Identification of Proteins that are Differentially Activated by Drug Cue Memory Extinction and Reconsolidation using Phosphoproteomics

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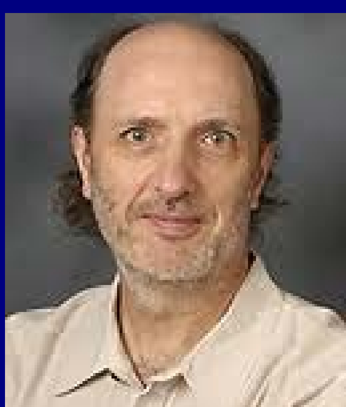
Thank You!

NIDA NATIONAL INSTITUTE ON DRUG ABUSE
The Science of Drug Abuse & Addiction

NIDA/Neuroproteomics Center Pilot Project Grant
K01DA031745
Future Support!

Jane Taylor
The Taylor Lab
Molecular Psychiatry at Yale



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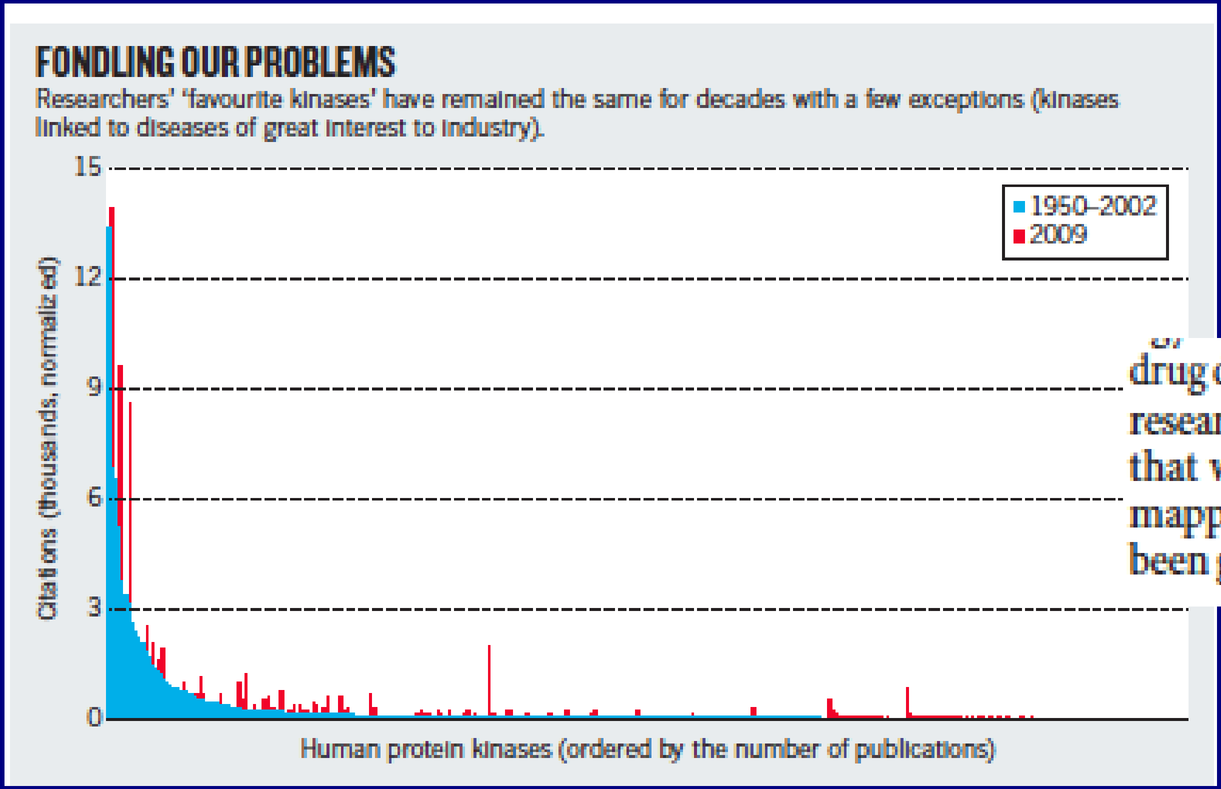
Lisa Chung

Too many roads not taken

Most protein research focuses on those known before the human genome was mapped. Work on the slew discovered since, urge **Aled M. Edwards** and his colleagues.

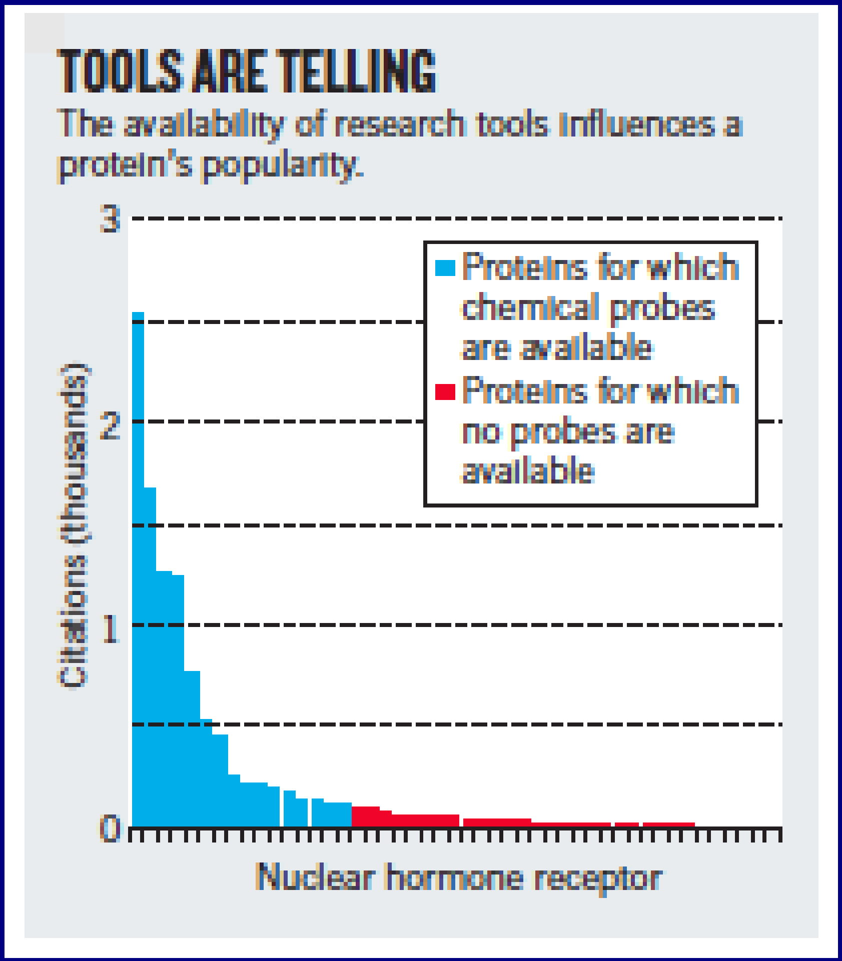


drug discovery. Yet more than 75% of protein research still focuses on the 10% of proteins that were known before the genome was mapped — even though many more have been genetically linked to disease.

Edwards et al., Comment, Nature, 2011

Too many roads not taken

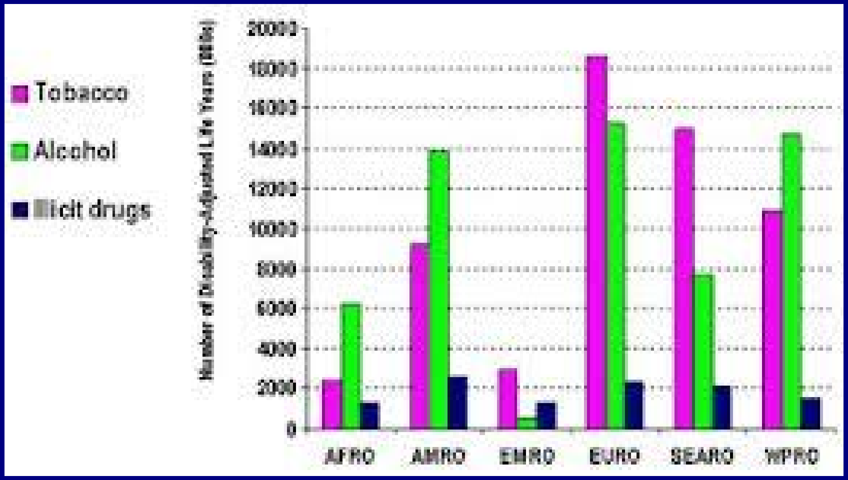
Most protein research focuses on those known before the human genome was mapped. Work on the slew discovered since, urge **Aled M. Edwards** and his colleagues.

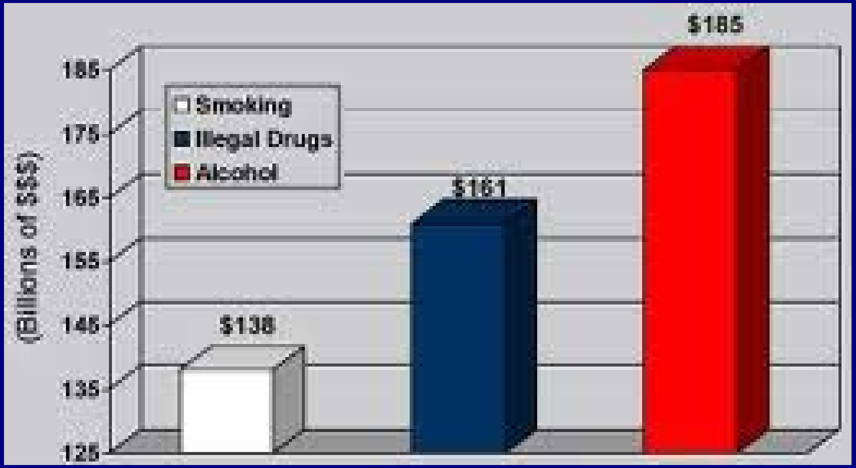


"Making protein-based research tools readily available must be a major objective in the decade to come."

