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Structure activity relationship of synaptic and junctional neurotransmission

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Abstract

Chemical neurotransmission may include transmission to local or remote sites. Locally, contact between 'bare' portions of the bulbous nerve terminal termed a varicosity and the effector cell may be in the form of either synapse or non-synaptic contact. Traditionally, all local transmissions between nerves and effector cells are considered synaptic in nature. This is particularly true for communication between neurons. However, communication between nerves and other effectors such as smooth muscles has been described as nonsynaptic or junctional in nature. Nonsynaptic neurotransmission is now also increasing recognized in the CNS. This review focuses on the relationship between structure and function that orchestrate synaptic and junctional neurotransmissions. A synapse is a specialized focal contact between the presynaptic active zone capable for ultrafast release of soluble transmitters and the postsynaptic density that cluster ionotropic receptors. The presynaptic and the postsynaptic areas are separated by the 'closed' synaptic cavity. The physiological hallmark of the synapse is ultrafast postsynaptic potentials lasting in milliseconds. In contrast, junctions are juxtapositions of nerve terminals and the effector cells without clear synaptic specializations and the junctional space is 'open' to the extracellular space. Based on the nature of the transmitters, postjunctional receptors and their separation from the release sites, the junctions can be divided into 'close' and 'wide' junctions. Functionally, the 'close' and the 'wide' junctions can be distinguished by postjunctional potentials lasting ~1 second and 10s of seconds, respectively. Both synaptic and junctional communications are common between neurons; however, junctional transmission is the rule at many neuro-non-neural effectors.

Keywords

postsynaptic potentials; junction potentials; smooth muscle neuromuscular transmission; autonomic ganglia; enteric nervous system; nonsynaptic neurotransmission; modes of neurotransmission

INTRODUCTION

What constitutes a synapse and synaptic neurotransmission continues to be a hotly debated issue. There are both structural and functional aspects to the definition of a synapse.

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Traditionally, investigators have used only a functional definition and consider any neurotransmission between the nerve and a target cell as synaptic neurotransmission. In recent years there have been considerable advances in our understanding of the morphology and molecular biology of synapses. Neurotransmission at the skeletal neuromuscular junctions via the 'motor endplate' is a type of a synapse but is not generally included in discussion of a synapse. Here we present our analyses of the existing structure-function relationship data to support a case for restricting the term synaptic transmission to the communication that involves morphologically defined synapses during an action potential. Nonsynaptic neurotransmission, also called 'volume' transmission, includes a continuum of wide spectrum of modes of transmission including junctional transmission and transmission to distant and remote sites via neurotransmitter release into the cerebrospinal fluid and blood capillaries (Agnati et al., 1986). Junctional transmission involves transmission between nerve terminals and juxtaposed membranes of target cells without synaptic contacts (Agnati et al., 1986; Vizi, 1984). Junctional transmission can be arbitrarily further divided into close junctional and wide junctional transmissions (Figure 1). This review is focused on relationship of structure and activity during synaptic and junctional neurotransmission.

BRIEF HISTORY

How nerves communicate with other neurons or targets has preoccupied physiologists since the middle of the nineteenth century. Excellent accounts of the history of different aspects of the synapse and neurotransmission have been provided by Cowan and Kandel (Cowan W.M., 2003), Bennett (Bennett, 1997), Sudhof (Sudhof, 2008a) and Lopez-Munoz and Alamo (Lopez-Munoz et al., 2009). The earliest morphological consideration of neural communication revolved around whether the cytoplasm of a nerve was continuous with the adjacent nerve or merely discontinuous and were led by Du Bois-Raymond (1877) and Kuhn (1862) [cited by (Bennett, 2005)]. The later theory, popularly known as the neuron theory, was championed by Cajal [see (Cajal, 1954)] and it was envisioned that the tips of axons of one neuron made contacts with dendrites and cell bodies of other neurons. Subsequently, the nature of these contacts became a subject of intense discussion. Sherrington proposed that these contacts were specialized and advocated the use of the term synapse in 1897 [see (Tansey, 1997)]. The term synapse was derived from Greek, *synapsis* "conjunction," from *synaptein*, from *syn*- "together" + *haptein* "to fasten" (Kandel et al., 2000).

Functionally, communication at the synapses was believed to take place via chemicals. However, experimental proof for the existence of chemical synaptic neurotransmission came later from studies on striated neuromuscular endplates, even though the term synapse is not traditionally used in that context. As early as 1877, Du Bois-Raymond had recorded electrical potentials in the striated muscles in response to the motor nerve stimulation and had suggested the existence of chemical transmission [see (Dierig, 2000)]. The concept of chemical transmission was advanced by the work of Langley, who introduced the idea of receptors on which chemicals acted (Langley, 1921). Evidence for chemical transmission was established further by Dale, Loewi, Feldberg and their colleagues by their work on physiology and pharmacology of the neurotransmitters noradrenaline and acetylcholine (Cowan W.M., 2003; Dale, 1952).

Even after the chemical nature of synaptic neurotransmission was well established, many investigators continued to doubt whether chemical signals could be conducted fast enough to explain the ultra-short synaptic potentials (see (Eccles, 1982). These doubts were laid to rest by the classical work of Katz and his colleagues, who showed that at the striated muscle neuromuscular junctions, acetylcholine (ACh) was released in a quantal fashion and produced ultra-fast postsynaptic responses (Katz, 2003). In addition to chemical synapses,

the existence of electrical synapses, called gap junctions, was also validated (Bennett, 1972b; Eccles, 1982; Furshpan et al., 1957).

Structurally, the use of electron microscopy in mid-1950s provided strong morphological basis of synapses in the central nervous system (CNS) (Couteaux, 1958; Palay et al., 1955) and the enteric nervous system (ENS) (Caesar et al., 1957; Richardson, 1958; Taxi, 1958). Further technical advances including high resolution electron microscopy and freeze fracture microscopy definitively identified and differentiated different types of specialized cell to cell contacts including synapses and gap junctions (Gray, 1976; Heuser et al., 1981; Peters et al., 1996). In the late 1950s, Whittaker and colleagues described the landmark techniques of the isolation of nerve varicosities (Whittaker, 1959). These techniques have allowed for the precise determination of synapse molecular anatomy and biochemistry (Morciano et al., 2009; Sudhof, 2008a).

In the CNS, distant nonsynaptic transmission to remote sites may include neuro-hormonal transmission where certain peptide neurotransmitters are released in blood capillaries of portal circulation and carried to distant targets (Kordon, 1985). Vizi and colleagues divided modes of neurotransmission into synaptic and nonsynaptic pathways (Vizi, 1984; Vizi et al., 2010; Vizi et al., 2004). On the other hand, Agnati and colleagues classified neurotransmission into wired transmission and volume transmission (Agnati et al., 1986; Agnati et al., 2010; Zoli et al., 1996). Wired transmission included neurotransmission at synapses and close juxtaposition of nerve terminals and the target cells. On the other hand, volume transmission included 3-dimensional diffusion of signals in the extracellular fluid (ECF) for distances larger than the synaptic cleft, including the CSF (Sykova et al., 2008; Vigh et al., 2004).

In the peripheral nervous system, local junctional transmission was recognized in the late 1960s and early 1970s (Bennett, 1997; Burnstock, 1986). Until then, all chemical neurotransmission was thought to involve synapses and the innervations of tissue were considered synonymous with the existence of a synapse. Later, it was observed that at smooth muscle neuromuscular junctions in the gut and other peripheral autonomic neuro-effector junctions, neurotransmission takes place in the absence of any synapses and it was suggested that at these sites, neurotransmission involved nonsynaptic transmission. Accordingly, nerve endings release their neurotransmitters in extracellular space in a manner similar to paracrine secretion. Target cells affected by a locally released transmitter even though located several hundreds to thousands of nanometers away from the release site are considered as being innervated [see (Burnstock, 2008)]. Thus, junctional transmission includes nonsynaptic wired neurotransmission and slightly overlaps the volume transmission (Vizi et al., 2010; Zoli et al., 1996).

NERVE TERMINALS AND VARICOSITIES

The nerve terminal is the terminal part of the axon filled with neurotransmitters and the location from which neurotransmitters are released. Nerve terminals may take different forms in different tissues. Nerve terminals appear like a button in the CNS, end plates in striated muscle and varicosities in many tissues including the gut (Jonakait et al., 1979). Buttons, endplates or varicosities all function to store and release neurotransmitters. In many peripheral tissues, the varicose axon branches in its proximal course and carries a covering of Schwann sheath, which is interrupted and finally lost in its most terminal part. The unmyelinated, preterminal axons with very long varicose branches are present in small axon bundles and varicose terminal axons are present as single isolated axons. The small axon bundles run parallel to and between muscle bundles and the "*en passage*" varicose axons are the main sources of innervations to the gut smooth muscle bundles (Bennett, 1997). Neuro-

neuronal synapses are also extensively present in the neuropil of the myenteric ganglia [see (Furness 2006)].

The varicose axons were first visualized for adrenergic terminals using fluorescence histochemistry described by Falck and colleagues (Falck et al., 1962; Hokfelt, 2010). These varicose axons resemble strings of beads with varicosities 0.5–2.0μ in diameter and 1 to 3 μ in length and separated by inter-varicosity axon 0.1 to 0.2 μ in diameter (Bennett, 1972a). The varicosities occur at 2–10 μm intervals and it has been estimated that a single adrenergic axon may have over 25000 varicosities on its terminal part (Bennett, 1972a). Use of dyes that penetrate and opacify terminal parts of the axons has revealed varicose endings in vagal preganglionic axons (Brookes S.J.H., 2006; Holst et al., 1997). On electron microscopy, varicosities similar to those on adrenergic fibers were also found on cholinergic axons (Richardson, 1966). Nitrergic varicosities on inhibitory motor axons in enteric muscles have recently been visualized using nitric oxide imaging in situ, providing evidence for the localized production of nitric oxide (Thatte et al., 2009) (Figure 2).

Electron microscopy reveals that varicosities are filled with vesicles. The vesicles can be divided into several different types based on their size, shape and electron density. They include: small clear vesicles (SCV), small dense core vesicles (SDCV) and large dense core vesicles (LDCV) (Song et al., 1995). Nerve varicosities that contain predominantly SCV show clear synapses but varicosities that contain mainly DCV of different sizes show no synaptic specialization (Basbaum, 1974; Heuser JE, 1977). The varicosities that contain a combination of vesicle types show an interesting distribution of vesicles types around the active zones; only the SCV cluster at the presynaptic zone and DCV are nested away from the synaptic zone. LDCV were not seen to cluster around and attach to the active zone (Cuadras, 1989; Lysakowski et al., 1999; Palay S.L., 1977; Smolen, 1988). Figure 3 presents some illustrative examples of various vesicle types and their relationship to synaptic specialization (Basbaum, 1974; Heuser JE, 1977; Klemm, 1995; Luff et al., 1987; Palay S.L., 1977). This unique distribution of vesicles in a varicosity appears to forecast that SCV and DCV vesicles may be released from synaptic and non-synaptic sites, respectively.

Different types of vesicles have been shown to contain different neurotransmitters (Table 1). SCV usually contain the so-called classical neurotransmitters that include glutamate, γ-aminobutyric acid (GABA), glycine, acetylcholine (ACh) and adenosine triphosphate (ATP). The vesicles are round in excitatory glutamatergic synapses, but flattened in inhibitory GABA synapses (Gray, 1959; Murphy et al., 1996; Takamori et al., 2000). Monoamines such as norepinephrine (NE), dopamine and serotonin or 5-hydroxytryptamine (5-HT) are typically contained in vesicles that appear SDCV when loaded with 5-hydroxy dopamine (5-OHDA). Dense core appearance of these vesicles is artifact of 5-OHDA loading. These vesicles are in fact clear, pleomorphic in nature and include small, flat or rounded and larger round, elongated or dumb-bell shaped vesicles (Hayakawa et al., 2008). Large dense core vesicles (LDCV) may contain a large variety of neuropeptides including cholecystokinin (CCK), galanin, neurokinin (NK), neuropeptide (NPY), neurokinin (NK) including substance P, opioids, oxytocin, somatostatin, vasoactive intestinal peptide (VIP) and vasopressin, and may also contain monoamines. ATP is contained in all types of vesicles, including SCV, pleomorphic adrenergic vesicles and LDCV (Table 1). In addition to vesicular neurotransmitters, varicosities also contain enzymes of nonvesicular neurotransmitters, an example of which is nNOSα, which generates nitric oxide (NO) (Rao et al., 2008; Thatte et al., 2009).

Neuropeptides are synthesized and packaged into the dense core vesicles at the Golgi complex in the nerve cell body and transported to nerve varicosities along axons via kinesin motors traveling on microtubules. Within the varicosity, LDCV mature and are filled with

ATP and other transmitters and are transported to the release site on the varicosity membrane by myosin motors traveling on actin tracts for exocytosis (Bridgman, 2009). Clear vesicles are also filled with transmitters in the varicosity and are transported to synaptic and nonsynaptic release sites on the varicosity membrane by myosin motors. It has recently been shown that the parent enzyme of the non-vesicular transmitter nitric oxide (NO), nNOSα, is transported to release sites on the varicosity membrane by motor proteins like myosin Va (Chaudhury et al., 2011).

Varicosities generally contain different types of vesicles containing different types of neurotransmitters, although one transmitter may predominate in some. Therefore, co-transmission is very common. ATP is a co-transmitter with most of the other transmitters including ACh, monoamines and neuropeptides. Moreover, various combinations of these neurotransmitters yield a large variety of chemically defined varicosities in different tissues (Chaudhury et al., 2011; Chaudhury et al., 2012; Lundberg, 1996; Mitsui et al., 2002; Qu et al., 2008).

Varicosities are in contact with all cell-types surrounding them, separated by the extravascular space. However, the close contact between a terminal button or varicosity of a neuron with a dendrite or other parts of a recipient neuron show membrane specializations called synapses (Colonnier, 1968; Gray, 1959). The site of contact between the nerve terminal and striated muscle is also a type of a synapse, although the word synapse is seldom used in this regard. In contrast, most of the varicosities making contacts with smooth muscles lack synaptic specialization, while some make synapse-like, close junctions (Burnstock, 2008; Gabella, 1995). Based on the presence or absence of synaptic specialization, varicosities can be divided into synaptic and nonsynaptic varicosities.

The release of all neurotransmitters across the varicosity membrane occurs at specialized areas of the membrane called release sites and includes both synaptic and nonsynaptic release sites (Haucke et al., 2011; Neher, 2010), although some investigators have considered all release sites to be synaptic (Vanden Berghe et al., 2007). The synaptic release sites are called presynaptic active zones (AZ) and are characterized by presence of readily releasable SCV on the AZ. The synaptic release sites are seen only in synaptic varicosities. On the other hand, nonsynaptic release sites are exclusively present on nonsynaptic varicosities, but they appear to be also present on the synaptic varicosities. The synaptic and nonsynaptic release sites share some common features to accomplish vesicular exocytosis. However, they differ substantially in details of their organization, types of vesicles they handle, their modes of exocytosis and sensitivity to stimulation (Ariel et al., 2012; Golding, 1994). Synaptic release sites primarily handle exocytosis from SCV and nonsynaptic release sites particularly handle exocytosis from LDCV (Cuadras, 1989; Hammarlund et al., 2008; Lysakowski et al., 1999; Thureson-Klein et al., 1986). The nonsynaptic release sites have molecular organization similar to that seen in endocrine cells (Stevens et al., 2011; Sudhof, 2008a; Tsuboi, 2009). For example, the deletion of synaptobrevin, SNAP25 or Munc18.1 results in the loss of all synaptic and junctional exocytosis (Verhage et al., 2000). They use both ‘kiss and run’ as well as ‘complete vesicular collapse’ modes of exocytosis that determine the types of transmitters that are released. ‘Kiss and run’ exocytosis predominantly releases fully soluble transmitters and full collapse exocytosis also releases proteinaceous neuropeptides from DCV (Fulop et al., 2005; Harata et al., 2006). The mode of exocytosis is determined by stimulus intensity and the type of transmitter released (Fulop et al., 2005). Nonsynaptic neurotransmitter release involves all peptide neurotransmitters (Salio et al., 2006; Zupanc, 1996), catecholamines (Basbaum, 1974; Stjarne et al., 1994) and nonvesicular transmitters (Steinert et al., 2008), as well as the classical synaptic transmitters (Olah et al., 2009; Sarter et al., 2009). Nonsynaptic varicosities may have distinct release sites for vesicular and non-vesicular transmitters such as nitric oxide (Chaudhury et al.,

2011; Chaudhury et al., 2012). Note that postsynaptic clustering scaffold proteins like PSD95 may actually exist at extrasynaptic sites, in addition to their prominent locations in the asymmetric synapses (Aoki et al., 2001) and nonsynaptic varicosities (Chaudhury et al., 2009).

