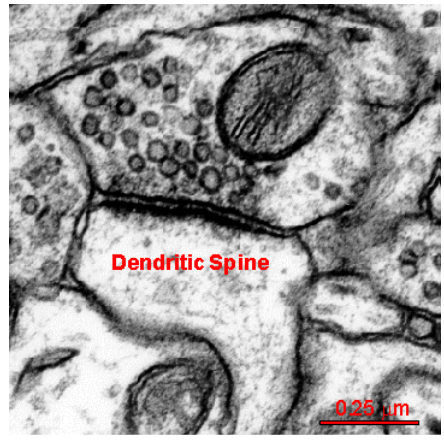
Lesson (9)

Inside the neuron V:

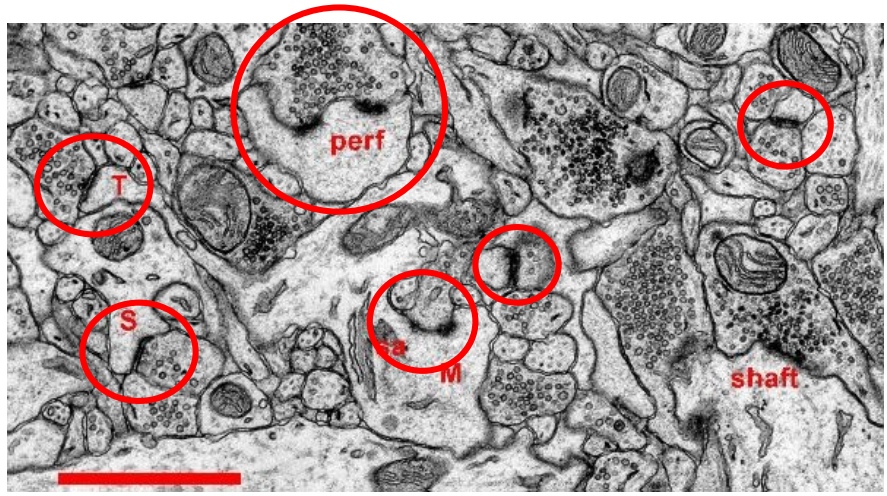
The postsynaptic density

The postsynaptic density (PSD)

The postsynaptic density (PSD) is a specialization of the cytoskeleton at the synaptic junction. It lies adjacent to the cytoplasmic face of the postsynaptic membrane, in close apposition to the active zone of the synapse and the docked synaptic vesicles in the presynaptic terminal.

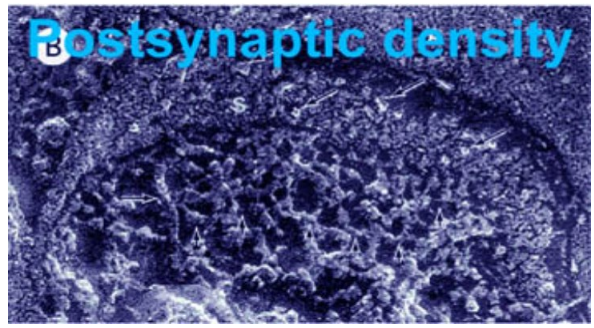


PSDs have large variety of shapes

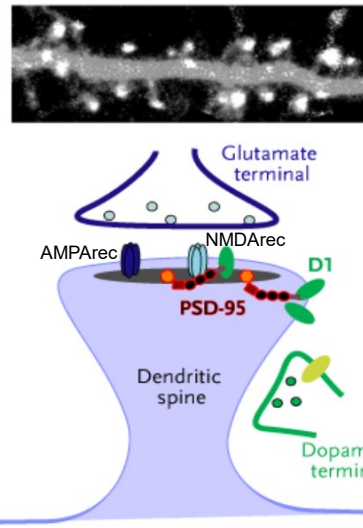


What is the PSD?

- A dense area on tip of spine head, occupying only 10-15% of total spine surface area.
- A membrane disk localizing glutamate receptors, protein kinases, and other signaling molecules associated with plasticity of synapses.
- PSD-95 is the most abundant protein.



What are the main components of PSD?



- A scaffolding protein named PSD95,
- AMPA and NMDA receptors,
- Other neurotransmitter receptors (e.g. D1 dopamine rec.)
- Adhesion molecules.
- Signalling proteins
- Cytoskeleton

This organization focuses the synaptic signal, increasing its efficiency, accuracy and speed.

Organization of the core structure of the postsynaptic density

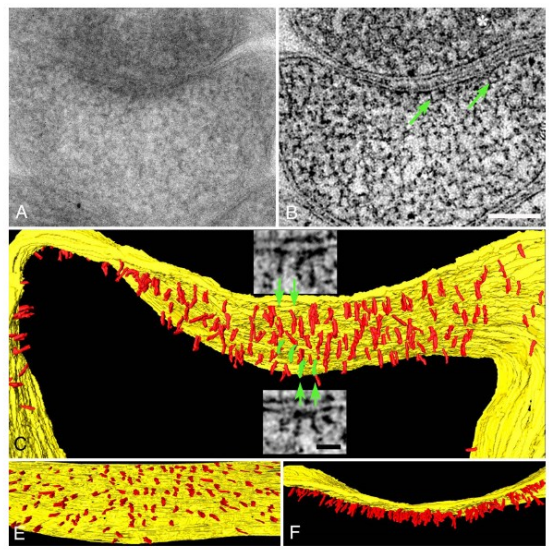
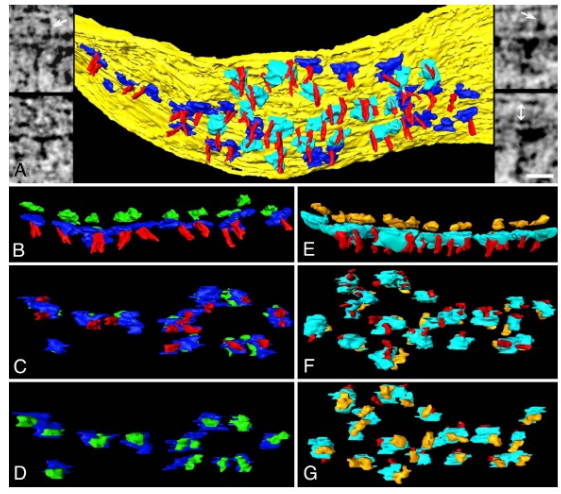


