Neurotransmitters, Drugs and Brain Function.
Edited by Roy Webster

ISBN: Hardback 0-471-97819-1 Paperback 0-471-98586-4 Electronic 0-470-84657-7

Section A

BASIC ASPECTS OF NEUROTRANSMITTER FUNCTION

Neurotransmitters, Drugs and Brain Function.
Edited by Roy Webster

ISBN: Hardback 0-471-97819-1 Paperback 0-471-98586-4 Electronic 0-470-84657-7

1 Neurotransmitter Systems and Function: Overview

R. A. WEBSTER

INTRODUCTION

> Analysis of Biological Function generally presumes that function at one level arises from the interactions of lower-level elements. It is often relatively straightforward to identify elements that may be involved and their individual interactions. Modern cell and molecular biology, in particular, is very efficient at identifying new molecules, and establishing which molecules 'talk to' one another. However, as the accumulation of such studies gradually reveals a complex network of interactions, its output — the biological function — becomes ever harder to understand and predict. The system is reduced to its elements, but it is not clear how to integrate it again. Yet this is the ultimate functional goal. (Brezina and Weiss 1997)

The molecules referred to are the neurotransmitters (NTs) and their receptors, found in the brain; the biological function is the activity of the brain itself. Our understanding of that must be the ultimate goal.

We have no such pretensions in this book but we do hope to help you to understand how neurotransmitters may be involved in brain function and more particularly how their activity is modified by disease and drugs. As the above quotation implies, this will mean considering the synaptic characteristics of each neurotransmitter, but before we do so, it is important to consider some more general and basic aspects of neurotransmitter function. Thus:

- What is a neurotransmitter and how did the concept of chemical transmission arise?
- Which substances are neurotransmitters? Can they be sensibly classified and how do we know they are transmitters?
- Which neurons and pathways use which neurotransmitters and how are they organised?
- How do neurotransmitters work? What effects do they have on neuronal activity?
- What is known about the receptors to which they bind?
- How are neurotransmitters released and how is this controlled?
- How can neurotransmitter function be modified?

Most of these points are considered in detail in subsequent chapters but some will be touched on collectively here.

CHEMICAL TRANSMISSION

We might start by considering what we understand by the term 'neurotransmitter'. According to the *Oxford English Dictionary* (2nd edition) it is:

> A substance which is released at the end of a nerve fibre by the arrival of a nerve impulse and by diffusing across the synapse or junction effects the transfer of the impulse to another nerve fibre (or muscle fibre or some receptor).

Other dictionaries carry similar definitions.

Based on this definition a neurotransmitter could be exemplified by actylcholine (ACh) released from motor nerves to excite and contract the fibres of our skeletal muscles. Indeed the synapses there, i.e. the junctions between nerve and muscle fibres, are anatomically and chemically geared to act as a fast relay station. Acetylcholine released rapidly from vesicles in the nerve terminal, on arrival of the nerve impulse, binds quickly with postsynaptic sites (receptors). When activated these open channels for sodium ions which pass through into the muscle fibre to depolarise its membrane and cause contraction. The whole process takes less than one millisecond and the ACh is rapidly removed through metabolism by local cholinesterase so that contraction does not persist and the way is cleared for fresh ACh to act. Anatomically there is a precise and very close relationship between the nerve ending and the muscle fibre at histologically distinct end-plates, where the receptors to ACh are confined. It is better than having the nerve directly linked to the muscle since the time lost through imposing a chemical at the synapse between nerve and muscle is insignificant and the use of a chemical not only facilitates control over the degree of muscle tone developed, but fortuitously makes it possible for humans to modify such tone chemically.

Blocking the destruction of ACh potentiates its effects while blocking the receptors on which it acts produces paralysis (neuromuscular blockade). Indeed it was the curare impregnated into the darts used by native South American hunters, so that they could paralyse and then easily kill their prey, that motivated Claude Bernand to investigate its actions at the end of the nineteenth century and so demonstrate the chemical sensitivity of excitable tissue that led to the concept of chemical transmission. He did a very simple experiment. He took a sciatic nerve gastrocnemious muscle preparation from a frog (not the actual quest of the hunters), placed the muscle in one dish of appropriate salt solution and extended the nerve into another. Not surprisingly, simple wire electrodes connected to an activated induction coil induced contractions of the muscle whether placed directly on the muscle or on the nerve to it. When, however, curare was added to the dish containing the muscle, direct stimulation of the muscle still induced a contraction, but activation of the nerve was ineffective. This was not due to any effect of curare on the nerve because when curare was added to the nerve rather than the muscle dish, stimulation of the nerve was still effective. Thus there had to be a chemically sensitive site on the muscle, where it was linked with the nerve, which was affected by the curare. This did not prove that a chemical had been released from the nerve but some years later (1916) Otto Loewi found that if he cannulated the ventricle of a frog's heart, isolated with its vagus nerve intact, then when this was stimulated not only did the heart slow, as expected, but if fluid withdrawn from the ventricle was subsequently reintroduced the heart slowed again. This suggested the release of a

chemical from the vagus, which was made even clearer by allowing the fluid perfused through one frog heart to drip onto a second one and establishing that when the first heart was slowed by stimulating its vagus the fluid from it also slowed the second heart when that was reached. Loewi did not identify the chemical, which he called vagustoff, but it was later shown to be acetylcholine (ACh), the first identified neurotransmitter (and it was also found to transmit the neural stimulation of skeletal muscle, which had been blocked by curare in the experiments of Bernard).

Now this brings us to the first problem with the dictionary definition of a neurotransmitter because in the heart ACh is not transmitting an excitatory impulse between nerve and muscle, it is causing inhibition. There are also other differences. Its cardiac effect, change in rate, occurs much more slowly, has nothing to do with the direct opening of any ion channel and is not blocked by curare. Thus the sites on cardiac muscle that are chemically sensitive to ACh, its so-called receptors, are different from those for ACh on skeletal muscle. In fact they are blocked by a different poison, namely atropine (from *Atropa belladonna*, Deadly Nightshade). These observations raise two important issues. First, it is the receptor which ultimately determines the effects of a neurotransmitter and second, since only the excitatory effects of ACh at the neuromuscular junction fulfil the original definition of a neurotransmitter in transmitting excitation, either acetylcholine cannot be considered to be a neurotransmitter in the heart, despite its effects, or the definition of a neurotransmitter needs modifying. We will return to this topic at the end of the chapter.

Thus a neurotransmitter can clearly have more than one effect and a moment's consideration of what is involved for your nervous system in effecting the processes that enable you to turn the pages of this book and read and remember some of its contents will make you realise just how much the nervous system has to achieve and how many different parts of it have to be involved and functionally integrated. This is without considering whether you feel content, anxious, or depressed and how that can affect your concentration and ability to read and learn or even turn over the pages. Clearly such processes must involve many different neural pathways and types of neuron producing different effects and presumably requiring a number of different chemicals (neurotransmitters). The importance and variety of such chemicals is also emphasised from a look at drug usage and the study of how they work.

There are many drugs that affect the nervous system for good (antidepressants, analgesics, anticonvulsants) and bad (toxins, poisons, drugs of abuse) and although it would be naive to think that any drug has only one effect, i.e. that an anti-epileptic drug will never cause any sedation, their demonstrably different primary effects, coupled with the diversity of their chemical structures, suggest that not only are drugs affecting different areas of the brain but as they are likely to do this at chemical synapses there must be a number of different chemical synapses and chemicals, i.e. neurotransmitters.

