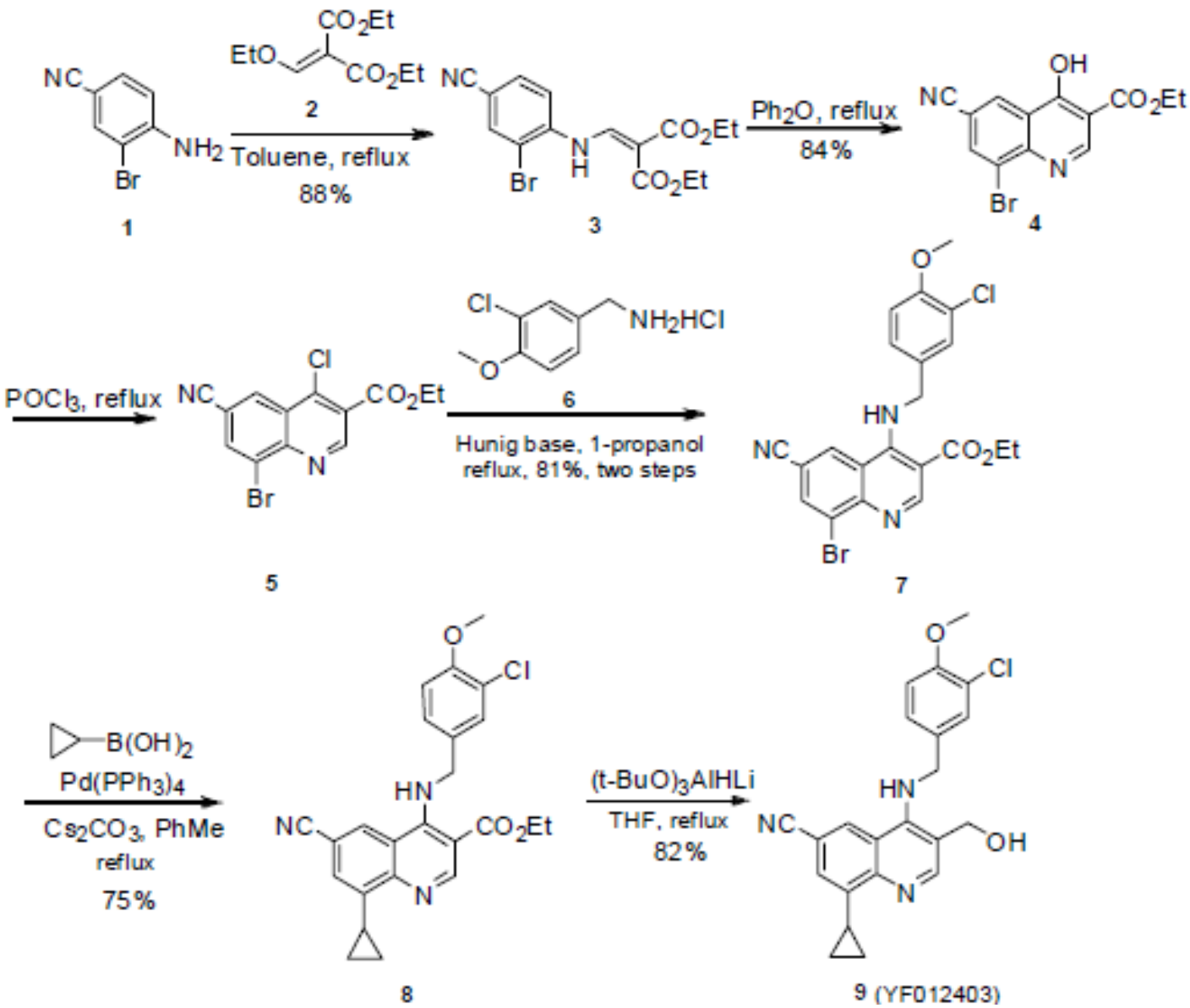
However, … Unknown,
selectivity for the remaining PDEs, *in vivo* efficacy in an AD model or other diseases, PK including BBB penetration, toxicity, and solubility.