Edwards et al., Comment, Nature, 2011

Drug Addiction





- Is a huge problem for the individual and for society.
- Most addicts go through many cycles of abstinence and relapse.
- Craving and relapse are driven by MEMORIES of drug use.

What memories?

People, Places, Things repeatedly associated with drug use

Can Drug-Associated Memories be Weakened to Reduce the Craving Response that Precipitates Relapse?

How can memories be manipulated?

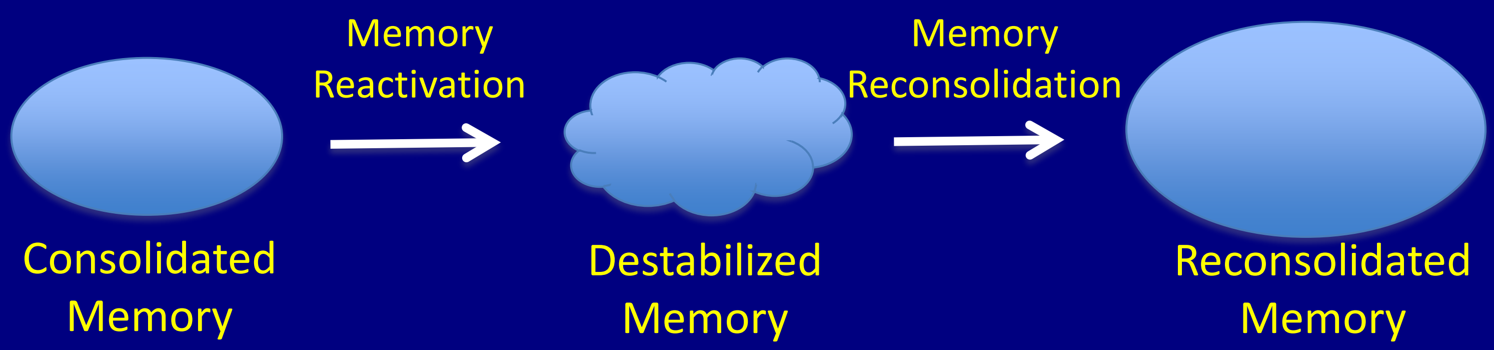
1. Disruption of Memory Reconsolidation
2. Extinction Training (Exposure Therapy)

What is reconsolidation?

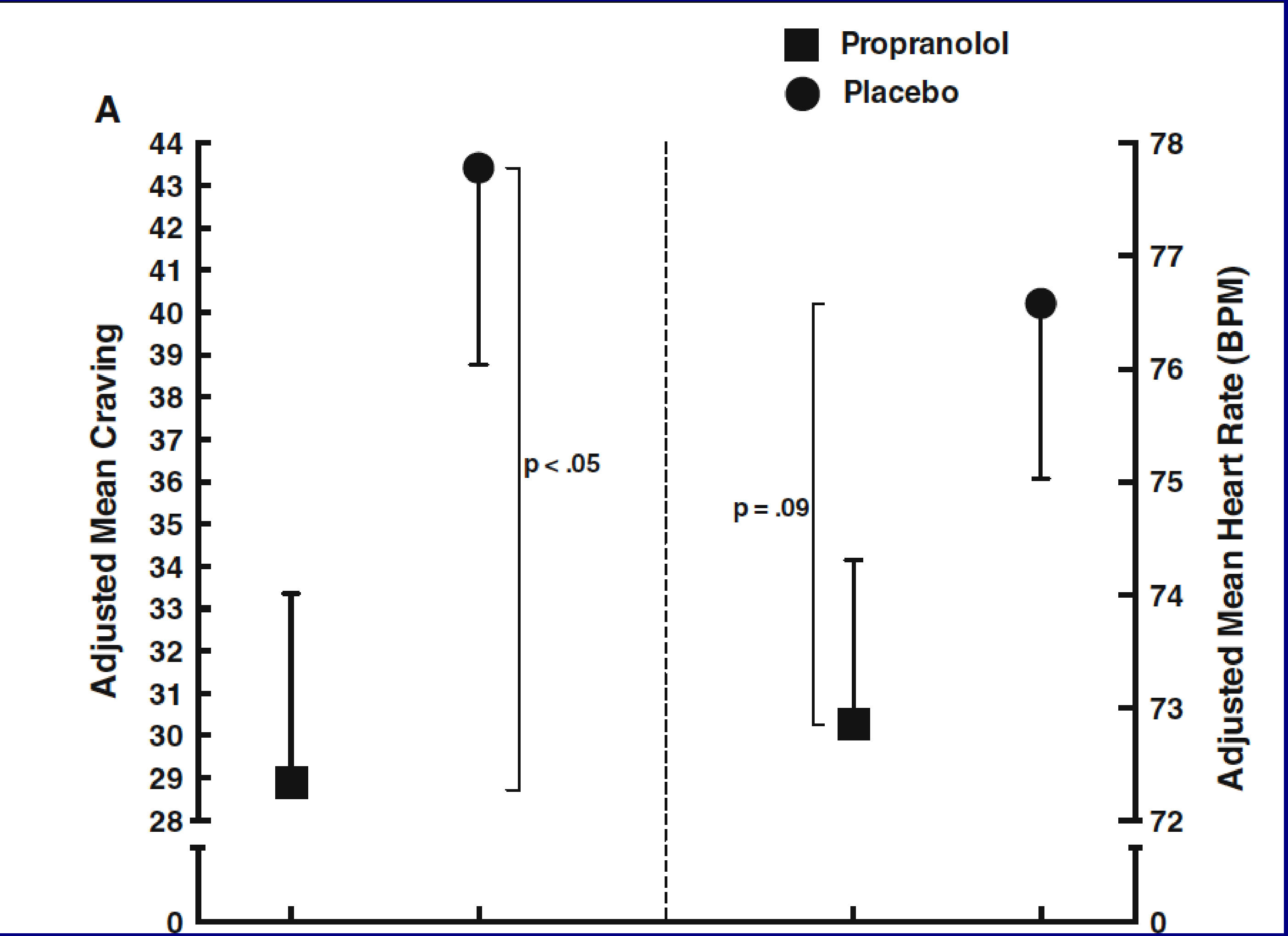
Process of restabilizing a memory into long-term storage after a reminder/destabilization event.

The purpose of reconsolidation is likely to allow weakening, strengthening, and/or incorporation of new information into a memory based on experience.

Reconsolidation can be interrupted during a brief period of time after memory reactivation with protein synthesis inhibitors, etc. to potentially "erase" the memory.



Disrupting Reconsolidation can Reduce Craving in Human Cocaine Addicts



Saladin et al., 2013

How can memories be manipulated?

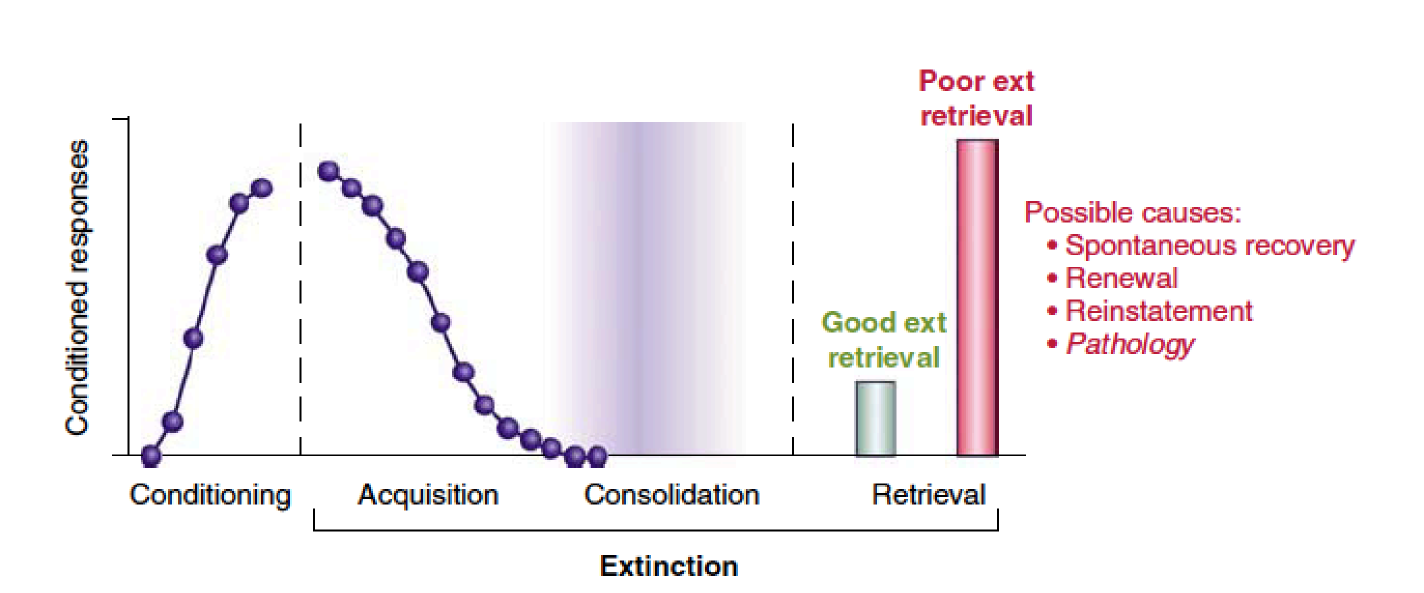
1. Disruption of Memory Reconsolidation
2. Extinction Training (Exposure Therapy)

Extinction

Learning that a cue no longer predicts reward by repeated cue presentation in the absence of the drug.

