WEEK 11

NEUROEPIGENETICS II

Neuroepigenetics

1) **The chromatin – Epigenetic basics (Lecture 1)**

   - Chromatin condensation
   - Regulation of chromatin structure
   - Environmental influences on epigenetics
   - Epigenetic inheritance

2) **Epigenetic dysregulation (Lecture 2)**

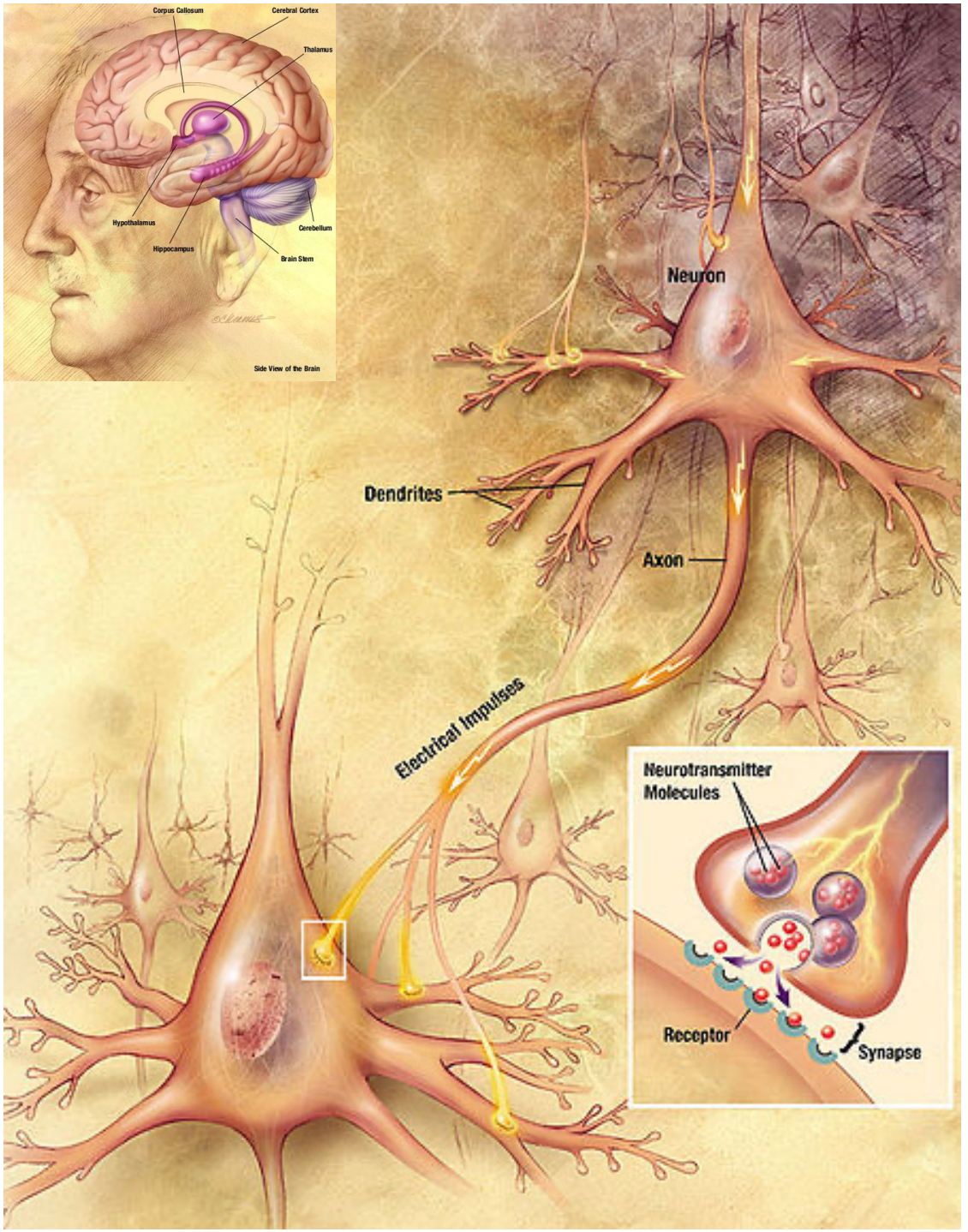
   - in AD

Learning objectives

At the end of today you will be up to date with

- How histone acetylation contributes to learning and memory
- How dysregulated histone acetylation contributes to cognitive decline in AD
- The concept of “cognitive epigenetic priming”, or how HDAC inhibitors work to improve cognition


Damage
ChIP
Water-Maze
Neurotoxic
Braak
H202
Priming
shRNA
Single-cell
HDACi
GR
HDAC2
Luciferase


Corpus Callosum
Cerebral Cortex
Thalamus
Hypothalamus
Cerebellum
Hippocampus
Brain Stem
Side View of the Brain
Neuron
Dendrites
Axon
Electrical Impulses
Neurotransmitter Molecules
Receptor
Synapse

Epigenetic memory?

How to **strengthen memory** ?

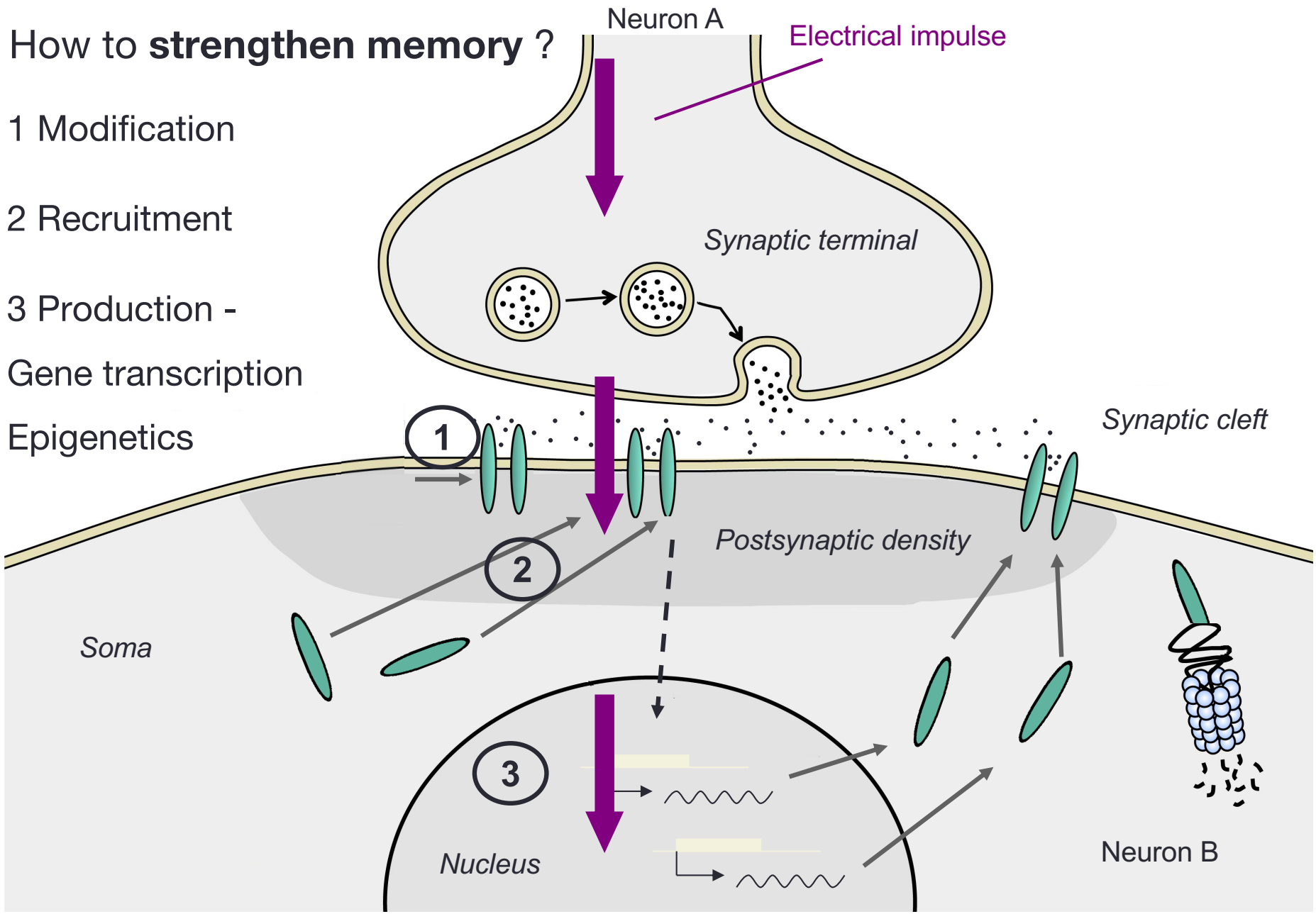
1 Modification

2 Recruitment

3 Production -

Gene transcription

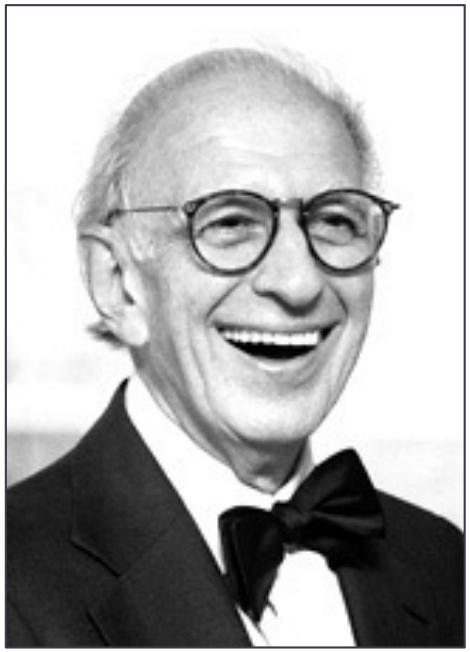
Epigenetics



REVIEW: NEUROSCIENCE

The Molecular Biology of Memory Storage: A Dialogue Between Genes and Synapses

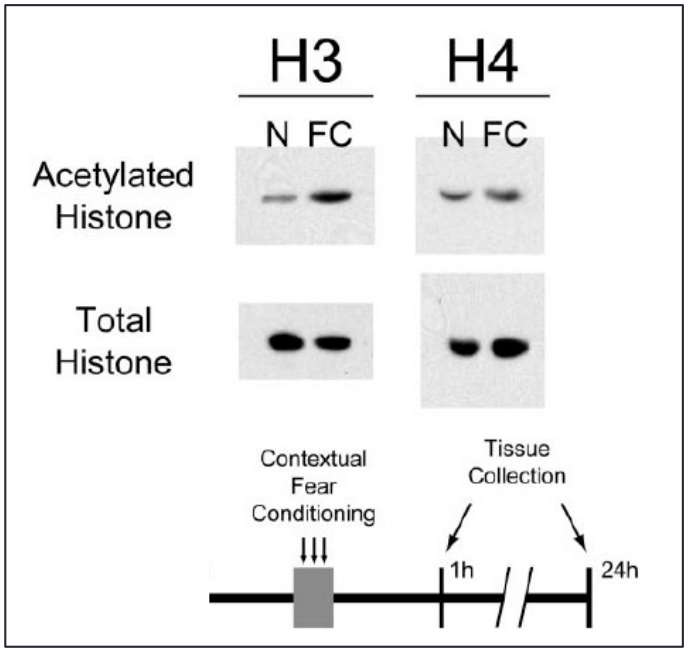
Eric R. Kandel*



Kandel, Science, 2001

Epigenetic memory?

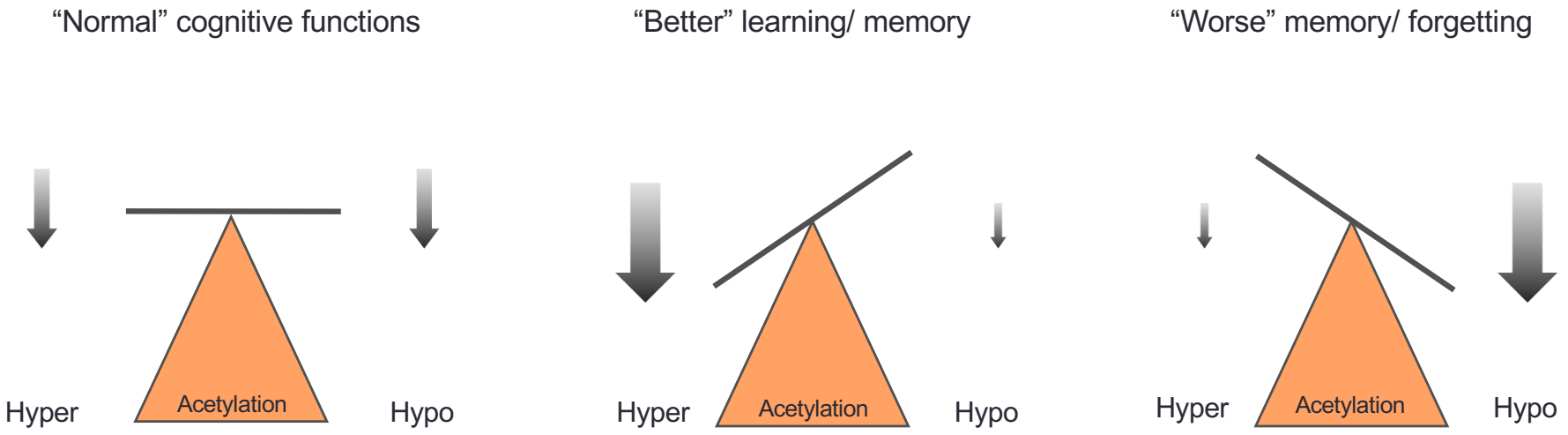
- **Epigenetic mechanisms**
  - = “on” or “above” the genes
  - Mechanisms regulating the compaction of the chromatin



