Chemical Synapses

Craig Garner, *University of Alabama, Birmingham, Alabama, USA*
Joanne Nash, *University of Alabama, Birmingham, Alabama, USA*

Chemical synapses are amongst the most elaborate junctions existing between two cells. They enable chemical neurotransmission to occur within the millisecond range.

Secondary article

Article Contents

Structures that Define Chemical Synapses

Vertebrate nervous systems are composed of two principal cell types: glia and neurons. Glial cells are the most abundant and are involved primarily in maintaining ion homeostasis in the brain. Information processing is performed by neuronal cells that form intricate networks of processes capable of conducting and transferring electrical information at high speed and high efficiency. Neurons are elongated, polarized cells that extend two types of processes, called axons and dendrites. Axons are long and thin, and can be up to 1 m in length. Dendrites are much shorter, tapered and highly branched processes often covered with small protrusions or thorns called dendritic spines. Contact sites between neurons (termed synapses: from the Greek 'to clasp') occur primarily between small swellings along axonal profiles, known as the presynaptic bouton, and the dendritic area of a target cell, termed the postsynaptic reception apparatus (postsynaptic junction). The extracellular space situated between the presynaptic and postsynaptic sides of these junctions is known as the synaptic cleft (**Figure 1**). While most presynaptic boutons can terminate on dendrites, they may also end on dendritic spines, the neuronal cell soma, the initial segment of an axon, called the axon hillock, or another presynaptic bouton. Each of these synapses has a specific name, such as axospinus, axodendritic, axoaxonic or even dendrodendritic, depending on whether the synapse is formed between an axonal presynaptic bouton, a spine, a segment of dendrite, another axon or between two dendrites, respectively. Synapses formed between the axon terminals of motor neurons and muscle cells are called neuromuscular junctions (NMJs) (**Figure 2**). (***see*** Cells of the nervous system.) (***see*** Neurons.) (***see*** Axons.) (***see*** Synapses.)

Intraneuronal signalling, originating primarily in the dendritic arbour or the soma of neuronal cells, takes the form of electrical impulses or action potentials which travel down the axon to the presynaptic boutons. Interneuronal signalling, occurring at synapses, can be either electrical or chemical in nature. Electrical synapses allow a direct electrical coupling between neurons through small pores or channels, called gap junctions, present in the plasma membranes of both cells. When open, these junctions allow the free passage of ions and small molecules in either direction. While gap junctions are commonly found between nonneuronal cells, such as glia and epithelia, they are rarely found between vertebrate neurons. This is probably due to the limited ability of electrical synapses to be dynamically regulated, compared with chemical synapses (see below). (***see*** Action potential: generation and propagation.) (***see*** Electrical synapses.)

As the name implies, signal transduction at chemical synapses involves the conversion of electrical signals that arrive at the presynaptic bouton into chemical signals that are perceived by the postsynaptic cell. As is discussed in detail below, these chemical signals or substances, called neurotransmitters, are released into the synaptic cleft by the presynaptic bouton, and act to initiate a second electrical signal in the postsynaptic cell by binding to, and opening, postsynaptic ligand-gated ion channels known as neurotransmitter receptors. Signalling across chemical synapses is mostly unidirectional and highly regulated, features that are essential for the establishment of complex highly plastic nervous systems. (***see*** Neurotransmitters.) (***see*** Neurotransmitter release from presynaptic terminals.) (***see*** Neurotransmitter receptors in the postsynaptic neuron.)

Clues to how chemical synapses enable interneuronal transmission came initially from high-resolution electron microscopy (EM) studies performed in the 1950s, and from electrophysiological studies. Ultrastructural studies revealed that central nervous system (CNS) synapses are asymmetrical cellular junctions composed of three morphologically distinct compartments: the presynaptic bouton filled with small clear synaptic vesicles (SVs), a synaptic cleft, and an electron-dense postsynaptic structure referred to as the postsynaptic density (PSD) (**Figure 1**). The presence of numerous SVs of uniform diameter (40–60 nm depending on neuronal type) within presynaptic boutons suggested that SVs were the morphological correlate of physiological quanta of signalling information being measured by electrophysiological methods. This concept is supported by subsequent studies showing that SVs contain high concentrations of neurotransmitter (3000–5000 molecules per vesicle), whose fusion with the

