Perspective

The Protein Biochemistry of the Postsynaptic Density in Glutamatergic Synapses Mediates Learning in Neural Networks.

Mary Kennedy

Biochemistry, **Just Accepted Manuscript** • DOI: 10.1021/acs.biochem.8b00496 • Publication Date (Web): 18 Jun 2018

Downloaded from <http://pubs.acs.org> on June 19, 2018

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

ACS Publications

is published by the American Chemical Society, 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

The Protein Biochemistry of the Postsynaptic Density in Glutamatergic Synapses Mediates Learning in Neural Networks.

by Mary B. Kennedy*, Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125
*kennedym@its.caltech.edu

Abstract

The strength of each excitatory synapse in the central nervous system is regulated by its prior activity in a process called synaptic plasticity. The initiation of synaptic plasticity occurs when calcium ions enter the postsynaptic compartment and encounter a subcellular structure called the postsynaptic density (PSD). The PSD is attached to the postsynaptic membrane just underneath the concentrated plague of neurotransmitter receptors. It is comprised of a core set of 30 to 60 proteins, approximately 20 of which are scaffold proteins. The rest include protein kinases and phosphatases, some of which respond to calcium ion; small GTPases and their regulators; chaperones; ubiquitins; and proteases. The assembly of the PSD involves competitive binding among a variety of specific protein binding sites to form a dynamic network. A biochemical challenge for the future is to understand how the dynamic regulation of the structure, composition, and activity of the PSD mediates synaptic plasticity, and how mutations in PSD proteins lead to mental and neurodegenerative diseases.

Excitatory synapses that use glutamate as neurotransmitter make up the majority of synapses in the brain. Typical pyramidal neurons have as many as 10,000 synapses impinging on their

dendrites and somas. When a synapse is active, it causes a small depolarization of the postsynaptic neuronal membrane that can add to those caused by all other active synapses on the neuron. When the total depolarization reaches a threshold, the neuron fires an action potential along its axon to activate its own presynaptic terminals. The “strengths” of each synapse, that is the size of the depolarization it causes when it is activated, varies depending on the synapse’s history. The pattern of activity at a synapse can increase or decrease its strength by a process called synaptic plasticity¹⁻³.

Fig. 1. Synaptic Plasticity includes the processes of LTP and LTD. LTP involves the addition of new synaptic AMPA-type glutamate receptors (AMPA), which causes larger depolarization upon activation of the synapses. It also includes an increase in the size of the head of the postsynaptic spine, supported by an increase in the size and branching of the actin cytoskeleton. Long-term depression (LTD) is a long-lasting decrease in synaptic strength that involves a decrease in the number of synaptic AMPARs and shrinkage of the spine head. The area of the PSD contains scaffold proteins and signaling molecules that organize and catalyze these changes. Adapted from Fig. 1 of Kennedy, M. B. (2013) Cold Spring Harb. Perspect. Biol. 8, a016824.

In particular, if a synapse is repeatedly activated presynaptically at the same time as the postsynaptic neuron is strongly depolarized (for example, by a back-propagating action potential), the synapse becomes stronger by a process called long-term potentiation (LTP, Fig.

1
2
3 1). Conversely, if a synapse is repeatedly activated presynaptically while the postsynaptic
4 neuron is weakly depolarized (~ 30 mV or less), the synapse becomes weaker by a process called
5 long-term depression (LTD). Surprisingly, although these processes adjust synaptic strength in
6 opposite directions, both are driven by the flux of calcium ion through a specialized glutamate
7 receptor called the NMDA-type glutamate receptor (NMDAR)⁴. A synapse is tipped toward
8 LTP when the flux of Ca^{2+} is large and peaks rapidly; it is tipped toward LTD when the flux of
9 Ca^{2+} is relatively small and continues in small amounts over several minutes¹. The flux of Ca^{2+}
10 through the NMDAR is linked to the pattern of synaptic activity by the rules governing opening
11 of the NMDAR channel^{5,6}. The behavior of the NMDAR is now well understood and the
12 structure of its channel has been determined at the atomic level⁷. However, an important
13 challenge still remaining for biochemists interested in studying the brain is to unravel the
14 complex biochemical mechanisms that underlie LTP and LTD, and that determine how they are
15 produced by differing levels of synaptic activity.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

