Introduction

1.1 History and Context

Within an evolutionary context, one can easily imagine that an ability to positively adjust decision-making processes relative to past experiences and environmental conditions would greatly increase the survival of an organism. Consequently, phylogenetically acquired mechanisms that allow an organism to adjust its behavior on the basis of information acquired ontogenetically have proven to be powerful adaptations. Information acquisition and utilization implies a close relationship between learning, memory, and behavior. Thus, it comes as no surprise that the biological mechanisms that underlie neural learning and memory have been intensely pondered and studied by philosophers, psychologists, and biologists since ancient times.

Evidence indicates that the number of neurons in an adult brain does not significantly increase with age, suggesting that the production of new neurons is not the cellular mechanism underlying learning and memory [1] . The theories put forth by Ramon y Cajal [2] and expanded upon by Donald Hebb [3] suggesting that changes in neuronal connectivity are the cellular and molecular basis of learning and memory have become the dominant paradigm for much of modern neuroscience. In particular, Hebb's neurophysiological postulate of learning states:

> *Let us assume that the persistence or repetition of a reverberatory activity (or "trace") tends to induce lasting cellular changes that add to its stability.... when an axon of cell A is near enough to*

> *excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased. [3]*

Hebb's postulate laid the initial theoretical framework that led subsequent researchers to identify long term potentiation- the cellular mechanism by which a neuron's excitatory response can be enhanced, or "potentiated," by previous high frequency stimulation, allowing neurons to respond more sensitively to subsequent low frequency stimulations.

Neural connectivity as a concept originated with Ramon y Cajal's identification of small protrusions extending away from the main dendritic axis (dendritic spines) and their contact points with the adjacent nerve fibers (axons; Fig 1.1a, panel a). However, the concept that the bulbous head of dendritic spines were likely to act as "receptors of current" was contributed by H.J Berkley (Fig 1.1a, panel b):

> *"The function of the gemmule is in all likelihood to receive nerve impulses from the ending of the numerous terminal nerve fibers that seem almost to touch them, and carry these impressions to the dendrite and by its medium on the cell body."*

Furthermore,

"…these spherical apparatus (terminal buttons) are closely adjusted against the bulbous tip of the gemmules, at times the application being so close as to give the impression of actual contact." [2]

In 1897, Sherrington united Ramon y Cajal's neuroanatomical and Berkley's physiological arguments into the single concept of the synapse. However, it was not until 1956 that the definitive identification of the synapse was made by Palay, and not until 1959 that dendritic spines were definitively identified as the postsynaptic units (Gray 1959 a, b); both utilized electron microscopes to illuminate these structures.

In 1959, Gray identified specific, conserved structural differences between the axo-somatic and axo-dendritic synapses [4]. In particular, he described the electron dense thickening of the postsynaptic membrane (Fig 1.1b):

"One very obvious feature of the cortical synapse is that in certain contacts with dendritic trunks of their spines a high proportion of the length over which the membranes are apposed shows a thickening and increased density. Also the thickening and density is much more pronounced in the post- than the pre-synaptic membrane." (p. 422, [4])

Ensuing biochemical fractionation methods were developed to isolate intact membranes containing the proteins of the postsynaptic densities (PSD) [5, 6] thus

ushering in an era during which the protein constituents of the PSD began to be identified.

Finally, in the late 1960s and early 1970s another set of seminal experiments were providing the first experimental description of long term potentiation. Specifically, in 1966, Lomo and colleagues reported that a series of conditioning trains of impulses could potentiate the size of synaptic potentials for periods ranging from 30 min to many hours. In their seminal work, Lomo and Colleagues wrote the following:

> *"Extracellular responses of dentate granule cells, evoked by repetitive stimulation of the entorhinal area or perforant path fibres, were recorded simultaneously with two microelectrodes. One electrode recording from the layer of perforant path synapses on the granule cell dendrites, the other from the layer of granule cell bodies.*
>
> *"After an initial depression, lasting for a few seconds, repetitive stimulation led to a large potentiated response, compared to the response evoked by a single volley. This effect, frequency potentiation, was seen as an increase of the amplitude and a decrease of the latency of the population spike and as an increase of the rate of rise and amplitude of the extracellular excitatory synaptic potentials.*

"This represents an example of a plastic change in a neuronal chain, expressing itself as a long-lasting increase of the synaptic efficiency. The effect, which may last for hours, is dependent upon repeated use of the system." [7]

Further experimentation by Bliss and Lomo showed that "one or more brief episodes of tetanic stimulation (15 sec $^{-1}$ for 10--15 sec) produces a potentiation of the monosynaptic response evoked by single shocks which may last for several hours." The full report of Lomo's work, published in 1973 [7] was the first quantitative description of LTP.

1.2 The Postsynaptic Density: Contents, Supramolecular Complexes, and Higher Order Structure

It has long been thought that synapses are the most suitable location of memory storage in the brain [3, 8]. Furthermore, many believed that a clear relationship existed between the morphology of dendritic spines and their functions during normal and diseased states [9]. However, the observation that spine head volume can increase and neck length vary during LTP [10, 11] helped establish the idea that there exist dynamic and plastic mechanisms within the spine that actively respond to synaptic activity. This work, together with that of Bliss and Lomo [7] helped lay the groundwork for the subsequent hypothesis that synaptic plasticity is critical to the underlying molecular basis of learning and memory.

1.2.1 Contents of the PSD

Understanding the activity dependent changes that occur in the PSD upon synaptic stimulation requires an integrated approach to identify its constituent proteins and then study their function and dynamic organization. Early efforts to identify components of the PSD utilized differential centrifugation and sucrose density gradients, followed by detergent extraction with non-ionic detergents such as Triton-X [6]. Protein separation and microsequencing techniques used by Mary Kennedy and co-workers resulted in the identification of numerous core PSD proteins, including αCaMKII [12], PSD-95 [13], the NR2B subunit of the NMDA receptor [14], Densin [15], SynGAP [16], and Citron [17]. Yeast two-hybrid screens using known PSD proteins as bait were used to identify other PSD proteins, among them GKAP [18-20], Shank [21, 22], GRIP/ ABP [23, 24], Homer [25], GRASP-1 [26], and SALMs (synaptic adhesion-like molecules) [27]. More recently, mass spectrometry methods have been used to detect a large number of putative PSD proteins [28-31]. Though proteomic based PSD identification studies have identified many proteins likely to play a role in postsynaptic reception, care needs to be taken when interpreting these findings. The spatial constraints, stoichiometry and organization of the PSD suggests that the number of different proteins in a single PSD is likely to be in the tens, not hundreds of different proteins [32, 33].