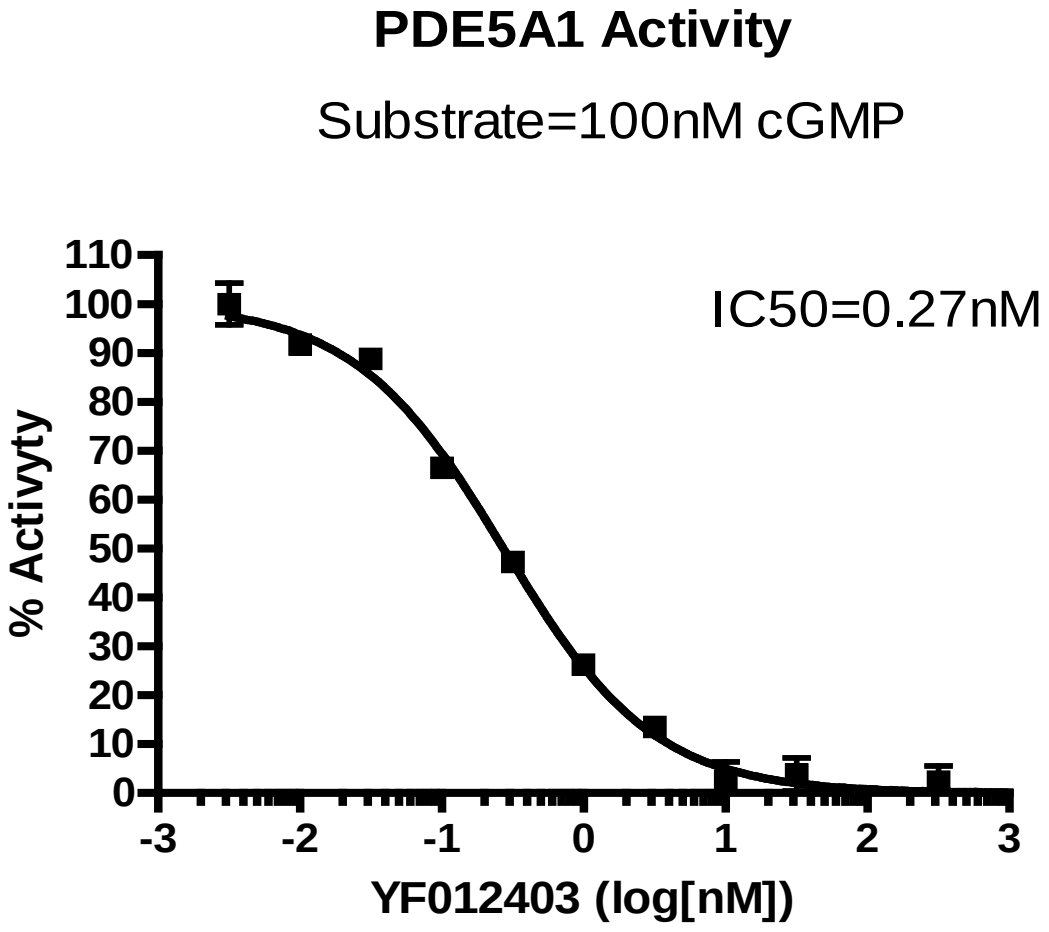
In addition only a few substituents on the quinoline ring were investigated and just one compound was dominant.

