

Keck/NIDA Neuroproteomics Center
April 26, 2013

Identification and Analysis of Protein Complexes Mediating Synapse Formation

Thomas Biederer

**Program in Cellular Neuroscience,
Neurodegeneration and Repair**

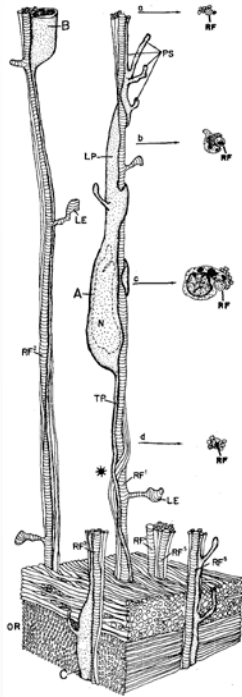
**Department of Molecular Biophysics
& Biochemistry**

Yale University



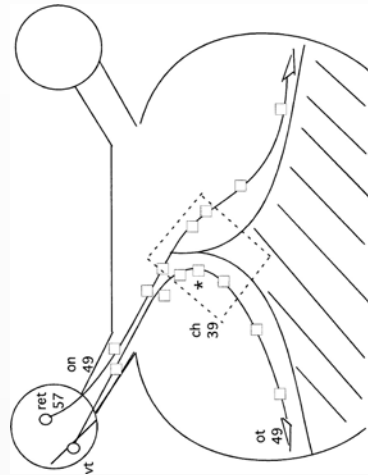
Surface interactions guide neuronal development

I. neuronal migration



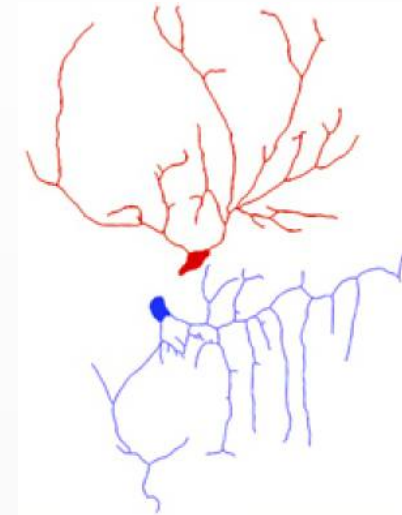
Rakic (1972)
J Comp Neurol 145:61

II. axonal growth cone guidance



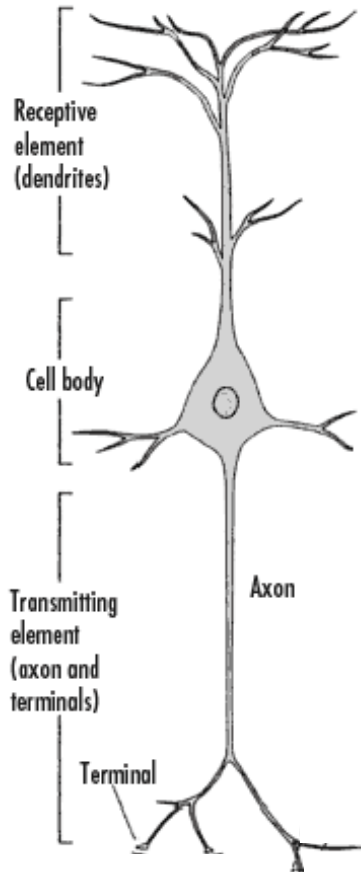
Mason and Erskine (2000)
J Neurobiol 44:260

III. dendritic differentiation



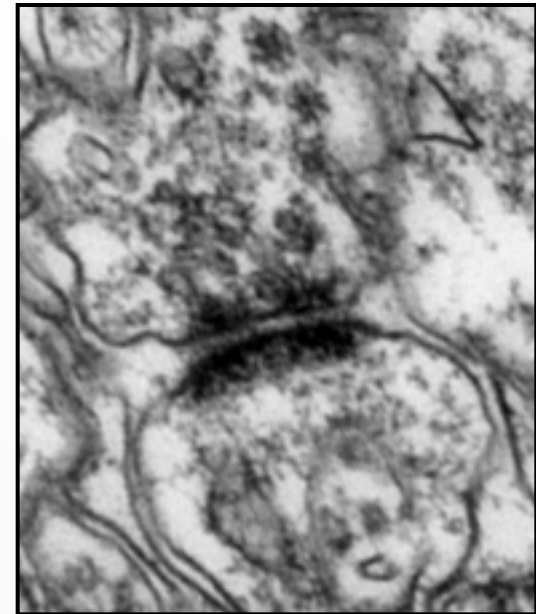
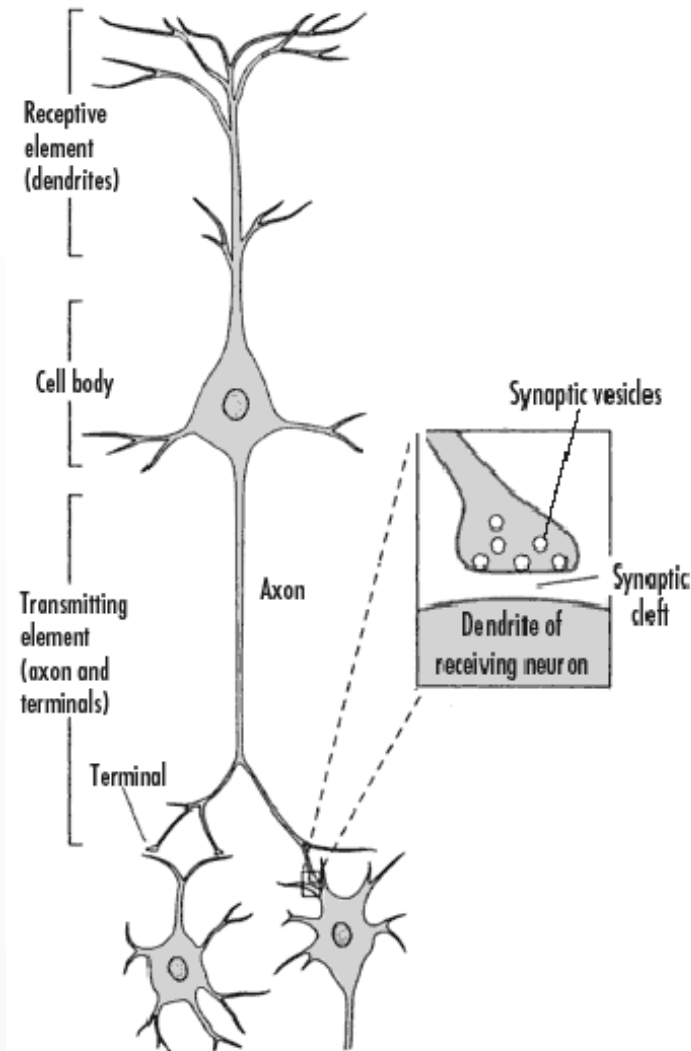
Matthews et al. (2007)
Cell 129:593