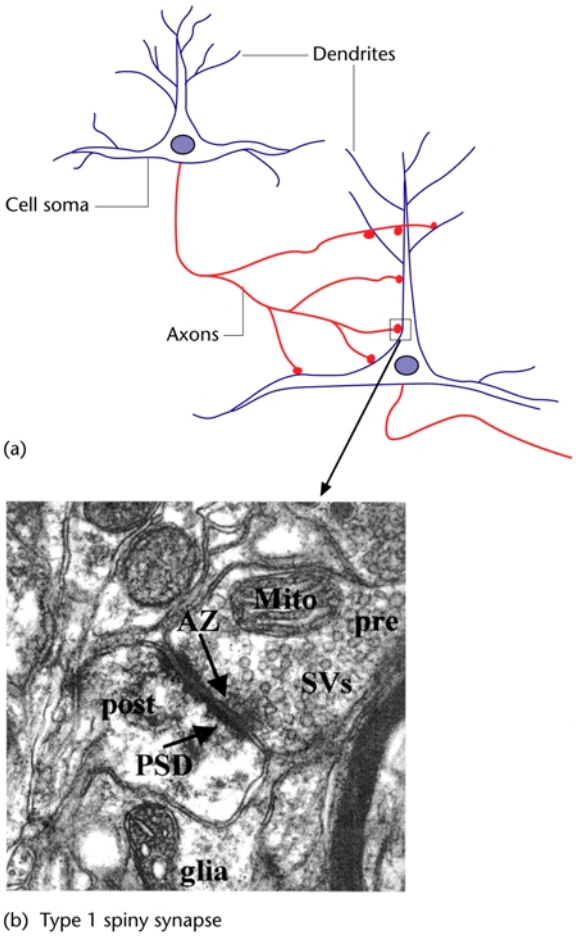

Figure 1 (a) Schematic diagram of two pyramidal-like neurons making type 1 glutamatergic synapses. The dendrites of these neurons are typically long tapered processes, whereas the single axon with multiple branches is of uniform calibre along its length. Axons typically make synapses *en passant* (in passage) with dendritic segments from many neurons. (b) Electron micrograph of an asymmetrical type 1 spiny synapse. The presynaptic junction (pre) is characterized by the presence of mitochondria (Mito), numerous synaptic vesicles (SV) and an active zone (AZ), where synaptic vesicles dock, fuse and release neurotransmitter into the cleft. The electron-dense material associated with the postsynaptic reception apparatus (postsynaptic density; PSD) contains a high concentration of neurotransmitter receptors.

presynaptic plasma membrane is activity dependent and results in the release of transmitter into the synaptic cleft in a millisecond time scale. Structural, molecular and cellular features of chemical synapses that allow neurons to communicate in a dynamic and plastic manner are the focus of this article. (***see*** History of neurochemistry.) (***see*** Synaptic vesicles: methods for preparation.) (***see*** Synaptic integration.)

Presynaptic Specialization: Synaptic Vesicles and the Active Zone

As discussed above, presynaptic boutons appear as axonal expansions or swellings (approximately 1 μm in diameter) which terminate on the somata, axon hillock or dendrites of other neurons. These expansions are filled with a variety of membrane organelles, including mitochondria, endosomal membranes and numerous small clear SVs. Mitochondria serve as a primary source of energy (in the form of adenosine triphosphate (ATP)), which is required during SV exocytosis and endocytosis, as well as for the uptake and synthesis of neurotransmitters. In contrast, endosomal membranes are thought to function in the sorting of SV proteins after their fusion and recycling with the plasma membrane. The best characterized of these membrane organelles are the SVs which contain, in addition to neurotransmitter, a collection of proteins that not only facilitate the fusion and recycling of SVs with the presynaptic plasma membrane, but also their refilling with neurotransmitter (see Synaptic vesicle cycle below). Nerve terminals are also filled with a plethora of soluble and cytoskeletal proteins that are involved in the establishment and maintenance of the synaptic junction as well as the docking, fusion and recycling of SVs. (***see*** Mitochondria: structure and role in respiration.) (***see*** Adenosine triphosphate.)

The most commonly used neurotransmitters at vertebrate CNS synapses are the amino acids glutamate, glycine and γ-aminobutyric acid (GABA). Other neurotransmitters include noradrenaline, dopamine, serotonin or acetylcholine, as well as a variety of neuropeptides. The modulatory neuropeptides are stored in large dense-core vesicles (LDCVs), which are found in low numbers at most CNS synapses. The major neurotransmitter at the NMJ is acetylcholine. (***see*** Amino acid neurotransmitters.) (***see*** Amine neurotransmitters.) (***see*** Peptide neurotransmitters and hormones.) (***see*** Acetylcholine.)

Depending on the type and location of the synapse, presynaptic boutons contain from a few hundred to several thousand SVs, which are clustered near the presynaptic plasma membrane juxtaposed to the PSD. These clustered SVs can be divided into two functionally distinct pools. Those situated more distally from the synaptic junction are part of a reserve pool of SVs. The second, smaller, pool of SVs, typically about 10 to 20 (usually about one-tenth of the total population of SVs in a particular synapse), are found in direct contact with the presynaptic plasma membrane (i.e. docked) at an area known as the active zone, and are considered as the release-ready pool of SVs. SV fusion, neurotransmitter release and SV recycling occurs specifically at the active zone of the presynaptic bouton. (***see*** Synaptic vesicle trac.)

