Primer

Neurotransmitters

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The nervous system processes sensory information and controls behavior by performing an enormous number of computations. These computations occur both within cells and between cells, but it is intercellular information processing, involving complex neural networks, that provides the nervous system with its remarkable functional capacity. The principal cells involved in information processing are neurons, of which there are hundreds, if not thousands of individual cell types based on morphology, location, connectivity and chemistry [1]. In addition to neurons, the other major kind of cell in the nervous system is the glia, which play critical support roles, but which are increasingly seen to function in some aspects of information processing.

To provide some idea of the magnitude of the information processing capacity of the human brain, its 10^{11} neurons make, on average, about 1000 connections or synapses, at which communication occurs with other neurons. The range of synapses per cell is very large; the Purkinje cells of the cerebellum may receive 100,000 contacts from input cells. Overall the human brain may contain between 10^{14} and 10^{15} synaptic connections.

The diverse chemical substances that carry information between neurons are called neurotransmitters. Otto Loewi discovered the first neurotransmitter in 1926 when he demonstrated that acetylcholine carried a chemical signal from the vagus nerve to the heart that slowed the cardiac rhythm. Since that time, more than one hundred substances and a far larger number of receptors have been implicated in synaptic transmission (Box 1). Because of the remarkably diverse effects of neurotransmitter-mediated signaling at the receptor and post-receptor levels, the number of neurotransmitters, as large as it is, vastly understates the complexity of signaling in the brain.

In the nervous systems of higher animals, only a small fraction of neurons are directly involved in transducing sensory information or controlling output cells, such as endocrine, smooth muscle or striated muscle cells. The vast majority form what Nauta [2] called the great intermediate net, which underlies the extraordinary computational power of the brain. The complex set of neuronal networks interposed between input and output neurons form the basis, *inter alia*, for learning complex motor sequences, for thought, emotion, for 'top down' behavioral control and, in humans, for such functions as language, writing poetry and planning wars.

In addition to performing present-oriented computations, the nervous system is plastic; it alters itself (forms memories) as it processes information, so that it can respond more adaptively in the future. The subtlety and complexity of the brain's outputs, along with its ability to change in response to new information, is supported by a rich set of mechanisms for cell–cell communication involving at an anatomical level, intricate but plastic local connections, larger scale neural circuits and overlying global regulatory systems; and at the chemical level, a large number of neurotransmitters with highly diverse mechanisms for decoding their informational content.

Because neurotransmitters play such a central role in brain function, neurotransmitter receptors and other proteins involved in neurotransmitter synthesis and inactivation are critical targets for the development of therapeutic drugs meant to treat psychiatric and neurologic disorders, pain, and a host of other ills [3]. Moreover, natural substances, such as cocaine, opiates, nicotine, ethyl alcohol and LSD, that can mimic or interfere with actions of neurotransmitters, exert potent effects on human behavior [3].

Box 1. Partial list of neurotransmitters organized by chemical properties.

Monoamines and acetylcholine
Acetylcholine
Dopamine
Norepinephrine
Epinephrine
Serotonin
Histamine

L-Amino acids
Glutamate
Aspartate
Gamma aminobutyric acid (GABA)
Glycine

D-Amino acid
D-Serine

Purines
Adenosine
Adenosine triphosphate (ATP)

Gases
Nitric oxide (NO)
Carbon monoxide (CO)

Lipids
Anadamide (endocannabinoid)
2-arachidonoylglycerol (2-AG) (endocannabinoid)

Peptides (very truncated list)
Enkephalins (endogenous opioid peptides)
Beta-Endorphin (endogenous opioid peptide)
Dynorphins (endogenous opioid peptides)
Substance P
Neuropeptide Y (NPY)
Peptide YY (PYY)
Orexin (also known as hypocretin)
Vasopressin
Oxytocin
Corticotrophin releasing hormone (CRH)
Somatostatin
Neurotensin
Bombesin
Galanin
Vasoactive intestinal polypeptide (VIP)
Bradykinin

Neurons are specialized to receive, process, and transmit information (Figure 1). As a first approximation, information is represented electrically within neurons and chemically (by neurotransmitters) between neurons. Once released, neurotransmitters diffuse across the synapse to bind to postsynaptic receptors. It should

Table 1. Properties of exemplary neurotransmitters.