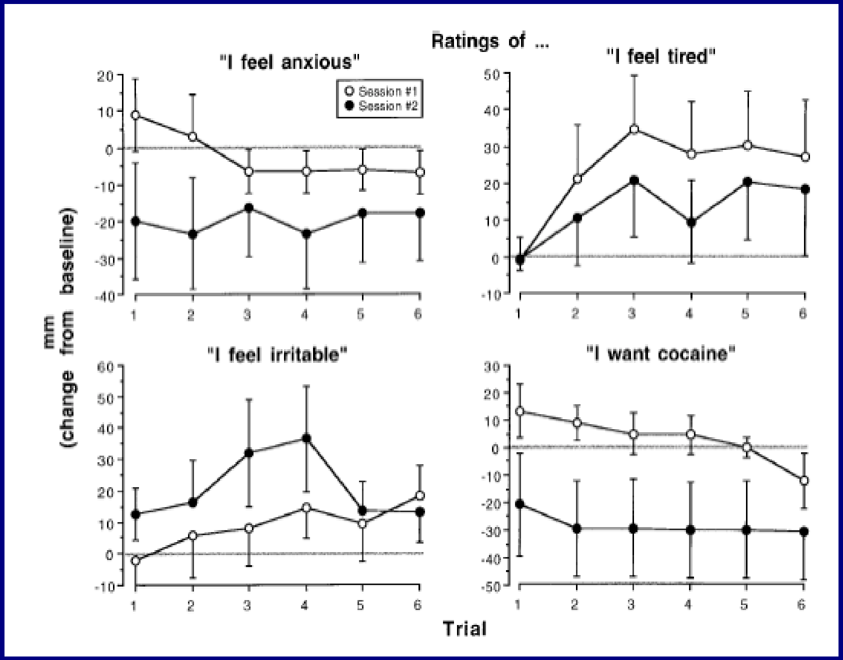
Formation of a new memory, not forgetting.

Extinction can be inhibited or enhanced with pharmacological manipulations.

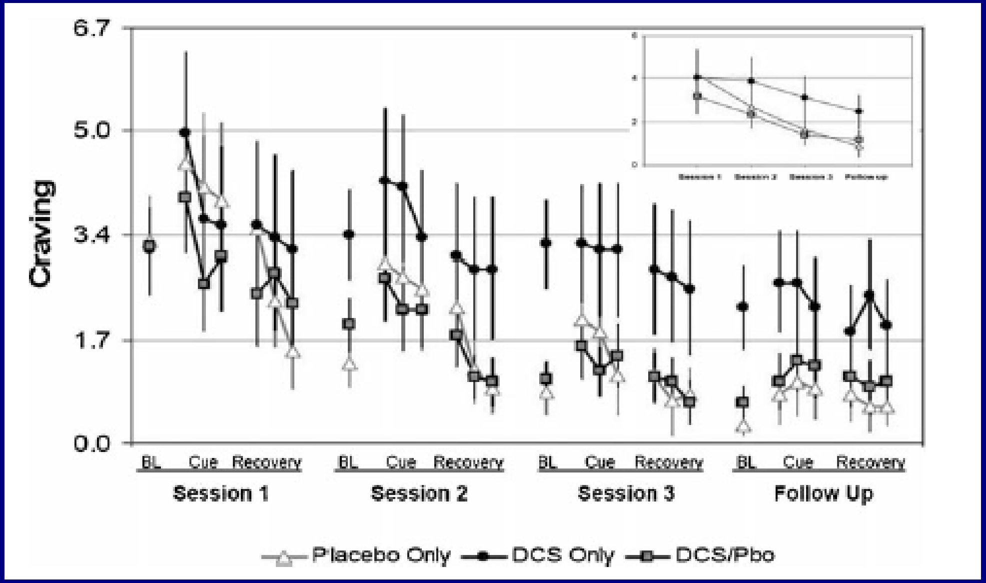


Quirk and Mueller, 2008

Craving can be Reduced in Human Cocaine Addicts with Extinction Training

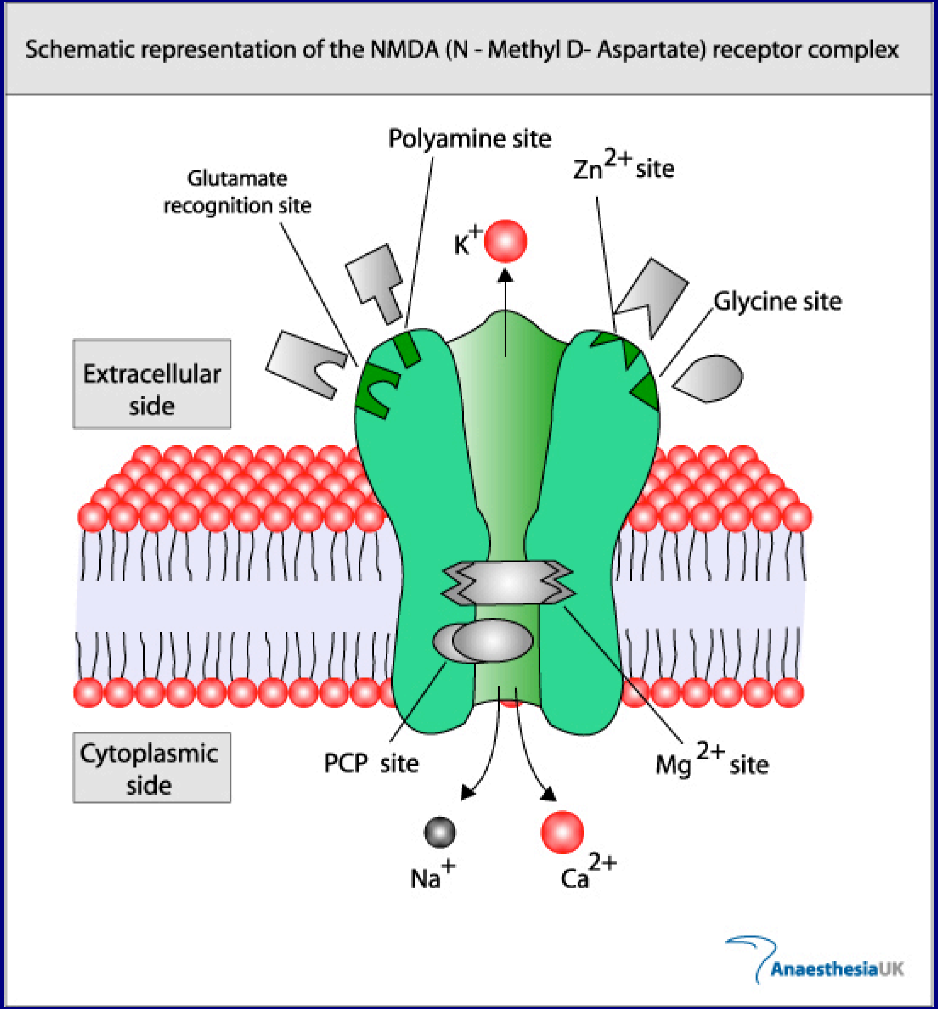


Foltin and Haney, 2000



Price et al., 2012

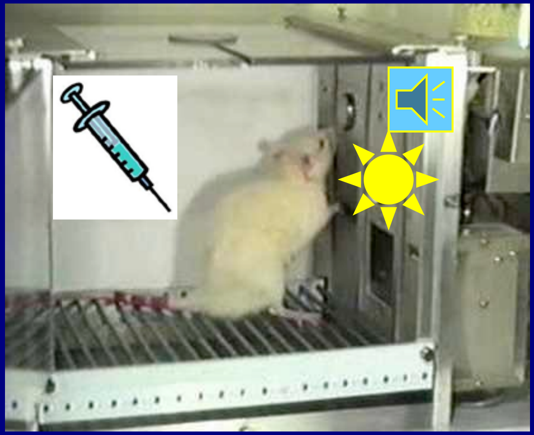
Can we enhance extinction to prevent relapse?



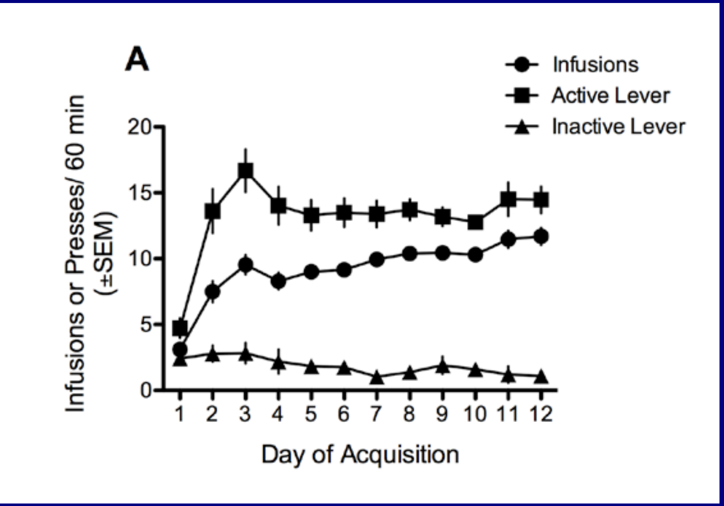
D-cycloserine (DCS)

-Partial agonist at the glycine site of the NMDA receptor.
-Increases efficiency of channel opening.
-Has shown efficacy as a "cognitive enhancer".

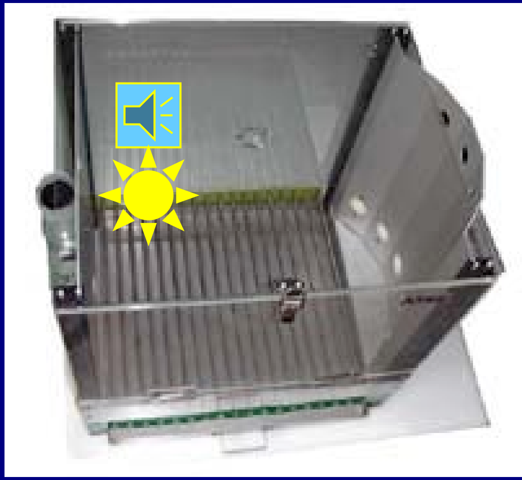
Can DCS enhance extinction learning to prevent relapse?



