Microanatomy of Dendritic Spines: Emerging Principles of Synaptic Pathology in Psychiatric and Neurological Disease

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Psychiatric and neurologic disorders ranging from mental retardation to addiction are accompanied by structural and functional alterations of synaptic connections in the brain. Such alterations include abnormal density and morphology of dendritic spines, synapse loss, and aberrant synaptic signaling and plasticity. Recent work is revealing an unexpectedly complex biochemical and subcellular organization of dendritic spines. In this review, we highlight the molecular interplay between functional domains of the spine, including the postsynaptic density, the actin cytoskeleton, and membrane trafficking domains. This research points to an emerging level of analysis—a microanatomical understanding of synaptic physiology—that will be critical for discerning how synapses operate in normal physiologic states and for identifying and reversing microscopic changes in psychiatric and neurologic disease.

Key Words: Dendritic spine, synaptic plasticity, membrane traffic, schizophrenia, addiction, mental retardation, postsynaptic density, actin cytoskeleton, endocytosis

Synapses are the principal sites of neuronal communication in the brain. These cell-cell contacts are eminently adaptable structures that are constructed, pruned, and modified throughout our lives. The growth and plasticity of neuronal circuitry is genetically guided, but it is experience that ultimately dictates the size, shape, number, and pattern of synaptic connections. At a cellular level, most excitatory synapses in the mammalian central nervous system occur onto micron-long protrusions from neuronal dendrites called dendritic spines (Hering and Sheng 2001; Fiala et al 2002b). Spines are quite distinctive when examined under the light microscope, appearing as knobby studs lining the shafts of dendrites (Figure 1A). Their striking morphology offers a powerful and quantifiable index of the brain's circuitry. Indeed, the search for the physical underpinnings of psychiatric disorders has focused on understanding the structural organization functional characteristics of spines that permit both appropriate and pathologic plasticity (Fiala et al 2002a).

Disruptions of Dendritic Spines Are Associated with Psychiatric and Neurologic Disorders

A broad variety of psychiatric diseases and neurologic disorders are accompanied by patterns of spine disruption (Huttenlocher 1970; Fiala et al 2002b). The major hereditary mental retardation syndromes, Fragile X and Down, are accompanied by changes in spine morphology, in particular a decrease in mature spines and an increase in elongated protrusions that resemble spine precursors called filopodia (Irwin et al 2000; Kaufmann and Moser 2000). Chronic administration of the psychostimulants cocaine and amphetamine produce increased spine density in the nucleus accumbens (Robinson and Kolb 1997, 1999), a brain region critical in mediating these drugs' addictive potential (Carlezon and Nestler 2002). Similarly, chronic nicotine exposure increases spines within the nucleus accumbens, but not in cortical areas unrelated to drug tolerance and reward (Brown and Kolb 2001).

Schizophrenia, on the other hand, is more commonly associated with fewer spines and synapses. Most notably, the spine density of cortical pyramidal neurons is reduced (Garey et al 1998), specifically in the dorsolateral prefrontal cortex (Glantz and Lewis 2000), a prominent area of cortical dysfunction in the disease (Lewis and Levitt 2002). Similarly, pyramidal neurons of the subicular cortex have reduced dendritic branching and a lower spine density (Rosoklija et al 2000). Spine size in striatal neurons is reduced (Roberts et al 1996). Markers of presynaptic terminals are also decreased in cortical and hippocampal regions (Harrison and Eastwood 1998; Harrison 1999). Since loss of neurons is not typical in schizophrenia, the decreased spine density is most likely not due to a degeneration of associated, presynaptic axons (Harrison 1999). Instead, normal development of cortical circuitry may be compromised by genetic disposition or early insult (Weinberger 1987; Lewis and Levitt 2002). Alternatively, the experience-dependent proliferation or stabilization of synapses may be disrupted in schizophrenia or normal synaptic pruning processes may be pathologically exaggerated (Feinberg 1982; Keshavan et al 1994). Thus, abnormal spine structure and plasticity restricted to critical brain areas frequently accompanies psychiatric and neurologic pathology. Understanding the implications of these region-specific defects will require a molecular understanding of spine function.

