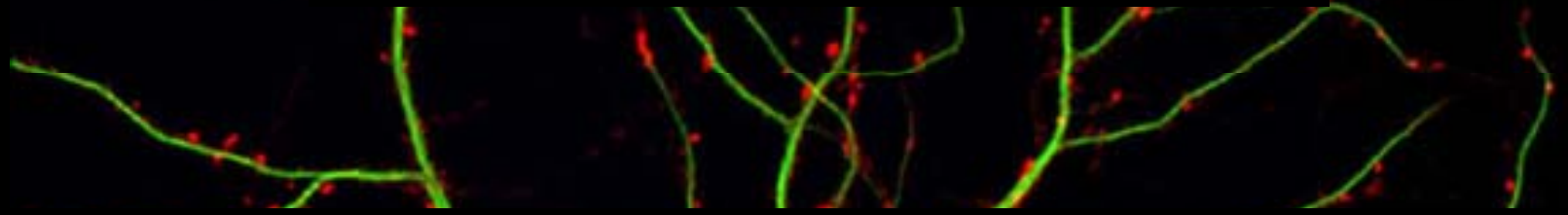
Regulation of Synaptic Structure and Function by Drugs of Abuse

Alexandre Stipanovich



1. Lfc: a molecular architect in spines
2. Identification of its phosphorylation sites
3. Regulation of the phosphorylation sites and putative function
4. New technique

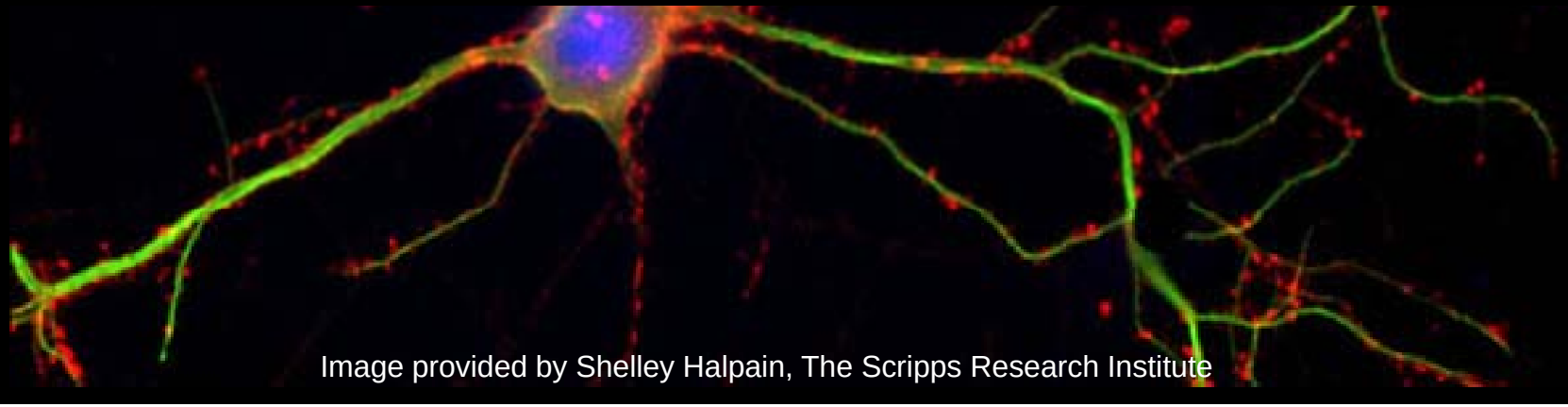
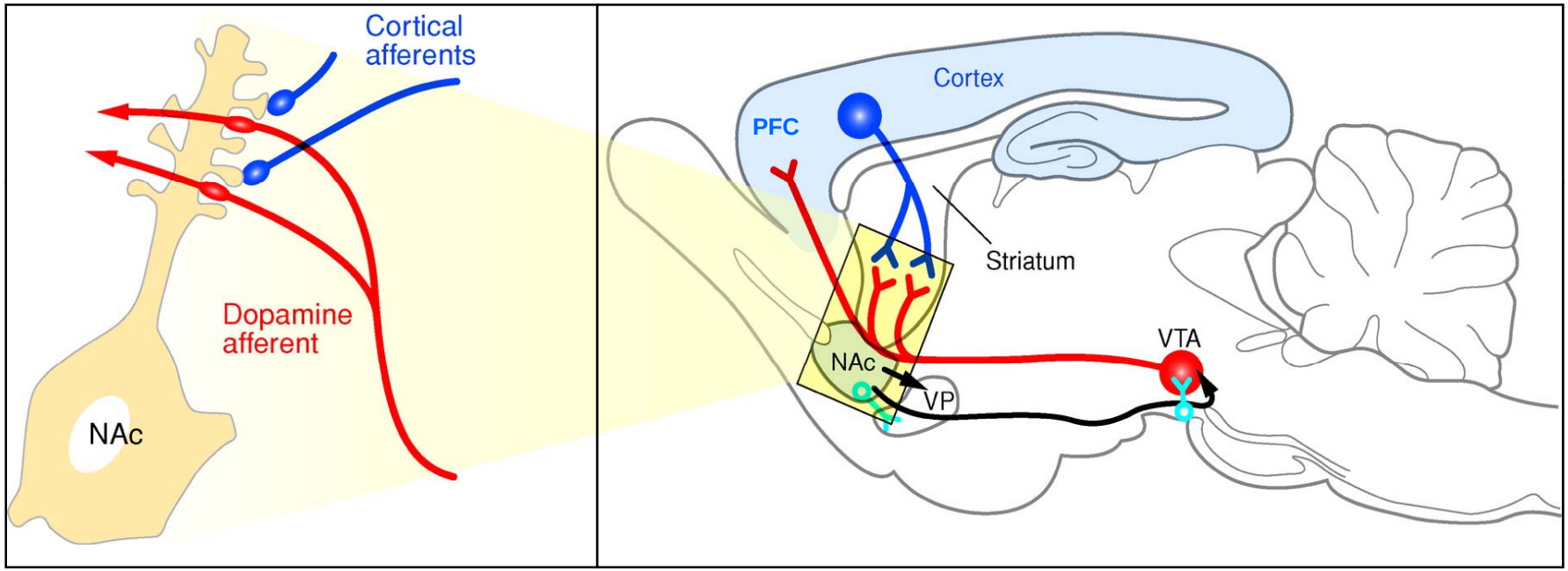