Training
Context A



120 cues
Cue Extinction
Context A or B

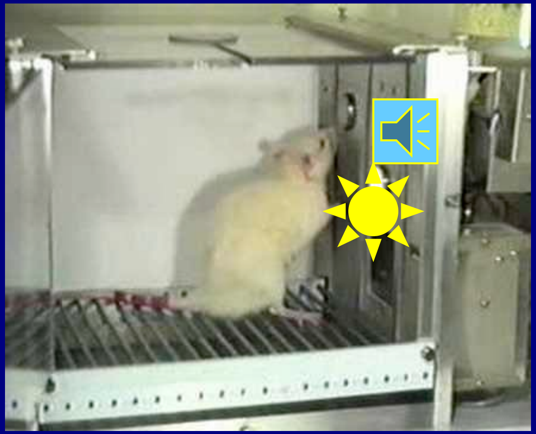


Vehicle

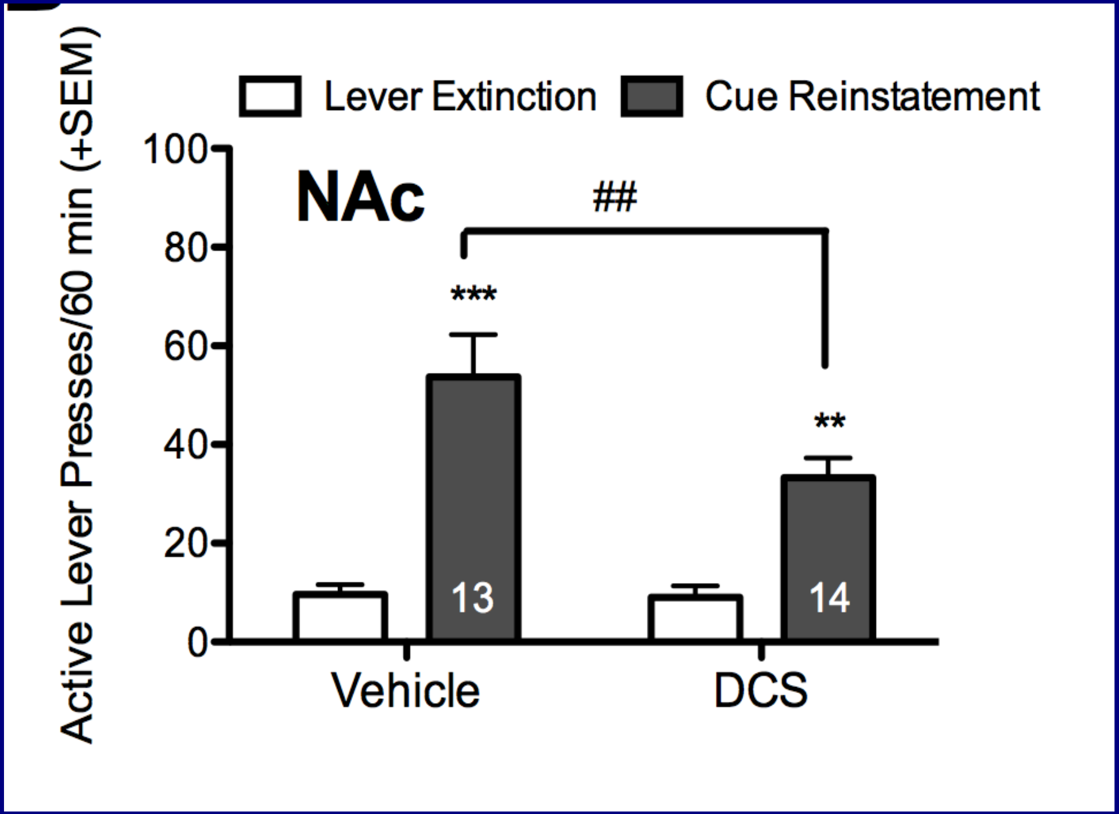
DCS

Torregrossa et al., 2010

DCS + Extinction Reduces Relapse in Rats

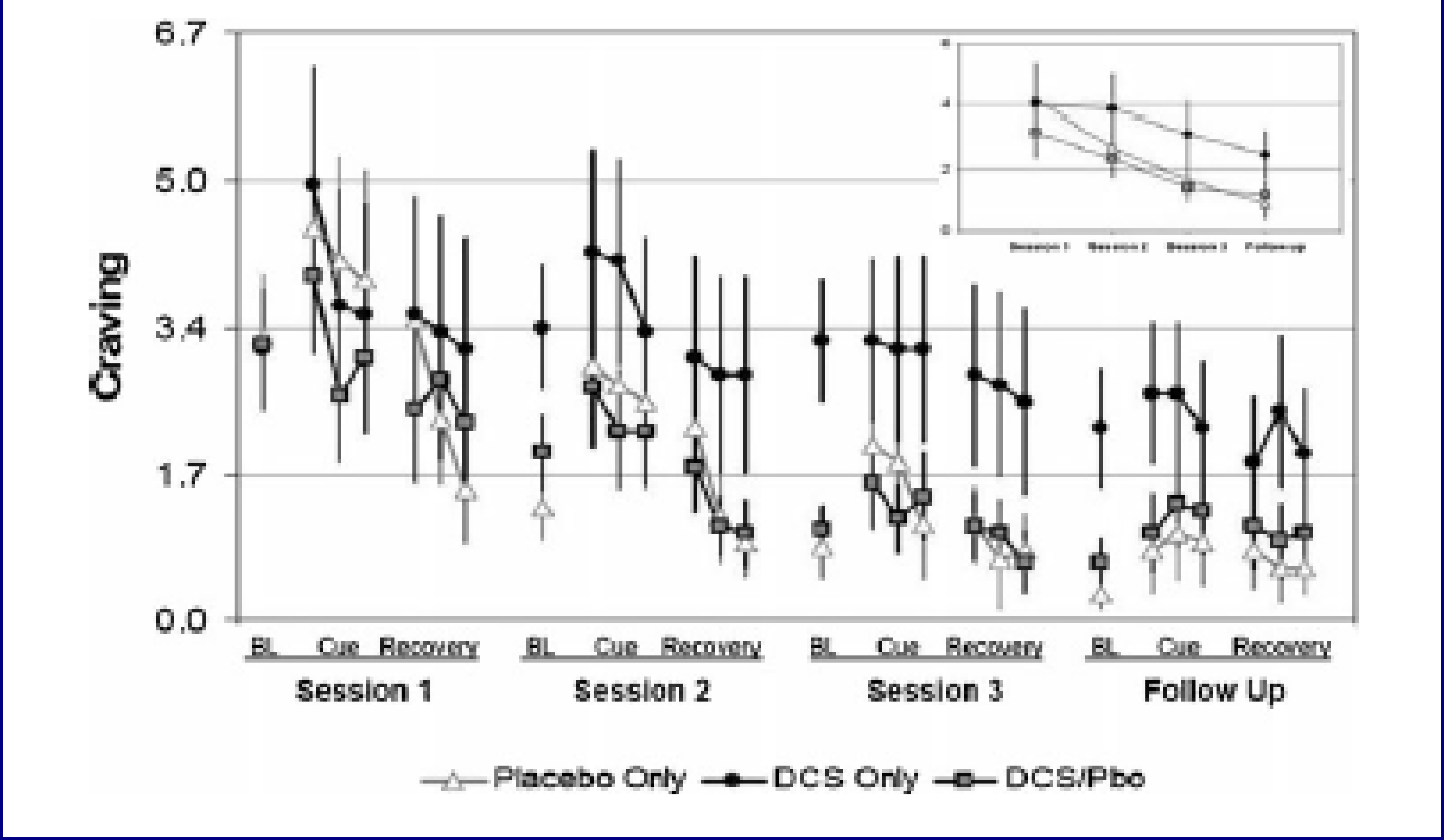


Cue-Induced Reinstatement
Testing
Context A



Torregrossa et al., 2010

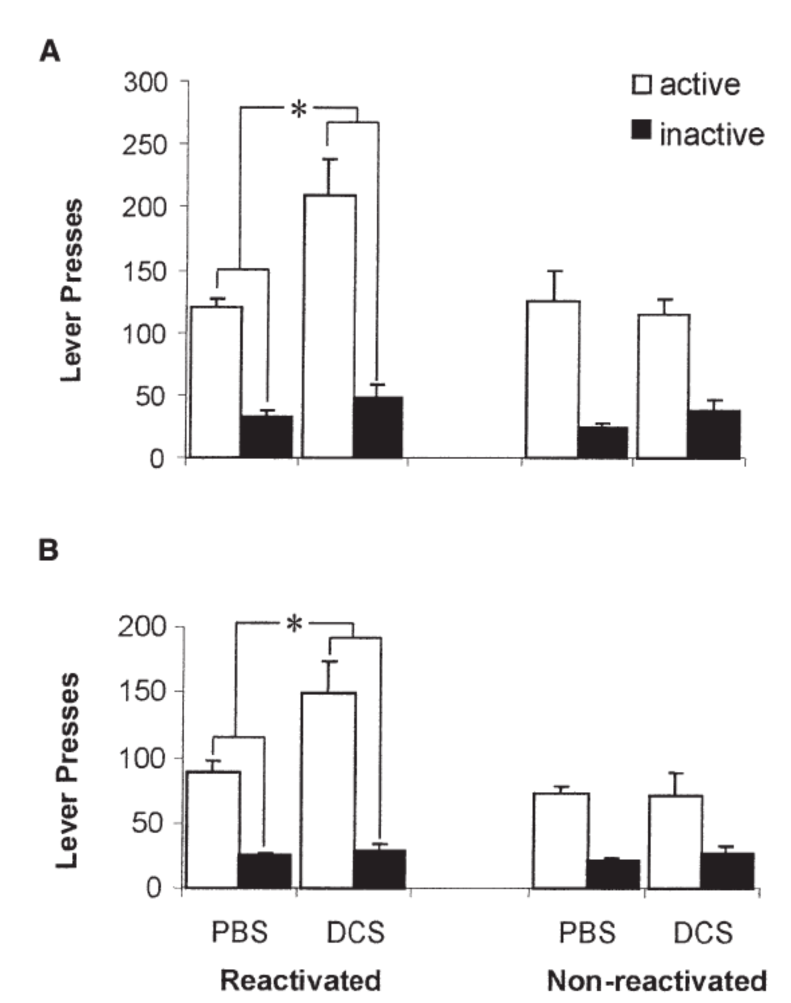
Clinical Efficacy of DCS?