Chemical class	Example	Cell of origin	Storage	Release and other mechanisms of initiating signaling	Inactivation	Receptors
Acetylcholine	Acetylcholine	Neurons	Vesicles	Depolarization	Enzymatic (acetylcholinesterase)	Ionotropic (nicotinic) and GPCR (muscarinic)
Amino acids	Glutamate	Neurons	Vesicles	Depolarization	Transporters	Ionotropic and GPCR
Monoamines	Dopamine	Neurons	Vesicles	Depolarization	Transporter	GPCRs
Peptides	Enkephalin	Neurons	Vesicles, often separate from small molecule neurotransmitters	Repetitive depolarization	Enzymatic (for many peptides relevant enzymes or other mechanisms are unknown)	GPCRs μ, δ, opiate receptors
D-Amino acids	D-Serine	Astrocytes (glia)	Unknown	Activation of serine racemase followed by release of resulting D-Serine by unknown mechanisms	Transporter	Modulatory (glycine) site on NMDA glutamate receptors
Purines	Adenosine	Neurons and probably others	ATP is stored in vesicles and may be rapidly hydrolyzed to adenosine after release; high basal extra-cellular levels represent unstored adenosine	(1) Liberation from diverse extracellular purines by ectoenzymes (2) Facilitated diffusion out of cells	Transporter	GPCRs
Gases	NO	Neurons	None	Ca^{2+}-dependent regulation of nitric oxide synthase (nNos) to produce NO for immediate diffusion	Diffusion. Perhaps enzymatic degradation	Many enzymes containing transition metals; such as soluble guanylyl cyclase
Lipids	Anadamide	Neurons	Unknown if stored	Probably released into synapse but diffusion mechanism in aqueous medium unclear	Transporter followed by hydrolysis	GPCRs (cannabinoid)

(1) These examples are not fully extendible to the class. For example the purine adenosine triphosphate (ATP), which despite its ubiquity as an energy carrying molecule can also carry information [10], is colocalized in vesicles with other neurotransmitters, such as, acetylcholine or dopamine, and is released by depolarization; while the purine adenosine is released by facilitated diffusion. ATP interacts with both ionotropic receptors (P2X receptors) and G-coupled receptors (P2Y receptors), while adenosine only interacts with G-coupled receptors. (2) As alluded to in the text, glia may, in some cases, be directly involved in neurotransmitter action: for example by inactivation of neurotransmitter, such as glutamate, or by release, for example of ATP or D-Serine [11].

be noted that, in addition to chemical synapses, which represent the vast majority, there are also electrical synapses, which permit the flow of ions between cells through gap junctions. Electrical synapses, which will not be further discussed here, permit simple electrical signals to pass between neurons, whereas chemical synapses, as will be seen, permit excitatory, inhibitory and complex biochemical information to pass between cells. There are, in fact, a wide variety of molecular structures that have been found to act as neurotransmitter receptors, but the most numerous are ligand-gated channels and G protein-coupled receptors (GPCRs; Figure 2 and Table 1).

The major structural elements of a chemical synapse are a presynaptic nerve terminal, a postsynaptic structure that contains neurotransmitter receptors and an appropriate intracellular signaling apparatus, and the synaptic cleft in between (Figure 3). At most chemical synapses, neurotransmitter is stored within synaptic vesicles, several thousand molecules per vesicle. Gaseous neurotransmitters, such as NO and CO, cannot be stored (Table 1). Synaptic vesicles are clustered at specialized regions of membrane within the presynaptic terminal called active zones, which also contain a high density of voltage-gated Ca^{2+} channels.

In a classical case of chemical transmission, a neurotransmitter is released by a presynaptic neuron and binds to receptors on the postsynaptic neuron; typically

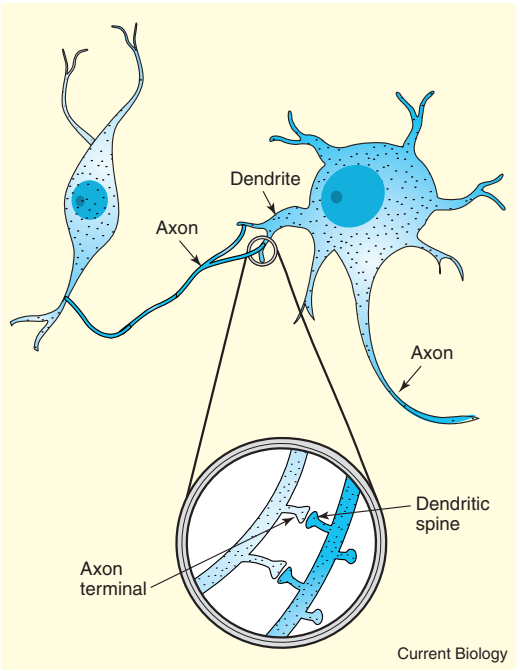

Figure 1. Chemical neurotransmission occurs across synapses.