Synapses connect neurons



Eric R. Kandel, *In Search of Memory*.
Norton, 2006

Synapses connect neurons



Eric R. Kandel, *In Search of Memory*.
Norton, 2006

Fine structure of excitatory synapses

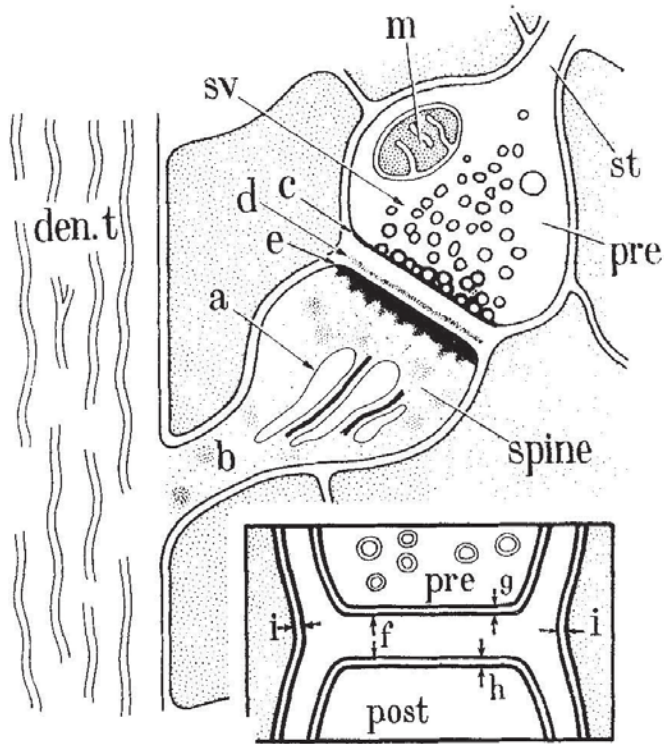
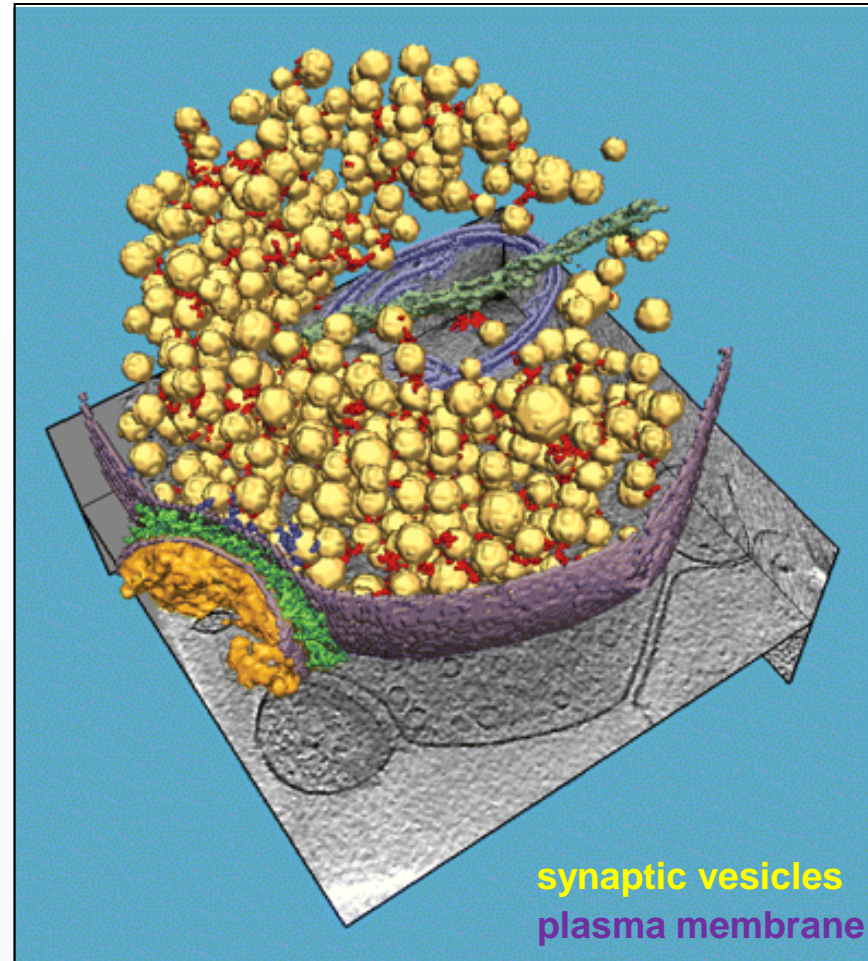


Fig. 1. Diagram of a synaptic contact on a dendritic spine, observed with the electron microscope after osmium tetroxide fixation. The stippled regions represent neuronal and glial processes of the neuropil

Inset. The opposed regions of the pre- and post-synaptic membranes seen after potassium permanganate fixation. The membranes (i) are of neighbouring processes of the neuropil

cryo-electron tomographic reconstruction of the presynaptic cytomatrix:



Gray (1959) *Nature* 183:1592-1593.

Fernández-Busnadiego et al. (2010)
J Cell Biol 188: 145–156.

Fine structure of excitatory synapses

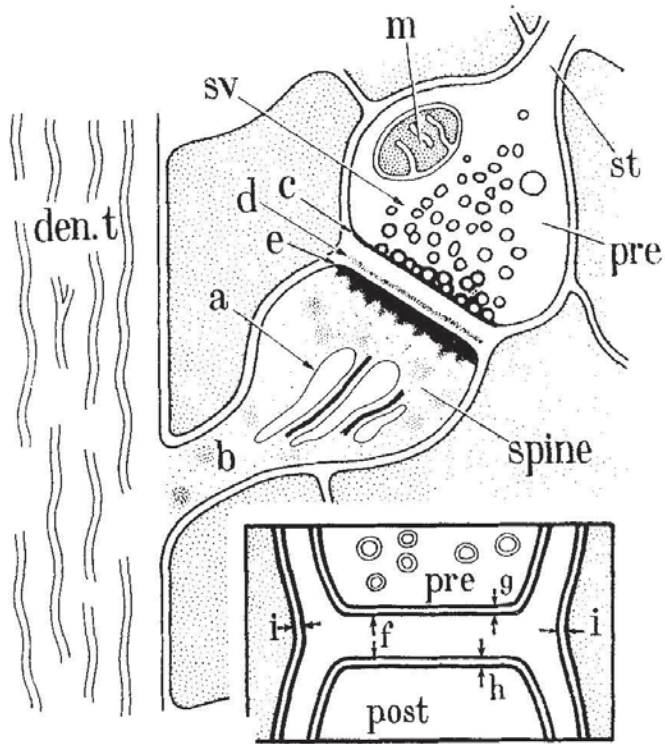
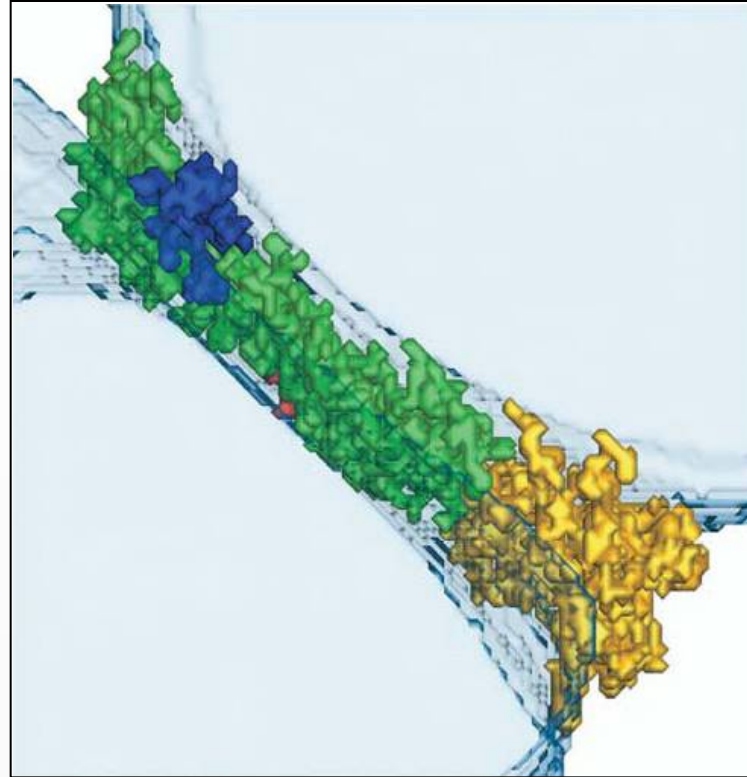