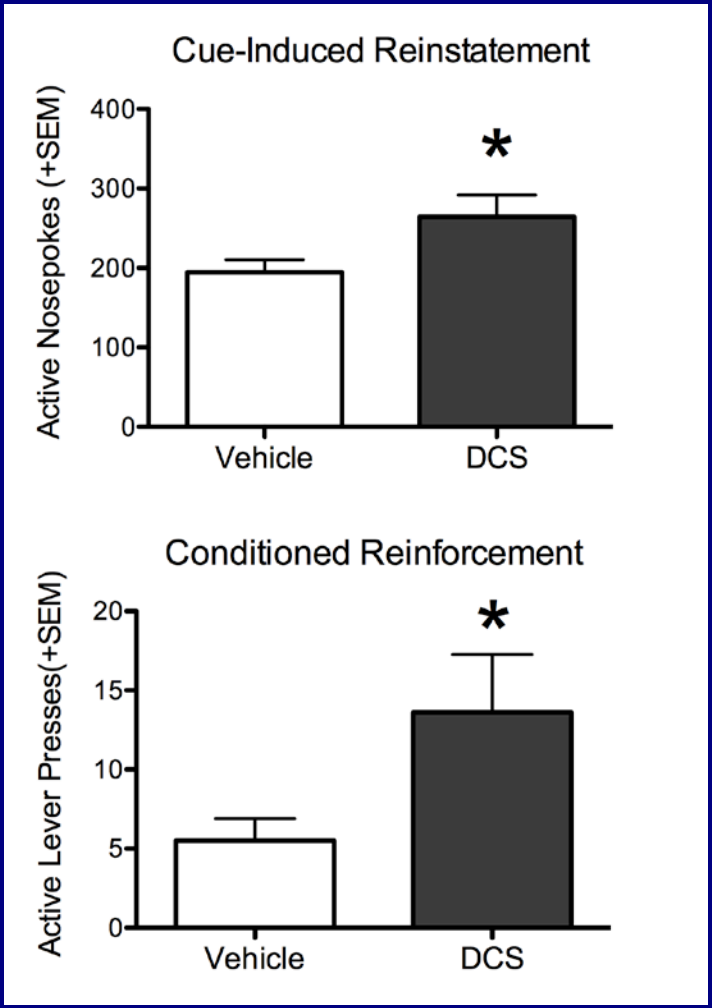
Price et al., 2012

Is DCS enhancing reconsolidation?

Cocaine-Intra LA DCS



Food-Systemic DCS



Lee et al., 2009

Summary

- DCS enhanced extinction training *might* be a viable treatment for addictive disorders.
- **<u>Unintentional enhancement of reconsolidation or inhibition of extinction may limit the use of these therapies.</u>**

Do extinction and reconsolidation processes engage different signaling cascades that can be selectively targeted for treatment?

Extinction and Reconsolidation are Distinct Processes. Is there selective signaling?

Selectively activated proteins?

Cue Exposure/Reminder

Consolidated Memory

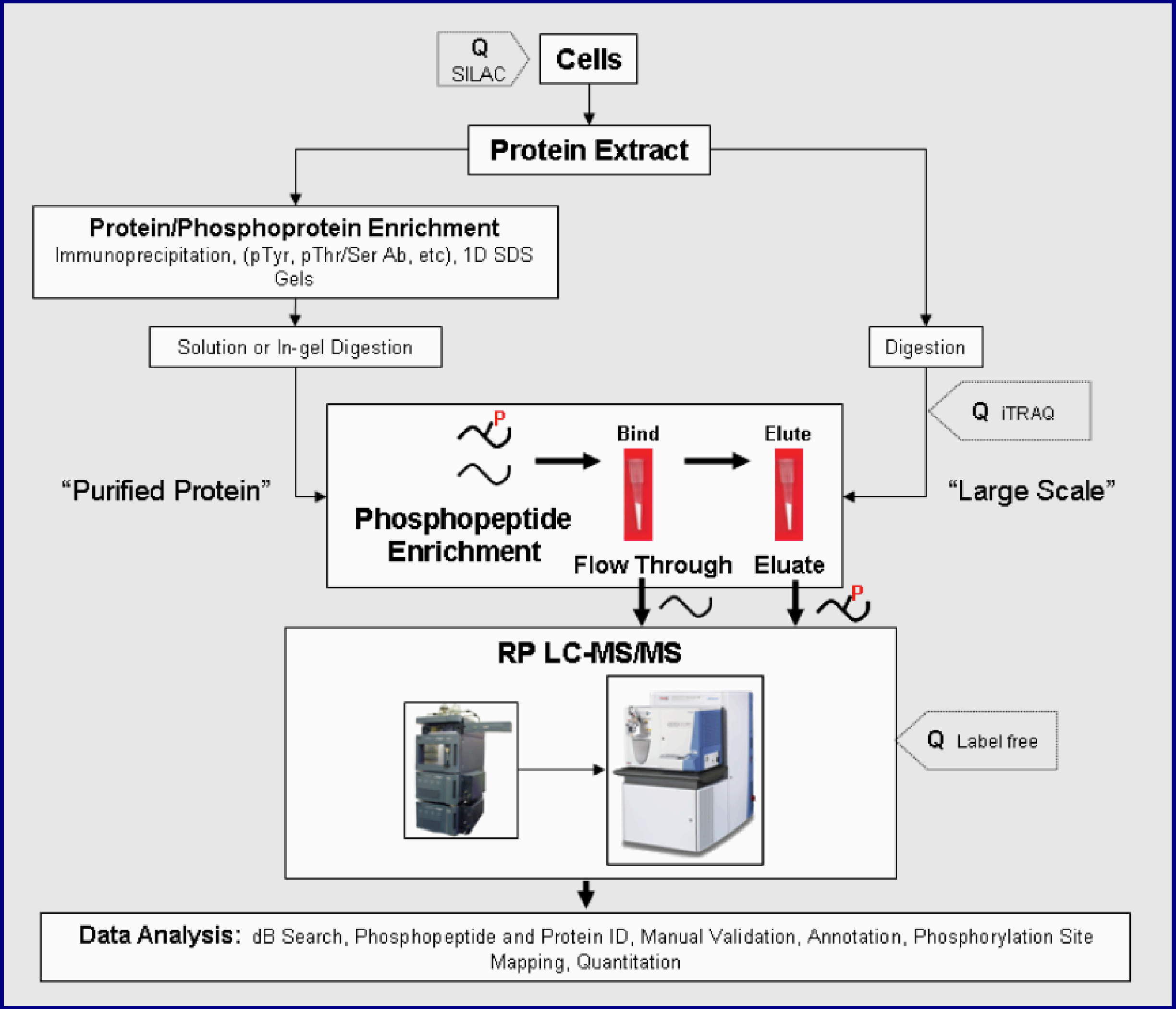
Reconsolidated Memory

NMDAR, Protein Synthesis, MAPK

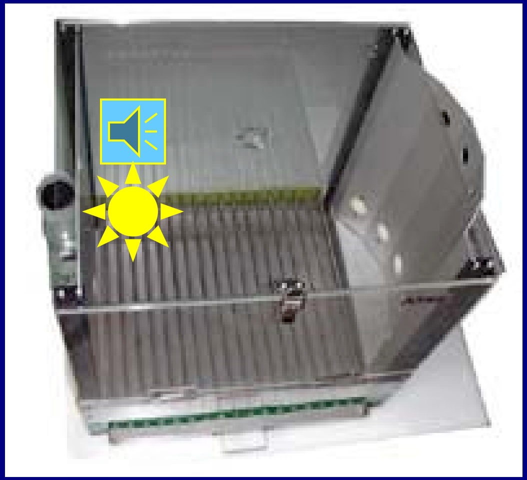
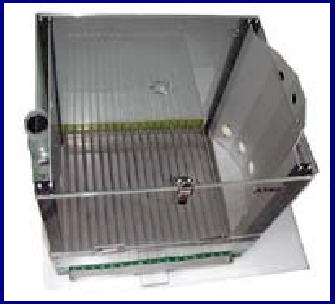
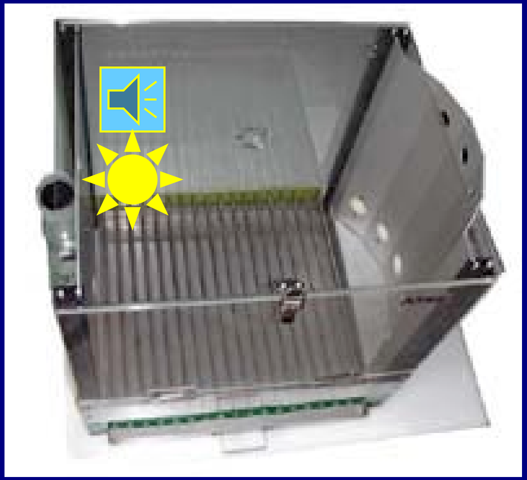
Consolidated Extinction Memory

Common proteins regulated in opposite directions?

Use of an unbiased, discovery based phospho-proteomics approach can answer this question.