Fig. 1. Vertical filaments at the PSD. (*A*) EM of a PSD in a mushroom-shaped dendritic spine. Structural details are obscured by overlap within this 120-nm-thick section. (*B*) Vertical filaments (green arrows) are apparent in a 1.5-nm-thick virtual section derived from the tomographic reconstruction of the section in *A*. The synaptic vesicle is indicated by an asterisk. (Scale bar: 100 nm.) (*C*) Rendering of vertical filaments (red) from the tomographic reconstruction. Vertical filaments, 5 nm in diameter and 20 nm long, contact the postsynaptic membrane (yellow). (*Insets*) Virtual sections from which particular vertical filaments (green) are segmented. (Scale bar: 20 nm.) (*D*) *En face* view showing uniform distribution of vertical filaments at the PSD. (*E*) Overlap of vertical filaments contributes to the typical thickened appearance of a PSD viewed in cross-section.

Chen et al. PNAS, March 18, 2008. vol. 105. 11:4453–4458

AMPAR-type (blue) and NMDAR-type cytoplasmic domains (cyan) associate with vertical PSD 95 filaments (red).



30–100AMPA receptors structures at PSD

16–25 NMDA receptors are arranged in a rhombic lattice in the central cluster of a typical PSD,

Fig. 3. Transmembrane structures at the PSD. (*A*) Cytoplasmic surface of the membrane (yellow) at the PSD, showing AMPAR-type (blue) and NMDAR-type cytoplasmic domains (cyan), and vertical filaments (red). (*Left Insets*) AMPAR-type structures in virtual sections from the tomographic reconstruction. Arrow points to a transcleft filament. (*Right Insets*) NMDAR-type structures. Arrow points to a transcleft filament, and double arrow (lower right) indicates the extent of synaptic cleft. (Scale bar: 20 nm.) (*B–D*) AMPAR-type structures shown in cross-section (*B*, extracellular domain green), *en face* from inside the spine (*C*), and *en face* from outside the spine (*D*). Cytoplasmic domains of AMPAR-type structures are contacted by vertical filaments (*C*). (*E–G*) NMDAR-type structures shown in cross-section (*E*, extracellular domain gold), *en face* from inside the spine (*F*), and *en face* from outside the spine (*G*). Cytoplasmic domains of NMDAR-type structures are contacted by one or two vertical filaments (*F*).

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Core structure of the PSD based on tomographic reconstructions.

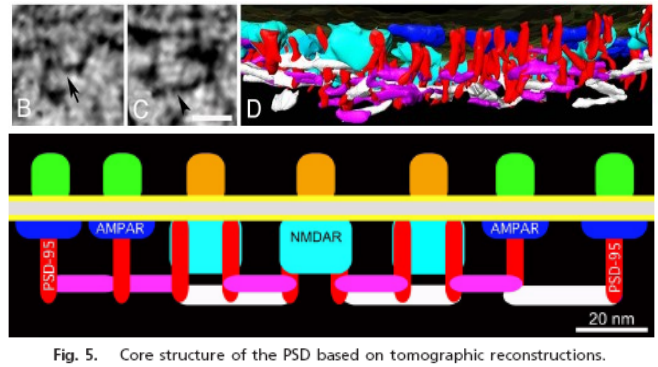
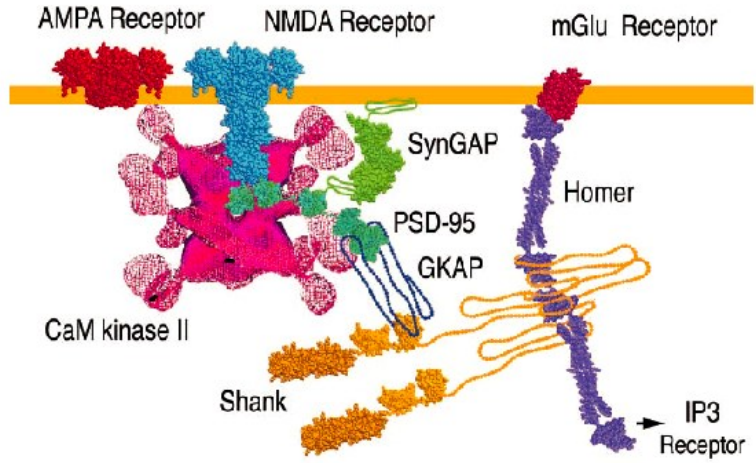


Fig. 5. Core structure of the PSD based on tomographic reconstructions. Components and spacings between components are drawn approximately to scale. Dominant structure is an array of vertical filaments containing PSD-95 (red). NMDAR-type structures, differentiated by their large cytoplasmic extensions (cyan), concentrate in the center of the PSD. AMPAR-type structures, differentiated by their flattened cytoplasmic aspects (blue), surround the NMDAR structures. Virtually all NMDAR- and AMPAR-type structures are contacted by vertical filaments. Short horizontal filaments (purple) link vertical filaments associated with both NMDAR- and AMPAR-type structures. Longer horizontal filaments (white) concentrated under the NMDAR-type structures cross-link the vertical filament meshwork.

