immunosurveillance depend on the ability of tumour cells to block DC maturation and functions, and thus the generation of tumour-specific cytotoxic responses. (*See* Atopy and Asthma; Autoimmune Disease; Autoimmune Disease: Pathogenesis; Rheumatoid Arthritis)

Given their central role in the immune system, DCs can represent an important target and a means for the manipulation of harmful or protective immunity. In particular, the possibility of generating large numbers of autologous DCs from precursors has made feasible and very attractive the use of DCs as natural adjuvants for inducing or boosting antitumour immune responses. (*See* Tumour Immunology)

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Dendritic Spines

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Introductory article

Article Contents

Dendritic spines are important sites of excitatory synaptic transmission and changes in the strength of these synapses are likely to underlie important higher brain functions such as learning and memory. Spines form biochemical compartments for isolating reactions that occur at one synapse from those at other synapses thereby providing a possible way to ensure the specificity of connections between neurons in the brain.

Spiny Projections from Dendrites Seen in the Light Microscope

Around the beginning of the twentieth century Ramon y Cajal first discovered the tiny protrusions, called dendritic spines, that stud the surfaces of neurons throughout the brain. **Figure 1a** illustrates a pyramidal cell, so named for the shape of its cell body. From the apex emerges a long dendritic trunk, the apical dendrite, which gives rise to many lateral dendrites. Several basillar dendrites emerge from the base of the cell body. At higher magnification (**Figure 1b**) the dendritic spines become visible along these dendrites. Cajal noticed that the dendritic spines have a diversity of shapes and, in one of his classic treatises, he described the spines as 'Stalcheln und Dornen' in reference to the long spicule-like spines and the shorter thorn-like spines that could be distinguished with light microscopy. (*See* Ramón y Cajal, Santiago; Neurons; Dendrites)

The Golgi silver-impregnation method (used to reveal the cell in **Figure 1**) is named after Camillo Golgi, who discovered it accidentally when some silver nitrate spilled on a brain he was dissecting. The Golgi method reveals in its entirety about 1 in every 1000 neurons, leaving the surrounding tissue unstained. If all of the cells were to be stained, then the tissue would be completely black and the individual cells would be indistinguishable from the surrounding cells. This Golgi preparation is identical to that which Cajal used more than a century ago, and is still widely used to obtain a first estimate of what happens to dendritic spines under a variety of conditions. The disadvantage is that it is still not known why the method works, and whether there is something special about the cells that stain. Nevertheless, much of our understanding about the diversity in neuronal structure has come from studying these beautiful preparations. (*See* Golgi, Camillo)

From the beginning, Cajal postulated that the spines were the points of contact between cells. Later, Gray discovered, using electron microscopy, that dendritic spines are the major sites of excitatory

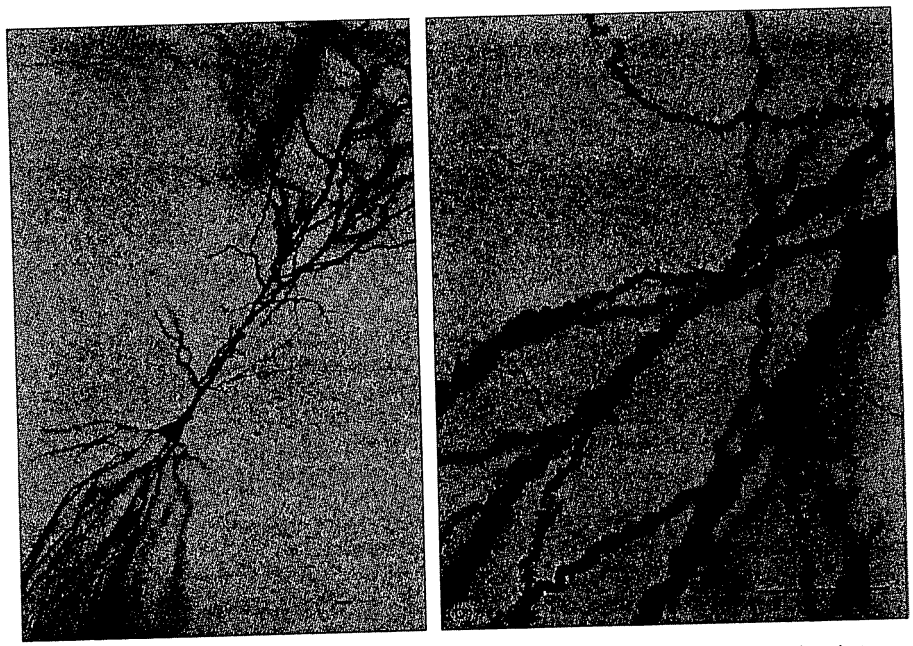

Figure 1 Pyramidal cell in hippocampal area CA1 of the rat brain, showing the typical appearance of the principal excitatory neurons that occur throughout the brain. (a) The low-magnification view of these cells shows an apical dendrite projecting towards the upper left quadrant of the figure. Many lateral dendrites emerge from the large apical dendrite. Several basillar dendrites project from the base of the cell soma. Dendritic spines are the tiny projections that stud the surface of these dendrites in both the apical and basillar dendritic arbour. (b) A higher magnification view of the dendrites reveals the tiny spines and a few of the thin axons passing through the dendritic arbour. Bars, 10 μm.