Experimental Design-
Initial Analysis of Amygdala and NAc

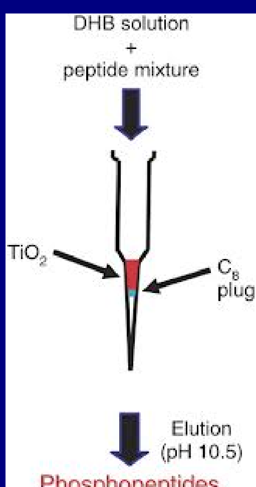
Extinction	Handled	Reconsolidation
		
120 cues	no cues	3 cues
15 min / 60 min	15 min / 60 min	15 min / 60 min

Experiment 1-Combined Discovery and Targeted Proteomics

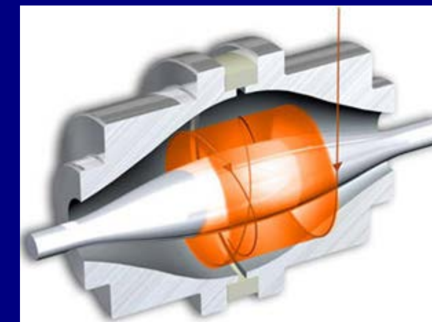
Amygdala Tissue

Homogenization

Trypsin Digestion



TiO2 Enrichment
For Phosphopeptides



Data- Identification of LOTS of Phosphopeptides

~80 Phosphopeptides Chosen for MRM

*Samples run in 3-4 biological replicates, technical triplicate, and randomized across a run.

Amygdala

Control vs Reconsolidation - Quantile Normalized Data			reconsolidation	extinction	reconsolidation	extinction
peptide	p-value	t-statistics	estimate (fold change)	estimate (fold change)	adjusted p-value	
GQGTASpPGSpVSDLAQTVK_2_ADCY9	0.038859	1.389705	0.648227861	-0.026508536	0.087433855	0.542336713
ALGSpLGGSpPSLPDQDK_2_AKA12	0.889197	-0.58847	-0.354114595	-0.728604712	0.941502441	0.37603572
MSpGFIYQGK_2_ARHG7	0.036311	1.449877	0.281387047	0.004766037	0.084334214	0.714548227
SSSpMAAGLER_2_BAIP2	0.827847	-0.01197	-0.005796648	0.159614436	0.907609271	0.904054138
SPQVLYSpPVSpPLSPHR_3_BSN	0.015052	1.32885	0.333062942	0.344514486	0.041683734	0.171275239
RASpLSDIGFGK_2_CKD18	0.630472	-0.44462	-0.091532919	-0.314974976	0.81060641	0.234533874
KVPLPGPGSpPEVK_2_CSKI1	0.011516	-3.05318	-0.530200503	-0.311931286	0.033166039	0.225642272
SRTSpVQTpEDDQLIAGQSAR_3_CTNA1	0.000115	3.045111	0.32137753	0.279900785	0.000922541	0.37603572
SRTpSpVQTpEDDQLIAGQSAR_3_CTNA1	0.320435	-0.06928	-0.013354601	0.017790466	0.490879072	0.176604658
RTSMGGTpQQQFVEGVR_3_CTNB1	0.769415	0.878366	0.327853731	-0.041940074	0.87933187	0.851396293
ALQSpPEHHIDPIYEDR_3_CTND2	0.761246	0.816507	0.196199704	0.134013352	0.87933187	0.98497389
MGQAGSTISpNSpHAQPFDFPDDNQNAK_3_CXA1	0.025981	1.201287	0.346684935	-0.70701096	0.064505726	0.29485909
M(ox)GQAGSTISpNSpHAQPFDFPDDNQNAK_3_CXA1	0.003377	1.774218	0.336513922	0.024905198	0.015614856	0.851396293
VAAGHELQPLAIVDQRPSSpRASpSpR_4_CXA1	2.20E-05	-3.62885	-0.513708235	-0.600709661	0.000264198	0.015915962
GAITpPPRSSpPANTCSPEVIHLK_3_DGKB	0.97889	0.3544	0.131541517	-0.154212696	0.984079829	0.376496616
ATAPQTQHVSpPMR_3_EF1D	0.718314	-0.37563	-0.231452459	-0.19488271	0.876586999	0.858091114
RNSpLPQIPTLNLESR_3_FAK2	0.005428	1.861413	0.304240482	0.190479447	0.020402545	0.079655403
EPSpLHEIGEK_2_FGF12	0.010094	-1.5345	-1.070387842	-0.07655568	0.030896362	0.474472487
HPPTpPPDPSGGLPR_3_GABR1	0.834228	0.453124	0.080572534	0.150261381	0.907609271	0.29485909
RHPPTpPPDPSGGLPR_3_GABR1	0.709161	-0.21476	-0.045196749	0.11188989	0.876586999	0.312781078
DPIEDINSpPEHIQR_3_GABR2	0.116979	-1.034	-0.220266953	0.178588033	0.221643602	0.225642272
HGSpGAESDYENTQSGEPLLGLEGK_3_GIT1	0.370495	0.173512	0.030246989	-0.364028497	0.544400453	0.023629321
NQSDLDDQHDYDSpVASpDEDTDQEPLPSAGATR_3_GIT1	0.004826	2.417925	0.219123269	0.101313164	0.019409008	0.436486686
LLYLTpSpAK_2_IF3M	0.741221	0.343621	0.111051457	0.234178396	0.87933187	0.694389226
EQESpSpGEEDNDLSPEER_2_IPP2	0.149267	1.075641	0.317019172	-0.090560445	0.268680111	0.98497389
ESSESTpNTpTpIEDEDTK_2_KCC2A	0.003356	1.770549	0.337970127	-0.062724393	0.015614856	0.905913219
ESSpESTNTTIEDEDTK_2_KCC2A	0.2349	-0.60106	-0.526570052	0.382570416	0.383886813	0.068487
HGTSpPVGDHGSLVR_3_KCNQ2	0.193721	-1.43755	-1.474816259	-0.289128475	0.332093063	0.29485909
HPPVLTpPPDQEVIR_3_KPCB	0.239929	-0.57604	-0.191986694	-0.172580532	0.383886813	0.376496616
TpFCGTPDYIAPEIIAYQPYGK_3_KPCG	0.001082	-2.71919	-0.444949233	-0.191582703	0.007792665	0.29485909
AAPALTpPPDR_2_KPCG	0.362738	0.406306	0.281360895	0.241375773	0.544106592	0.714548227
EAAEAEPAEPGSpPSAETEGASASSTSSPK_3_MARCS	6.36E-05	1.369588	0.264571014	0.213940773	0.000571986	0.066586091
GEAAAERPGEAAVASpSPSK_3_MARCS	0.26463	-1.21256	-1.966776807	0.214677334	0.414203342	0.562103314
DQQNLPFGVTPASpPSGHSQGR_3_MARK2	2.80E-06	2.070381	0.215736996	0.278576869	0.000100898	0.070777239
SDSVLPASHGHLPQAGSpLER_3_MINK1	0.084032	1.270319	0.161494282	0.119590006	0.168063454	0.088062766
VADPDHDHTGFLTpEYpVATR_3_MK01	0.065389	0.967874	0.156326135	0.241733186	0.142667808	0.225642272
TAGTSFMMTpPYpVVTR_2_MK10	0.017728	1.061559	0.521376506	-0.245675722	0.045585546	0.376496616
EIEDLSQSQSpPESpETDYPVSTDTR_3_NBEA	0.008589	2.6279	0.45369318	0.080642778	0.02944804	0.491829211
TPLENVPGNLSpPIKDPDR_3_NBEA	0.004852	-1.53832	-0.290581826	-0.042362861	0.019409008	0.29485909