Core structure of the PSD based on protein-protein interactions



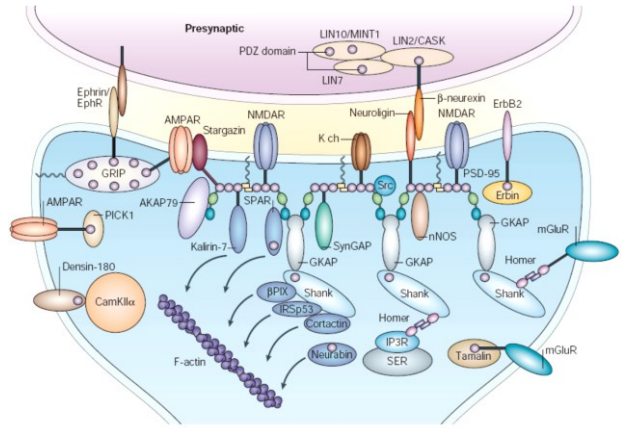


Figure 3 | **A schematic diagram of the organization of PDZ proteins at a mammalian excitatory synapse.** The main PDZ-containing proteins of a glutamatergic synapse are shown, focusing on the postsynaptic density. PDZ domains are indicated by purple circles. The C-terminal cytoplasmic tails of membrane proteins are indicated by black lines. Specific protein–protein interactions are indicated by the overlap of proteins. Only a subset of known protein interactions is illustrated. Although not shown, LIN2, LIN7 and LIN10 are also present postsynaptically, and many of the proteins of the postsynaptic domain are also present in the presynaptic terminal. Green and blue ellipses in PSD-95 represent SH3 and GK domains, respectively. Crooked lines indicate palmitoylation of PSD-95 and GRIP. Grey arrows indicate binding and/or regulatory actions of proteins on the actin cytoskeleton. AKAP79, A-kinase anchor protein 79; AMPAR, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor; βPIX, PAAK-interactive exchange factor; CaMKIIα, α-subunit of Ca^{2+}/calmodulin-dependent protein kinase II; GK, guanylate kinase-like domain; EphR, ephrin receptor; ErbB2, EGF-related peptide receptor; GKAP, guanylate kinase-associated protein; GRIP, glutamate-receptor-interacting protein; IP3R, IP3 receptor; IRSp53, insulin-receptor substrate p53; K ch, potassium channel; LIN7, lin7 homologue; LIN10, lin10 homologue; mGluR, metabotropic glutamate receptor; NMDAR, NMDA (*N*-methyl-D-aspartate) receptor; nNOS, neuronal nitric oxide synthase; PICK1, protein interacting with C kinase 1; PSD-95, postsynaptic density protein 95; SER, smooth endoplasmic reticulum; SH3, Src homology 3 domain; Shank, SH3 and ankyrin repeat-containing potein; SPAR, spine-associated RapGAP; SynGAP, synaptic Ras GTPase-activating protein.

Protein Organization of the Postsynaptic Density

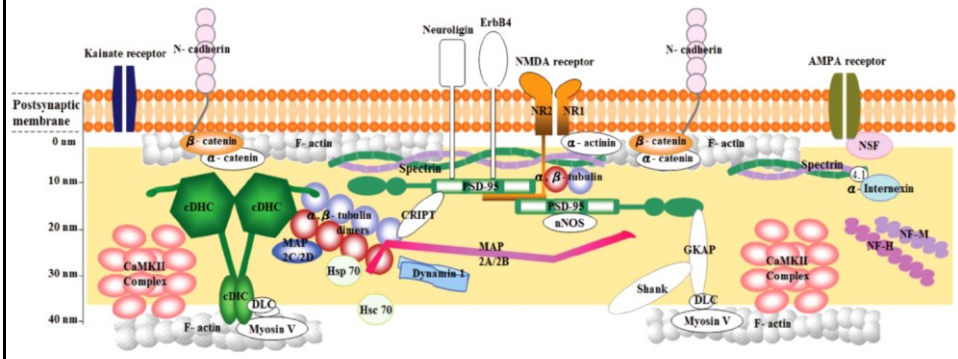
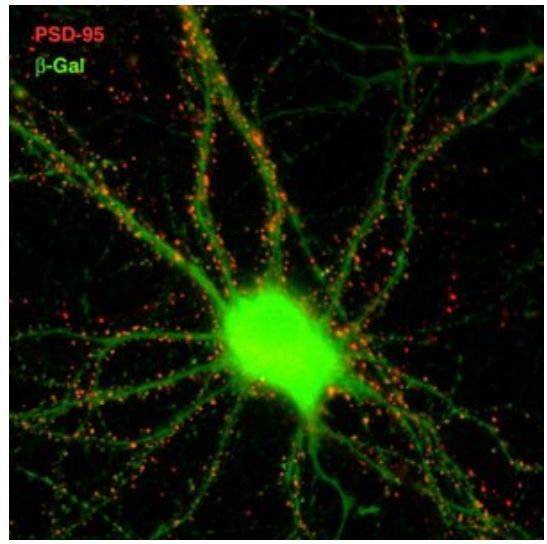


FIG. 8. **A molecular model of the PSD.** The PSD is assumed to be a disk-shaped protein complex of ~41 nm in thickness spanning from beneath the postsynaptic membrane to the cytoplasm of dendritic spines. A layer of phospholipid membrane is added to the PSD model to indicate the side of the PSD that faces the postsynaptic membrane. The cDIC, AMPA receptors, kainate receptors, N-cadherin, β-catenin, NSF, heat shock 70-kDa protein 8 (a member of hsc70), and actin reside primarily in the space within 3.5 nm from the surface, whereas the α,β-tubulin subunits, cDHC, MAP2A/2B, MAP2C/2D, spectrin, neurofilament heavy subunit (*NF-H*), neurofilament medium subunit (*NF-M*), heat shock 70-kDa protein 12A (a member of hsp70), α-internexin, dynamin-1, and PSD-95 reside primarily in a region deeper than 3.5 nm from the surface (*yellow* colored) of the PSD. α-CaMKII subunits form large complexes sitting at the edge of the cytoplasm-facing surface of the PSD. NMDA receptors reside on the postsynaptic membrane-facing surface of the PSD and are in close association with large proteins that also reside on the postsynaptic membrane. *DLC*, light chain of dynein; *nNOS*, neuronal nitric-oxide synthase; *GKAP*, guanylate kinase-associated protein.

Liu et al., 2006. Molecular & Cellular Proteomics 5:1019–1032.



Rat **hippocampal neuron** in culture expressing beta-Gal to visualize the dendrites, and immunostained for beta-Gal (green) and **PSD-95** (red), a protein enriched in postsynaptic structures, the dendritic spines.

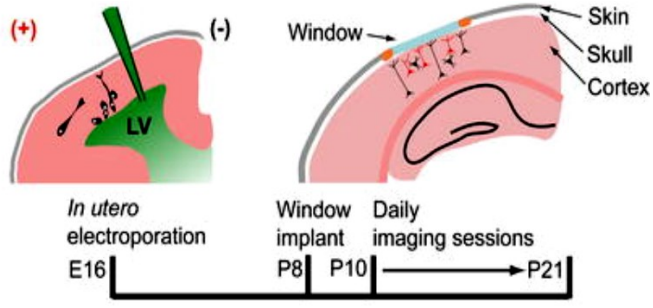
PSD-95 is known to last only for hours

-How do synapses maintain their size and strength with unstable constituents?