In addition to the presence of SVs, the active zone is characterized by the presence of a fine filamentous network, which extends 1–200 nm distally from the

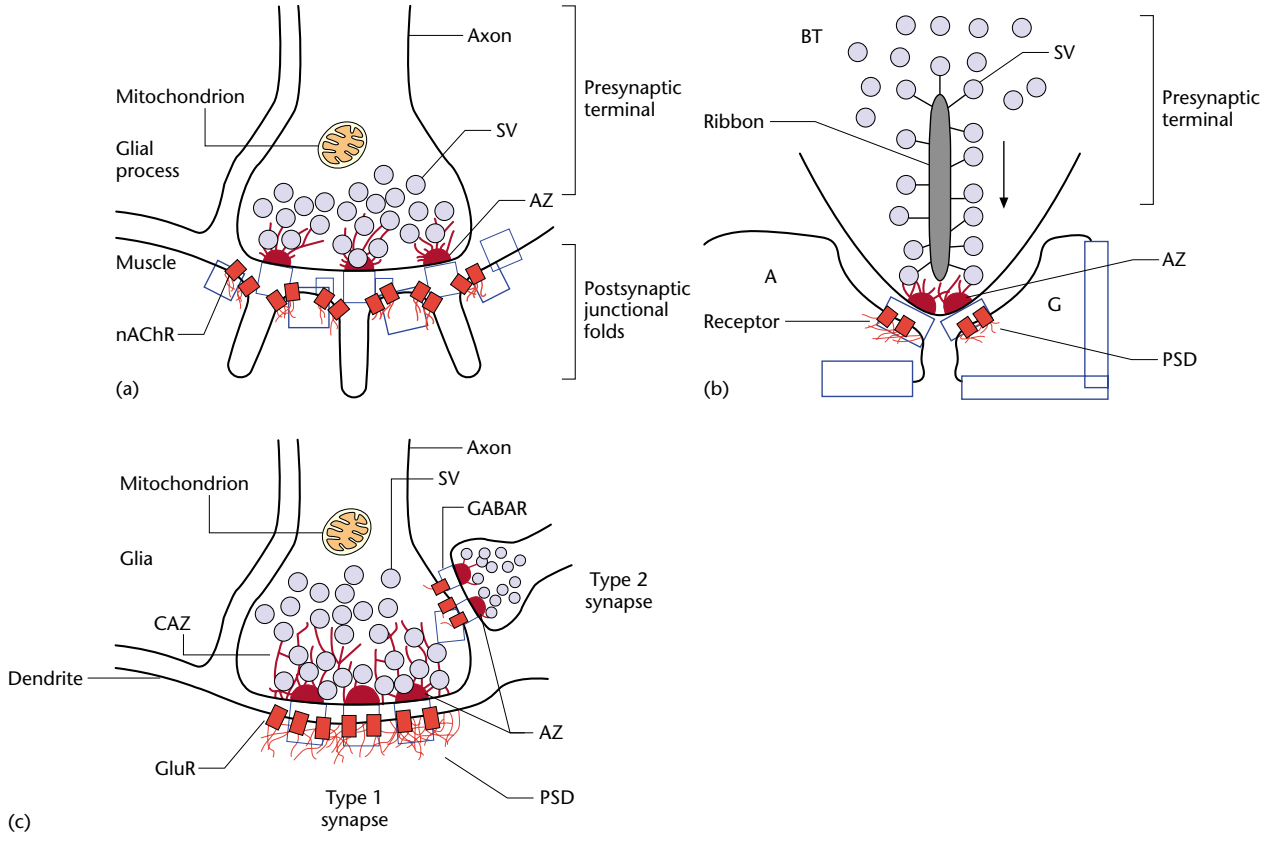

Figure 2 Schematic diagrams of synapses found in the vertebrate nervous system. (a) A neuromuscular junction (NMJ) formed between spinal cord motor neurons and muscle cells. The presynaptic bouton containing mitochondria and numerous synaptic vesicles (SVs) is found to terminate directly on the surface of the muscle and to release the neurotransmitter acetylcholine (ACh). The postsynapse is characterized by the presence of many junctional folds. Nicotinic acetylcholine receptors (nAChRs) are concentrated at the apex of the junctional folds, juxtaposed to the presynaptic active zone (AZ). Glial cell processes ensheathe the terminal, isolating the NMJ from the surrounding milieu. (b) A dyadic ribbon synapse formed between a cone bipolar cell nerve terminal (BT), an amacrine cell (A) and a ganglion cell (G). The presynaptic nerve terminal is filled with synaptic vesicles that become associated with the synaptic ribbon as they move progressively down (arrow) towards the active zone. The postsynaptic densities (PSDs) of the amacrine and ganglion cells are situated juxtaposed to the active zones. (c) Conventional excitatory glutamatergic axodendritic and inhibitory γ-aminobutyric acid (GABA)ergic axoaxonic synapses are found in the central nervous system. Type 1 glutamatergic synapses have a pronounced, thick, electron-dense postsynaptic reception apparatus (PSD), whereas type 2 GABAergic synapses do not. As at other synapses, the presynaptic nerve terminals of both types contain numerous synaptic vesicles and mitochondria. Associated with the active zones of the four types of synapse shown is a dense matrix of cytoskeletal proteins (CAZ), which serves to guide synaptic vesicles toward their docking and fusion sites at the plasma membrane. GABAR, GABA receptor.