Recent work by Heintz and co-workers [33] used a mix of genetic engineering, biochemical fractionation, affinity purification, and mass spectrometry to identify the contents of the parallel fiber/ Purkinje cell PSD. This experimental approach identified approximately 60 proteins enriched in these PSDs. Previously identified PSD proteins such as Homer3, Shank1, Shank2, PSD-95, PSD-93, α and βCaMKII, and δ-catenin

where identified. However, other previously unidentified proteins were also isolated. This work suggests that a core group of PSD proteins are likely to exist at most PSDs. Moreover, the bulk proteomic identification of numerous proteins from whole brain PSDs speaks more to the diversity of synapses, their spatial and functional specificity, and their complex signaling pathways than the general contents of a PSD.

Identifying the protein-protein interactions and atomic structure of individual PSD proteins is necessary for modeling the spatial geometry of this integrated macromolecular complex. Together with data generated from systematic EM immunogold analysis [34], electron microscopy [35], EM tomography [36], and solid phase chemical cross-linking methods [37], a comprehensive 3-dimensional model of the PSD is beginning to emerge. Two major characteristics of all current models are 1) the clustering and scaffolding of supramolecular complexes immediately adjacent to the membrane, and 2) a matrix of proteins forming a platform upon which these supramolecular complexes sit.

1.2.2 Supramolecular Complexes of the PSD

The supramolecular complexes immediately adjacent to the membrane are composed of transmembrane proteins (receptors, ion channels, and adhesion molecules) and their downstream effectors (G-proteins, kinases, phosphatases, signaling proteins, as well as cytoskeletal and adaptor proteins). Three such supramolecular complexes are 1) the NMDA receptor complex, 2) the mGluR complex, and 3) the AMPA receptor complex. The main scaffolding protein for the NMDA complex is PSD-95. Homer specifically complexes the mGluR complex, while GRIP1/ APB and PSD-95 each work to cluster and localize AMPA receptors with potential downstream signaling pathways.

Cho et al. [38], identified the first PSD scaffolding protein, PSD-95. With its three PDZ domains, a SH3 domain, and a guanylate kinase domain, PSD-95 is the prototypical PSD scaffolding molecule, allowing for multiple and simultaneous protein-protein interactions. PSD-95 has been shown to nucleate a NMDA receptor signaling complex [39, 40] and synaptic adhesion complexes [41] within the PSD. Though PSD-95 has not been shown to nucleate AMPA receptor signaling complexes, it has been shown to localize and target AMPA receptors via Stargazin [42-44]. Furthermore, Gerrow et al. [45] showed that mobile, preformed complexes of PSD-95, GKAP, and Shank are transported to dendritic positions primed for synapse development. Such evidence suggests that PSD-95 acts as a major scaffolding protein for modular clusters of supramolecular complexes i.e., the PSD-95/ NMDA receptor complex and the PSD-95/ adhesion molecule complex.

A second major PSD scaffolding molecule, Homer, is known to interact with and cluster the group 1 metabotropic receptors (mGluR1 and mGluR5) and inositol trisphosphate receptors (IP3R) [22, 25, 46]. The Homer protein has two major splice variants, long and short [47]. The long form contains two major domains, 1) the N-terminal EVH1 PDZ-like target-binding domain that mediates interactions with mGluR and IP3R [22, 25, 48] and 2) the C-terminal self-assembly coiled-coil/ leucine zipper domain that mediates self-dimerization [49]. The short form of Homer contains only the N-terminal EVH1 PDZ-like target-binding domain and exhibits activity regulated expression, placing it in the family of immediate early genes [25, 50]. Furthermore, this short form of Homer (a.k.a. Homer1a) exerts a dominant-negative activity by impairing self-dimerization and Homer-mediated multi-protein complexes [49, 51-53].

The third scaffolding protein that mediates clustering of particular PSD proteins into supramolecular complexes is GRIP/ ABP (AMPA binding protein). GRIP1 and ABP/ GRIP2 were independently identified using the C-terminal tail of GluR2/3 as bait in yeast two-hybrid screens [23, 24]. In addition to binding and clustering GluR2/3 receptors, GRIP1 also binds to GRASP-1, a neuron specific guanine nucleotide exchange factor (GEF). Recent work by Ye et al. [54] shows that GRASP-1 is able to bind both JNK and the upstream kinase MEKK1 in neurons, and that these interactions facilitate JNK signaling. Though a complex specifically containing AMPA, GRIP1, and GRASP-1 has yet to be shown, or the direct activation of AMPA receptors leading to the activation of the JNK pathway scaffolded by GRASP-1, the suggestion that GRIP1 scaffolds an AMPA receptor complex is intriguing. Finally, like PSD-95, GRIP family proteins can cluster synaptic adhesion molecules into larger supramolecular complexes [55-57].

1.2.3 Higher Order Structure of the PSD

Current models of the PSD suggest that the supramolecular complexes adjacent to the membrane sit on a platform matrix formed by the protein Shank. Shank was originally identified by using the C-terminal residues of GKAP, a PSD protein that specifically co-localizes with PSD-95 and directly interacts with its guanylate kinase domain [19-21, 58], as bait in a yeast two-hybrid screen [21]. Shank contains multiple protein-protein binding domains, including numerous N-terminal ankyrin repeats, SH3 and PDZ domains, conserved proline-rich clusters, and a SAM (sterile alpha motif) domain at its C-terminus [21]. X-ray crystallography and electron microscopy showed that Shank can form polymer sheets through self-association of the SAM domain, and

that helical fibers formed by Shank can be crossed linked by Zn^{2+} [35]. More recently, Hayashi et al. [59] demonstrated that interactions between multimerized Shank and tetramerized Homer form higher-order-polymerized complexes with a mesh-like network structure. This higher-order structure was proposed to form the core structural framework and binding platform for PSD supramolecular complexes.