33 The set of signaling enzymes that initiates LTP and LTD resides in a structure called the
34 postsynaptic density (PSD) attached to the plaque of receptors located at the synaptic cleft^{8,9}.
35 The emerging view of this structure suggests that it constitutes a type of “membraneless
36 compartment”^{10,11} in the classical cell biological sense¹²⁻¹⁴. The PSD is comprised of a set of
37 scaffold proteins that concentrate and direct the reactions of a network of regulatory enzymes^{9,}
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Studies of the molecular composition of the PSD were initiated by the work of Philip
Seikevitz and Carl Cotman, who used cell fractionation techniques to isolate a subcellular
fraction from brain homogenates enriched in structures with the approximate thickness and
length of PSDs observed in the electron microscope e.g.,¹⁶. After a brief period of controversy as

to whether the PSD fraction represented a subcellular structure or simply an uninteresting protein aggregate, the issue was settled when my laboratory identified a new scaffold protein, PSD-95, highly enriched in the PSD fraction, and showed that it is localized nearly exclusively at the postsynaptic membranes of excitatory synapses¹⁷. These findings were “pre-genome” and involved micro-sequencing of proteins from PSD fractions separated on SDS-gels followed by cDNA cloning of the full length coding region of PSD-95. We raised antibodies against the protein and established its subcellular location by immunocytochemistry. Through the next decade, several core proteins that are highly localized in the postsynaptic density of intact tissue were identified in the PSD fraction by many laboratories^{8, 9, 18, 19}. The completion of the genome sequencing projects in ~2003 left us with a reasonably accurate list of the major core PSD proteins. Since then, a major task has been to understand how the machinery comprised of these proteins works to regulate synaptic strength.

Before outlining some of the biochemical opportunities for studying this molecular machinery, I’ll address an often-voiced notion that the PSD contains so many individual proteins that it seems an impossibly complex structure to decipher. Early proteomic studies employing mass spectrometry, resulted in identification of one to two hundred proteins said to be associated with postsynaptic machinery^{20, 21}. However, these conclusions should to be tempered by two considerations. First, like any fractionation technique, cell biological methods for isolating postsynaptic proteins, or postsynaptic density proteins, are capable only of enriching stable structures that are believed to represent the subcellular organelle of interest. They never result in completely pure preparations. On the other hand, the detection of proteins by proteomic mass spectrometry is extremely sensitive and so will identify minor and/or contaminating components as well as major constituents of any subcellular fraction. The early proteomic studies of synapses

Fig. 2. The PSD includes four major classes of scaffold proteins that are found only, or primarily, in the PSD. **MAGUKs** (Membrane-Associated GUanylate Kinases) contain three PDZ (PSD-95, Discs Large, ZO1) domains, an SH3 (Src Homology 3) domain, and a GUK (Guanylate Kinase-like) domain. **SAPAPs** (Synapse-Associated Protein-Associated Proteins) or **GKAPs** (Guanylate Kinase-Associated Proteins) contain a domain of five 14-residue repeats that bind directly to the GUK domain of PSD-95, a GH1 (region of homology to a *C. elegans* protein) domain, and a 7 residue PDZ ligand at the C-terminus that associates with the PDZ domain of SHANKs. **SHANKs** (SH3 domain, ANKyrin repeats) contain multiple Ankyrin repeats at the N-terminus, an SH3 domain, a PDZ domain that binds to SAPAPs, an extended proline-rich disordered domain, and an N-terminal SAM (Sterile Alpha Motif) that can self-associate. **Homers** contain an EVH1 domain that binds metabotropic and IP3 receptors, and a coiled-coil domain by which the Homers multimerize. Only Homer-1a lacks the coiled-coil domain.

did not distinguish between highly abundant, enriched proteins in the fraction and more minor proteins. In addition, the high number of proteins includes individual members of families of closely related proteins that contain several homologues. More refined studies, that take advantage of quantitative methods ^{22, 23}, and consideration of immunocytochemical studies to cross-check the subcellular localizations of proteins, point to a smaller set of proteins that constitute the “core” PSD proteins. Investigators differ on the exact size of this set, but it appears to me to be no smaller than ~30 and no larger than ~60. This set of proteins is likely present in nearly all PSD structures in excitatory postsynaptic spines, some at high abundance and others at just a few copies.