electrochemical communication between neurons, via synapses. Throughout most brain regions, more than 90% of all excitatory synapses occur on dendritic spines; understanding the function of dendritic spines is therefore one of the major questions today in neurobiology. (*See* Synapses; Glutamatergic Synapses: Molecular Organization)

Modern studies of dendritic spines can be summarized around attempts to answer four key questions: How big are the spines and what do they contain? How do they interact with the structures that surround them in the brain? How are new spines generated, during development and in the adult nervous system? Are changes in spine structure involved in more global brain processes, such as learning and memory? Many new methods have been developed to answer these questions, including computer-assisted reconstruction from serial sections at the electron microscopic level and functional imaging using confocal and two-photon microscopy. In combination with electrophysiological analyses of neurons, these imaging approaches are beginning to give new insights into the functions of dendritic spines and their synapses. In addition, new molecular labels have made it possible to probe the identity of molecules that could mediate these functions in the dendritic spines. (*See* Vertebrate Central Nervous System; Learning and Memory; Imaging: An Overview)

Distinctive Structural Features of Dendritic Spines

Spine morphology

Dendritic spines have a wide variety of sizes, shapes and subcellular compositions, both within and across brain regions. Most spines can be classified by their shapes as stubby, thin or mushroom-shaped (**Figure 2a**). Occasionally, dendritic spines have multiple branches, each of which can be innervated by the same presynaptic axon or by different axons depending on brain region. Sometimes one or more of the branches have no presynaptic axon.

Three-dimensional reconstructions of dendritic spines have revealed their total size to range more than 100-fold. Neighbouring spines on the same short segment of dendrite can express this full range of dimensions (**Figure 2b**). This finding provided the first clue that individual spines might act as separate units.

For all spines, the postsynaptic density occupies only about 10–15% of the total spine surface area, suggesting that the nonsynaptic membrane must be present in a particular proportion to support synaptic function. In addition, the volume and surface area of the spine are proportional to the area of the synapse on its head, to the