The multiple protein domains of Shank have been shown to bind the different PSD supramolecular complexes immediately adjacent to the membrane either by direct binding or via adaptor proteins [32]. In particular, the PSD-95/ NMDAR and the PSD-95/ synaptic adhesion molecule complexes can be directly linked to the Shank scaffolding via GKAP [21]. Homer is known to directly bind Shank, thus linking the mGluR/ Homer complex to the underlying Shank scaffold matrix [22]. Consequently, Shank is able to cross-link Homer and PSD-95 complexes in the PSD. Finally, though no interaction has been demonstrated between the GRIP/ AMPA complex and Shank, AMPA receptor complexes can interact with PSD-95 through the C-terminal PDZ binding motif of Stargazin, a transmembrane AMPA receptor regulatory protein [42, 60]. Ultimately, the ability of Shank to tether and nucleate numerous PSD supramolecular complexes supports the current models of a laminar organization of the postsynaptic density.

1.3 Densin is a Core Component of the Postsynaptic Density

Densin was first identified as a highly enriched protein in the insoluble PSD fraction following extraction with *N*-lauroyl sarcosinate (sarcosyl), thus defining it as a "core" PSD protein [15]. A 167kDa glycosylated protein originally thought to be brain

specific, Densin has been subsequently shown to be expressed at low levels in kidney [61-63], Testis [64], and the pancreas [61].

Densin contains two indisputable protein domains, 1) a N-terminal leucine-rich repeat (LRR) domain consisting of 16 canonical LRRs, and 2) a C-terminal PSD-95/ Dlg/ ZO-1 (PDZ) domain (Fig 1.3). Other proteins with this arrangement of domains were subsequently identified and designated a new protein family call LAP proteins (LRR and PDZ domains), of which Densin is the founding member [65]. Initial sequence analysis of Densin also identified a RGD motif, a Mucin homology domain and a putative transmembrane domain (Fig 1.3).

1.3.1 Cellular Localization and Tissue Expression

Immunofluorescent double labeling of Densin and Synapsin I, a presynaptic marker, reveals that Densin is localized to the synapse (Fig. 1.4a, top panel). Furthermore, co-staining neuronal cultures with antibodies to Densin and PSD-95 reveals a tight colocalization of these two proteins, supporting the hypothesis that Densin is a postsynaptic density protein (Fig. 1.4a, bottom panel). Interestingly, Densin was also identified in the axon initial segment (Fig. 1.4a, bottom panel) suggesting that it may play a role in the macromolecular complex of the axon hillock where action potentials are generation. Comparison of in situ hybridization images of Densin with those of αCaMKII (high expresser) and the NR2C subunit of the NMDA receptor (low expresser) show that Densin is highly expressed in all forebrain regions (Fig 1.4b; images taken from the Allen Brain Map, Allen Institute for Brain Science http://www.brain-map.org/). Though there is not always a direct relationship between gene and protein expression

patterns, these studies suggest that the Densin protein is likely to be widely expressed in the forebrain.

1.3.2 CaMKII Phosphorylation of and Association with Densin

Apperson et al. [15] showed that Densin is specifically phosphorylated by endogenous CaMKII within the PSD. Subsequent work by Strack et al. [66] identified Ser^{1397} and Walikonis et al. [67] identified Ser^{1293} and Ser^{1397} as sites phosphorylated by CaMKII. Both studies independently identified the region between the putative transmembrane domain and the PDZ domain as the region of CaMKII binding, with Walikonis specifically demonstrating that Densin directly interacts with the association domain of CaMKII. Walikonis et al. [67] also demonstrated that autophosphorylation of CaMKII on Thr^{286} significantly increases its binding affinity for Densin; non-phosphorylated CaMKII has a reduced, but still significant binding affinity for Densin. Furthermore, the phosphorylation of Densin by CaMKII does not dramatically alter the binding affinity between the two proteins. Strack et al. [66] similarly demonstrated that autophosphorylation of CaMKII is not required for its interaction with Densin, and that binding of CaMKII to either Densin or the NR2B tail of the NMDA receptor is non-competitive. Taken together, these data suggest that Densin may act as a docking site for CaMKII even after dephosphorylation of the kinase, and that phosphorylation of Densin by CaMKII may act to modulate binding interactions between Densin and other proteins in the PSD. Finally, Strack et al. [66] and Jiao et al. [68] further demonstrated that the exon containing the CaMKII binding site (exon 24) has a developmentally regulated

splice variant, suggesting that alternative splicing may act as a mechanism to regulate the docking of CaMKII to Densin.

1.3.3 Protein-Protein Interactions Between Densin and Other PSD Proteins

In addition to identifying CaMKII as a binding partner for Densin in a yeast two-hybrid screen, Walikonis et al. also identified α-actinin as a binding partner for the PDZ domain of Densin. Biochemical studies of the interactions between Densin, CaMKII, and α-actinin not only confirms that α-actinin and Densin bind to distinct regions within CaMKII, but that CaMKII and α-actinin interact with each other; thus, these proteins can form a ternary complex.

Using the PDZ domain of Densin as bait in a yeast two-hybrid screen, Ohtakara and coworkers [69] identified Maguin-1 as a binding partner of Densin. Maguin-1 directly interacts with the PDZ domain of Densin via a canonical class I PDZ binding motif, THV, located at its C-terminus. Maguin-1 was previously shown to interact with PSD-95, S-SCAM (synaptic scaffolding molecule) and Raf-1, an activator of MAPK/ ERK signal transduction [70, 71]. Immunoprecipitation studies revealed that Densin, Maguin-1, and PSD-95 interact in a complex, while immunofluorescent imaging demonstrated tight co-localization of all three at the tips of spines in dissociated hippocampal cultures. A C-terminal leucine rich region allows Maguin-1 to self-associate. This self-association allows Maguin-1 to simultaneously interact with both Densin and PSD-95. Taken together, these results suggest that Maguin-1 may link Densin to the PSD-95 receptor signaling complexes and to the MAPK/ ERK signaling pathway, both of which are known to be involved in synaptic plasticity [32, 72].