YF012403

PCT/US,2008 appl.61/140,315

YF012403 was easily prepared in six steps



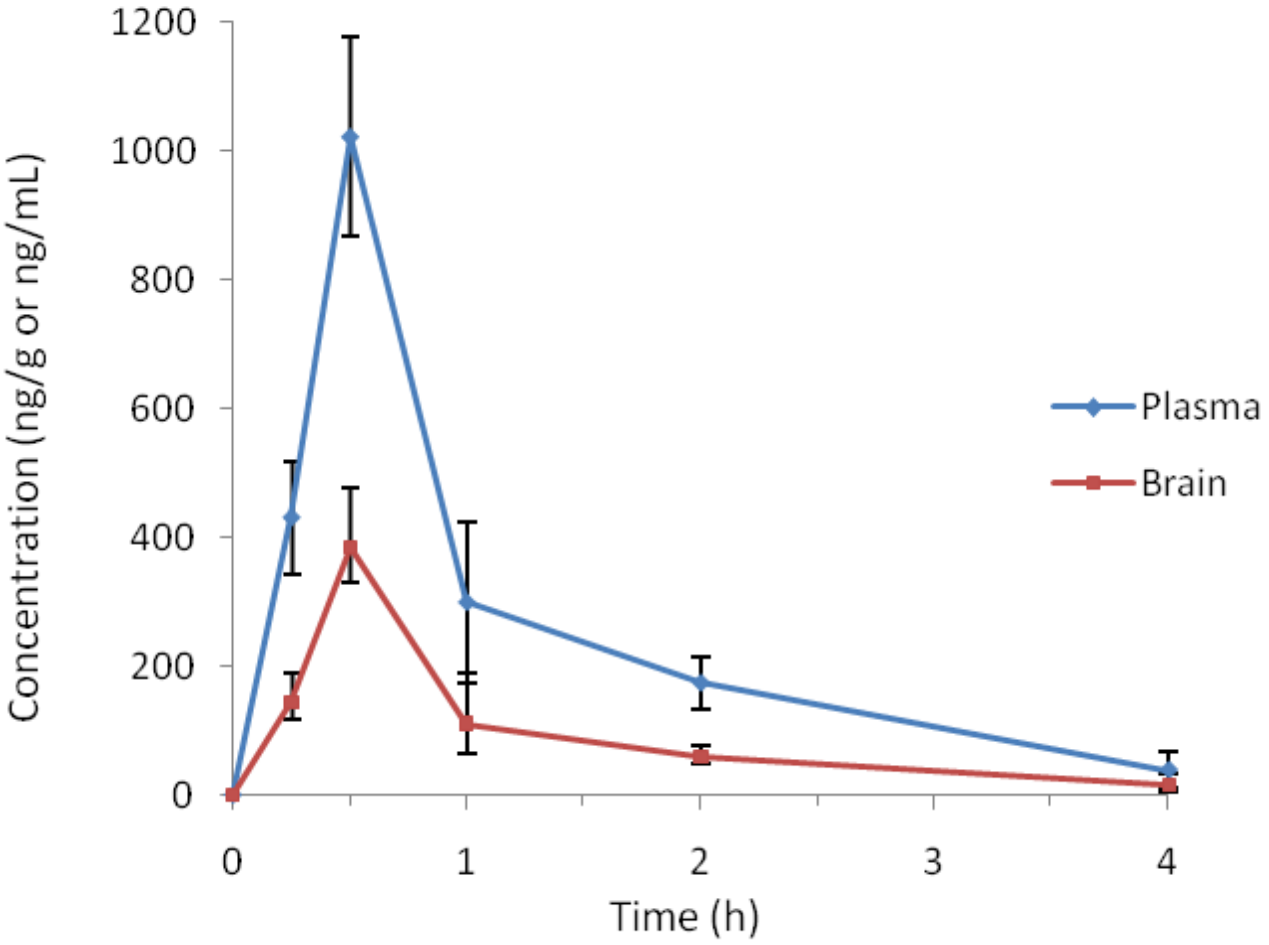

PDE5A1 Activity
Substrate=100nM cGMP
IC50=0.27nM
% Activyty
110
100
90
80
70
60
50
40
30
20
10
0
-3
-2
-1
0
1
2
3
YF012403 (log[nM])