NEUROTRANSMITTER CLASSIFICATION

The following substances, listed alphabetically, have been widely implicated and generally accepted as neurotransmitters in the central nervous system (CNS), although some, such as glutamate, are much more important than others, e.g. adrenaline. Some

classification is appropriate and the simplest and most commonly used is that based on chemical structure with the substances grouped as follows:

	Chemical group	Examples
A	Choline ester	Acetylcholine (ACh)
B	**Monoamines**	
	Catechol	Dopamine (DA), noradrenaline (NA) (adrenaline)
	Indole	5-Hydroxytryptamine (5-HT, serotonin)
	Imidazole	Histamine (HIST)
C	**Amino acids**	
	Acidic	Glutamate (GLT)
	Basic	γ-Aminobutyric acid (GABA), glycine
D	**Peptides**	Enkephalins, endorphins, cholecystokinin, substance P (Many others have been implicated)
E	**Purines**	Adenosine triphosphate (ATP), adenosine

In addition to the above it is now clear that the following substances may have an important central action but whether they can be classified as true neurotransmitters is uncertain:

F	**Steroids**	Pregnenalone, dehydroepiandrosterone
G	**Nitric oxide**	(A gas but it is always in solution in the brain)
H	**Eicosanoids**	Prostaglandins

A glance at the structure of the classical neurotransmitters (Fig. 1.1) shows that apart from the peptides (D) (and purines, E), most of them are fairly simple chemicals. Some authors therefore divide them into small (e.g. A, B, C) and large (peptides, D) molecular NTs. Although we will see that peptides certainly have some properties different from other NTs, in that they rarely have a primary neurotransmitter function and usually just complement the actions of those NTs in groups A–C, to put them in a class of their own and group all the others together simply on the basis of molecular size is inappropriate and misleading since it elevates the peptides to a status that is neither proven nor warranted.

NEURONS: STRUCTURE AND ENVIRONMENT

The neurons from which NTs are released number more than 7 billion in the human brain. Each (Fig. 1.2) consists of a cell body, the soma or perikaryon, with one major cytoplasmic process termed the axon, which projects variable distances to other neurons, e.g. from a cortical pyramidal cell to adjacent cortical neurons, or to striatal neurons or to spinal cord motoneurons. Thus by giving off a number of branches from its axon one neuron can influence a number of others. All neurons, except primary sensory neurons with cell bodies in the spinal dorsal root ganglia, have a number of other, generally shorter, projections running much shorter distances among neighbouring neurons like the branches of a tree. These processes are the dendrites. Their

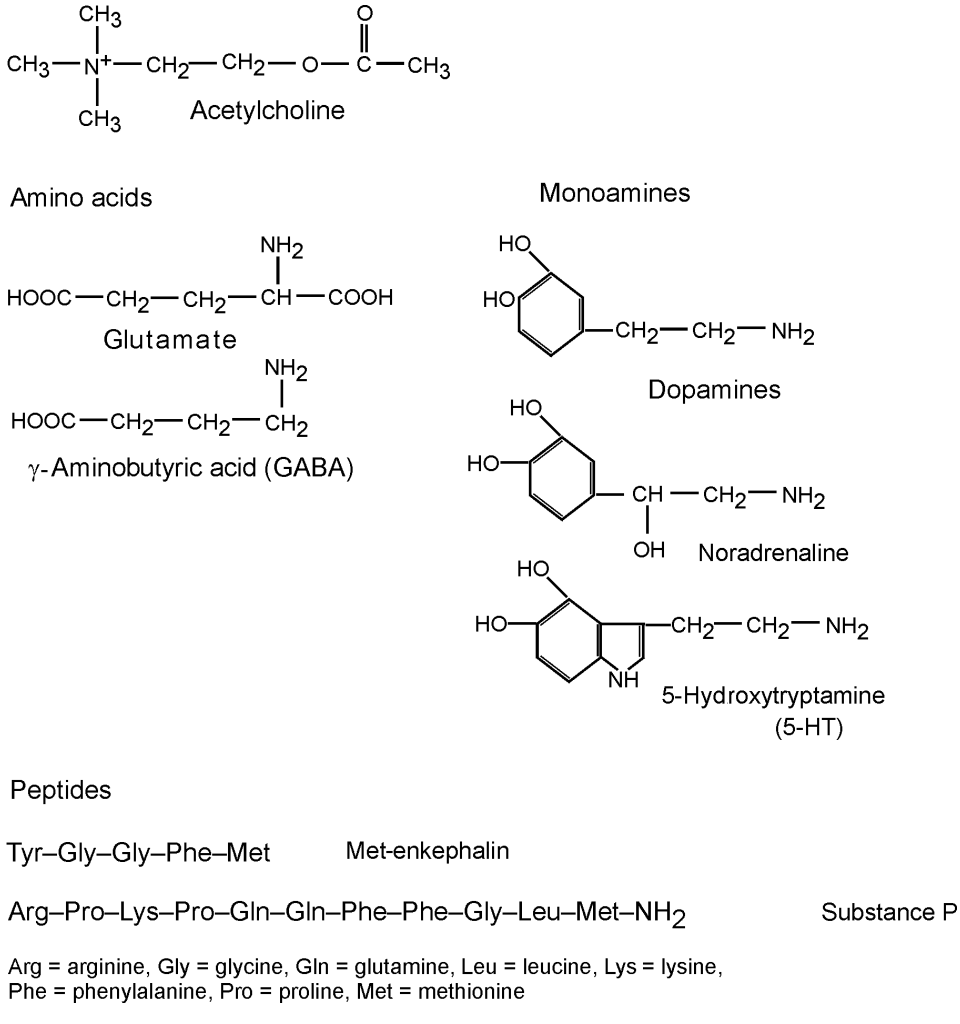
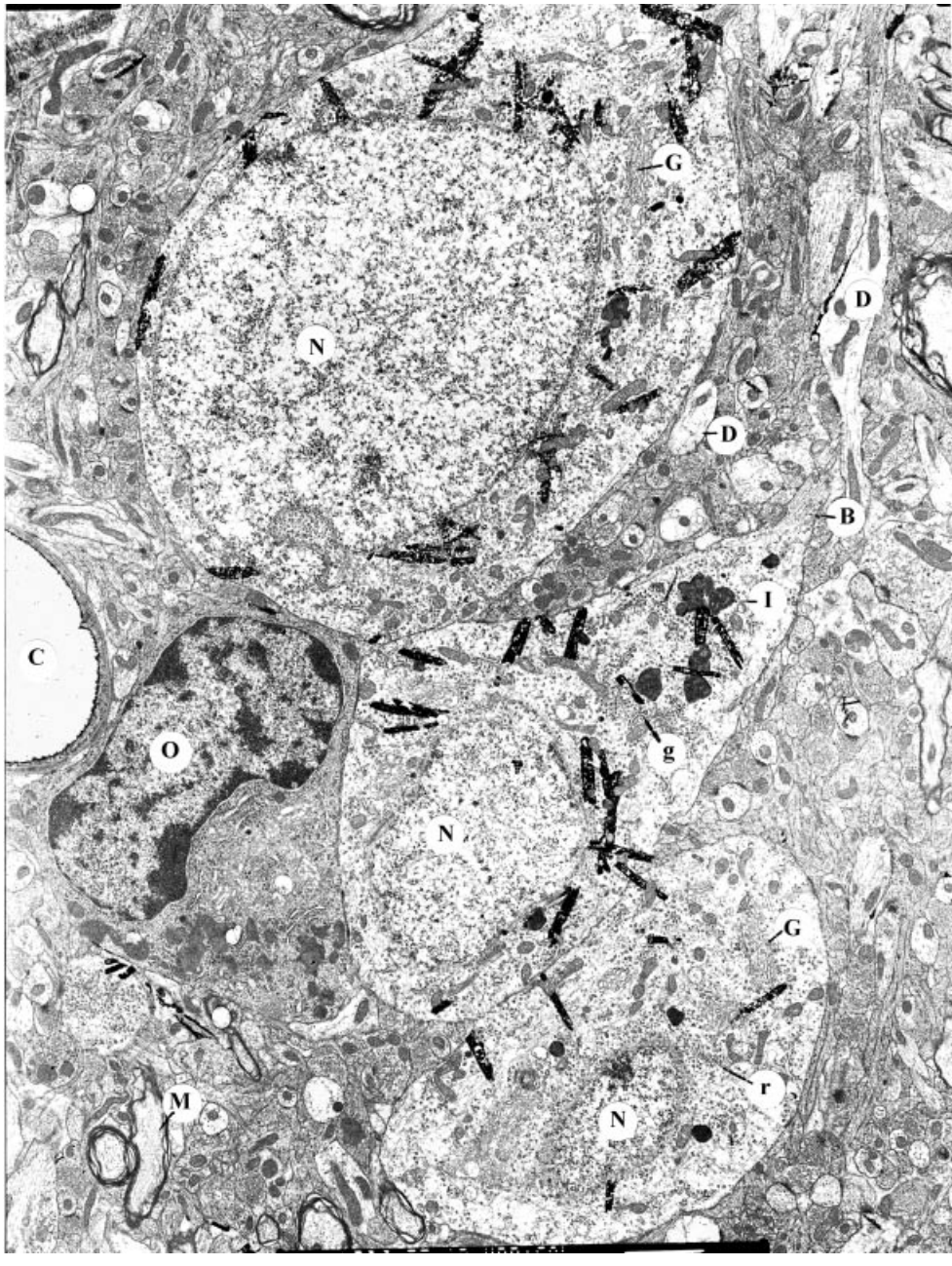