The PDZ domain of Densin was also shown to interact directly with the C-terminal SWV PDZ binding motif of δ-catenin/ NPRAP [73]. Izawa and co-workers [73] showed the following: 1) Densin, δ-catenin/ NPRAP, and N-Cadherin co-immunoprecipitate with each other, 2) all three proteins co-localize at the tips of spines in dissociated hippocampal cultures, and 3) δ-catenin/ NPRAP specifically mediates the association of Densin and N-Cadherin. These results provide strong evidence that Densin is linked to cadherin-catenin complexes and that it may be involved in organizing synaptic cell-cell junctions.

Another PSD protein found to interact with Densin is Shank. Quitsch et al. used the SH3 domain of Shank as bait in a yeast two-hybrid screen. Of the approximately one million clones screened, only the C-terminus of Densin, residues 1125-1542, was consistently identified. Further analysis determined that the membrane proximal region of Densin was require for binding to Shank. However, the putative transmembrane region and the PDZ domain enhance the interaction between Densin and Shank such that, in their absence, Densin and Shank no longer interact.

Though only identified in the glomerular podocytes of kidney thus far, two additional cell adhesion complex proteins known to directly interact with Densin are β-catenin and nephrin [62, 63]. In addition, Heikkila and co-workers [63] isolated δ-catenin and α-actinin from the same yeast two-hybrid screen using the entire putative intracellular region of human Densin. Immunoprecipitation studies showed that β-catenin, P-cadherin, and Densin precipitate as a complex. Ahola and co-workers [62] identified Densin as a key component of the nephrin mediated slit diaphragm complex of the kidney; the slit diaphragm complex is critical for the maintenance of podocyte

cytoarchitecture and connections to the cytoskeleton. Though these protein-protein interactions have yet to be identified in the brain, the fact that Densin directly interacts with nephrin and complexes with β-catenin and P-cadherin suggests a conserved role for Densin as part of cell adhesion complexes.

1.3.4 Functional Analysis of Densin.

The only analysis of Densin's function in neurons to date is the work by Quitsch and co-workers [74]. Overexpression of full length Densin in primary hippocampal cultures caused a striking increase in dendritic branching and in the number of branch points. Quitsch and co-workers further demonstrated that this enhanced branching phenotype could be induced only by overexpression of constructs containing the LRR domain of Densin. Interestingly, co-overexpression of Shank was able to abrogate Densin induced branching. However, the increased branching patterns induced by Densin overexpression are not reversed by Shank when the C-terminal region of Densin is not part of the construct. Thus, the ability of Shank to abrogate Densin induced enhancement of dendritic branching requires direct interaction between Shank and the C-terminal domain. Finally, Quitsch and co-workers demonstrated that the overexpression of Densin resulted in an increased number of presynaptic synaptophysin clusters. They interpreted these results to mean that more presynaptic release sites are formed when Densin is over-expressed. Consequently, these results, in combination with known interactions of Densin with adhesion complexes in the PSD suggest that Densin may play a crucial role in synaptogenesis as well as in dendritic arborization.

1.3.5 Membrane Topology of Densin

A recent review by Thalhammer and collegues [75] proposed a new, membrane associated, entirely cytosolic topology for Densin. Recent analysis of the *in vivo* phosphorylation state of PSD preps found that Densin contained a cluster of phosphorylation sites positioned N-terminal to the proposed transmembrane domain. These findings prompted Thalhammer and colleagues to suggest that the original transmembrane topology of Densin was incorrect, and that Densin was in fact entirely cytosolic. They further purport that current bioinformatic analysis does not support the existence of a signal peptide or other sequence motifs that suggests a transmembrane domain. Moreover, they argue that the inability to surface biotinylate the proposed extracellular portion supports its intracellular position. Finally, they suggest with much hand-waving, that the experimental evidence for glycosylation was "quite weak." On this point, we take serious issue.

Two enzymatic digestions were done to support the glycosylation state of Densin. The first enzymatic digestion used neuraminidase from *Arthrobacter ureafasciens.* Neuraminidases are glycohydrolases that catalyze the hydrolysis of sialic acid-α-ketosides [76]. When Densin was subjected to a neuraminidase digestion, its molecular weight was significantly shifted from ~180kDa to 148kDa. Neuraminidase from *Arthrobacter ureafasciens* has been reported to be very efficient and highly specific for hydrolyzing α-2,6 linked sialic acid [77-79]. Thalhammer and colleagues argue that "only prolonged incubation with neuraminidase suggested the presence of sialic acid modified residues." The 24 hour, 37^0C incubation that was conducted is a standard protocol and is supported by voluminous research and protocol development. As such,

Thalhammer and colleagues are quite mistaken in their biochemical protocol assumptions.

Second, these authors argue that "digestion with O-sialoglycoprotein endoprotease suggested possible positioning of O-sialoglycosylation within the Mucin domain." The suggestion here is that digestion with the O-sialoglycoprotein endoprotease from *Pasteurella haemolytica* lacks in specificity of its action. The *P. haemolytica* O-sialoglycoprotein endopeptidase cleaves only proteins that are heavily sialyated, in particular those with sialylated serine and threonine residues [80, 81]. It does not cleave unglycosylated proteins, desialylated glycoproteins or glycoproteins that are only N-glycosylated [82-84]. Ultimately, this is a highly specific endopeptidase. The observed cleavage products were identified by immunoblot to be Densin.

Colbran and colleagues recently published an extensive profile of alternatively spliced species of Densin [68]. Though it has yet to be shown, the numerous slice variants of Densin suggest that both a transmembrane and a entirely cytosolic topology is possible. Thalhammer and colleagues never once suggested this possibility-- a serious scientific oversight.

Based on current hard evidence, not inference alone, we believe that Densin may adopt alternative membrane topologies, and that both a transmembrane and a cytosolic membrane-associated orientation may exist.