>>the investigators addressed this issue by studying the movement of PSD-95.

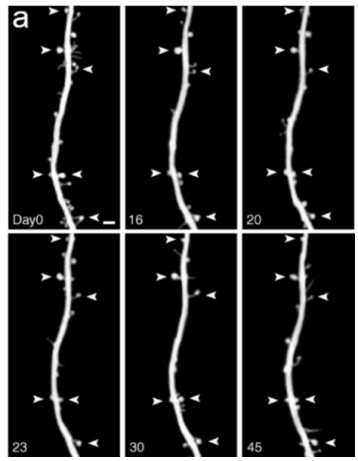
Rapid Redistribution of Synaptic PSD-95 in the Neocortex In Vivo
Noah Gray et. al. PLOS Biology 2006

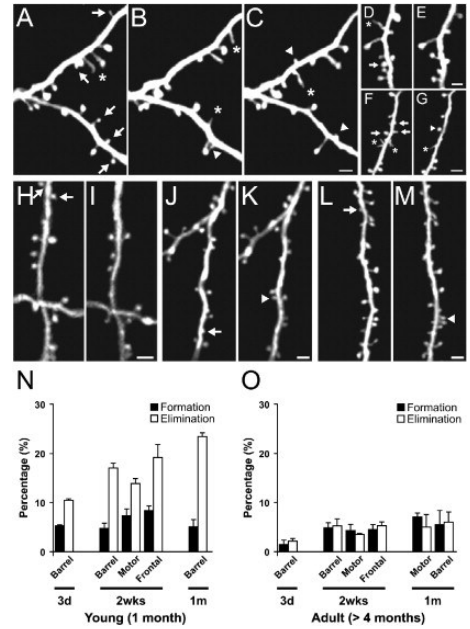
In vivo experiment



Procedure: DNA Constructs: RFP mCherry and GFP-PSD-95
Electroporation: transfection at E16 into L2/3 pyramidal cells
Surgery: implant imaging window at P8
Imaging: done daily at P10-P21 with 2-photon microscope

Wen Biao Gan
Langone Medical Centre
New York University





Neuron, Vol. 27, 219–225, August, 2000, Copyright ©2000 by Cell Press

Multicolor "DiOlistic" Labeling of the Nervous System Using Lipophilic Dye Combinations

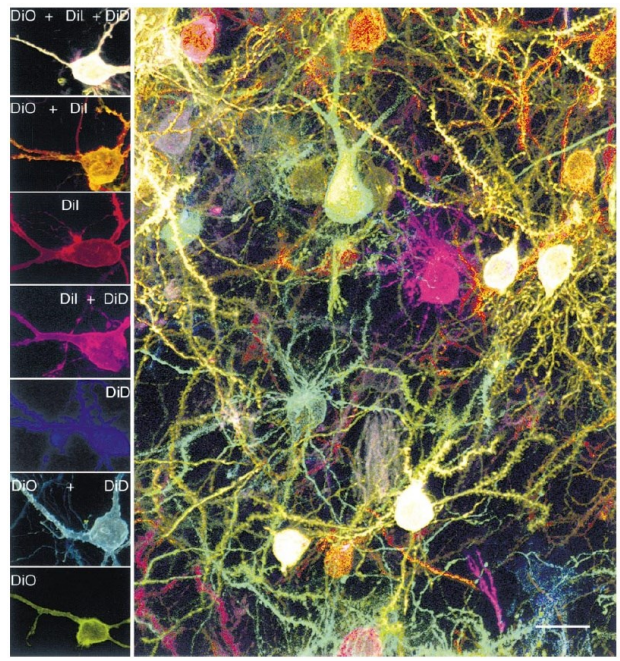


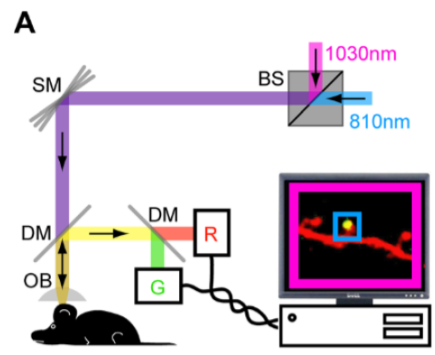
Figure 1. Multicolor Labeling of Cortical Neurons with Lipophilic Dye–Coated Particles

Methods

- During the second week of life, dendritic spines begin to stabilize.
- Photoactivatable-GFP (pa-GFP) was used to monitor diffusion of one PSD-95 protein.
- pa-GFP is a type of GFP that is not normally fluorescent but can be 'photo-activated' by a specific wavelength of light (810nm)

Rapid Distribution of Synaptic PSD-95

- PSD-95 was tagged with paGFP.
- Dendrite activated at 1030nm wavelength.
- Used a 2-photon excitation at 810nm, converting the dark paGFP into bright fluorophore:

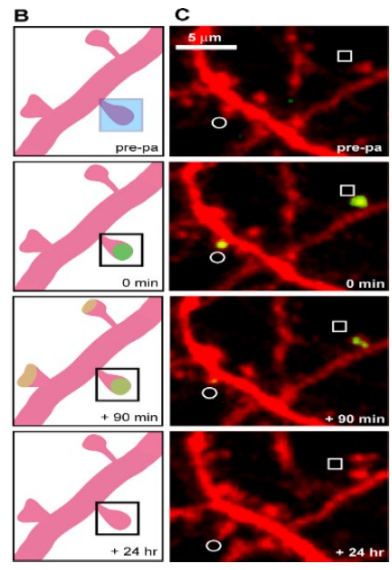


Key Points

- Spines seem to appear and disappear on daily basis, which is consistent with high rates of synaptic formation and elimination during developmental stage in mice.
- Dendritic spines and their PSDs are quite stable around second postnatal week.
- Stabilization also conserves the sizes of the spines.