Four classes of scaffold proteins comprise most of the central scaffold that holds together the postsynaptic density; MAGUKs, SHANKs, SAPAPs, and HOMERs (Fig. 2). The MAGUK (Membrane-Associated GUanylate Kinase) family, includes PSD-95 (SAP90), PSD-93 (Chapsyn), SAP97, and SAP102 ^{24, 25}. Each contains three PDZ domains that are the business end of the MAGUK scaffolds because they bind to receptors and to a variety of signaling enzymes that regulate synaptic strength. Each PDZ domain binds a 5 to 7 residue motif usually occurring at the C-terminus of ligand proteins. The guanylate kinase domains (which are inactive) bind directly to the SAPAPs (also called GKAPs), which in turn link via their C-terminus to the PDZ domain in SHANK ²⁶. The affinity of SAPAPs for the PDZ domain is strengthened by additional binding between residues adjacent to the PDZ ligand and the PDZ domain in SHANK ²⁷. The SHANKs reside about 12 nm more distal to the membrane than the MAGUKs ²⁸. They can adopt sheet-like tertiary structures mediated by association of their SAM domains, suggesting that they form an underlying “base” to which the more proximal scaffold proteins are anchored ^{29, 30}. The HOMERs multimerize to form rod shapes bearing protein-

Fig. 3. Cartoon of the Organization of the Postsynaptic Density (PSD). Signaling proteins are immobilized near receptors at the synapse by a core group of scaffold proteins including PSD-95, GKAP, SHANK, and Homer. Electron microscopic immunocytochemistry has revealed an approximate organization of the scaffold proteins (see text). However, the precise organization of individual proteins and the mechanisms by which the dynamics of the structure are regulated remain to be determined. Adapted from Fig. 4 of Kennedy, M. B. (2013) *Cold Spring Harb. Perspect. Biol.* 8, a016824.

binding EVH domains at either end^{31, 32}. They link metabotropic glutamate receptors, IP3 receptors, and TRP channels to the SHANK scaffold³³.

The emerging picture of how these core proteins are arranged is still poorly resolved (Fig. 3). Thus, an important challenge for biochemists is to understand at the structural level how the core proteins interact specifically with each other and how dynamic changes in the organization of the PSD occur as synaptic strength is altered in response to activity. These issues represent a fertile opportunity for biochemists willing to tackle the complexity of excitatory synaptic machinery in the CNS. The conceptual and methodological challenges are similar to those posed by many

other dynamic cytosolic protein machines, including chromosomes, spindles, kinetochores, ribosomes, and spliceosomes. How do the individual scaffold proteins interact with each other and how is their interaction regulated? How does competition for binding to certain protein binding domains shape the ultimate configuration of the machinery?³⁴ What role do liquid-liquid phase transitions¹¹ play in formation of the “membraneless” cellular compartment. Finding solutions to these questions takes on additional salience because mutations in several of the proteins most central to the PSD can cause or contribute to Alzheimer’s disease³⁵, mental illnesses³⁶, autism spectrum disorders³⁷, and intellectual disability³⁸.