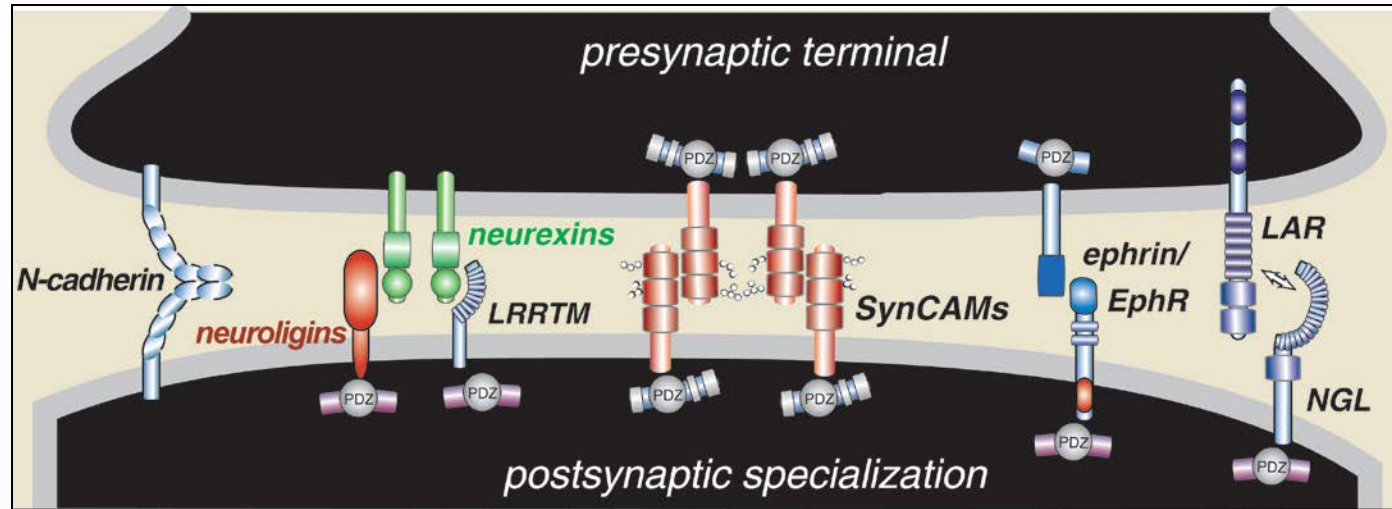
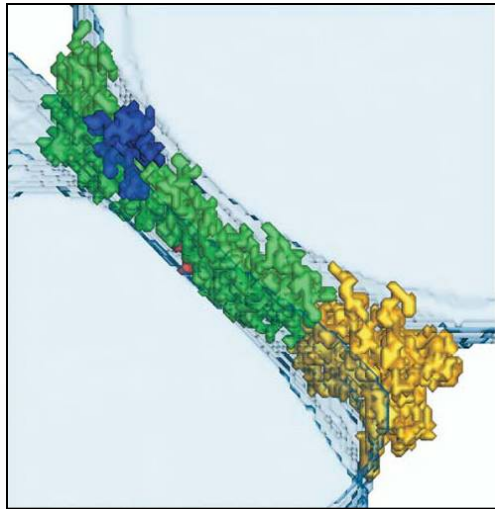
Fig. 1. Diagram of a synaptic contact on a dendritic spine, observed with the electron microscope after osmium tetroxide fixation. The stippled regions represent neuronal and glial processes of the neuropil

Inset. The opposed regions of the pre- and post-synaptic membranes seen after potassium permanganate fixation. The membranes (i) are of neighbouring processes of the neuropil

cryo-electron tomographic reconstruction of the synaptic cleft:

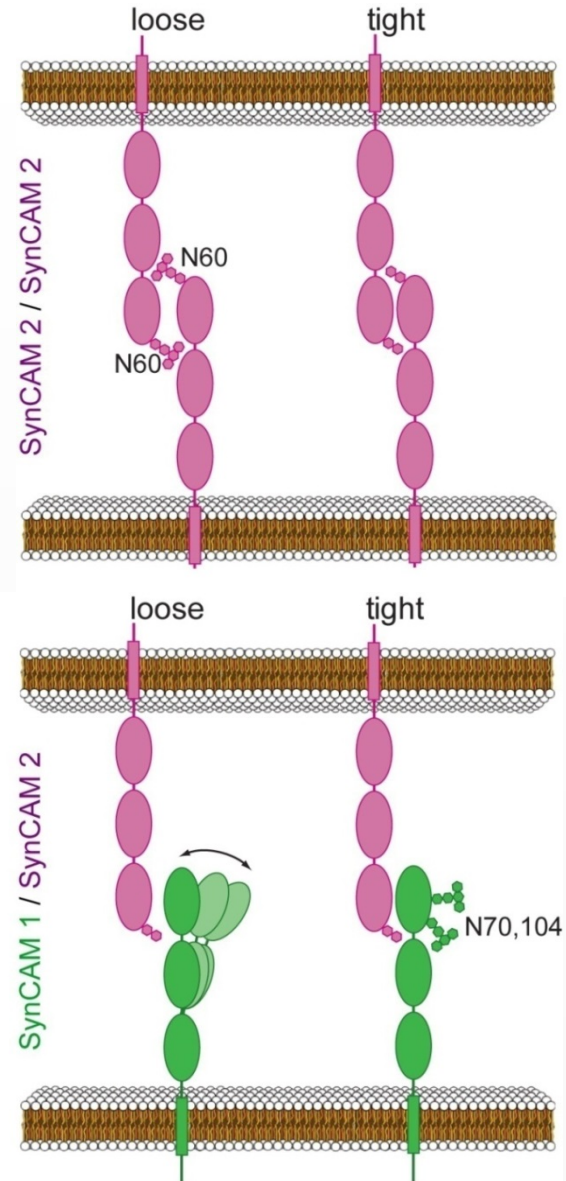
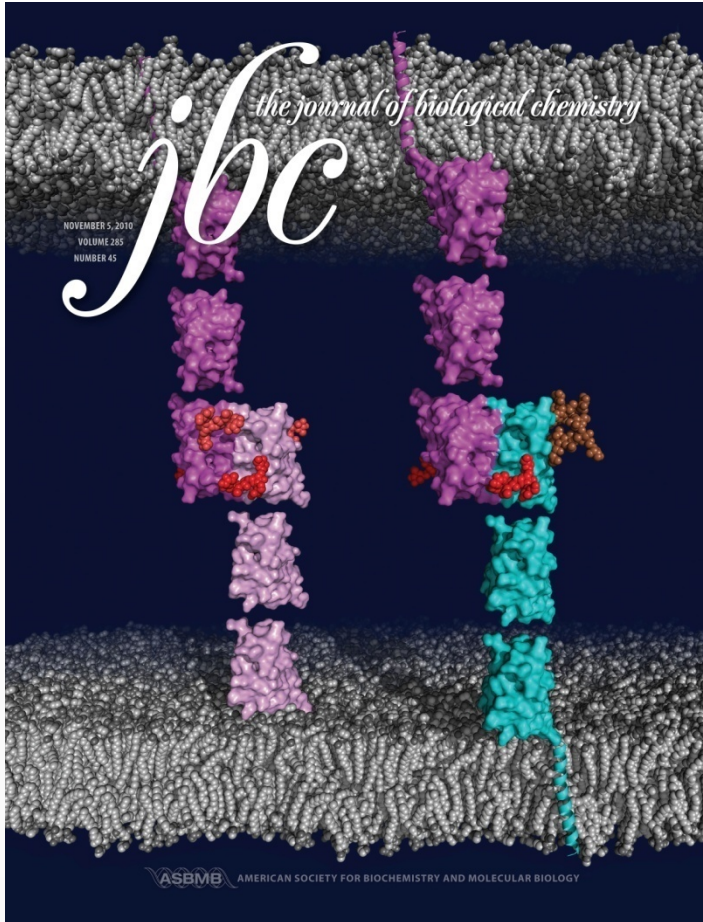