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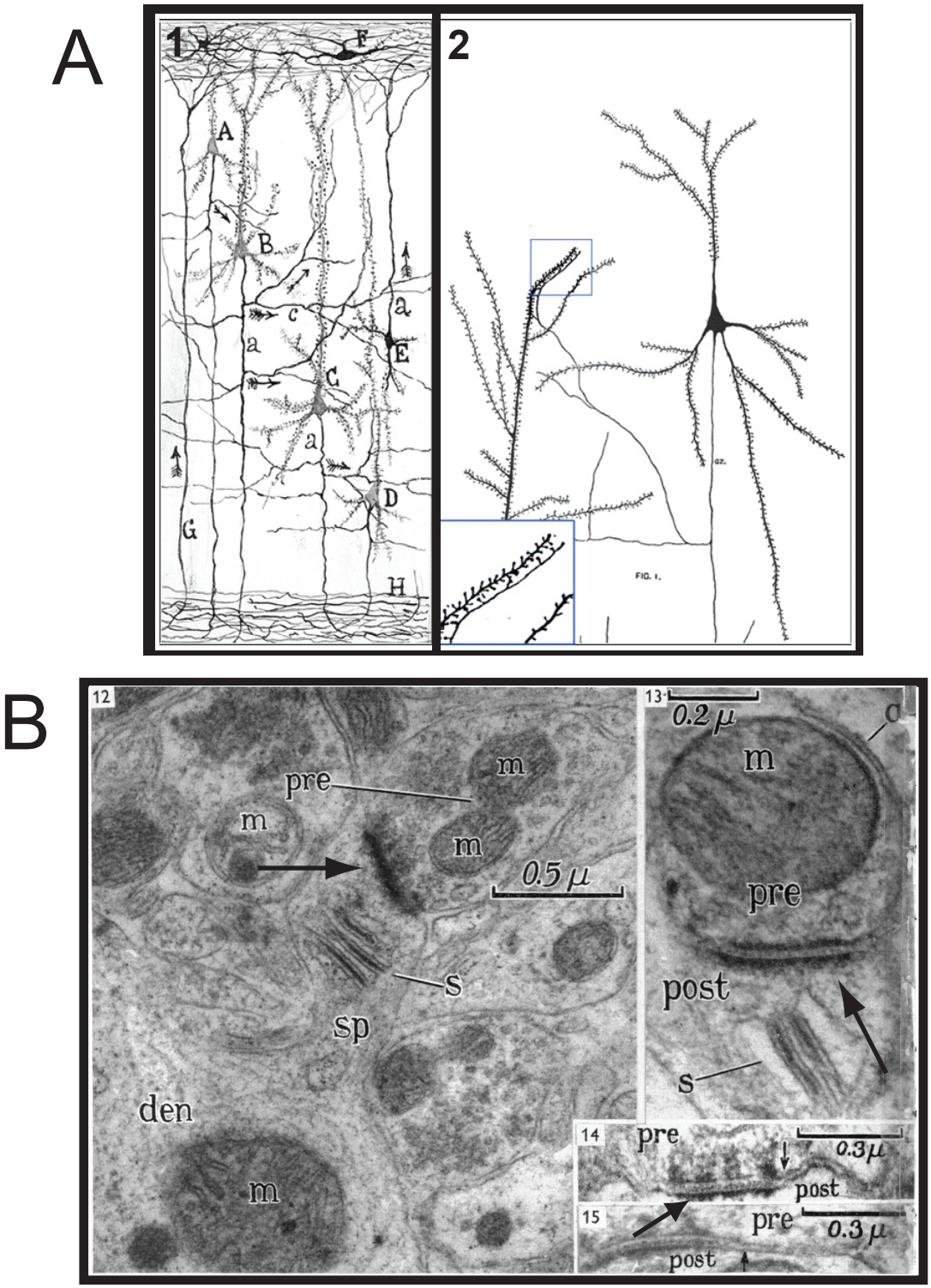
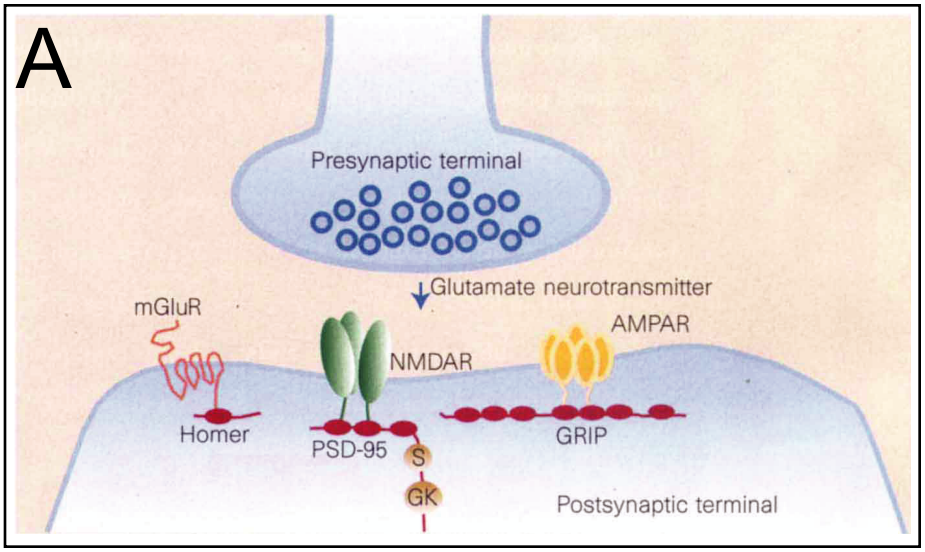


Figure 1.1 Hand drawing and electronmicrograph of synaptic connections between excitatory dendritic spines and axonal nerve fibers. (A) Comparative drawings from Ramon y Cajal (panel 1) and Berkley (panel 2) showing connections of axons with dendritic spines. Adapted from Garcia-Lopez et al. [2007]. (B) Electronmicrograph of presynaptic terminals forming glutamatergic synapses with dendritic spines. All panels show Gray's Type 1 asymmetric synapses with electron dense thickenings at the postsynaptic terminal (arrows). Thickenings represent postsynaptic densities as defined ultrastructurally. Adapted from Gray [1959] Abbreviations: pre, presynaptic process; post, postsynaptic process; m, mitochondria; s, spine apparatus; sp, dendritic spine; den, dendrite