Results

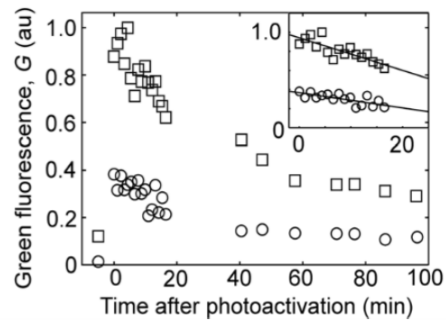
- Revealed green puncta in photoactivated spines.
- Within those spines, PSD-95-paGFP fluorescence decayed at an exponential time, proceeded by a long fluorescent tail.
- Exponential time lasted 10mins and paGFP was no longer detectable after a day.



Results

- Exponential decay reflects the escaping (unbound) PSD-95 to PSDs in individual dendritic spines.
- The long tail shows the PSD-95 trapped in the dendrite.

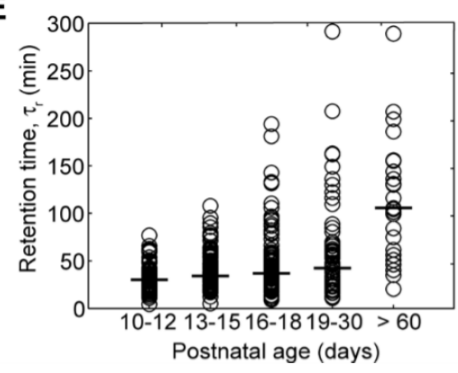
D



Results

- Retention time (T_r) is the time constant for the exponential component of the fluorescence decay.
- It measures the average time over where PSD-95 molecules as associated in an individual spine.
- Retention time increased as number of PSDs decreased.

E



Key Points

With increasing developmental age:

- PSD-95 are bound to PSDs for approximately an hour, significantly shorter than the lifetime of dendritic spines and their PSDs and the PSD-95.
- PSD-95 became less dynamic in the dendritic spines.
- PSDs decreased as development increased.
- However, retention time in PSDs increased.

What mechanisms keep PSD-95-paGFP, thus determining the retention time?

2 possible reasons:

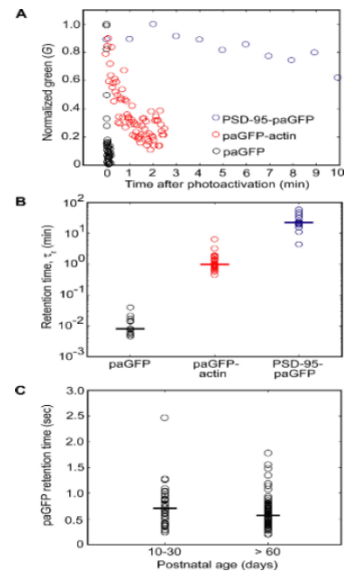
- Retention time of PSD-95-paGFP could reflect unbinding of PSD-95 from PSD.
- PSD-95 could be trapped in the spine head of diffusional compartmentalization by the narrow spine neck.

Measured retention times for other proteins not known to be concentrated in the PSD:

- Cytoplasmic paGFP – determined by spine geometry alone.
- paGFP-actin – depended on cycling of actin in dendritic spines.
- PSD-95-paGFP

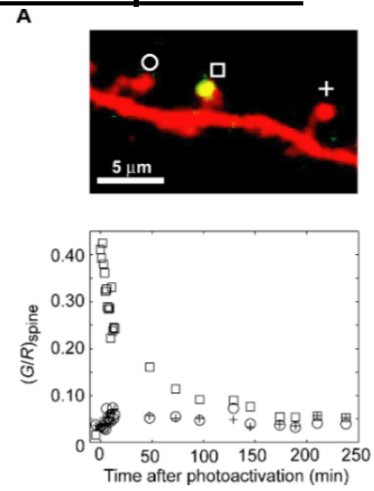
Results

- The retention times for paGFP were 1000 x shorter than PSD-95-paGFP and independent of developmental age.
- However, paGFP increased with spine volume.
- The retention time for paGFP-actin was intermediate.



Are PSD-95 exchanged between PSDs in different spines?

- Fluorescence appeared in neighboring spines after photoactivating PSD-95-paGFP in a single spine
- Spread of PSD-95 was bidirectional, and did not seem to involve obvious transport particles.
- PSD-95-paGFP spread rapidly over small distances.



Key Points

- PSD-95 spreads from PSD to PSD by diffusion.
- Hence, PSDs share a common pool of PSD-95.

Synapse-Specific Capture and Retention

What governs the diffusional exchange of PSD-95 between spines?

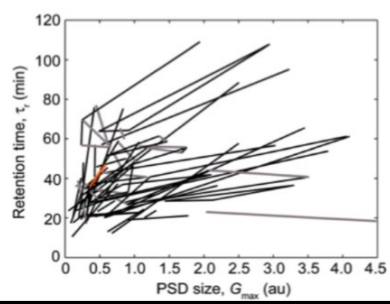
After PSD-95 unbinds from a PSD it rapidly diffuses along the dendritic shaft until captured by other PSDs.

PSD-95 content of a PSD is roughly proportional to the PSD size(57,58)

Stable sets of PSD-95 binding sites at individual PSDs could explain the stability of PSD-95 clusters

Results Review

- PSD-95 is retained by individual PSDs for a very short time
- PSD-95 unbinds and diffuses rapidly within the dendrite and binds to other PSDs
- PSD-95 visit dozens of PSDs before degradation; synapses are sharing dendritic PSD-95
- Larger spines have longer PSD-95 retention times



Results Review

- PSD-95 is retained by individual PSDs for a very short time in comparison
- PSD-95 unbinds and diffuses rapidly within the dendrite and binds to other PSDs
- PSD-95 visit dozens of PSDs before degradation; synapses are sharing dendritic PSD-95
- Larger spines have longer PSD-95 retention times
- Spine stability increases with developmental age