References

- [1] Sjöström, P. J., and Nelson, S. B. (2002) Spike timing, calcium signals and synaptic plasticity, *Curr. Opin. Neurobiol.* 12, 305-314.
- [2] Ho, V. M., Lee, J. A., and Martin, K. C. (2011) The cell biology of synaptic plasticity, *Science* 334, 623-628.
- [3] Kennedy, M. B. (2013) Synaptic signaling in learning and memory, *Cold Spring Harb. Perspect. Biol.* 8, a016824.
- [4] Mayer, M. L. (2017) The structure and function of glutamate receptors: Mg(2+) block to X-ray diffraction, *Neuropharmacology* 112, 4-10.
- [5] Mayer, M. L., Westbrook, G. L., and Guthrie, P. B. (1984) Voltage-dependent block by Mg²⁺ of NMDA responses in spinal cord neurones, *Nature* 309, 261-263.
- [6] Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., and Prochiantz, A. (1984) Magnesium gates glutamate-activated channels in mouse central neurones, *Nature* 307, 462-465.
- [7] Mayer, M. L. (2006) Glutamate receptors at atomic resolution, *Nature* 440, 456-462.

- [8] Kennedy, M. B. (1997) The postsynaptic density at glutamatergic synapses, *Trends Neurosci.* 20, 264-268.
- [9] Kennedy, M. B. (2000) Signal-processing machines at the postsynaptic density, *Science* 290, 750-754.
- [10] Hyman, A. A., and Simons, K. (2012) Cell biology. Beyond oil and water--phase transitions in cells, *Science* 337, 1047-1049.
- [11] Li, P., Banjade, S., Cheng, H. C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J. V., King, D. S., Banani, S. F., Russo, P. S., Jiang, Q. X., Nixon, B. T., and Rosen, M. K. (2012) Phase transitions in the assembly of multivalent signalling proteins, *Nature* 483, 336-340.
- [12] Yang, Y., Tao-Cheng, J. H., Bayer, K. U., Reese, T. S., and Dosemeci, A. (2013) CaMKII-mediated phosphorylation regulates distributions of SynGAP- α 1 and - α 2 at the postsynaptic density, *PLoS One* 8, e71795.
- [13] Zeng, M., Shang, Y., Araki, Y., Guo, T., Huganir, R. L., and Zhang, M. (2016) Phase Transition in Postsynaptic Densities Underlies Formation of Synaptic Complexes and Synaptic Plasticity, *Cell* 166, 1163-1175 e1112.
- [14] Kennedy, M. B., and Mastro, T. L. (2017) Liquid Phase Transition in the Postsynaptic Density?, *Trends Biochem. Sci.* 42, 2-4.
- [15] Sheng, M., and Kim, E. (2011) The postsynaptic organization of synapses, In *Cold Spring Harb. Perspect. Biol.* 2011/11/03 ed., p a005678.
- [16] Cohen, R. S., Blomberg, F., Berzins, K., and Siekevitz, P. (1977) The structure of postsynaptic densities isolated from dog cerebral cortex I. overall morphology and protein composition, *J. Cell Biol.* 74, 181-203.
- [17] Cho, K.-O., Hunt, C. A., and Kennedy, M. B. (1992) The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein, *Neuron* 9, 929-942.
- [18] Boeckers, T. M., Kreutz, M. R., Winter, C., Zuschratter, W., Smalla, K. H., Sanmarti-Vila, L., Wex, H., Langnaese, K., Bockmann, J., Garner, C. C., and Gundelfinger, E. D. (1999) Proline-rich synapse-

associated protein-1/cortactin binding protein 1 (ProSAP1/CortBP1) is a PDZ-domain protein highly enriched in the postsynaptic density, *J. Neurosci.* 19, 6506-6518.

[19] Sheng, M., and Hoogenraad, C. C. (2007) The postsynaptic architecture of excitatory synapses: a more quantitative view, *Annu. Rev. Biochem.* 76, 823-847.

[20] Husi, H., Ward, M. A., Choudhary, J. S., Blackstock, W. P., and Grant, S. G. N. (2000) Proteomic analysis of NMDA receptor-adhesion protein signaling complexes, *Nat. Neurosci.* 3, 661-669.

[21] Peng, J., Kim, M. J., Cheng, D., Duong, D. M., Gygi, S. P., and Sheng, M. (2004) Semiquantitative proteomic analysis of rat forebrain postsynaptic density fractions by mass spectrometry, *J. Biol. Chem.* 279, 21003-21011.