In vitro activity of YF012403 against PDE1-11

Compound		PDE1	PDE2	PDE3	PDE4	PDE5	PDE6	PDE7	PDE8	PDE9	PDE10	PDE11
YF012403[a]	IC50 (nM)	>10^4	>10^4	>10^4	>10^4	0.27	339	>10^4	>10^4	>10^4	>10^4	>10^4
	PDEX/PDE5	>10^4	>10^4	>10^4	>10^4	1	1256	>10^4	>10^4	>10^4	>10^4	>10^4
YF016203[a]	IC50 (nM)	>10^4	>10^4	>10^4	>10^4	0.40	5100	>10^4	>10^4	>10^4	>10^4	>10^4
	PDEX/PDE5	>10^4	>10^4	>10^4	>10^4	1	12750	>10^4	>10^4	>10^4	>10^4	>10^4
Sildenafil[b]	IC50 (nM)	1500	35000	15000	20000	2.20	9.5[a]	78000	>10^4	5600	6800	6100
	PDEX/PDE5	682	15909	6818	9091	1	4	35455	>10^4	2545	3091	2773
Vardenafil[b]	IC50 (nM)	300	3100	380	3800	1.00	11.0[c]	1900	57000	680	880	240
	PDEX/PDE5	300	3100	380	3800	1	11	1900	>10^4	680	880	240
Tadalafil[b]	IC50 (nM)	>10^4	>10^4	>10^4	9200	1.2	5200[d]	74000	>10^4	>10^4	19000	10
	PDEX/PDE5	>10^4	>10^4	>10^4	7667	1	4333	61667	>10^4	>10^4	15833	8

1. a) Data obtained by BPS Bioscience; b) Graeme L. Card, et. al. *Structure*, **2004** , *12*, 2233-2247; c) I Saenz de Tejada, et al., *International Journal of Impotence Research*, **2001**, *13*, 282-290; d) Alain, Daugan, et. Al, *Journal of Medicinal Chemistry*, **2003**, *46*, 4533-4542.
2. Two compounds, YF012403 and YF016203, were picked up based on the SAR for selectivity profiling.