cytoplasmic face of the presynaptic plasma membrane into the cytoplasm. Freeze-fracture–deep-etch EM studies have shown that these cytoskeletal filaments are in direct contact with SVs, and are thought to play a role in directing SVs to fusion sites. Another hallmark of active zones is the presence of voltage-gated calcium and potassium channels, which can be found in ordered arrays in the presynaptic plasma membrane in close proximity to sites of SV fusion. Calcium influx through these channels after nerve terminal depolarization plays a direct role in the fusion of SVs with the plasma membrane (see below). Potassium channels function in membrane repolarization after the action potential. (***see*** Axonal transport and the neuronal cytoskeleton.) (***see*** Calcium channels in presynaptic terminals.) (***see*** Voltage-gated potassium channels.)

The cytoskeletal matrix assembled at active zones, known as the CAZ, contains a cocktail of synaptic proteins thought to be involved in organizing the active zone (e.g. localizing calcium channels), defining neurotransmitter release sites and modulating synaptic transmission (Garner *et al*., 2000a). For example, the CAZ proteins Munc13 and Munc18 have been shown to be involved in the priming of SVs, before exocytosis. Others, such as Piccolo, Bassoon and Rim, are likely to play organizational roles, localizing components of the exocytotic and endocytotic machinery at the active zone. Interactions between components of the cytoskeleton, such as actin, and proteins protruding from SVs (e.g. synapsins) are required for the retention of SVs in the reserve pool. The size and structural organization of the CAZ varies depending on synaptic type. For example, the

CAZ present at the NMJ is organized in rows opposed to the postsynaptic junctional folds (**Figure 2a**). Within synapses of the CNS, the CAZ appears as an array of tuffed material, each about 50 nm apart. At some synapses within the retinal and auditory system (e.g. ribbon synapses) the CAZ extends into the presynaptic bouton as a ribbon of material (**Figure 2b**). Here, SVs are clustered along both sides of the ribbon, which is thought to facilitate the sequential movement of SVs towards the active zone at the plasma membrane (**Figure 2b**). (***see*** Endocytic and exocytic transport assays.)

Flanking the active zone is a region of the presynaptic plasma membrane known as the perisynaptic plasma membrane, which is morphologically less remarkable than the active zone, but is none the less important for nerve terminal function. These membranes contain numerous voltage-gated ion channels involved in the depolarization of nerve terminals, as well as neurotransmitter receptors and transporters. The former, generally inhibitory in nature, act to hyperpolarize the nerve terminal plasma membrane. This blocks calcium influx through voltage-gated calcium channels, which is necessary for SV exocytosis. As discussed below (see The synaptic cleft), neurotransmitter transporters, found concentrated in the perisynaptic plasma membrane, play a fundamental role in the reuptake and clearance of neurotransmitter after release into the synaptic cleft. (***see*** Action potential: ionic mechanisms.) (***see*** GABA as a neurotransmitter.) (***see*** Glycine as a neurotransmitter.) (***see*** Neurotransmitter transporters.) (***see*** Calcium and neurotransmitter release.)

Synaptic Vesicle Cycle

Release of neurotransmitter is repetitive and takes place in a millisecond time scale. In mature synapses, this occurs in the absence of any new protein synthesis, implying that nerve terminals are self-contained, regenerating subcompartments. Autonomy is achieved through the recycling of SVs and their components in nerve terminals, involving several distinct steps (Sudhof, 1995). The first involves the docking of SVs, filled with neurotransmitter, at the active zone. This is mediated by the formation of a docking complex between SV and plasma membrane proteins called vesicle (v) and target (t) SNAREs (SNAP (*S*-nitroso-*N*-acetylpenicillamine) receptors), respectively (Sudhof, 1995). Following nerve terminal depolarization, voltage-gated N- and P/Q-type calcium channels are activated, which leads to an increase in the local concentration of calcium at the active zone. Raised calcium levels can be detected by the calcium sensor SV protein synaptotagmin, which is thought to keep SVs in a docked/primed state, ensuring that SV fusion does not occur in absence of calcium. Numerous proteins, such as Munc13, Munc18, complexins, NSF (*N*-ethylmaleimide-sensitive fusion protein) and α-SNAP, have been shown to facilitate the formation of the SNARE complex and SV fusion (Sudhof, 1995). Following exocytosis, SV proteins are retrieved from the synaptic plasma membrane via clathrin-mediated endocytosis (De Camilli and Takei, 1996). In general, endocytosis vesicles can be directly refilled with neurotransmitter and used in subsequent exocytotic events. Alternatively, they may pass through the endosomal membrane present in nerve terminals to ensure that they have the correct composition of SV proteins. (***see*** Synaptic vesicle trac.) (***see*** Synaptic vesicle proteins.) (***see*** Synaptic vesicle fusion.) (***see*** Calcium channels.)