Figure 1.1 The chemical structures of the main neurotransmitters. The relatively simple structure of acetylcholine, the monoamines and the amino acids contrasts with that of the peptides, the simplest of which are the enkephalins which consists of five amino acids; substance P has eleven

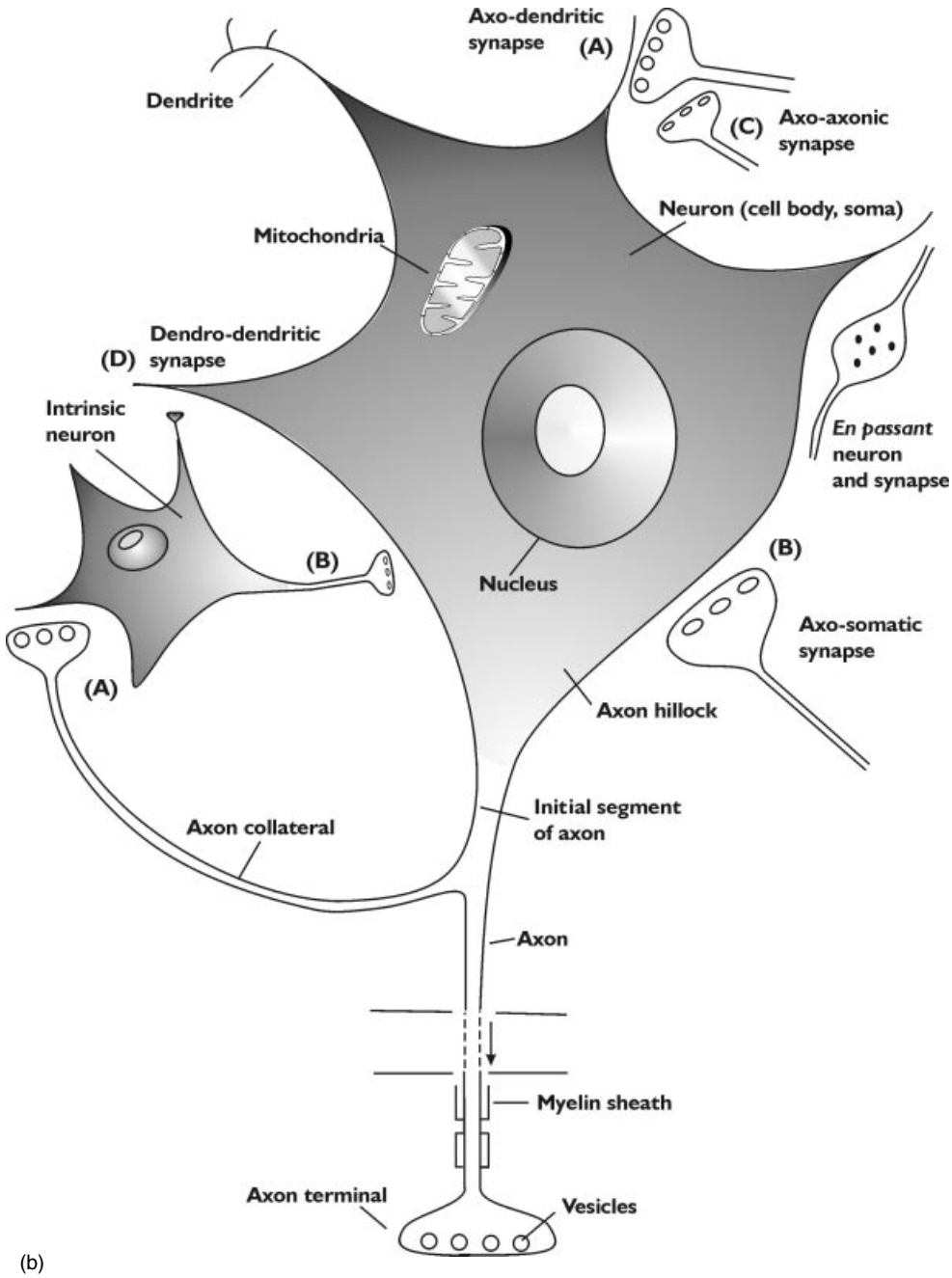
absence from sensory, i.e. initiating, neurons immediately suggests that their function is associated with the reception of signals (inputs) from other neurons. Neuron cell bodies vary in diameter from 5 μm to 100 μm and axons from 0.1 μm to 10 μm, although these are enlarged at their terminal endings. Axons are generally surrounded by an insulating myelin sheath which is important for the propagation of action potentials generated in the neurons and gives the axons and the pathways they form a white colour which contrasts with the grey appearance of those areas of the CNS dominated by the presence of neuron cell bodies and their dendrites.

The axon terminals of one neuron synapse with other neurons either on the dendrites (axo-dendritic synapse) or soma (axo-somatic synapse). Synapses on another axon



(a)

Figure 1.2 (a) Electron micrograph of three neuronal cell bodies in the anterior thalamic nucleus of the rat retrogradely labelled with horseradish peroxidase conjugated with cholera toxin B (dark bars) injected into the posterior cingulate cortex. N = nucleus of neurons, O = nucleus of oligodendrocyte, C = capillary, D = dendrite, G = Golgi apparatus, M = myelinated fibre, r = ribosome, l = lipofuscin pigment, g = granular endoplasmic reticulum. Picture kindly provided by Professor A. R. Lieberman (University College London). Reproduced by permission of Springer-Verlag GmbH & Co. KG, from Wang *et al.*, *Exp. Brain Res.* **126**: 369–382 (1999)



(b)

Figure 1.2 (b) Schematic representation of a neuron. The main features of a neuron are shown together with different synaptic arrangements (A) axo-dendritic, (B) axo-somatic, (C) axo-axonic and (D) dendro-dendritic. For more detail see section on 'Morphological correlates of synaptic function' and Fig. 1.7

terminal are also found (axo-axonal) and occasionally even between dendrites (dendro-dendritic) (see Fig. 1.2(b)). The morphology of synapses is considered later.

Like other cells, a neuron has a nucleus with genetic DNA, although nerve cells cannot divide (replicate) after maturity, and a prominent nucleolus for ribosome synthesis. There are also mitochondria for energy supply as well as a smooth and a rough endoplasmic reticulum for lipid and protein synthesis, and a Golgi apparatus. These are all in a fluid cytosol (cytoplasm), containing enzymes for cell metabolism and NT synthesis and which is surrounded by a phospholipid plasma membrane, impermeable to ions and water-soluble substances. In order to cross the membrane, substances either have to be very lipid soluble or transported by special carrier proteins. It is also the site for NT receptors and the various ion channels important in the control of neuronal excitability.