Control of Synaptic Function by Spine Morphology

Spine morphology is intimately linked to synaptic function (Sorra and Harris 2000; Hering and Sheng 2001; Yuste and Bonhoeffer 2001), and so alteration of spine shape and size during disease likely has diverse functional effects. Most conspicuously, larger spines have larger synapses and support stronger synaptic transmission (El-Husseini et al 2000; Murthy et al 2001). In addition, spine morphology plays an important role in synaptic plasticity, since the large spine head and the constricted spine neck which separates the synapse from the dendritic shaft helps to create an autonomous compartment where biochemical signals rise and fall independent of neighboring synapses (Nimchinsky et al 2002). Synaptic activity can trigger intracellular calcium increases that are compartmentalized within single spines (Finch and Augustine 1998; Mainen et al 1999; Wang et al 2000; Sabatini

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Received June 16, 2003; revised October 3, 2003; accepted October 8, 2003.

0006-3223/04/$30.00
doi:10.1016/j.biopsych.2003.10.006

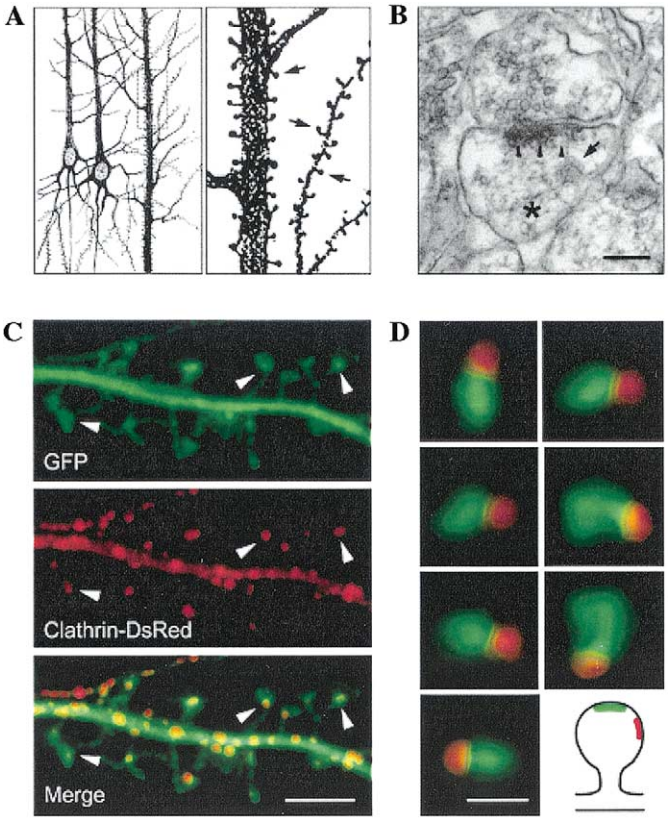

Figure 1. Morphology and microanatomy of dendritic spines. **(A)** Pyramidal neurons in the cerebral cortex, showing their dendrites densely studded with spines. The boxed region in the left panel is shown at higher power in the right panel. Arrows indicate spines. (Adapted from Ramon y Cajal [1904].) **(B)** An electron micrograph of a dendritic spine (bottom structure, asterisk) and associated presynaptic nerve terminal (top structure) from the hippocampus of an adult rat. The arrowheads indicate the postsynaptic density. The arrow indicates an invaginated coated pit at the lateral endocytic zone. Scale bar, 200 nm. (Micrograph courtesy of Bence L. Rácz and Richard J. Weinberg.). **(C)** Machinery for protein trafficking is found within spines. Co-expression in a cultured hippocampal neuron of GFP to fill the cell cytosol (top) and the major endocytic coat protein clathrin tagged with the red fluorescent protein DsRed (middle) demonstrates that most spines (arrowheads) contain the machinery of endocytosis (bottom). Scale bar, 3 μm. **(D)** Endocytic zones in spines are spatially and molecularly distinct from the PSD. Each image panel shows a 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). The diagram summarizes the relative locations of the PSD and the endocytic zone within spines. Scale bar, 1 μm. GFP, green fluorescent protein; PSD, postsynaptic density.

et al 2002). Accordingly, calcium-activated kinases and phosphatases that trigger synaptic plasticity (Morishita et al 2001; Thiels and Klann 2001; Lisman et al 2002) can be recruited and activated on a single-spine level. Thus, the spine is an independent computational unit of the brain, and spine morphology controls synaptic signaling pathways that may be disrupted in disease.