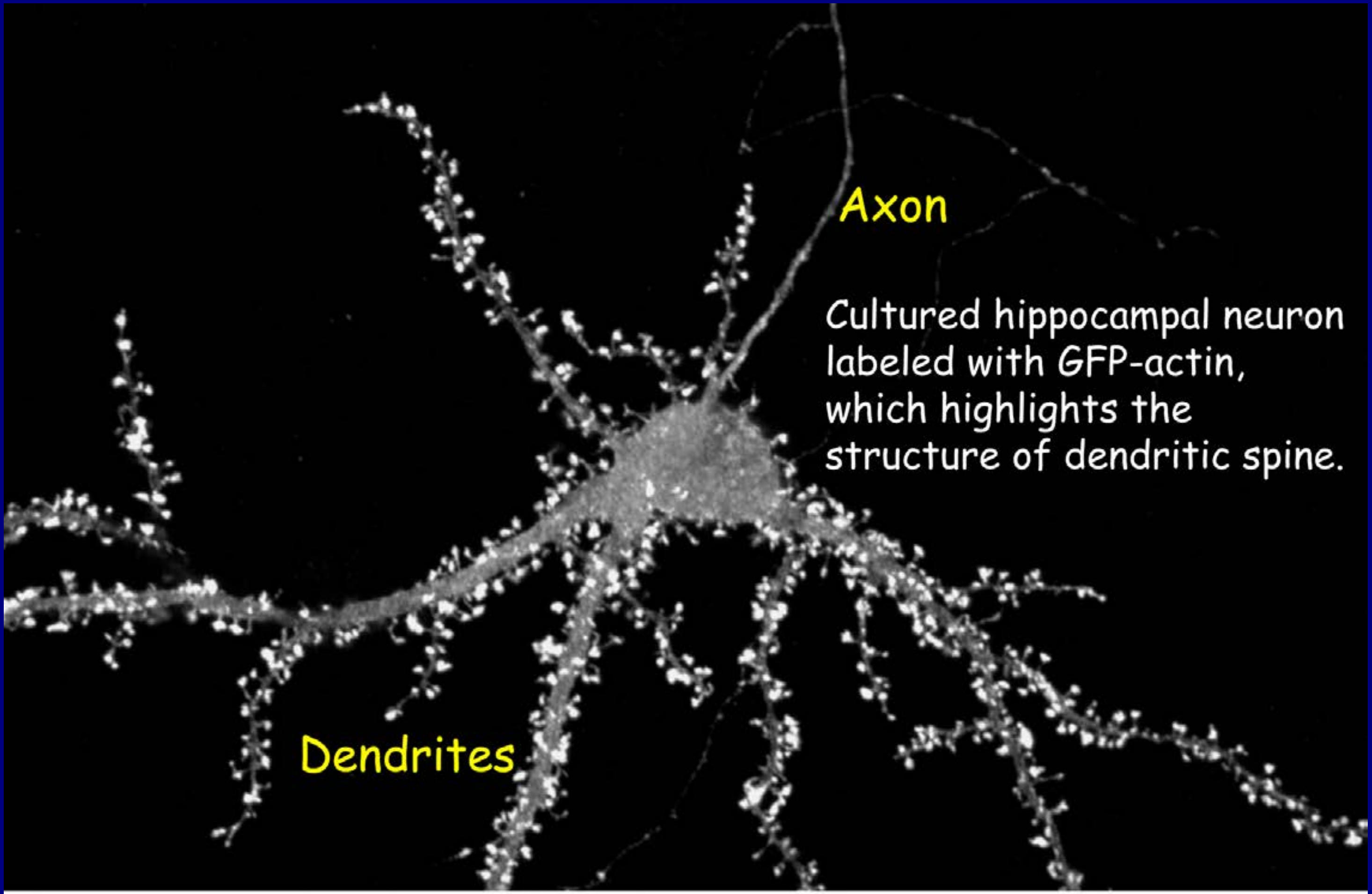
Amygdala

DESpKEPIVEVR_2_NCAM1	0.711619	-0.52227	-0.099182535	-0.04971831	0.876586999	0.98497389
ITNHEDGSpPVNEPNETTpPLTEPEK_3_NCAM2	0.535424	-0.65166	-0.382143766	0.485815142	0.700918754	0.562103314
ITNHEDGSpPVNEPNETTPLTEPEK_3_NCAM2	0.98408	-0.98209	-0.177303651	0.027379192	0.984079829	0.29485909
HSQLSDLYpGK_2_NMDE2	0.965472	0.412792	0.150039842	0.241333808	0.984079829	0.98497389
TVSETPAVPPVSpEDEDDDDDATPPPVIAPRPEHTK_4_PAK1	0.445302	-1.61765	-0.243896292	0.005494375	0.616572448	0.905913219
QPSpEEEIIK_2_PEA15	0.844581	-0.02027	-0.00952966	0.104568663	0.907609271	0.904054138
SEPSSPDHGSSpAIEQDLAALDAEMTQK_3_PLCB1	0.408275	0.110661	0.029935485	-0.041244436	0.587915661	0.29485909
VNLKSpPSpSEEVQGENAGR_3_PLCB1	0.504619	0.534331	0.212926058	0.213523596	0.685519724	0.402960896
HGGGIVADLSpQQSpLK_3_PP1R7	0.001316	1.737971	0.399635832	0.046268081	0.008611547	0.29485909
IAESHLQTISNLSENQASpEEEDELGELR_3_PPR1B	0.214972	-0.31651	-0.226399689	-0.130547376	0.359953208	0.402960896
LPEEGGSSpRAEDSSpEGHEEEVLGGHGEK_4_PTPRN	0.001851	0.793738	0.262578122	0.085755261	0.010250362	0.29485909
KEESpEESpEDDM(ox)GFGLFD_2_RLA1	1.59E-06	2.656804	0.390772359	0.321142528	0.000100898	0.078768835
KEESpEESpDDDM(ox)GFGLFD_2_RLA2	6.15E-06	2.220685	0.281136814	0.186899489	0.000147488	0.078768835
WHQLQNENHVSSpD_2_RP3A	0.417726	-0.39668	-0.300291619	-0.527639278	0.589730388	0.621854035
RFSSpPHQSpLLSIR_3_SCN2A	1.68E-05	2.851437	0.542315906	0.173769084	0.000242454	0.474472487
RAPSpPVVSpPTELSK_2_SHAN2	0.017508	0.865856	0.234808694	0.280744562	0.045585546	0.272281607
SRSpPSpPSpPLPSPSPGSGPSAGPR_3_SHAN3	0.033462	2.202769	0.680656804	0.259423581	0.080309743	0.402960896
SESpMGSpLLCDEGSK_2_SRBS2	0.00347	0.728472	0.29307922	0.200259148	0.015614856	0.130802815
DSGSSSVFAESpPGGK_2_SRCN1	0.009407	1.914563	0.285008731	0.112168902	0.030785901	0.225642272
RGSpDELTVPR_2_SRCN1	0.010299	-1.59292	-0.26959234	-0.127616046	0.030896362	0.59290364
SSpGATpPVSGPPPPAVSSTPAGQPTAVSR_3_SRCN1	0.959747	0.974992	0.379683678	0.296371902	0.984079829	0.562103314
RFSpNVGLVHTSER_3_SRCN1	3.07E-05	-1.13815	-0.191466727	-0.071702251	0.000315554	0.020474862
KAESpEELEIQKPQVK_3_SRCN1	0.167963	-1.68027	-0.302232301	-0.064685566	0.294959395	0.904054138
ESVPEFPLSpPPK_2_STMN1	0.530299	1.067928	0.208092142	-0.018643857	0.700918754	0.627488831
RASpGQAFELILSpPR_3_STMN1	0.001482	1.064474	0.374916615	0.140422927	0.008893004	0.280061557
DLSpLEEIQK_2_STMN1	0.796541	-0.0663	-0.017869129	-0.012350774	0.896109123	0.621854035
HSAILASpPNPDEK_2_STX1A	0.101069	-1.71718	-0.272301184	-0.235311287	0.196675157	0.29485909
TAKDSpDDDDDVTVTVDR_3_STX1A	0.751154	2.143851	0.19628734	0.299550156	0.87933187	0.07399745
LHQVYpFDAPSCVK_3_SYPH	0.129579	-0.65614	-0.283111869	0.112975621	0.239223144	0.763448222
DQALKDDDAETGLTpDGEEK_3_SYT1	0.082807	-2.02264	-0.249764451	0.149295803	0.168063454	0.630210458
SEGSpPVLPHEPSK_3_TNIK	1.09E-05	-3.86966	-0.620596609	-0.093113015	0.000196935	0.434162457
GGAPLPPSGSpK_2_VIAAT	0.081918	-1.50795	-0.704217797	-0.1676	0.168063454	0.781796861

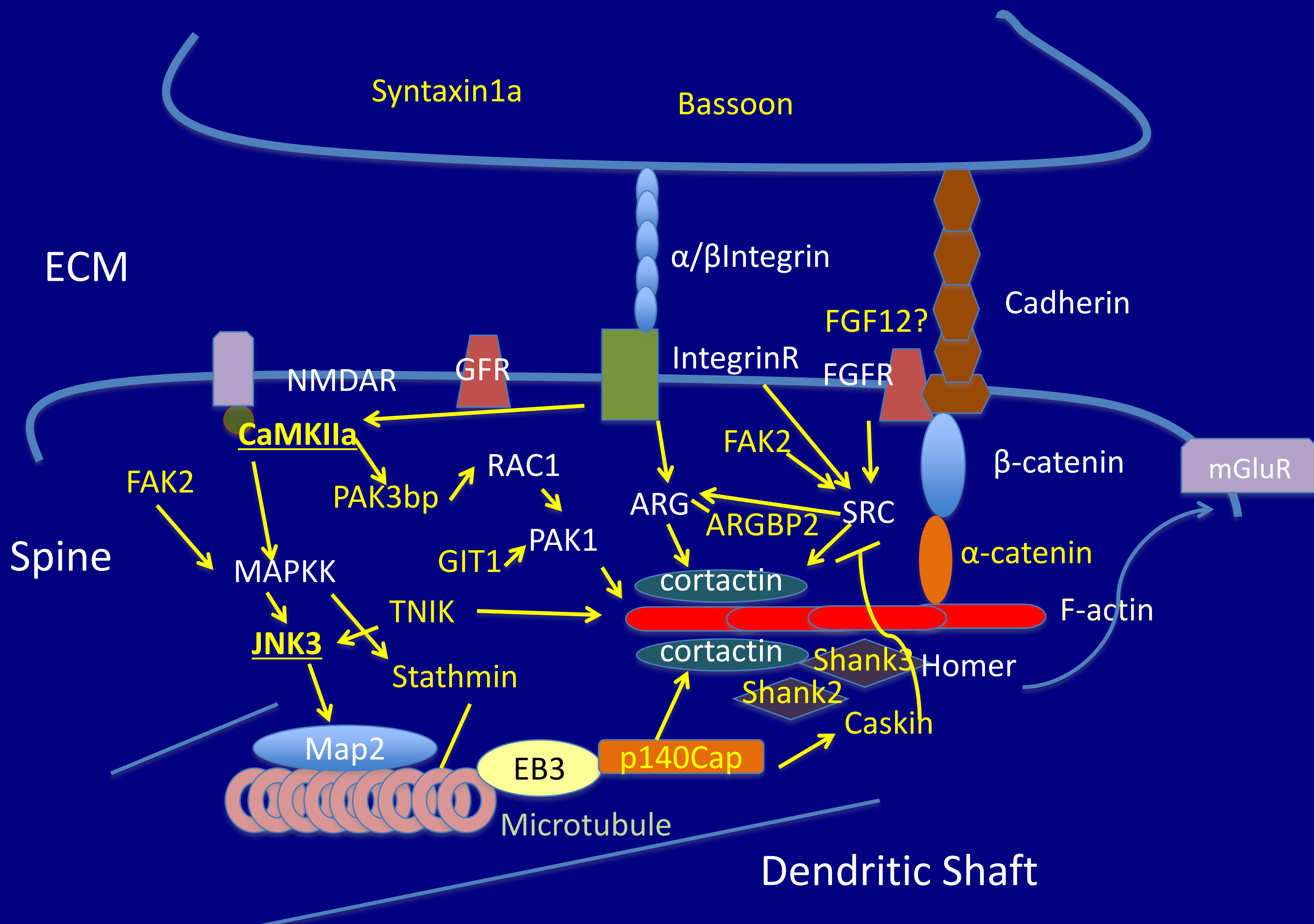
Amygdala Analysis Summary

Analysis of Amygdala Phosphoprotein Regulation in Response to Extinction vs. Reconsolidation		
	Number of Phosphopeptides	Examples
Differential Regulation	6	MK10, KCC2A*, CXA1*
Same Regulation	16	BSN, FAK2, RLA1, RLA2
Reconsolidation Only	21	CTNA1, FGF12, KPCG
Extinction Only	3	GIT1, KCC2A*, STX1A
No Regulation	37	AKA12, DGKB, IF3M
	*Two different peptides from the same protein	