The mechanism and machinery involved in the synthesis, refilling and uptake of neurotransmitter depends on the type of transmitter released. At most synapses, neurotransmitter can be synthesized locally within the bouton and then pumped into SVs by specific vesicle transporters. Following their release into the cleft, these neurotransmitters are recycled by plasma membrane transporters found, for example, in the perisynaptic plasma membrane. One exception is at cholinergic synapses, where acetylcholine always requires *de novo* synthesis prior to SV refilling because of its rapid degradation upon release by acetylcholine esterase in the synaptic cleft. In contrast to neurotransmitters, SV proteins are synthesized in the soma on the rough endoplasmic reticulum and then anterogradely transported to nerve terminals along microtubules, utilizing motor proteins such as kinesins. Unlike SVs, LDCVs containing neuropeptides are one-shot vesicles and are not recycled or refilled after fusing with the plasma membrane. Instead, components of LDCVs are synthesized in the cell soma, including the precursor proteins that give rise to the neuropeptides. (***see*** Acetylcholine.) (***see*** Protein synthesis in neurons.) (***see*** Motor proteins.)

Postsynaptic Specialization: the Postsynaptic Density

The postsynaptic reception apparatus is situated in the postsynaptic plasma membrane and juxtaposed to the presynaptic active zone. EM studies have revealed that the postsynaptic reception apparatus has a high avidity for heavy metals, and as such appears as an electron-dense structure termed the postsynaptic density (PSD). As is the case in the presynaptic junction, a dense meshwork of cytoskeletal filaments is required for the structural organization of the PSD (Garner *et al*., 2000b). This dense network extends into both the synaptic cleft (see The synaptic cleft below) and the dendritic spine head. In the late 1950s Gray demonstrated that, within the CNS, PSDs are either thick with pronounced PSDs or thin with only a little associated electron-dense material. Generally, synaptic junctions with thick, pronounced PSDs are asymmetrical with respect to the presynaptic junction.

Asymmetrical synapses are classified as type 1 synapses, and are usually associated with excitatory glutamatergic synapses. The PSD at type 1 synapses is approximately 50–60 nm thick and 300–500 nm in diameter. Synapses with thin PSDs are known as symmetrical type 2 synapses, and appear to function as inhibitory synapses, utilizing either GABA or glycine as neurotransmitter. Generally, type 2 inhibitory synapses are located on the cell soma or on the axon hillock, whereas excitatory synapses are located all along the dendritic arbour.

While type 1 synapses can be found directly on dendritic shafts, they are often found at the tips of dendritic spines on neurons throughout the CNS. Spiny neurons are generally found in brain regions involved in more complex brain function, where synaptic plasticity is likely to play a major role in modulating neuronal function. Although the exact role of dendritic spines is unknown, they are thought to create microenvironments within dendrites that act as an extra mechanism for modulation of synaptic plasticity. Three distinct spine morphologies have been described: short and stubby spines, long thin spines, and large mushroom-shaped spines. At present, the functional significance of these different shapes is unclear, although changes in spine morphology can be correlated to changes in synaptic activity, indicating a fundamental role for spines in activity-dependent mechanisms (Matus, 1999). (***see*** Dendritic spines.) (***see*** Synaptic plasticity: short term.) (***see*** Synaptic plasticity as a mechanism of learning.)

Organization of Receptors at the Postsynaptic Density

The main function of the PSD is to anchor neurotransmitter receptors of the correct type at the postsynaptic plasma membrane, and to ensure that following receptor activation that the appropriate signalling cascade is activated, allowing a physiological neuronal response. Until recently, little was known about the molecular mechanisms responsible for ensuring appropriate receptor function; however, in recent years tremendous progress has been made in defining the molecular constituents that promote the clustering, anchoring and modulation of neurotransmitter receptors at synapses (Garner *et al*., 2000b). (***see*** Neurotransmitter receptors in the postsynaptic neuron.)

Three types of protein have been shown to be critical for the assembly of PSD. The first are cell adhesion molecules (CAMs) that are involved in cell–cell recognition and cell–cell adhesion. The second type of protein includes components of the cortical membrane cytoskeleton such as actin, spectrin, adducin and protein 4.1, which associate with the cytoplasmic tails of the CAMs, stabilizing adhesion between the active zonal plasma membrane and the postsynaptic reception apparatus. The third type of protein belongs to a superfamily of so-called adaptor proteins that interact both with the actin/spectrin cortical cytoskeleton and with distinct subtypes of neurotransmitter receptors (Garner *et al*., 2000b). (***see*** Adhesive specificity and the evolution of multicellularity.) (***see*** Cytoskeleton.) (***see*** Actin and actin filaments.)