Microtubules (about 20 nm in diameter) and solid neurofilaments (10 nm) extend from the cell body into the axon and are found along its length, although not continuous. They give structure to the axon but are not involved in the transport of material and vesicles to the terminal, which despite its high level of activity does not have the facility for molecular synthesis possessed by the cell body. Such transport is considered to be fast (200–400 mm per day), compared with a slower transport (1 mm per day) of structural and metabolic proteins. Although axonal flow is mainly towards the terminal (ortho or anterograde) there is some movement (fast) of waste material and possibly information on synaptic activity back to the cell body (retrograde). The neuron is obviously very active throughout the whole of its length.

In addition to neurons the CNS contains various neuroglia (often just called glia). These can outnumber neurons by up to 10:1 in some areas and include star-like astrocytes with their long cellular processess which not only enable them to provide structural support for the nerve cells but also facilitate NT degradation and the removal of metabolites. Oligodendrites are glial cells which are involved in myelin formation and although they also have long processes, these are spirally bound rather than extending out as in the astocytes.

Neurons and glia are bathed in an ion-containing protein-free extracellular fluid which occupies less of the tissue volume (20%) in the brain than in other organs because of the tight packing of neurons and glia. In fact the whole brain is really suspended in fluid within its bony casing. The brain and spinal cord are covered by a thin close-fitting membrane, the pia mater and a thicker loose outer membrane, the dura mater. In the space between them, the subarachnord space, is the cerebrospinal fluid (CSF). This also flows into a series of ventricular spaces within the brain as well as a central canal in the cord and arises mainly as a secretion (ultra filtrate) of blood from tufts of specialised capillaries (the choroid plexus), which invaginate the walls of the ventricles. While the CSF is contiguous with the extracellular fluid within the brain and contributes to it, much of this fluid comes directly from the copious network of capillaries found throughout the brain. In fact neurons are never far from a capillary and their high metabolic rate means that despite contributing only 2% towards body weight, the nervous system receives 15% of cardiac output.

In most parts of the body, substances, other than large molecular ones like proteins, are filtered from the blood into the extracellular space through gaps between endothelial cells in the capillary wall. Such gaps are much narrower, almost non-existent, in brain capillaries and it is likely that any filtering is further reduced by the manner in which astrocytes pack around the capillaries. This constraint is known as the

blood–brain barrier (BBB). It protects the brain from inappropriate substances, including all NTs and many drugs. To enter the brain as a whole is therefore almost as difficult for a substance as entering a neuron and again it has to be either very lipid soluble, when it can dissolve in and so pass through the capillary wall, or be transported across it.

NEUROTRANSMITTER FUNCTION

BASIC CIRCUITRY

In a classical neural pathway, such as that depicted in Fig. 1.3, neuron A must excite neuron B and at the same time inhibit neuron C in order to optimise the excitation of B. It could achieve this with one NT able to activate receptors linked to different events on B and C. Of course, neuron C would have other inputs, some of which would be excitatory and if the same NT was used it could activate the inhibitory mechanism on C as well. Also, the NT released from A might be able to stimulate as well as inhibit neuron C (Fig. 1.3(a)). Even the provision of separate receptors linked to excitation and inhibition would not overcome these problems since both would be accessible to the NT. One possible solution, used in the CNS, is to restrict the NT to the synapse at which it is released by structural barriers or rapid degradation. Also the inputs and receptors linked to excitation could be separated anatomically from those linked to inhibition and, in fact, there is electrophysiological and morphological evidence that excitatory synapses are mainly on dendrites and inhibitory ones on the soma of large neurons (Fig. 1.3(b)). Nevertheless, the problem of overlap would be eased if two NTs were released, one to activate only those receptors linked to excitation and another to evoke just inhibition, i.e. place the determinant of function partly back on the NT (Fig. 1.3(c)). This raises a different problem which has received much consideration. Can a neuron release more than one NT?

It was generally assumed that it cannot and this became known as Dale's Law. During his studies on antidromic vasodilation he wrote (1935) 'When we are dealing with two different endings of the same sensory neuron, the one peripheral and concerned with vasodilation and the other at a central synapse, can we suppose that the discovery and identification of a chemical transmitter at axon reflex dilation would furnish a hint as to the nature of the transmission process at a central synapse. The possibility has at least some value as a stimulus to further experiments'.

This it certainly has been and in the last few years much evidence has been presented to show that more than one substance (but not necessarily more than one conventional NT) can co-exist in one nerve terminal. This does not disprove Dale's Law (so called), since he was referring to 'a' not 'the' NT and to different endings of one neuron. In fact he was simply saying that if a neuron uses a particular transmitter at one of its terminals it will use it at another, although he did not add, irrespective of whether or not it uses more than one NT. This makes good sense especially since it is difficult to conceive how a neuron could achieve, let alone control, the release of different NTs from different terminals, unless the NTs were synthesised solely at the terminals independently of the cell body. In that way different substances might be released from different terminals of a neuron by arriving action potentials without the neuron having to do anything special