Concentration-Time curve of YF012403 in mouse brain tissue and plasma



PK parameters of YF012403 in mouse brain tissue and plasma

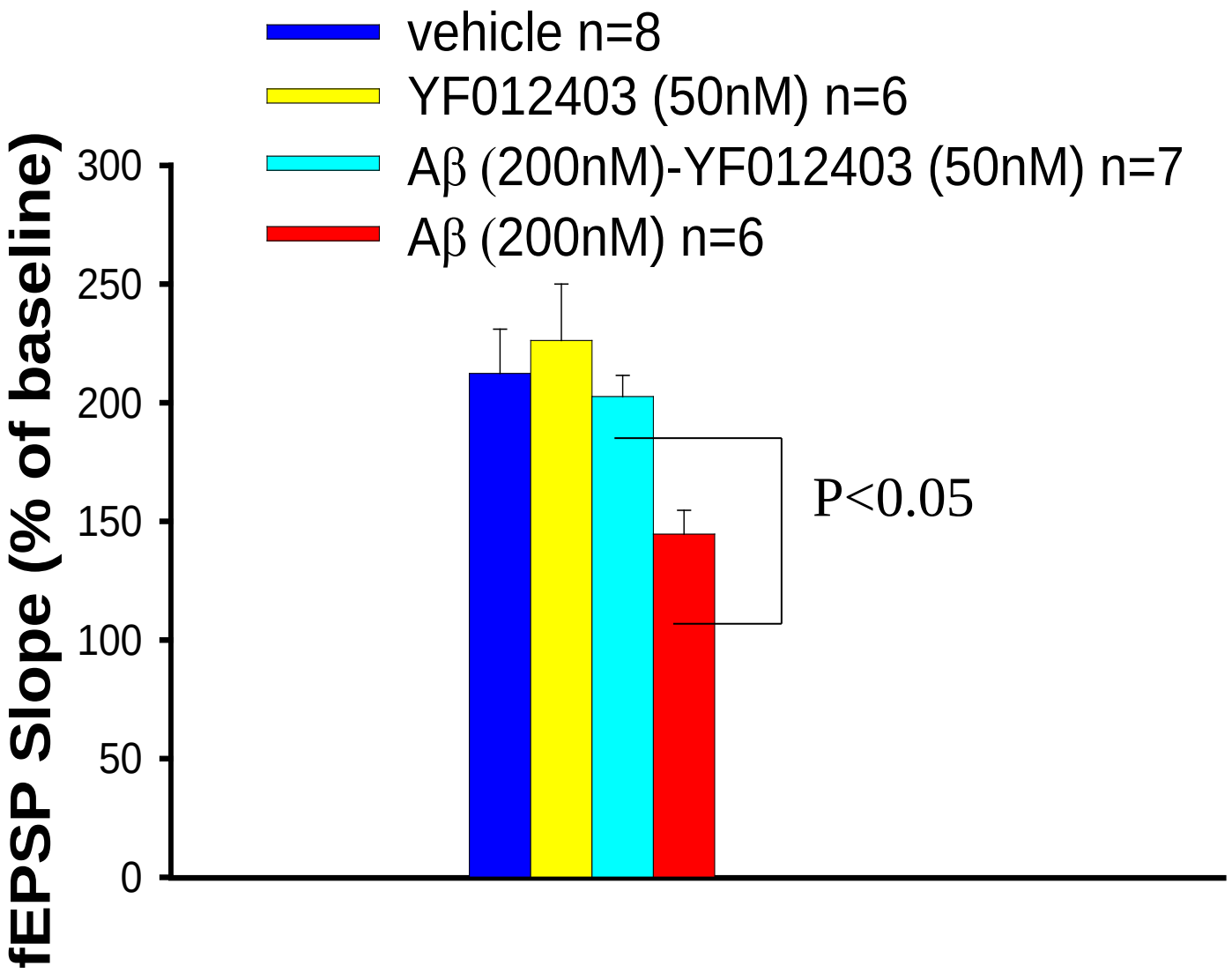
Parameters		YF012403		
		Brain	Plasma	Ratio*
T_{max}	(h)	0.5	0.5	-
C_{max}	(ng/mL or ng/g)	385	1022	0.38
AUC_{0-t}	(ng·h/mL or ng·h/g)	418	1014	0.41
$AUC_{0-\infty}$	(ng·h/mL or ng·h/g)	420	1133	0.37
t1/2	(h)	1.04	1.33	-
MRT	(h)	1.66	1.61	-