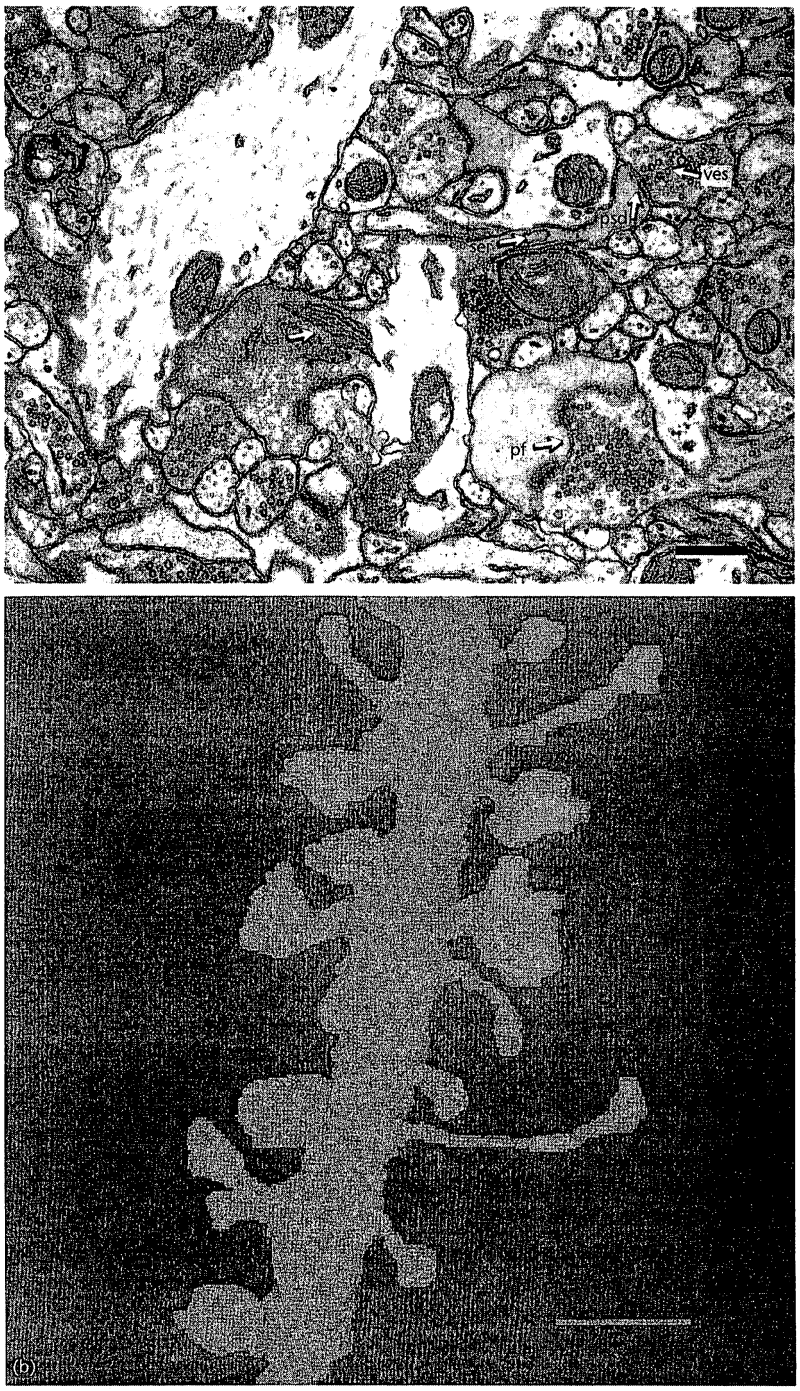

Figure 2 [*Figure is also reproduced in colour section.*] (a) Electron micrograph of a single thin section (0.05 μm thick) through dendritic spines in the stratum radiatum of hippocampal area CA1. In this fortuitous section, three spines were sectioned parallel to their longitudinal axis, revealing spines of the stubby (red), thin (blue) and mushroom (green and yellow) morphologies. Each of the presynaptic axons associated with these four spines is coloured purple. The postsynaptic density ('psd' in the thin blue spine) occurs on the spine head immediately adjacent the synaptic cleft and to a presynaptic axonal bouton that is filled with round vesicles (ves). The thin blue spine contains a small tube of smooth endoplasmic reticulum (ser) in its neck. A spine apparatus (sa) is visible in the green mushroom spine. A perforated postsynaptic density (pf) is evident on the head of the yellow mushroom spine. About 30 serial sections are needed to reconstruct each of the largest dendritic spines. Bar, 0.5 μm. (b) Three-dimensional reconstruction of an 8-μm segment of a CA1 dendrite, illustrating the many different types of spines that occur along its length. This dendrite was outlined in 89 serial sections to obtain the reconstruction, which is then computer-generated. Bar, 1 μm.

volume of smooth endoplasmic reticulum it contains and to the number of vesicles in the presynaptic bouton.

Synapses

As indicated above, dendritic spines are the major postsynaptic target for excitatory synapses throughout the central nervous system. These excitatory synapses are characterized by a thickened postsynaptic density (PSD) occurring on the spine head, across from a presynaptic axon containing round clear vesicles (**Figure 2a**). Three-dimensional reconstruction from serial electron microscopy reveals that some of these PSDs are uniform discs, referred to as macular PSDs. Other PSDs have electron-lucent regions, which create the appearance of perforations in the PSD, and these synapses are termed perforated PSDs (**Figure 2a**). Perforated PSDs occur preferentially on the large mushroom-shaped dendritic spines. Over 30 different proteins, known to be involved in synaptic transmission or maintaining the integrity of the synapse, have been identified in biochemically-enriched fractions of the PSD. The PSD is discussed in detail elsewhere in this encyclopedia. (*See* Synapse Formation)

In some brain regions a second synapse occurs directly on the spine neck. This second synapse differs from the primary excitatory synapse. Its structure is different, in that there is not a thickened PSD and the presynaptic bouton contains smaller, round and flattened vesicles. Immunostaining shows these synapses to contain inhibitory and modulatory substances that could alter the strength of the excitatory synapse occurring on the spine head. (*See* Synaptic Vesicles: Methods for Preparation)

Organelles

Most spines contain an organelle called smooth endoplasmic reticulum (**Figure 2a**), which is involved in the local regulation of the ionic milieu of the spine, especially calcium. The amount of smooth endoplasmic reticulum is proportional to the spine volume and occupies about 10–20% of the total spine volume. In large dendritic spines, the smooth endoplasmic reticulum is laminated with dense-staining material into a structure known as the spine apparatus (**Figure 2a**). Polyribosomes have been localized in dendritic spines, leading to the hypothesis that they could be sites for local protein synthesis that does not require the nucleus of the cell. Mitochondria are not usually found in dendritic spines, although large and complex dendritic spines occasionally have mitochondria in them. Coated vesicles and multivesicular bodies, which are usually involved in endocytosis and degradation, are occasionally found in dendritic spines of the normal brain and their frequency appears to increase during periods of synapse formation, as well as under pathological conditions of synapse degradation. (*See* Cell Staining: Fluorescent Labelling of the Endoplasmic Reticulum; Protein Export from the Endoplasmic Reticulum to the Cytosol: Methods; Eukaryotic Ribosomes: Assembly; Messenger RNA: Interaction with Ribosomes)