Image provided by Shelley Halpain, The Scripps Research Institute

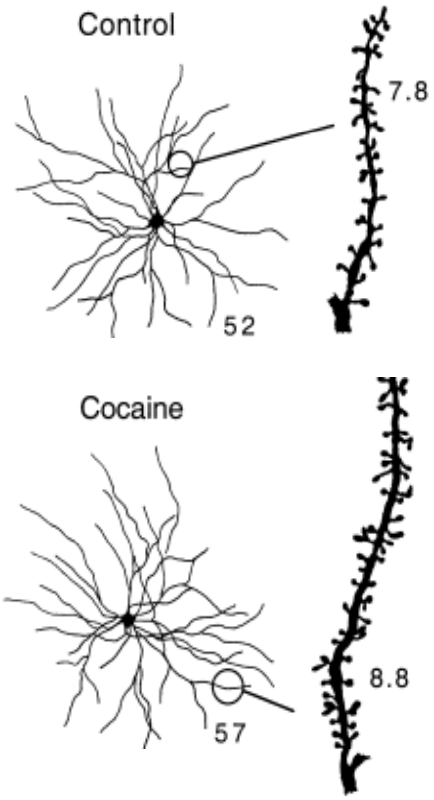
Dopaminergic inputs to striatum and prefrontal cortex



Drug Addiction and Neural (Structural) Plasticity

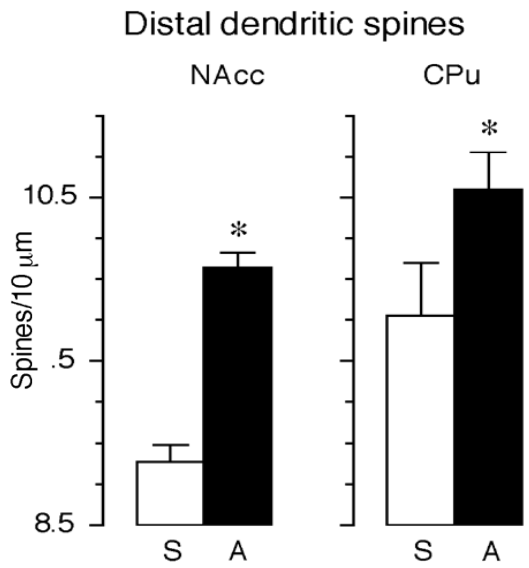
- Dendritic spine changes associated with chronic psychostimulant exposure
- These changes are persistent
- Mechanism by which this occurs not understood
  - role of Cdk5 and MEF2
- Functional role is not clear
  - may be part of a negative-feedback response to limit the effects of psychostimulants

