Presentation 6 – James Bibb

SOUTHWESTERN

Neuronal Signal Transduction in Gulf War Illness

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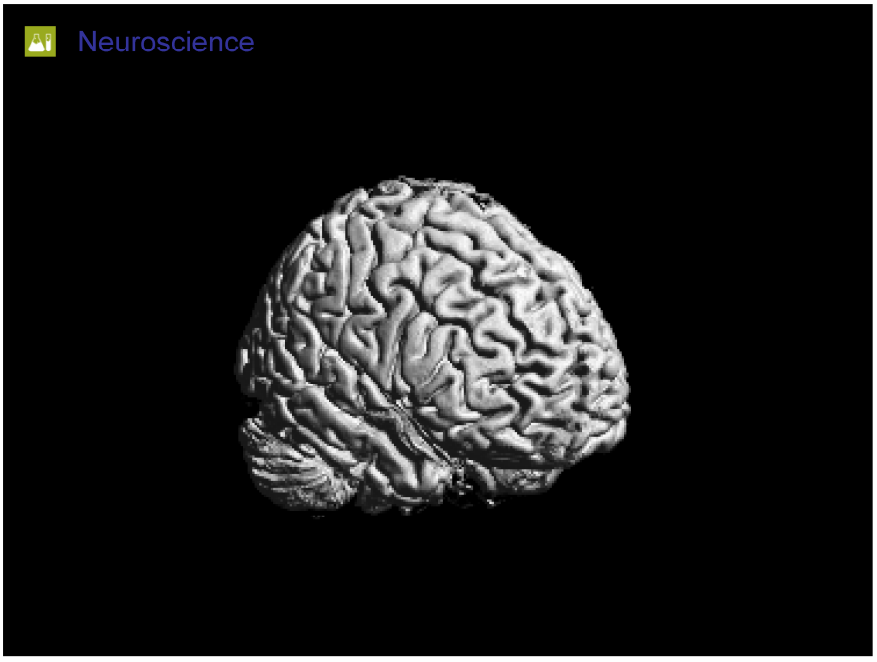
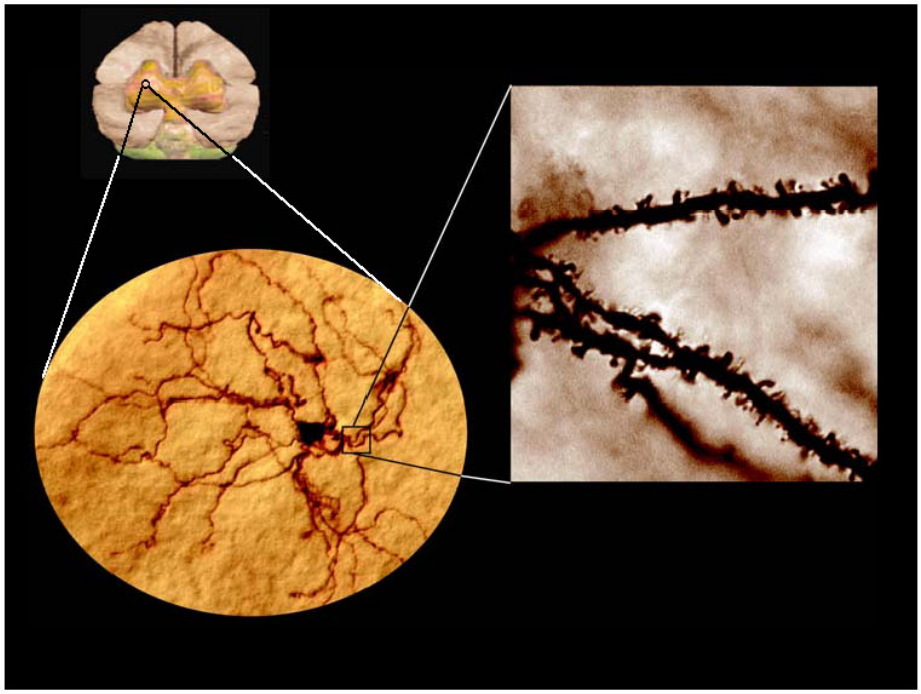
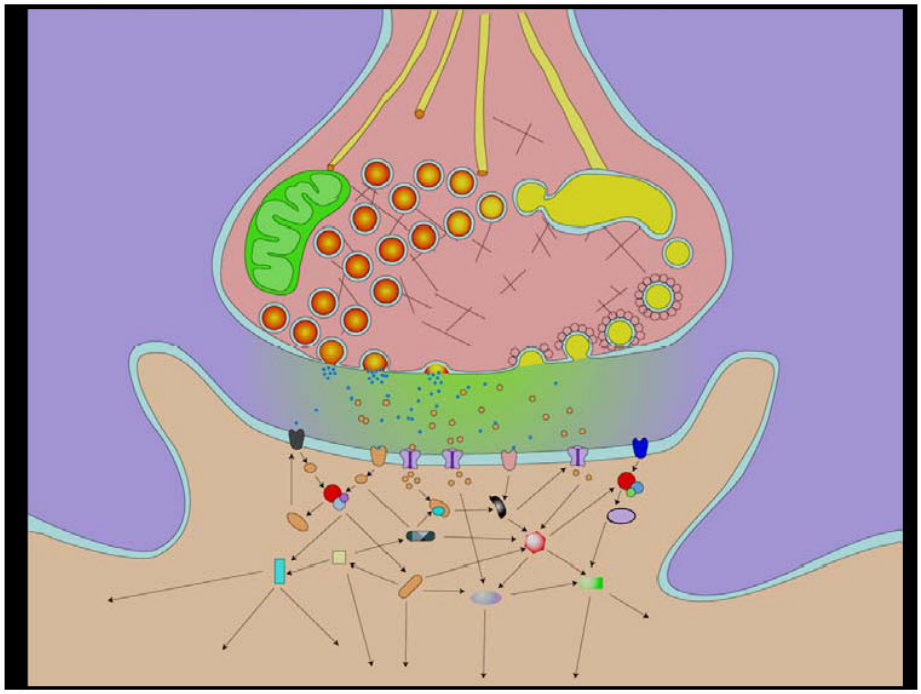
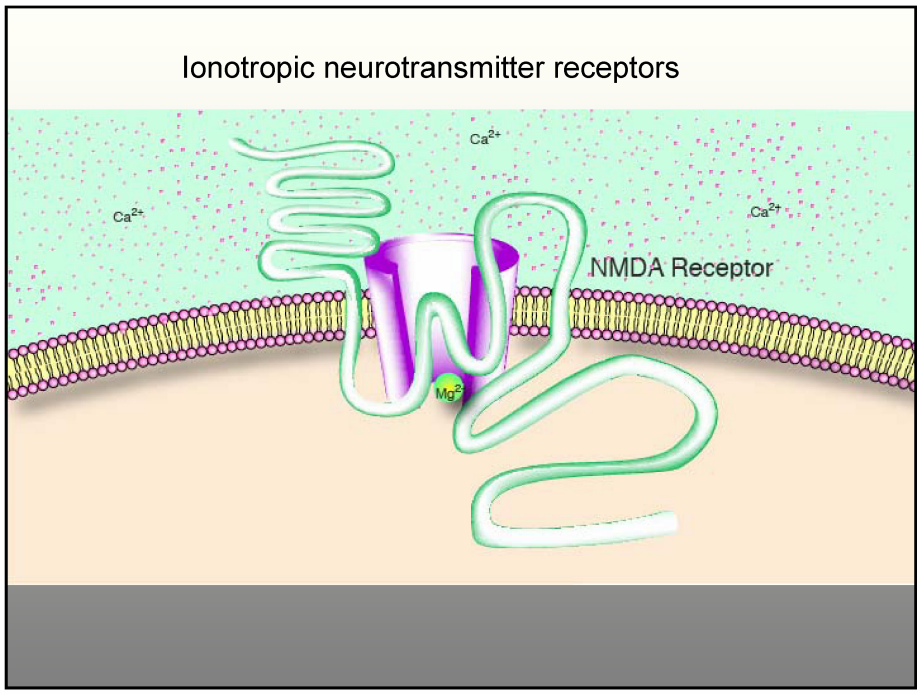
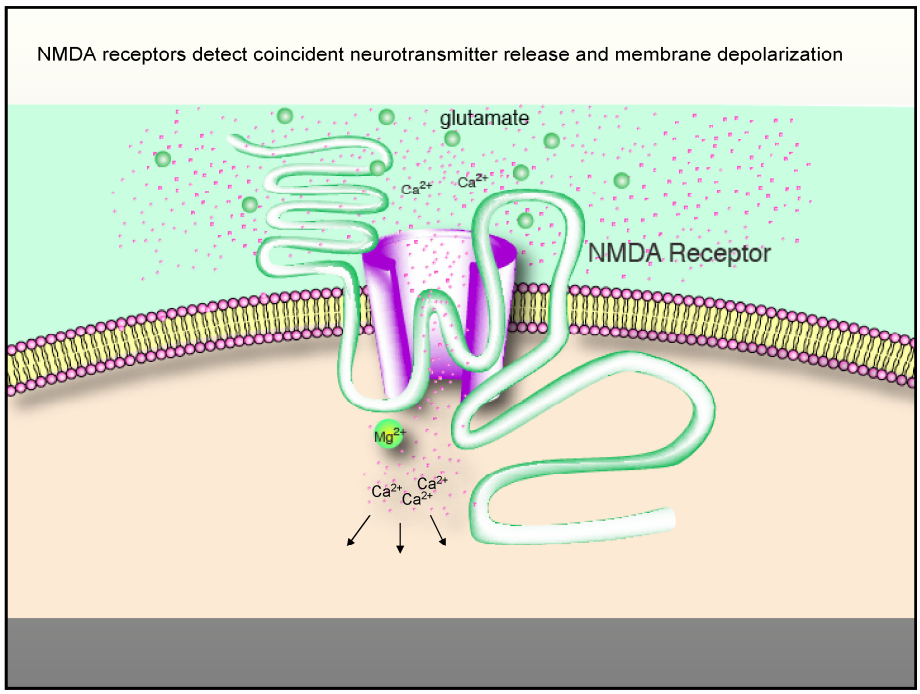
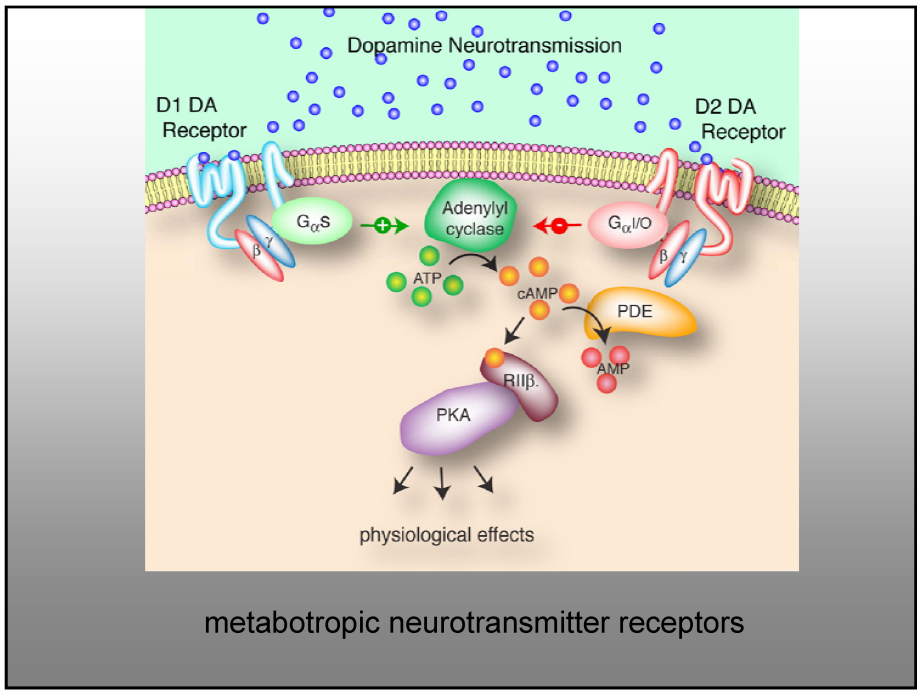