The Actin Cytoskeleton and Spine Shape

Spine morphology is determined by a number of factors, the most well established of which is the actin cytoskeleton. Spines and their filopodial precursors are rich in filamentous actin and display substantial actin-dependent motility (Matus 2000). Many signals such as extracellular guidance cues and growth factors that control dendritic morphology do so via the Rho GTPases (Nakayama and Luo 2000), a family of highly conserved proteins that link extracellular signals to control of the actin cytoskeleton (Etienne-Manneville and Hall 2002). Activation of different Rho family members can modify spine shape and can stimulate either spine extension (Nakayama et al 2000; Ethell et al 2001; Irie and Yamaguchi 2002; Penzes et al 2003) or withdrawal (Tashiro et al 2000; Murai et al 2003).

The link between spine actin and disease may be an important one. The protein responsible for Fragile X syndrome, fragile X mental retardation protein (FMRP), binds directly to a Rac effector, and known consequences of genetic deletion of FMRP in mice include immature spine morphology (Comery et al 1997; Braun and Segal 2000; Nimchinsky et al 2001) reminiscent of spine changes in Fragile X patients (Irwin et al 2000). Furthermore, a number of single gene mutations identified as producing nonsyndromic mental retardation are in proteins known to regulate members of the Rho family. These include a RhoA GTPase activating protein (GAP) called oligophrenin 1, a Rac effector named PAK3, and a Rac guanine nucleotide exchange factor (GEF) called αPIX (Ramakers 2002). It will be important to assess spine morphology and synaptic plasticity in animals that bear mutations in these proteins. Intriguingly, another downstream target of the RhoA pathway, LIM kinase, is known to control actin dynamics and is mutated in Williams syndrome, leading to mental retardation and visuospatial cognitive deficits (Frangiskakis et al 1996; Bellugi et al 1999). Interestingly, mice lacking LIM kinase have abnormally small, thin spines (Meng et al 2002), although it is not yet known whether Williams syndrome patients have similarly disrupted spine morphology. In summary, one working hypothesis is that misregulation of Rho-dependent cytoskeletal dynamics in spines contributes to various disorders by disrupting spine morphology and preventing appropriate synaptic function and plasticity.

The Microanatomical Organization of Spines

Although spine size and shape have long been recognized as key attributes of synapse function that are altered during disease (Fiala et al 2002b), new techniques and recent findings are defining the arrangement of molecular components and membrane domains within spines—what we term the "microanatomy" of spines. Indeed, spines contain highly specialized protein complexes, structural elements, intracellular membrane compartments, and cell surface microdomains, which together orchestrate molecular adaptation at synapses. The most prominent microanatomical feature in spines is the postsynaptic density (PSD) (Figure 1B) (Kennedy 2000; Sheng and Kim 2002). The PSD is a proteinaceous matrix at the postsynaptic membrane (Husi et al 2000; Kennedy 2000). At the core of this matrix lie multidomain molecules such as PSD-95 and shank that link neurotransmitter receptors, intracellular signaling proteins, and the actin cytoskeleton to create a dynamic scaffold within the spine (Kennedy 2000; Sheng and Kim 2002). The precise positioning of the PSD at the synapse, and even of proteins within the PSD (Valtschanoff and Weinberg 2001), is essential for coordinating and regulating synaptic strength and plasticity. Most importantly, by situating receptors immediately across the synapse from sites of neurotransmitter release, the localization of the PSD assures rapid and efficient postsynaptic responses to presynaptic neurotransmitter release. Increasing the level of postsynaptic scaffolding molecules triggers spine growth, hastens spine maturation, and results in stronger synapses (El-