Cytoskeleton

In the dendrite proper, microtubules are a prominent cytoskeletal element, involved in basic structure as well as the transport of organelles, such as mitochondria, and proteins. In contrast, the cytoskeleton of dendritic spines is usually devoid of microtubules except for the very largest spines in a few brain regions, such as hippocampal area CA3. Spine cytoplasm is characterized instead by a loose network of filaments comprised of actin and actin-binding proteins. The actin filaments are longitudinally situated in the spine neck, while those in the head are organized into a lattice. The actin filaments have been thought to provide both the scaffolding for supporting spine shape and a mechanism whereby the shape can be rapidly altered in a calcium-dependent fashion. Molecules found in the spine cytoplasm which could bind with the actin cytoskeleton, usually in a calcium-dependent manner, include calmodulin, myosin, brain spectrin (fodrin), microtubule-associated protein (MAP)-2 and a host of synaptic molecules located in the PSD, such as α-actinin, which may be crucial for mediating changes in the strength of synaptic transmission. (*See* Axonal Transport and the Neuronal Cytoskeleton; Cytoskeleton; Tubulin and Microtubules; Hippocampus; Actin and Actin Filaments)

Presynaptic vesicles

The axonal boutons which form synapses with dendritic spines have numerous round clear vesicles (**Figure 2b**). The membrane surrounding the vesicles contains specific molecules involved in filling the vesicles with neurotransmitter, docking them with the presynaptic membrane and fusing the vesicle with the membrane, thereby releasing the neurotransmitter, usually glutamate, into the synaptic cleft. The total number of vesicles can range from as few as 1–3 to more than 2000 in each axonal bouton. This number is proportional to spine volume, the volume of the smooth endoplasmic reticulum and the area of the PSD on the dendritic spine. An area of active research is to determine how variation in vesicular size, composition and distribution influences the properties of synaptic transmission at dendritic spine synapses. (*See* Synapse Formation; Synaptic Vesicle Traffic)

Synaptic cleft

Between the membranes of the presynaptic axon and the postsynaptic dendritic spine is a gap, the synaptic cleft, which is about 10–20 nm wide and is filled with a dense-staining material. The composition of this dense-staining

material is not known; however, it is likely to contain cell adhesion molecules which span the two membranes and serve both structural and molecular signalling functions.

Connections between dendritic spines and astrocytic processes

Astrocytes are glial cells in the central nervous system which are involved in regulating ions, glucose and neurotransmitter concentration in the extracellular space. In some brain regions, such as the cerebellum, astrocytic processes surround the spine and presynaptic axon, thereby tightly controlling the escape of molecules from the synaptic cleft. In other brain regions there is a less conspicuous interdigitation of tiny astrocytic processes adjacent to some, but not all, of the spines. At some spines the astrocytic processes also form cell adhesion junctions where there can be direct signalling between the spines and glia during synaptic transmission. One important role of the astrocytes is to protect the neuron from calcium-induced neurotoxicity. This regulation could also be aided by smooth endoplasmic reticulum, which is found on either side of the cell adhesion junctions between spines and the astrocytic processes. (*See* Astrocytes and Brain Signalling)

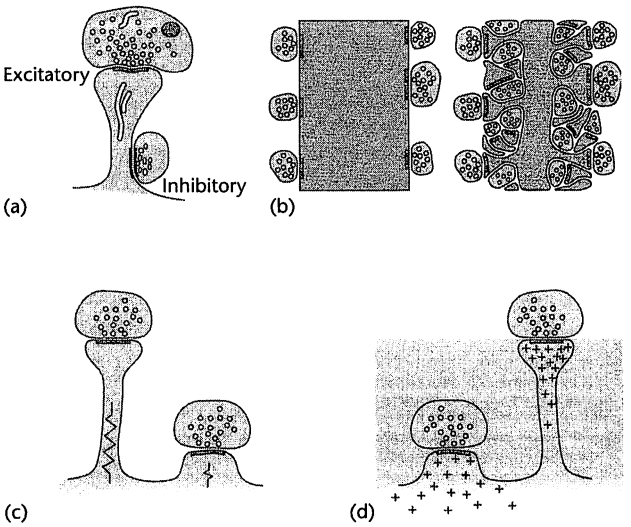

Figure 3 Functions of dendritic spines: (a) modulation; (b) density; (c) resistance; and (d) compartmentalization. See text for descriptions of each possible function.

Dendritic Spines as Postsynaptic Targets

As indicated above, dendritic spines are the major site of excitatory synapses, with more than 90% of all excitatory synapses occurring on spines. In some brain regions (e.g. portions of cortex and striatum) up to 30% of the spines also have an inhibitory and/or modulatory synapse occurring on the neck or at the base of the spine (**Figure 3a**). In this location, an inhibitory input could 'veto' or modify the strength of the excitatory input by altering the amount of synaptic charge that reaches the postsynaptic cell through the spine neck. Since excitatory, inhibitory and modulatory events can all occur on an individual spine, the spine is thought to be the smallest multifunctional integrative unit in the brain.