The probability of transmitter secretion has been investigated in sympathetic nerve terminals. It was found that the varicosities of single sympathetic nerve terminals showed different probabilities of transmitter secretion which correlated with different syntaxin zones and synaptotagmin content of different varicosities (Brain et al.,1997).

CONTACTS BETWEEN NERVE TERMINALS AND EFFECTOR CELLS

The contact between a nerve terminal and the target cell is a 3 dimensional structure, but is often described in two dimensions. Typically the bare nerve terminal and the target receptor bearing membrane are less than 200 nm apart. The nature of contact between nerve terminal and effector cell has been investigated by light microscopy, electron microscopy and confocal microscopy. Light microscopy is limited by its resolution (~200nm) and its two dimensional capability. Confocal microscopy has advantage of providing information in 3 dimensions, but is limited by its resolution (~200nm). Confocal microscopy, however, remains a convenient method of scanning for contacts between nerve terminals and effector cells (Mann et al., 1997). Electron microscopy may have the needed spatial resolution but is only effective in two dimensions unless tedious serial sections are obtained. High resolution electron microscopy and freeze fracture techniques are needed to unequivocally distinguish between synapses and synapse-like close contacts.

POSTSYNAPTIC AND POSTJUNCTIONAL RECEPTORS

Postsynaptic receptors are almost exclusively ligand-gated ion channels (ionotropic receptors) for glutamate, GABA, ACh, certain monoamines and ATP, which are designed for eliciting very rapid response to a classical transmitter (Sudhof et al., 2008). Nonsynaptic postjunctional receptors are mostly G-protein coupled metabotropic receptors that produce a slower response. They include metabotropic receptors for the classical neurotransmitters, monoamines, norepinephrine, purines and peptide transmitters (Kandel et al., 2000). Postjunctional receptors also include some ionotropic receptors such as nicotinic receptors in the central nervous system (CNS) (Dani et al., 2007) as well as the autonomic nervous system (ANS) (Fernandes et al., 2010). For example, in chicken ciliary ganglion, postganglionic neurons have been show to possess a high concentration of nicotinic receptors at the synapse and a lower concentration of nicotinic receptors outside the synapse (ectopic site) (Coggan et al., 2005). Similar observations have been made in the skeletal neuromuscular junctions, as well as autonomic neuromuscular junctions in heart and blood vessels (Hirst et al., 1992; Sargent et al., 1989). Interestingly, the synaptic and nonsynaptic nicotinic receptors was found to be molecularly different, α3-nAChR in synapses and α7-nAChR outside the synapse, each having different functional behavior (Coggan et al., 2005).

POSTSYNAPTIC AND POSTJUNCTIONAL POTENTIALS

Neurotransmitters released from nerve terminals produce changes in the membrane potential of target cells that are called either postsynaptic or junction potentials, terminologies coined based on the investigators' belief as to whether they are due to synaptic or junctional transmission. In the CNS, the ANS and the enteric nervous system (ENS), these electrical voltages are traditionally called postsynaptic potentials. At the smooth muscle neuromuscular junction, these potentials are celled junction potentials. These potentials propagate passively and may result from either depolarization (called excitatory potential) or hyperpolarization (called inhibitory potential). The effector cells may show only excitatory,

inhibitory or both excitatory and inhibitory potentials. Moreover, they are graded and show temporal and spatial summation resulting in net change in membrane potential. Duration of the action potential generated by Na^+ channels as in neurons is around 1ms. On the other hand, duration of the action potential generated by Ca^{2+} channels in muscles may be as long as 100ms (Kandel et al., 2000).

Nerve stimulation simultaneously releases many neurotransmitters that produce overlapping postsynaptic or postjunctional potentials in the effector cells. Individual potential changes are pharmacologically isolated by the use of receptor antagonists of the overlapping potentials. The potentials are also classified based on their time courses including latency, time to peak, decay time and total duration. These potentials can be arbitrarily grouped into those lasting ~10–50ms, ~250ms-2s, and several seconds to minutes.

The ultrafast potential lasting 10–50 ms is only seen in synapses that are present between neurons. It is not seen at other neural targets such as smooth neuromuscular junctions. The synaptic potential is called fast postsynaptic potential (fPSP) or simply postsynaptic potential (PSP). PSP may be excitatory called EPSP or inhibitory called IPSP. Both of these are mediated by ionotropic responses, but the IPSPs have slower time courses than fast EPSPs with durations up to 150 ms. The slow potentials lasting several seconds to minutes are seen in neurons as well as non-neural targets. They have been traditionally called sPSP in the neurons and may be excitatory or sEPSP and inhibitory or sIPSP. At the smooth muscle neuromuscular junction, these slow potentials have been called sJP and may be excitatory or sEJP and inhibitory or sIJP. The potentials lasting 250ms-2s have been recognized at the neuromuscular junctions for a long time and have also been recognized in the neurons (Kobayashi et al., 1968). At the smooth muscle neuromuscular junction, they are called fast junction potentials that may be excitatory or fEJP or inhibitory or fIJP. In the ENS, potentials with similar durations have recently been recognized but are called intermediate PSP, because the true synaptic ultrafast PSP has been called fPSP. Monro et al. (Monro et al., 2004) have described purine mediated fast, intermediate and slow PSP in submucous as well as myenteric neurons (Gwynne et al., 2009a) (Figure 4). A critical review of the available data suggests that the potential lasting 10ms-50ms is truly a synaptic response. iPSPs and sPSPs in neurons have origins that are similar to fJPs and sJPs seen at neuromuscular junctions.

In neurons, excitability induced electrical signals facilitate neurotransmitter release. In smooth muscles, the released neurotransmitters influence the contractile state of smooth muscles by a process called electromechanical coupling. Neurotransmitters may also cause muscle contraction or relaxation by directly affecting contractile processes without any change in membrane potential by a process called pharmacomechanical coupling. However, transmitters usually cause muscle contraction or relaxation via both electromechanical and pharmacomechanical coupling mechanisms (Bolton et al., 1986; Somlyo et al., 1968). Therefore, membrane potentials may not fully reflect the effect of a transmitter on muscle.

The time course of the synaptic/junctional response is dependent upon several factor including: 1) time course of neurotransmitter release which itself is dependent upon the nature of the neurotransmitter and the release mechanism; 2) characteristics of the space where the neurotransmitter is released such as ‘closed’ or ‘open’ space and the distance between the release site; and 3) the target receptors; and density, distribution and kinetics of the receptor and the signaling pathway. These individual factors are appropriately packaged together into synaptic and junctional modes of neurotransmission with distinct structural units that can be distinguished by the time course of the response. Morphological and molecular components of the synapses and correlative synaptic potentials are best described in the CNS and are being investigated in the ANS including the ENS. On the other hand,

structural and functional features of the junctional neurotransmission are best described at the peripheral autonomic neuromuscular junctions.

SYNAPTIC NEUROTRANSMISSION

Synaptic neurotransmission exclusively involves the so called synaptic varicosities. Sometimes, conclusions about the presence of a synapse are made on light or confocal microscopy. High resolution electron microscopy with proper tissue fixation and orientation is necessary for identification of a synapse (Colonnier, 1968; Gray, 1976; Westphal et al., 2008). Synaptic transmission typically occurs between neurons. The advances in molecular anatomy and pharmacology of a synapse, which have been made in the CNS, are largely lacking in the study of the peripheral autonomic nervous system including the ENS and AG. Therefore, structure to function relationship of synaptic neurotransmission is mostly derived from studies in the CNS neurons.

CENTRAL NERVOUS SYSTEM

On electron microscopy, a synapse is recognized as a focal area of less than a square micrometer that is characterized by variably thickened presynaptic and postsynaptic membranes, separated by a synaptic cleft (Peters et al., 1996). However, the prominence of the thickening may vary in different types of synapses (Klemann et al., 2011) and there are many pitfalls in definitively identifying a synapse from other synapse-like specializations (Gray, 1976). The use of freeze fracture or freeze etching techniques is necessary for the unequivocal identification of a synapse from other synapse-like junctions (Gray, 1976; Peters et al., 1996).

Presynaptic membrane specialization—The focal area of membrane at the synapse in the presynaptic terminal or varicosity is called the presynaptic “active zone”. It is characterized by the presence of a synaptic grid and SCV docked on the membrane. The synaptic grid was initially described by Gray in 1963 and subsequently in more detail by others using careful transverse and tangential sections of synapses (Gray, 1963; Pfenninger et al., 1972). The grid is also called cytomatrix of active zone (CAZ) and has been described in detail (Gray, 1963; Phillips et al., 2001; Zamorano et al., 2001). It appears as an interrupted presynaptic thickening on a transverse section of a synapse and closely spaced hexagonal electron dense areas in a tangential section (Gray, 1963) (Figures 5A& 5B).

Recent studies have further established the focal nature of the presynaptic active zone and the presynaptic grid. Figure 6A shows a localized synaptic active zone with attached SCV that can be easily distinguished from the extensive nonsynaptic region of the varicosity (Siksou el al 2007). Figures 6B and 6C present a model of presynaptic web or grid at the active zone (Phillips et al 2001). The presynaptic grid consists of dense fibrils and particles. The grid contains a fibrillar component that projects into the cytosol and a particulate component that forms a sievelike structure (Bloom et al., 1968a; Bloom et al., 1968b; Landis et al., 1988). The grid particles are 50–80nm and are located 50–100nm apart. They are connected to each other by 10nm fibrils forming a web or sieve-like grid. They are also connected to the PSD across the synaptic cleft via adhesion molecules. The presynaptic active zone shows secretory granules and voltage dependent Ca^{2+} channels (Haucke et al., 2011). The active zone grid is thought to serve several functions: 1) exclude larger-sized DCVs from the active zone and allow only SCV access to the active zone; 2) guide SCV towards the presynaptic membrane; 3) register the active zone in perfect alignment with the postsynaptic density; and 4) provide housing for proteins involved in exo-endocytosis of SCV (Ariel et al., 2012; Gray, 1963; Pfenninger et al., 1972; Waites et al., 2011; Zamorano et al., 2001).

The presynaptic active zone is characterized by the presence of SCV docked on the cytoplasmic side of the synaptic membrane. Such membrane docked SCV are not seen in nonsynaptic release sites, at least during thorough review of widely available electron photomicrographs. SCV attached to the cytoplasmic side of the membrane at the synaptic cleft were first observed at motor endplates in striated muscles (Peters et al., 1996). Similar observations were made in the varicosities at the glutamatergic synapse in the CNS (Peters et al., 1996).

The proteome and the 3D cytomatrix of the presynaptic active zone in CNS neurons reveal several hundred proteins that serve specific functions during the release of neurotransmitters (Morciano et al., 2009; Siksou et al., 2007). Three major protein complexes with overlapping functions define the presynaptic active zone (Figure 7). 1) The first complex is largely structural that includes proteins associated with the presynaptic grid and registration with the postsynaptic membrane. It includes: cell adhesion molecules such as neurexins, ephrin and SynCAM that bind with postsynaptic proteins, neuroligins and N-cadherin, ephrin receptor and NCAM, respectively,to maintain the structure of a synapse. Out of these adhesion proteins, neuroligins and neurexins may be unique to a synapse, and N-cadherin and integrins anchor together the perisynaptic areas around the synapse to “lock-in” the synaptic cavity (Fannon et al., 1996).. Cytoskeletal proteins, such as piccolo, bassoon, ERC/Cast, liprins; PDZ domain containing proteins such as CASK (calcium/calmodulin-dependent serine protein kinase), veli and mint, which help cluster transmembrane proteins including VDCCs (Fejtova et al., 2006). The filamentous component of the grid includes actin, spectrin and structural proteins such as bassoon, piccolo and rim. The filaments help regulate the delivery of SCV to their presynaptic location (Cingolani et al., 2008; Hilfiker et al., 1999). The particle is a supramolecular structure formed by structural proteins and is rich in proteins involved in synaptic vesicular exocytosis and endocytosis (Phillips et al., 2001; Zamorano et al., 2001). 2) The second complex is involved in synaptic vesicle docking and fusion. It includes Rim, Rab3a and Munc13, components of the SNARE complex, including syntaxin, SNAP 25, and Munc18 and VDCCs. 3) The third complex is involved in synaptic vesicle endocytosis. It includes clathrin, dynamin and a family of SH3-domain-containing adaptor proteins (Dittman et al., 2009; Sorensen, 2009).

A large number of proteins are involved in the exocytosis of SCV at the presynaptic active zone (Haucke et al., 2011; Sudhof et al., 2008). In varicosities, SCV anchors to the actin forming fibrils in the core of the varicosity via synapsin (Hilfiker et al., 1999). These vesicles form the ‘reserve pool’ of the SCV (Rizzoli et al., 2005). Phosphorylation of synapsin detaches SVC from the actin grid and vesicles enter the ‘recycling pool’ as they are guided to the varicosity membrane with the help of motor proteins like myosin Va (Bridgman, 2009; Rizzoli et al., 2005). Tethering and docking proteins of the active zone include the RIM complex that includes RIM, ELKS, Munc13, α-liprins, piccolo, and bassoon. Rab proteins including Rab3 and Rab27 are GTPases that mediate the anchoring of vesicles to the RIM proteins. Docking of the vesicles to the active zone is also facilitated by active zone actin (Cingolani et al., 2008).

Neurotransmitters contained in secretory vesicles are released by exocytosis as packets in a quantal fashion (Katz, 2003). The steps involved in synaptic exocytosis have been described by Sudhof and colleagues (Sudhof, 2008a) (Figure 8). After vesicle docking, the priming proteins force the docked vesicles and the active zone of the varicosity membrane together and prepare them for immediate exocytosis. These vesicle constitute the so called ‘release ready pool’(Sudhof, 2008a). The priming proteins include the SNARE complex, Munc18.1 and complexin. The SNARE complex includes synaptobrevin on synaptic vesicle and SNAP25 and syntaxin-1 on the plasma membrane. Both the SNARE complex and clasp like Munc18 are necessary for early stages of priming (Sudhof, 2008a). A soluble protein,

complexin, binds with the SNARE complex for further priming and preparing of the vesicles for Ca^{2+}-induced exocytosis (Tang et al., 2006). Membrane-associated actin plays the additional regulatory role of providing physical and molecular barrier to priming and therefore possibly acts to hinder exocytosis (Cingolani et al., 2008).

At the presynaptic active zone, there are two types of Ca^{2+}-induced exocytosis. At resting Ca^{2+} concentrations, there is a spontaneous low-rate neurotransmitter release called "asynchronous exocytosis" that is synaptotagmin and complexin independent. With Ca^{2+} influx, associated with an invading action potential, there is a rapid neurotransmitter release called "synchronous release", which is synaptotagmin and complexin dependent and accounts for >90% of total transmitter release. During synchronous release, the rate of neurotransmitter release increases >10,000 fold in less than a millisecond. This amazing speed is consistent with the view that Ca^{2+} acts as a trigger for the release of the vesicles that are already docked and primed at the presynaptic active zone (Sudhof, 2008a). Genetic deletions of synaptotagmin-1 and complexin cause the loss of synaptic synchronous vesicular release while normal asynchronous vesicular exocytosis is preserved (Tang et al., 2006).

Endocytosis and recycling events follow exocytosis (Lang et al., 2008) (Figure 9). The prominent form of exo-endocytosis in synaptic neurotransmission is "kiss and run type" or Ω fusion, as it is the fastest type of recycling (Harata et al., 2006). In kiss and run type exo-endocytosis, complete fusion of the vesicle does not occur. After complete fusion of the vesicles, endocytosis of synaptic vesicles involves clathrin coating, membrane deformation, budding, fission and uncoating that occurs in the perisynaptic region of the varicosity membrane (Dittman et al., 2009).