Figure 1.2 Organization of receptor-scaffold and macromolecular complexes in the postsyanptic density (PSD). (A) Excitatory postsynaptic clusters of glutamate receptors with their asociated scaffolding molecules, as mediated by PDZ or PDZ-like protein-interaction domains. Metabotropic glutamate receptors (mGluR), NMDA receptors (NMDAR), and AMPA receptors (AMPAR) bind to the PDZ domains of Homer, PSD-95, and GRIP, respectively. The unbound protein domains of Homer, PSD-95, and GRIP are available to bind other synaptic proteins, thus forming supramolecular signaling complexes. PSD domains are in red, S, SH3 domain; GK, guanylate kinase domain (from Sheng, 1997). (B) Schematic diagram of the laminar network of major supramolecular complexes immediately adjacent to the membrane and the supermolecular complex of the PSD nucleated by the Shank platform matrix. Contacts between proteins indicate an established interaction between them. Domain structure is shown only for PSD-95 (PDZ doamin, red; SH3 domain, dark green; GK domain, light blue) and GRIP1. Other scaffolding molcecules are shown in yellow, signaling enzymes are shown in light green, and actin binding proteins are shown in pink. CaMKII is shown as a dodecamer. Unnamed gray shapes denote PSD proteins that are not specifically illustrated, but are known to interact with with the particular PSD proteins shown. Abbreviations: AKAP150, A-kines anchoring protein 150 kDa; CAM, cell adhesion molecule; Fyn, a Src family tyrosine kinase; GKAP, guanylate kinase-associated protein; H, Homer; IRSp53, insulin receptor substrate 53 kDa; KCh, K+ channel; nNOS, neuronal nitric oxide synthase; RTK, receptor tyrosine kinase (e.g., ErbB4; SPAR, spine-associated RapGAP). Figure adapted from Sheng and Hoogenradd [2007].


A
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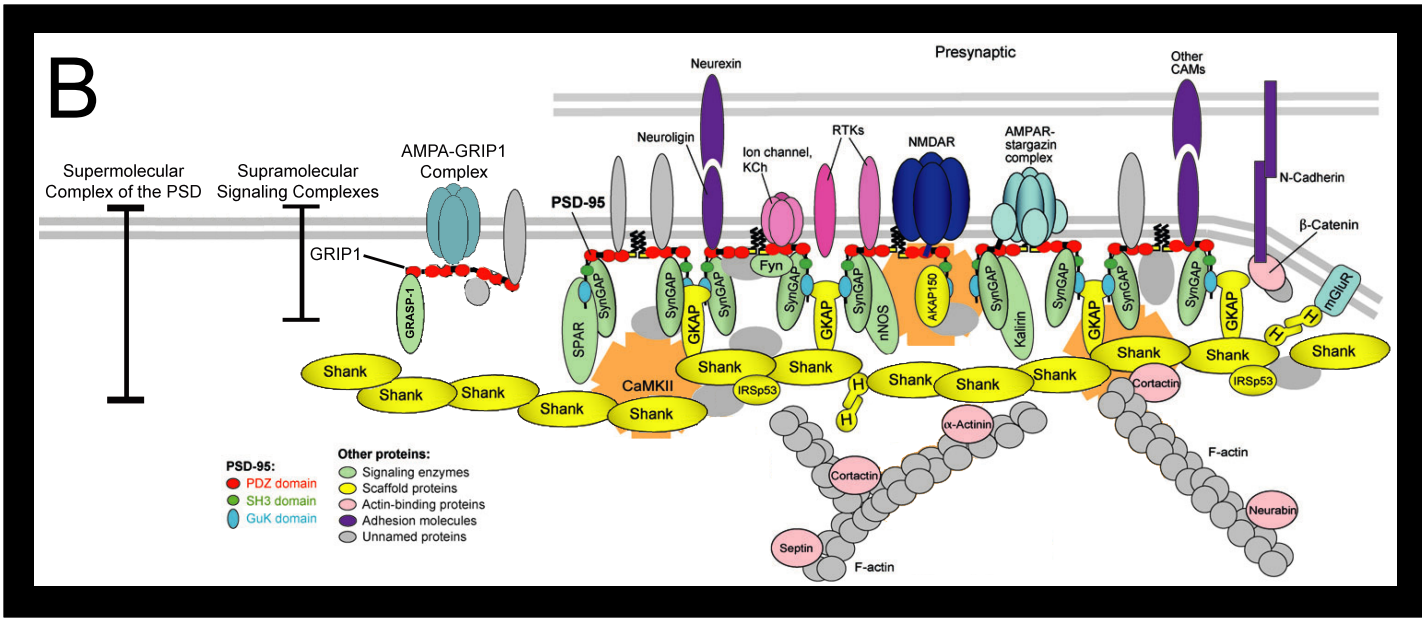
Glutamate neurotransmitter
mGluR
AMPAR
NMDAR
Homer
PSD-95
S
GK
GRIP
Postsynaptic terminal


B
Supermolecular Complex of the PSD
Supramolecular Signaling Complexes
AMPA-GRIP1 Complex
GRIP1
GRASP-1
PSD-95
Neurexin
Neuroligin
Ion channel, KCh
RTKs
NMDAR
AMPAR-stargazin complex
Presynaptic
Other CAMs
N-Cadherin
β-Catenin
mGluR
Fyn
SynGAP
GKAP
SPAR
nNOS
AKAP150
Kalirin
Shank
CaMKII
IRSp53
Cortactin
α-Actinin
F-actin
Septin
Neurabin
PSD-95:
PDZ domain
SH3 domain
GuK domain
Other proteins:
Signaling enzymes
Scaffold proteins
Actin-binding proteins
Adhesion molecules
Unnamed proteins