from Levenson et al. 2004

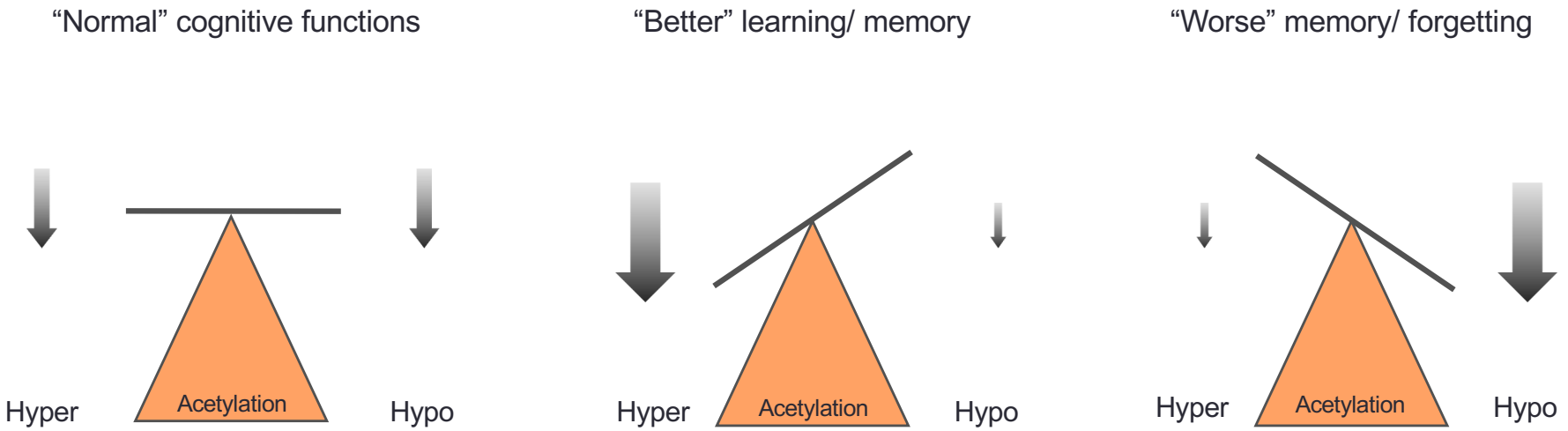
Histone acetylation and memory

- **Histone acetylation and cognitive processes** (Kandel, Sweatt, Abel and others)
  - Increased histone acetylation facilitates synaptic plasticity, learning and memory
  - Decreased histone acetylation constrains synaptic plasticity, learning and memory
    - Reduced attraction between the acetylated (-$COCH_3$) lysine residue on histones (that is positively charged) and the partially negatively charged DNA, thereby facilitating gene transcription



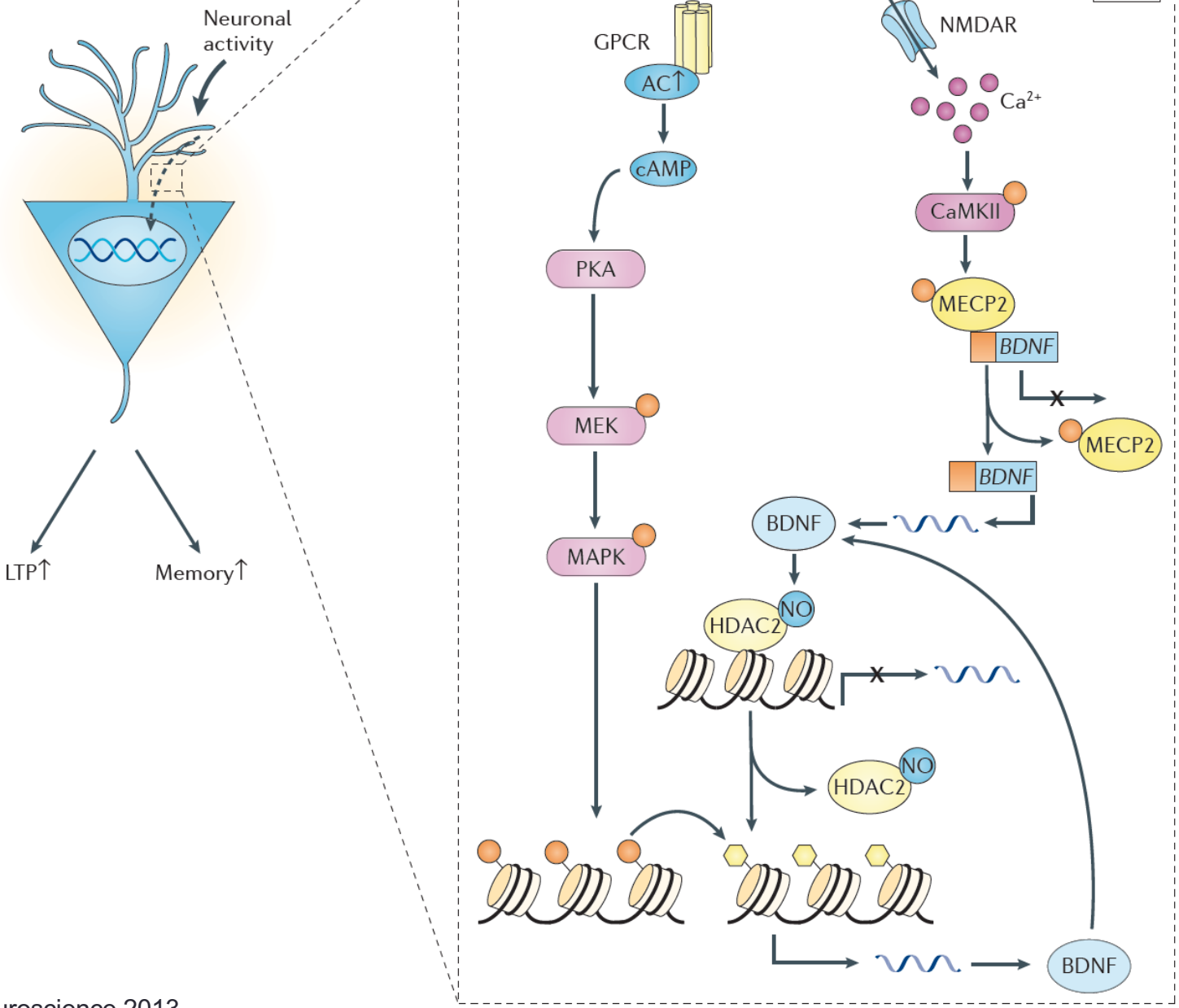
Histone acetylation and memory

- **Histone acetylation and cognitive processes** (Kandel, Sweatt, Abel and others)
  - Catalyzed by **H**istone **A**cetyl **T**ransferases (HATs) and **H**istone **Deac**etylases (HDACs)
    - **HAT**s favor learning and memory Korzus et al., Neuron, 2004; Alarcon et al., Neuron, 2004; Oliveira et al., L&M, 2007
    - **HDAC2** blocks learning and memory Guan et al., Nature, 2009



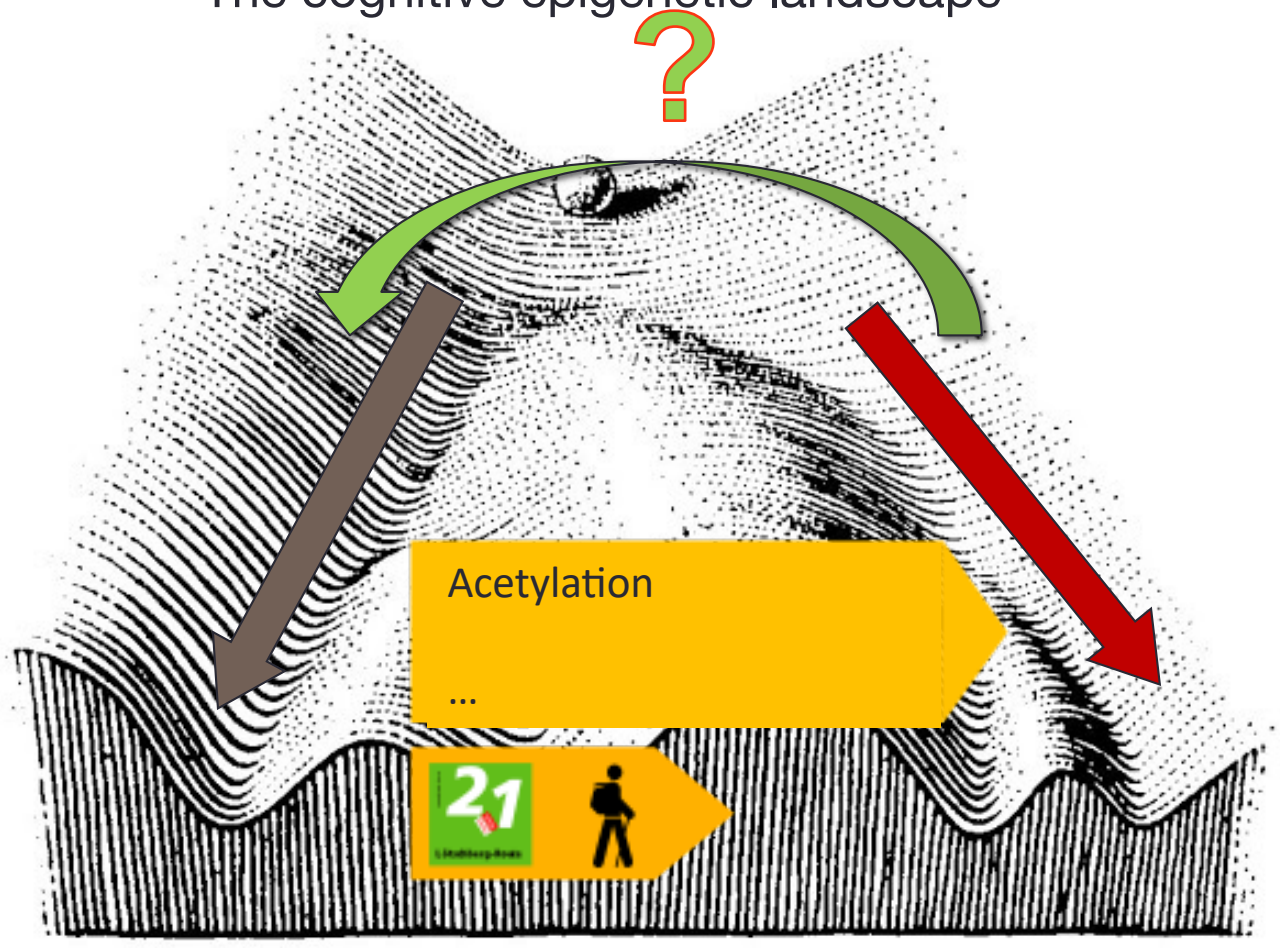
Histone acetylation and memory

- **Histone acetylation:**



Histone acetylation for learning and memory

"The cognitive epigenetic landscape"



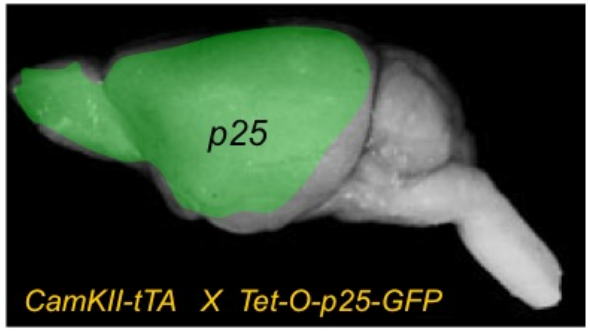
Normal functioning

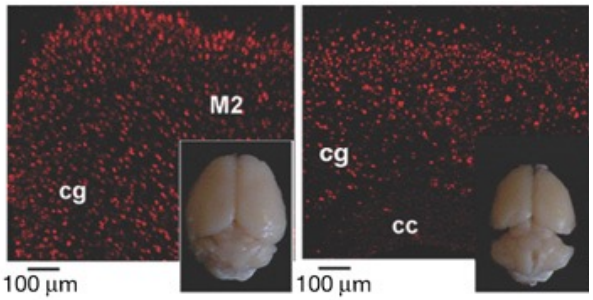
Impaired functioning

Is histone acetylation involved in the cognitive decline associated with AD?