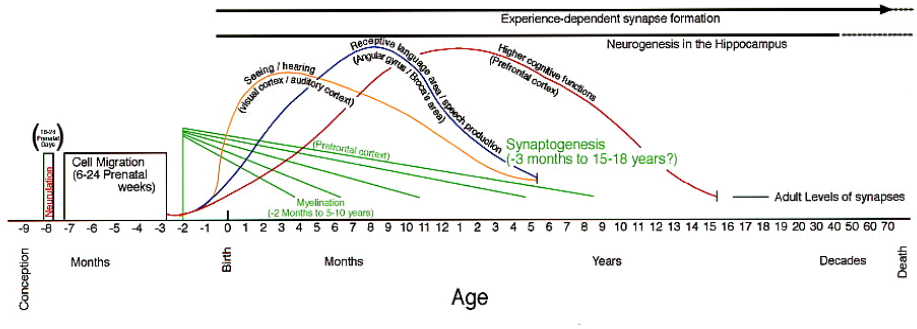
Interpretations

- [PSD-95 binders] > [PSD-95] = competition
- PSD-95 levels may determine synaptic strength and combined with other PSD molecules determine synaptic size
- Redistribution of PSD-95 could play a role in synaptic plasticity (vs. gene alteration)
- Redistribution of PSD-95 (or other PSD molecules) contributes to induction and maintenance of LTP

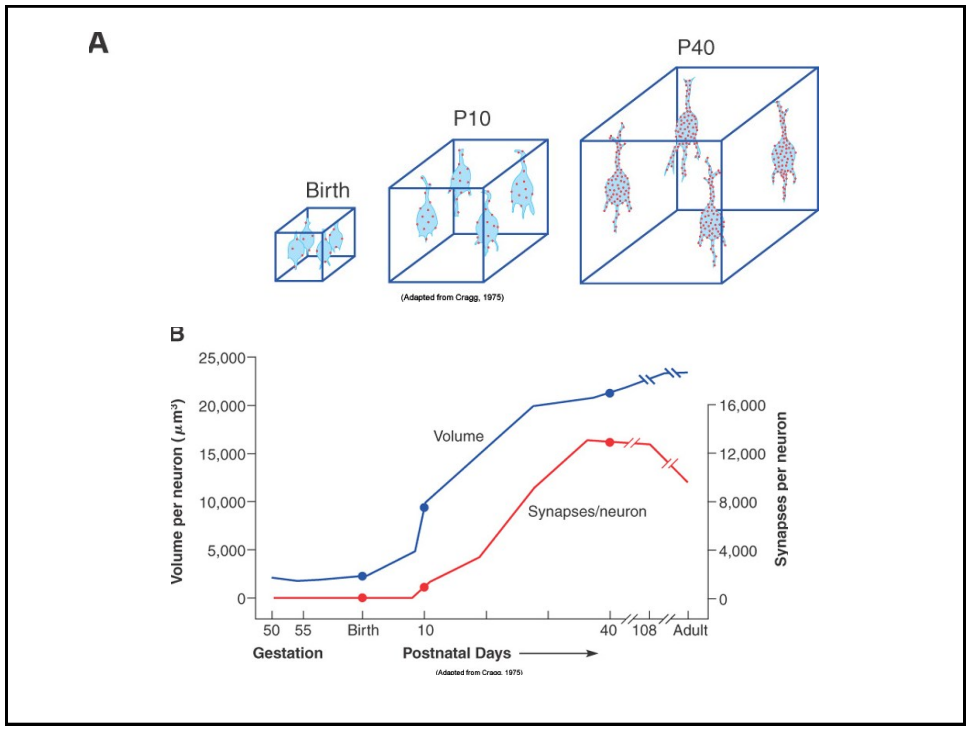
Molecular mechanisms of CNS synaptogenesis

Craig C. Garner, R. Grace Zhai, Eckart D. Gundelfinger and Noam E. Ziv

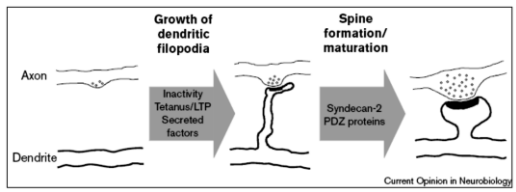
Human Brain Development



Thompson, R. A., & Nelson, C. A. (2001). Developmental science and the media: Early brain development. *American Psychologist, 56(1),* 5-15.

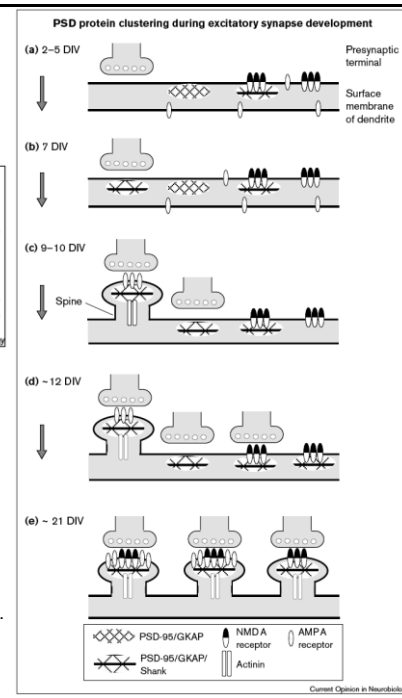


PSD protein dynamics during synaptogenesis



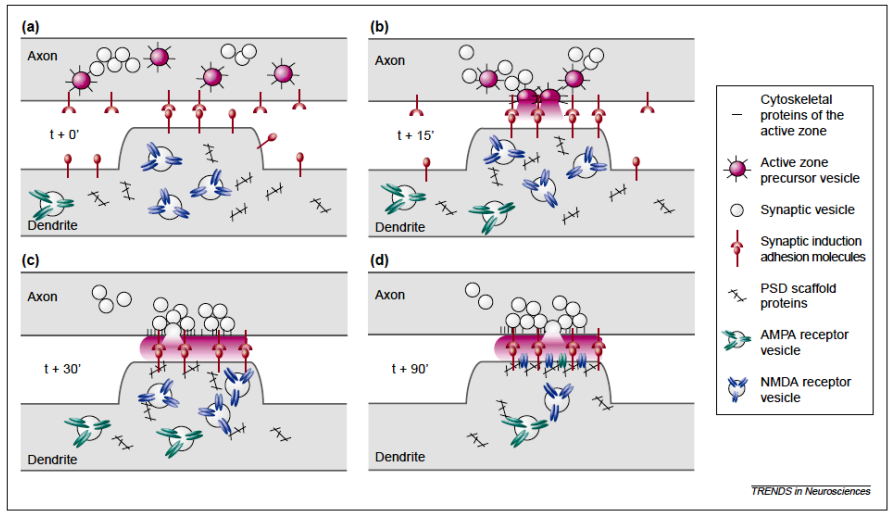
A model for the regulation of dendritic spine development. Dendrites send out long thin processes (filopodia) that seek out and form synapses with nearby axons. Formation of filopodia is stimulated by high levels of synaptic activity (e.g. tetanus or other LTP-inducing stimuli) or by profound inactivity, presumably in conjunction with local secreted factors.