Golgi-Cox staining

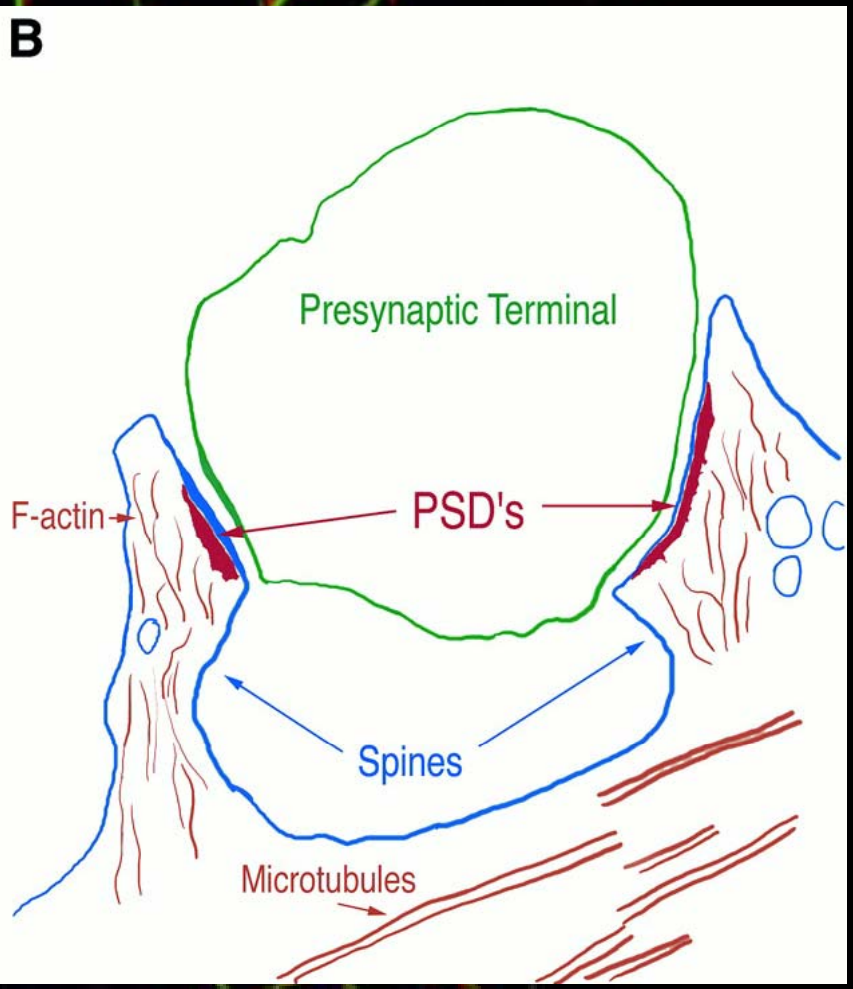


Cocaine (4 weeks)
TE Robinson & B Kolb (1999)