Select adhesion molecules span the synaptic cleft



Lucic et al. (2005)
Structure 13:423

N-glycans differentially regulate SynCAM adhesion

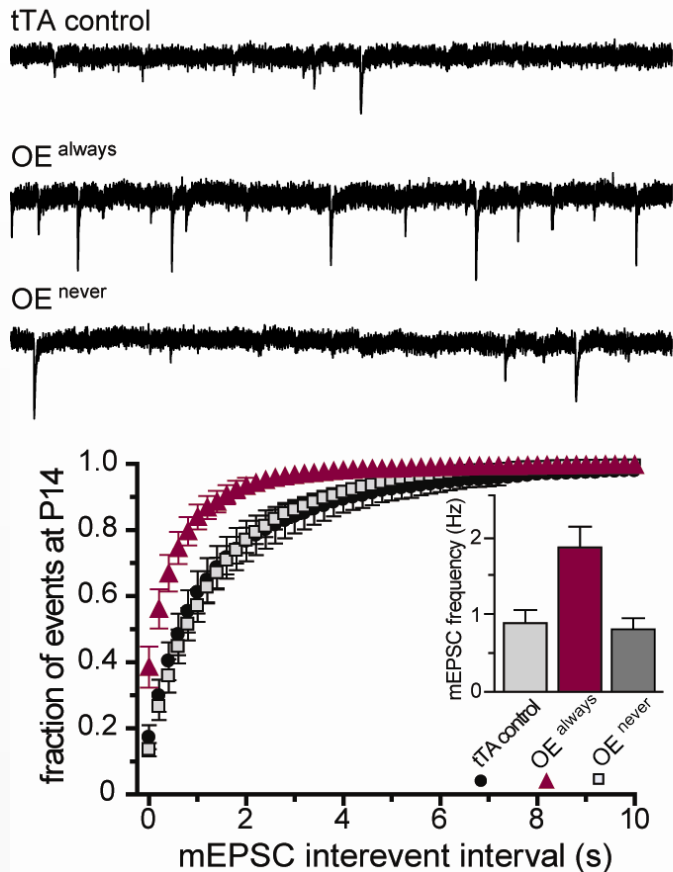


Fogel et al. (2010) *J Biol Chem* 285: 34864-74.

Glycoproteomics with TuKiet Lam, Keck/NIDA Neuroproteomics Center

SynCAM 1 promotes functional excitatory synapses

whole cell patch recording of mEPSCs from CA3-CA1 synapses at P14:



Robbins et al. (2010) *Neuron* 68:894-906.

Alexander Krupp

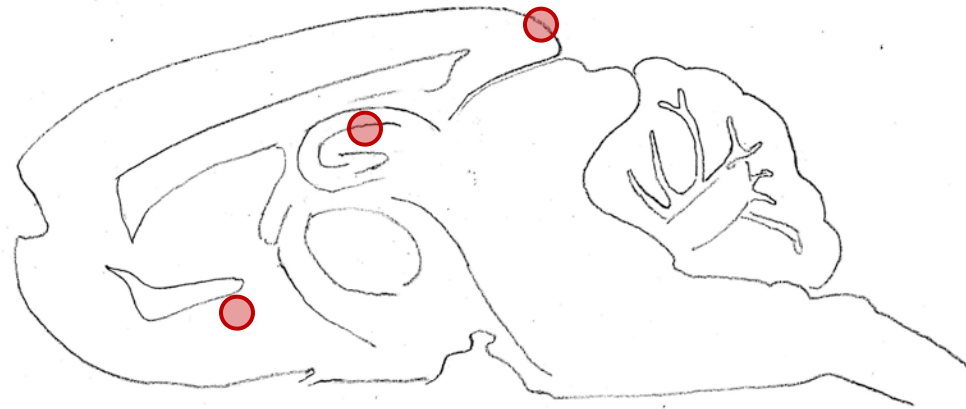
Current studies of synapse-organizing mechanisms

hippocampus:

- molecular/cellular studies of synaptogenic mechanisms
- test hippocampus-dependent behaviors in mouse models with altered synaptogenesis

sensory cortex:

- roles of synapse-organizing mechanisms in the experience-dependent remodeling of circuits



retina:

- structural and physiological studies of synapses from EM to circuits

nucleus accumbens:

- control of excitatory input onto inhibitory neurons
- synaptic remodeling by drugs of abuse

Current studies of synapse-organizing mechanisms

hippocampus:

- molecular/cellular studies of synaptogenic mechanisms
- test hippocampus-dependent behaviors in mouse models with altered synaptogenesis

translational relevance:

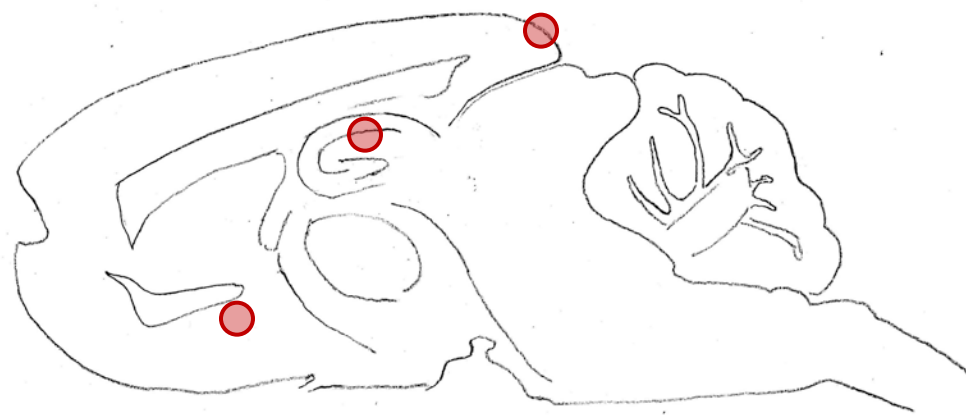
- *mechanistic analysis of mutations linked to developmental disorders*

sensory cortex:

- roles of synapse-organizing mechanisms in the experience-dependent remodeling of circuits

translational relevance:

- *insights into critical window aberrations in developmental disorders*



retina:

- structural and physiological studies of synapses from EM to circuits

nucleus accumbens:

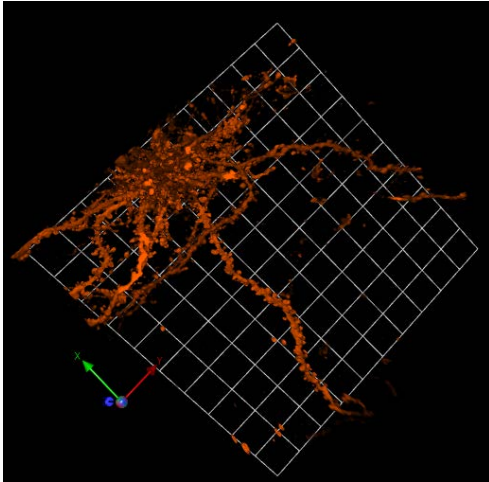
- control of excitatory input onto inhibitory neurons
- synaptic remodeling by drugs of abuse

translational relevance:

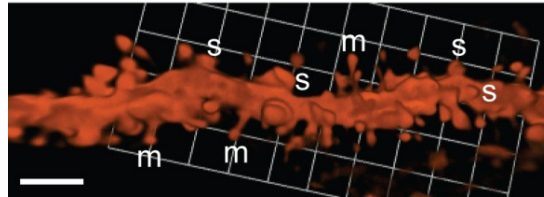
- *synaptic alterations in addictive behaviors*

Loss of SynCAM 1 alters cocaine-induced spine structure changes in medium spiny neurons