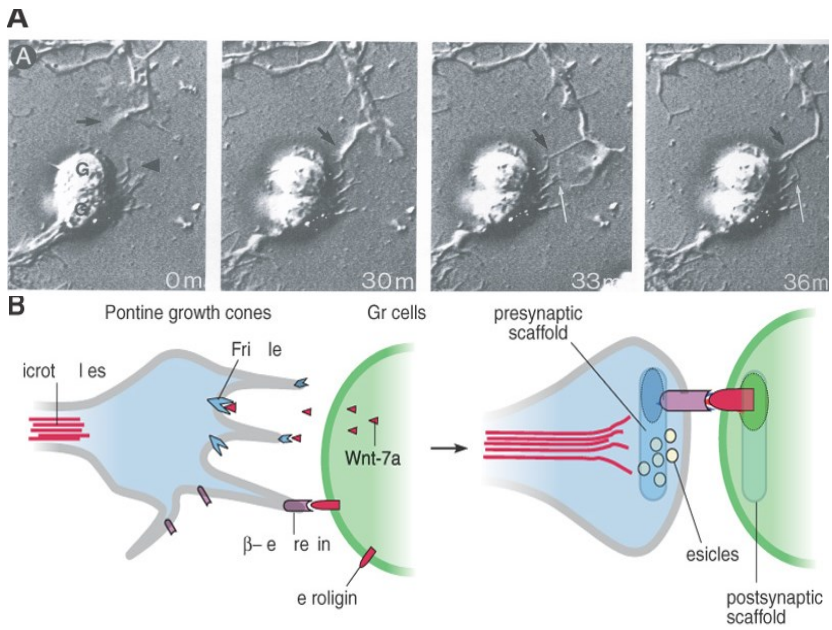
Postsynaptic PDZ proteins are critical for the development of filopodia into mature spines (a representative mature mushroom-shaped spine is shown). The maintenance of mature spines depends on lowlevel stimulation of the AMPA receptor.



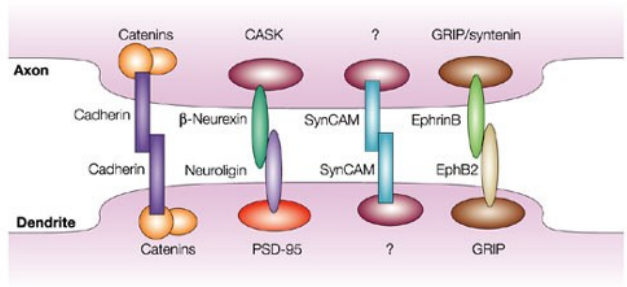
Current Opinion in Neurobiology 2000, 10:125–131

Review TRENDS in Neurosciences Vol.25 No.5 May 2002 245



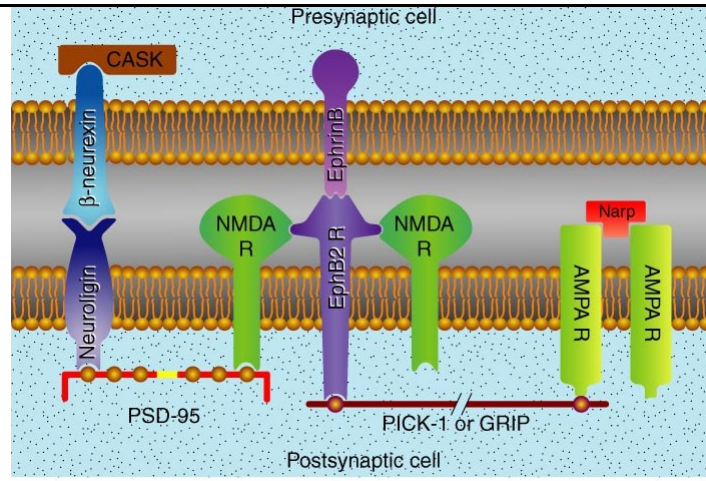


Trans-synaptic protein interactions implicated in synaptic contact/adhesion & synapse development: Some players



Nature Reviews | Molecular Cell Biology

Homophilic interactions: The carboxy-terminal cytoplasmic tails of -neurexin, neuroligin, EphB2, ephrinB and SynCAM (synaptic cell-adhesion molecule) bind to specific PDZ and ZO-1-domain-containing proteins, which can assemble large protein complexes that are associated with the cell-surface membrane protein.
Zheng Li & Morgan Sheng 2003



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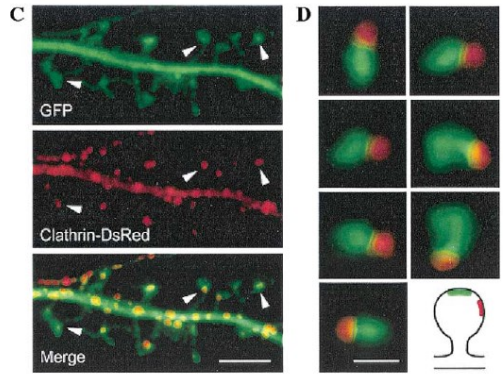
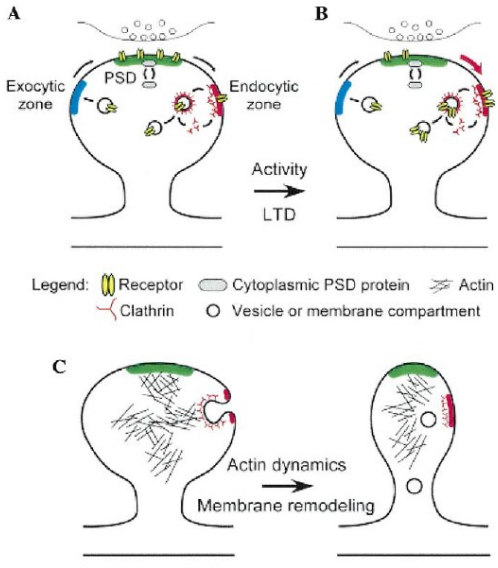
Presynaptic and postsynaptic elements at glutamatergic synapses.
The PDZ-containing protein PSD-95 binds to **NMDA** receptors, PICK-1 and GRIP, bind to **AMPA** receptors. Presynaptic β–neurexin binds to postsynaptic Neuroligin which is associated with NMDA receptors via PSD-95 to align pre- and postsynaptic sites. Pre-synaptic ephrinB binds to postsynaptic EphB2 receptors, clustering NMDA receptors. EphB2 receptors bind to PICK-1 & GRIP linking NMDA and AMPA receptors. Interactions between Narp & AMPA receptors have been established by *in vitro* binding and immunoprecipitation experiments; the importance remains to be determined.