Ventral and dorsal striatum

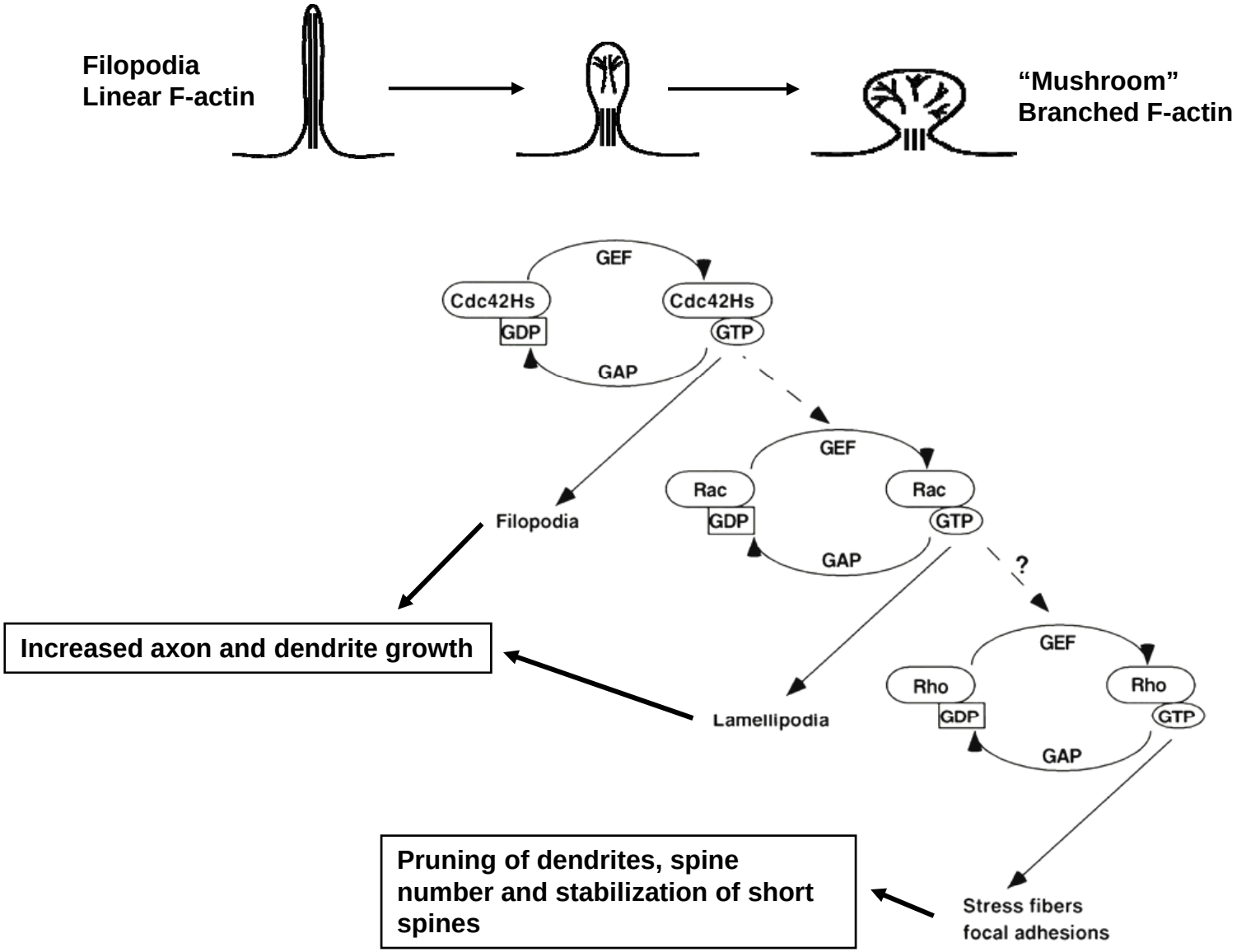


Spines and cytoskeleton



Microtubule-associated protein MAP2 Actin filament DNA

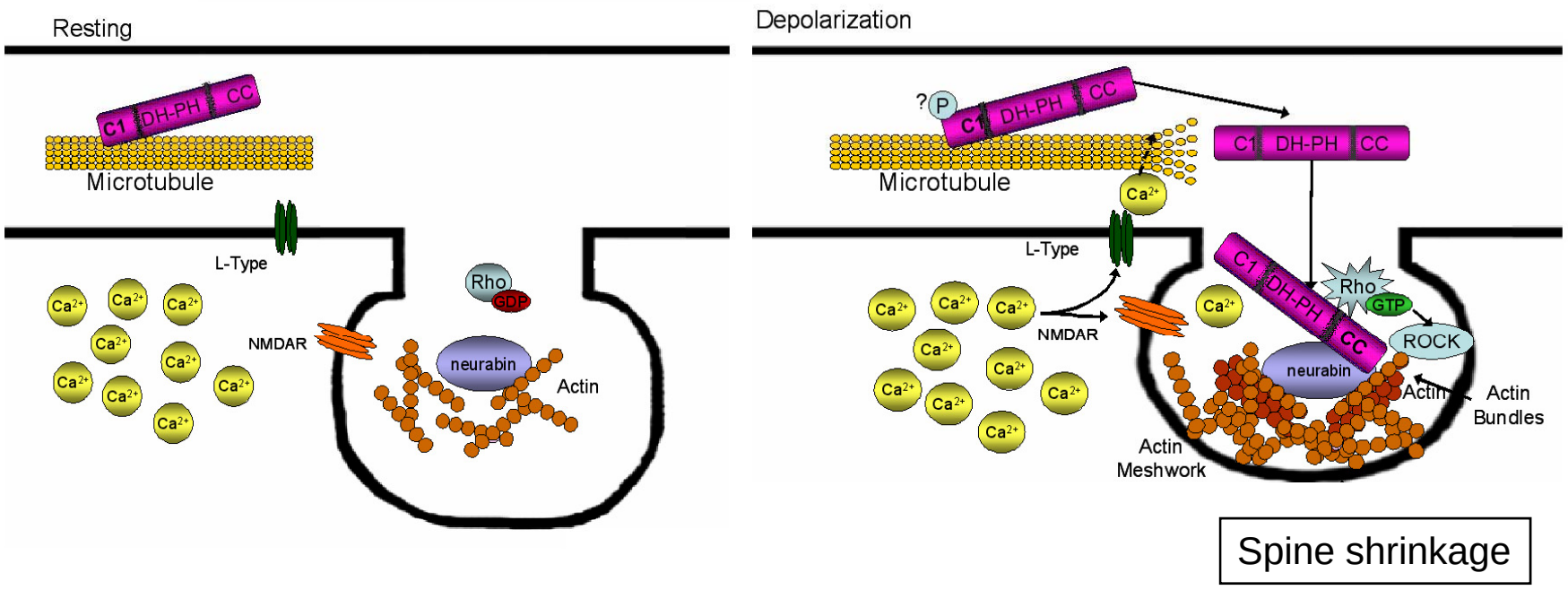
Small GTPases and regulation of dendritic morphogenesis



(adapted from Tashiro, Miden and Yuste, 2000)