It had been postulated by Ramón y Cajal that spines could increase the surface area available for new synapses to form; however, most of the area on the dendritic shaft between spines does not have synapses and ample room is available for more synapses to occur, even in the absence of more dendritic spines. Spines do allow relatively thin dendrites to reach multiple axons as they weave through the neuropil (**Figure 3b**). For nonspiny dendrites to attain the same reach to the meandering axons, they would need to be thicker than spiny dendrites (which typically they are) and to occupy a significantly greater volume of the neuropil. Thus, spiny dendrites allow more synaptic connections to be compacted into a limited brain volume.

Dendritic Spines as Electrical Compartments

Amplification of voltage in the spine head

Even a small constriction in the dendritic spine neck poses a resistive barrier. Such a resistive barrier results in a build-up of charge in the spine head near to the synapse. Measurements of dendritic spine necks have been used in computer simulations to determine that they have sufficient resistance to delay complete charge transfer for up to 100 ms after the initiation of a synaptic event. This 100-ms delay allows a transient amplification of voltage at the spine synapse. In this way, the spine neck constriction facilitates the opening of voltage-dependent channels in response to less synaptic activation than would be required to depolarize similar synapses on nonspiny dendritic shafts. For example, this transient amplification is important because it facilitates the activation of the *N*-methyl-D-aspartate glutamate receptor-channel complexes, which require sufficient depolarization to let calcium in. The postsynaptic elevation in calcium is an important first step in cellular processes involved both in learning and memory, as well as in pathological states when spines are lost and there is excess synaptic activation and calcium influx, leading to neurotoxicity (**Figure 3c**). (*See* Voltage-gated Potassium Channels; NMDA Receptors; Learning and Memory)

Sharing of postsynaptic potential

The narrow dimensions of the spine neck have long been thought to attenuate the flow of current from the synapse on the spine head to the dendrite. Morphological evidence suggests, however, that most spine necks are wide enough and short enough to allow 85–100% of the charge to be transferred to the postsynaptic dendrite within about 100 ms. Conversely, since the spines are small relative to the dendrite, any current occurring in the dendrite will easily pass to the spine. Thus, linear summation of current occurs amongst coactivated spine synapses via preservation of charge transfer through the dendrites connecting them. Other models endow the spine with active membrane, which would boost further the amount of synaptic current shared among neighbouring spines. (*See* Membrane Potential)

Dendritic Spines as Biochemical Compartments

At least some spine necks are sufficiently narrow to slow diffusion of small ions from the activated synapses such that their concentrations may build up to levels that activate second messenger cascades. Mathematical modelling provides plausible insights into molecules that could be sequestered in spine heads, even though there is no absolute barrier to diffusion between the spines and their parent dendrites. For example, three conditions could achieve local compartmentalization of calcium, an important second messenger. First, the narrow spine neck could provide a restricted diffusion path, thereby limiting calcium ion flux into or out of some spine heads. Second, even a small increase in spine calcium could trigger a subsequent release from intracellular calcium stores and amplify the calcium signal. Third, calcium pumps could extrude the calcium ions, which might otherwise pass through the spine neck, thereby preventing transfer to the parent dendrite.

Given the diversity in spine morphologies, not all spines would be expected to restrict diffusion. Similarly, the distribution of intracellular calcium stores and pumps might not be the same on all spines and dendrites. Recent visualization with confocal and two-photon microscopy reveals prolonged sequestration of calcium following synaptic events at living dendritic spines. The biochemical compartmentalization of ions and other molecules in spine heads may also serve an important role in protecting the neuron during synaptic activation. For example, by restricting high concentrations of calcium from the postsynaptic dendrite, excitotoxic cell damage such as microtubule breakdown and mitochondrial swelling can be prevented. Since dendritic spines rarely have microtubules or mitochondria, a high calcium concentration in the spine head is less likely to have a detrimental effect (**Figure 3d**). (*See* Calcium and Neurotransmitter Release)

Postsynaptic Integration in Dendritic Spines

Cajal and his contemporary, Tanzi, postulated that changes in spine structure or number would be important factors in synapse formation during development and synapse modification during learning and memory in the mature nervous system. As indicated above, several molecular mechanisms exist that could mediate changes in spine and synapse structure. For example, the neurotransmitter glutamate causes breakdown of brain spectrin (fodrin) by the neuron specific protease, calpain I. Since fodrin is a structural protein of the (spine) cytoskeleton, its breakdown could allow the spine to change shape during synaptic transmission. The viscosity of the spine cytoplasm is regulated by the degree of actin polymerization, which in turn is regulated by calcium. Thus, rapid changes in spine structure could also occur in response to actin polymerization and depolymerization. Similarly, actin could serve to stabilize spine structure through its binding to the subplasmalemmal cytoskeleton or to alter spine structure through 'contraction'.