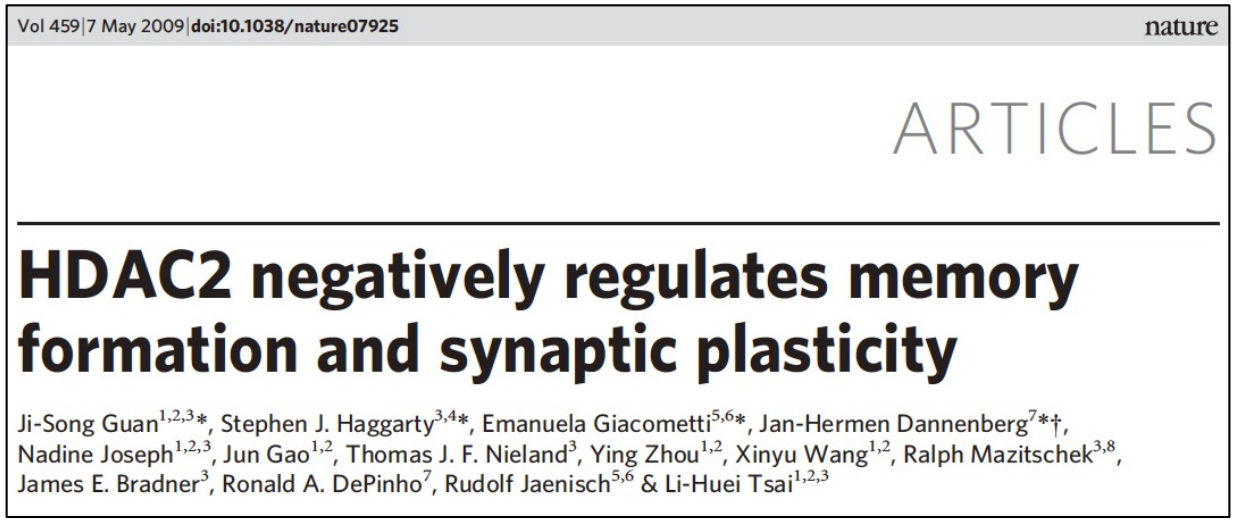
Epigenetic Mechanisms gone wrong

- **Mouse mouse model of AD** Cruz et al., 2003/2006; Fischer et al., 2005
  - Caused by aberrant activity of **C**yclin-dependent **K**inase **5** (CdK5) due to its association with **p25**
    - forebrain-specific expression of p25-GFP
  - Brain mass and neuronal loss
  - Amyloid plaques
  - Learning and memory deficits





Epigenetic Mechanisms gone wrong



Vol 459|7 May 2009|doi:10.1038/nature07925 nature

ARTICLES

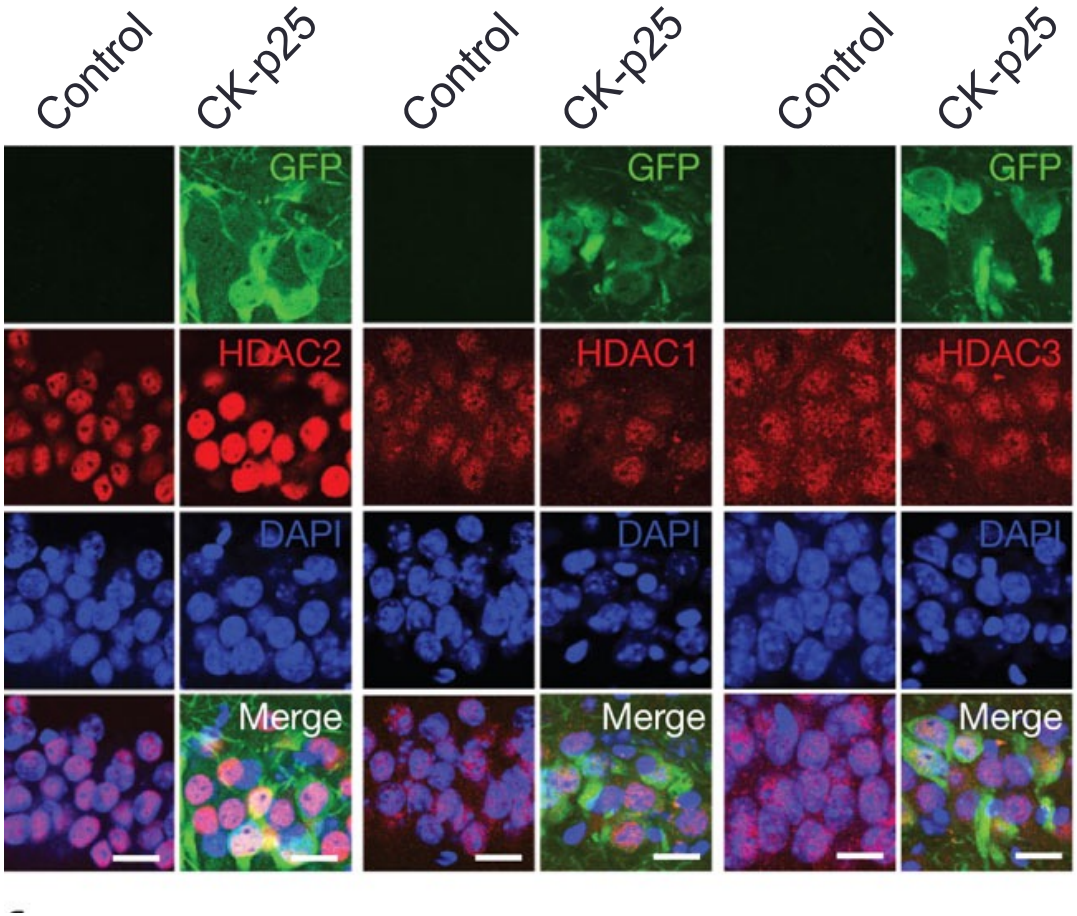
HDAC2 negatively regulates memory formation and synaptic plasticity

Ji-Song Guan[1,2,3]*, Stephen J. Haggarty[3,4]*, Emanuela Giacometti[5,6]*, Jan-Hermen Dannenberg[7]*†, Nadine Joseph[1,2,3], Jun Gao[1,2], Thomas J. F. Nieland[3], Ying Zhou[1,2], Xinyu Wang[1,2], Ralph Mazitschek[3,8], James E. Bradner[3], Ronald A. DePinho[7], Rudolf Jaenisch[5,6] & Li-Huei Tsai[1,2,3]

- **Do epigenetic mechanisms contribute to cognitive decline in AD?**

- **Does HDAC2 contribute to cognitive decline in AD?**

- **HDAC2 levels are elevated in the AD mouse brain**

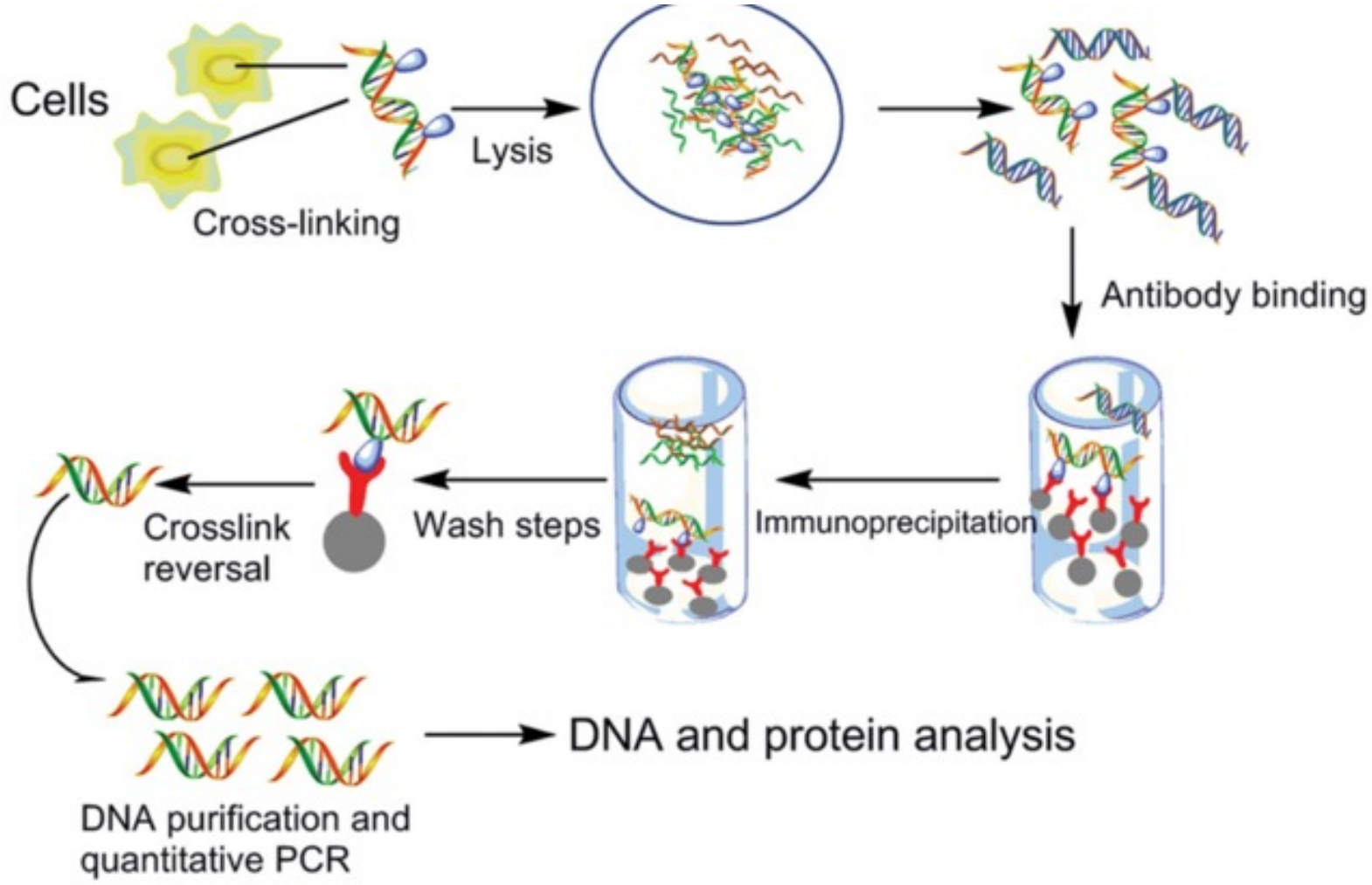


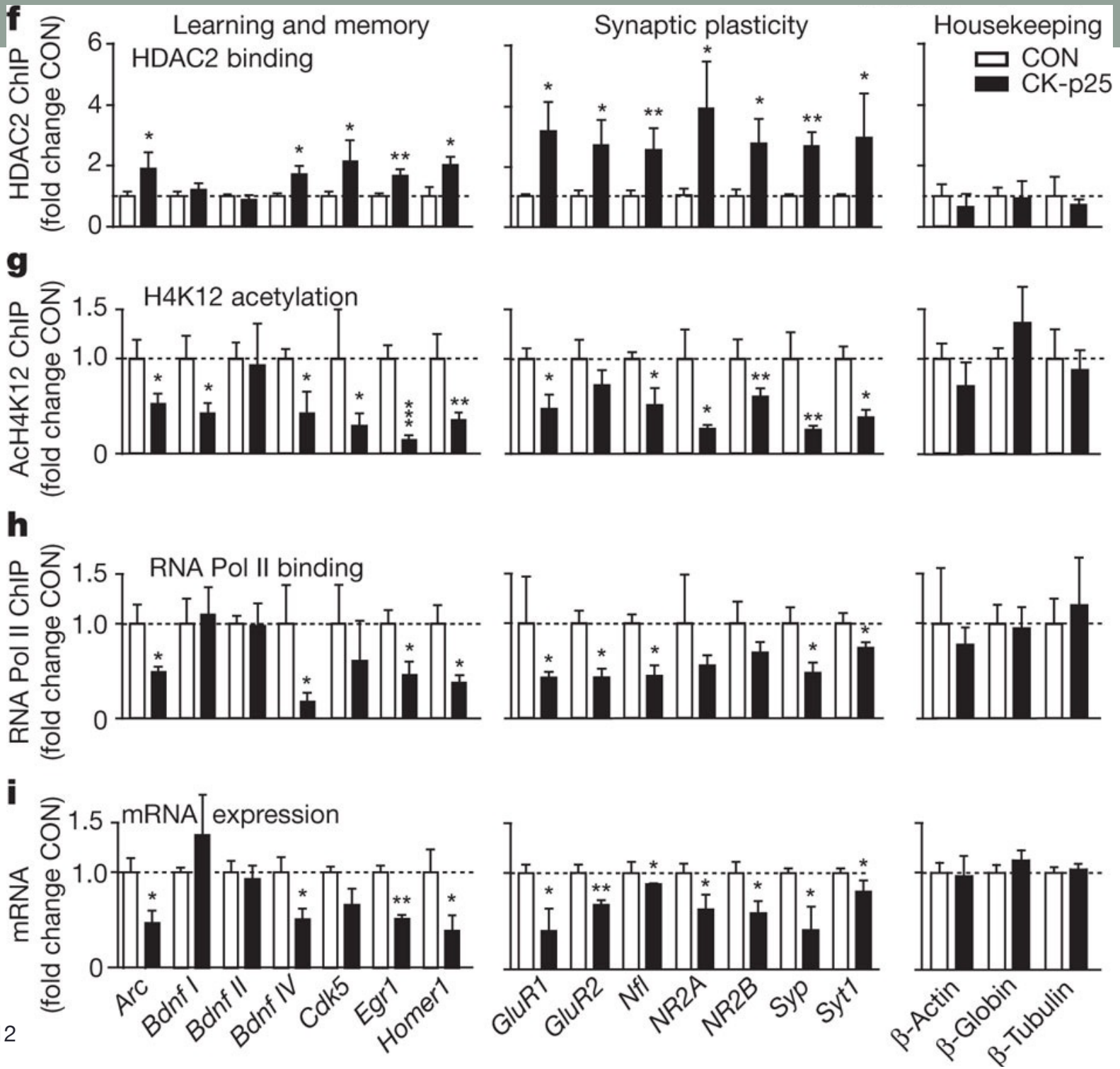
- **Consequences of elevated HDAC2 levels in the AD mouse brain:**
  1) **More HDAC2 binding in the promoter region of genes important for learning and memory**
  2) **Less histone acetylation in the same promoter region**
  3) **Less binding of the RNA polymerase responsible for mRNA transcription of these genes**
  4) **Less mRNA transcription of these genes**

1-3 measured by chromatin immunoprecipitation (ChIP): Next slide

An epigenetic blockade of cognitive functions

Chromatin Immunoprecipitation (ChIP)




f
Learning and memory
Synaptic plasticity
Housekeeping
HDAC2 binding
HDAC2 ChIP (fold change CON)
CON
CK-p25
g
H4K12 acetylation
AcH4K12 ChIP (fold change CON)
h
RNA Pol II binding
RNA Pol II ChIP (fold change CON)
i
mRNA expression
mRNA (fold change CON)
Arc
Bdnf I
Bdnf II
Bdnf IV
Cdk5
Egr1
Homer1
GluR1
GluR2
Nfl
NR2A
NR2B
Syp
Syt1
β-Actin
β-Globin
β-Tubulin