Contribution of the Rho GEF Lfc in the regulation of dendritic morphogenesis

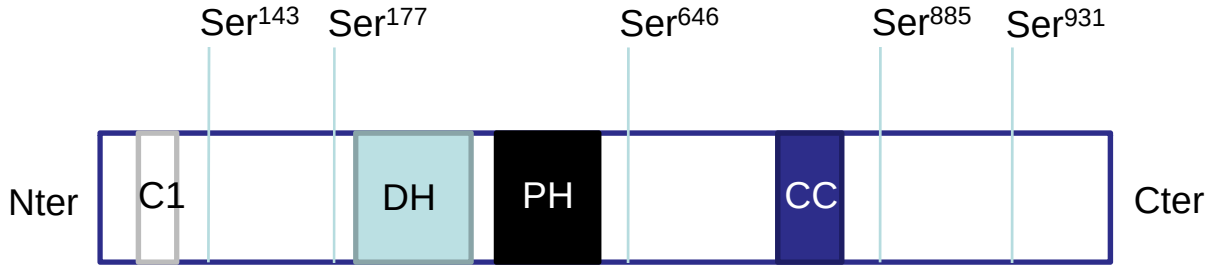
RhoA GEF, Lfc C1 DH-PH CC



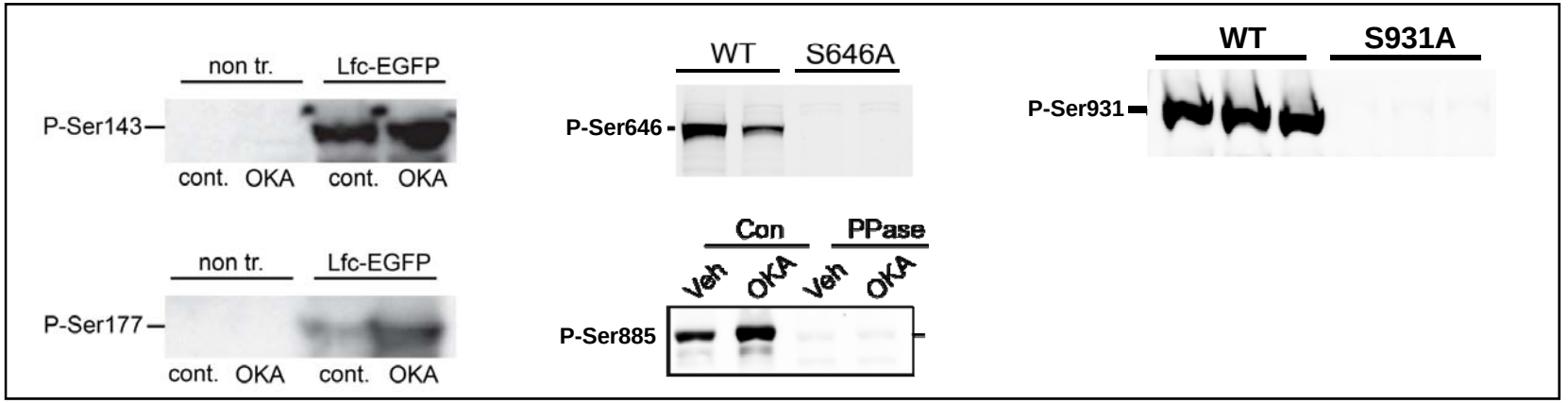
Ryan et al 2005

Strategy for identification and analysis of multiple phosphorylation sites

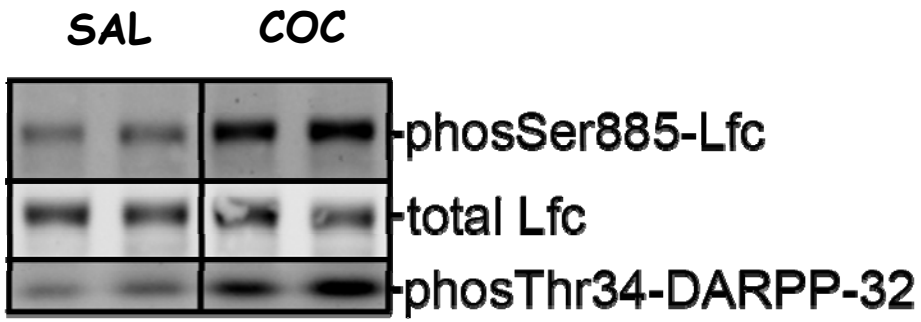
- **Express LFC-HA in N2a cells in culture (30 x 25cm2 plates)**
- **Incubate with protein phosphatase inhibitors**
- **Immunoprecipitate, SDS-PAGE, in gel digestion**
- **TiO2- enrichment, MS/MS identification of phosphopeptides**

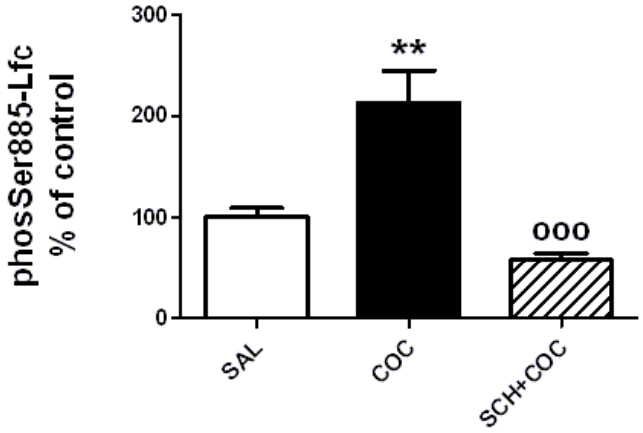


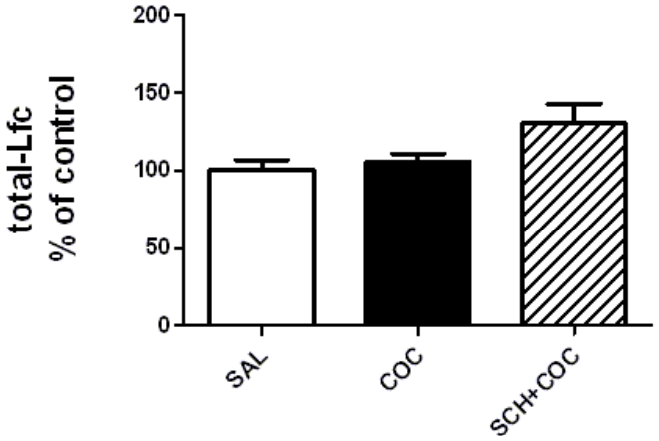
- **Repeat analysis (3 x 25cm2 plates)**
- **Prepare phospho-specific antibodies to each site**
- **Characterize phosphorylation in neuronal preparations and in vivo**