Husseini et al 2000; Pak et al 2001; Sala et al 2001; Penzes et al 2003), emphasizing the tight coupling between molecular composition, spine morphology, and synapse function. Furthermore, recent studies have shown that the PSD exists as an interconnected network of functional protein ensembles, which undergoes remarkably rapid turnover and ubiquitin-dependent remodeling (Ehlers 2003a). Such rapid advances in our understanding of PSD microanatomy should allow for increasing insights into structural and functional defects in psychiatric disease.

Protein Trafficking Within Spines

The strict positioning of synaptic proteins and neurotransmitter receptors at the PSD is the most celebrated example of spine microanatomical organization. More recently, however, it has become clear that spines contain additional specialized domains that are devoted to delivering, maintaining, or removing synaptic proteins from the PSD proper. In particular, many synaptic receptors are dynamically transported to and from the postsynaptic membrane, and controlling the number of receptors present at the synapse has emerged as a critical function played by spines (Moss and Smart 2001; Carroll and Zukin 2002; Malinow and Malenka 2002; Wenthold et al 2003). For example, at excitatory synapses in the brain, glutamate receptor numbers are regulated by changing synaptic activity levels over both short and long time scales (Rao and Craig 1997; O'Brien et al 1998; Ehlers 2000; Turrigiano and Nelson 2000; Shi et al 2001; Malinow and Malenka 2002). Changes in the number of postsynaptic glutamate receptors, in turn, controls synaptic strength, and it is these changes in synaptic strength that are thought to underlie many forms of learning, memory acquisition, and behavioral plasticity (Martin and Morris 2002) and may be altered in psychiatric disease.

Controlling the number of synaptic receptors requires machinery for protein trafficking and transport. Such machinery—including mRNAs, ribosomes, coat proteins, and intracellular vesicles—conducts the synthesis and insertion of new proteins and the removal of receptors from the plasma membrane. The rapid and nearly autonomous regulation of synaptic transmission at individual spines strongly suggests that many of these functions must be carried out within spines themselves. Protein synthesis in spines has yet to be demonstrated, but substantial evidence has accrued that synthesis of at least some proteins takes place within dendrites (Steward and Levy 1982; Weiler et al 1997; Job and Eberwine 2001; Steward and Schuman 2001; Miller et al 2002; Ostroff et al 2002; Horton and Ehlers 2003). Indeed, some neurons possess a distributed and discontinuous arrangement of dendritic Golgi that seems uniquely designed to serve the membrane protein synthesis and processing requirements of dendritic subregions (Horton and Ehlers 2003). Of particular interest is the demonstration of dendritic RNA localization and protein synthesis for several proteins involved in synaptic plasticity, such as the calcium/calmodulin-dependent protein kinase IIa (CaMKIIα) (Mayford et al 1996), brain-derived neurotrophic factor (BDNF) (Tongiorgi et al 1997), and Arc (Steward and Worley 2001). Thus, control of dendritic protein synthesis by activity (Casadio et al 1999; Aakalu et al 2001; Job and Eberwine 2001; Havik et al 2003) may be a particularly rapid means of modifying synaptic proteins and may provide an input-specific means of regulating synaptic function, for instance, during memory consolidation (Miller et al 2002). This model remains unproven, but consistent with it, synaptic activity that induces long-term potentiation (LTP) of synaptic strength mobilizes polyribosomes to subsets of dendritic spines (Ostroff et al 2002). It will be important to determine the mechanisms that direct the positioning of mRNAs, protein synthesis machinery, and secretory organelles in dendrites and spines.