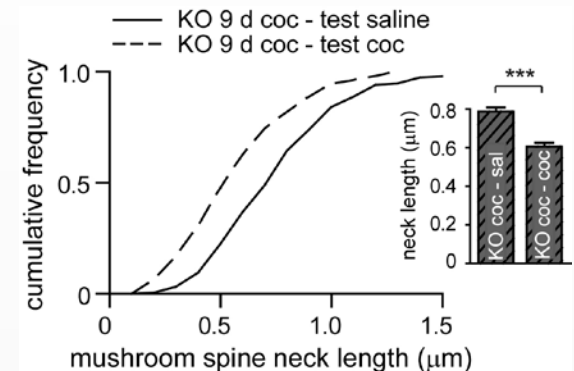
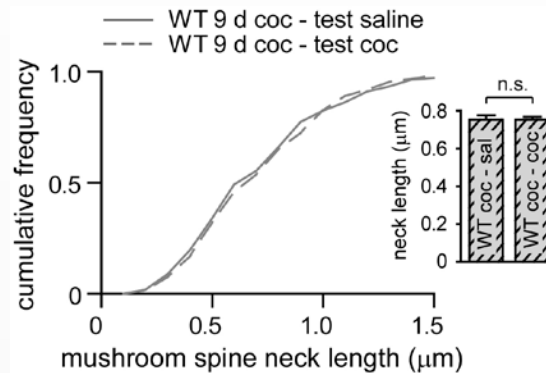
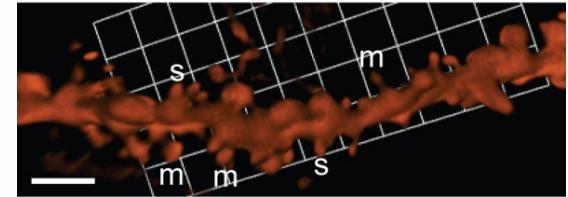
medium spiny neuron in nucleus accumbens:



cocaine-withdrawn wild-type after cocaine challenge



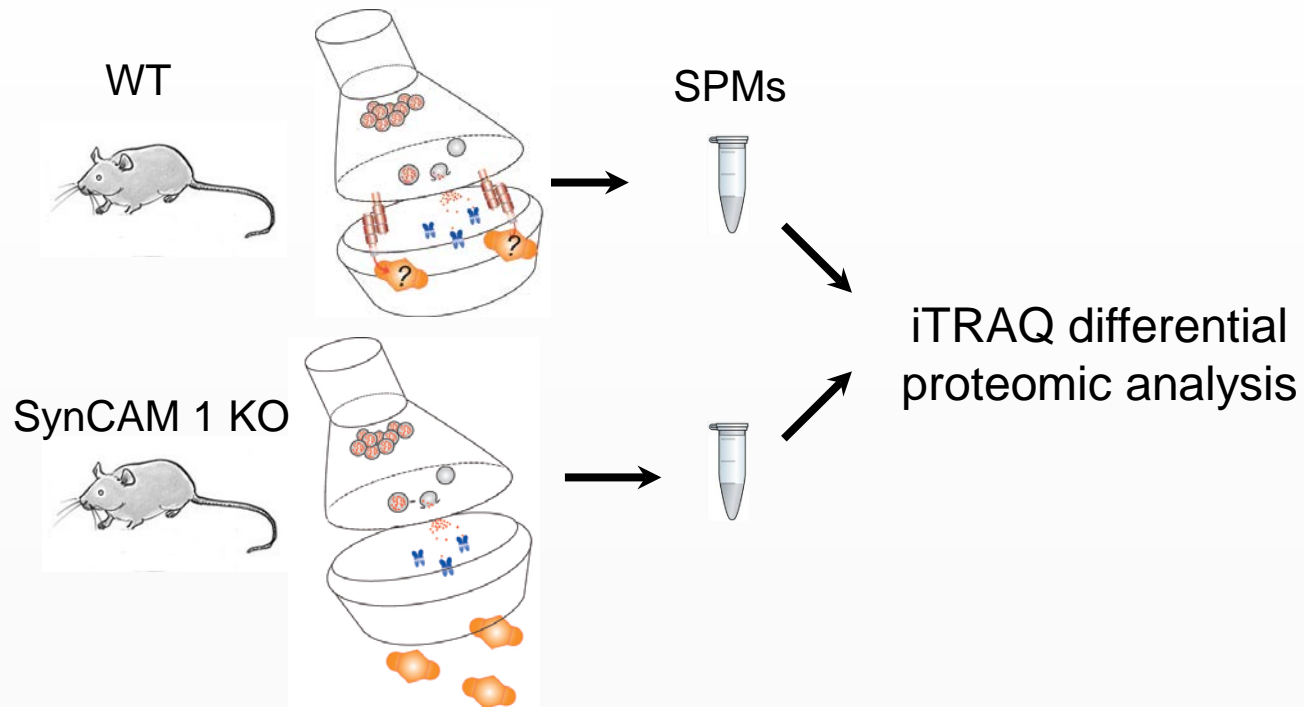
cocaine-withdrawn SynCAM 1 KO after cocaine challenge



- synapse-organizing adhesion molecules can modulate cocaine effects on spine structures in nucleus accumbens and vulnerability to behavioral actions of cocaine
- spines of medium spiny neurons show previously unknown structural responses to cocaine

- 1. Proteomic studies of postsynaptic signaling**
2. Identify novel signaling molecules that underlie concerted actions of synapse-organizing proteins

A proteomic screen for synaptogenic signaling proteins



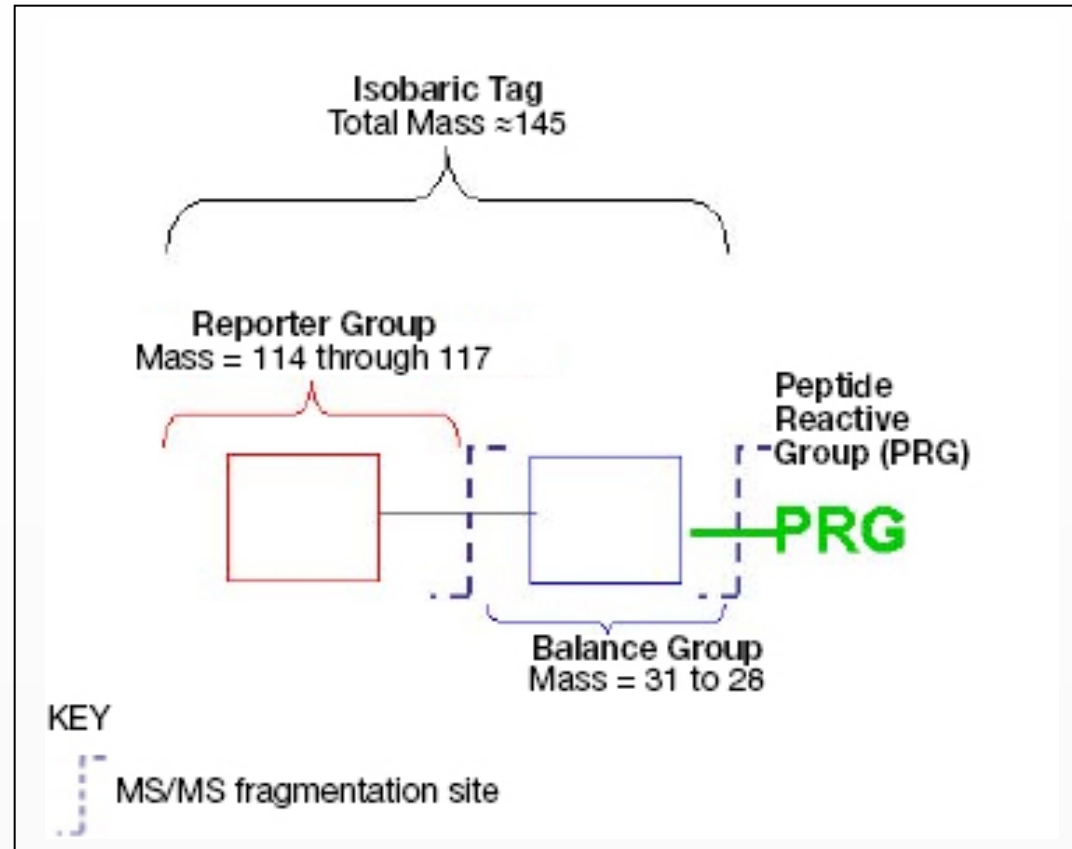
Proteomic analysis of synaptic composition by iTRAQ