The type of neurotransmitter released from the presynaptic bouton governs the type of transmembrane receptor found postsynaptically, which in turn governs which families of adaptor proteins constitute that particular PSD. For example, when the inhibitory transmitter glycine is released presynaptically, glycine receptors are found clustered at the postsynaptic membrane. Clustering of glycine receptors is mediated by the adaptor protein Gephrin, thus the PSDs of glycinergic synapses contain gephrin. A similar organizational structure is observed at GABAergic, glutamatergic and cholinergic synapses; less is known about dopaminergic or serotonergic synapses. Genetic studies have shown that loss of specific adaptor proteins can interfere with the synaptic localization of specific neurotransmitter receptors. For example, at the cholinergic neuromuscular junction, the nicotinic acetylcholine receptor (nAChR) has been shown to interact with the adaptor protein rapsyn. Disruption of the gene encoding rapsyn in mice interferes with the postsynaptic recruitment of nAChR (Sanes and Lichtman, 1999). (***see*** Glycine receptors.) (***see*** Nicotinic acetylcholine receptors in muscle.)

The most well studied synapses in the CNS are excitatory glutamatergic synapses (Blackstone and Sheng, 1999; Garner *et al*., 2000b). Four families of glutamate receptors are found postsynaptically. These include AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate), NMDA (*N*-methyl-D-aspartate), kainate and the metabotropic receptors. AMPA, NMDA and kainate receptors are excitatory ionotropic receptors, which are ligand-gated ion channels. Following activation, ionotropic receptors have an immediate and rapid time course of action, which decays quickly. Metabotropic glutamate receptors (mGluRs) are guanine nucleotide-binding (G) protein-coupled receptors and are thought to play a modulatory role at the glutamatergic synapse. There are several subtypes of mGluRs, some of which evoke an excitatory response, whereas others are inhibitory. Following activation of these receptors, downstream signalling mechanisms follow a slower time course of activation and inactivation compared with ionotropic receptors. In general, ionotropic glutamate receptors have been located in the centre of the PSD, whereas metabotropic glutamate receptors are located perisynaptically. (***see*** Glutamate as a neurotransmitter.)

Given that each transmembrane receptor interacts with adaptor proteins specific for that receptor, and that glutamatergic synapses contain four subtypes of glutamate receptor, it is not surprising that a plethora of adaptor proteins has been identified in the PSD of type 1 glutamatergic synapses (Blackstone and Sheng, 1999;

Garner *et al*., 2000b). The hallmark of many of these adaptor proteins is the presence of 1–13 copies of a small modular unit called the PDZ domain (named after the three proteins initially found to contain them PSD95/synapse associated protein (SAP) 90-disc large giant lethal (DLG)-zona occludens (ZO) 1). PDZ domains are comprised of 90 amino acid residues that have the capacity to bind ligand- and voltage-gated ion channels, cell adhesion molecules, as well as small regulatory proteins such as kinases and phosphatases by their C-terminal tails. (***see*** Glutamatergic synapses: molecular organization.)

Two subfamilies of PDZ domain-containing proteins have been characterized that interact with subclasses of glutamate receptors. The first subfamily is composed solely of PDZ domains, and includes glutamate receptor interacting protein (GRIP)/AMPA receptor binding protein (ABP) 1 and multiple PDZ domain-containing protein (MUPP) 1. The second are members of the SAP90/PSD95 family of membrane-associated guanylate kinases (MAGUKs). This family includes SAP90/PSD95, SAP102, SAP97, PSD93/Chapsyn110, neuron-specific synaptic scaffolding molecule (SCAM) 1 and CaMK/SH3/guanylate kinase domain protein (CASK) (Garner *et al*., 2000b). In general, MAGUKs are composed of one to five PDZ domains, an src homology 3 (SH3) domain and a guanylate kinase (GUK)-like domain. SH3 and GUK domains also interact with proteins within the PSD, broadening the repertoire of proteins that interact with these multidomain proteins. Each MAGUK appears to contain targeting sequences that direct them to postsynaptic membranes of type 1 glutamatergic synapses. Functionally, these features are thought not only to promote the localization and clustering of postsynaptic glutamate receptors, but also to facilitate the assembly of macromolecular signalling complexes that modulate both receptor activity and downstream signalling. (***see*** Protein–protein interactions.) (***see*** Protein kinases: physiological roles.) (***see*** AMPA receptors.)