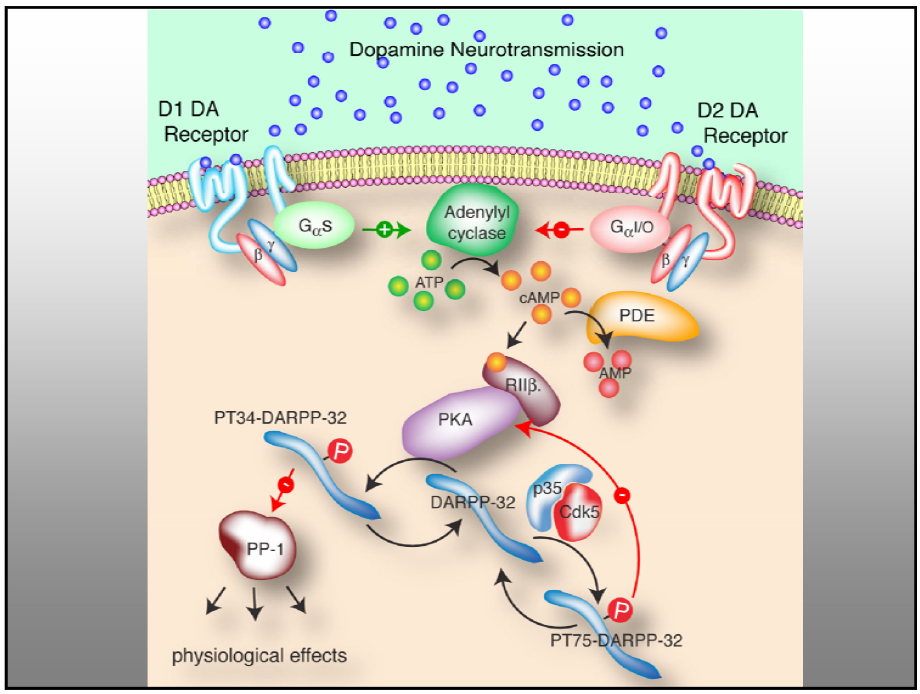





Ionotropic neurotransmitter receptors
Ca^{2+}
Ca^{2+}
Ca^{2+}
NMDA Receptor
Mg^{2+}