Neurons are asymmetric cells with dendrites acting as the major, but not the only, receptive region of the cell. Neurotransmitter receptors are found not only on dendrites, but also on cell bodies and presynaptic terminals. Axons are the information transmitting structures that release neurotransmitters from their terminals. Shown here is the classical arrangement for communication with the axon of the presynaptic cell contacting a dendritic spine (see inset), a feature of many neuronal cell types, but axons may also contact cell bodies and other axons. As described in the text, a chemical message is received by the post-synaptic neuron and is converted into an electrical signal within dendrites. The electrical information would then be integrated within dendrites and the cell body, and if it reaches an adequate threshold of depolarization, the electrical information is transmitted down the axon of the receiving neuron to its presynaptic terminal (not shown). In the presynaptic terminal, the cycle of synaptic transmission is renewed as the electrical signal of the action potential is converted into a new chemical signal. While the most common direction of communication is from presynaptic neuron to postsynaptic neuron in a circuit, as shown here, both autocrine-like feedback onto 'autoreceptors' and retrograde neurotransmission occur. In the latter cases, gaseous and lipid neurotransmitters such as endocannabinoids may play an important role [12]. (Adapted with permission from [3].)

these receptors are localized on dendrites — filamentous extensions from the neuron's cell body that are the primary receiving structures of the cell (Figure 1). Chemical information is converted by receptors and associated proteins into electrical information by the activation of ion channels. In the simplest case, such as nicotinic acetylcholine receptors or γ-aminobutyric acid ($GABA_A$) receptors, the channel is an intrinsic component of the neurotransmitter receptor itself (Figure 2).

At rest the neuronal membrane is polarized, bearing a negative charge. By regulating ion channels, neurotransmitter binding can activate ion fluxes across the membrane. Depending on which types of channel are activated, either 'hyperpolarizing' negative charges or 'depolarizing positive charges' may enter the cell. The balance of negative and positive charge is integrated within the dendrites and cell body of the neuron, and if a threshold of depolarization is reached, specialized voltage-gated Na^+ channels open in succession, generating a wave of depolarization down the axon, an 'action potential'.

When an action potential arrives at the distal end of the axon — the presynaptic terminals — the inrush of positive charge activates voltage-sensitive Ca^{2+} channels. Ca^{2+} entry then initiates processes by which vesicles fuse with the presynaptic cell membrane releasing neurotransmitter into the synaptic cleft. When neurotransmitters, such as acetylcholine or glutamate, activate cation (for example Na^+ or Ca^{2+}) channels, and are thus depolarizing, they can be described as excitatory; when neurotransmitters, such as GABA, activate anion (for example Cl^-) channels, they can be described as inhibitory.

As noted above, neurotransmitters often have more than one receptor. For many neurotransmitters, the receptors may be structurally and functionally quite distinct. Thus, in addition to having receptors that are ligand-gated channels, acetylcholine, glutamate and GABA also have receptors that are coupled to G proteins. Most of the receptors for monoamine neurotransmitters (Box 1) and all known receptors for neuropeptides are G protein-coupled receptors, sometimes described by the ungainly term, metabotropic receptors (meant to contrast them with ionotropic receptors). G protein-coupled receptors activate biochemical cascades involving G proteins, second messengers and protein kinases (Figures 2 and 3). The activation of a G protein-coupled receptor may lead to changes in membrane potential, but the onset is slower than that caused by a ligand-gated channel because in this case the channel is a separate molecule that must be activated by a G protein, second messenger or protein kinase, in all cases a multistep process.

In some cases, activation of a G protein-coupled receptor has no significant effect on the membrane potential, but by activating protein kinases that phosphorylate ion channels, for example, it may alter the subsequent responsiveness of the neuron to excitatory or inhibitory neurotransmission. By activating second messenger systems, protein kinases and gene expression, G protein-coupled receptors and those ligand-gated channels that permit entry of Ca^{2+} (in its own right a second messenger) can also initiate functional [4] and structural changes in neurons [5] that provide the substrate for long-term memory.