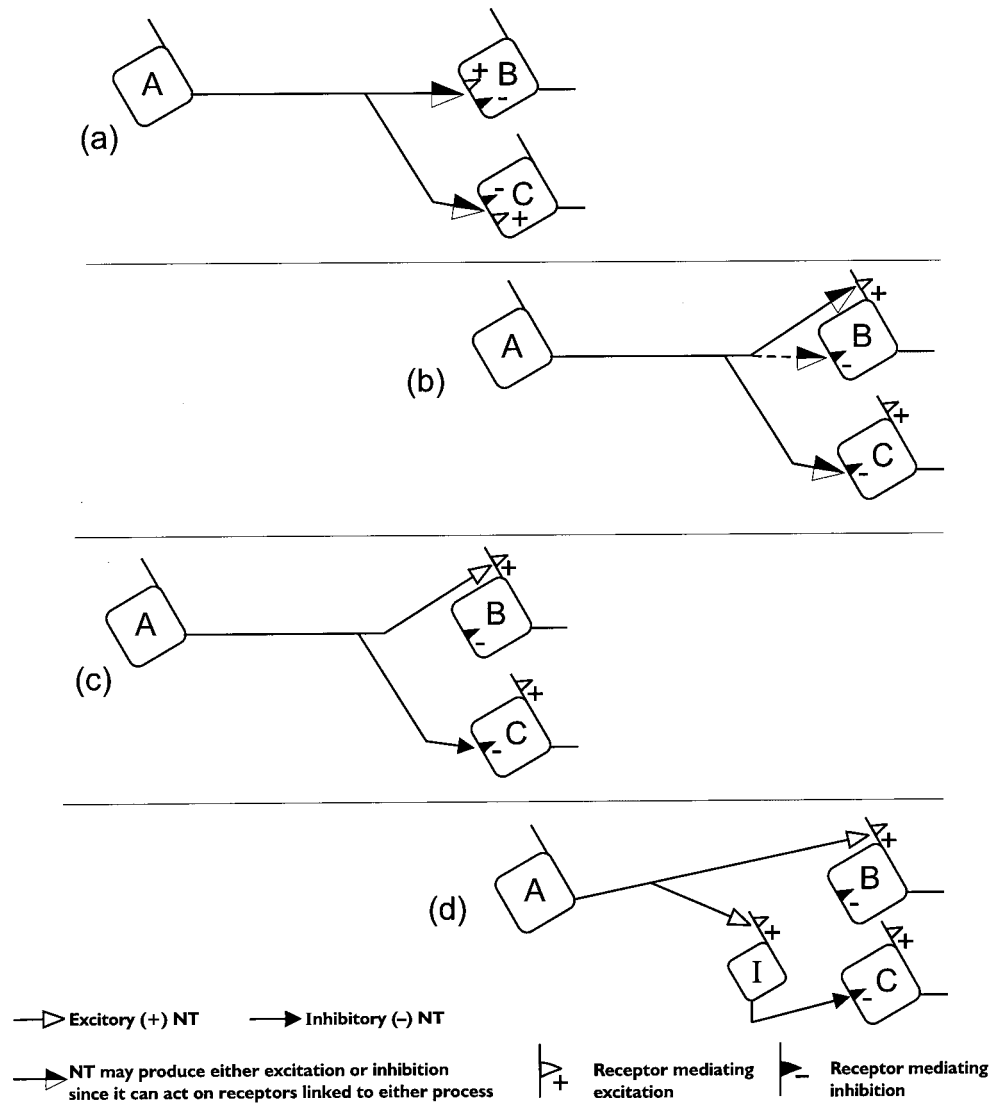


Figure 1.3 Some possible basic neurotransmitter–synaptic arrangements for the excitation and inhibition of different neurons. (a) The single NT activates neuron B and inhibits neuron C by being able to activate both excitatory and inhibitory receptors or, more probably, acting on one receptor linked to both events. There is potential, however, for the NT to activate any inhibitory receptors that may be on B or excitatory receptors on C. (b) The same NT is used as in (a) but the excitatory receptors are now only on dendrites and separated from the inhibitory receptors only on the soma. There is less chance of unwanted mixed effects. (c) Neuron A releases distinct excitatory and inhibitory NTs from its two terminals each acting on specific and morphologically separated receptors. But this depends on a neuron being able to release two NTs. (d) Neuron A releases the same NT from both terminals. It directly excites B but inhibits C through activating an inhibitory interneuron (I) which releases an inhibitory NT onto specific receptors on C. This last scheme (d) is clearly more functional and is widely used

to achieve it. Thus neuron A (Fig. 1.3) could then conceivably always release one NT at B and another at C or even two NTs at both but probably could not vary their release independently at different (or the same) synapses.

Fortunately there is another way in which one neuron can excite and inhibit different neurons using just one NT. Neuron A could excite B and inhibit C by the introduction of an inhibitory interneuron the activation of which by A, using the same excitatory NT as at B, automatically inhibits C (Fig. 1.3(d)). This form of inhibition is quite common in the CNS and in fact much inhibition is mediated by these so-called short-axon interneurons and a neuron may inhibit itself through feedback via an axon collateral synapsing onto an adjacent inhibitory short-axon interneuron (Fig. 1.2).

It might therefore be possible to set up a CNS with two NTs exerting fast excitatory and inhibitory effects through different receptors, situated on different parts of the neuron provided those were the only effects wanted. But this is not so. One neuron can receive hundreds of inputs and its activity and responsiveness is in fact balanced by such inputs producing different effects at differing speeds by using different NTs. So what are these different effects and how are they produced?

NEURONAL EXCITABILITY

POSTSYNAPTIC EVENTS

The neuronal membrane normally has a resting membrane potential around $-70\,mV$ (inside negative in respect of outside) with Na^+ and Cl^- concentrated on the outside and K^+ on the inside prepared to move down their concentration gradients when the appropriate ion channels are opened (Fig. 1.4). On arrival of an excitatory impulse the Na^+ channels are opened and there is an increased influx of Na^+ so that the resting potential moves towards the so-called equilibrium potential for Na^+ ($+50\,mV$) when Na^+ influx equals Na^+ outflux but at -60 to $-65\,mV$, the threshold potential, there is a sudden increase in Na^+ influx. This depolarisation leads to the generation of a propagated action potential. The initial subthreshold change in membrane potential parallels the action of the excitatory transmitter and is graded in size according to the amount of NT released. It is known as the excitatory postsynaptic potential (EPSP) and lasts about 5 ms.

An inhibitory input increases the influx of Cl to make the inside of the neuron more negative. This hyperpolarisation, the inhibitory postsynaptic potential (IPSP), takes the membrane potential further away from threshold and firing. It is the mirror-image of the EPSP and will reduce the chance of an EPSP reaching threshold voltage.

Such clear postsynaptic potentials can be recorded intracellularly with microelectrodes in large quiescent neurons after appropriate activation but may be somewhat artificial. In practice a neuron receives a large number of excitatory and inhibitory inputs and its bombardment by mixed inputs means that its potential is continuously changing and may only move towards the threshold for depolarisation if inhibition fails or is overcome by a sudden increase in excitatory input.

Not all influences on, or potentials recorded from, a neuron have the same time-course as the EPSP and IPSP, which follow the rapid opening of Na^+ and Cl^- ion channels directly linked to NT receptors. There are also slowly developing, longer lasting and smaller non-propagated (conditioning) changes in potential most of which appear to have a biochemical intermediary in the form of G-proteins linked to the activation (Gs) or

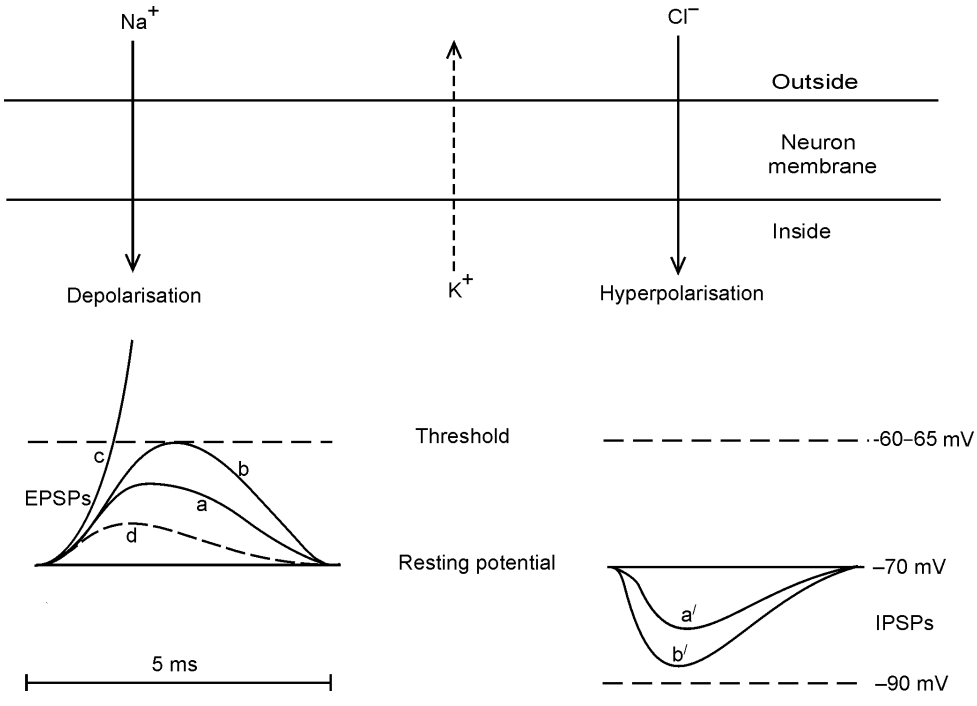


Figure 1.4 Ionic basis for excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs). Resting membrane potential (-70 mV) is maintained by Na^+ influx and K^+ efflux. Varying degrees of depolarisation, shown by different sized EPSPs (a and b), are caused by increasing influx of Na^+. When the membrane potential moves towards threshold potential (60–65 mV) an action potential is initiated (c). The IPSPs (a′b′) are produced by an influx of $C1^-$. Coincidence of an EPSP (b) and IPSP (a′) reduces the size of the EPSP (d)

inhibition (Gi) of adenylate cyclase and cyclic AMP production or IP_3 breakdown (see Chapter 2). They can be excitatory (depolarising) or inhibitory (hyperpolarising) generally involving the opening or closing of K^+ channels. This can be achieved directly by the G-protein or second messenger but more commonly by the latter causing membrane phosphorylation through initiating appropriate kinase activity.