A third gene family of multidomain scaffold proteins has been recently found, which links together different subtypes of glutamate receptors in the PSD (e.g. ProSAP/Shank). For example, ProSAP/Shank has the ability to bind to Homer (a synaptic protein that binds to mGluRs), as well as PDZ domain-containing proteins, which interact with NMDA receptors. Thus, ProSAP/Shank promotes the formation of macromolecular complexes containing both NMDA and metabotropic glutamate receptors (Blackstone and Sheng, 1999; Garner *et al*., 2000b) (**Figure 3**). (***see*** NMDA receptors.) (***see*** Metabotropic glutamate receptors.)

The Synaptic Cleft

The synaptic cleft is a narrow space, about 20–30 nm wide, situated between the presynaptic and postsynaptic plasma membranes. The synaptic cleft has several major roles in facilitating neurotransmission. These include maintaining the active zone and postsynaptic reception apparatus in close register and at a fixed distance, as well as the removal of neurotransmitter. The cleft contains a collection of anterograde and retrograde signalling molecules that are important for both the formation and maintenance of synaptic junctions. Each of these features is discussed individually. (***see*** Synapse formation.) (***see*** Retrograde signalling.)

The synaptic cleft is narrow enough to allow the neurotransmitter, released from the presynaptic nerve terminal, to diffuse across to the postsynaptic plasma membrane in a few milliseconds. This feature is essential for rapid intercellular signalling. At the same time, the cleft is large enough to permit the neurotransmitter to diffuse quickly away from the receptor after initial binding. At the majority of synapses, diffusion coupled with active reuptake by transporters is thought to be the primary mechanism responsible for the removal of neurotransmitter from the cleft. However, at some synapses (e.g. cholinergic synapses) neurotransmitter is degraded enzymatically within the cleft by acetylcholinesterase, removing acetylcholine from the synaptic cleft and further limiting its length of action. (***see*** Cellular neuromodulation.) (***see*** Synapses.)

Ultrastructural and biochemical studies have shown that the synaptic cleft is filled with an electron-dense extracellular matrix (ECM). Rod-like filaments traversing the synaptic cleft and extending into both the presynaptic bouton and the PSD suggest that the cleft provides a structural link between the CAZ and PSD, holding them in close register. While current knowledge of the molecular composition of the synaptic cleft and these bridging molecules is still quite limited, three types of cleft protein have been identified and characterized. The first type is proteins of the ECM including collagen, laminin and fibronectin. The second type includes several subtypes of CAMs, such as integrins, members of the immunoglobulin G superfamily of CAMs such as neural cell adhesion molecule (N-CAM), and members of the cadherin family of calcium-dependent CAMs (N- and R-cadherin) (Shapiro and Colman, 1999). In addition, metalloproteases, which modulate cell–cell adhesiveness, have been found in the cleft. (***see*** Extracellular matrix.) (***see*** Integrin superfamily.)

Some of the most interesting molecules found in the synaptic cleft are those involved in signalling between the presynaptic bouton and the PSD. The most well characterized are those found at the NMJ, including agrin, laminin β1 and neuregulin (Sanes and Lichtman, 1999). Agrin is an ECM protein that is released by motor neurons, sending an anterograde signal which induces postsynaptic differentiation, and ultimately the clustering of postsynaptic AChRs. Laminin β1 is an ECM protein that is secreted into the synaptic cleft by the muscle cell, affecting the assembly of the presynaptic junction. Finally, trophic factors such as neuregulin in the cleft act on postsynaptic tyrosine kinase receptors to modulate the transcription of genes encoding synaptic junctional proteins. At CNS