Receptor down-regulation is an established aspect of many forms of use-dependent plasticity, and a large body of evidence indicates that synaptic receptors are removed from the cell surface during synaptic plasticity via internalization or endocytosis (Ehlers 2000; Carroll et al 2001; Moss and Smart 2001; Roche et al 2001; Snyder et al 2001; Vissel et al 2001; Carroll and Zukin 2002; Lee et al 2002; Malinow and Malenka 2002; Nong et al 2003; Wenthold et al 2003). Recent studies have shed light on exactly where this removal occurs within spines themselves. Endocytosis of most cell surface receptors is mediated by the protein clathrin at specialized sites on the plasma membrane called coated pits (Conner and Schmid 2003). Clathrin forms a coat that concentrates cargo destined for internalization and helps to pinch off cargo-laden vesicles from the membrane. Ultrastructural evidence suggests the presence of clathrin-coated pits within spines (Toni et al 1999; Cooney et al 2002), but they are rarely observed due, in part, to their transience (Gaidarov et al 1999; Blanpied et al 2002) and to the difficulty of identifying coated structures on anything but distinctively curved membranes. In cultured neurons, on the other hand, immunocytochemically localized clathrin as well as the expression of green fluorescent protein (GFP)-tagged clathrin has revealed that clathrin assembles at discrete puncta on the membrane throughout dendrites and notably within most dendritic spines (Blanpied et al 2002; Blanpied et al 2003). Clathrin puncta in spines colocalize with other endocytic proteins, and it is at these punctate microdomains where cargo molecules destined for internalization enter the cell (Blanpied et al 2002). Thus, spines contain the requisite molecular machinery for local endocytosis.

Microanatomical Localization of Endocytic Zones in Dendritic Spines

The spatial configuration of clathrin coats at the light microscopic level has given the first insight into the organization of protein trafficking machinery within spines. Remarkably, clathrin coats in spines are present at a stereotypical location in relation to the synapse (Blanpied et al 2002). Clathrin puncta almost never form directly at the synapse but instead are segregated from the synaptic membrane, typically forming several hundred nanometers away (Figure 1C, 1D). The presence and stability of the clathrin coat zone is not perturbed by changes in synaptic activity or by activation of glutamate receptors, similar to the manner in which the PSD arises and persists independent of synaptic activation. The enduring presence of clathrin at membrane regions adjacent to, but distinct from, the PSD establishes the presence of a trafficking domain in spines dedicated to endocytosis. This arrangement parallels the spatial coordination of membrane traffic in presynaptic nerve terminals, where clathrin-mediated synaptic vesicle endocytosis is typically separated from the active zones where exocytosis takes place (Miller and Heuser 1984; Teng et al 1999; Brodin et al 2000; Gundelfinger et al 2003). The spatially reliable association between the PSD and the endocytic zone suggest that the two are molecularly linked, perhaps through common scaffolding molecules or through shared regulation of local membrane lipid composition. Alternatively, the actin cytoskeleton, by binding both to proteins in the PSD and to those associated with endocytosis (Schafer 2002; Gundelfinger et al 2003), may coordinate these two functional