Endocytic zones in spines are spatially and molecularly distinct from the PSD



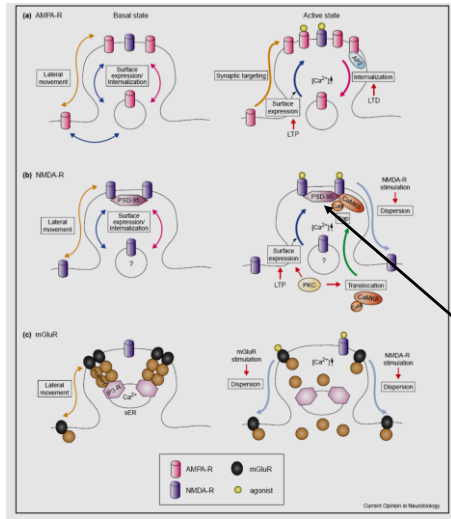
Single spine from neurons transfected with both clathrin-DsRed (red) and the postsynaptic density protein PSD-95 tagged with GFP(green).
Note the adjacent and largely nonoverlapping nature of the PSD (green) and the endocytic zone (red).. Scale bar, 1 micron. GFP, green fluorescent protein; PSD, postsynaptic density.

BIOL PSYCHIATRY 2004; 55:1121–1127
Thomas A. Blanpied and Michael D. Ehlers



The positioning of the endocytic zone near to but distinct from the PSD suggests a general model of synaptic membrane traffic in which receptors move into synaptic membranes via perisynaptic regions

Figure 2. Role of spine microanatomical zones in synaptic plasticity and spine morphologic dynamics. **(A)** Proposed model whereby spines contain a number of domains dedicated to protein trafficking. Internalization of synaptic receptors and other membrane proteins occurs at the endocytic zone (red), where clathrin and endocytic proteins recycle (Blanpied et al 2002). Receptors are hypothesized to be inserted in the membrane at an exocytic zone (green) and moved laterally into the synapse, whereas cytosolic proteins can be directly added to and removed from the PSD (green). Equipped with functional domains to insert, stabilize, and remove receptors, each spine retains autonomous control over the strength of transmission at its synapse. **(B)** Activity-dependent synaptic plasticity, such as long-term depression (LTD), produces a net decrease in the number of synaptic receptors. The presence of a constitutively operating endocytic zone suggests that synaptic receptors translocate from the PSD to the endocytic zone before endocytosis. **(C)** A model for coordinated regulation of actin and endocytosis during spine morphologic change. We propose that actin-dependent changes in spine shape may involve membrane removal by endocytosis. PSD, postsynaptic density.



Dynamics of glutamate receptors and PSD components at a glutamatergic synapse at basal and active states. To simplify the model, protein molecules involved in the dynamics of the three types of glutamate receptors are independently depicted with their associated/signaling proteins and their regulatory mechanisms triggered upon neuronal stimulation (an increase in intracellular Ca^{2+} = 'active state'). **(a)** Basal state: the number of synaptic AMPA-Rs remains nearly constant through a balance of 'surface expression' and 'internalization' between plasma membrane and transport vesicles. 'lateral movement' of AMPA-Rs between synaptic and extrasynaptic membranes is also involved [42••]. Active state: glutamate stimulation leading to LTP causes an increase in synaptic AMPA-Rs by 'surface expression' from transport vesicles as well as 'synaptic targeting' from the

(Figure 1 Legend continued) extrasynaptic site. NMDA-R-dependent 'internalization' of AMPA-Rs provides a possible mechanism for LTD. Direct association of the clathrin adaptor protein complex (AP2) with AMPA-R is essential for this internalization [47••]. **(b)** Basal state: NMDA-Rs in transport vesicles are rapidly recruited to ('surface expression') and internalized from ('internalization') the synaptic membrane [15••]. The nature of the vesicular pool in the spine and the delivery mechanisms of NMDA-Rs are not well understood (see update). NMDA-Rs can also move on the neuronal surface between synaptic and extrasynaptic sites ('lateral movement'), at the basal state. Active state: although neuronal stimulation does not affect the distribution of synaptic NMDA-Rs, PKC activation or LTP induction can increase NMDA-R 'surface expression' through SNARE-dependent exocytosis [43,45••]. PKC activation also induces rapid 'dispersion' of NMDA-Rs from synaptic sites (not shown), whereas in contrast, CaMKII accumulates in the PSD [44•]. Reversible CaMKII 'translocation' is also achieved through NMDA-R stimulation, and requires CaM binding. Direct binding ('trap') of CaM-CaMKII to NMDA-R is sustained [60,62••]. **(c)** Basal state: mGluR clusters themselves are highly mobile about perisynaptic areas ('lateral movement') [68••]. PSD-Zip45 (Homer 1c) binds to both type-I mGluRs (mGluR1/5) and IP_3-Rs on the surface of Ca^{2+}-enriched smooth endoplasmic reticulum (sER). Multimerization of PSD-Zip45 molecules leads to the clustering of mGluRs, and enhances IP_3-R-dependent increases in cytoplasmic Ca^{2+}. Active state: agonist binding to mGluR5 promotes mGluR5 cluster 'dispersion' [68••]. NMDA-R stimulation entails both a slow increase in intracellular Ca^{2+} and subsequent mGluR cluster 'dispersion' [66].

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The lability of AMPA relative to NMDA receptors is thought to contribute to synaptic plasticity

PSD-95 family members might stabilize the NMDA receptors arrayed in the center of the PSD while allowing AMPA receptors arrayed around the periphery to turn over more quickly.