NMDA receptors detect coincident neurotransmitter release and membrane depolarization
glutamate
Ca^{2+}
Ca^{2+}
NMDA Receptor
Mg^{2+}
Ca^{2+}
Ca^{2+}
Ca^{2+}


Dopamine Neurotransmission
D1 DA Receptor
D2 DA Receptor
$G_{\alpha}S$
Adenylyl cyclase
$G_{\alpha}I/O$
ATP
cAMP
PDE
AMP
RIIβ.
PKA
physiological effects
metabotropic neurotransmitter receptors


Dopamine Neurotransmission
D1 DA Receptor
D2 DA Receptor
$G_{\alpha}S$
Adenylyl cyclase
$G_{\alpha}I/O$
ATP
cAMP
PDE
AMP
RIIβ.
PKA
PT34-DARPP-32
DARPP-32
p35
Cdk5
PP-1
PT75-DARPP-32
physiological effects

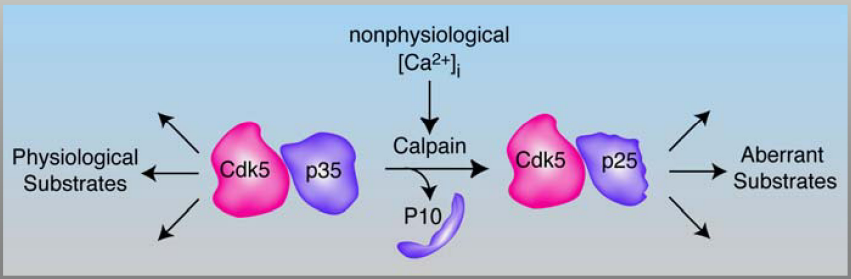
Role of Cdk5 in CNS Function and Disease

Normal

- Development
  - Cortical lamination
  - Neuromuscular junction formation
- Cytoskeletal dynamics
- Synaptic vesicle cycle
- Neurotransmitter receptor trafficking
- Synaptic plasticity
- Learning and memory

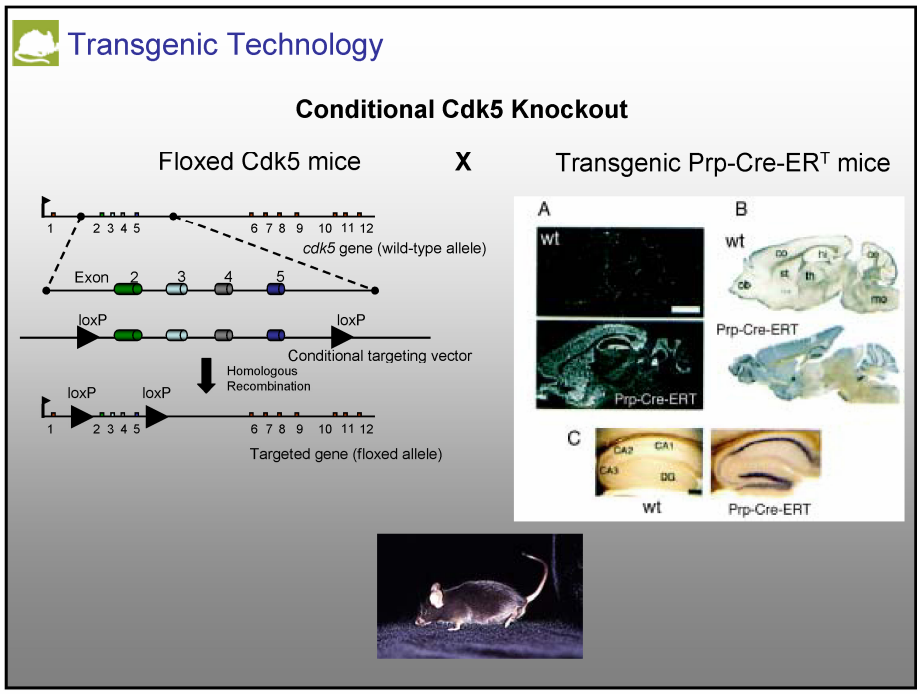
Diseases

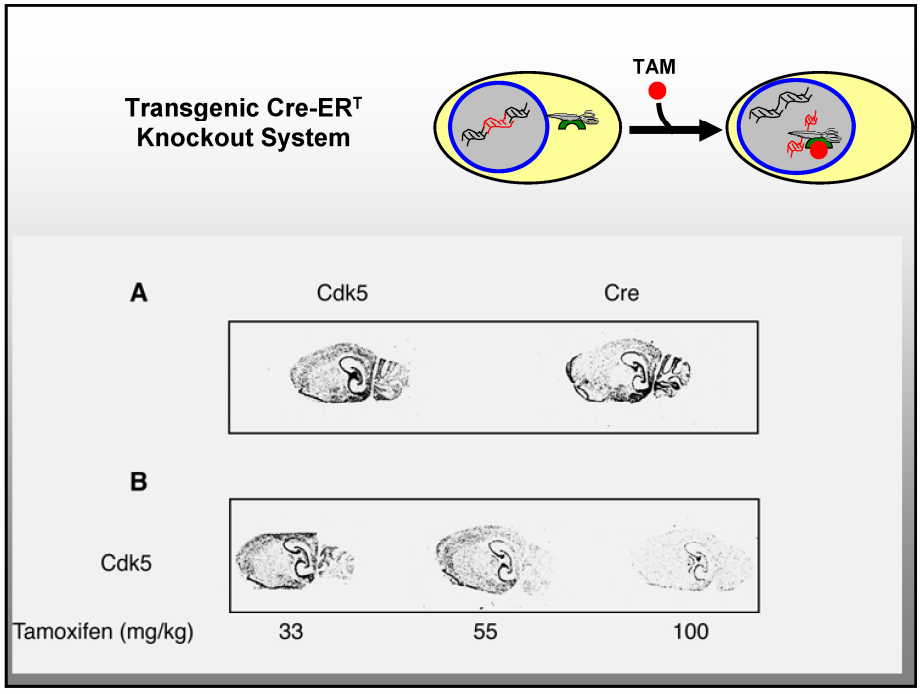
- Neurotoxicity
- Neurodegeneration
  - Alzheimer's disease
  - Parkinson's disease
  - Amyotrophic lateral sclerosis
- Stroke
- Neuropsychiatric disorders
- Drug Addiction
- Developmental disorders



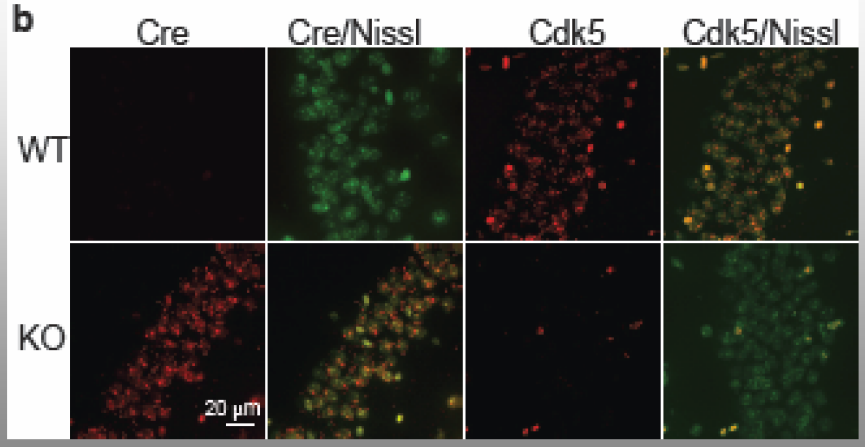
STUDY OF HOW ALTERED NEURONAL SIGNAL TRANSDUCTION CONTRIBUTES TO GULF WAR ILLNESS