Results from many studies suggest that spines undergo changes in structure with synaptogenesis during development, with the behavioural changes associated with learning and memory, and under pathological conditions associated with neural dysfunction. One compelling example concerns the appearance and disappearance of dendritic spines during the oestrus cycle in female rats. This cycle is mediated by the gradual rise and decline of oestrogen and a corresponding sharper peak and decline in progesterone. In rats, this cycle lasts only 5 days, and the disappearance of 30% of the spines occurs within less than a 24-h period from the peak to decline of progesterone. Such a natural cycle in spine and synapse number reflects the incredible rate at which the neurons control their functional state in response to the global hormonal state of the animal.

During development the number of synapses and dendritic spines increases steadily, and in some cortical regions of higher mammals the total number of spines appears to peak during adolescence, declining to a plateau in early adulthood. This peak is thought to occur during some critical period and its duration is lengthened by preventing synaptic activation in the brain, suggesting that synaptic transmission mediates the pruning of excess synapses. (*See* Developmental Biology of Synapse Formation)

A phenomenon called long-term potentiation (LTP) has been discovered in most brain regions and is thought to represent the cellular basis of learning and memory. LTP is expressed as an enhanced synaptic response following a brief, high-frequency stimulation of the synapses. The enhanced response can last indefinitely, depending on the exact experimental paradigm. The enduring nature of LTP has led to the idea that perhaps it is mediated by an increase

in spine and synapse number and/or size. Many studies have been carried out but controversy remains as to whether new spines and synapses form or whether the geometry of existing spines and synapses changes. The reason for this controversy is that small changes in number and size may be sufficient to mediate dramatic changes in neuronal function such as LTP. Subtle differences in the way scientists have investigated this question have led to conflicting results, with some laboratories claiming changes in synapse number, and spine size, while others show no comparable changes. Several new approaches are under development to ascertain which structural effects are specific to synaptic plasticity, such as LTP, and which structural effects occur with other forms of synaptic activity. More robust sampling procedures have been developed to make appropriate distinctions between changes in size and number of synapses. Another powerful approach will be to combine confocal or two-photon microscopy while visualizing living dendrites with reconstruction from serial electron microscopy to identify and measure the activated synapses. As these new approaches are applied to the problem of synaptic plasticity they should help to resolve whether changes in the structure and/or number of particular spines and synapses are involved in the functional synaptic plasticity. (*See* Long-term Potentiation; Long-term Depression and Depotentiation)

Summary

Much is now known about the diversity in the structure of dendritic spines and their synapses. Much remains to be learned about how variation in structure might be involved in variation in synaptic function. The challenge for the next generation of neuroscientists will be to discover the full range of molecules residing in dendritic spines and whether the compartments formed by the spines are crucial for the molecular function. Ultimately we will need to learn exactly how the spine compartments work in conjunction with signalling the cell nucleus when changes in synaptic input have occurred. Obtaining this understanding will be important for elucidating the causes of mental retardation, where dendritic spines are drastically affected, either remaining as immature, very long and very thin structures or disappearing altogether.

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Dengue Fever Viruses

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Secondary article

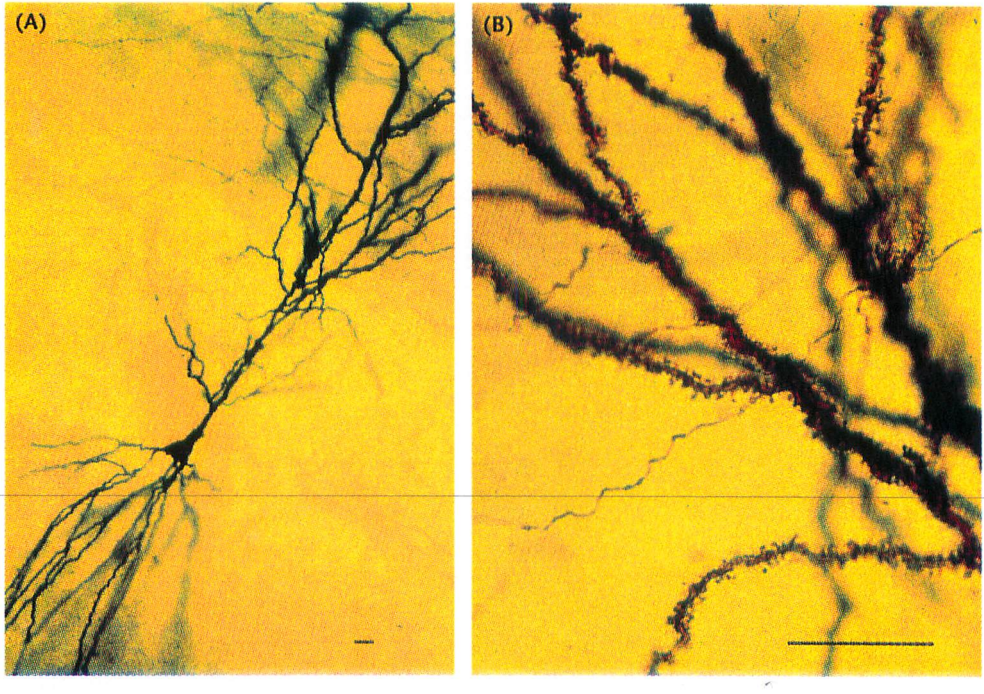
Article Contents

Dengue viruses are the most important arboviruses causing disease in humans. Over half of the world's population live in areas of risk, and an estimated 50–100 million cases occur each year.