synaptic plasma membrane proteins:

iTRAQ analysis of preparations from SynCAM 1 knock-out brains vs. controls after isobaric tag labeling

multiplexing of four different samples in a single LC/MS/MS experiment

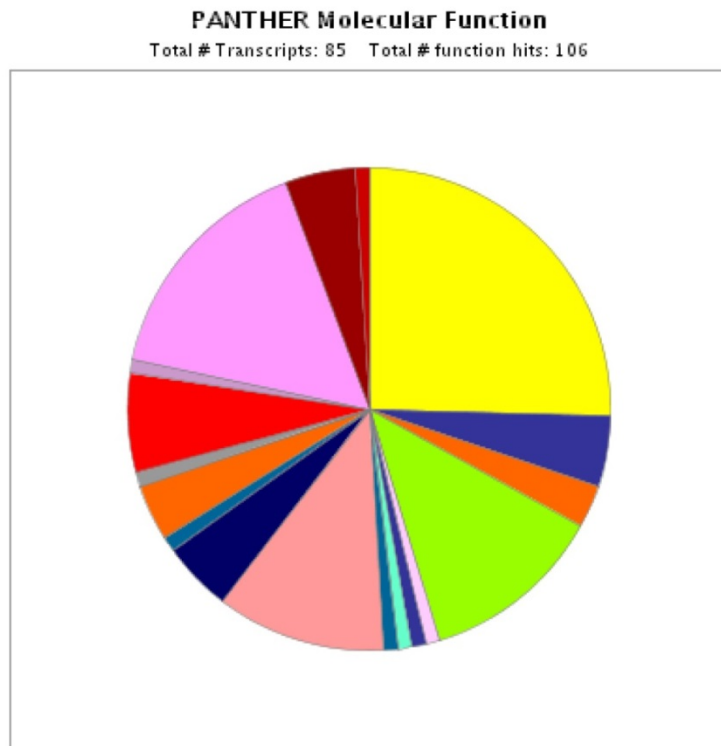
relationships can be quantified by comparing the MS peak area of one reporter group peak to another



from: Applied Biosystems iTRAQ Reference Guide

Synaptic plasma membranes lacking SynCAM 1 display altered protein composition

tabulated:
molecular function down-regulated



iTRAQ with Chris Colangelo,
Keck/NIDA Neuroproteomics Center

Color	Panther Category	Transcripts(Trans)	% Trans to Total Trans
Yellow	Cell adhesion molecule (MF00040)	27	31.8%
Dark Blue	Cell junction protein (MF00276)	5	5.9%
Orange	Chaperone (MF00077)	3	3.5%
Light Green	Cytoskeletal protein (MF00091)	13	15.3%
Light Purple	Hydrolase (MF00141)	1	1.2%
Dark Blue	Ion channel (MF00024)	1	1.2%
Cyan	Isomerase (MF00166)	1	1.2%
Blue	Membrane traffic protein (MF00267)	1	1.2%
Light Red	Miscellaneous function (MF00197)	12	14.1%
Dark Blue	Molecular function unclassified (MF00208)	5	5.9%
Blue	Oxidoreductase (MF00123)	1	1.2%
Orange	Phosphatase (MF00113)	4	4.7%
Grey	Protease (MF00153)	1	1.2%
Red	Receptor (MF00001)	7	8.2%
Light Purple	Select calcium binding protein (MF00188)	1	1.2%
Pink	Select regulatory molecule (MF00093)	17	20.0%
Dark Red	Transferase (MF00131)	5	5.9%
Red	Transporter (MF00082)	1	1.2%

Synaptic scaffolding molecules are altered by loss of SynCAM 1

Membrane Organizing Scaffolding Molecules

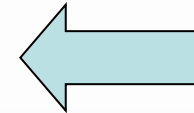
<u>Protein Score</u>	<u>Protein ID</u>	<u>Protein Name</u>	<u>Percent Coverage</u>	<u># Distinct Peptides in Ratios</u>	<u>115/114 ratio</u>	<u>115/114 P value</u>	<u>117/114 ratio</u>	
19.55	IPI00122094	Gene_Symbol=Dlg4 Isoform 2 of Discs large homolog 4 indistinguishable		23.99	<u>8</u>	0.7524	0.1502	0.7371
4.63	IPI00344142	Gene_Symbol=Lin7a Lin-7 homolog A indistinguishable		32.19	<u>3</u>	1.1532	0.6564	0.7540
4.02	IPI00313899	Gene_Symbol=Cpne9 Copine-9 indistinguishable		12.3	<u>2</u>	0.8192	0.5576	0.7730
19.52	IPI00224626	Gene_Symbol=Sept7 cell division cycle 10 homolog		35.24	<u>6</u>	0.9680	0.9484	1.2498
2.08	IPI00678465	Gene_Symbol=Dlg1 similar to Disks large homolog 1 (Synapse-associated protein 97) (SAP-97) (Embryo-dlg/synapse-associated protein 97) (E-dlg/SAP97) isoform 16 indistinguishable		16.63	<u>1</u>	-	1.0000	1.4084
1.06	IPI00459542	Gene_Symbol=EG435601 similar to PDZ domain containing 7		11.97	<u>1</u>	1.0596		1.5323

Red reduced
Green increased

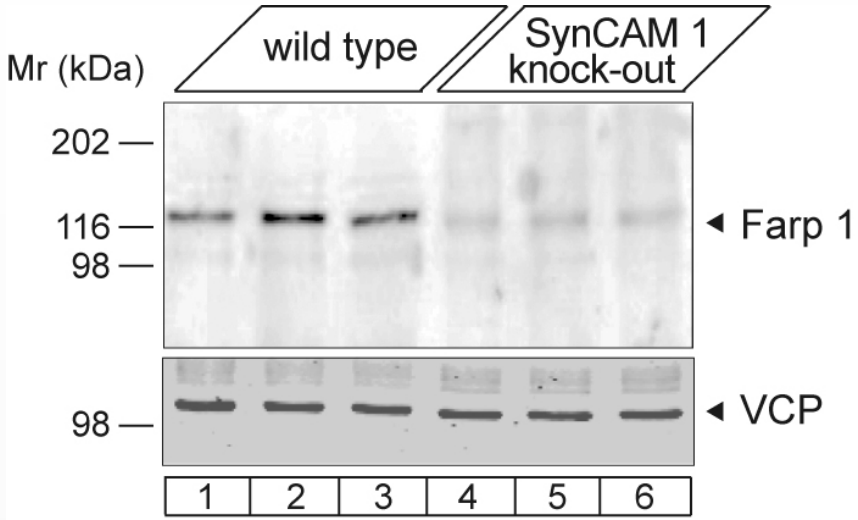
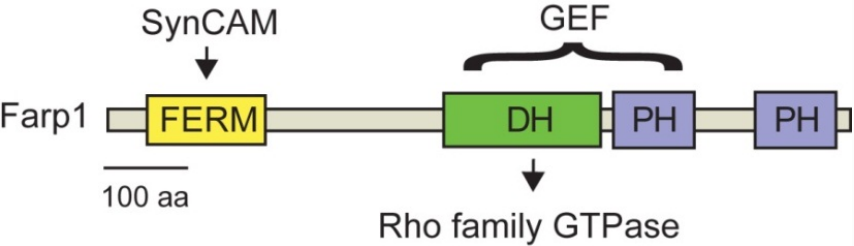
Proteins involved in signal transduction are altered by loss of SynCAM 1