- Protein Biochemistry
- Neuropharmacology
- Neurophysiology
- Transgenic Technology
- Behavioral Studies

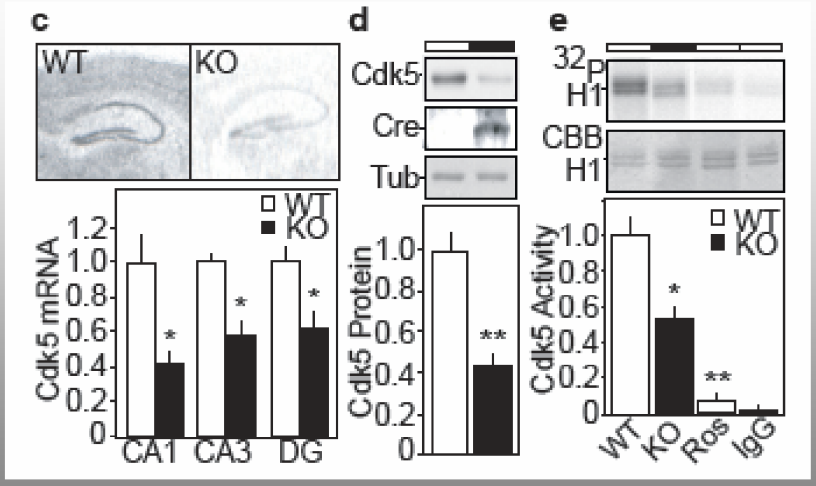

Transgenic Technology
Conditional Cdk5 Knockout
Floxed Cdk5 mice
X
Transgenic Prp-Cre-ERT mice
1 2 3 4 5 6 7 8 9 10 11 12
cdk5 gene (wild-type allele)
Exon 2 3 4 5
loxP
loxP
Conditional targeting vector
Homologous Recombination
loxP
loxP
1 2 3 4 5 6 7 8 9 10 11 12
Targeted gene (floxed allele)
A
wt
Prp-Cre-ERT
B
wt
Prp-Cre-ERT
C
CA2
CA1
CA3
DG
wt
Prp-Cre-ERT


Transgenic Cre-ERT
Knockout System
TAM
A
Cdk5
Cre
B
Cdk5
Tamoxifen (mg/kg)
33
55
100

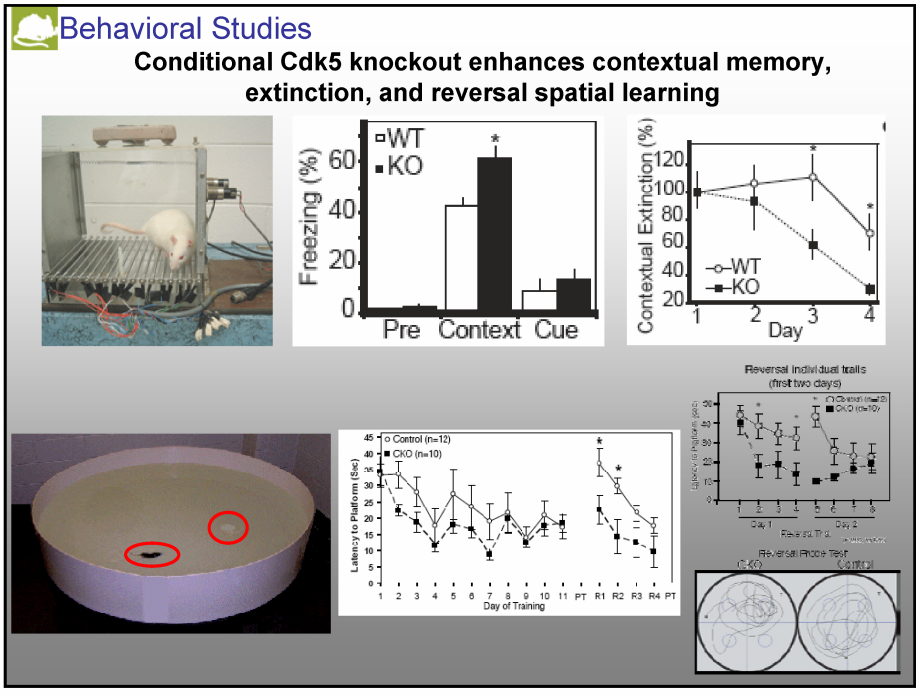
Conditional Cdk5 knockout in CA1 pyramidal neurons



Conditional Cdk5 knockout in the hippocampus

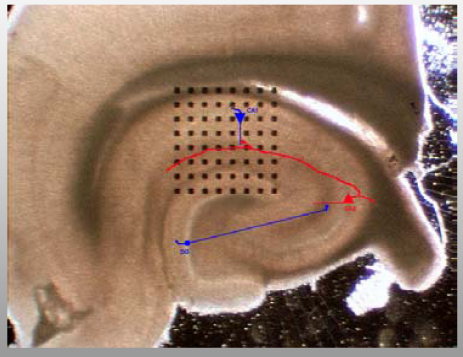


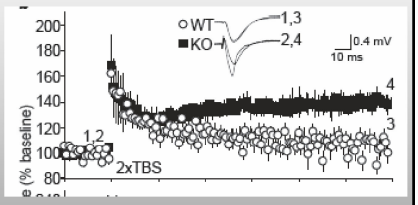
QuickTime™ and a
Sorenson Video decompressor
are needed to see this picture.



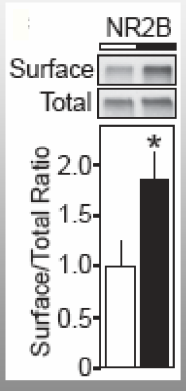
Neurophysiology

Loss of Cdk5 increased synaptic plasticity as assessed by whole field multielectrode array analysis

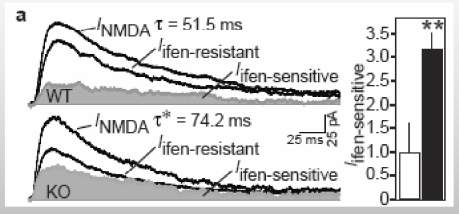




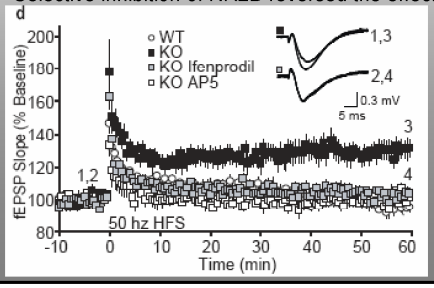
Loss of Cdk5 increasedNR2B subunit receptor levels