Regulation of the phosphorylation sites

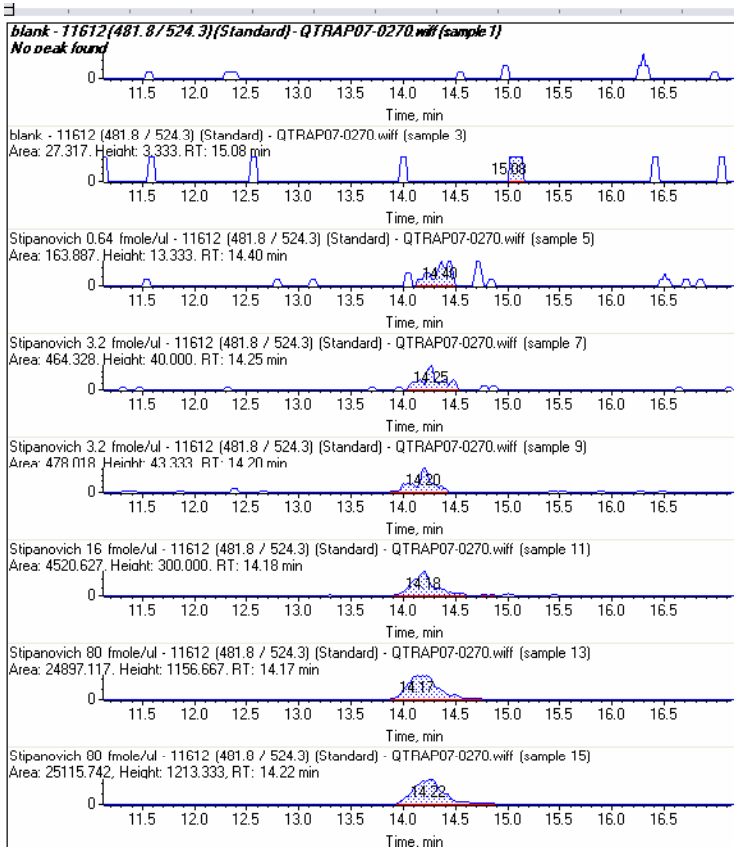






Multiple Reaction Monitoring scanning (MRM)

Standard Curve with Ser885 heavy peptide



0.64 fmol/ul

3.2 fmol/ul

3.2 fmol/ul

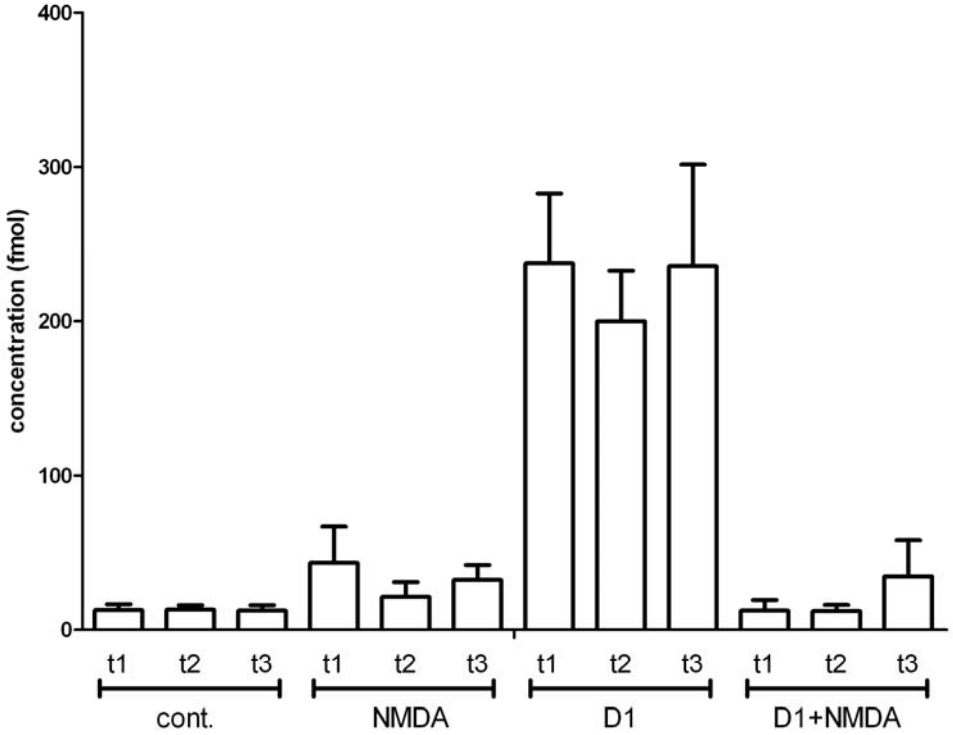
16 fmol/ul

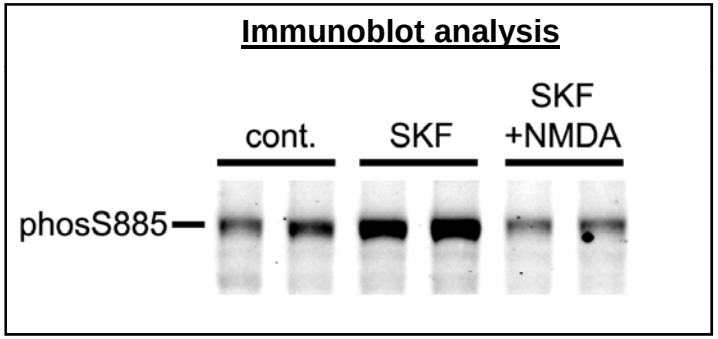
80 fmol/ul

80 fmol/ul

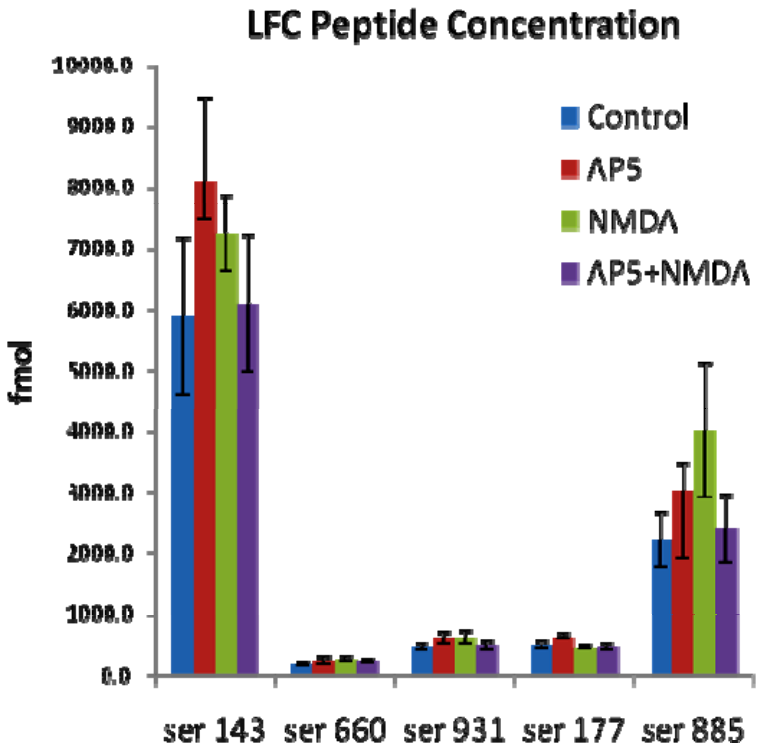
- **Acutely dissected striatal slice preparation**
- **Lyse protein sample in 8M Urea and digest with protease**
- **TiO2-affinity enrichment**
- **Quantitative MRM analysis of Lfc phosphopeptides**