Thus the activity of a neuron can be controlled in a number of ways by NTs activating appropriate receptors (Fig. 1.5). Two basic receptor mechanisms are involved:

(1) **Ionotropic** Those linked directly to ion channels such as those for Na^+ (e.g. ACh nicotinic or some glutamate receptors) or Cl^- (GABA), involving fast events with increased membrane conductance and ion flux.
(2) **Metabotropic** Those not directly linked to ion channels but initiating biochemical processes that mediate more long-term effects and modify the responsiveness of the neuron. With these the first messenger of synaptic transmission, the NT, activates a second messenger to effect the change in neuron excitability. They are normally associated with reduced membrance conductance and ion flux (unless secondary to

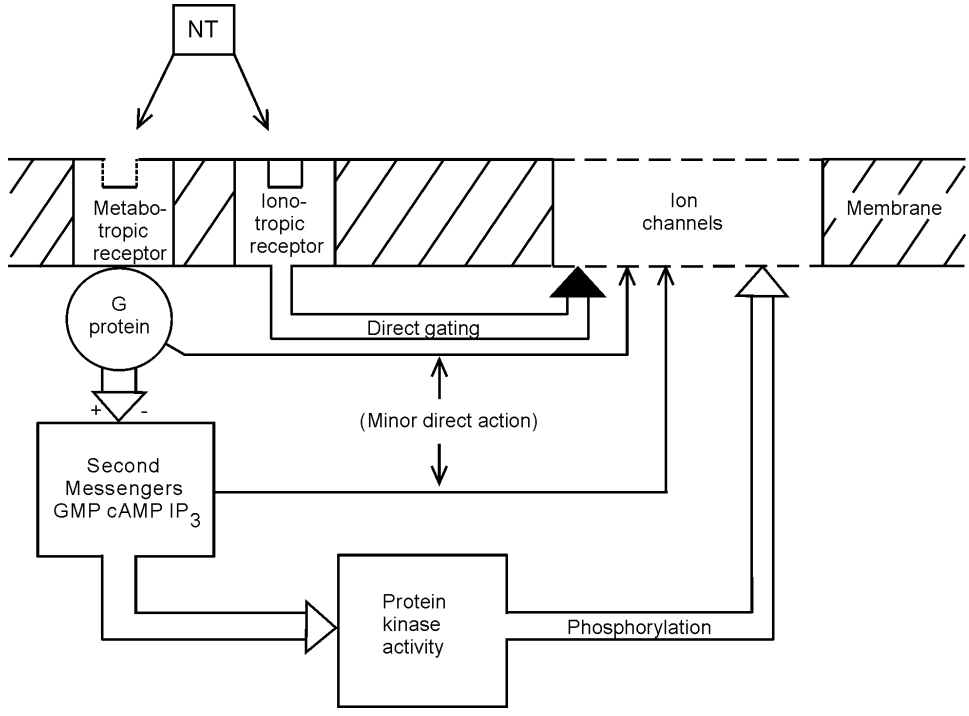


Figure 1.5 The degree of ion channel opening can be controlled (gated) either directly (ionotropic effect) or indirectly (metabotropic effect). In the former the neurotransmitter combines with a receptor that is directly linked to the opening of an ion channel (normally Na^+ or $C1^-$) while in the latter the receptor activates a G-protein that can directly interact with the ion channel (most probably K^+ or Ca^{2+}) but is more likely to stimulate (Gs) or inhibit (Gi) enzymes controlling the levels of a second messenger (e.g. cAMP, GMP, IP_3). These in turn may also directly gate the ion channel but generally control its opening through stimulating a specific protein kinase that causes phosphorylation of membrane proteins and a change in state of the ion channel. The latter (metabotropic) effects may either open or close an ion channel (often K^+) and are much slower (100s ms to min) than the ionotropic ones (1–10 ms). A variety of neurotransmitters, receptors, second messengers and ion channels permits remarkably diverse and complex neuronal effects

an increased Ca^{2+} conductance) and may involve decreased Na^+ influx (inhibitory) or K^+ efflux (excitatory). Some amines (e.g. noradrenaline) may increase K^+ efflux (inhibitory).

These two basic mechanisms could provide a further classification for NTs, namely fast and slow acting, although one NT can work through both mechanisms using different receptors. The slow effects can also range from many milliseconds to seconds, minutes, hours or even to include longer trophic influences. What will become clear is that while one NT can modify a number of different membrane ion currents through different mechanisms and receptors, one current can also be affected by a number of different NTs. The control of neuronal excitability is discussed in more detail in Chapter 2.

PRESYNAPTIC EVENTS

So far we have assumed that a NT can only modify neuronal activity by a postsynaptic action. Recently, interest has also turned to presynaptic events. It has been known for many years that stimulation of muscle or cutaneous afferents to one segment of the spinal cord produces a prolonged inhibition of motoneuron activity without any accompanying change in conductance of the motoneuron membrane, i.e. no IPSP. Such inhibition is probably, therefore, of presynaptic origin and is, in fact, associated with a depolarisation of the afferent nerve terminals and a reduction in release of the excitatory NT. If it is assumed that the amount of NT released from a nerve terminal depends on the amplitude of the potential change induced in it, then if that terminal is already partly depolarised when the impulse arrives there will be a smaller change in potential and it will release less transmitter (Fig. 1.6). There is no direct evidence for this concept from studies of NT release but electrophysiological experiments at the crustacean neuromuscular junction, which has separate excitatory and inhibitory inputs, show that stimulation of the inhibitory nerve, which released GABA, reduced the EPSP evoked postsynaptically by an excitatory input without directly hyperpolarising (inhibiting) the muscle fibre. Certainly when GABA is applied to various *in vivo* and *in vitro* preparations (spinal cord, cuneate nucleus, olfactory cortex) it will produce a depolarisation of afferent nerve terminals that spreads sufficiently to be recorded in their distal axons.

Such presynaptic inhibition can last much longer (50–100 ms) than the postsynaptic form (5 ms) and can be a very effective means of cutting off one particular excitatory input without directly reducing the overall response of the neuron. How GABA can produce both presynaptic depolarisation and conventional postsynaptic hyperpolarisation by the same receptor, since both effects are blocked by the same antagonist, bicuculline, is uncertain (see Chapter 2) although an increased chloride flux appears to be involved in both cases. If nerve terminals are depolarised, rather than hyperpolarised by increased chloride flux, then their resting membrane potential must be different from (greater than) that of the cell body so that when chloride enters and the potential moves towards its equilibrium potential there is a depolarisation instead of a hyperpolarisation. Alternatively, chloride efflux must be achieved in some way.

This form of presynaptic inhibition must be distinguished from another means of attenuating NT release, i.e. autoinhibition. This was first shown at peripheral noradrenergic synapses where the amount of noradrenaline released from nerve terminals is reduced by applied exogenous noradrenaline and increased by appropriate (alpha) adrenoceptor antagonists. Thus through presynaptic (alpha) adrenoreceptors, which can be distinguished from classical postsynaptic (alpha) adrenoreceptors by relatively specific agonists and antagonists, neuronal-released noradrenaline is able to inhibit its own further (excessive) release. It is a mechanism for controlling the synaptic concentration of noradrenaline. This inhibition does not necessarily involve any change in membrane potential but the receptors are believed to be linked to and inhibit adenylate cyclase. Whether autoinhibition occurs with all NTs is uncertain but there is strong evidence for it at GABA, dopamine and 5-HT terminals.