A larger portion of NMDAR current is mediated by NR2B

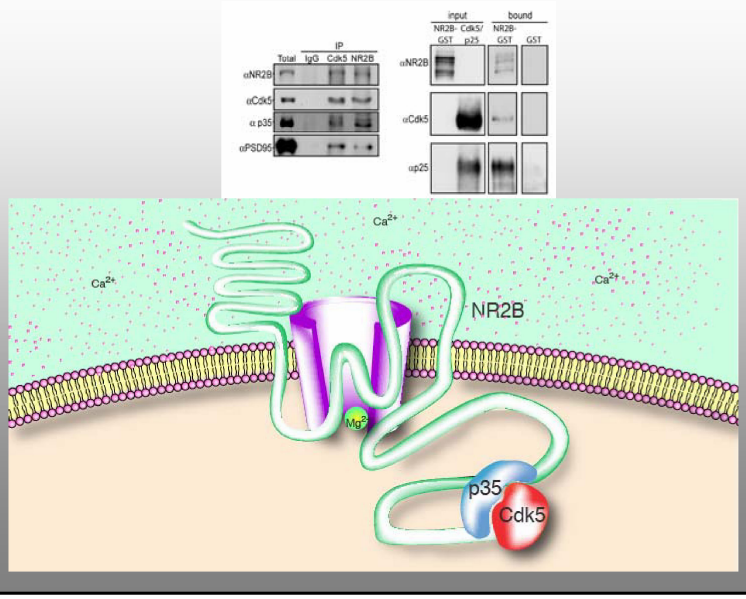


Selective inhibition of NR2B reversed the effect

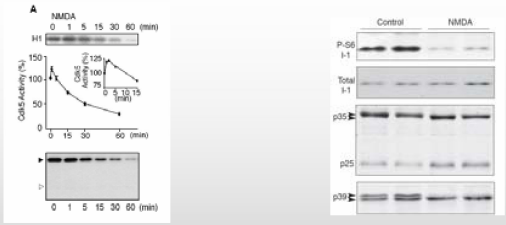


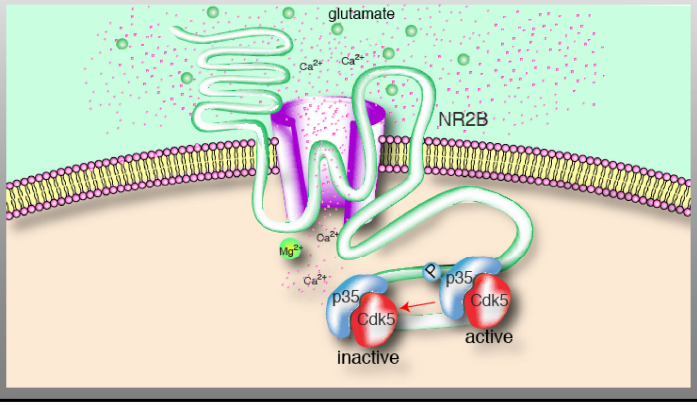
Protein Biochemistry

Cdk5 and NR2B occur in a complex and bind to one another



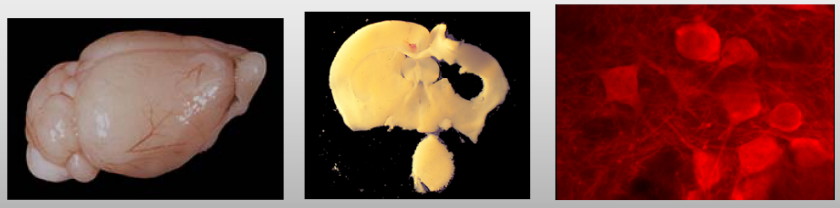
NMDAR activation inactivates Cdk5



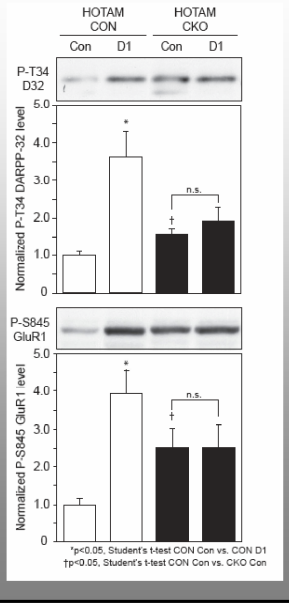


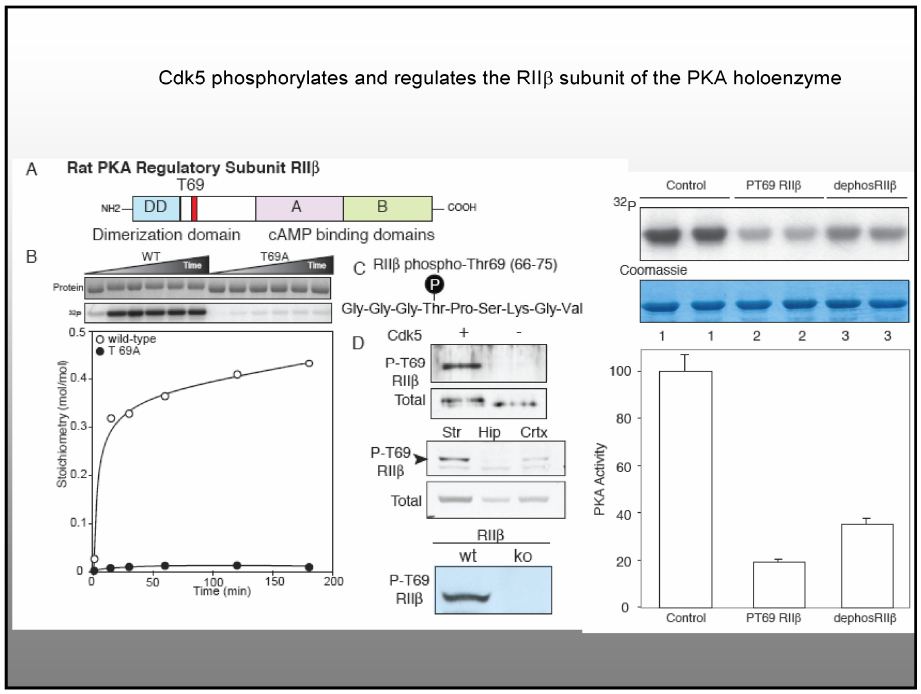
Neuropharmacology

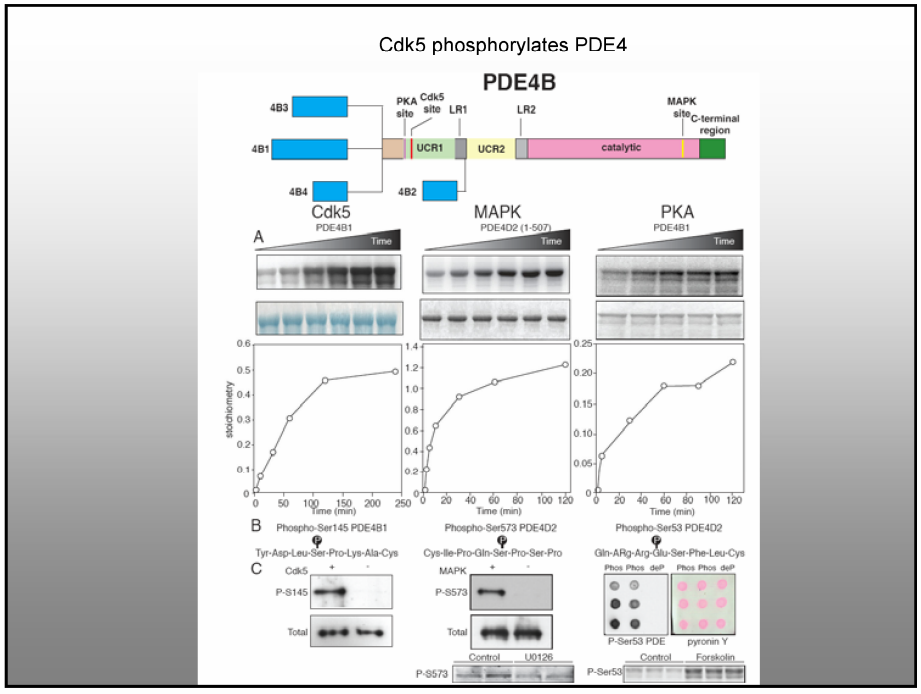
Acute slice preparations

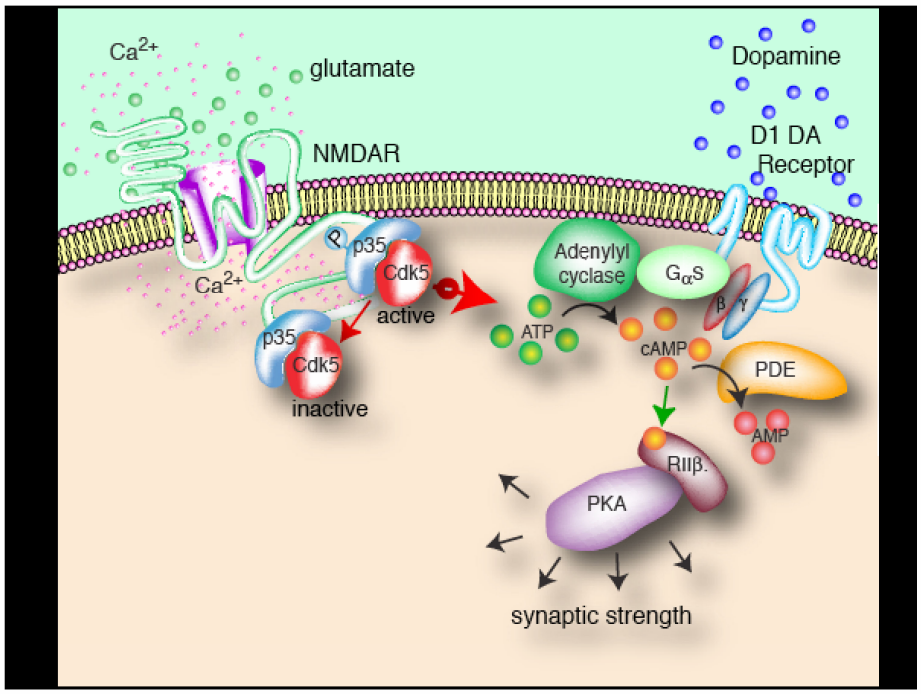


PKA activity is increased in conditional Cdk5 knockouts

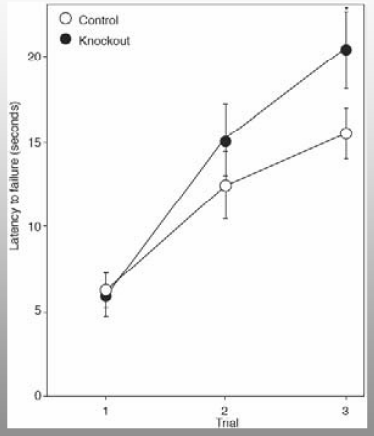


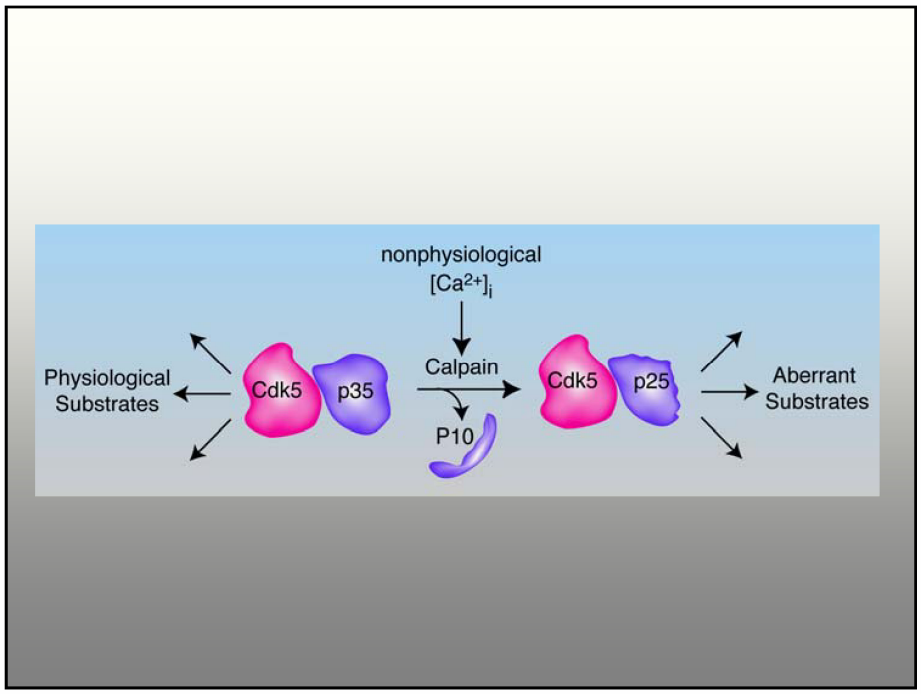

Cdk5 phosphorylates and regulates the RIIβ subunit of the PKA holoenzyme