Not all released neurotransmitter molecules find a receptor to which to bind. In order to maintain the fidelity of synaptic transmission, the action of excess neurotransmitter in the synapse must be rapidly terminated to avoid diffusion of high concentrations of neurotransmitter to inappropriate

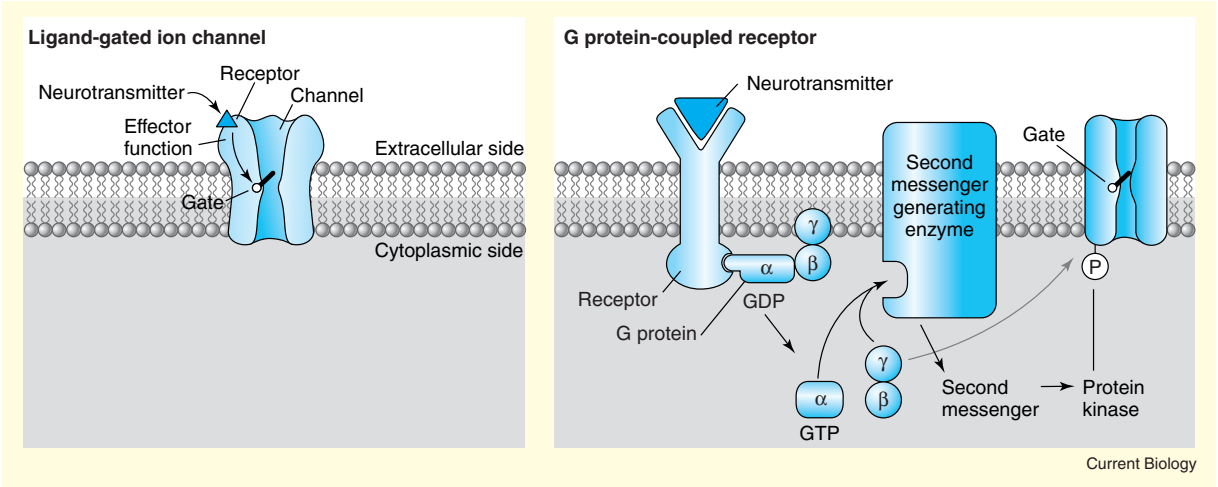

Figure 2. Neurotransmitter receptors.

As more substances are recognized to act as neurotransmitters, the diversity of molecules that serve as receptors also grows. For example, NO receptors are metal-containing enzymes. By far the most numerous neurotransmitter receptors are ligand-gated channels (left) and G protein-coupled receptors (right). Both have an extracellular ligand binding domain and a mechanism to convert extracellular ligand binding to a cellular signal. The ligand-gated channels do this by opening a central pore that permits ions to pass. The G-coupled receptors transmit a signal across the membrane to activate trimeric G proteins and their cognate signaling cascades, most often activating or inhibiting second messenger generating enzymes. (Adapted with permission from [3].)

synapses. The most common mechanism of neurotransmitter inactivation involves neurotransmitter-specific transporters, which may be located on the presynaptic terminal (as in the case, for example, of norepinephrine, serotonin and dopamine), or in some cases on neighboring glial cells (as with some glutamate transporters).

Less commonly, neurotransmitters are inactivated by enzymatic degradation, as is the case for acetylcholine, which is degraded by a highly active ectoenzyme acetylcholinesterase, located within cholinergic synapses (Table 1). Several peptide neurotransmitters have been shown to be degraded by enzymes but, unlike small molecule neurotransmitters, peptides may diffuse for substantial distances within the nervous system before finding targets or being degraded [6]. Neurotransmitters with a long diffusion radius are obviously not involved in the kind of precise point-to-point information transfer that might characterize the basic functions of the visual system, for example.

Historically, the problem of determining which molecules actually function as neurotransmitters has not been a trivial one. This problem is illustrated by the observation that both glutamate and ATP can serve as neurotransmitters. The vast preponderance of both molecules is in other cellular pools where they act, for example, as building blocks of proteins and nucleic acids, respectively, or in intermediary metabolism. Thus, there has long been a concern with setting experimental criteria for determining that a substance was a neurotransmitter.

There is no full agreement on the experimental criteria, but one representative set (adapted from [7]) would make the following stipulations: (1) the presynaptic neuron contains the appropriate synthetic enzymes to produce the substance, or in the case of neuropeptides, expresses the appropriate gene(s); (2) the substance is released upon stimulation of the presynaptic neuron; (3) when the substance is applied experimentally to the postsynaptic cell, it mimics normal synaptic transmission; (4) there is a local mechanism to terminate the action of the putative transmitter; (5) postsynaptic cells contain receptors for the putative neurotransmitter; (6) antagonists that block the effects of the substance when applied experimentally also block the effect of normal neurotransmission at relevant synapses.

In truth many substances broadly considered to be neurotransmitters have not fully met all of these criteria. For example, many peptides lack selective antagonists, and in some cases mechanisms of inactivation remain to be discovered. The difficulty of characterizing transmission at a particular set of synapses is also complicated by the recognition, dating from the 1970s, that neurons often release more than one neurotransmitter from the same presynaptic terminals, most often a small molecule together with one or more peptides [8]. The recognition that gases such as NO and CO can function as neurotransmitters means that not all neurotransmitters are stored in vesicles — these gases diffuse across membranes as soon as they are synthesized. Indeed, not all putative neurotransmitters are synthesized in neurons: D-Serine is synthesized in the protoplasmic astrocytes that surround synapses [9].