Synaptic cavity—A synaptic cleft separates the pre- and post- synaptic membranes. The usual width of the synaptic cavity is 20–30 nm (De Camilli et al., 2003) (Figure 10). At the motor endplate, which is a type of synapse at the skeletal neuromuscular junction, the synaptic cavity is as wide as 100 nm (Cowan W.M., 2003). The synaptic cavity in the CNS is usually wider than that of the adjoining junctional space (De Camilli et al., 2003). Therefore the proximity of membranes of the prejunctional varicosity and the target cell is not a reliable morphological marker of a synapse. The synaptic cavity is characterized by anchoring proteins that bind the presynaptic and postsynaptic membranes together (Fannon et al., 1996). In freeze-substituted and in deep etched material, 4–6 nm diameter fibrils can be seen to bridge between the pre- and postsynaptic membranes (Gray, 1976). The synaptic cavity also contains short fibrillar structures that are 4–6 nm in diameter and 8–15 nm in length that may represent ionotropic receptors in the membrane of the postsynaptic element. The concentration of neurotransmitters in the synaptic cleft may reach millimolar range and decays rapidly with a time constant, t_{decay} of 0.1–1 ms.

Postsynaptic specialization—Presynaptic nerve terminals make focal synaptic contacts with different parts of the postsynaptic neuron including dendrites, cell bodies and axons. The post synaptic membrane is highly specialized and precisely aligned with the presynaptic release site.

The postsynaptic site is characterized by the postsynaptic density, which is a dense plate of actin frame work and scaffolding proteins called PSD proteins, bearing transmembrane ionotropic channel receptors (Okabe, 2007). The size, shape, and prominence of the postsynaptic density differ at different synapses (Klemann et al., 2011). The postsynaptic density may be oval, perforated or annular in shape and is prominent in glutamatergic excitatory synapses (Okabe, 2007). Glutamatergic synapses have a prominent postsynaptic density, whereas GABA inhibitory synapses have a less prominent postsynaptic density.

Glutamatergic synapses with postsynaptic prominence are celled asymmetrical synapses whereas GABA inhibitory synapses without postsynaptic prominence have been called symmetrical synapses (Colonnier, 1968; Gray, 1963; Peters et al., 1996).

The main scaffolding protein at the glutamatergic synapse is PSD95, which assembles ionotropic glutamate receptors, nNOSα and neuroligins. PSD95 is dynamically anchored to the membrane via palmitoylation. The proteome of the postsynaptic density of a glutamatergic synapse includes several hundred proteins (Feng et al., 2009) (Figure 11). The nature of the synapse-associated proteins may differ in different types of synapses. At the cholinergic synapse in the parasympathetic ganglion, PSD93 may play an important role in the stabilization of nicotinic receptors (Conroy et al., 2003; Neff et al., 2009; Parker et al., 2004). The ionotropic receptors are anchored directly or indirectly to the postsynaptic density by scaffolding proteins containing PDZ binding domains (Conroy et al., 2003; Kim et al., 2004). Ionotropic receptors are ligand gated ion channels that allow for the rapid activation of ionic currents in the postsynaptic membrane. Note the interesting observation that despite the diversity of the neurotransmitter-specific receptors, there is huge homology in the scaffolding proteins in different varieties of the synapses.

Postsynaptic potentials—The postsynaptic electrical response is very fast and called the fPSP. The fPSP is characterized by a small delay of 1–3 ms and lasts up to 30 ms. Duration of the postsynaptic current can vary more than an order of magnitude depending upon the kinetics of the postsynaptic receptors (Scimemi et al., 2009). In the CNS, the fPSP may be either depolarizing (excitatory) or hyperpolarizing (inhibitory) in nature (Kandel et al., 2000). The fPSP correlates with the structural features of a synapse such as the release-ready vesicles, a synaptic cavity where sudden high concentrations of released transmitter can be achieved and rapidly responding transmitter-gated ion channels. The fEPSP and fIPSP may be surrogate electrophysiological markers of a synapse.

The fast excitatory postsynaptic potentials (fEPSP) in the CNS are usually due to glutamate acting on its ionotropic receptors called NMDA and non-NMDA (AMPA and kainate) receptor (Frank, 2011). The non-NMDA ionotropic receptors are permeable to Na^+ and K^+ and are responsible for fast early peak of the fEPSP. The NMDA receptor has the additional property of being voltage dependent and Ca^{2+} permeable. NMDA ionotropic receptor contributes to the late component of the fEPSP and the Ca2+ mediated effects in the postsynaptic neuron. The time constant of decay of fEPSPs mediated by AMPA receptors is 1–2 ms and is much longer for NMDA receptor, which reflects the channel kinetics.

The fast inhibitory postsynaptic potential (fIPSP) in the CNS is mediated by GABA and glycine acting on their ionotropic receptors, respectively, that conduct Cl^- (Arancibia-Carcamo et al., 2006). The influx of Cl^- in central neurons causes membrane hyperpolarization (Smith et al., 2010).

ENTERIC NERVOUS SYSTEM

The electron microscopic details of ENS and CNS synapses are very similar. However, the molecular anatomy and physiology of synapses in the ENS remain to be fully investigated. Functionally, synapses of ENS exhibit synaptic fast excitatory potentials similar to those of the CNS synapses, but the chemical nature of their synaptic transmitters are different (Bornstein J.C., 2002; Galligan, 2002a; Galligan, 2002b; Gwynne et al., 2007). Fast IPSPs have not been described in the enteric neurons.

In the ENS, presynaptic varicosities contain several different types of vesicles (Baumgarten et al., 1970; Gabella, 1972; Richardson, 1966). In the myenteric plexus of the guinea pig ileum, Gabella (Gabella, 1979) reported that the nerve profiles that contained SCV showed

synaptic specializations that varied from a moderate thickening of pre- and post-synaptic membranes to prominent dense presynaptic projections. In the ENS, SCV are filled with ACh. This is in contrast to the CNS where most SCV are filled with amino acids. Nerve profiles that mainly contained LDCV are nonsynaptic and contain neuropeptides such as VIP and SP as well as ATP (Furness, 2006). Moreover, LDCV were not observed closer than 200 nm to the presynaptic membrane (Baumgarten et al., 1970; Gabella, 1979) (Figure 12). These observations are similar to those made in the CNS neurons.

Hayakawa and colleagues (Hayakawa et al., 2008) reported that in the neuropil of myenteric ganglia in rat duodenum, half of the nerve terminals contained pleomorphic vesicles. A considerable number of tyrosine hydroxylase immunoreactive terminals made asymmetrical synaptic contacts with dendrites, spine or soma of myenteric ganglia. It was also found that 16% of the total number of axosomatic terminals showed tyrosine hydroxylase immunoreactivity. These studies show that myenteric neurons receive direct input from adrenergic terminal containing pleomorphic vesicles, which contain NE, 5HT and also ATP. However, it is unclear if there are well defined synapses between the adrenergic nerve terminals and the myenteric neurons.

Furness and colleagues have investigated connections between the immunohistochemically identified enteric neurons, using high resolation confocal microscopy and electron microscopy. They have described many synapses in these circuits (Li and Furness 2000; Pompolo and Furness 1998; Mann et al., 1997; Portbury et al. 1995). Such studies will help define the relative abundance and the role of synapses in well defined neural circuits in the ENS.

The enteric neurons can be divided based on morphology, neurochemistry, pharmacology and function. Electrophysiologically, they can be divided into AH neurons that are characterized by prolonged hyperpolarization following their action potentials and S-neurons that show prominent fast EPSPs (Bornstein J.C., 2002; Galligan et al., 2000; Lomax et al., 1999). AH neurons have Dogiel type II type morphology and serve as intrinsic primary sensory neurons (IPANs). S neurons serve as interneurons, motor neurons and secretomotor neurons. However, some AH neurons also receive synaptic input and serve as interneurons.

Limited information is currently available on the molecular anatomy and biochemistry of neurotransmitter release from the active zone and nature and physiology of postsynaptic density in the enteric synapses. However, the synaptic adhesion molecules, neurexins and neuroligins that are known to be present in the CNS synapse, have also been reported in the ENS (Gershon and Ratcliffe, 2004). Moreover, several types of ligand gated ion channels are expressed in the enteric neurons. These include: nACh receptors, P2X receptors, 5-HT_3 receptors, GABA-A receptors, NMDA-and AMPA receptors and glycine receptors (Galligan, 2002a). All these receptors are expressed in AH neurons and many of them are localized to extrasynaptic sites. In all S-type and some AH neurons, nACh receptors, P2X receptors and 5-HT3 receptors mediate fast synaptic potentials (Galligan, 2002b). The functional role of GABA, glutamate and glycine receptors in the enteric neurons is unclear at this time.

In contrast to the paucity of structural data, a considerable amount of functional data is available in the ENS. In the ENS, fast EPSPs are observed, which have a latency of 1 to 3 ms and last around 30 ms (Galligan, 2002a; Gwynne et al., 2007). This response pattern is very similar to synapse-mediated fPSPs in the CNS. However, unlike the central neurons, the enteric neurons exhibit only fEPSPs, no fIPSPs. fEPSPs are present in 70% of the myenteric and 90% of submucous neurons (Gwynne et al., 2007). Fast EPSPs are

uncommon in AH neurons, but are present in almost all musculomotor neurons, secretomotor neurons and interneurons (Castelucci et al., 2002; Gwynne et al., 2007).

Acetylcholine acting on nicotinic receptors is the major synaptic neurotransmitter in the ENS. However, P2X and $5HT_3$ receptor mediated purinergic and serotoninergic neurotransmission also plays a role (Galligan 2002b; Monro et al., 2002) (Figure 13). The nicotinic receptors on the enteric neurons have subunit composition of α3α5β4 that is effectively blocked by hexamethonium (Galligan, 2002b; Zhou et al., 2002). Hexamethonium-sensitive fEPSPs are demonstrable in almost all S-type neurons (Zhou et al., 2002). Consistent with this observation, most of the identified enteric neurons express nicotinic receptors and nicotinic fEPSP (Bian et al., 2004; Gwynne et al., 2007; Kirchgessner et al., 1998; Monro et al., 2002; Nurgali et al., 2004; Zhou et al., 2002). More recent quantitative studies revealed that exclusive nicotinic transmission was seen in only one quarter of unselected myenteric S-type neurons. However, in the majority (> three quarters) of neurons, nicotinic transmission appeared to account for a portion of the fEPSP. Despite the paucity of information on the molecular biology of nicotinic synapses, there appear to be important differences between synaptic proteins at nicotinic and glutamatergic synapses (Conroy et al., 2003; Neff et al., 2009; Parker et al., 2004).

ATP or a related purine and one of the several subtypes of P2X receptors contribute to the fEPSP in two-thirds of unselected enteric neurons (Galligan, 2002b; Ren et al., 2008). Until recently, purinergic nerves involved in specific locations could not be identified because reliable markers of purinergic varicosities or vesicle had not been available. However, recently, a vesicular ATP/ADP transporter has been identified as the protein, SLC17A9 (Sawada et al., 2008). Immunolocalization of SLC17A9 should enhance our ability to identify purinergic nerve terminals, as has been recently shown in enteric varicosities (Chaudhury et al., 2012). ATP is co-stored with most other neurotransmitters including acetylcholine. The involvement of P2X receptors in fEPSP is inferred by pharmacologic studies and by studies in mice lacking P2X2 receptor (Burnstock, 2007; Galligan et al., 1994; Ren et al., 2008). Ionotropic P2X receptors and purinergic fEPSP have been found to be widely distributed in the ENS (Castelucci et al., 2002).

AUTONOMIC GANGLIA

Motor neurons in sympathetic and parasympathetic ganglia receive synaptic inputs from preganglionic neurons. Many neurons in prevertebral sympathetic ganglia receive additional synaptic inputs from intestinofugal neurons located in the enteric plexuses. Interestingly, only a minority of varicosities make synaptic contacts (Gibbins et al., 2006). The presynaptic varicosities are filled with SCV containing predominantly ACh. The postsynaptic nicotinic receptors are of different subunit compositions (Skok, 2002). Little information on electron microscopy and the molecular nature of synapses in the autonomic ganglia is currently available. Electrical potentials in the postsynaptic neuron elicited by presynaptic nerve stimulation in the autonomic ganglia are similar to those described in neural synapses in the CNS and the ENS. They are characterized by fEPSP with synaptic delays of less than a millisecond and usually lasts over 10 to 30 milliseconds (Ascher et al., 1979). The fEPSP is mediated by ACh acting on nicotinic receptors and the duration of fEPSP may depend on the subunit composition of nicotinic receptors (Skok, 2002). It has been shown that in chicken ciliary ganglion, α3-nAChR are concentrated at the PSD and α7-nAChR migrate laterally to nonsynaptic areas (Coggan et al., 2005). ATP and other transmitters, acting via their ionotropic receptors, also play a role in synaptic transmission in the autonomic ganglia (Galligan et al., 2000; Nakazawa, 1994).

JUNCTIONAL NEUROTRANSMISSION

In the past, based on the dogma that all neurotransmission between motor varicosities and smooth muscles were synaptic in nature, all types of electrical responses to nerve stimulation in smooth muscle cells were called postsynaptic potentials (Bennett, 1997).

Morphology of the Junctions is quite different from that of the synapses. Electron microscopic studies of the neuro-smooth muscle junctions show that the bare *en passage* nerve varicosities in the axon bundle lie at variable distances from the smooth muscle cells, with the apposition interval varying from 100–200 nm and greater than 2 mm (Rogers et al., 1966; Thaemert, 1966). Studies using serial EM sections have provided us with useful limited information on the contacts between the bare varicosities and the smooth muscles(Hirst et al., 1992; Klemm, 1995; Luff, 1996; Luff et al., 1987). Klemm (Klemm 1996) reported that reconstruction of serial sections of neuromuscular junctions of the longitudinal muscle, reveal two types of contacts. These contacts were called large and small contacts, respectively. In the large contacts, the bare varicosities and the smooth muscles were separated by ~60nm and the small contacts the two were separated by ~400nm . These varicosities show no synaptic specializations of the membranes (Thaemert, 1966). Therefore, neurotransmission between motor nerves and smooth muscle bundles is nonsynaptic in nature. In contrast to the synaptic cavity, the junctional space is not ‘closed’ and is –’open’ to extracellular space. Overall, nonsynaptic junctional space between the neural release site and the postjunctional receptors may show variable degrees of separation (from <20 nm to >2000 nm) between the release site on the prejunctional nerve terminal and the postjunctional receptors on the target cell.

Electrical potentials in smooth muscle cells in response to motor nerve stimulation are called junction potentials rather than postsynaptic potentials (Bennett, 1997). Functionally, junctional neurotransmission is distinguished from synaptic transmission by the time course of the response. Whereas synaptic transmission is measured in milliseconds, junctional transmission is measured in second to minutes. The time course of the junctional potential has been divide into two most frequently observed time courses representing ‘close and ‘wide’ junctional transmissions. The “close” junctional transmission associated with fast junction potential and “wide” junctional transmission associated with slow junction potential. The slow electrical potentials reach a peak in about 150 ms and then declines with a time constant between 250 and 500 msec. These responses typically last several seconds to minutes and may be hyperpolarizing or inhibitory and depolarizing or excitatory, and have been called slow EJP or slow IJP, respectively. The time scale of the response is dependent on many factors including distance of anatomic separation of the junctional space. The time course is determined by the nature of the neurotransmitters and kinetics of their release, distance between the release site and the postjunctional receptors, and nature of the receptors and the signaling pathway. Nature of the receptors play a critical role in the time course with metabotropic receptors being associated with much slow time courses than ionotropic receptors, a natural consequence of the way these receptors operate. The fast and the slow junction potentials are usually observed together in the same target cell.

Nonsynaptic junctional transmission is the only mode of transmission involving the varicosities that show no synaptic contacts that includes almost all nerve terminals whose target is not a neuron. Most smooth muscles exhibit both fast and slow junction potentials typically mediated by different classes of metabotropic receptors (although some P2X receptors are involved) with different kinetics (Bennett, 1972a). However, the synaptic varicosities are not only involved in synaptic transmission, but also partake in junctional neurotransmission. Junctional transmission also coexists with synaptic transmission in the central and the peripheral nervous systems including the enteric nervous system. In these

cases, the neurotransmitter producing junctional, extrasynaptic effects may represent the neurotransmitter that may overflow out of the synaptic cavity or released from extrasynaptic release sites. All receptors involved in junctional transmission are present in the extrasynaptic region.

CLOSE JUNCTIONAL NEUROTRANSMISSION

The close junctional neurotransmission is characterized by synapse like close contact between the prejunctional release site and the postjunctional receptors. However, unlike the synapse, the junctional space is open to the extravascular space; the prejunctional release site lack the distinguishing features of the presynaptic active zone and release of the soluble transmitters; and the post junctional receptors include metabotropic receptors or slower acting ionotropic receptors. Functional marker of close junctional transmission is post junctional potential lasting ~1 second.