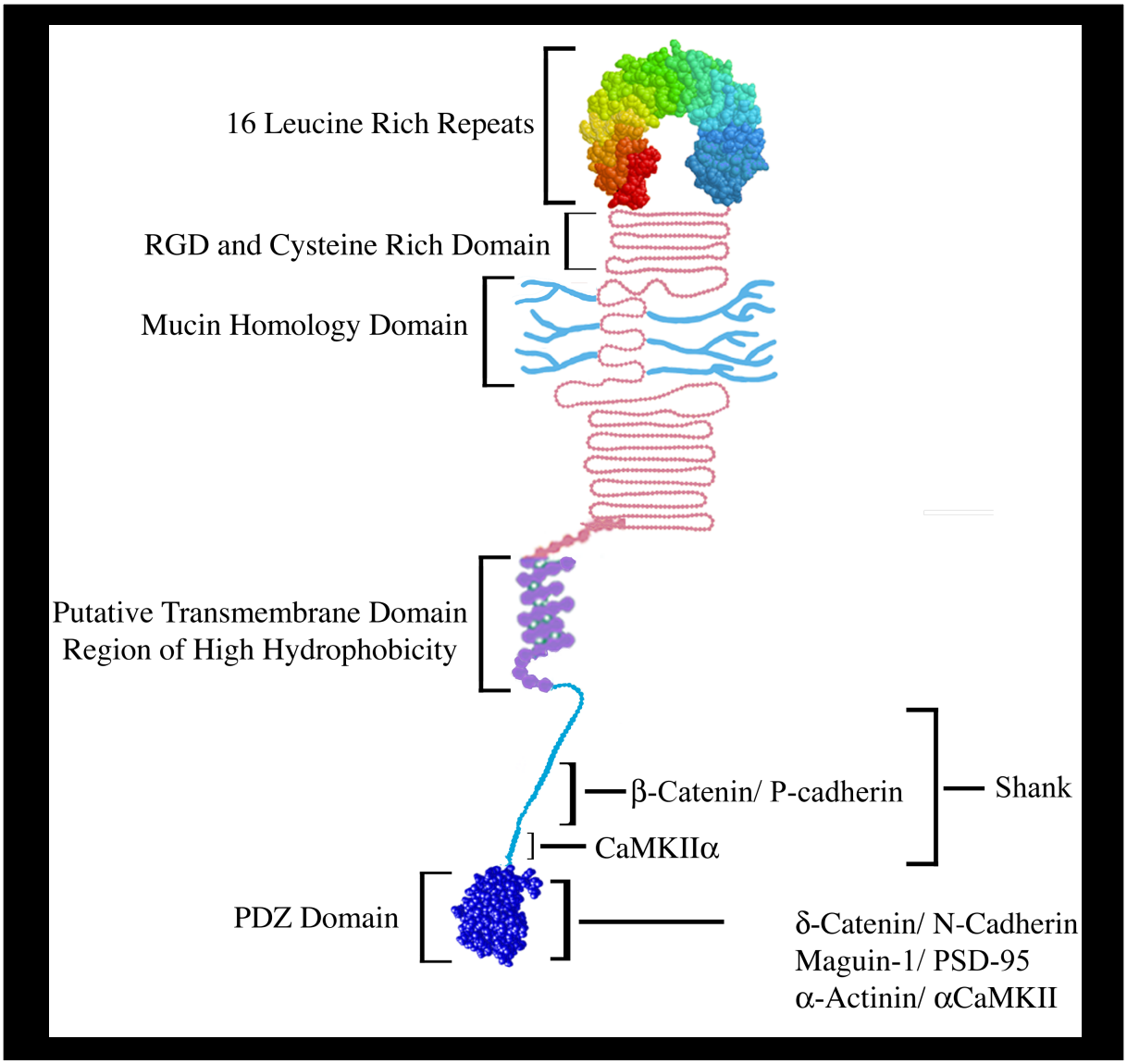
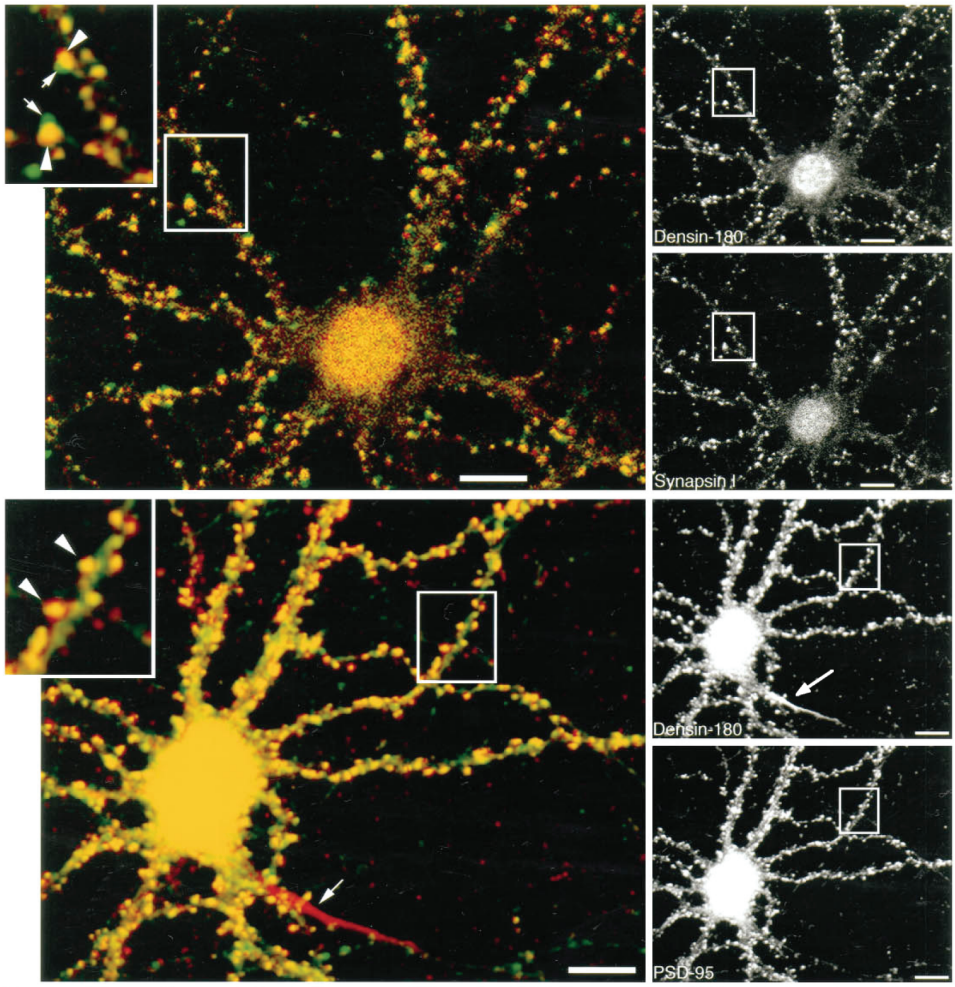