Signaling Molecules

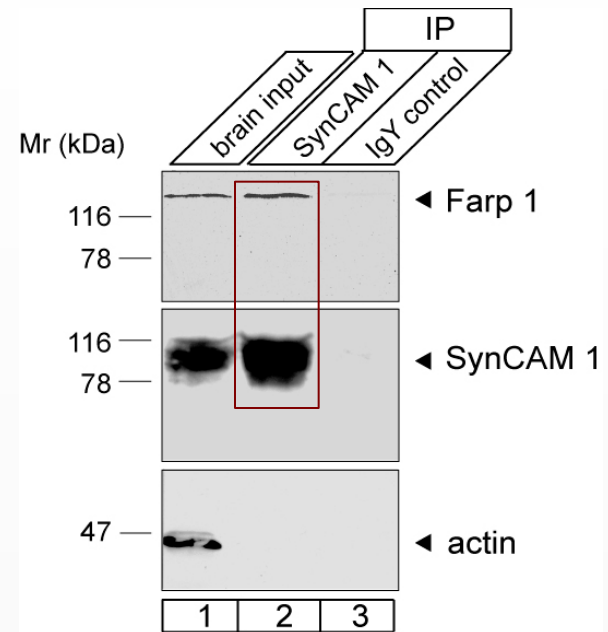
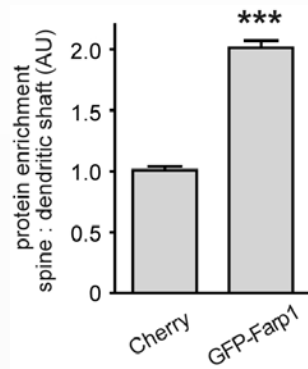
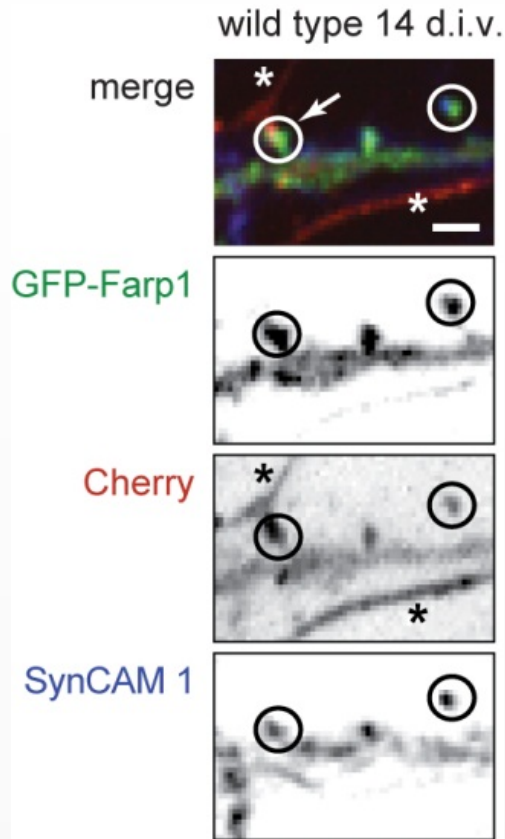
<u>Protein Score</u>	<u>Protein ID</u>	<u>Protein Name</u>	<u>Percent Coverage</u>	<u># Distinct Peptides in Ratios</u>	<u>115/114 ratio</u>	<u>115/114 P value</u>	<u>117/114 ratio</u>
1.38	IPI00356904	Gene_Symbol=Farp1 FERMRhoGEF (Arhgef) and pleckstrin domain protein 1 indistinguishable			7.16	1 0.9016	0.5420
28.47	IPI00621806	Gene_Symbol=Camk2a Isoform Alpha CaMKII of Calcium/calmodulin-dependent protein kinase type II alpha chain indistinguishable			44.77	7 0.9482 0.8152	1.6210
4.04	IPI00115875	Gene_Symbol=Pik4ca Phosphatidylinositol 4-kinase, catalytic, alpha polypeptide			14.52	2 - 1.0000	2.2685



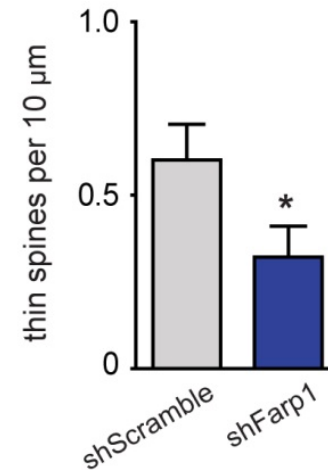
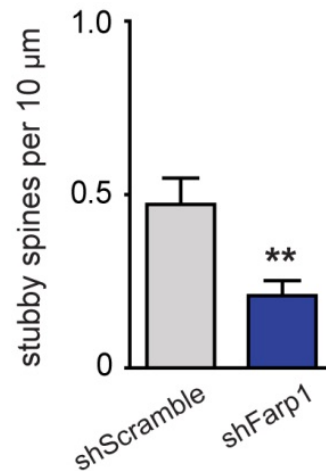
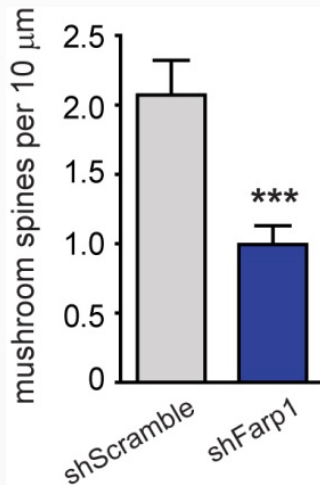
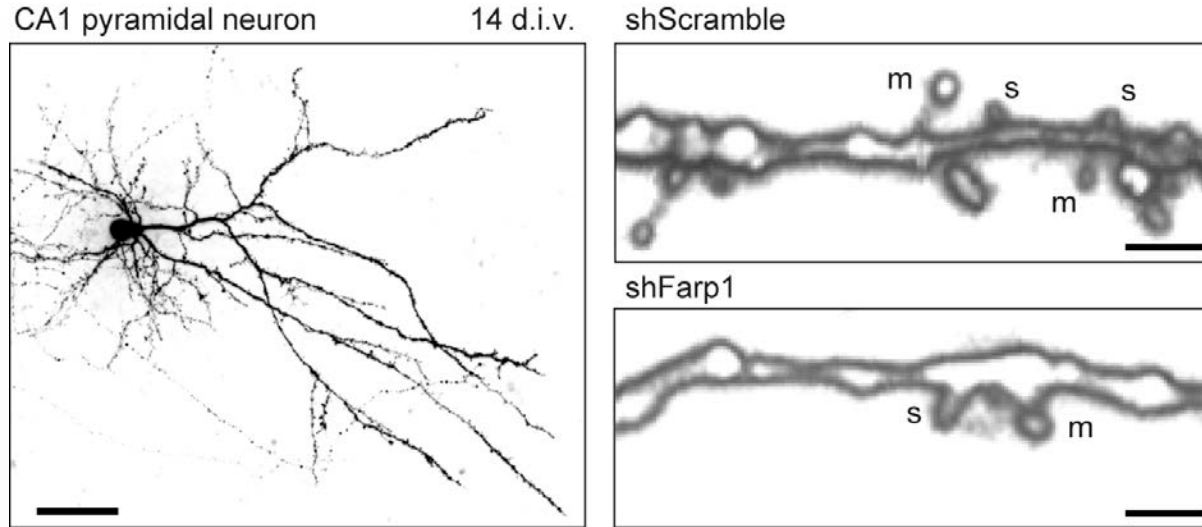
Farp1 protein amounts are reduced in SynCAM 1 KO



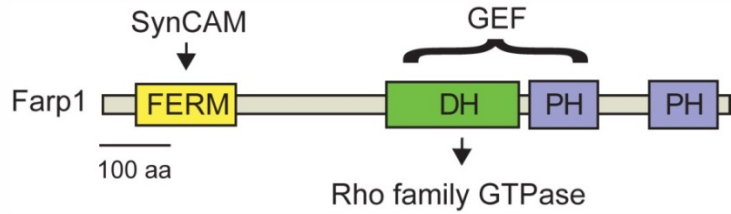
SynCAM 1/Farp1 form a complex at synapses