At many neuroeffector contacts, there is a very close juxtaposition of membranes of the nerve terminal and the effector cell. Electron microscopic evaluation of thin serial sections of an entire varicosity is necessary to define all contacts of a varicosity with effector cells in 3 dimensions (Hirst et al., 1992; Luff, 1996). However, random sections have identified very close (< 20 nm) junctions at some smooth muscle neuromuscular junctions (Hirst et al., 1992; Richardson, 1962). This interval is similar to or even smaller than that of a synapse.

At the close neuromuscular junctions, there may be some presynaptic specialization but indistinct postsynaptic membrane specialization and without an organized synaptic cavity. Brain et al. (1997) reported that adrenergic varicosities in the vas deferens possessed active zones about 250 – 700 nm in diameter, that are delineated by syntaxin, SNAP25 and N-type calcium channels and vesicular protein VAMP (Brain et al., 1997). These focal zones of release were precisely opposed to large clusters of P2X1 receptors on the smooth muscle membrane (Bennett et al., 1995), suggesting the presence of close contact between the adrenergic nerve ending and the smooth muscle. These junctions may have some resemblance to synapses in the CNS (Colonnier, 1968) and, therefore, have been called “synapse-like junctions.” Close junctional transmitters may also include synaptic transmitters that leak out of the synaptic cleft and act on extra-synaptic receptors located outside the synapse (Scimemi et al., 2009).

Several studies have reported localized clustering of receptors on the postjunctional membrane at the close junctions and small patches of diffusely distributed receptors at the distant junctions (Bennett et al., 1995; Hansen et al., 1998). Moreover, the nature of the receptors at the synapselike close junctions also appears to be different from those at the synapse and nonsynaptic distant junctions. Postjunctional receptors at the synapse-like close junctions are mostly metabotropic ACh and ATP receptors, ionotropic receptors of ATP and certain types of monoamine receptors.

Functionally, the close junctional transmission appear to be associated with whose time course (~ 1 sec) is much faster than the slow junction potentials at the smooth muscle neuromuscular junctions. In the neurons, this time course is intermediate between the time courses of the fPSPs and sEPSPs.

CLOSE JUNCTIONAL NEUROTRANSMISSION AT DIFFERENT SITES

Close junctions or fJP and its equivalent have been described in smooth muscles of vas deferens, urinary bladder, blood vessels and gut, as well as neurons of the ENS, AG and the CNS.

SMOOTH MUSCLES OF VAS DEFERENS, URINARY BLADDER AND BLOOD VESSELS

In the vas deferens, there is a predominance of close contacts between the adrenergic varicosities and smooth muscles, but no synapses (Bennett, 1972a; Burnstock et al., 2010). However, release sites are precisely apposed to large clusters of P2X1 receptors on the smooth muscle membrane (Bennett et al., 1995; Brain et al., 1997). High-resolution microscopy and biochemical and molecular studies of these pre-junctional varicosity membrane have not been performed. Functional studies have shown that in the mouse or rat vas deferens, sympathetic nerve stimulation produces a fEJP, which reaches a peak value in around 20 ms and decay with a time constant between 20 and 100ms, with an overall duration of around 1 sec. The fEJP is mediated by ATP acting on P2X purino-receptors.

In the urinary bladder, a close relationship between nerve terminals and smooth muscles in the absence of clear synaptic contacts has been recognized for a long time (El-Badawi, 1988). Gabella (1995) reported that in rat detrusor muscle, part of the varicosities in axons may be devoid of Schwann cells and varicosity membranes come within 10 nm to 100nm of the muscle membrane (Gabella, 1995). When there is no Schwann cell covering, the entire nerve terminal may be in contact with the membrane of the smooth muscle. Hansen and colleagues (1998) reported that P2X1 receptors on detrusor muscle formed clusters of two sizes (Hansen et al., 1998). One of them forms a large ellipse of around 1 micrometer and small spherical clusters of around 0.4 micrometer. Whereas the larger clusters were found juxtaposed to the nerve varicosities, smaller ones were not. The post-junctional clusters of P2X1 receptors without a synapse may represent a pattern of close contacts. It has also been shown that the varicosities contain both ACh and ATP. Functional studies have shown that in the rat urinary bladder, parasympathetic (cholinergic) nerve stimulation elicits a fEJP that lasts around 500 ms to 1 sec and is mediated by ATP acting on P2X1 purino-receptors (Burnstock, 2007; Hashitani et al., 2000).

The vascular smooth muscles of the resistance arteries and veins are richly innervated by sympathetic nerves. Terminals of sympathetic nerves make close contacts with smooth muscles (Luff, 1996). Adrenergic varicosities are filled with pleomorphic clear vesicles which appear as SDCV upon loading with 5-OHDA and contain 5-HT and ATP in addition to NE. Sympathetic nerve stimulation also elicits fEJPs that are mediated by ATP acting on P2X receptors in these smooth muscles (Luff, 1996). Stjarne and colleagues (Stjarne et al., 1994) reported that although there are no morphological synapses between adrenergic nerve terminals and vascular smooth muscles, small amounts of NE release by low frequency stimulation results in its actions on muscle cells close to and directly facing the varicosity via α2 receptors whereas high discharge rate results in actions of NE in more distant α1 receptors.

SMOOTH MUSCLES AND PDGFRα+ INTERSTITIAL CELLS IN THE GUT

In the gut, nerve terminals make close junctions with smooth muscles and transmural stimulation produces fast junction potentials (Bennett, 1972a). In the guinea pig tenia ceci, electrical field stimulation (EFS) elicits an ATP and P2X receptor mediated fEJP (Zhang et al., 2005). At enteric neuromuscular junctions in most of the gut, transmural stimulation elicits fEJPs which are mediated by ACh acting on metabotropic muscarinic M3 receptors (Inoue et al., 1993; Maggi et al., 1997). Gut smooth muscles also exhibit fIJPs which have a similar time course to the fEJP. The fIJP is mediated by ATP or related purines acting on P2Y1 receptors (Gallego et al., 2012; Goyal, 2011).

At the contact sites between nerve terminals and fibroblast-like cells positive for platelet derived growth factor receptor α fibroblast-like cell (PDGFRα+FLC), synapse-like close contacts (<20 nm) have been described using confocal microscopy that has resolution of

only ~200 nm (Kurahashi et al., 2011). No molecular morphological evidence supporting the presence of synapses between the nerve varicosities and PDGFRα+FLC is currently available. Moreover, synaptic potentials have not been reported from these cells. The functional role of these cells in purinergic fIJP recorded from smooth muscle cells is not fully understood (Goyal et al., 2013).

ENTERIC NERVOUS SYSTEM, AUTONOMIC GANGLIA AND CENTRAL NERVOUS SYSTEM

In the synaptic varicosities that mediate true ultrafast synaptic potentials, junctional potential lasting few seconds have not been well recognized and have been given different names. In the ENS, synapse-like close junctions have also been described between adrenergic varicosities and enteric ganglia in guinea pigs as well as humans, which have a rich supply of adrenergic varicosities. On electron microscopy, 5-OHDA loaded adrenergic varicosities were found to contain SDCV and make nonsynaptic contacts with enteric neurons (Llewellyn-Smith et al., 1984). It is now known that 5-OHDA loading causes artifactual appearance of dense core vesicle and destroys synaptic structures (Hayakawa et al., 2008). Recent studies have shown that the adrenergic terminals are filled with pleomorphic clear vesicles and make synapse-like close contacts with enteric neurons (Hayakawa et al., 2008). There are limited studies examining synapse-like close junctions of other motor varicosities of enteric neurons (Mann et al., 1997; Pompolo et al., 1995; Young, 1983; Young et al., 1995). Electrophysiological studies have shown that submucous and myenteric neurons in guinea pigs exhibit an EPSP and IPSP lasting 150 ms to 2.5sec. This duration is longer than the longest fEPSPs (<50 ms) and much shorter than that of the sPSPs (several seconds). Therefore, it was named “intermediate” EPSP (iEPSP). Intermediate EPSP is mediated by ATP acting on P2Y receptors; and interestingly, iIPSP is also mediated by ATP (Gwynne et al., 2007; Monro et al., 2002). The time courses of iEPSPs or iIPSPs are similar to that of the fast EJPs at the neuromuscular junctions that have synapse-like close contacts. Further studies are needed to establish the association of close junctions with iEPSPs and iIPSPs in the enteric neurons.

In the autonomic ganglia, prejunctional nerve stimulation produces inhibitory and excitatory post synaptic potentials that last ~1 second each. Although they are called slow, their time course may resemble the ‘fast’ potentials described in other systems (Dun et al, 1993). The so called ‘slow’ IPSP is due to catecholamines that exert their effect by stimulating adrenergic receptirs that are metabotropic in nature (Eranko et al., 1980). This sEPSP is expressed after the sIPSP (Adams et al., 1986; Gibbins et al., 2006; Mochida et al., 1988). It is mediated by ACh acting on muscarinic M1 receptors.

In the CNS, postsynaptic potentials lasting 200ms-1.6 sec and time to peak duration of 10ms-120ms have been described in subsets of neurons in the CNS (Benardo, 1994; Byrne, 2009). Moreover, phasic release of ACh, at the scale of seconds, produces precisely defined cognitive responses (Sarter et al., 2009). These potentials lasting a few seconds may be due to close junctional transmission. However, further studies are needed to test this speculation.

WIDE JUNCTIONAL NEUROTRANSMISSION

The wide junctional neurotransmission is characterized by widely separated prejunctional release site and the postjunctional receptors. The junctional space is freely continuous with the extravascular space; the prejunctional release sites have features similar to the neuroendocrine cells and can also release NE and peptide neurotransmitters; and the post junctional receptors include metabotropic receptors, including those for neuropeptides that are diffusely distributed at distant sites. Functional marker of close junctional transmission is post junctional potential lasting several seconds.

Almost all tissues that exhibit close junctional neurotransmission also show wide junctional neurotransmission. Thus, wide junctional transmission has been described in many smooth muscles such as vas deferens, urinary bladder, blood vessels, gut as well as the nervous systems including ENS, autonomic ganglia and the CNS. Wide junctional neurotransmission is also seen in all other innervated cells including epithelial and secretory cells (Burnstock, 2008; Owyang et al., 2004), endocrine cells (Grunditz et al., 1988), endothelial cells (Draid et al., 2005) and immune cells (Selmeczy et al., 2008). It is interesting to note that the effect of cholinergic nerves on the immune cells is mediated by nicotinic receptors which may explain the beneficial effect of smoking in ulcerative colitis (Ben-Horin et al., 2008)

SMOOTH MUSCLES OF VAS DEFERENS, URINARY BLADDER AND BLOOD VESSELS

In the vas deferens, there is a predominance of close contacts between the adrenergic varicosities and smooth muscles. Functional studies have shown that in the mouse or rat vas deferens, sympathetic nerve stimulation produces a sEJP mediated by NE acting on α-adrenergic receptors, in addition to a fEJP mediated by ATP acting on P2X purino-receptors (Muir et al., 1988).

In the urinary bladder, there are large areas of distant junctions, in addition to the predominance of close junctions (El-Badawi, 1988; Gabella, 1995). Functional studies have shown that apart from producing ATP mediated fEJPs, parasympathetic nerve stimulation also produces sEJPs. The sEJP lasts several seconds and is mediated by ACh acting on metabotropic M3 muscarinic receptors (Krell et al., 1981). The sEJP was associated with late contraction (El-Badawi, 1988; Krell et al., 1981).

In resistance blood vessels and veins, adrenergic innervation to smooth muscle is very prominent. Adrenergic varicosities are filled with pleomorphic clear vesicles containing NE, 5-HT and ATP. Whereas ATP produces fEJPs, NE acting on α-adrenoreceptors mediates slow EJPs. It has been shown that α adrenergic receptors are only located on the junctional regions of the smooth muscle membrane and adrenergic neurotransmission is junctional in nature (Luff, 1996).

SMOOTH MUSCLES AND c-KIT+ INTERSTITIAL CELLS IN THE GUT

In the gut, prejunctional nerves endings contain neuropeptides including VIP and NK, transmitters such as ACh and ATP, and nNOSα that is the enzymatic source of nonvesicular transmitters such as NO. ACh and NK are colocalized in the excitatory varicosities and VIP and nNOSα are colocalized in inhibitory varicosities. Electrical field stimulation of intramural nerves in the gastrointestinal muscle strips produces sEJPs that are mediated by NK acting on its metabotropic receptors (Furness, 2006). On the other hand, sIJPs are mediated by the VIP and the nonvesicular neurotransmitter NO that stimulates NO-sensitive G-cyclase (Mashimo et al., 1996; Thatte et al., 2009). VIP may act presynaptically to enhance NO release and may also cause relaxation without directly contributing to the genesis of the inhibitory potential (Mashimo et al., 1996; Van Geldre et al., 2004).

There has been considerable recent debate regarding the nature of the contacts between enteric varicosities and interstitial cells of Cajal (ICC) or c-kit positive interstitial cells and their role in junction potentials recorded from the smooth muscles. Based on anatomic location and function, two main types of ICC have been described: myenteric ICC (ICC-MY) and intramuscular ICC (ICC-IM) (Sanders et al., 2010). ICC-MY are present around the myenteric plexus and thought to be pacemaker cells for slow waves in the smooth muscles cells (Hirst et al., 2006). Calcium imaging studies in the colon have shown that ICC-MY is innervated by nitrergic and cholinergic nerve terminals, though the nature of the contacts has not been well defined. (Bayguinov et al., 2010).

ICC-IM is located in between the smooth muscle cells. Enteric nerves have been reported to make synaptic contacts with ICC-IM. These contacts include areas of electron dense lining on the inner aspect of the varicosity membrane without any postsynaptic density on the membrane of ICC. Such contacts were not reported between the nerves and the smooth muscles (Horiguchi et al., 2003; Sanders et al., 2010). Most of these recent studies that used confocal microscopy (resolution ~200 nm) to identify ~20 nm synapses or close-contacts raise concerns about their validity. Moreover, careful comparative studies report that the contacts between the nerve terminals and the ICC are not truly synaptic nature and are similar to those between nerve terminal and smooth muscle cells (Mitsui et al., 2002) (Figure 14). Synaptic ultrafast potentials have not been recorded in the ICC. It has been proposed that electrical signals generated in the ICC are passively transferred to the smooth muscles. If enteric nerve stimulation produced synaptic potential in the ICC and transferred to the smooth muscle, ultrafast synaptic potential should be recorded in the smooth muscle. However, cholinergic sEJPs and nitrergic sIJPs are recorded in smooth muscle cells. Slow junction potentials are hallmark of junctional neurotransmission. Moreover, recent studies have shown that the nitrergic junction potentials are actively generated in the smooth muscle cells (He and Goyal, 2012). The functional role of ICC-IM in neuromuscular transmission is currently unproven.

ENTERIC NERVOUS SYSTEM

Neurons in the enteric ganglia receive rich projections of varicosity-bearing axons from many different populations of neurons. Some varicosities contain SCV or a mixture of SCV and LDCV. These varicosities show synaptic specialization. The varicosities containing a mixture of SCV and LDCV revealed that in the region of the varicosity near the presynaptic active zone, only SCV and LDCV were present (Figure 11) (Baumgarten et al., 1970; Gabella, 1979). These observations are similar to those made in the CNS neurons. The SCV contain ACh and ATP that may participate in synaptic neurotransmission. LDCV are filled with neuropeptides such as VIP and NK and LDCV that do not participate in synaptic neurotransmission and their release may be at non-synaptic contacts or junctions.

Stimulation of nerves innervating enteric neurons also elicit sPSPs. Slow EPSPs and sIPSPs are mediated by tachyinins, mGluR1, mGluR5 and other receptor subtypes including muscarinic and 5-HT7 receptors and somatostatin, respectively [see (Gwynne et al., 2007)]. These neuropeptides are contained in LDCV, released at nonsynaptic junctions and produce the slow potentials by stimulating their metabotropic receptors. ACh, ATP and 5-HT produce fIPSP by acting on their ionotropic receptors and sEPSP by stimulating metabotropic receptors on postjunctional membranes (Foong et al., 2010). NE produces sIPSPs in submucosal neurons acting via α2-adrenoceptors (Bornstein et al., 2002).