A
Rat PKA Regulatory Subunit RIIβ
T69
NH2
DD
A
B
COOH
Dimerization domain
cAMP binding domains
B
WT
T69A
Time
Protein
32P
C
RIIβ phospho-Thr69 (66-75)
P
Gly-Gly-Gly-Thr-Pro-Ser-Lys-Gly-Val
D
Cdk5
+
-
P-T69 RIIβ
Total
Str
Hip
Crtx
RIIβ
wt
ko
Stoichiometry (mol/mol)
wild-type
T 69A
Time (min)
Control
PT69 RIIβ
dephosRIIβ
32P
Coomassie
PKA Activity


Cdk5 phosphorylates PDE4
PDE4B
4B3
4B1
4B4
4B2
PKA site
Cdk5 site
LR1
LR2
MAPK site
C-terminal region
UCR1
UCR2
catalytic
Cdk5
PDE4B1
MAPK
PDE4D2 (1-507)
PKA
PDE4B1
A
Time
stoichiometry
Time (min)
B
Phospho-Ser145 PDE4B1
Phospho-Ser573 PDE4D2
Phospho-Ser53 PDE4D2
Tyr-Asp-Leu-Ser-Pro-Lys-Ala-Cys
Cys-Ile-Pro-Gln-Ser-Pro-Ser-Pro
Gln-ARg-Arg-Glu-Ser-Phe-Leu-Cys
C
Cdk5
MAPK
P-S145
P-S573
Total
Control
U0126
Phos
dePhos
P-Ser53 PDE
pyronin Y
Forskolin
P-Ser53