Reconsolidation regulates proteins associated with dendritic spines



(By H.-F. Wang)



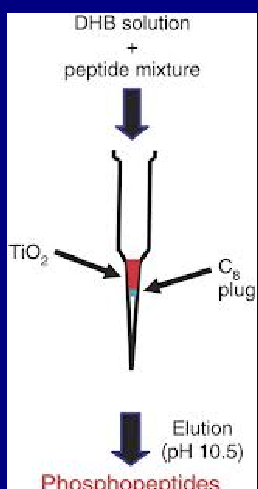
Syntaxin1a
Bassoon
ECM
α/βIntegrin
Cadherin
FGF12?
IntegrinR
FGFR
NMDAR
GFR
CaMKIIa
FAK2
RAC1
FAK2
β-catenin
mGluR
PAK3bp
ARG
SRC
ARGBP2
Spine
PAK1
GIT1
MAPKK
α-catenin
cortactin
TNIK
F-actin
JNK3
cortactin
Shank3
Homer
Stathmin
Shank2
Caskin
Map2
EB3
p140Cap
Microtubule
Dendritic Shaft

Experiment 2-Label Free Quantitation
Nucleus Accumbens

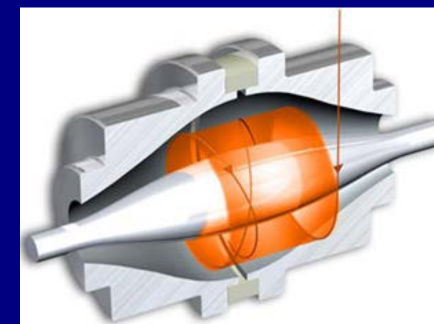
Nucleus Accumbens Tissue

Homogenization

Trypsin Digestion

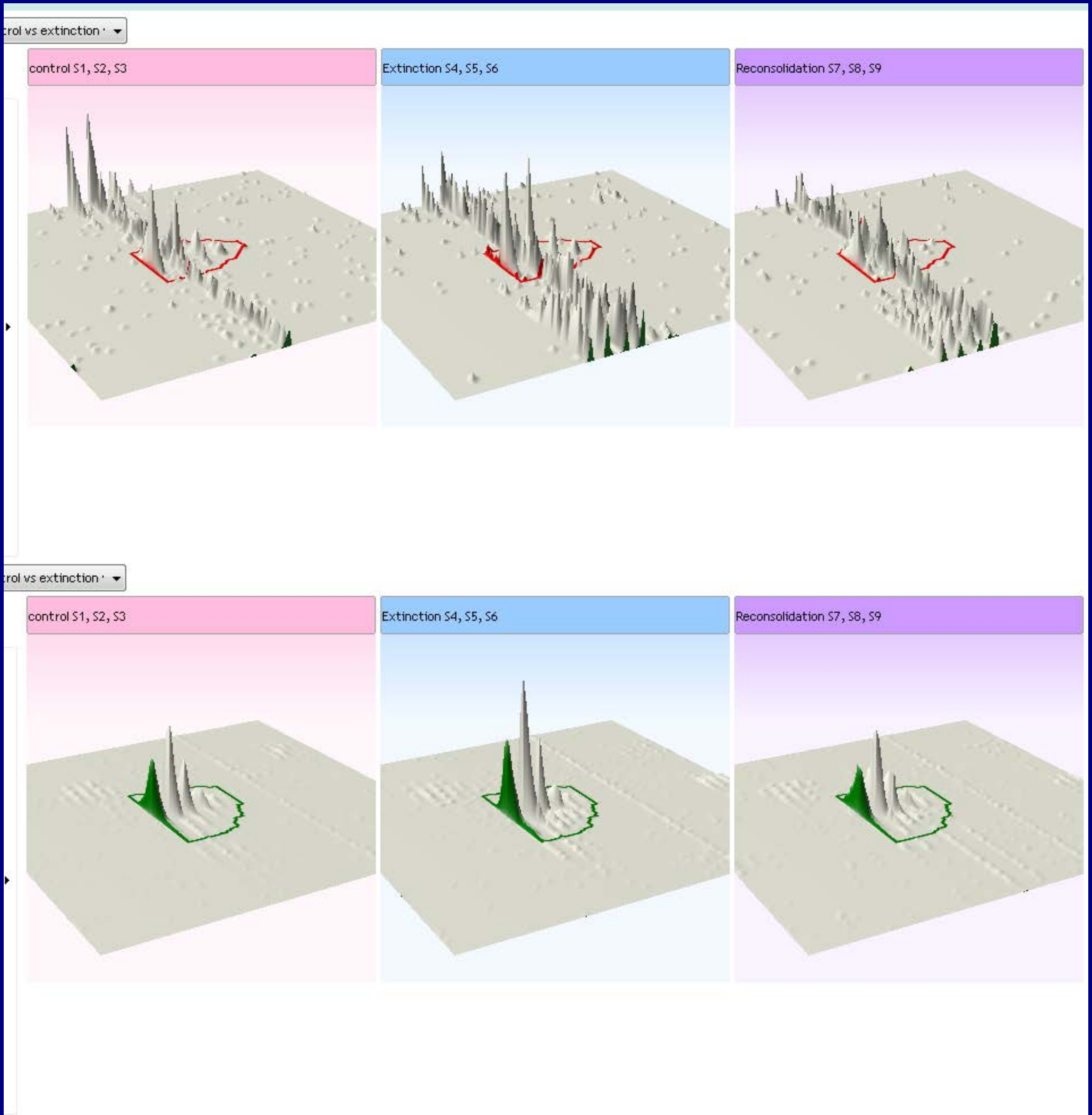


TiO2 Enrichment
For Phosphopeptides

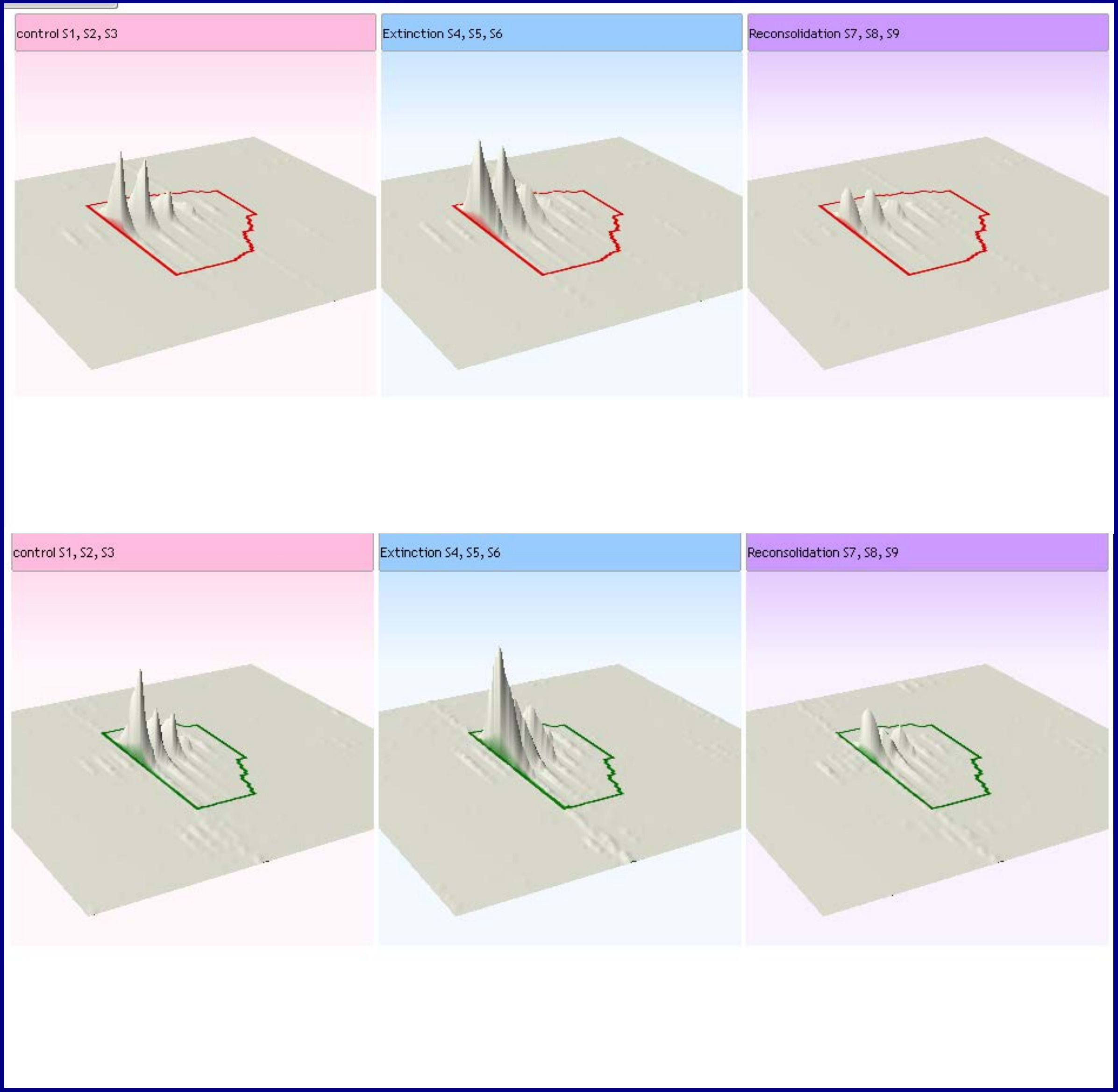


Data- Identification of LOTS of Phosphopeptides

pNCAM1-S784 AAFSKDESKEPIVEVR



pGAP43-S96 KEGDGSATTDAAPATSPK



Summary

- Discovery based, unbiased proteomics is an exciting method for identifying novel proteins involved in a process.
- Label free methods are particularly helpful in identifying PTMs involved in specific signaling cascades.
- Follow up targeted proteomics on "interesting" peptides are a valuable addition to the workflow.
- Once the proteomics analysis is complete, the hard work begins.