Epigenetic Mechanisms gone wrong

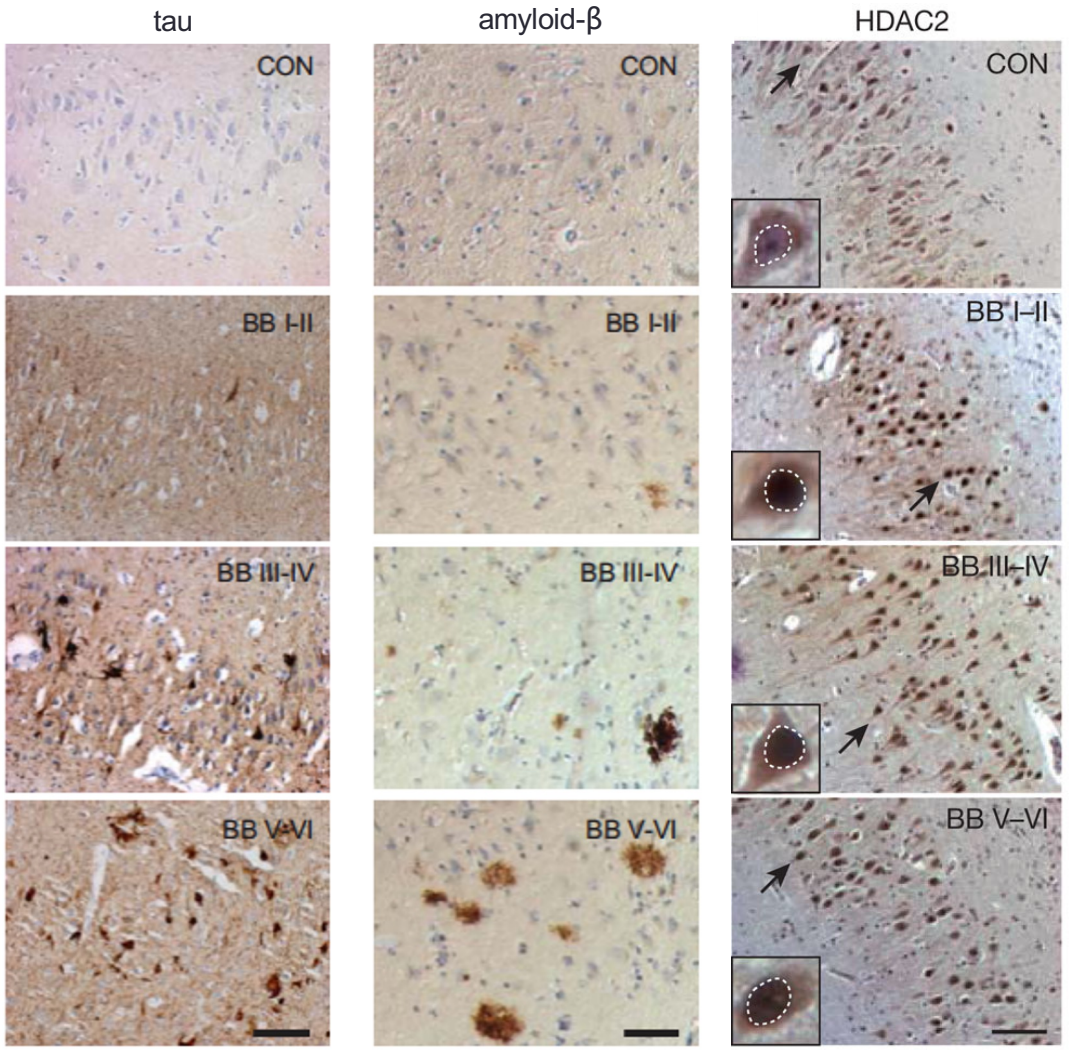
- **HDAC2 in the human AD brain**

CON: No AD pathologies

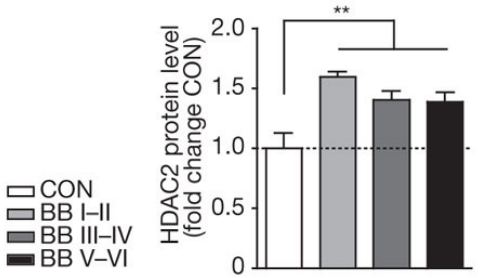
BBI-II: Mild cognitive impairment

BBIII-IV: Moderate AD

BBV-VI: Severe AD

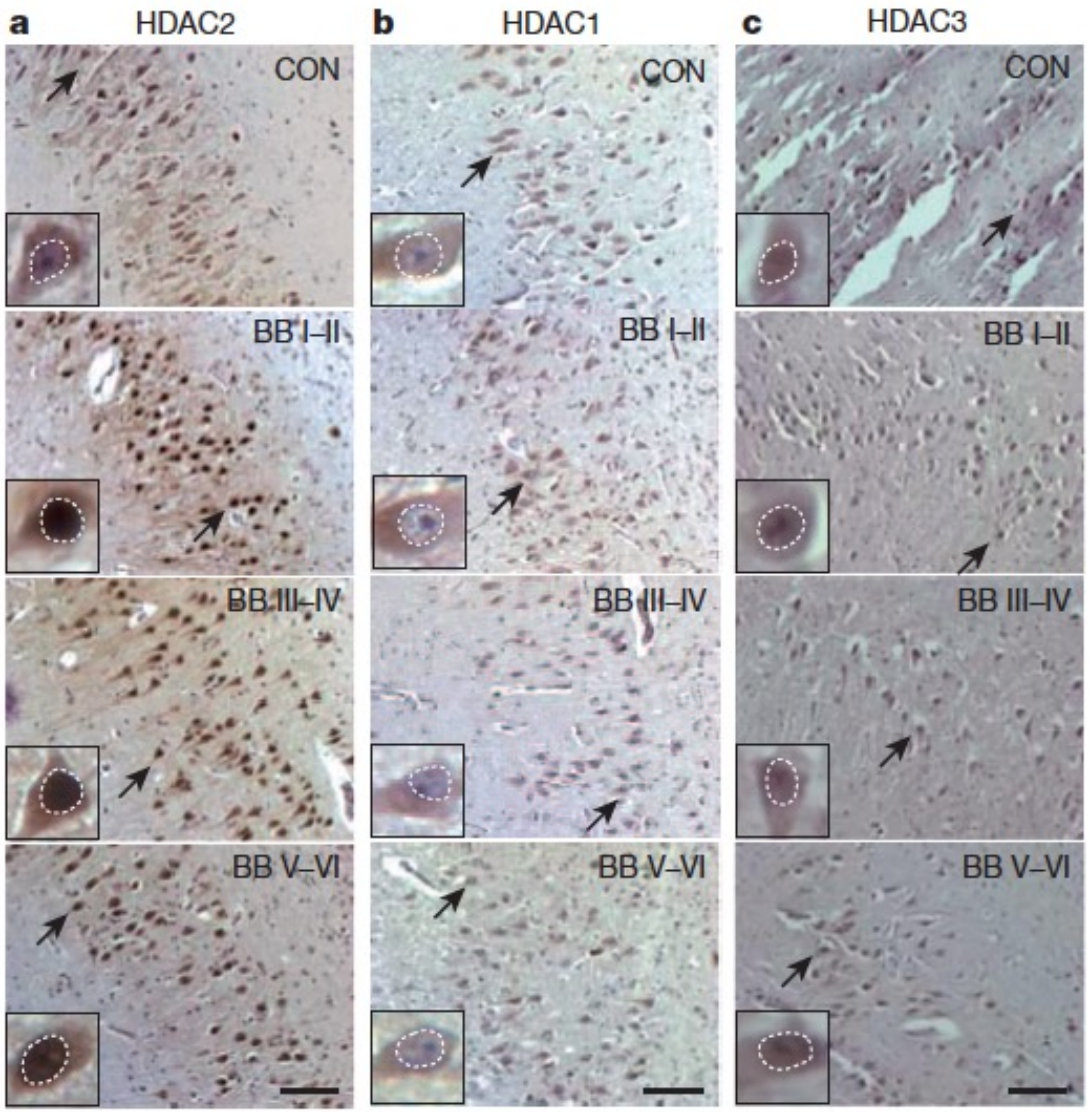


A similar epigenetic blockade in the human AD brain?



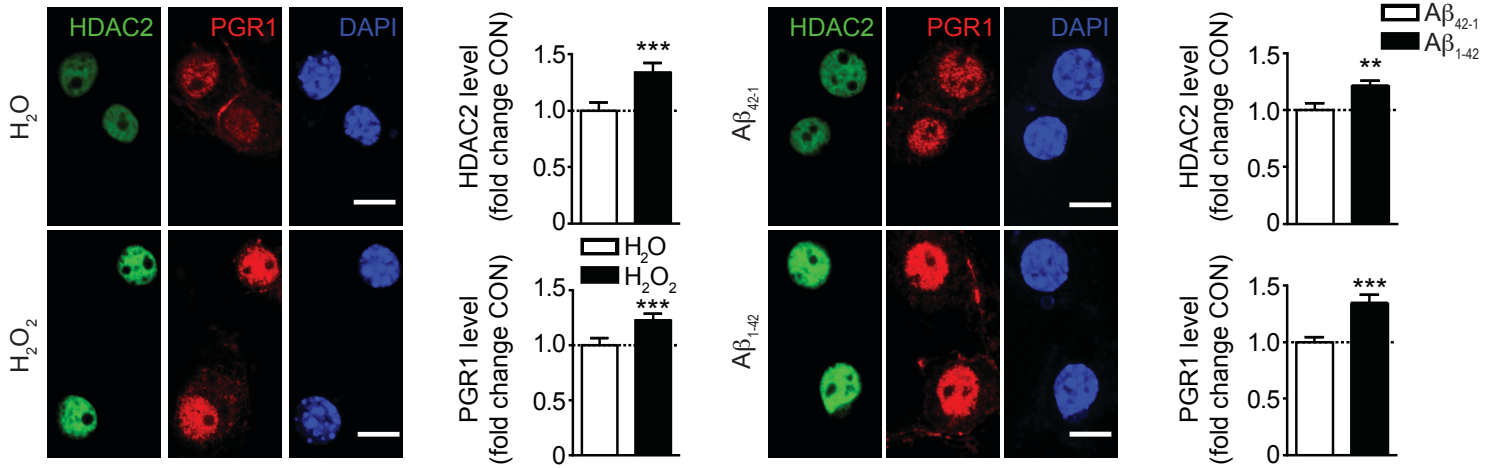
Epigenetic Mechanisms gone wrong

- The increase for HDAC2 was specific, and not observed on structurally related HDAC1 and HDAC3



Epigenetic Mechanisms gone wrong

- **What are the mechanisms behind the upregulation of HDAC2?**
  - Responsive to oxidative stress (H_2O_2) and neurotoxic insults (amyloid-β)
  - transcriptionally regulated via glucocorticoid receptor element (GRE)?



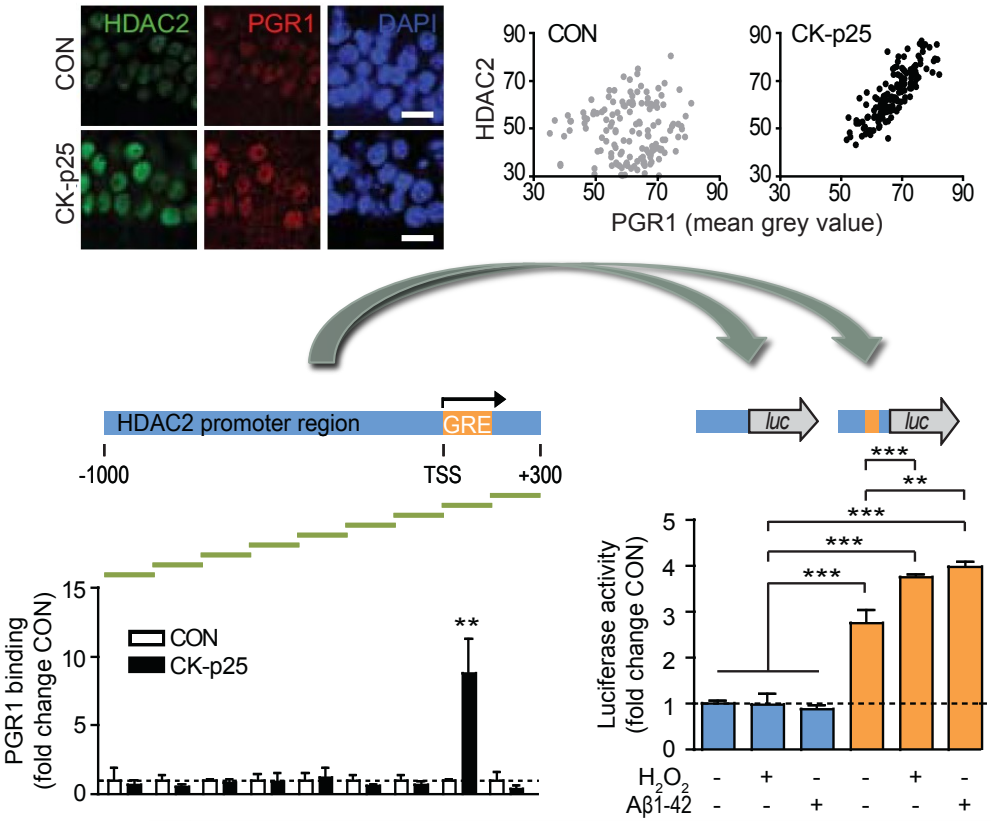
Transcription factor binding sites in HDAC2 promoter?