*Ratio = brain/plasma

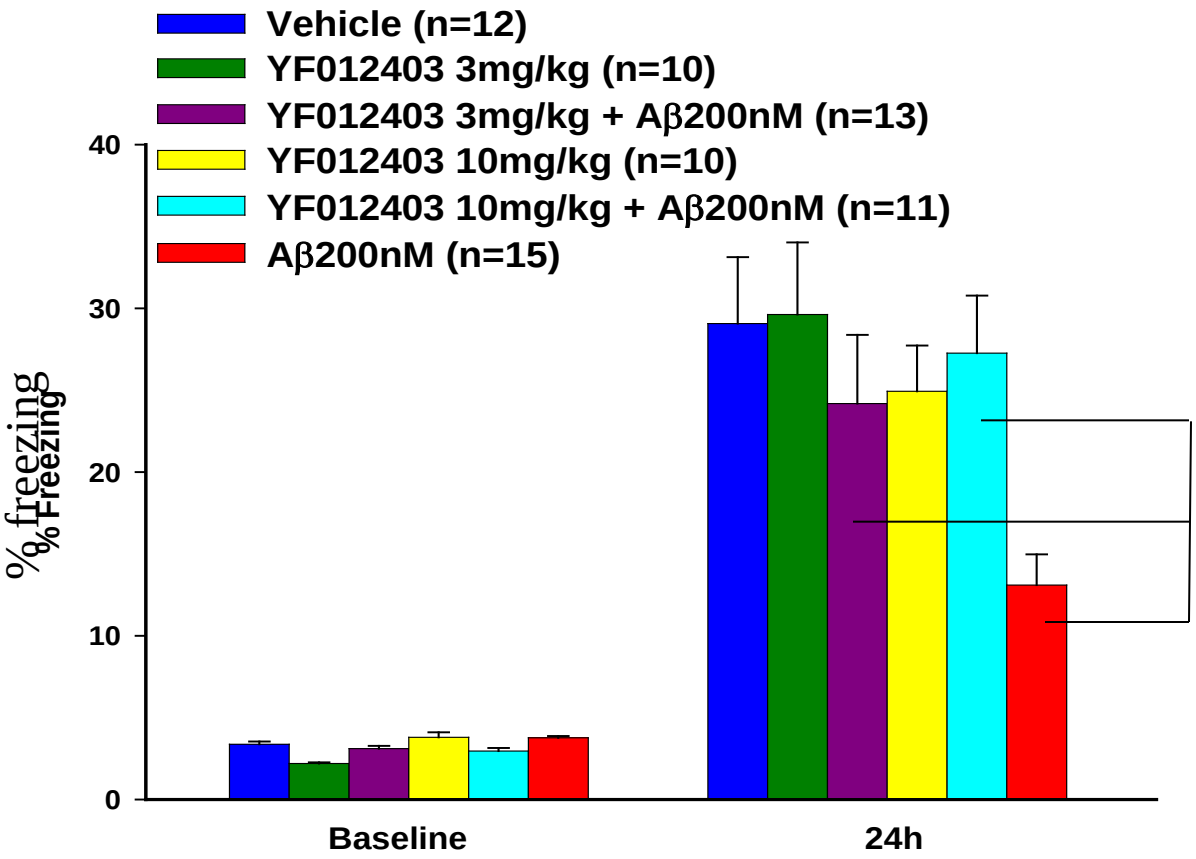
** 50 mg/kg, p.o.; Vehicle for YF012403 is 0.5% methylcellulose aqueous solution; Vehicle for sildenafil is 0.2M HCl.

AUC: Area Under Curve ; MRT: Mean Residence Time.