Classification

Dengue viruses belong to the family *Flaviviridae*, genus *Flavivirus*. There are four serotypes: DEN-1, DEN-2, DEN-3 and DEN-4. They belong to a larger, heterogeneous group of viruses called arboviruses. This is an ecological classification, which implies that transmission between vertebrate hosts including humans is dependent upon haematophagous (blood-sucking) arthropod vectors. (*See* Human Pathogenic Viruses)

There are over 70 antigenically related viruses in the genus *Flavivirus*, including the type species, *Yellow fever virus*. The genus includes several antigenic complexes, including the dengue complex, the Japanese encephalitis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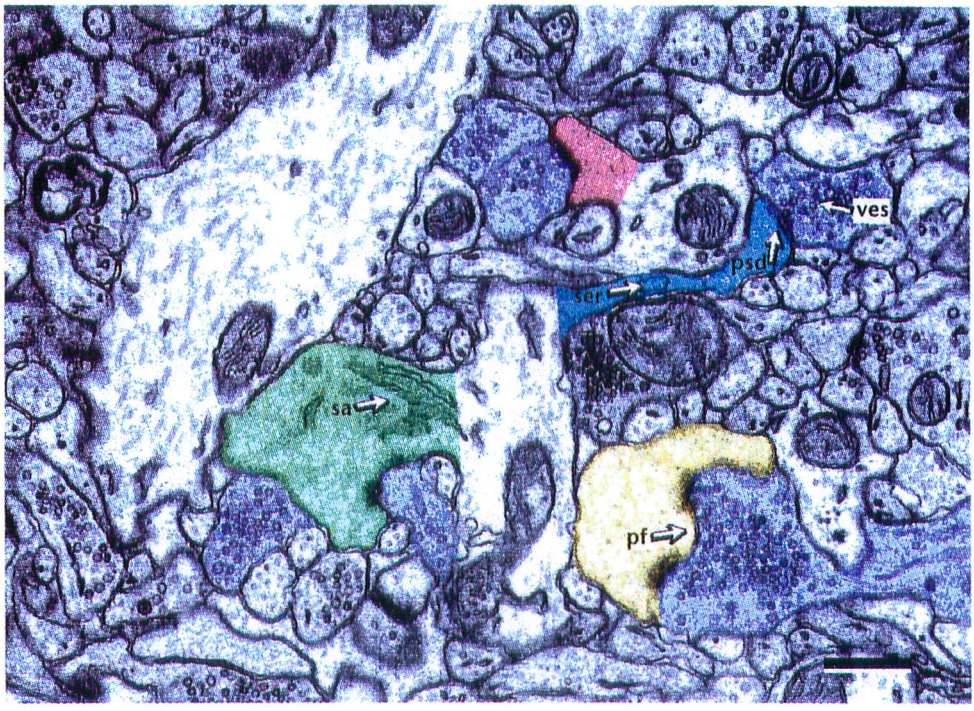