Glucocorticoid receptor (GR1)

- stress hormone receptor
- transcription factor
- activated by phosphorylation

Epigenetic Mechanisms gone wrong

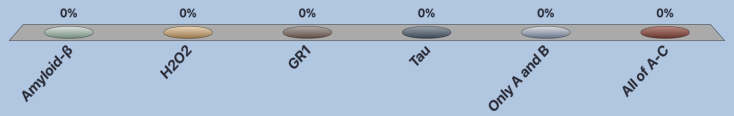
- **What are the mechanisms behind the upregulation of HDAC2?**
  - Correlation between phosphorylated, i.e. activated GR and HDAC2 also *in vivo*
  - PGR binds to the *HDAC2* promoter region



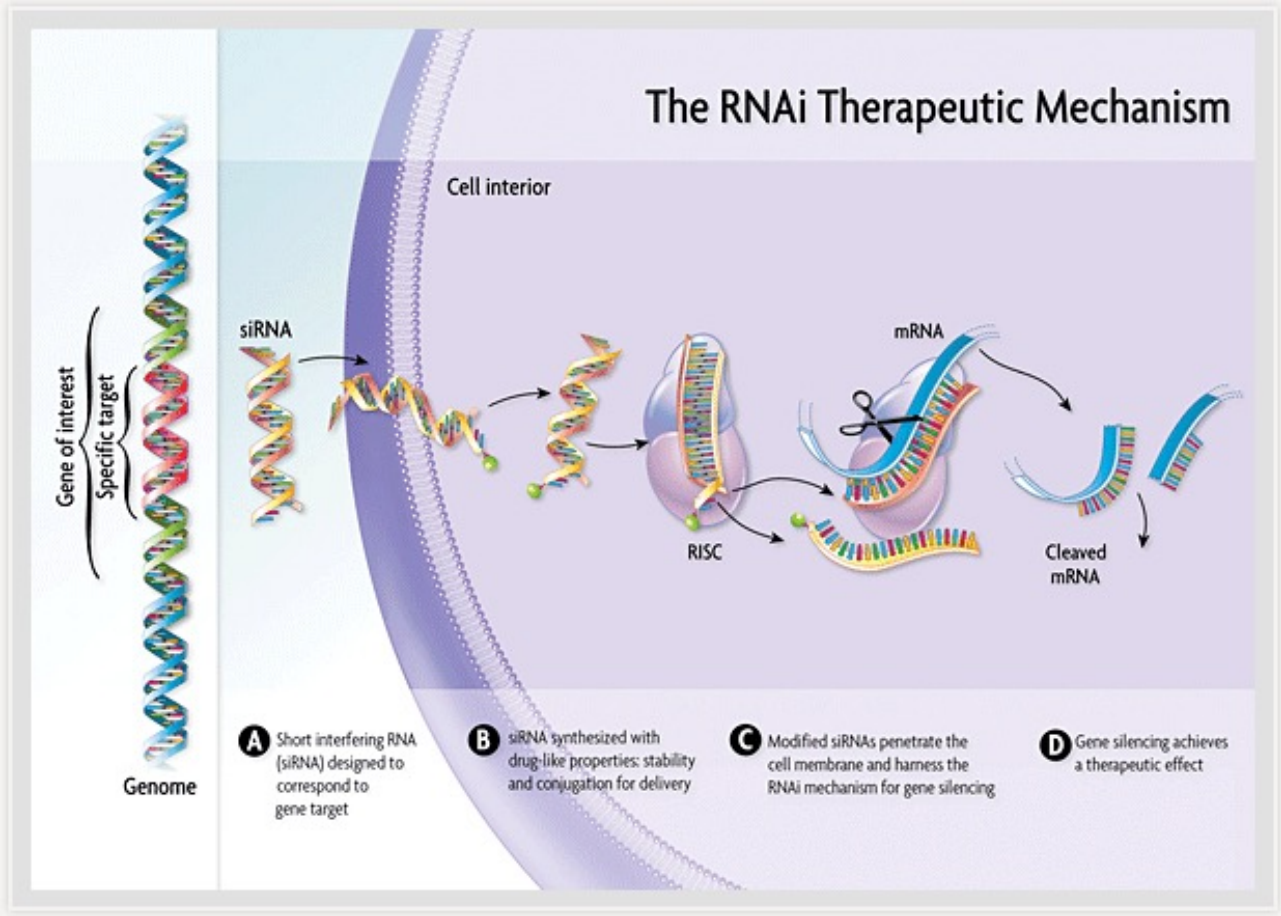
Neurotoxic insults drive the upregulation of HDAC2 via stress hormone receptors.

What mediates the upregulation of HDAC2?

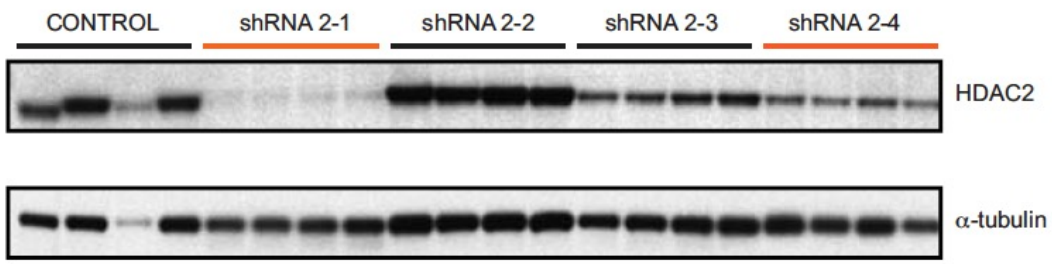
A. Amyloid-β
B. H_2O_2
C. GR1
D. Tau
E. Only A and B
F. All of A-C



- **Functional importance for learning and memory?**
  - **RNAi expression to reduce elevated HDAC2 levels**

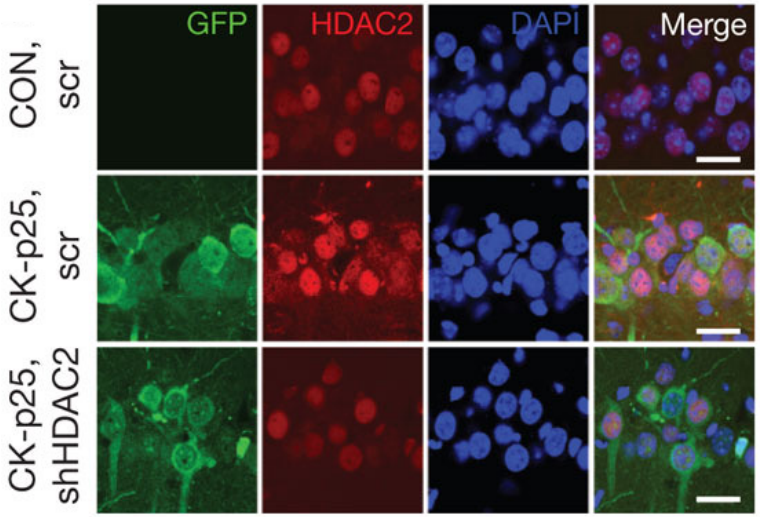


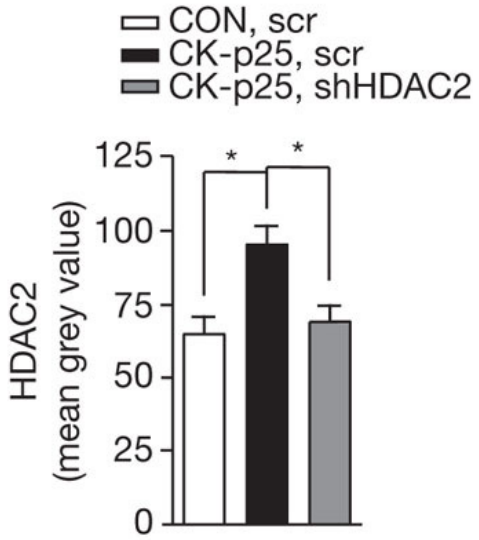
- **Functional importance for learning and memory?**
  - **RNAi expression to reduce elevated HDAC2 levels**
    - **Testing different shRNA constructs (different sequences)**



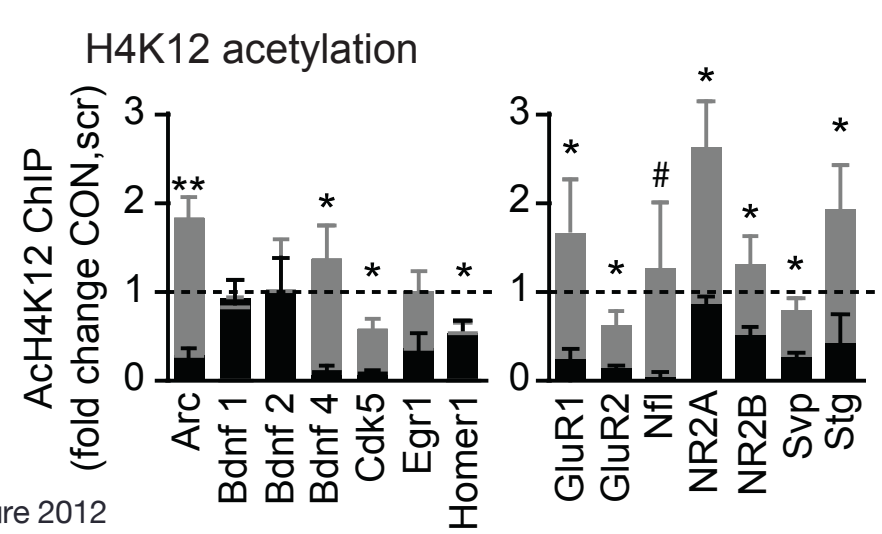
Epigenetic Mechanisms gone wrong

- **RNAi-mediated reversal of HDAC2 levels?**

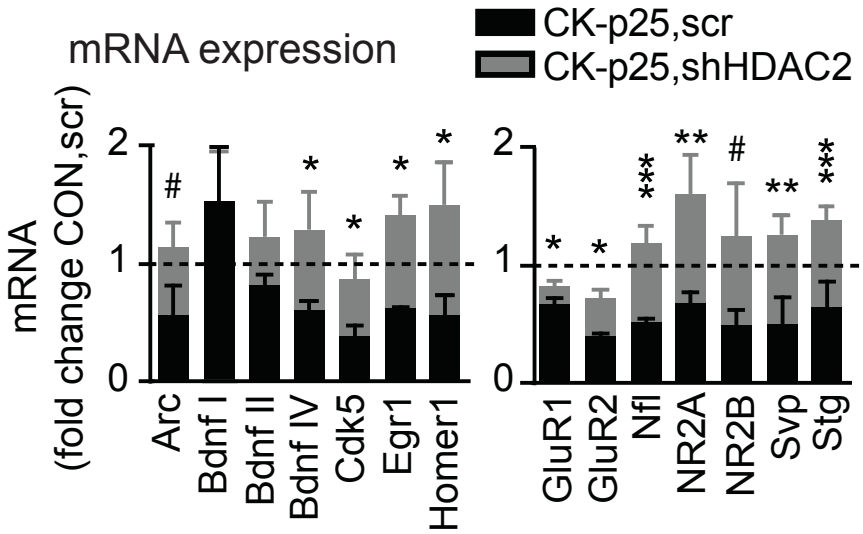




Restored histone acetylation

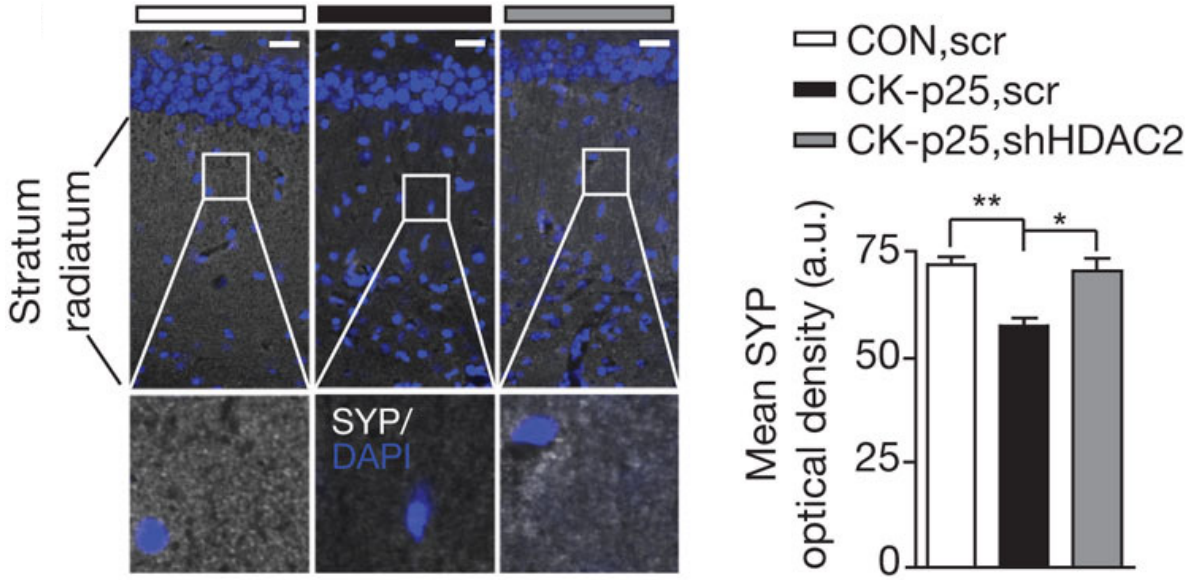


Restored plasticity-related gene expression



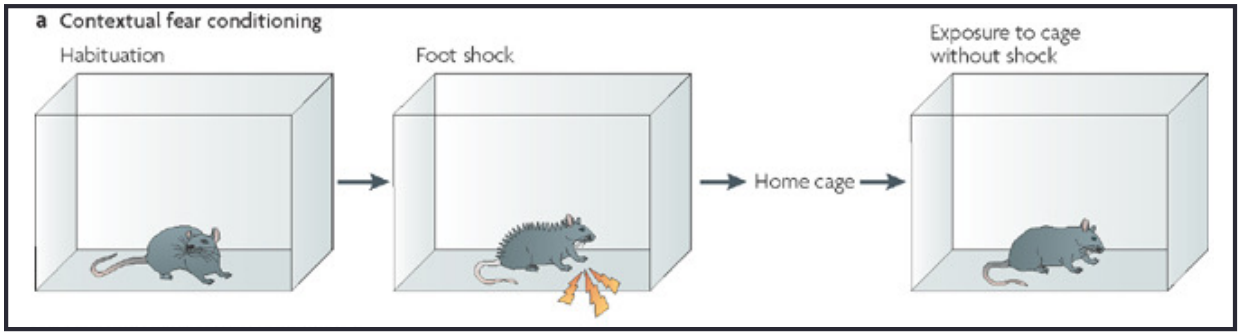
Epigenetic Mechanisms gone wrong

- **Restored structural plasticity**
  - as assessed by synaptophysin (SYP) IHC, a marker of synaptic density