Durations of the sEPSPs vary from tens of second to minutes and are dependent on many factors including temporal summation of stimuli affecting transmitter release, anatomic distribution of the receptors, and properties of the metabotropic receptors which is the dominant factor. The ionic basis of sEPSP is different in different tissues (Wood et al., 2004). Slow EPSP are seen in virtually all enteric neurons. As many as twenty different neurotransmitters are candidates for producing sEPSP. However, functional studies identify a handful of transmitters. They include NK acting on NK1 and NK3 receptors, 5-HT acting on 5HT1P, 5HT7, ACh acting on M1 muscarinic receptors and ATP acting on P2Y1 receptors.

AUTONOMIC GANGLIA

In the autonomic ganglia, a potential called ‘late slow’EPSP is expressed that lasts over 30 to 60 seconds to several minutes (Dun et al, 1993). Its time course may resemble the sEPSP

described in other systems. It is mediated by neuropeptides. A variety of peptides including SP, VIP, NPY, ENK, CGRP and angiotensin have been localized to SIF nerve cell bodies or nerve fibers (Tanaka et al., 1996). They have been shown that the neuropeptides are e released on nerve stimulation and may mediate the 'late slow' EPSP. The action of neuropeptides is exerted on their respective metabotropic receptors involving a large field of action. ACh and the peptides may be released from the same varicosity or interneuron in certain ganglia (Samano et al., 2006).

CENTRAL NERVOUS SYSTEM

In the CNS, nonsynaptic neurotransmission is now increasing been recognized and has also been called 'volume' transmission (Agnati et al., 1986; Vizi, 1984). It includes a wide spectrum of modes of transmissions varying from transmission to remote sites via neurotransmitter release into the cerebrospinal fluid, blood capillaries and junctional transmission. The junctional neurotransmission involves transmission between nerve terminals and juxtaposed membranes of target cells without synaptic contacts

Junctional neurotransmission in the CNS is mediated by various neuropeptides, catecholamines, purines as well as classical neurotransmitters. Junctional transmitters include those released from the release sites on the nonsynaptic membrane of the varicosities and also those that may leak out of the synaptic cleft to exert effects on extra-synaptic receptors (Clark et al., 2002; Scimemi et al., 2009). In the CA1 pyramidal cells, repetitive electrical stimulation of stratum oriens produces a series of fEPSPs followed by an inhibitory potential. With higher intensity stimulation these potentials were followed by a sEPSP lasting 20–30s (Cole et al., 1984). It has also been reported that GABA released from synaptic cleft or GABA release from nonsynaptic release sites of neuroglia can affect extrasynaptic receptors to exert their effect (Olah et al., 2009).

In the CNS, most of the cholinergic buttons in the CNS have been shown to be nonsynaptic and release acetylcholine (ACh) into the extracellular space (Coggan et al., 2005; Sarter et al., 2009; Vizi et al., 2004). Non-synaptically released ACh exerts its effect by stimulating muscarinic M1 receptors which have been localized to nonsynaptic sites (Yamasaki et al., 2010). Moreover, nicotinic acetylcholine receptors have been shown to be localized outside the synapses at postjunctional and presynaptic sites (Vizi et al., 2004). These postjunctional nicotinic receptors are of high affinity and are of different subtypes than the synaptic nicotinic receptors (Conroy et al., 1995; Fernandes et al., 2010). Nonsynaptic nicotinic transmission plays a role in both neural plasticity and cellular learning processes, as well as in long-term changes in basic activity through fast activation, desensitization of receptors, and fluctuations of the steady-state levels of ACh. The effects of smoking and nicotine in the CNS are mediated by the nonsynaptic nicotinic receptors (Lendvai et al., 2008).

MODULATION OF SYNAPTIC AND JUNCTIONAL NEUROTRANSMISSION

Both synaptic and nonsynaptic neurotransmission are highly regulated. These regulatory mechanisms are major targets of therapy to enhance neurotransmission. The modulation of neurotransmission is affected by two broad mechanisms (Vizi et al., 2010).

One of the important mechanisms of modulation of synaptic activity is by the termination of the action of the neurotransmitter. It is brought about by: 1) Rapid reuptake or enzymatic degradation of the transmitter. Reuptake by transporters located outside the synapse on the presynaptic terminal and glial cells are major regulators of glutamate and GABA neurotransmission and are targets of therapy in glutamatergic and GABA-ergic neurotransmission in the CNS (Bunch et al., 2009; Madsen et al., 2010). Inhibitors of the active reuptake of monoamine (monoamine transporters) inhibit the transport of

norepinephrine, dopamine and 5-HT and play important roles in augmenting actions of these monoamines. Nonselective serotonin uptake inhibitors such as tricyclic antidepressants (TCA) and selective serotonin uptake inhibitors (SSRI) are extensively used in treatment of anxiety, depression and irritable bowel syndrome (Haenisch et al., 2011; Kaminski et al., 2011). 2) Enzymatic degradation such as that of ACh by cholinesterase that may be present within the synapse or outside the synapse. Cholinesterase inhibitors are used to enhance cholinergic neurotransmission in Alzheimer disease (Geula et al., 2004). 2) The synaptic strength is strongly modulated by excitatory or inhibitory presynaptic receptors. Presynaptic receptors may be the targets of the transmitter released by the same terminal (autocrine) or from a postsynaptic site (retrograde neurotransmission). Mediators that modulate presynaptic receptors may also be derived from different nerve terminals or autocoids released by other cell types. At glutamatergic central synapses, NO serves as a retrograde modulator of synaptic transmission. Similarly, at GABA synapses in the CNS, endocannabinoids have shown to be retrograde neurotransmitters that modulate the activity of the GABA synapses (Lovinger, 2008; Steinert et al., 2008). Retrograde neurotransmitters, NO and cannabinoids, have been shown to play an important role in the long term plasticity of their respective synapses (Galligan, 2009; Lovinger, 2008).

The ‘presynaptic’ receptors usually include both metabotropic and ionotropic receptors (Marchi et al., 2010; Parnas et al., 2010) and their actions are usually brought about by increasing or decreasing activity of the presynaptic Ca^{2+} channels and the regulation of cycling of the neurotransmitter vesicles and by regulation of the size of the pool undergoing exocytosis (Sudhof, 2008a).

In the gut, there are several examples of modulation of neurotransmitter release by presynaptic receptors. Neurotransmitter release by nicotinic fEPSPs has been shown to be augmented by presynaptic muscarinic M1 receptors (North et al., 1985a). Such synergistic augmentation of neurotransmitter release may be necessary for full expression of a functional response. It has been reported that a combination of nicotinic and muscarinic antagonists was necessary to abolish vagal stimulation mediated relaxation of the lower esophageal sphincter relaxation (Goyal et al., 1975). Presynaptic excitation of VIP autoreceptors at smooth muscle neuromuscular junctions may act to enhance NO release or ACh release (He et al., 1993; Lundberg, 1996; Van Geldre et al., 2004). The activation of presynaptic CCK receptors has been shown to facilitate nicotinic synaptic neurotransmission in the gall bladder (Mawe, 1991). Activation of 5-HT_4 receptors enhances ACh release and augments synaptic transmission via presynaptic actions (Neal et al., 2006; Tonini et al., 1989), though they may also inhibit synaptic transmission via 5HT1A receptors. (North et al., 1980) and inhibit neurotransmission at nerve endings of the intrinsic sensory neurons (Schikowski et al., 2002). 5-HT_4 receptor antagonists and partial agonists have been shown to enhance synaptic ACh release and enhance sensory input and thus act as prokinetic agents to enhance gastrointestinal transit (Fang et al., 2008; Schikowski et al., 2002).

The inhibition of synaptic and nonsynaptic neurotransmission by the stimulation of presynaptic inhibitory receptors is due to a wide variety of transmitters and chemical agents. Some of the metabotropic presynaptic inhibitory receptors include: 5-HT_1 receptors, M2 muscarinic receptors, α2 adrenergic receptors, A1 adenosine receptors, H3 histamine receptors, receptors for pancreatic polypeptide family (PP, NPY, PYY) and receptors for inflammatory mediators such as H3 histamine, IL-1β, IL-6 and platelet activating factor. Presynaptic inhibition by norepinephrine (NE) acting on α2 receptors occurs at both fast EPSP and slows EPSP in the enteric neurons and neuro-effector junctions and is responsible for inhibition of intestinal motility and secretion during states of adrenergic stimulation (North et al., 1985a; North et al., 1985b; Wood, 2006). Presynaptic inhibition by inflammatory mediators may be responsible for the suppression of intestinal motility in

states of intestinal inflammation. Retrograde transmission by endocannabinoids may be an important inhibitory modulators of synaptic neurotransmission in the ENS (Galligan, 2009).

CONCLUSIONS

Traditionally, all chemical communication between neurons had been assumed to be synaptic in nature. In the last few decades, there have been important advances in our understanding of the molecular anatomy, biology and physiology of synapses in the neurons that suggest a strong structure-function correlation. Based on structure to function relationship studies, this review proposes three types of contacts between nerve terminals and target cells. They include: synapse, close junction and wide junction (Table 2). Morphologically, a synapse is: 1) a small, less than 1 micrometer square area of close contact between specialized active zone of the varicosity and the PSD on the postsynaptic membrane. 2) The synaptic membranes are separated by a 20–30nm ‘closed’ synaptic cavity which makes it unaccessible to exogenous chemicals. 3) The active zone of the varicosity membrane is characterized by the presynaptic grid and membrane docked ‘release ready’ small clear vesicles with exclusion of the large vesicles. 4) The postsynaptic membrane has a postsynaptic density, bearing densely packed ionotropic receptors. 5) The molecular anatomy of a synapse is designed to support a pinpoint, precise, and fast neurotransmission. 6) Electrophysiological hallmark of a synapse is a fPSP that lasts ~30ms. 7) Synapses are typically present between neurons in the CNS, the ANS and the ENS.

Nonsynaptic, junctional transmission can be divided into close and wide junctional transmissions. The close junctional neurotransmission is characterized by: 1) synapses-like narrow junctional space separating the prejunctional release site and the postjunctional receptors. However, the junctional space is open to the extravascular space. 2) The post junctional receptors include metabotropic receptors or slower acting ionotropic receptors. 3) The neurotransmitters include the synaptic transmitters that leak out of the synapses and those released from the prejunctional release sites. The prejunctional release sites lack the distinguishing features of the presynaptic active zone. 4) The prejunctional transmitters are soluble transmitters including the classical transmitters and others such as catecholamines. 5) Functional marker of close junctional transmission are fIJPs as described in the smooth muscles, and iPSPs in neurons (this distinguishes it from the synaptic ultrafast potential that has been called fPSP). The fIJPs or iPSPs last round ~1 second or 30-fold longer than the fPSP. 6) The close junctional transmission may be seen along with the synaptic potentials in neurons and in the absence of synaptic potentials at the smooth muscle neuromuscular junctions.

The wide junctional neurotransmission is characterized by: 1) wide junctional space separating the prejunctional release site and the postjunctional receptors, ‘open’ to the extravascular space. 2) Metabotropic receptors with slow kinetics that are widely distributed on the post junctional membrane. 3) The prejunctional release sites resemble peptide release sites seen in neuroendocrine cells and non-vesicular release sites such as nitric oxide release sites. 4) The neurotransmitters include all types of transmitters including neuropeptides and nonvesicular neurotransmitters such as nitric oxide. 5) Functional markers of the wide junctional transmission are sJPs or sPSPs (in neurons) that may last 10s of seconds. 6) In neurons, the sJPs are often seen with the fPSPs, whereas at smooth muscle neuromuscular junctions, sJPs are observed in association with the fJPs. Slow junctional transmission is very widespread among almost all tissues. Salient differences between neurotransmission at a synapse, close junction and wide junctions are summarized in table 2.

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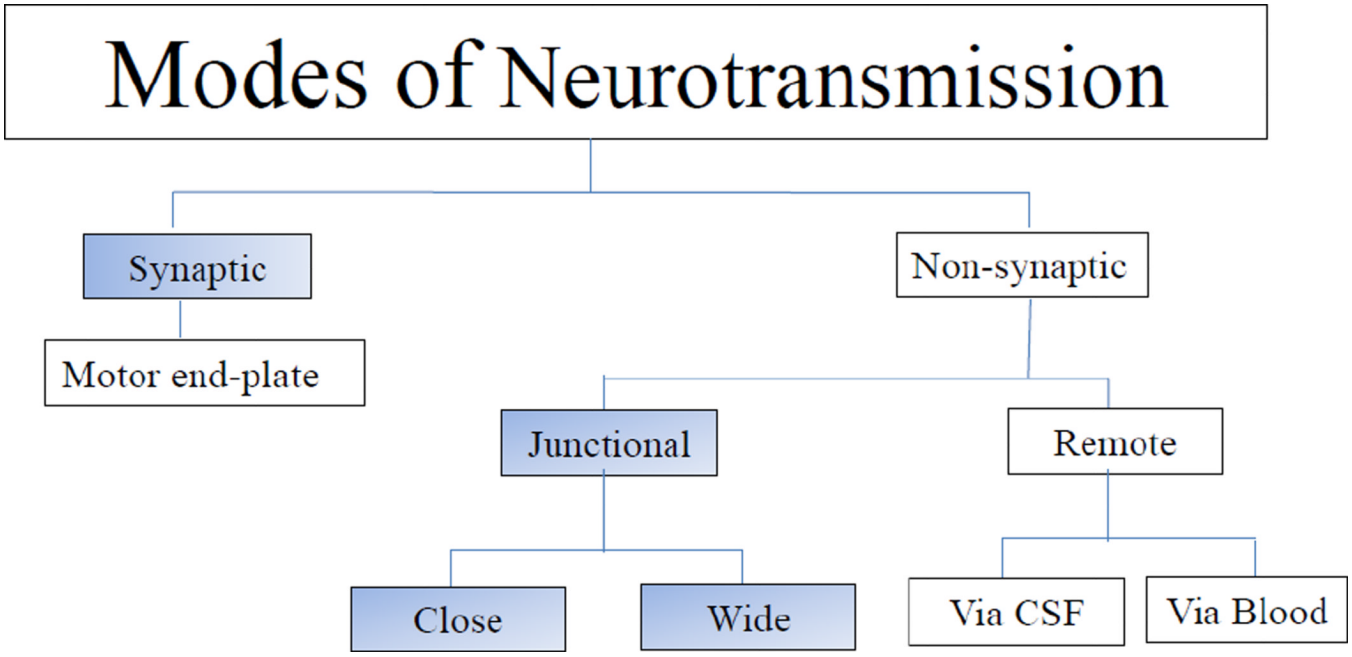

Figure 1.
Flow diagram of different modes of neurotransmission.

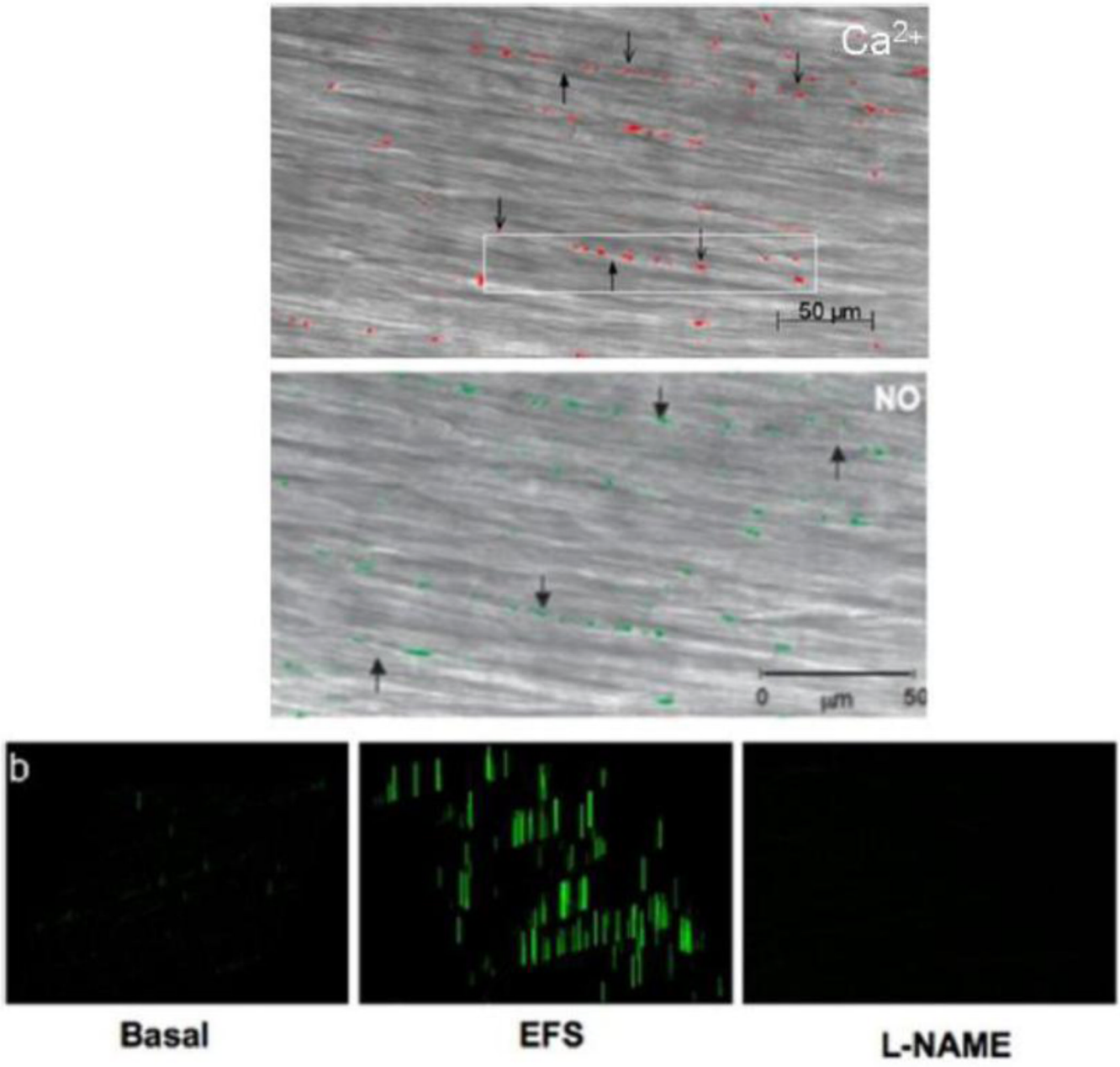

Figure 2.