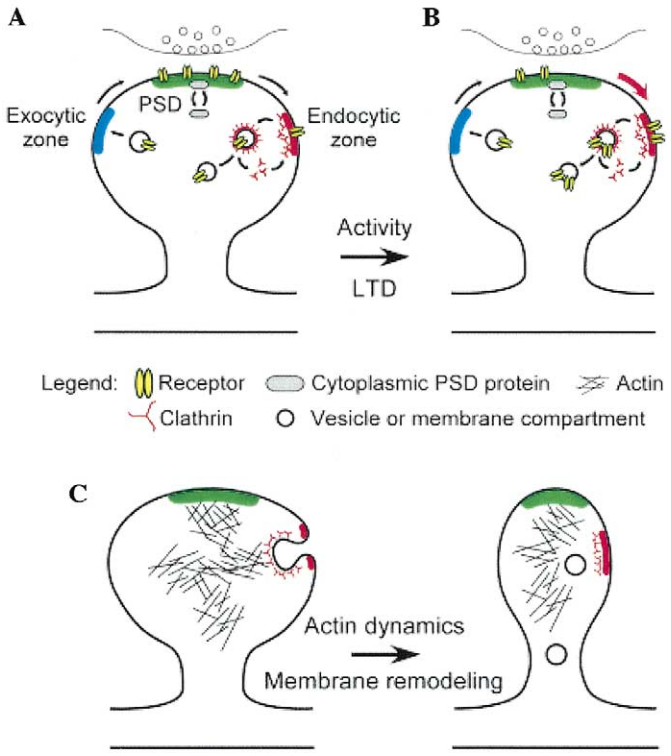

Figure 2. Role of spine microanatomical zones in synaptic plasticity and spine morphologic dynamics. **(A)** Proposed model whereby spines contain a number of domains dedicated to protein trafficking. Internalization of synaptic receptors and other membrane proteins occurs at the endocytic zone (red), 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.

domains. Consistent with a role of the cytoskeleton in organizing the microanatomy of spine protein trafficking, actin is directly involved in spatial positioning of coated pits in the membrane of nonneuronal cell lines (Gaidarov et al 1999). Further definition of the spatial and molecular relationship between the PSD and the endocytic zone will be essential to understanding their intertwined role in synapse development, function, and plasticity.

The spatial confinement of the endocytic cycle (Gaidarov et al 1999; Blanpied et al 2002) is a fundamental mechanism by which neurons may control the protein composition of very local membrane domains. The positioning of the endocytic zone near to but distinct from the PSD suggests a general model of synaptic membrane traffic (Figure 2A, 2B) in which receptors move into synaptic membranes via perisynaptic regions (Choquet and Triller 2003), whereas intracellular components of the PSD are more likely to be moved into and out of the PSD directly (Shen and Meyer 1999). In this model, the dense matrix of the PSD would not be disrupted by the formation of coats directly on the postsynaptic membrane, as would be required if the ongoing trafficking of receptors occurred directly into or out of the synapse. Exocytic traffic presumably occurs away from the synapse proper as well, and we postulate the existence of such an exocytic zone, which has yet to be experimentally identified. In favor of this idea, recent studies suggest that synaptic delivery of glutamate receptors is a two-step process of plasma membrane insertion and synaptic accumulation (Chen et al 2000; Passafaro et al 2001; Lee et al 2002), although the mechanisms that determine the points of exocytosis are unknown.

Localized endocytosis may also contribute to spine morphologic plasticity. As discussed above, spine morphology is intimately linked to actin dynamics. Another prominent role for actin is in coordinating the removal of membrane via the clathrin endocytic machinery. Indeed, clathrin adaptors and other endocytic proteins directly bind to actin or actin-regulatory proteins (Gaidarov et al 1999; Qualmann et al 2000; Blanpied et al 2002; Schafer 2002; Conner and Schmid 2003) and clathrin dynamics are controlled by actin (Gaidarov et al 1996; Gundelfinger et al 2003). Notably, periods of heightened spine morphologic change are accompanied by the appearance of clathrin-coated vesicles (Toni et al 2001). Thus, localized membrane retrieval at the endocytic zone may be one way that actin coordinates spine morphologic change (Figure 2C). It will be important for future experimental studies to determine whether clathrin-mediated endocytosis does, in fact, contribute to spine growth, movement, and morphology.

The Role of Endocytosis in Addiction

Receptor endocytosis plays notable roles in the physiologic basis of drug abuse and addiction. Many drugs of abuse, including the opiates, cannabinoids, and nicotine, produce their effects as agonists at cell surface receptors. Activation of many receptors leads to their removal from the membrane by clathrin-mediated endocytosis (Claing et al 2002; Sorkin and Von Zastrow 2002; Dani et al 2001; St John and Gordon 2001). This endocytic control is involved in receptor down-regulation and the acute termination of signaling (Claing et al 2002) and may directly modulate tolerance that develops to most drugs with addictive potential (Bohn et al 2000; Tsao et al 2001; He et al 2002).