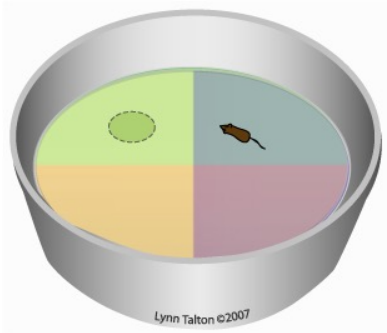


Epigenetic Mechanisms gone wrong

- **Restored cognitive capacities**
  - Associative memory: Pavlovian Fear Conditioning

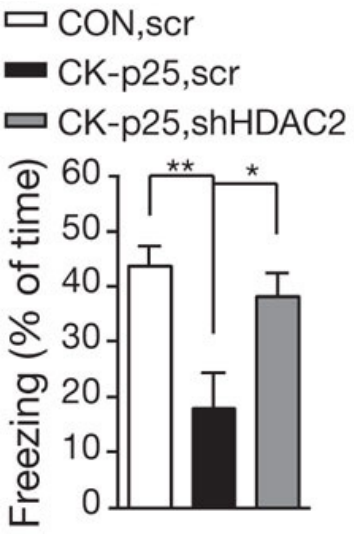


  - Spatial memory: Water Maze



Epigenetic Mechanisms gone wrong

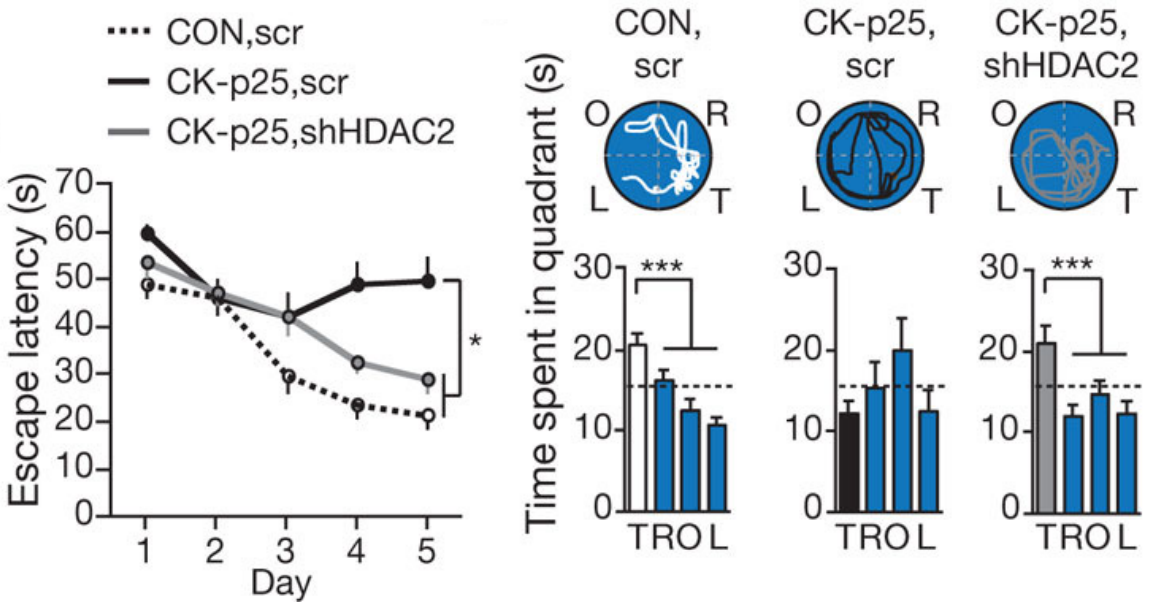
- **Restored cognitive capacities**
  - Associative memory: Fear Conditioning



Epigenetic Mechanisms gone wrong

- **Restored cognitive capacities**
  - Spatial Memory: Water Maze

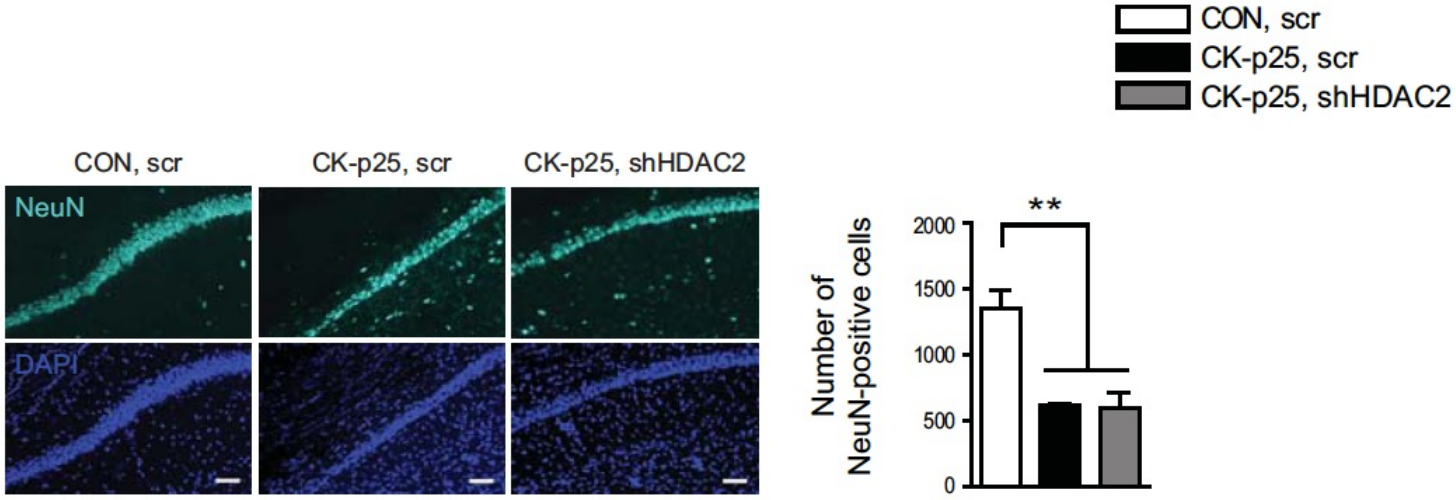
Training/Acquisition Testing/24h memory



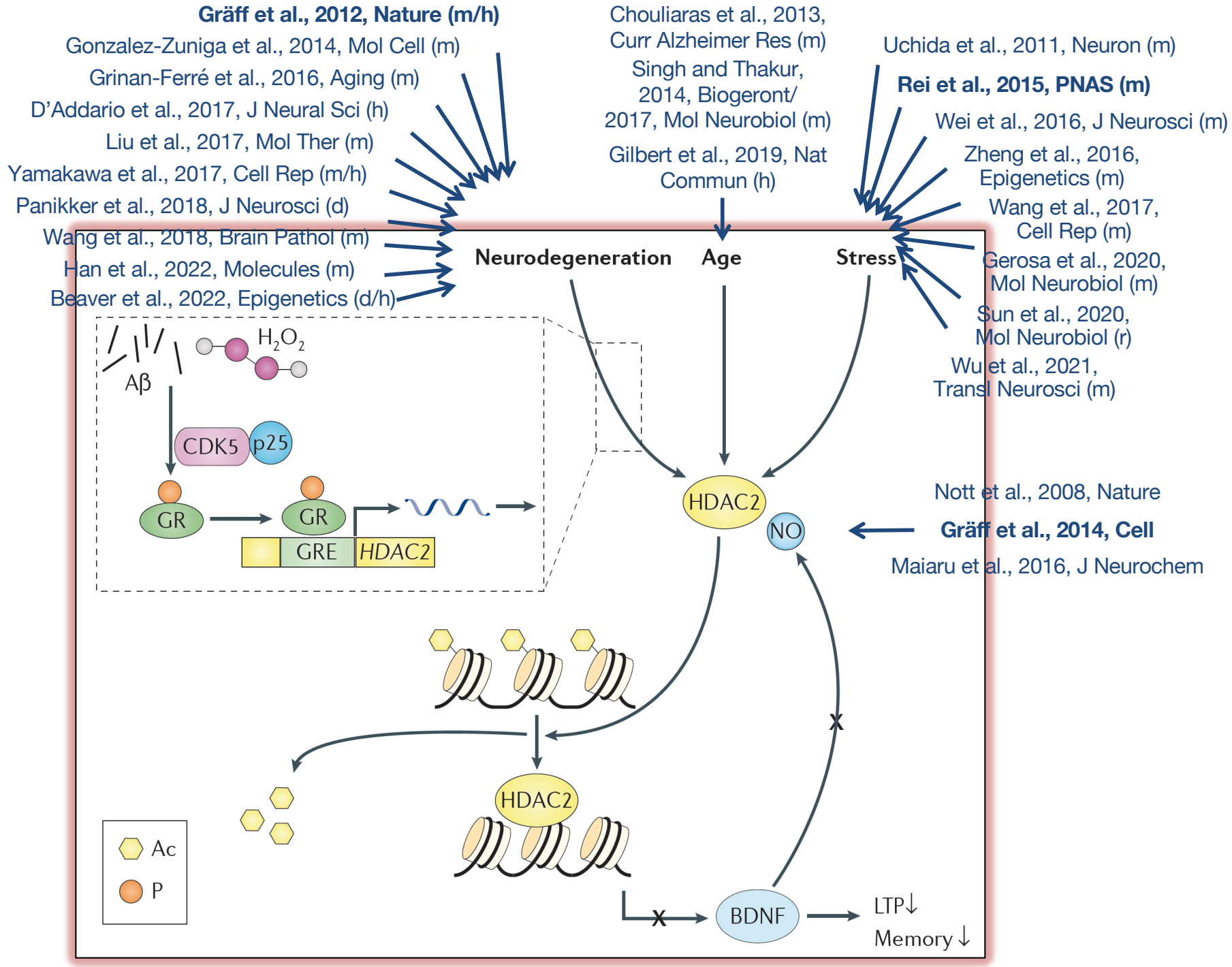
The epigenetic blockade on cognitive functions is potentially reversible.

Epigenetic Mechanisms gone wrong

- **Despite persistent neuronal loss.**
  - Number of neurons (NeuN staining)

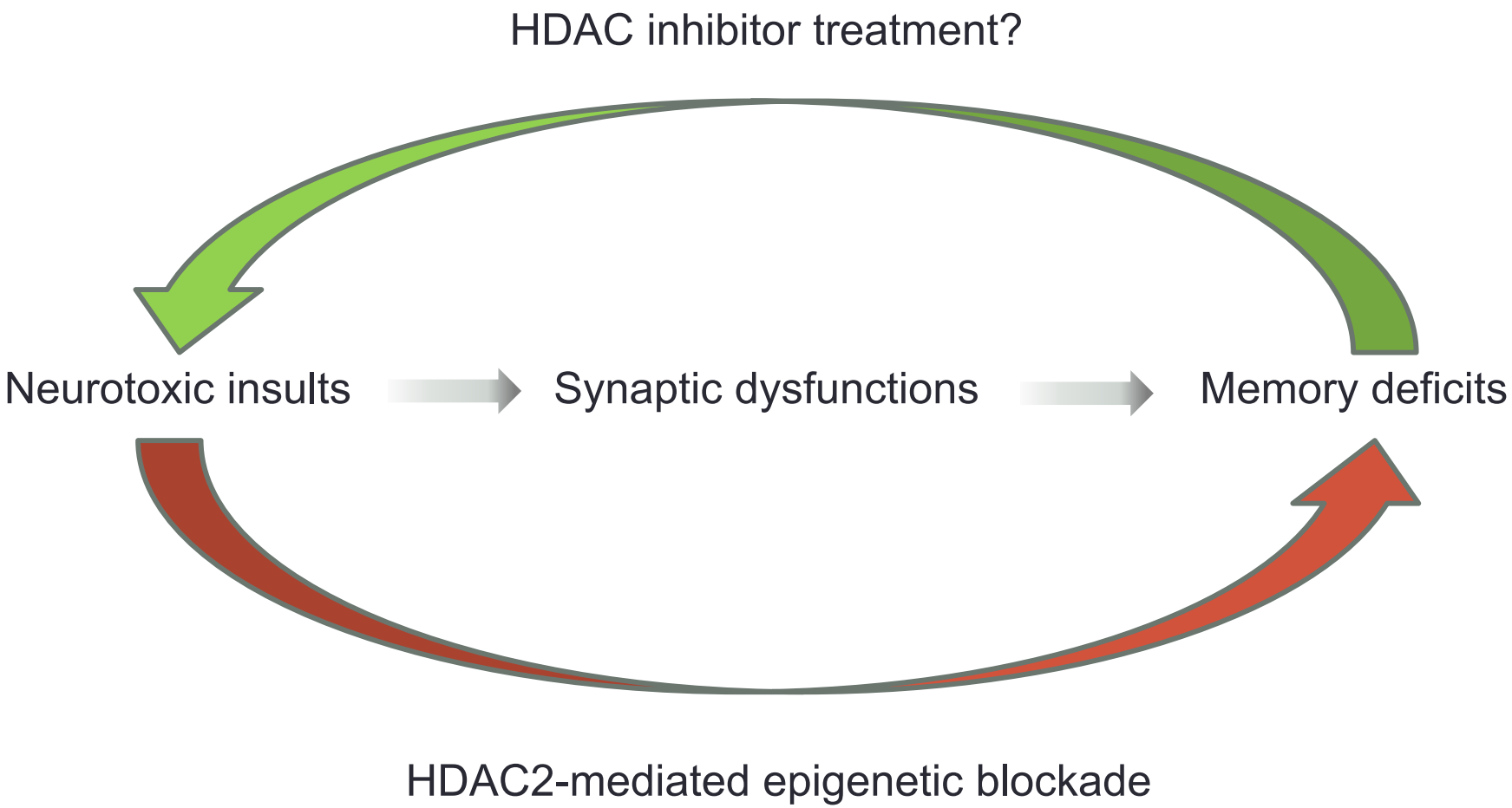


Even in a severely degenerated brain,
neuronal plasticity is not entirely lost, but merely constrained.