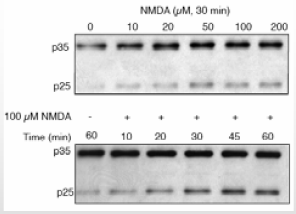


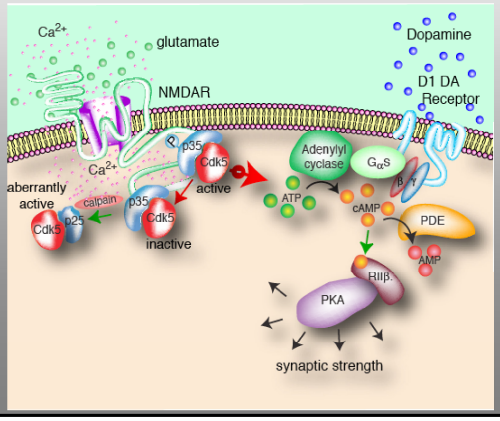
Conditional Cdk5 knockouts have improved motor learning



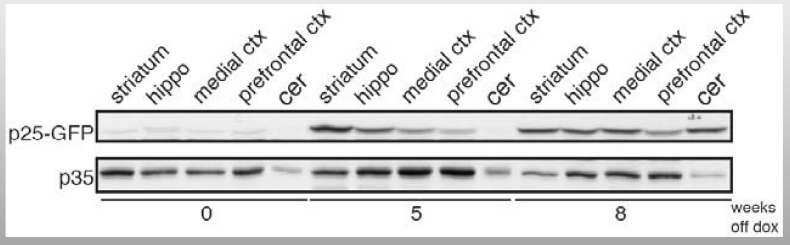


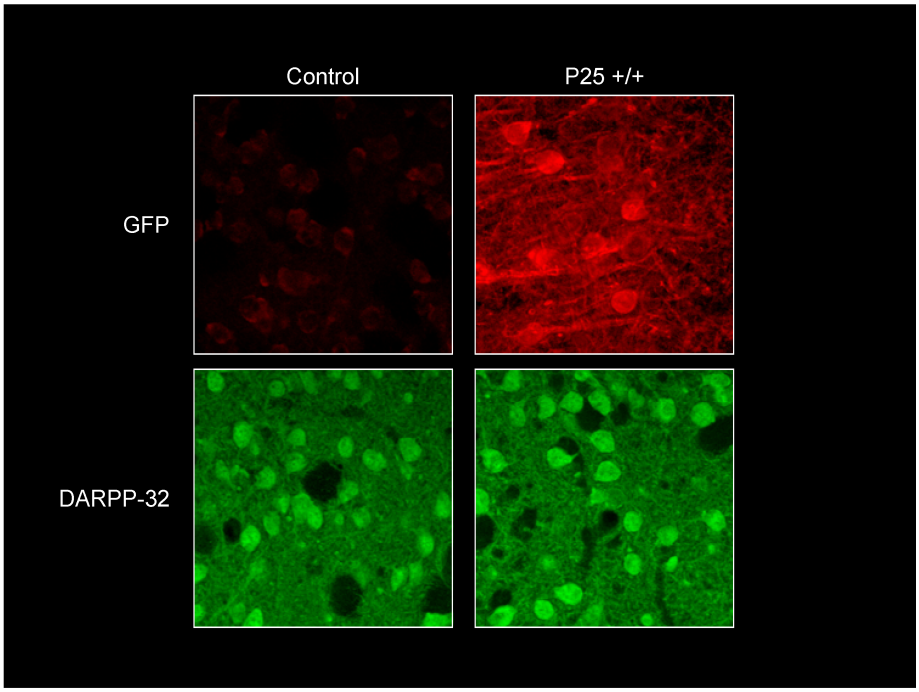
NMDA induces p25 generation in mouse striatal slices



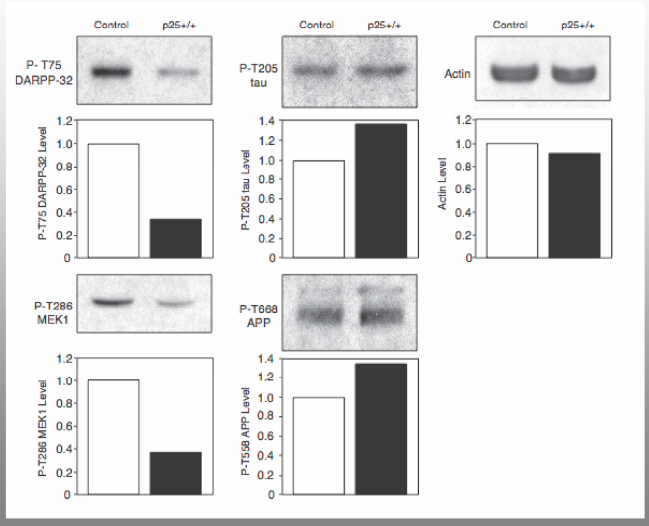


Inducible p25 expression in NSE/p25-GFP mice

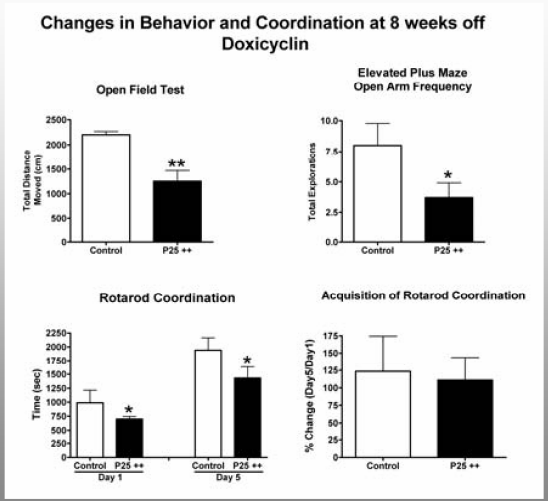




Effect of p25 over-expression on Cdk5-dependent phosphorylation of physiological and aberrant substrates



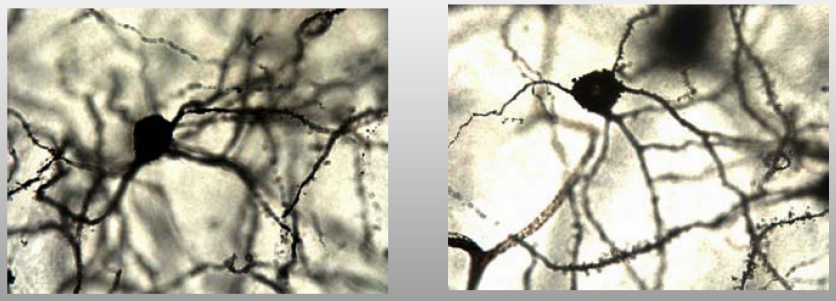
Changes in Behavior and Coordination at 8 weeks off Doxicyclin