Multiple Reaction Monitoring (MRM) analysis on striatal slices

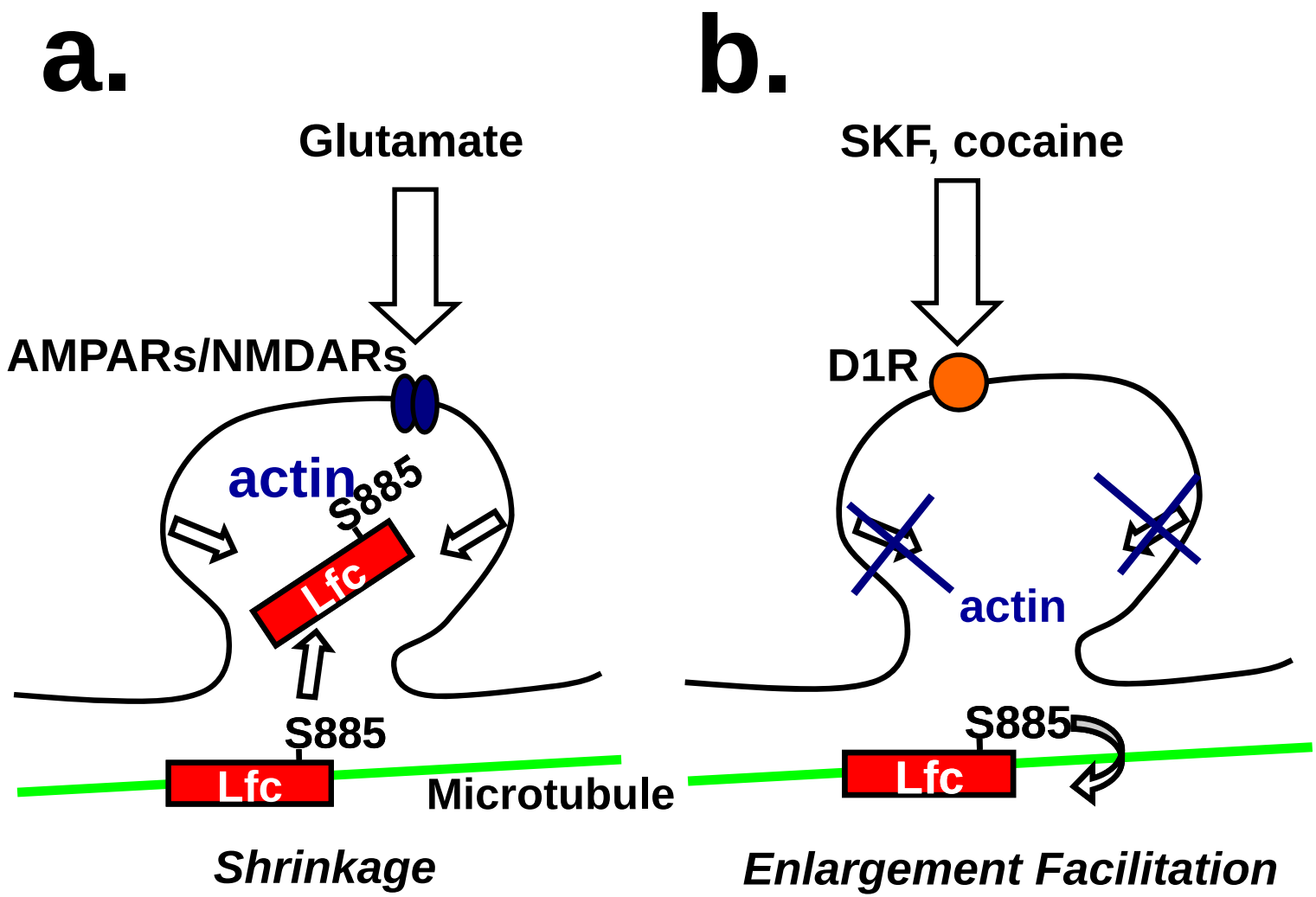




Multiple Reaction Monitoring (MRM) analysis on striatal slices



Phosphorylation of Ser885: a possible impact on spine shape



New technique: polyphosphorylated heavy peptide (the Ser931 site is part of a multi phosphorylated domain)