Nitrergic varicosities in circular smooth muscle strip of the mouse gastric fundus. Top panel shows calcium fluorescence in the varicosities. Note that the nerve varicosities are visible as beaded structures lying along the bed of smooth muscle cells (thin arrows). The inter-varicosity axons are also clearly visible, giving a beads-on-string appearance (thick arrows). Middle panel shows DAF fluorescence indicating nitric oxide production. Note that the nerve varicosities (green) are appear as beaded structures, similar to those seen by calcium fluorescence. Bottom panel shows profiling of DAF signals. Note small signals in the basal state, intense signals after EFS and loss of signals after pretreatment with L-NAME, indicating their nitrergic nature. The fluorescence images were obtained using multiphoton

microscope in muscle strips preloaded with calcium orange and DAF-2DA. EFS was applied under nonadrenergic noncholinergic conditions (From (Thatte et al., 2009), by permission).

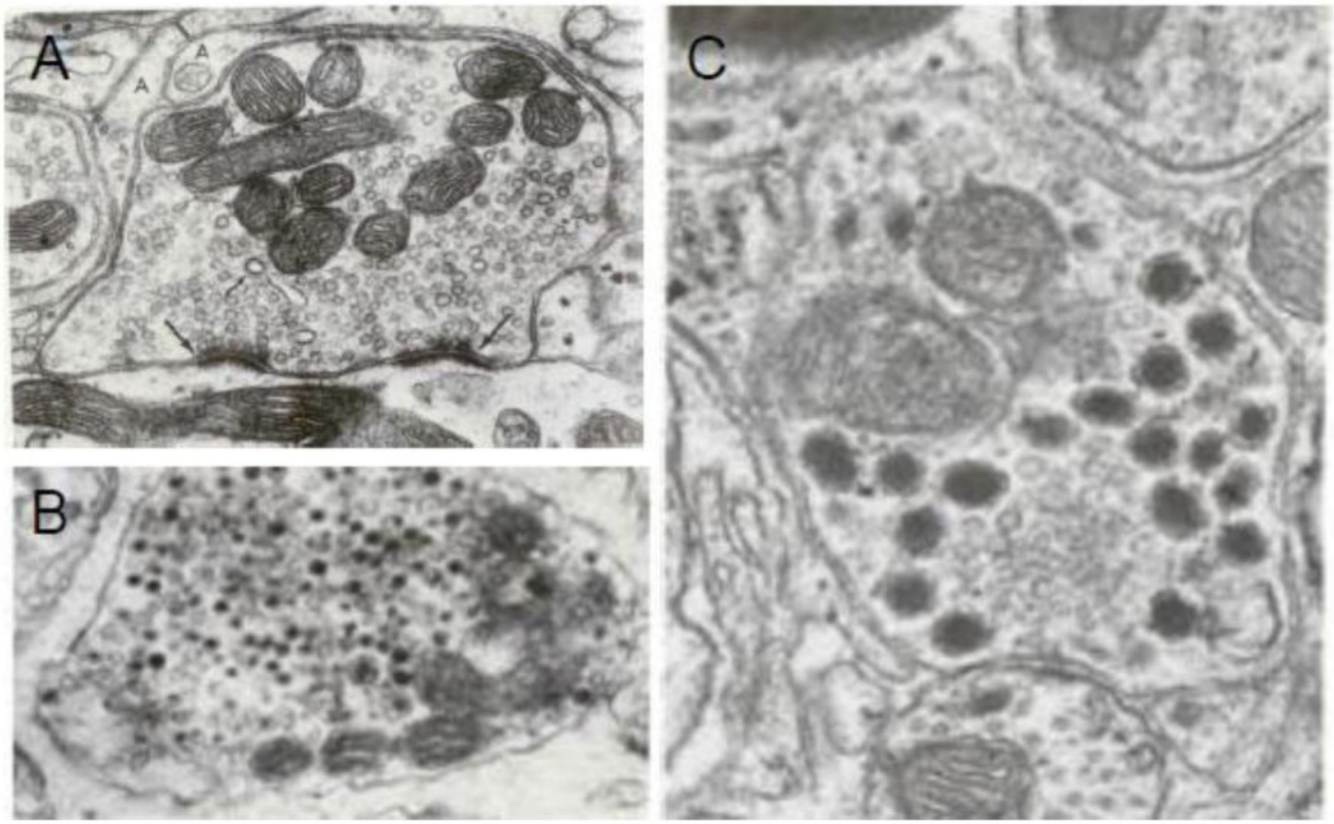

Figure 3.

Ultrastructure of nerve varicosities containing different types of vesicles and their relationship to synaptic specialization in the CNS neurons. (**A**) Shows a varicosity loaded with SCV many of which are in close association with the varicosity membrane at presynaptic membrane specialization (large arrow). The apposing postsynaptic membrane possesses postsynaptic density. This synapse is on a dendrite in superior olive, X 60.000 (From (Heuser JE, 1977), with permission). The specialized active zone is for active synaptic exocytosis. (**B**) Shows a varicosity with DCV of various sizes. Specialized presynaptic zone with docked vesicles are not found in such varicosities. This illustration represents an adrenergic varicosity in rat vas deferens, X 110,000; (From (Basbaum, 1974), with permission). (**C**) shows a varicosity containing SCV and LDCV. Note that the synaptic junction is characterized by some widening of cleft and pre- and post-synaptic plaques. Also note an interesting distribution of the vesicles in the varicosity: whereas SCV are clustered around the presynaptic specialization, the LDCV are seen away from the synapse, X77000; From dentate nucleus of cerebellum of *Macaca mullata* (From (Palay S.L., 1977), with permission).

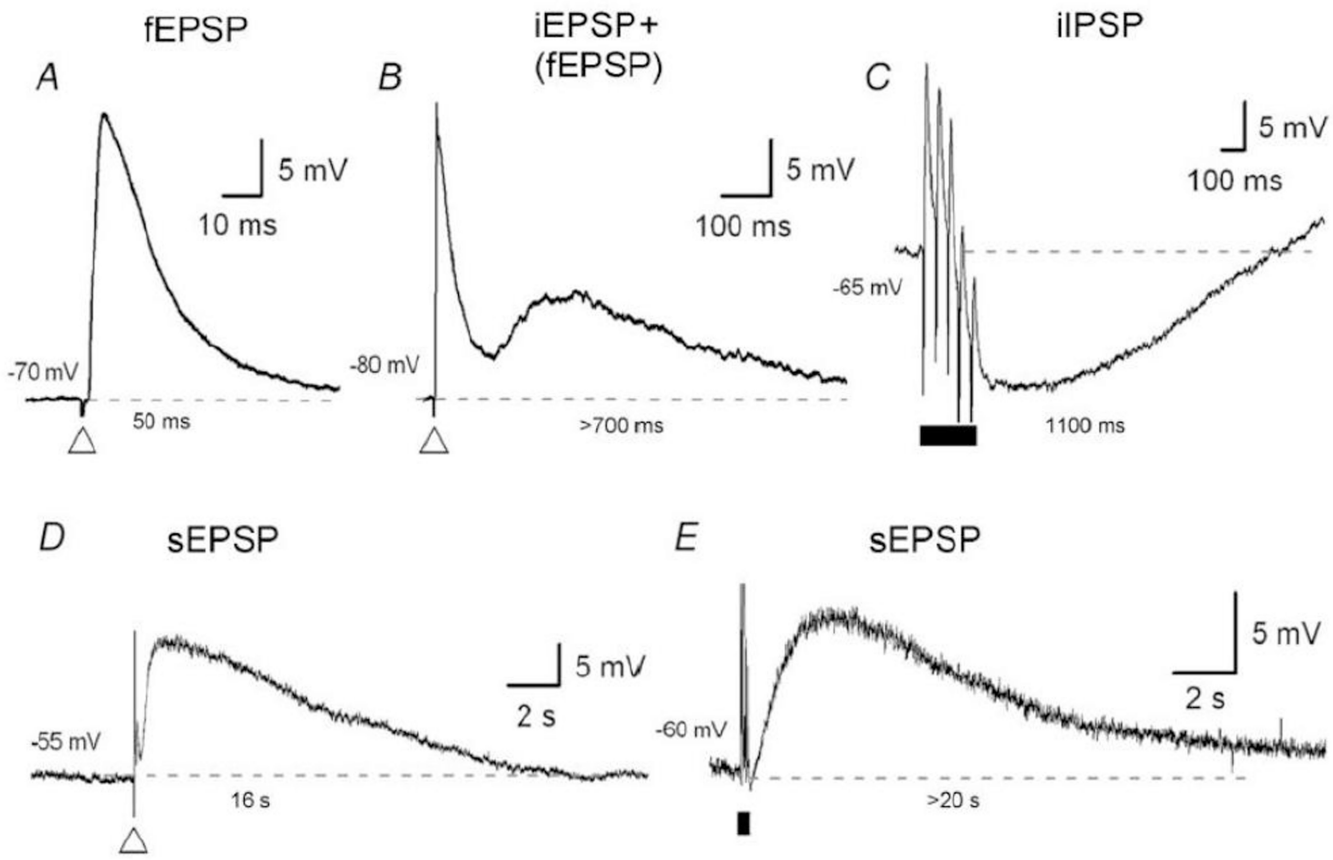

Figure 4.

Three time courses of postsynaptic potentials in enteric neurons. (**A**) Shows fast excitatory postsynaptic potential (fEPSP) with duration of ~50ms. (**B**) Shows intermediate postsynaptic potentials (IPSP) having duration of ~600 ms; **B1**) Shows intermediate excitatory postsynaptic potential (iEPSP) and **B2**) Shows inhibitory postsynaptic potential (iIPSP). The iIPSP shown here was elicited by a train of stimuli; however, a single pulse also produces a similar IPSP. (**C**) Shows slow post synaptic potential (sPSP) with a duration of >12,000 ms. **C1**) shows sEPSP elicited by a single pulse and **C2**) shows a sEPSP elicited by a train of stimuli. Note long duration of this sEPSP. Thus durations of intermediate potentials are 20-times longer and that of sEPSPs is >400-times longer than that of the fEPSP. The time course of iIPSP and sIPSP are similar to those of fastJP and slowJP respectively (From (Monro et al., 2004), with permission).

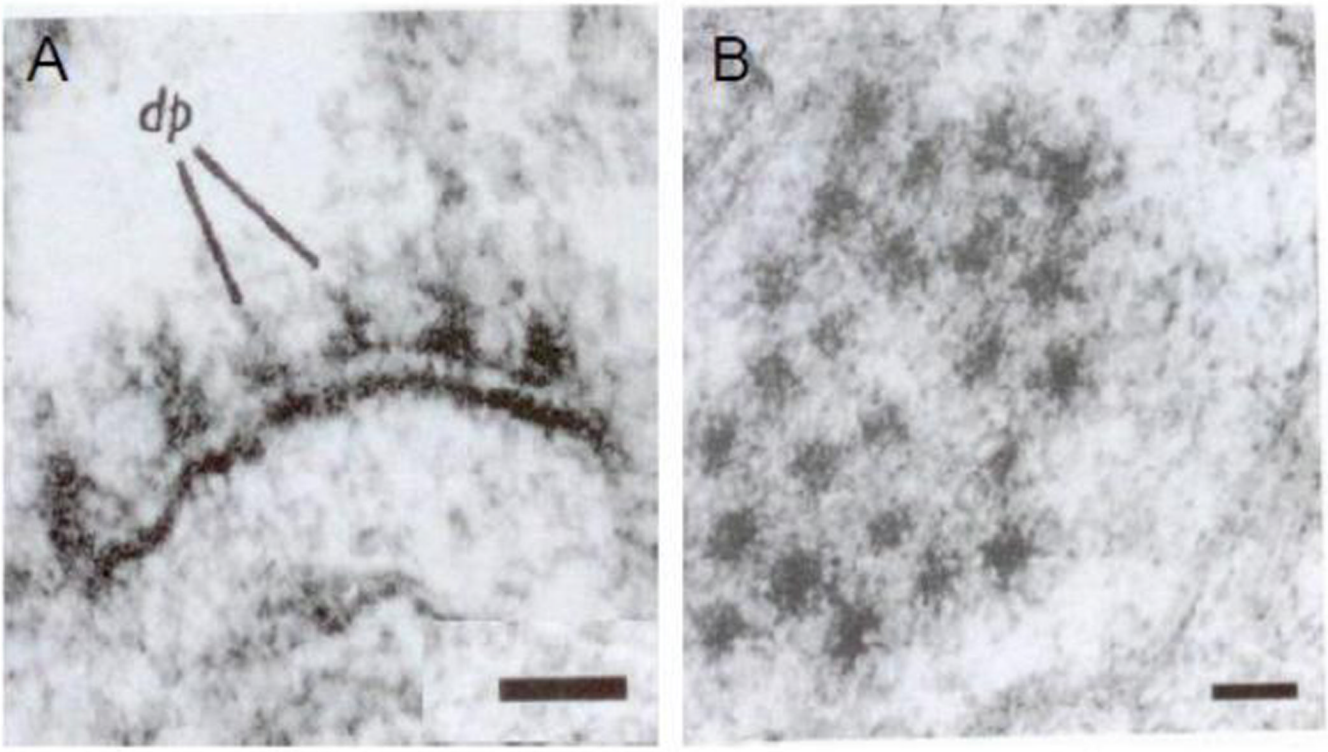

Figure 5.

The presynaptic active zone (**A**) and (**B**) are electron micrographs of cat spinal cord synapse cut perpendicularly (A) and tangentially (B), respectively. Note the regularly arranged dense projections in the presynaptic active zone. The electron density of the grid was enhanced by the use of 1% phosphotungstic acid. Vesicle membranes were not preserved by the fixative; Scale bar 100nm (From (Gray, 1963), with permission).