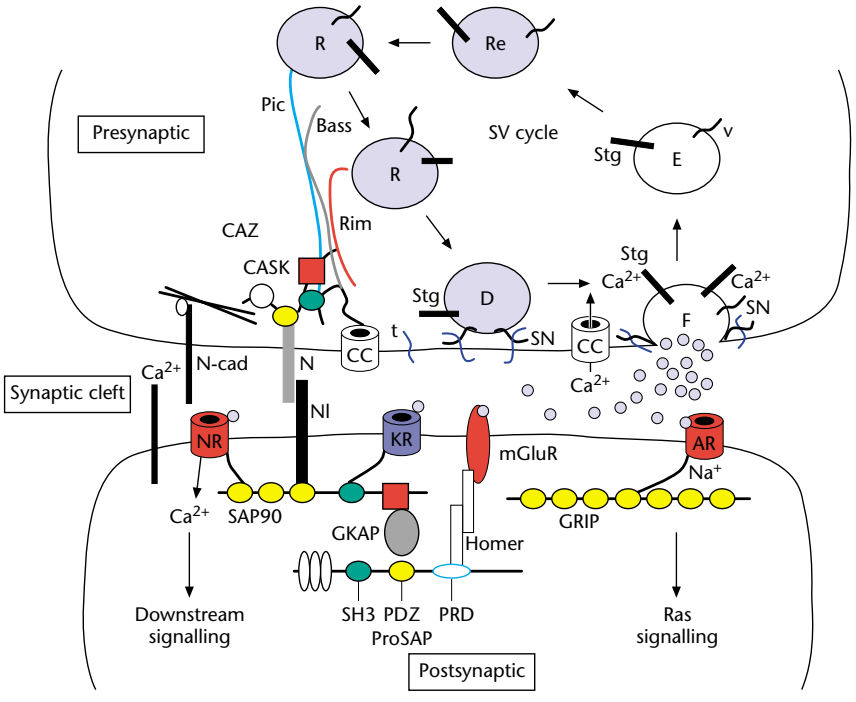

Figure 3 Schematic representation of synaptic proteins that have been localized at excitatory synaptic junctions. PDZ domain-containing proteins are thought to be the central organizing molecules that create macromolecular signalling complexes at both the presynaptic and postsynaptic junctions, thus defining the active zone (AZ) and the postsynaptic density (PSD). Cell adhesion molecules (CAMs) such as N-cadherin (N-cad) are thought to hold the presynaptic and postsynaptic plasma membranes close together in a calcium-dependent mechanism. Other CAMs such as β-neurexin (N) and neuroligin (Nl) are thought to keep the PDZ domain-containing scaffold proteins CaMK/SH3/guanylate kinase domain protein (CASK) and SAP90/PSD95, respectively, in register at the presynaptic and postsynaptic plasma membrane. The presynaptic cytoskeletal matrix proteins assembled at the active zone (CAZ), including CASK, Piccolo (Pic), Bassoon (Bass) and Rim, are thought to help define the AZ as the neurotransmitter release site by clustering voltage-gated calcium channels (CCs) and the machinery involved in exocytosis and endocytosis, as well as guiding synaptic vesicles (SVs) in the reserve pool (R) to the docked pool (D). After fusion (F), these vesicles undergo endocytosis (E) and refilling (Re) with neurotransmitter. Synaptic vesicle docking is mediated by the formation of the SNARE complex (SN) between the target (t) SNAREs, syntaxin and SNAP-25, and the vesicle (v) SNARE, synaptobrevin. The synaptic vesicle protein, synaptotagmin (Stg), is thought to be the calcium sensor that triggers SV exocytosis in a calcium-dependent manner. In the postsynaptic plasma membrane of glutamatergic synapses, four types of glutamate receptor are found, including NMDA (NR), kainate (KR), AMPA (AR) and metabotropic glutamate receptor (mGluR). The multidomain PSD proteins synapse-associated protein (SAP) 90/PSD95, guanylate kinase-associated protein (GKAP)/synapse-associated protein-associated protein (SAPAP), ProSAP/Shank, Homer and GRIP/ABP1 are thought to use their modular structures to scaffold these and other regulatory proteins in the postsynaptic junction. Four types of subdomain found in these proteins are shown, including the PDZ, SH3 and proline-rich (PRD) domains. These domains interact with, for example, the C-terminal tails of channels and CAMs, coupling the glutamate receptors to downstream signalling pathways. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate; ABP, AMPA receptor binding protein; GRIP, glutamate receptor interacting protein; mGluR, metabotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; SNAP, *S*-nitroso-*N*-acetylpenicillamine; SNARE, SNAP receptor.

synapses, transsynaptic signalling proteins have not been well characterized. None the less they are expected to play fundamental roles in determining which class of receptors is clustered at a given nascent synapse. (***see*** Postsynaptic membranes at the neuromuscular junction: molecular organization.) (***see*** Excitation-contraction coupling.) (***see*** Trophic support.)

Summary

Chemical synapses are undoubtedly amongst the most elaborate junctions existing between two cells. They enable chemical neurotransmission to occur within the millisecond range. This high rate of transmission is achieved through the specialization of three subsynaptic compartments. For example, the presynaptic bouton has evolved into a very sophisticated machine capable of packaging neurotransmitters into SVs and then releasing them into the synaptic cleft in response to a depolarizing action potential. In contrast, the postsynaptic junction has evolved into a complex neurotransmitter reception apparatus that responds rapidly, following release of neurotransmitter into the synaptic cleft. The PSD is a dynamic structure, being able to alter its responsiveness to neurotransmitter, and thus the strength of any given synaptic connection; this is known as synaptic plasticity. The synaptic cleft ensures that postsynaptic receptors are

actived at the appropriate level, by maintaining the active zone and PSD in close register and removing neurotransmitter from the cleft. In addition, the cleft is a repository for anterograde and retrograde signalling molecules affecting both presynaptic and postsynaptic differentiation. Finally, the cytoskeletal matrix assembled at both the active zone and postsynaptic reception apparatus appears to play fundamental organizational roles in the establishment of these membrane specializations through the formation of macromolecular protein signalling complexes. (***see*** Action potential modulation.) (***see*** Heterosynaptic modulation.) (***see*** Receptor transduction mechanisms.)

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