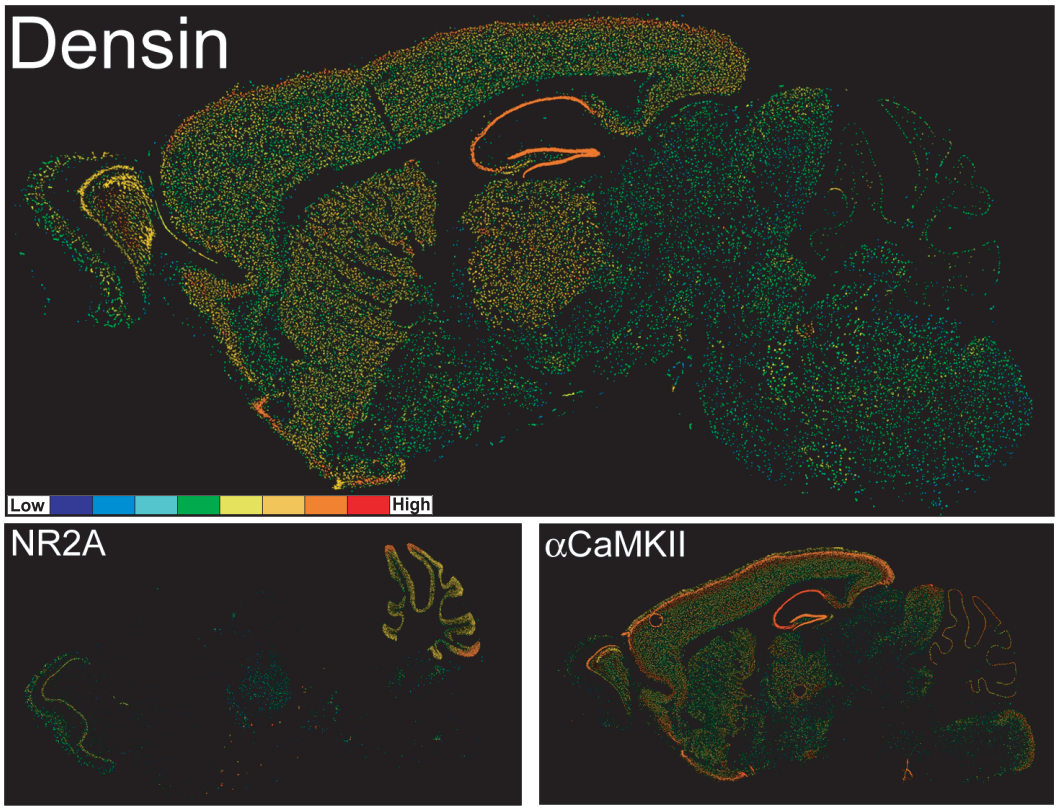
Figure 1.3 Densin protein domains and binding partners. The hypothetical structure of the Densin leucine rich repeat motif and the PDZ domain is based on homologous protein structures. The LRR structure is composed of 16 contiguous LRRs, and is immediately flanked on both sides by clusters of cysteine residues. The mucin-like repeats are thought to serve as sites for O-linked sugars (Apperson et al., 1996). The putative transmembrane domain was hypothesized due to the prediction of an amphipathic helix-like structure between amino acids 1223 to 1246. The region downstream from the putative transmembrane domain is known to bind Shank (Quitsch et al.,2005), CaMKII (Walikonis et al., 2001 and Strack, et al., 2000) and the β-catenin/ P-Cadherin complex in the kidney (Heikkila et al., 2007),. A PDZ domain is located at the C-terminal and is known to interact with the δ-catenin/ N-Cadherin complex (Inagaki et al., 2002), the α-Actinin/ αCaMKII ternary complex (Walikonis et al., 2001), and the Maguin-1/ PSD-95 complex (Ohtakara et al., 2002).

Figure 1.4 Immunocytochemical localization of Densin in 14-21 DIV hippocampal cultures (Apperson et al., 1996) and in situ hybridization of Densin in adult C57B/6J mouse (Allen Brain Map, Lein et al., 2007). (A- top panel) Double staining of Densin (red)/ Synapsin I (green; top panel) and Densin (red)/ PSD-95 (green; bottom panel). Overlay of dual channel confocal images (left) show that Densin (top panel, large arrowheads) and Synapsin I (top panel, small arrowheads) overlap. Small inset boxes on right show single channel images of Densin and Synapsin I. Overlay of dual channel images of Densin and PSD-95 show precise co-localization at punctal positions at spine tip structures along dendrites (arrowheads in inset box, bottom panel) as well as localization of Densin at the axon initial segment/ axon hillock (arrowhead in dual and single Densin channel images, bottom panel). Single channel images of Densin and PSD-95 are shown in bottom right panels. (B) In situ hybridization heat maps of Densin, NR2C and αCaMKII at saggital level 12-13 are shown. Densin gene expression is shown in comparison to NR2C (low gene expression in forebrain) and αCaMKII (high gene expression in forebrain). Densin is highly expressed in all regions except the cerebellum. In contrast, NR2C is highly expressed in the cerebellum, but not in the forebrain, while αCaMKII is highly expressed throught all brain regions. Heat map color scale indicates level of gene expression.

A


Densin-180
Synapsin I
Densin-180
PSD-95

B


Densin
Low
High
NR2A
αCaMKII