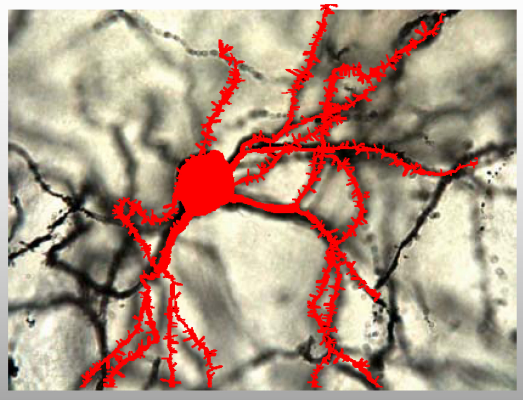
Histological Studies

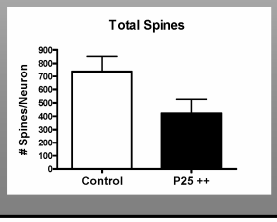
Neuro-morphological effects aberrant Cdk5 activity

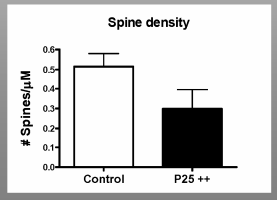
Control | **P25 +/+**



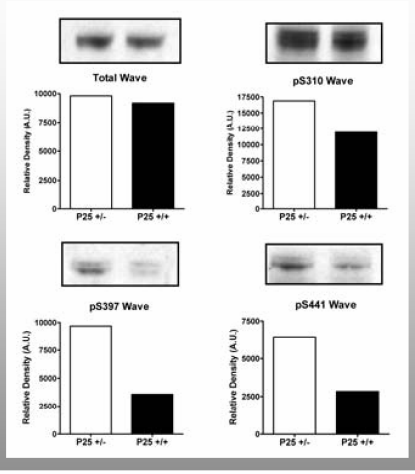
Aberrant Cdk5 activity causes decreases in number of spines and spine density







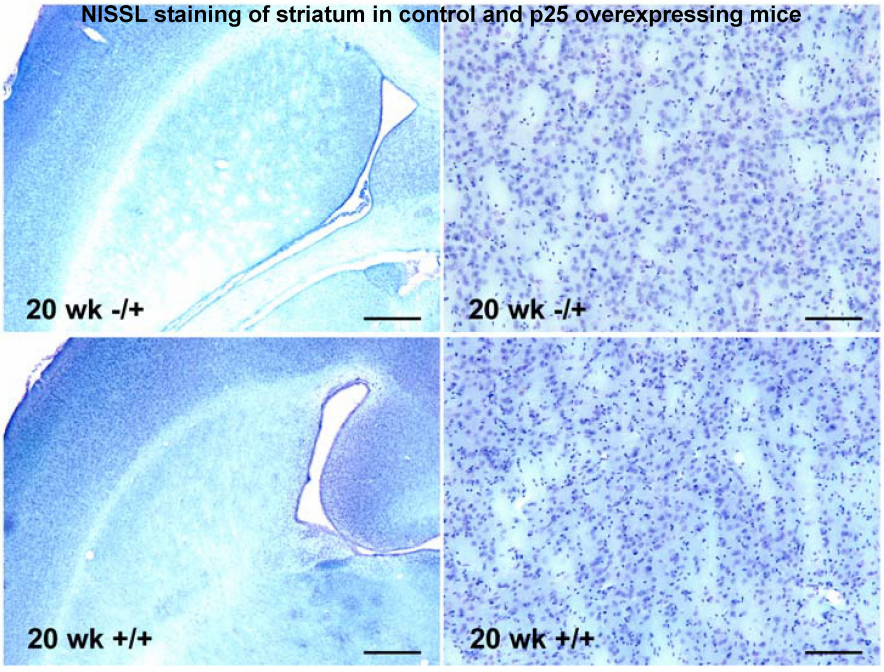
Decrease in Cdk5-dependent phosphorylation of on WAVE1 in p25 over-expressing mice

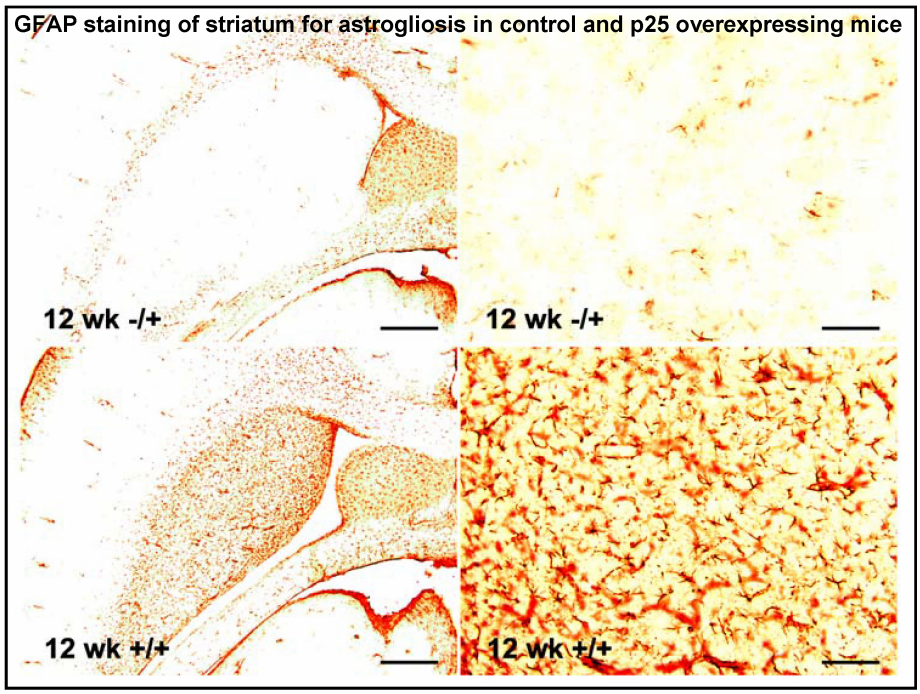


Phosphorylation of WAVE1 regulates actin polymerization and dendritic spine morphology

Yong Kim[1], Jee Young Sung[1], Ilaria Ceglia[1], Ko-Woon Lee[1], Jung-Hyuck Ahn[1], Jonathan M. Halford[1], Amie M. Kim[1], Seung P. Kwak[2], Jong Bae Park[3], Sung Ho Ryu[3], Annette Schenck[4], Barbara Bardoni[4], John D. Scott[5], Angus C. Nairn[1,6] & Paul Greengard[1]

NISSL staining of striatum in control and p25 overexpressing mice





CONCLUSIONS

- Aberrant Cdk5 activity (*i.e.*, p25 generation) may serve as a biomarker of Gulf War illness
- Detection of p25 or the processes contributing to its generation may serve as an important diagnostic tool for Gulf War illness
- Prevention of p25 generation may serve as a preventative or therapeutic treatment for Gulf War illness

STUDY OF ALTERED NEURONAL SIGNAL TRANSDUCTION IN GULF WAR ILLNESS

- Assess the delayed effects of nerve agent exposure upon dopamine signaling, its regulation by Cdk5, and aberrant Cdk5 activity
- Delineate the mechanisms by which nerve agents affect these signaling pathways
- Develop methods of detection and pharmacological means of intervention