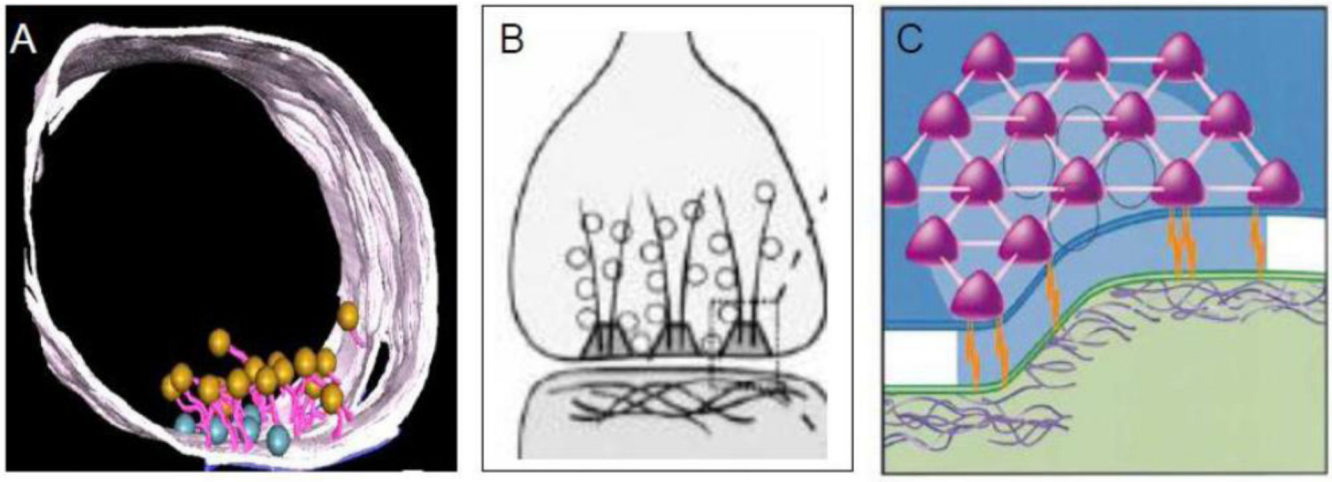

Figure 6.

The presynaptic active zone and the synaptic grid (**A**) is a 3D reconstruction of transverse section through a varicosity Note that active zone with docked vesicles at the varicosity membrane (blue) forms a small localized zone of the varicosity membrane (From (Siksou et al., 2007), with permission). (**B**) Represents a model of the presynaptic grid. Fibrillar components originating at the presynaptic membrane project into the cytoplasm. The presynaptic particles with spaces in between are present next to the membrane. (**C**) Three-dimensional model of the presynaptic grid formed by the particles. The particles form a hexagonal array with sieve like formation. The particle grid supports selective access of small vesicles to the plasma membrane for their subsequent fusion The particles are linked to PSD of the postsynaptic cell across the synaptic cleft by adhesion molecules. Presynaptic particles also contain components necessary for the retrieval of vesicle membrane proteins after their fusion with the plasma membrane. (From (Phillips et al., 2001), with permission).

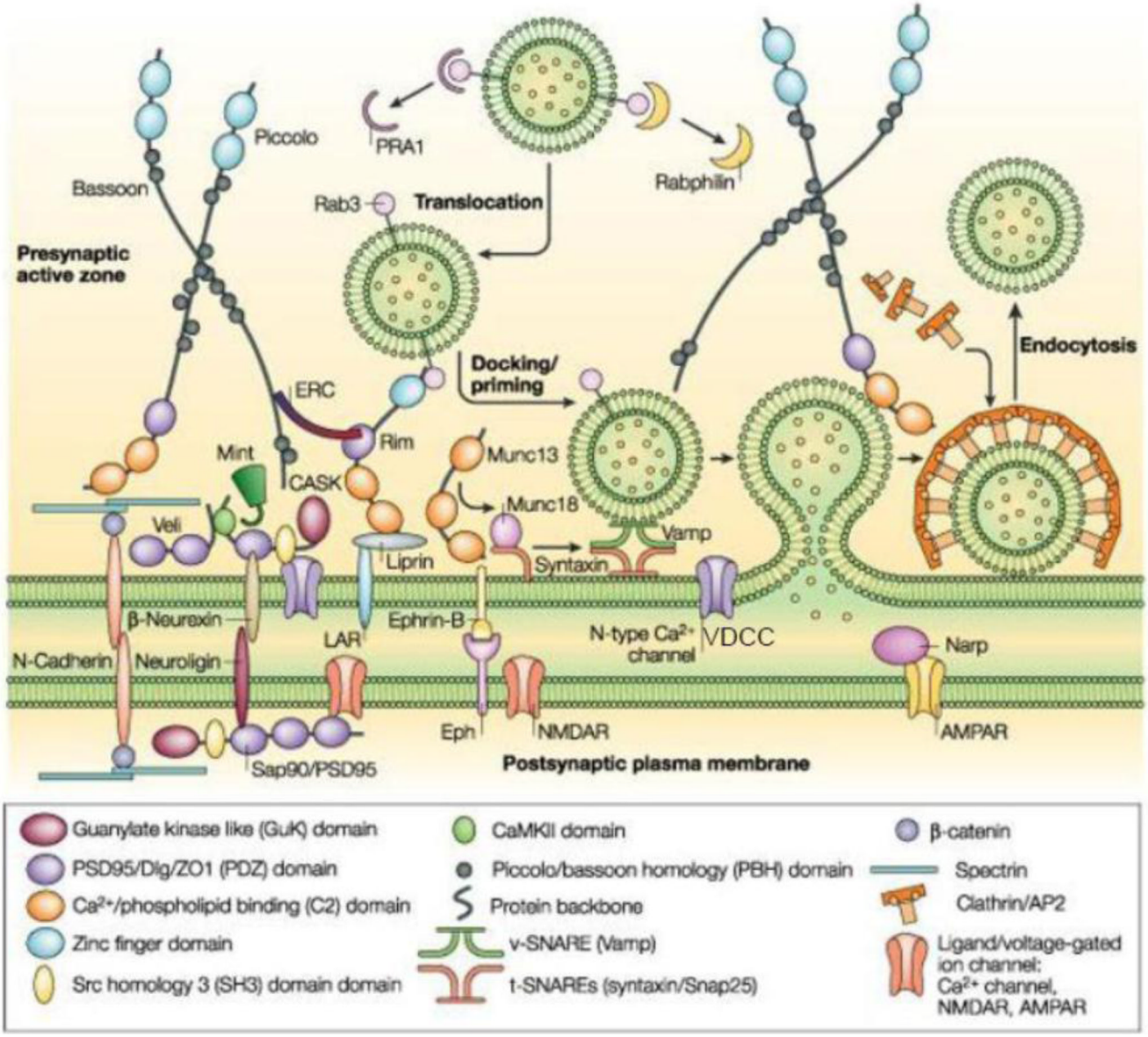

Figure 7.

Molecular anatomy of the presynaptic active zone. Three distinct complexes help to define the active zone (described in text). The first complex is largely structural, and is thought to hold the active zone in register with the postsynaptic density (PSD) and clusters calcium channels within the active plasma membrane. The second complex is involved in synaptic vesicle docking and fusion. The third complex is involved in synaptic vesicle endocytosis. (From (Qui, 2004), with permission).

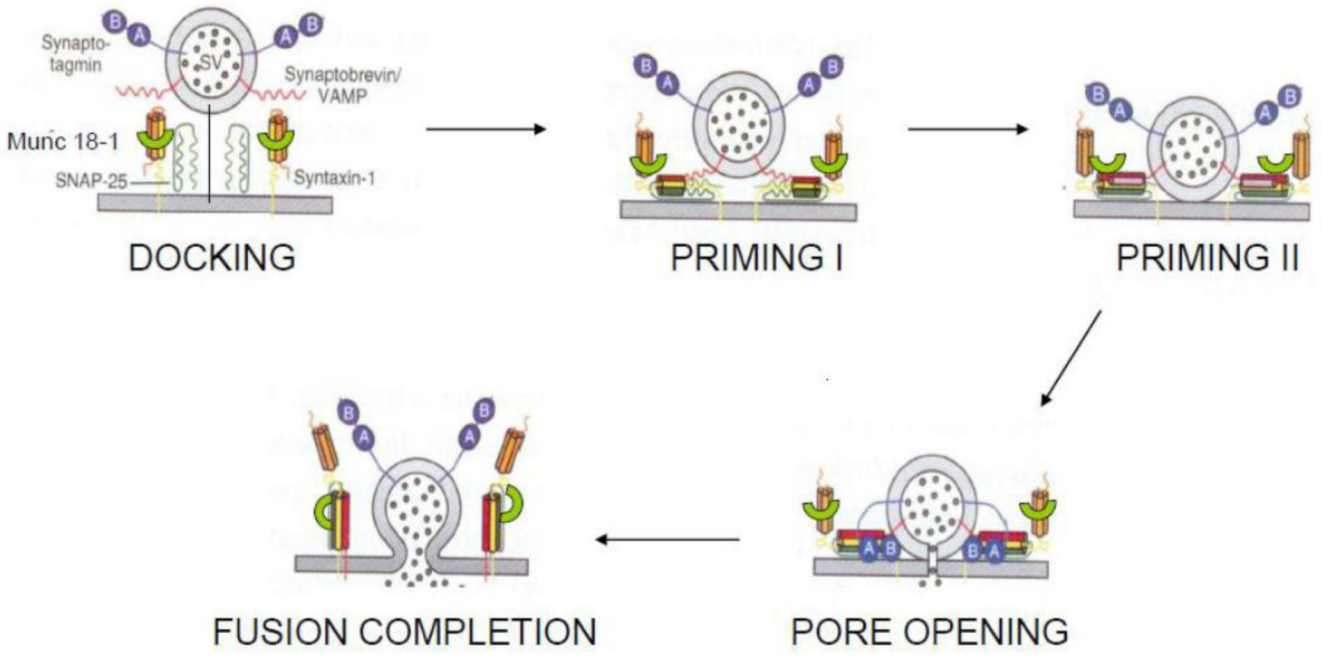

Figure 8.

Schematic diagram of key steps involved in exocytosis of vesicles at the active zone. For details, refer text. (Modified from (Tang et al., 2006) and (Sudhof, 2008b), with permission).

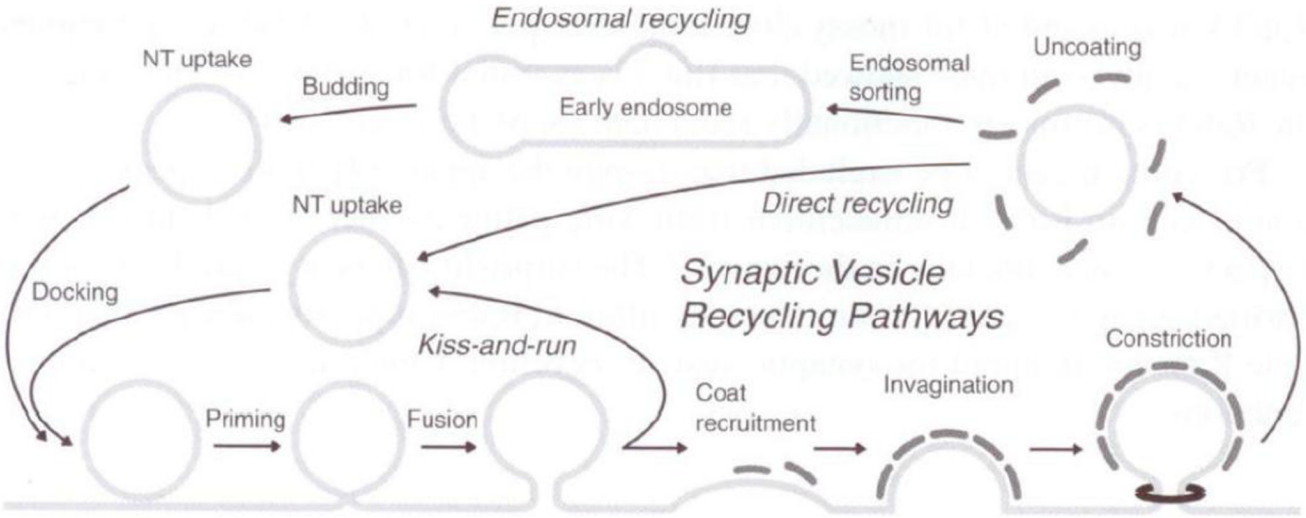

Figure 9.
Recycling of the synaptic vesicle. For details, refer text (From (Lang et al., 2008), with permission).

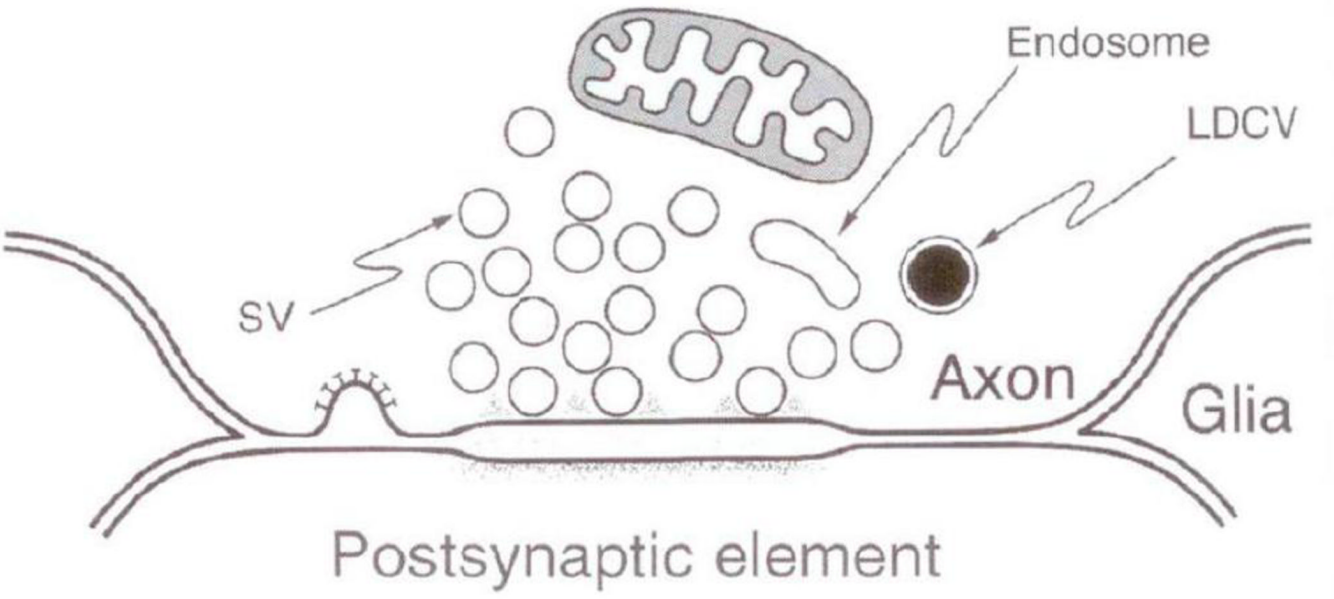

Figure 10.
Schematic illustration of synaptic cleft Synaptic cleft separates the presynaptic active zone with SCV docked on the varicosity membrane and synaptic grid and the postsynaptic density. Note that the synaptic cleft may be wider (20–30nm) than the adjoining nonsynaptic, junctional space (10–20nm). However, width of the junctional space is highly variable (From De Camilli P, 2003, with permission).