[22] Chen, X., Vinade, L., Leapman, R. D., Petersen, J. D., Nakagawa, T., Phillips, T. M., Sheng, M., and Reese, T. S. (2005) Mass of the postsynaptic density and enumeration of three key molecules, *Proc. Natl. Acad. Sci. U. S. A.* 102, 11551-11556.

[23] Dosemeci, A., Makusky, A. J., Jankowska-Stephens, E., Yang, X., Slotta, D. J., and Markey, S. P. (2007) Composition of the synaptic PSD-95 complex, *Mol. Cell. Proteomics* 6, 1749-1760.

[24] Kornau, H.-C., Seeburg, P. H., and Kennedy, M. B. (1997) Interaction of ion channels and receptors with PDZ domain proteins, *Curr. Opin. Neurobiol.* 7, 368-373.

[25] Zhu, J., Shang, Y., and Zhang, M. (2016) Mechanistic basis of MAGUK-organized complexes in synaptic development and signalling, *Nat. Rev. Neurosci.* 17, 209-223.

[26] Sheng, M., and Kim, E. (2000) The Shank family of scaffold proteins, *J. Cell Sci.* 113, 1851-1856.

[27] Zeng, M., Shang, Y., Guo, T., He, Q., Yung, W. H., Liu, K., and Zhang, M. (2016) A binding site outside the canonical PDZ domain determines the specific interaction between Shank and SAPAP and their function, *Proc. Natl. Acad. Sci. U. S. A.* 113, E3081-3090.

[28] Valtschanoff, J. G., and Weinberg, R. J. (2001) Laminar organization of the NMDA receptor complex within the postsynaptic density, *J. Neurosci.* 21, 1211-1217.

- [29] Baron, M. K., Boeckers, T. M., Vaida, B., Faham, S., Gingery, M., Sawaya, M. R., Salyer, D., Gundelfinger, E. D., and Bowie, J. U. (2006) An architectural framework that may lie at the core of the postsynaptic density, *Science* 311, 531-535.
- [30] Hayashi, M. K., Tang, C., Verpelli, C., Narayanan, R., Stearns, M. H., Xu, R. M., Li, H., Sala, C., and Hayashi, Y. (2009) The postsynaptic density proteins Homer and Shank form a polymeric network structure, *Cell* 137, 159-171.
- [31] Beneken, J., Tu, J. C., Xiao, B., Nuriya, M., Yuan, J. P., Worley, P. F., and Leahy, D. J. (2000) Structure of the Homer EVH1 domain-peptide complex reveals a new twist in polyproline recognition, *Neuron* 26, 143-154.
- [32] Hayashi, M. K., Ames, H. M., and Hayashi, Y. (2006) Tetrameric hub structure of postsynaptic scaffolding protein homer, *J. Neurosci.* 26, 8492-8501.
- [33] Worley, P. F., Zeng, W., Huang, G., Kim, J. Y., Shin, D. M., Kim, M. S., Yuan, J. P., Kiselyov, K., and Muallem, S. (2007) Homer proteins in Ca²⁺ signaling by excitable and non-excitable cells, *Cell Calcium* 42, 363-371.
- [34] Walkup, W. G. t., Mastro, T. L., Schenker, L. T., Vielmetter, J., Hu, R., Iancu, A., Reghunathan, M., Bannon, B. D., and Kennedy, M. B. (2016) A model for regulation by SynGAP- α 1 of binding of synaptic proteins to PDZ-domain 'Slots' in the postsynaptic density, *Elife* 5, e16813.
- [35] Mucke, L., and Selkoe, D. J. (2012) Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction, *Cold Spring Harb. Perspect. Med.* 2, a006338.
- [36] Ripke, S., and Sanders, A. R., and Kendler, K. S., and Levinson, D. F., and Sklar, P., and Holmans, P. A., and Lin, D. Y., and Duan, J., and Ophoff, R. A., and Andreassen, O. A., and Scolnick, E., and Cichon, S., and St Clair, D., and Corvin, A., and Gurling, H., and Werge, T., and Rujescu, D., and Blackwood, D. H., and Pato, C. N., and Malhotra, A. K., and Purcell, S., and Dudbridge, F., and Neale, B. M., and Rossin, L., and Visscher, P. M., and Posthuma, D., and Ruderfer, D. M., and Fanous, A., and Stefansson, H., and Steinberg, S., and Mowry, B. J., and Golimbet, V., and De Hert, M., and Jonsson, E. G., and Bitter, I., and Pietilainen, O. P., and Collier, D. A., and Tosato, S., and Agartz, I., and Albus, M.,