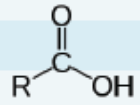
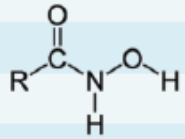
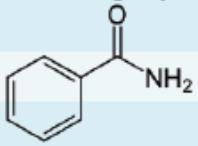
updated from Gräff and Tsai, 2013, Nat Rev Neurosci

- **Can this be overcome? The case of HDAC inhibitors**

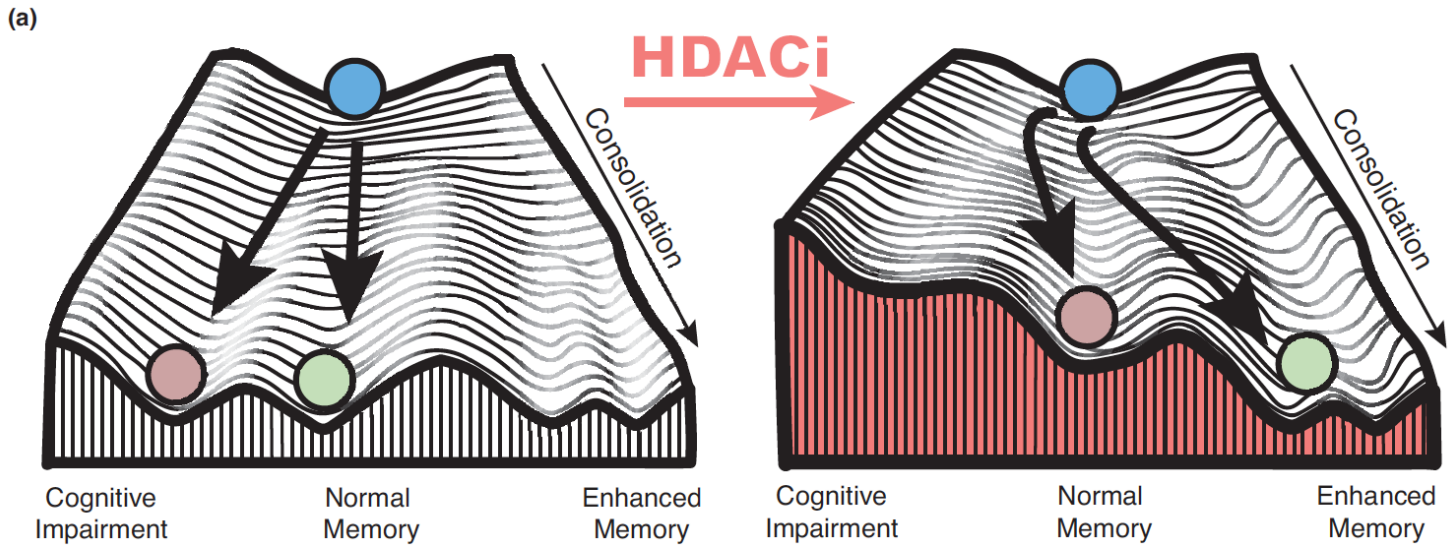


- **Can this be overcome? The case of HDAC inhibitors**

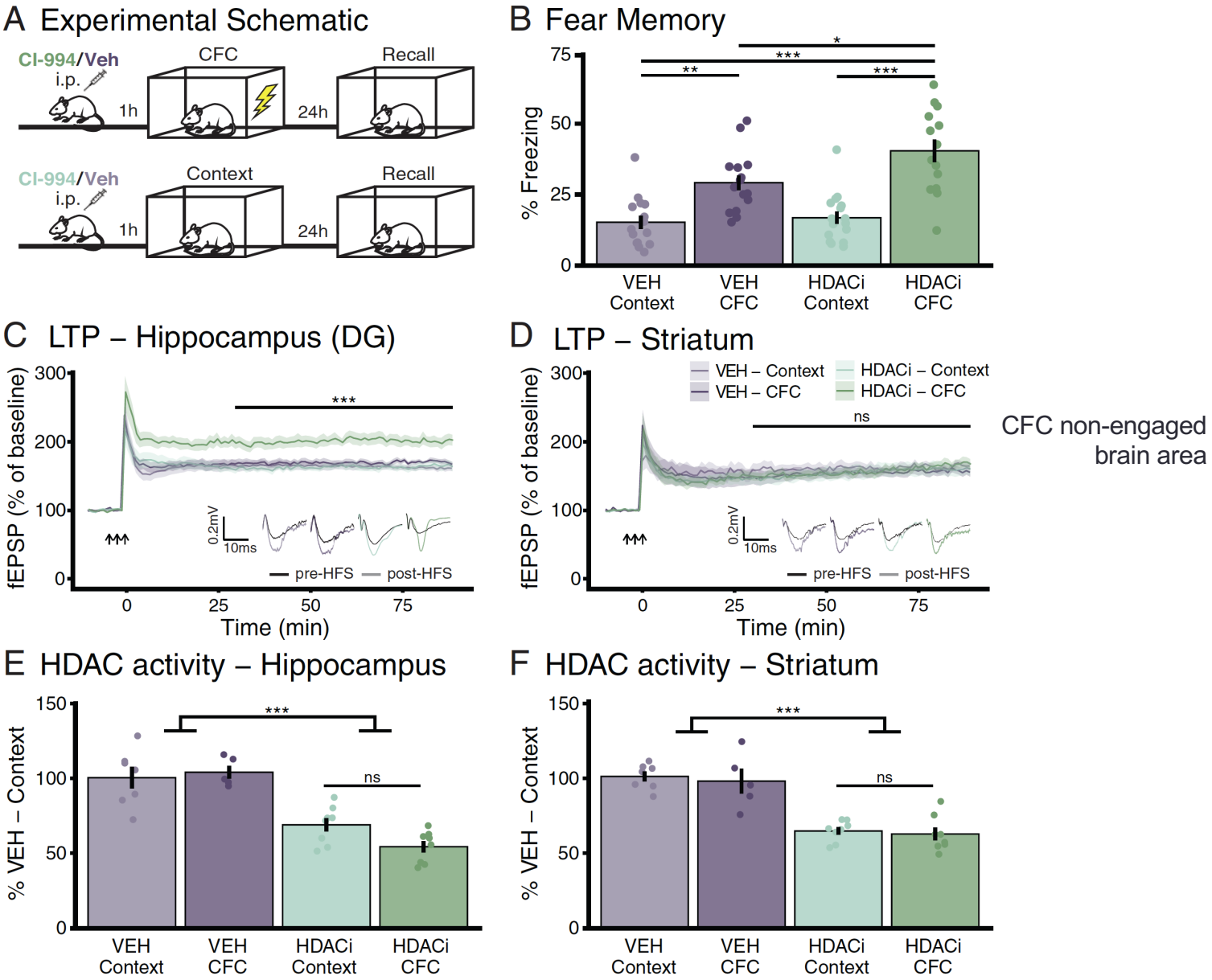
Table 2 HDAC inhibitors used as treatments against cognitive decline or as pure cognitive enhancers

Structural Class	Selectivity	HDACi	Cognitive Process: Treatment of decline	Cognitive Process: Enhancement	Refs
Carboxylic acids 	Class I	NaB	Rescue of contextual fear and spatial memories in CK-p25 mice		(73)
			Rescue of fear memory in APP/PS1 mouse models		(51; 74)
			Rescue of object memory in $CBP^{KIX/KIX}$-mice		(72)
				Enhancement of contextual memory in mice, rats, and crabs	(6; 29; 62; 73)
				Enhancement of spatial learning	(73)
				Enhancement of object recognition and location memory in mice and rats	(72; 81; 82)
		PB	Rescue of contextual fear memory in APP/PS1 mice		(74)
			Rescue of associative memories in Tg2576 mice		(75)
		Valproic Acid	Rescue of contextual fear memory in APP/PS1 mice		(74)
				Enhancement of spatial memory in rats	(79; 80)
Hydroxamic acid 	Class I and II	TSA	Rescue of object memory deficits in CBP <HAT>-mice		(40)
			Rescue of contextual fear memory in APP/PS1 mice		(50)
				Enhancement of fear potentiated startle in rats	(76)
				Enhancement of contextual memory in mice and crabs	(25; 29; 77)
				Enhancement of cued fear memory reconsolidation	(24)
				Enhancement of object memory	(83)
		SAHA	Rescue of contextual fear memory in $CBP^{+/-}$ mice		(39)
Benzamide group 	Class I (mostly HDAC3)	MS-275		Enhancement of contextual fear memory	(62)
				Enhancement of object memory	(83)
		RGFP 136		Enhancement of object location memory	(63)