There is also the interesting possibility that presynaptic inhibition of this form, with or without potential changes, need not be restricted to the effect of the NT on the terminal from which it is released. Numerous studies in which brain slices have been loaded with a labelled NT and its release evoked by high K^+ or direct stimulation show

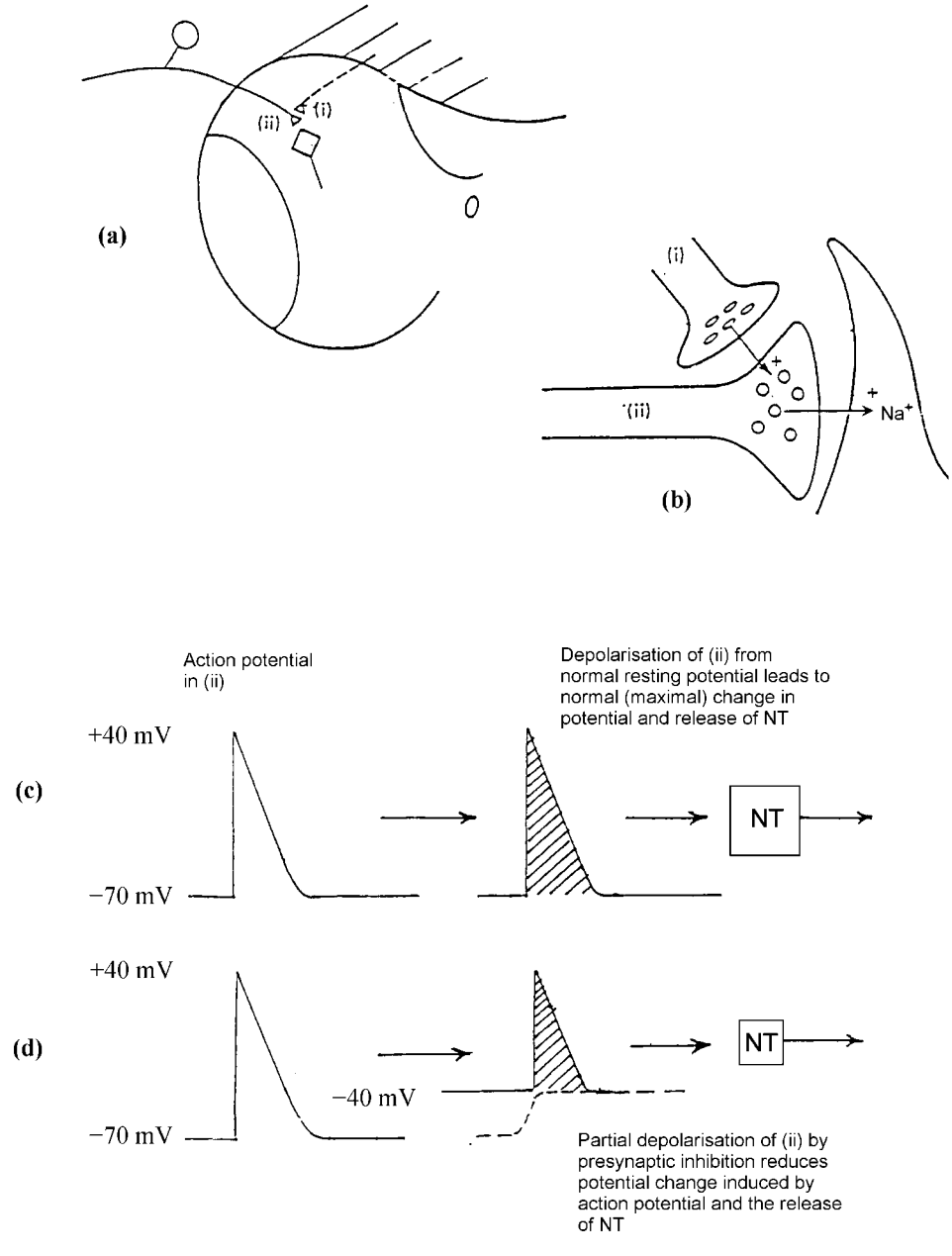


Figure 1.6 Presynaptic inhibition of the form seen in the dorsal horn of the spinal cord. (a) The axon terminal (i) of a local neuron is shown making an axo-axonal contact with a primary afferent excitatory input (ii). (b) A schematic enlargement of the synapse. (c) Depolarisation of the afferent terminal (ii) at its normal resting potential by an arriving action potential leads to the optimal release of neurotransmitter. (d) When the afferent terminal (ii) is already partially depolarised by the neurotransmitter released onto it by (i) the arriving acting potential releases less transmitter and so the input is less effective

that such release can be inhibited by a variety of other NTs. A noradrenergic terminal has been shown to possess receptors for a wide range of substances, so-called heteroceptors (see Langer 1981, 1997) and although this may be useful for developing drugs to manipulate noradrenergic transmission it seems unlikely that *in vivo* all of the receptors could be innervated by appropriate specific synapses or reachable by their NT. They may be pharmacologically responsive but not always physiologically active (see Chapter 4).

CONTROL OF SYNAPTIC NT CONCENTRATION

Having briefly discussed the presynaptic control of NT release it is necessary to consider how the concentration of a NT is controlled at a synapse so that it remains localised to its site of release (assuming that to be necessary) without its effect becoming too excessive or persistent.

Although one neuron can receive hundreds of inputs releasing a number of different NTs, the correct and precise functioning of the nervous system presumably requires that a NT should only be able to act on appropriate receptors at the site of its release. This control is, of course, facilitated to some extent by having different NTs with specific receptors so that even if a NT did wander it could only work where it finds its receptors and was still present in sufficient concentration to meet their affinity requirements. Normally the majority of receptors are also restricted to the immediate synapse.

Nevertheless, from release (collection) studies we know that enough NT must diffuse (overflow) to the collecting system, be that a fine probe *in vivo* or the medium of a perfusion chamber *in vitro*, to be detected. Thus one must assume that either the concentration gradient from the collecting site back to the active synaptic release site is so steep that the NT can only reach an effective concentration at the latter, or it is not unphysiological for a NT to have an effect distal from its site of release.

Released NT, if free to do so, would diffuse away from its site of release at the synapse down its concentration gradient. The structure of the synapse and the narrow gap between pre- and postsynaptic elements reduces this possibility but this means that there must be other mechanisms for removing or destroying the NT so that it, and its effects, do not persist unduly at the synapse but are only obtained by regulated impulse controlled release. In some cases, e.g. ACh, this is achieved by localised metabolising enzymes but most nerve terminals, especially those for the amino acids and monoamines, possess very high-affinity NT uptake systems for the rapid removal of released NT. In fact these are all Na^+- and Cl^--dependent, substrate-specific, high-affinity transporters and in many cases their amino-acid structure is known and they have been well studied. Transport can also occur into glia as well as neurons and this may be important for the amino acids. Of course, a further safeguard against an excessive synaptic concentration of the NT is the presence of autoreceptors to control its release.

Thus there are mechanisms to ensure that NTs neither persist uncontrollably at the synapse nor produce dramatic effects distal from it. Studies of glutamate release always show a measurable basal level (1–3 μM), although this may not all be of NT origin, and yet it is very difficult to increase that level even by quite intense stimulation. Whether this is a safeguard against the neurotoxicity caused by the persistent intense activation

of neurons by glutamate (see Chapter 9), or just to ensure that neurons remain responsive to further stimulation is unclear, as is the mechanism by which it is achieved.

Despite the above precautions, it is still possible that NT spillover and extrasynaptic action may occur and indeed could be required in some instances. Thus the diffusion of glutamate beyond the synapse could activate extrasynaptic high-affinity NMDA or metabotropic receptors (Chapter 9) to produce long-lasting effects to maintain activity in a network. This may be important in long-term potentiation and memory effects. Crosstalk between synapses could also act as a back-up to ensure that a pathway functions properly (see Barbour and Hausser 1997).