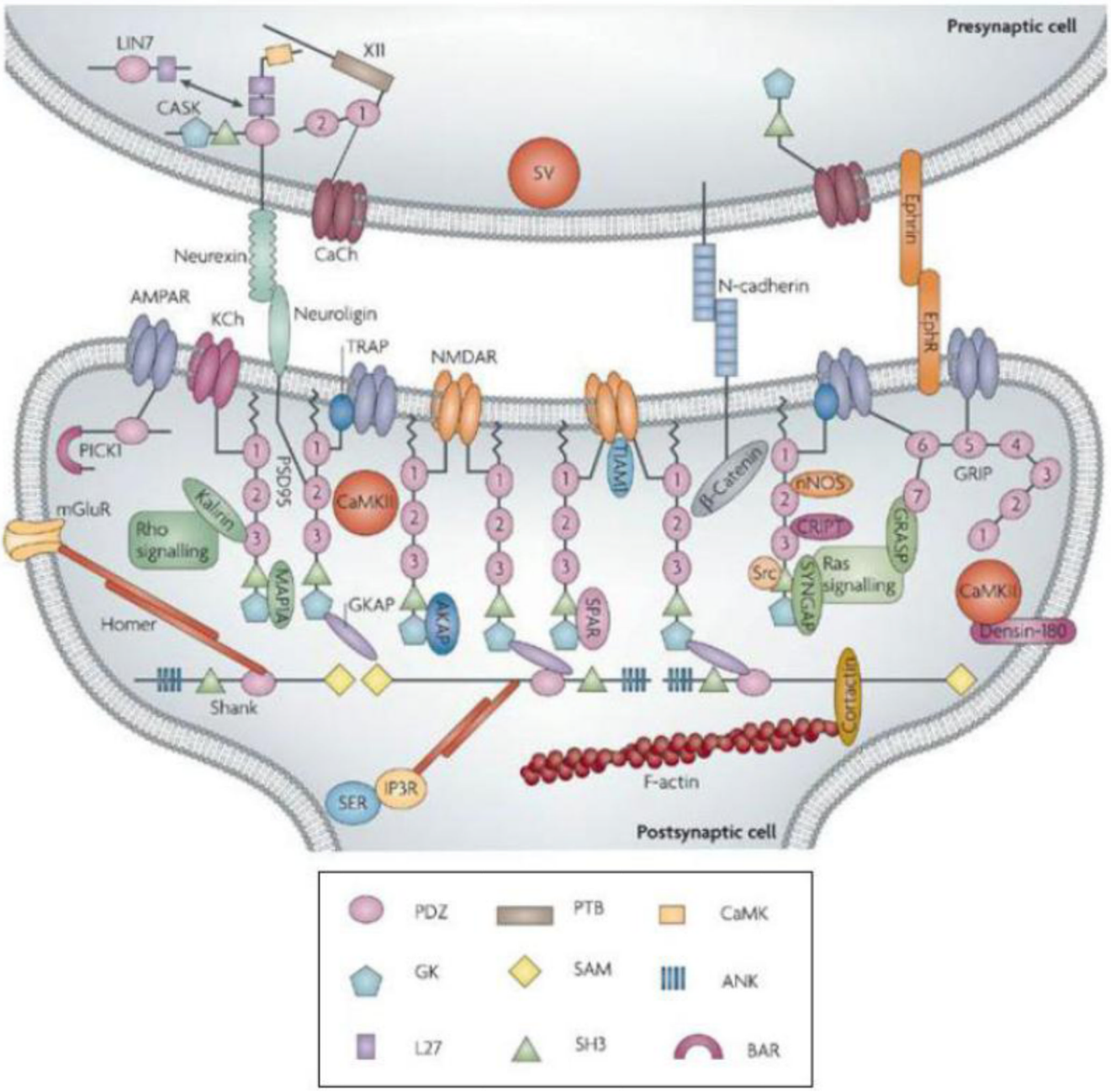

Figure 11.
Molecular anatomy of the postsynaptic density For details, refer text (From Feng et al., 2009, with permission).

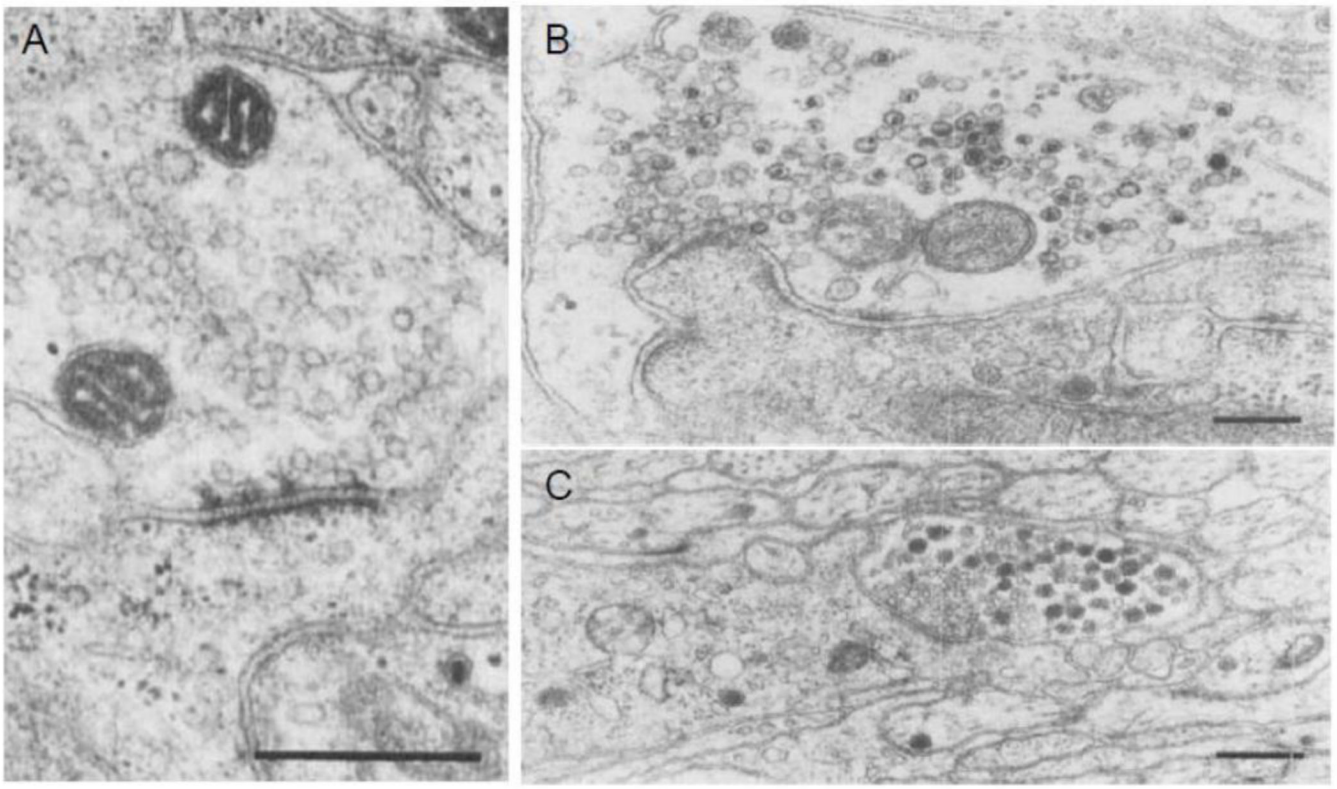

Figure 12.

Distribution of vesicles around synaptic specialization in enteric varicosities. (**A**) Shows a varicosity containing SCV and presynaptic active zone with prominent cytoplasmic projections. Also note the associated postsynaptic density on a dendrite. (From myenteric plexus of the guinea pig ileum) Marker: 0.5 μ*m*. (**B**) Shows a varicosity containing SGV contacting with an intramural neuron. Note the absence of well-defined synapse. Marker 0.2 μ*m* (From myenteric plexus of the guinea pig ileum). (**C**) Shows a nerve varicosity with different types of synaptic vesicles. Note that SCV are clustered around an area of active zone and the SCV seem to be interposed between the DCV and the active zone. Marker: 0.5 μ*m* (From submucosal enteric plexus of the guinea pig ileum). Note that the distribution of the vesicles in enteric varicosities is similar to that in the CNS varicosities (From (Gabella, 1979), with permission).

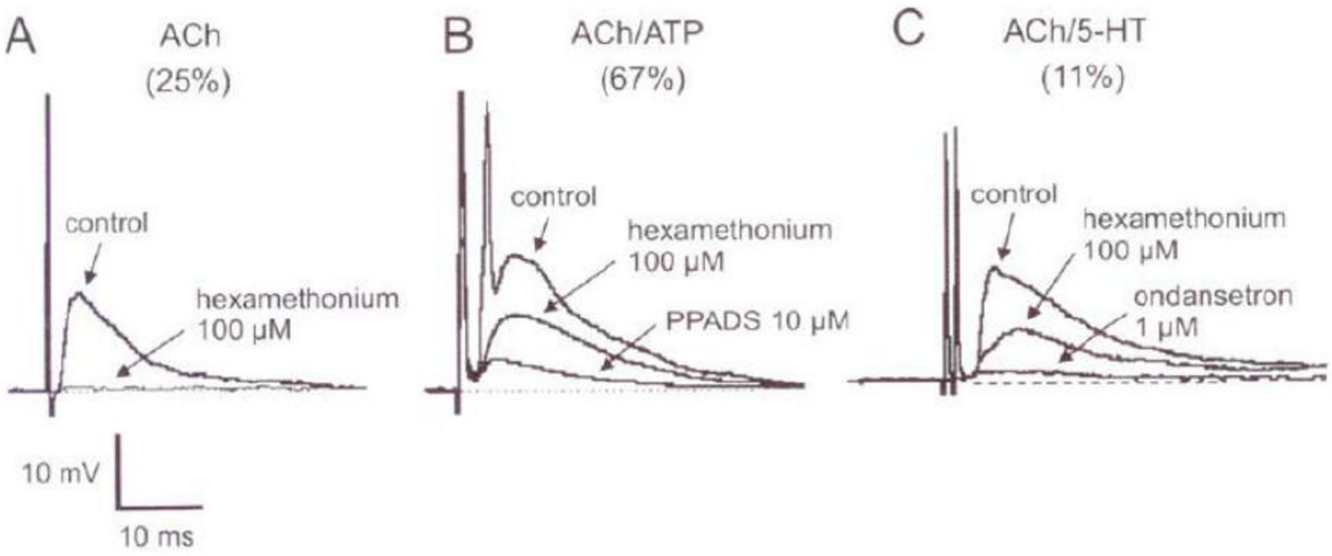

Figure 13.

Pharmacological identification of chemical nature of the fast EPSP in S neurons the myenteric plexus of guinea pig ileum. (**A**) An exclusive cholinergic fEPSP that is blocked by hexamethonium. (**B**) A mixed cholinergic and purinergic fEPSP that is blocked only be a combination of hexamethonium and ionotropic P2X receptor antagonist, PPADS (**C**) A mixed cholinergic and serotonergic fEPSP that is only blocked by a combination of hexamethonium and ondansetron. The percentages of synaptic responses of each type are indicated. (From Galligan, 2002a, with permission).

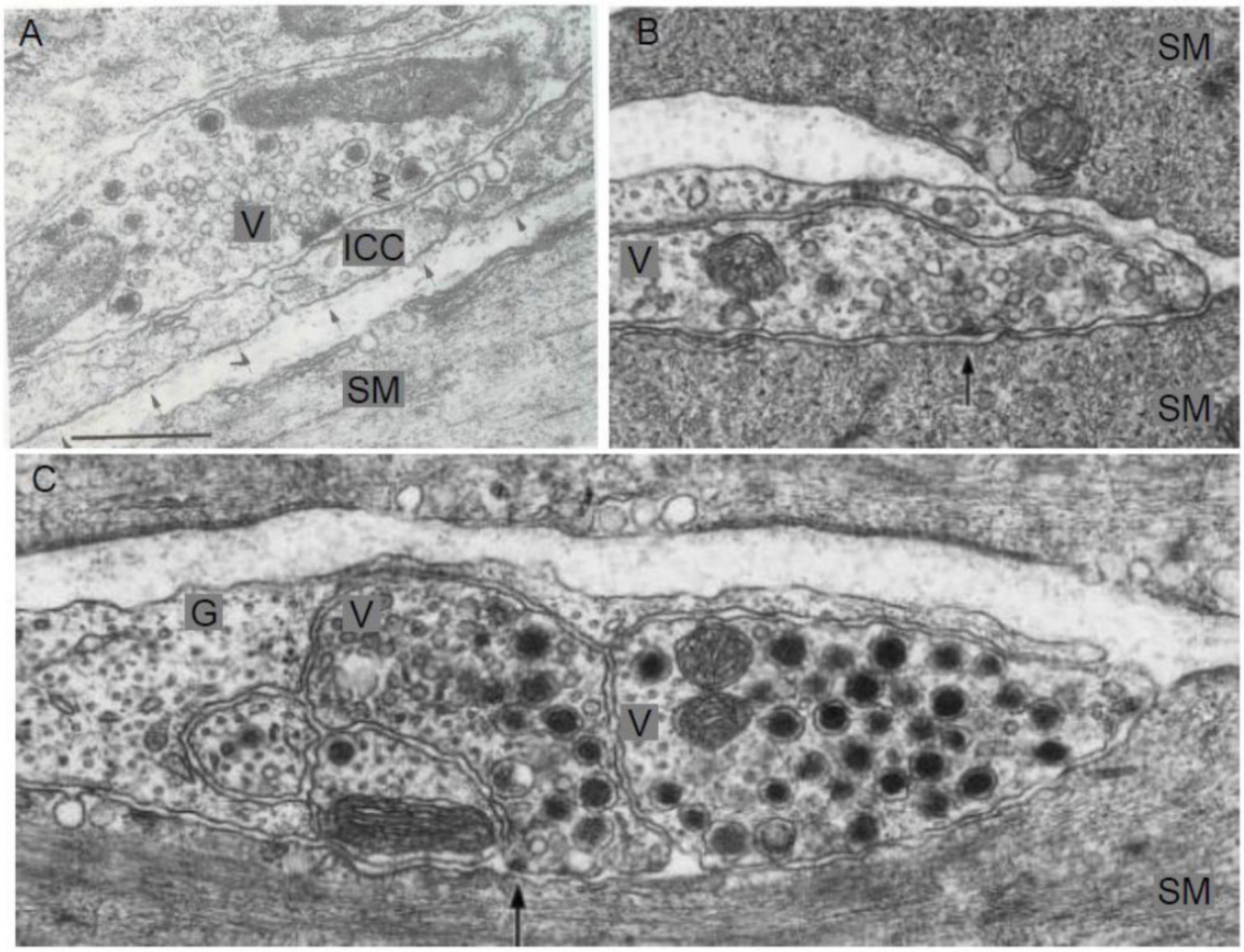

Figure 14.

Close contacts between nerve varicosities and ICC and smooth muscle cell in the gut. (**A**) Shows a varicosity containing SCV and some LDCV. This varicosity makes a close ~20nm contact with a thin process of ICC and end of a smooth muscle cell. Also note membrane densities on the presynaptic membrane but no postsynaptic specialization. The section in a single plane section gives the illusion that the thin processes of ICC form a barrier between the varicosity and the smooth muscle cell that is seen several hundred nm away (scale, 0.5μ) (**B**) Shows 10–20nm close contact between a circular muscle and a varicosity filled with SCV The presynaptic electron-dense lining is indicated by an arrow, X41,000. (**C**) Shows close contact 10–20nm between a longitudinal muscle cell and two axon terminals: one containing a mixture of SCV and LDCV and the other containing only LDCV. Electron dense lining is marked by an arrow, X28,000 (A is from Rumessen et al., 1982, with permission; B and C are from Mitsui et al., 2002, with permission).

Table 1

Vesicle types and main neurotransmitters they contain

Vesicle type	Size	Neurotransmitter
Small clear vesicle (SCV)	30–60 nm	Classical: ACh, GABA, Glutamate, ATP
Pleomorphic clear vesicle (PCV) or Small dense core vesicle (SDCV)	50–90 nm	Amines: NE, Dopamine, 5HT, Histamine, ATP
Large dense core vesicle (LDCV)	90–130 nm	Peptides: CCK, CGRP, GRP, DYN, ENK, GAL, PACAP, PYY, NPY, SOM, TK,VIP Also: NE, Dopamine, 5-HT, Histamine, ATP
None		nNOS (NO)

Table 2

Summary of key structure-activity differences in neurotransmission at synapse nonsynaptic, close and wide junctions

	SYNAPSE	JUNCTION	
		CLOSE	WIDE
STRUCTURE			
Nature of the contact	~1 μm^2 closed cavity	Open to extracellular space	Open to extracellular space
Separation of release sites and their target receptors	20 nm –30 nm	10 nm –20 nm	>100nm- >2000 nm
Morphology	Pre- and postsynaptic specialization	Subtle pre- and post-junctional specialization	Some pre- and postjunctional specialization
Transmitter release site	Actin grid & Membrane docked SCV (Active zone)	No actin grid or membrane docked SCV	No actin grid or membrane docked SCV
Vesicles	SCV	SCV, SDCV (pleomorphic)	DCV, nonvesicular, other
Transmitters released	Classical transmitters (amino acids, ACh, ATP)	Classical transmitters and 5HT	Neuropeptides, nonvesicular and others
Postsynaptic/ postjunctional receptor clustering	Dense clustering in the synapse receptors	Localized areas of clustering	Widespread distribution
Typical receptor type	Ionotropic, for classical transmitters	Metabotropic for classical transmitters	Metabotropic for neuropeptides and all other transmitters
ACTIVITY			
Potentials (synaptic varicosities)	fPSP (fEPSP, fIPSP)	iPSP (iEPSP, iIPSP)	sEPSP (sEPSP, sIPSP)
Potentials (nonsynaptic varicosities)	none	fJP (fEJP, fIJP)	sJP (sEJP, sIJP)
Duration of response	10 ms– 30 ms	250ms-2.5s	several second - minutes
EFFECTOR CELL TYPE			
Usual effector cell types	Neurons	Neurons, smooth muscles	Neurons, smooth muscles, secretory cells