Understanding the mode of action of HDAC inhibitors



Cognitive epigenetic priming

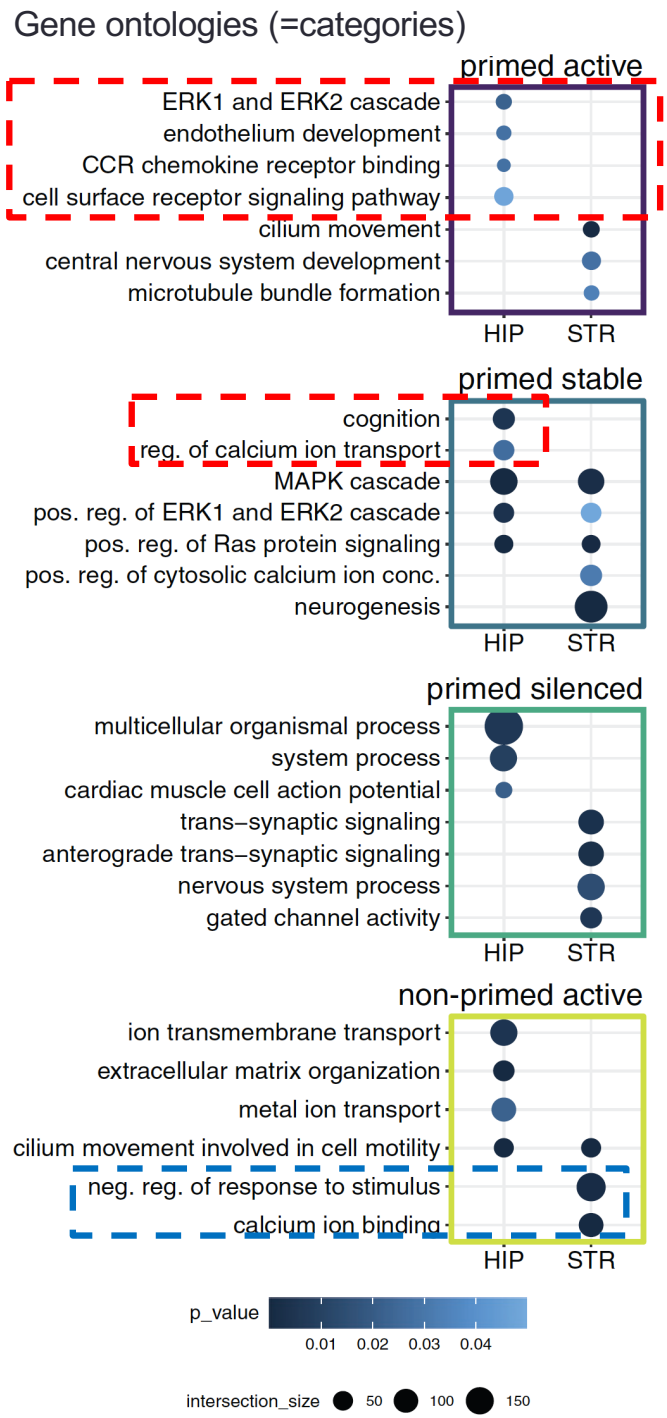


CFC-engaged brain area

CFC non-engaged brain area

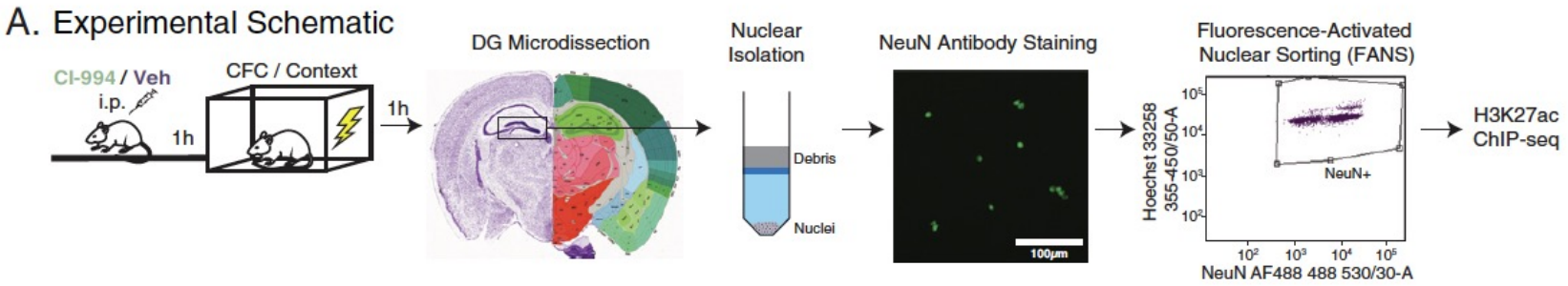
Cognitive epigenetic priming

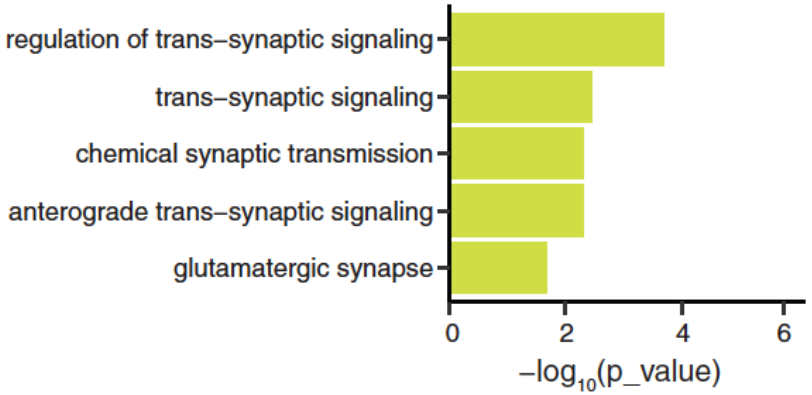
Differential gene expression (whole brain area)



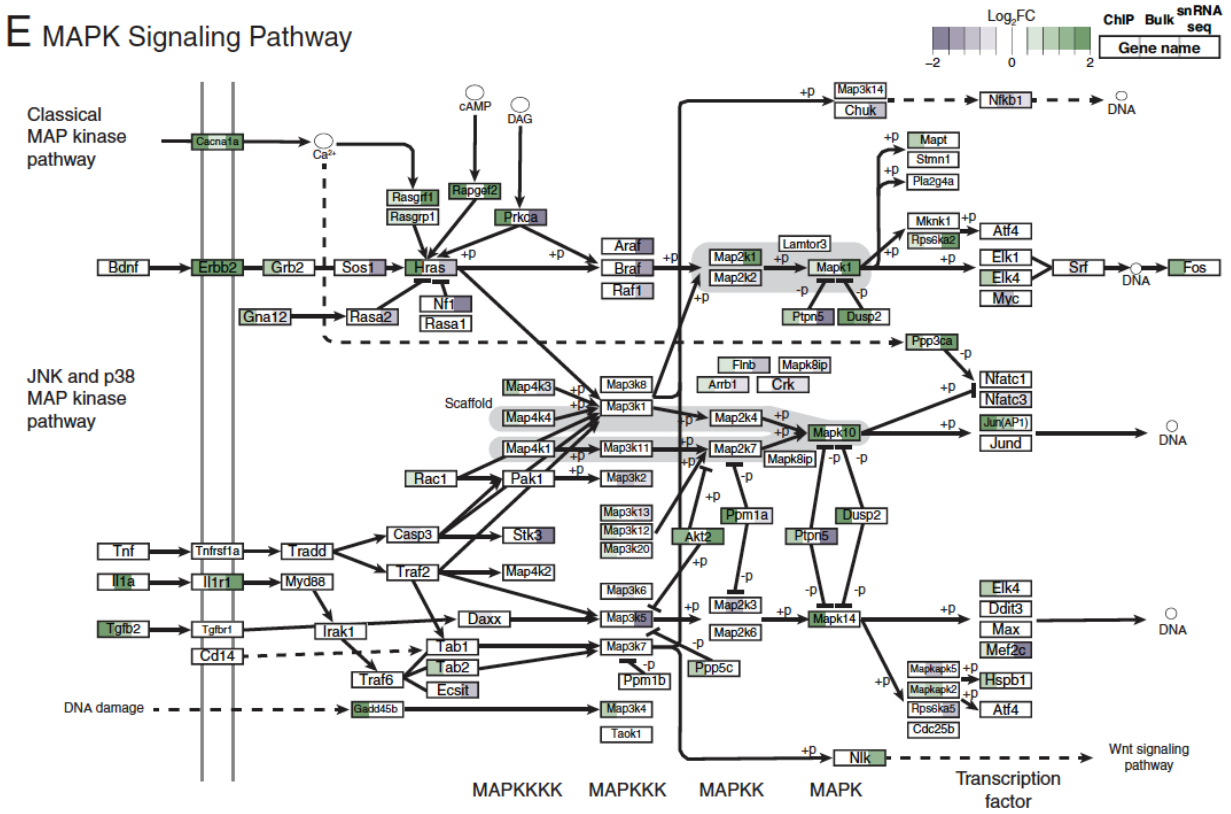
Cognitive epigenetic priming

ChIP with H3K27ac:





Cognitive epigenetic priming



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Cite
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Share
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2 MAPK, CREB and zif268 are all required for the consolidation of recognition memory.
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Cite
Philos Trans R Soc Lond B Biol Sci. 2003 Apr 29;358(1432):805-14. doi: 10.1098/rstb.2002.1224.
Share
PMID: 12740127 Free PMC article. Review.

3 Ras-related and MAPK signalling in neuronal plasticity and memory formation.
Mazzucchelli C, Brambilla R.
Cite
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Share
PMID: 11130460 Review.

The Potential of HDAC Inhibitors as Cognitive Enhancers

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

- **”VALPRO” (NCT0278913):**
  - HDACi valproic acid to support exposure therapy for arachnophobia
- **“VostatAD01” (NCT03056495):**
  - Determine tolerability of HDACi SAHA in patients with mild AD

Cognitive epigenetic priming

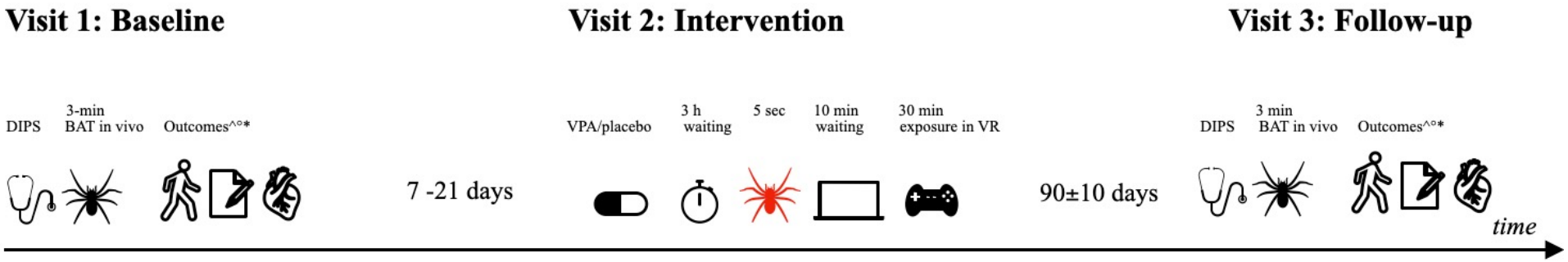
- **Can we test this in humans?**
  - Use of valproic acid (VPA) instead of CI-994
    - pan-HDAC inhibitor
    - FDA approved against bipolar disorder and epilepsy
  - Arachnophobia
  - Virtual reality setting

Dorothée Benz

Dominique de Quervain

Cognitive epigenetic priming

- **Experimental setting:**



DIPS=structured clinical interview (DSM-IV)

BAT=behavioral approach test

Outcomes included:

Objective measurements: SCL, skin conductance levels; startle reaction; heart rate

Subjective measurements: FSQ, fear of spiders questionnaire, SBQ, spider's belief questionnaire

= reactivation cue 

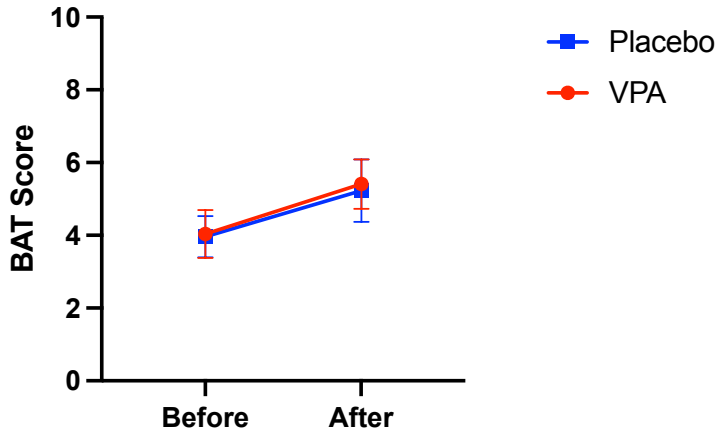
Schicktanz et al., under review

Cognitive epigenetic priming

- **Results:**

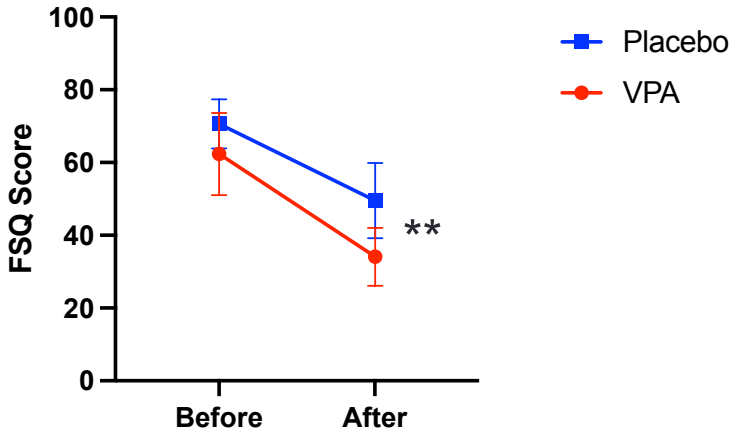
similar results for SBQ

Objective measurements – Approach behavior

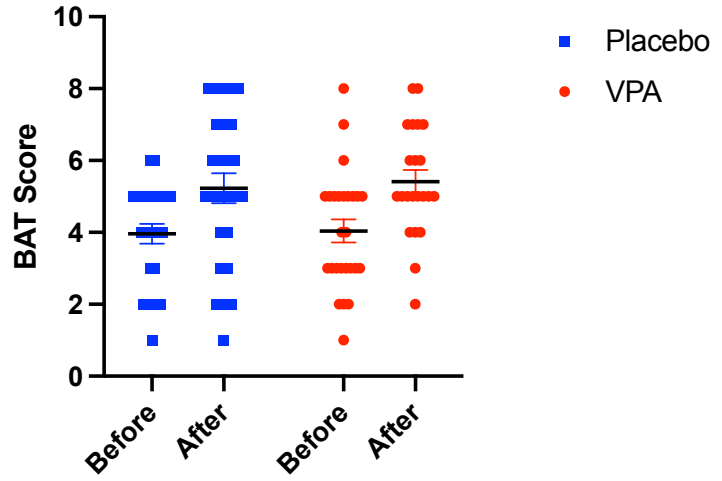


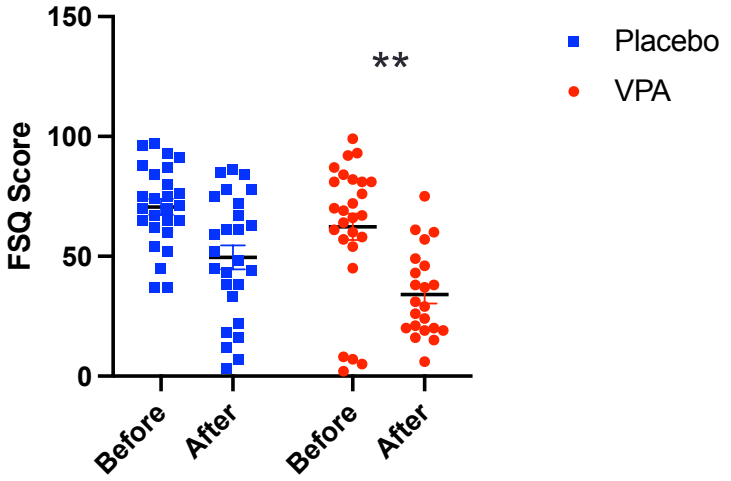
Cohen's d=0.23
p=0.46

Subjective measurements – Fear of spiders



Cohen's d=0.95
p=0.003





Schicktanz et al., under review

Cognitive epigenetic priming – to be continued

Kamilla
Woznika Miskowiak

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- “VostatAD01” (NCT03056495): ✓
  - Determine tolerability of HDACi SAHA in patients with mild AD
- **New grant to test HDACi SAHA in patients with major depressive disorder**