9181	ARHGEF2		4	163 93	rho/rac guanine nucleotide exchange factor (GEF) 2	SVSTTNIAGHFNDES#PLGLR
9181	ARHGEF2	1		174 10	rho/rac guanine nucleotide exchange factor (GEF) 2	ILSQS#TDSLNMR
9181	ARHGEF2	1	1	645 26	rho/rac guanine nucleotide exchange factor (GEF) 2	SES#LESPRGER
9181	ARHGEF2		2	648 35	rho/rac guanine nucleotide exchange factor (GEF) 2	SESLES#PRGER
9181	ARHGEF2		1	695 11	rho/rac guanine nucleotide exchange factor (GEF) 2	EPALPLEPDSGGNT#SPGVTANGEAR
9181	ARHGEF2	4	1	696 20	rho/rac guanine nucleotide exchange factor (GEF) 2	EPALPLEPDSGGNTS#PGVTANGEAR
9181	ARHGEF2	12	4	886 45	rho/rac guanine nucleotide exchange factor (GEF) 2	S#LPAGDALYLSFNPPQPSR
9181	ARHGEF2	1		894 19	rho/rac guanine nucleotide exchange factor (GEF) 2	SLPAGDALY#LSFNPPQPSR
9181	ARHGEF2	4	2	932 500	rho/rac guanine nucleotide exchange factor (GEF) 2	QELGS#PEER

9181	ARHGEF2	1		940 31	rho/rac guanine nucleotide exchange factor (GEF) 2	LQDS#S#DPDTGSEEEGSSRLS#PPHS#PR
9181	ARHGEF2	1		941 27	rho/rac guanine nucleotide exchange factor (GEF) 2	LQDS#S#DPDTGSEEEGSSRLS#PPHS#PR
9181	ARHGEF2	1		953 7	rho/rac guanine nucleotide exchange factor (GEF) 2	LQDSSDPDTGSEEEGSS#RLSPPHS#PR
9181	ARHGEF2	8	2	956 29	rho/rac guanine nucleotide exchange factor (GEF) 2	LQDS#S#DPDTGSEEEGSSRLS#PPHS#PR
9181	ARHGEF2	9	2	960 58	rho/rac guanine nucleotide exchange factor (GEF) 2	LQDS#S#DPDTGSEEEGSSRLS#PPHS#PR

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Aknowledgements

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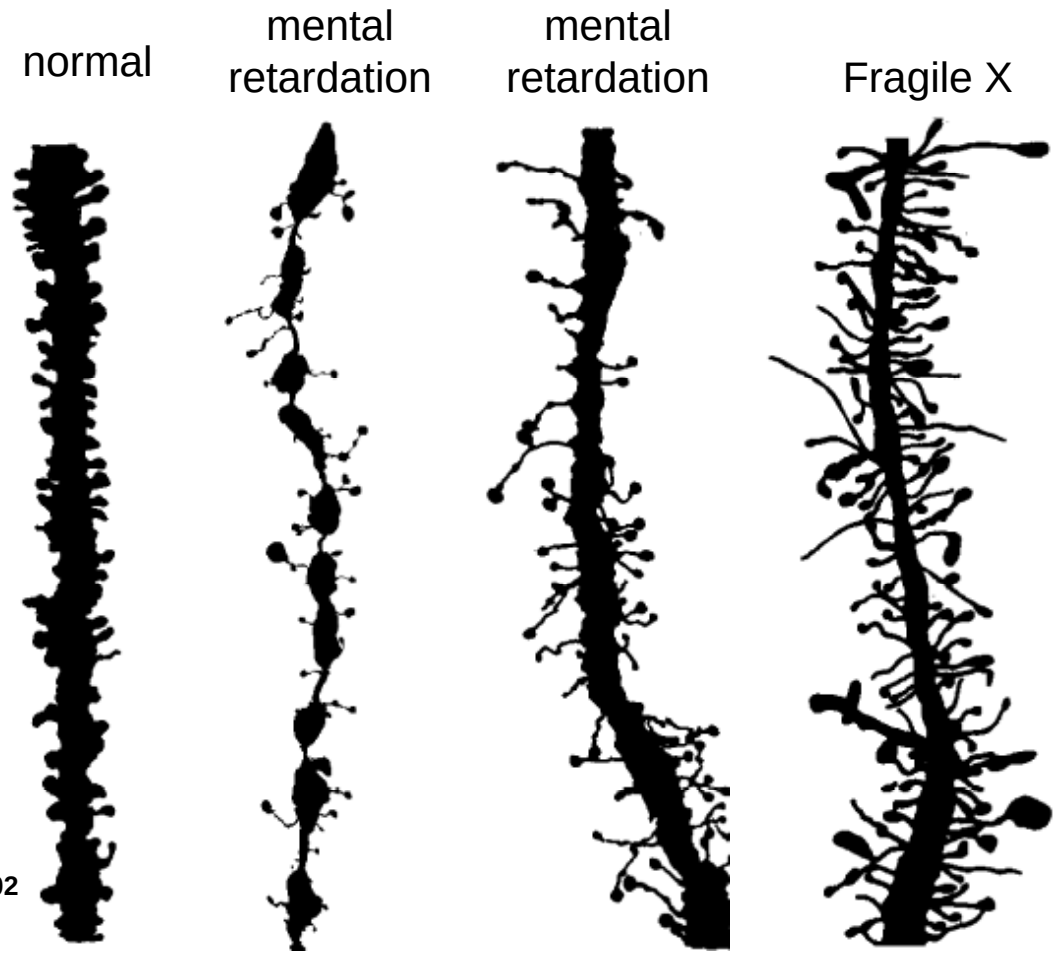
Dendritic Spine Pathology



Fiala et al 2002