and Alexander, M., and Amdur, R. L., and Amin, F., and Bass, N., and Bergen, S. E., and Black, D. W.,
and Borglum, A. D., and Brown, M. A., and Bruggeman, R., and Buccola, N. G., and Byerley, W. F., and
Cahn, W., and Cantor, R. M., and Carr, V. J., and Catts, S. V., and Choudhury, K., and Cloninger, C. R.,
and Cormican, P., and Craddock, N., and Danoy, P. A., and Datta, S., and de Haan, L., and Demontis, D.,
and Dikeos, D., and Djurovic, S., and Donnelly, P., and Donohoe, G., and Duong, L., and Dwyer, S., and
Fink-Jensen, A., and Freedman, R., and Freimer, N. B., and Friedl, M., and Georgieva, L., and Giegling,
I., and Gill, M., and Glenthøj, B., and Godard, S., and Hamshire, M., and Hansen, M., and Hansen, T.,
and Hartmann, A. M., and Henskens, F. A., and Hougaard, D. M., and Hultman, C. M., and Ingason, A.,
and Jablensky, A. V., and Jakobsen, K. D., and Jay, M., and Jurgens, G., and Kahn, R. S., and Keller, M.
C., and Kenis, G., and Kenny, E., and Kim, Y., and Kirov, G. K., and Konnerth, H., and Konte, B., and
Krabbendam, L., and Krasucki, R., and Lasseter, V. K., and Laurent, C., and Lawrence, J., and Lencz, T.,
and Lerer, F. B., and Liang, K. Y., and Lichtenstein, P., and Lieberman, J. A., and Linszen, D. H., and
Lonnqvist, J., and Loughland, C. M., and Maclean, A. W., and Maher, B. S., and Maier, W., and Mallet,
J., and Malloy, P., and Mattheisen, M., and Mattingsdal, M., and McGhee, K. A., and McGrath, J. J., and
McIntosh, A., and McLean, D. E., and McQuillin, A., and Melle, I., and Michie, P. T., and Milanova, V.,
and Morris, D. W., and Mors, O., and Mortensen, P. B., and Moskvina, V., and Muglia, P., and Myin-
Germeys, I., and Nertney, D. A., and Nestadt, G., and Nielsen, J., and Nikolov, I., and Nordentoft, M.,
and Norton, N., and Nothen, M. M., and O'Dushlaine, C. T., and Olincy, A., and Olsen, L., and O'Neill, F.
A., and Orntoft, T. F., and Owen, M. J., and Pantelis, C., and Papadimitriou, G., and Pato, M. T., and
Peltonen, L., and Petursson, H., and Pickard, B., and Pimm, J., and Pulver, A. E., and Puri, V., and
Quested, D., and Quinn, E. M., and Rasmussen, H. B., and Rethelyi, J. M., and Ribble, R., and Rietschel,
M., and Riley, B. P., and Ruggeri, M., and Schall, U., and Schulze, T. G., and Schwab, S. G., and Scott,
R. J., and Shi, J., and Sigurdsson, E., and Silverman, J. M., and Spencer, C. C., and Stefansson, K., and
Strange, A., and Strengman, E., and Stroup, T. S., and Suvisaari, J., and Terenius, L., and Thirumalai, S.,
and Thygesen, J. H., and Timm, S., and Toncheva, D., and van den Oord, E., and van Os, J., and van
Winkel, R., and Veldink, J., and Walsh, D., and Wang, A. G., and Wiersma, D., and Wildenauer, D. B.,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

and Williams, H. J., and Williams, N. M., and Wormley, B., and Zammit, S., and Sullivan, P. F., and O'Donovan, M. C., and Daly, M. J., and Gejman, P. V., and Schizophrenia Psychiatric Genome-Wide Association Study, C. (2011) Genome-wide association study identifies five new schizophrenia loci, *Nat. Genet.* 43, 969-976.

