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

Hyman et al. 2006
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

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

Golgi-Cox staining

Ventral and dorsal striatum
Distal dendritic spines
NAcc
CPu

Spines/10 μm

S A

8.5 9.5 10.5 11.5

*
Spines and cytoskeleton

(from Kennedy, Science 2000)

Microtubule-associated protein MAP2

Actin filament

DNA
Small GTPases and regulation of dendritic morphogenesis

Filopodia
Linear F-actin

“Mushroom”
Branched F-actin

Increased axon and dendrite growth

Pruning of dendrites, spine number and stabilization of short spines

(adapted from Tashiro, Miden and Yuste, 2000)
Contribution of the Rho GEF Lfc in the regulation of dendritic morphogenesis

RhoA GEF, Lfc

Spine shrinkage

Ryan et al 2005
Strategy for identification and analysis of multiple phosphorylation sites

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

- Repeat analysis (3 x 25 cm² plates)
- Prepare phosho-specific antibodies to each site
- Characterize phosphorylation in neuronal preparations and in vivo
Regulation of the phosphorylation sites

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**Graphs:**
- **Left:** Percentage of control for phosSer885-Lfc.
  - SAL: 100%
  - COC: 200%
  - SCH+COC: 0%
- **Right:** Percentage of control for total Lfc.
  - SAL: 100%
  - COC: 100%
  - SCH+COC: 150%
Multiple Reaction Monitoring scanning (MRM)

Standard Curve with Ser885 heavy peptide

0.64 fmol/ul
3.2 fmol/ul
16 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

![Graph showing concentration of proteins over time with error bars]

**Immunoblot analysis**

- **cont.**
- **SKF**
- **SKF + NMDA**

![Immunoblots showing phosphorylation of S885]

- **phosS885**
Multiple Reaction Monitoring (MRM) analysis on striatal slices
Phosphorylation of Ser885: a possible impact on spine shape

a. Glutamate
   AMPARs/NMDARs
   actin
   Lfc
   $885$
   Microtubule
   Shrinkage

b. SKF, cocaine
   D1R
   actin
   Lfc
   $885$
   Enlargement Facilitation
**New technique: polyphosphorylated heavy peptide**

*(the Ser931 site is part of a multi phosphorylated domain)*

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Dephouere N.; Gygi S. *et al.*, 2008
Aknowledgements

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Dendritic Spine Pathology

normal mental retardation mental retardation Fragile X

Fiala et al 2002