YF012403 ameliorates the LTP deficit in $A\beta_{42}$-treated slices

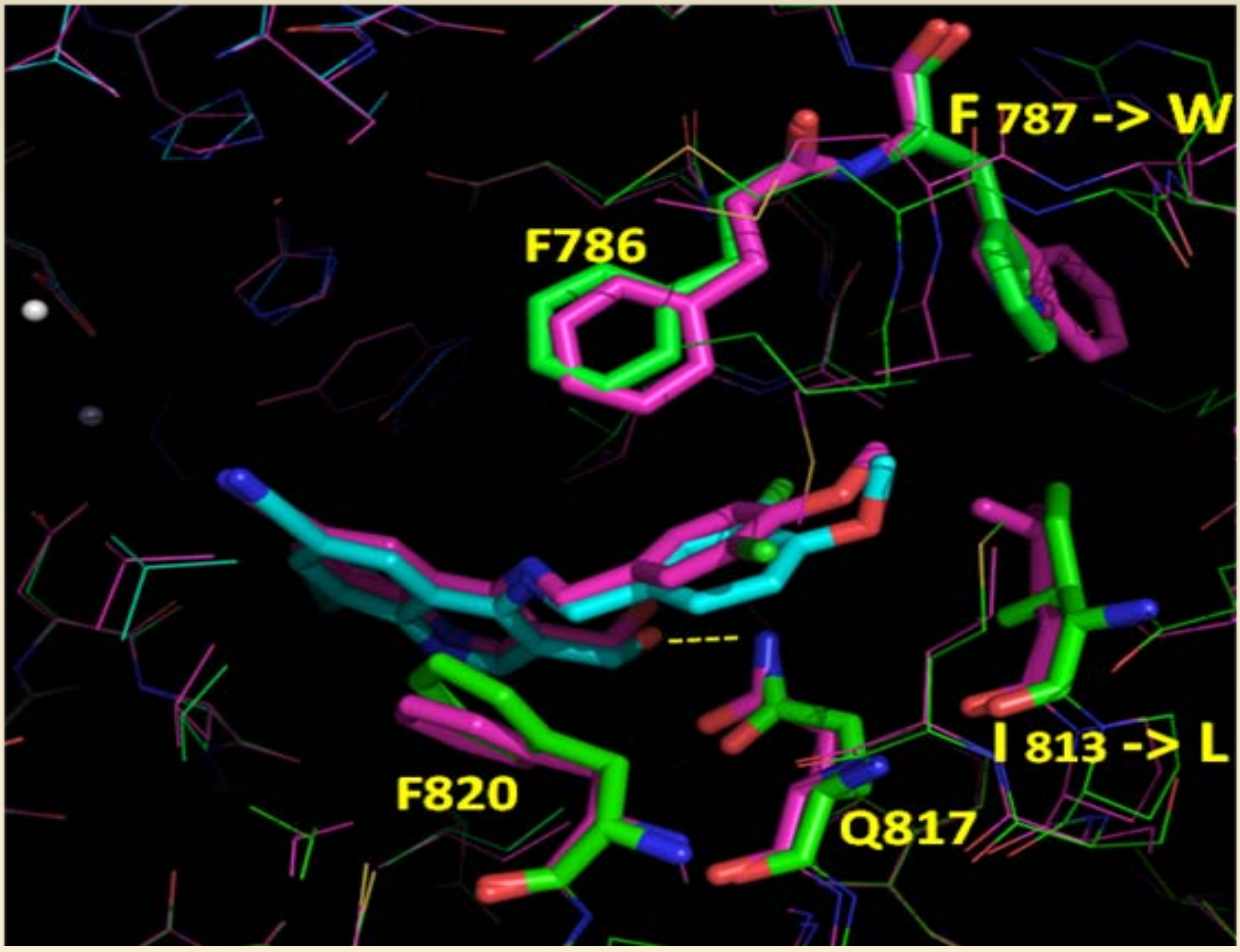


YF012403 ameliorates the contextual fear memory deficit in $A\beta_{42}$-infused mice.



P<0.05

YF012403 binding structure with human PDE5 and PDE6 protein (in purple) determined by molecular docking



Experimental Plan

We will focus our research design on modifications of YF012403 to optimize its druggability

The primary benzylic alcohol at the 3-position (C3) is very likely to be oxidized by microsomes generating benzaldehyde and consequently causing first-pass metabolism problems and severe side effects due to subsequent conjugate addition to proteins

A cyclopropyl group at the 8-position (C8) may not be stable *in vivo* by undergoing ring opening, and thus representing an electrophilic liability

CONCLUSIONS

-PDE5 inhibition rescues synaptic and memory dysfunctions by amyloid-β elevation.

-The quinoline scaffold was selected for high potency and selectivity of known compounds with this scaffold.

Based on the results of a biological screening against synaptic dysfunction, only changes resulting in improvement compared to YF012403 activity against PDE5 are being advanced further.

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Based on SAR analysis we propose new scaffolds meeting the following criteria:

1) A fused ring system with an H-bond acceptor or donor

2) Readily synthesized from readily available starting materials

3) Sufficient sites that can be modified to generate a relatively large number of compounds for screening

4) Novel composition of matter with no impediment for development