Attempts have also been made to find conceptual groupings of neurotransmitters. Perhaps the

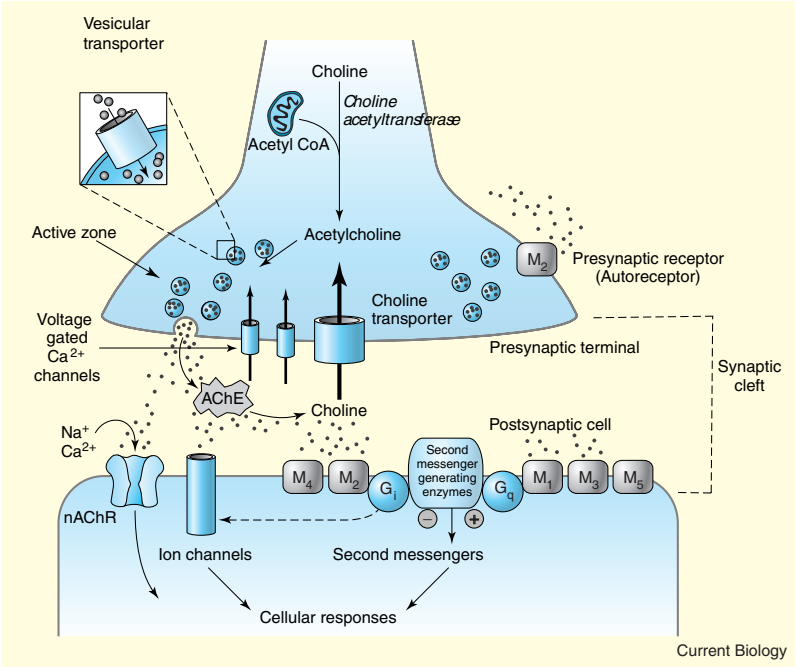

Figure 3. Key features of a cholinergic synapse.

The major features of a synapse are the presynaptic and postsynaptic terminals and the cleft. Within the presynaptic terminal, neurotransmitter is contained in vesicles typically docked along the plasma membrane in a region known as the active zone. When an action potential signal invades the presynaptic terminal, voltage-sensitive Ca^{2+} channels found at high density near the area of the active zone open, and the inrush of calcium initiates the complex process by which vesicles rapidly fuse with the membrane of the presynaptic terminal, dumping neurotransmitter into the synapse. Neurotransmitter diffuses across the synapse, where it finds appropriate neurotransmitter receptors. Acetylcholine has receptors that are ligand-gated channels, the nicotinic acetylcholine receptors (nAChR), and also muscarinic receptors, which are G-coupled (the five subtypes known are depicted as M1–M5). The presynaptic terminal also contains the biosynthetic machinery to produce the neurotransmitter, and in this case, a transporter for the precursor, choline. Neurotransmitter is loaded into vesicles via a vesicular transporter. The action of acetylcholine in the synaptic cleft is terminated by the ectoenzyme, acetylcholinesterase (AChE). (Adapted with permission from [3].)

least problematic groupings are those based on the chemical nature of the substances (Box 1 and Table 1 are organized in this fashion). Chemical groupings are far from perfect with respect to function, but proposed functional groupings have been hampered by the fact that neurotransmitters have multiple receptors and that it is the receptor rather than the neurotransmitter that actually determines function. The most broadly disseminated proposal was to reserve the term neurotransmitter for those substances that produced rapid changes in the membrane potential of the receiving neuron (via ligand-gated channels), and to apply the term neuromodulator to substances that initiated slower-onset biochemical changes in postsynaptic neurons (via G protein-coupled receptors, for example). The term neuromodulator was never really successful, however, because a large number of neurotransmitters induce both excitatory or inhibitory postsynaptic electrical potentials as well slow-onset biochemical changes. For example, glutamate is the most significant excitatory neurotransmitter in the brain, acting at several families of receptors that are ligand-gated channels, but it also initiates complex biochemical functions within neurons via its metabotropic receptors, which are G protein-coupled receptors. Serotonin (5 hydroxytryptamine or 5-HT) has thirteen known receptors, twelve of which are G-coupled receptors and one, the 5-HT3 receptor, that is a ligand-gated channel. In short, serotonin might be called a neuromodulator, except when it isn't... Unfortunately for those scientists with an intense need for simple classifications, evolution was a tinkerer that has reused signaling molecules to different effect in many different contexts.

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