Drugs of abuse initially act on or through diverse receptors, but their addictive potential may result from common downstream consequences (Nestler 2001; Saal et al 2003). One prominent effect shared by psychostimulants is an increase in the strength of glutamatergic synaptic drive onto midbrain dopaminergic neurons (Wolf 1998; Carlezon and Nestler 2002). This effect greatly outlasts the presence of the drugs themselves (Ungless et al 2001) and is thought to underlie the development of sensitization, a progressive increase in drug rewarding properties presumably leading to craving during addiction (Wolf 1998; Hyman and Malenka 2001). This drug-induced synaptic plasticity appears to be mediated by a process similar to long-term potentiation (Ungless et al 2001; Saal et al 2003). Remarkably, psychostimulants such as amphetamine may facilitate this synaptic potentiation by interfering with long-term depression (LTD) in the same cells (Jones et al 2000; Gutlerner et al 2002). These forms of synaptic plasticity are controlled by glutamate receptor trafficking, and glutamate receptor endocytosis is, in particular, critical for LTD (Carroll et al 2001; Gutlerner et al 2002; Lee et al 2002). One attractive possibility is that acute drug exposure or chronic addictive states alter the microanatomy of endocytic zones in spines, thereby altering glutamate receptor

abundance and synaptic efficacy at neural circuits involved in reward. Restoring clathrin-mediated endocytosis at spine endocytic zones may thus be useful in terminating the chronic upregulation of glutamate receptors associated with sensitization. Interestingly, despite the fact that a conserved cell biological mechanism (i.e., clathrin/dynamin-dependent endocytosis) is involved in many types of plasticity, the signaling cascades that initiate synaptic plasticity, such as LTD, differ across cell types and across synaptic populations (Mulkey et al 1994; Gutlerner et al 2002; Chung et al 2003). Thus, it will clearly be important to examine in much more detail the molecular properties of dendritic spines in cell types, including dorsal and ventral striatal medium spiny neurons and various neuronal subclasses in the prefrontal cortex and amygdala, that have been implicated in psychiatric illnesses. Activation or suppression of particular signaling pathways by drugs or during psychiatric illness may modify spine function in specific cell types or specific brain circuits, thereby providing attractive targets for future therapies.

New Technology and Future Directions

Further progress linking the disorder of spines in disease with biochemical or molecular therapeutic targets will require a more complete understanding of the cell biological foundations of spine function and plasticity. A number of new tools are permitting rapid advances in these areas. The twin technologies of confocal/multiphoton microscopy and GFP protein labeling for imaging live neurons allow visualization of single spines at high spatial resolution in culture, in tissue slices, and in vivo over timescales from milliseconds to months (Sabatini et al 2001; Yuste and Bonhoeffer 2001; Grutzendler et al 2002; Trachtenberg et al 2002) and are spurring the development of new optical approaches to the biochemistry of living spines (Wouters et al 2001; Zhang et al 2002; Lippincott-Schwartz and Patterson 2003). Correlated light microscopic and ultrastructural detail via electron tomography (Martone et al 2002) should help bridge molecular and functional analyses. Particularly in combination with genetic models of disease, these new methods should help further define the microanatomy of spines and determine how the dynamic morphology of spines subserves neuronal plasticity. In addition, new paradigms of synaptic plasticity in psychiatrically relevant brain areas (Jones et al 2000; Gutlerner et al 2002; Saal et al 2003) should help to focus the attention of basic research on clinically important phenomena. Further, systematic assessment of the proteins present in the PSD (Husi et al 2000; Walikonis et al 2000; Ehlers 2003a) has demonstrated activity-dependent turnover of PSD protein ensembles (Ehlers 2003a) and suggests that a quantitative proteomics approach to generate a molecular fingerprint of synaptic function and dysfunction will help to identify protein imbalances in numerous psychiatric disorders.

The microanatomical, structural, and molecular organization of synapses and spines is finally beginning to be elucidated. From such studies, we can expect great progress in identifying the common principles that link synaptic function with psychiatric health and disorder.

We thank Juliet Hernandez, April Horton, and Michele Pucak for helpful comments on the manuscript. This work is partially supported by the National Institutes of Health (MDE) a grant from the Ruth K. Broad Biomedical Research Foundation (MDE), and a Young Investigator Award from the National Alliance for Research on Schizophrenia and Depression (TAB).

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