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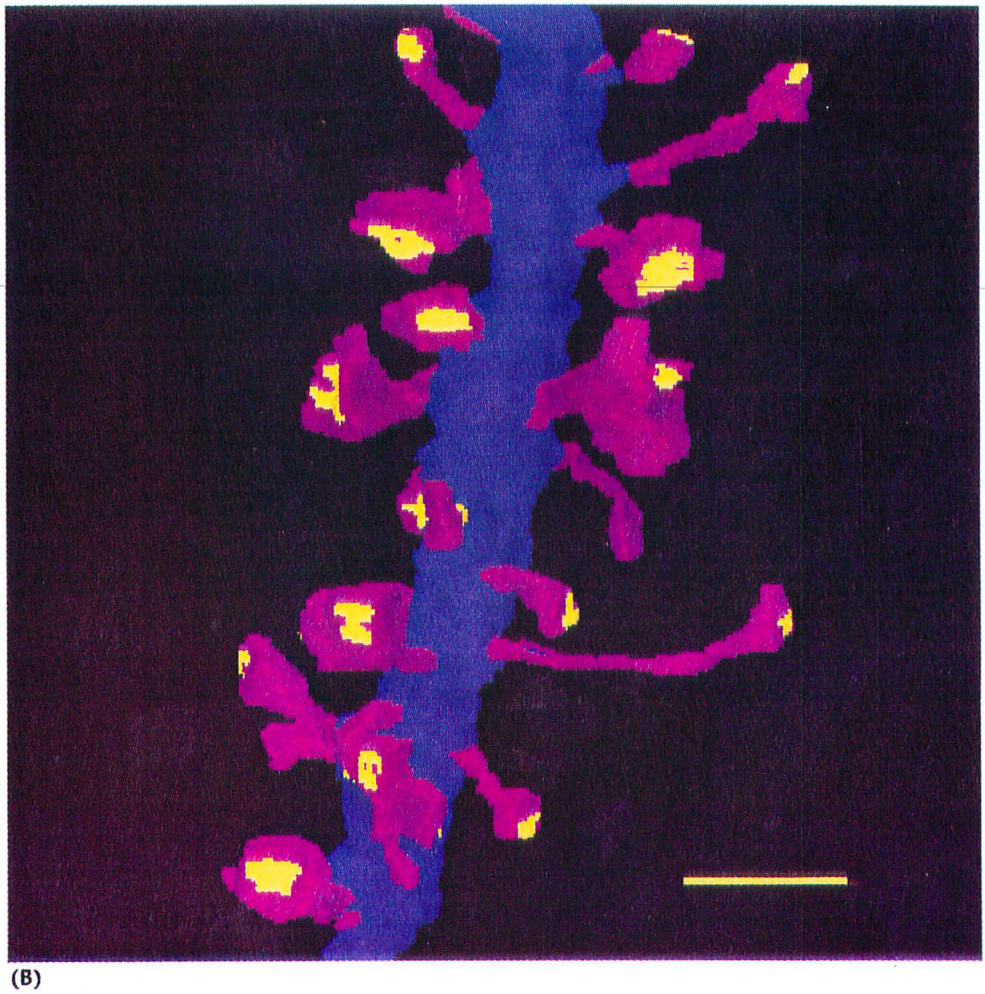

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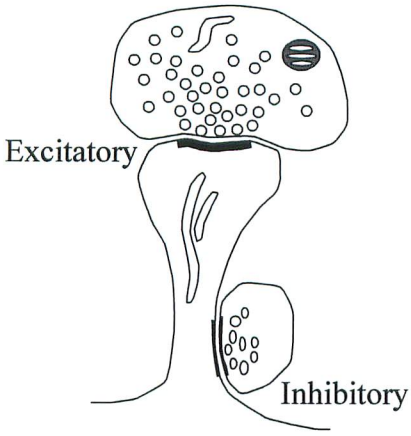
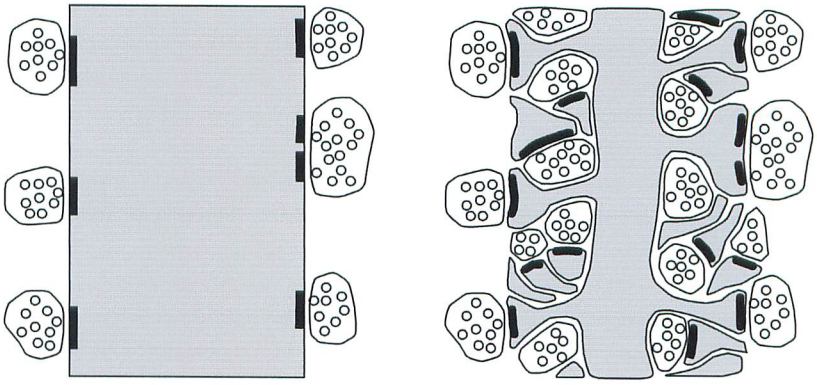
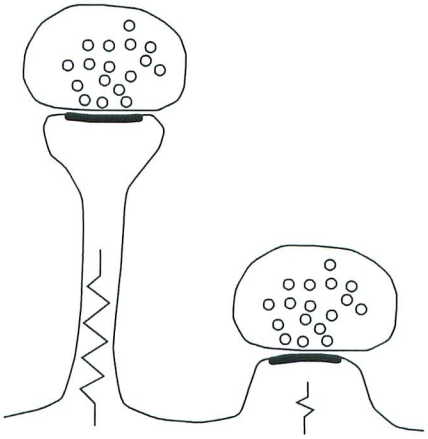


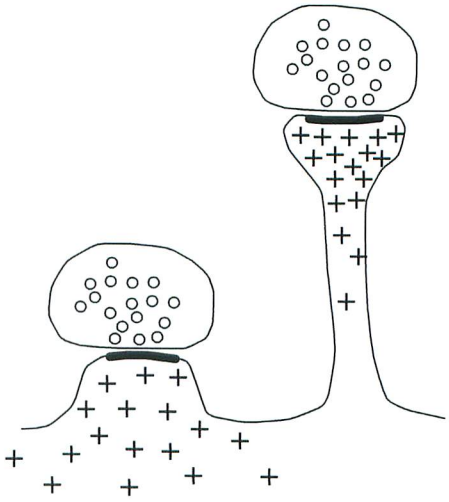
Modulation



Density



Resistance



Compartmentalization