[37] Zoghbi, H. Y., and Bear, M. F. (2012) Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities, *Cold Spring Harb. Perspect. Biol.* 4, a009886.

[38] Berryer, M. H., Hamdan, F. F., Klitten, L. L., Moller, R. S., Carmant, L., Schwartzentruber, J., Patry, L., Dobrzyniecka, S., Rochefort, D., Neugnot-Cerioli, M., Lacaille, J. C., Niu, Z., Eng, C. M., Yang, Y., Palardy, S., Belhumeur, C., Rouleau, G. A., Tommerup, N., Immken, L., Beauchamp, M. H., Patel, G. S., Majewski, J., Tarnopolsky, M. A., Scheffzek, K., Hjalgrim, H., Michaud, J. L., and Di Cristo, G. (2013) Mutations in SYNGAP1 cause intellectual disability, autism, and a specific form of epilepsy by inducing haploinsufficiency, *Hum. Mutat.* 34, 385-394.

Fig. 1. Synaptic Plasticity includes the processes of LTP and LTD. LTP involves the addition of new synaptic AMPA-type glutamate receptors (AMPARs), which causes larger depolarization upon activation of the synapses. It also includes an increase in the size of the head of the postsynaptic spine, supported by an increase in the size and branching of the actin cytoskeleton. Long-term depression (LTD) is a long-lasting decrease in synaptic strength that involves a decrease in the number of synaptic AMPARs and shrinkage of the spine head. The area of the PSD contains scaffold proteins and signaling molecules that organize and catalyze these changes. Adapted from Fig. 1 of Kennedy, M. B. (2013) Cold Spring Harb. Perspect. Biol. 8, a016824.

101x106mm (300 x 300 DPI)

Fig. 2. The PSD includes four major classes of scaffold proteins that are found only, or primarily, in the PSD. MAGUKs (Membrane-Associated GUanylate Kinases) contain three PDZ (PSD-95, Discs Large, ZO1) domains, an SH3 (Src Homology 3) domain, and a GUK (Guanylate Kinase-like) domain. SAPAPs (Synapse-Associated Protein-Associated Proteins) or GKAPs (Guanylate Kinase-Associated Proteins) contain a domain of five 14-residue repeats that bind directly to the GUK domain of PSD-95, a GH1 (region of homology to a *C. elegans* protein) domain, and a 7 residue PDZ ligand at the C-terminus that associates with the PDZ domain of SHANKs. SHANKs (SH3 domain, ANKyrin repeats) contain multiple Ankyrin repeats at the N-terminus, an SH3 domain, a PDZ domain that binds to SAPAPs, an extended proline-rich disordered domain, and an N-terminal SAM (Sterile Alpha Motif) that can self-associate. Homers contain an EVH1 domain that binds metabotropic and IP3 receptors, and a coiled-coil domain by which the Homers multimerize. Only Homer-1a lacks the coiled-coil domain.

203x212mm (300 x 300 DPI)

Fig. 3. Cartoon of the Organization of the Postsynaptic Density (PSD). Signaling proteins are immobilized near receptors at the synapse by a core group of scaffold proteins including PSD-95, GKAP, SHANK, and Homer. Electron microscopic immunocytochemistry has revealed an approximate organization of the scaffold proteins (see text). However, the precise organization of individual proteins and the mechanisms by which the dynamics of the structure are regulated remain to be determined. Adapted from Fig. 4 of Kennedy, M. B. (2013) Cold Spring Harb. Perspect. Biol. 8, a016824.

137x85mm (300 x 300 DPI)

86x44mm (300 x 300 DPI)