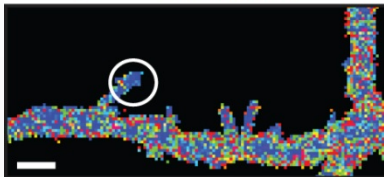
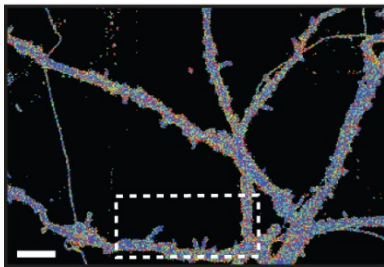
Farp1 is required for normal spine numbers of CA1 neurons in organotypic slice culture



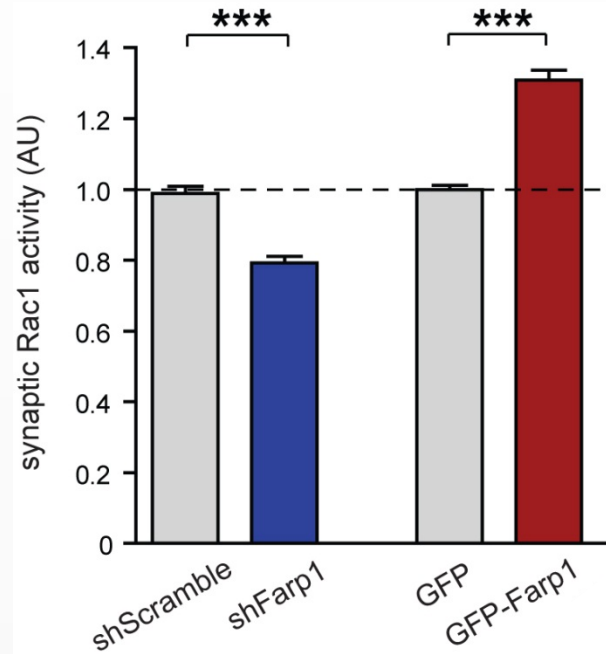
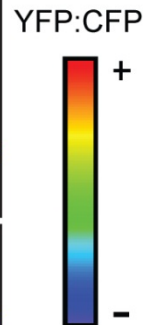
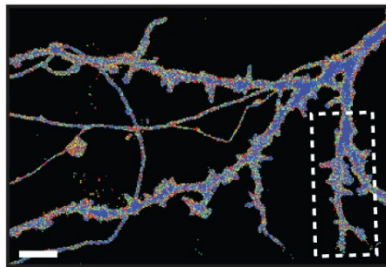
Farp1 activates Rac1 in dendritic spines



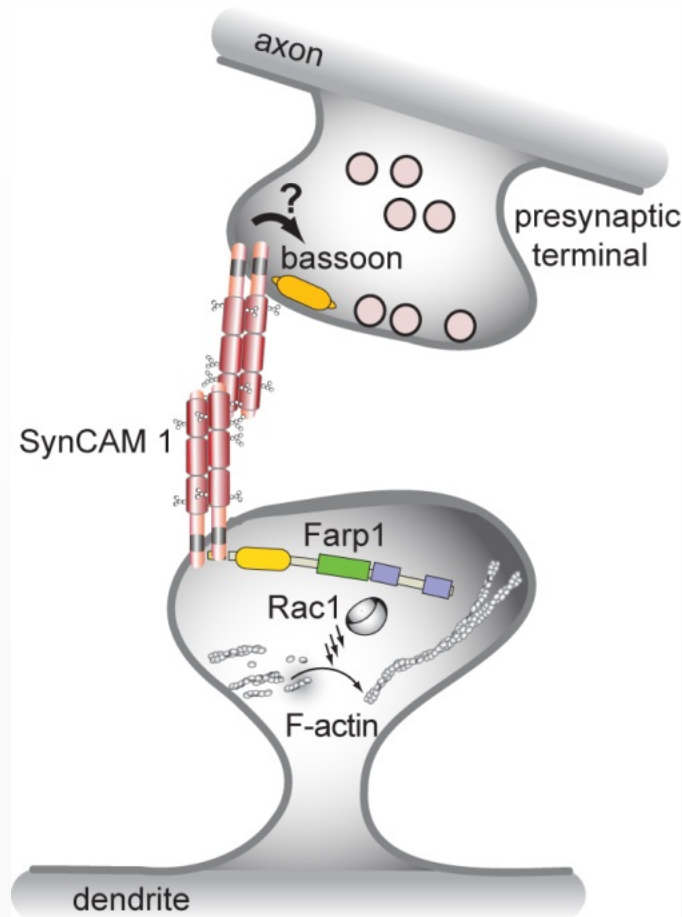
Raichu-Rac1 probe 9 d.i.v.



Raichu-Rac1 + Farp1



Trans-synaptic SynCAM adhesion and signaling organize excitatory synapses

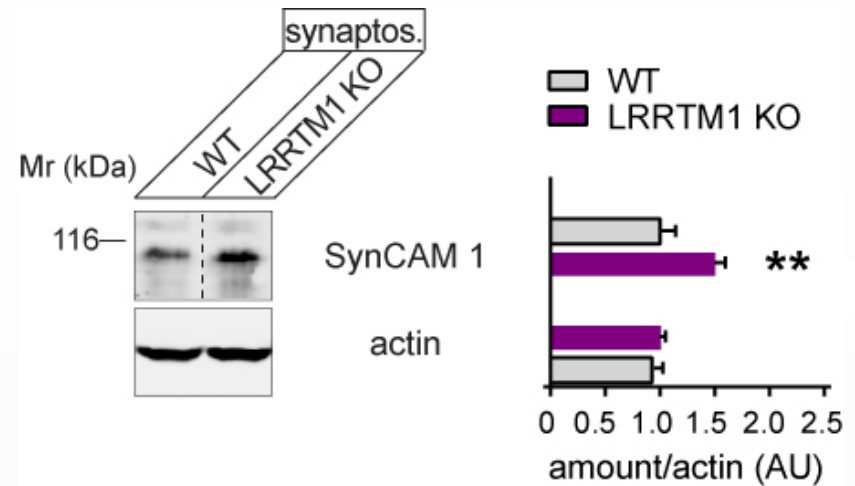


1. Proteomic studies of postsynaptic signaling
2. **Identify novel signaling molecules that underlie concerted actions of synapse-organizing proteins**

Identify synapse-organizing proteins through proteomics of mouse models

- screen protein changes in purified synaptic membranes of mice lacking combinations of synapse-organizing adhesion molecules

- identify the protein composition of synapse-inducing SynCAM complexes after affinity purification of epitope-tagged SynCAM 1 from the brain of transgenic mice overexpressing SynCAM 1



Acknowledgements

Lucas Cheadle

Santino Butler

Rachel Jeffrey, Ph.D.

Karen Perez de Arce, Ph.D.

Adema Ribic, Ph.D.

Kellie Park, M.D./Ph.D.

Fabian Laage-Gaupp

Yuling Lei



Yale SCHOOL OF MEDICINE


Cellular Neuroscience, Neurodegeneration and Repair


universität**bonn**
Valentin Stein

 **UNIVERSITÉ
DE GENÈVE**

Dominique Muller

Yale MB&B 
Yorgo Modis

Yale Psychiatry 
Marina Picciotto
Jane Taylor

 **Yale/NIDA** NATIONAL INSTITUTE
ON DRUG ABUSE
NEUROPROTEOMICS CENTER

Angus Nairn
Ken Williams
TuKiet Lam
Chris Colangelo

Funding Support:

NIH/NIDA 2R01 DA018928, NIH/NIDA R21 DA034492, and NIH/NIDA P30 DA018343