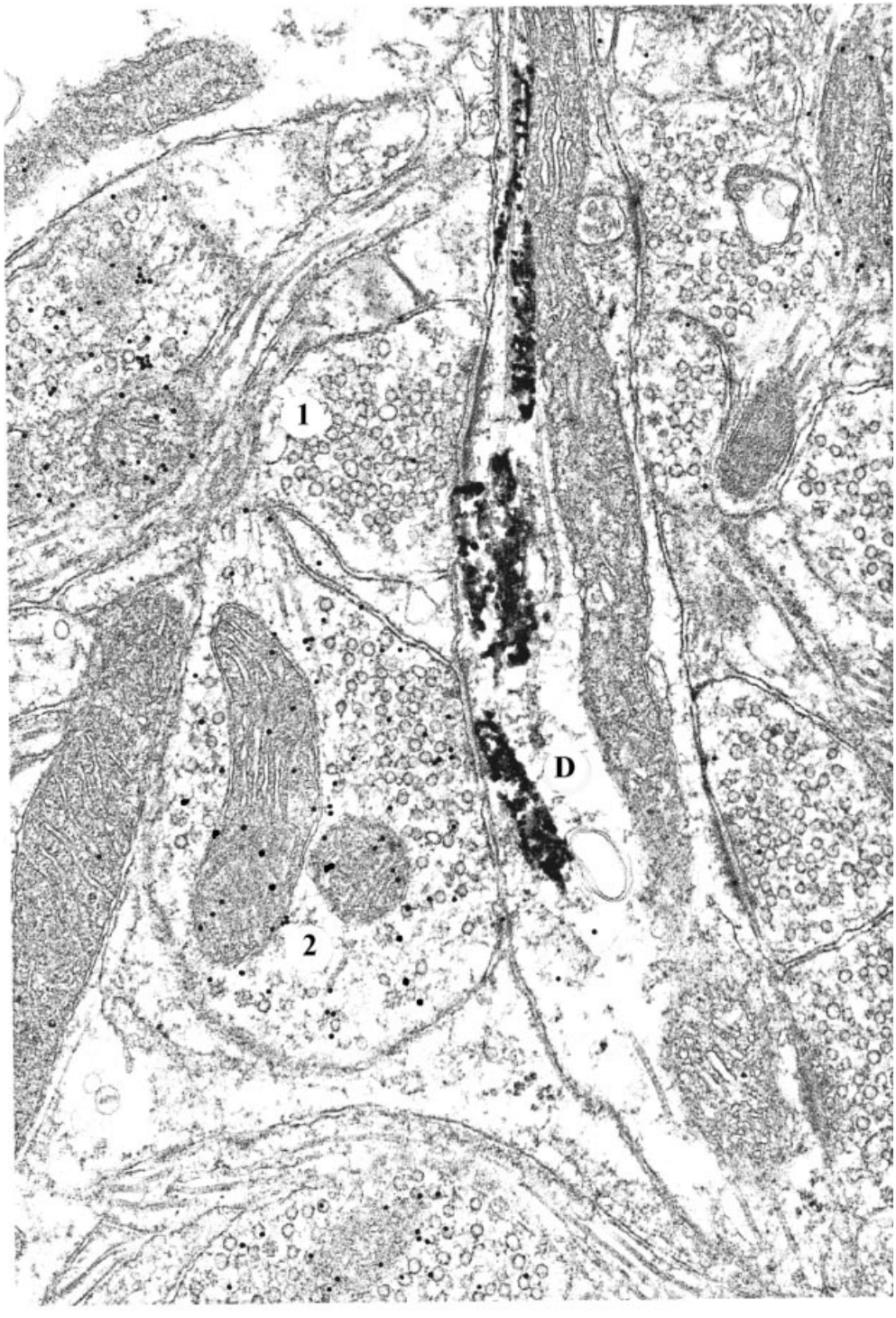
MORPHOLOGICAL CORRELATES OF SYNAPTIC FUNCTION

Obviously different NTs have different synaptic actions and it is of interest to see to what extent there are morphological correlates for these differing activities.

As mentioned previously, an axon generally makes either an axo-dendritic or axo-somatic synapse with another neuron. Gray (1959) has described subcellular features that distinguish these two main types of synapse. Under the electron microscope, his designated type I synaptic contact is like a disk (1–2 μm long) formed by specialised areas of opposed pre- and postsynaptic membranes around a cleft (300 Å) but showing an asymmetric thickening through an accumulation of dense material adjacent to only the postsynaptic membrane. They are now often referred to as asymmetric synapses (Fig. 1.7). A type II junction is narrower (1 μm) with a smaller cleft (200 Å) and a more even (symmetric) but less marked membrane densification on both sides of the junction. In addition the presynaptic vesicles are generally large (300–600 Å diameter), spherical and numerous at the asymmetric type I synapse but smaller (100–300 Å), fewer in number and somewhat flattened or disk-like at the symmetric type II. Vesicles of varying shape can sometimes be found at both synapses, and while some differences are due to fixation problems, the two types of synapse described above are widely seen and generally accepted. They appear to be associated with fast synaptic events so that type I synapses are predominantly axo-dendritic, i.e. excitatory, and utilise glutamate while type II axo-somatic synapses are inhibitory generally utilising GABA, although the separation is not absolute. Asymmetric excitatory synapses outnumber GABA inhibitory symmetric synapses by up to 4:1, even though at such synapses there is usually only one actual synaptic junction whereas at the symmetrical inhibitory synapse there can be a number of such junctions — presumably to ensure adequate inhibitory control.

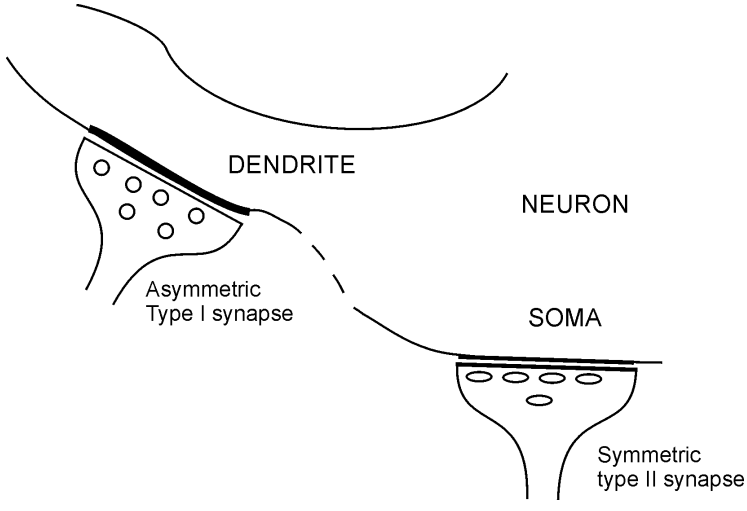
Unfortunately in routine EM (electron microscope) preparations one cannot identify the NT at individual synapses although structural features (shape of vesicle, asymmetric or symmetric specialisations) may provide a clue. At cholinergic synapses the terminals have clear vesicles (200–400 Å) while monoamine terminals (especially NA) have distinct large (500–900 Å) dense vesicles. Even larger vesicles are found in the terminals of some neuro-secretory cells (e.g. the neurohypophysis). One terminal can contain more than one type of vesicle and although all of them probably store NTs it is by no means certain that all are involved in their release.

Anatomical evidence can also be presented to support the concept of presynaptic inhibition and examples of one axon terminal in contact with another are well documented. These do not show the characteristics of either type I or II synapses but



(a)

Figure 1.7 *Caption opposite*



(b)

Figure 1.7 *continued* Different types of synaptic contact. (a) Symmetric and asymmetric synapses. The electromyograph from the anterior nuclear complex of the adult rat thalamus shows two terminals 1 and 2 establishing synaptic contact on the same dendrite. The electro-dense material in the dendrite is a HRP reaction product and identifies the dendrite as that of a thalamocortical projection neuron (the tracer-cholera toxin B conjugated to HRP was injected into the cingulate cortex). Terminal 1 makes a prominent Gray type I (asymmetric) and terminal 2 a Gray type II (symmetric) synaptic contact. The latter is also labelled with gold particles indicating that despite the spherical vesicles obtained in the fixation procedure, it contains GABA since the material was immunoreacted with antibody against GABA (post-embedding immunogold method). The picture was kindly provided by Professor A. R. Lieberman (University College London). Reproduced from Wang *et al.*, *Brain Res. Bull.* **50**: 63–76 (1999) published by Elsevier Science. (b) Schematic representation of asymmetric (Gray type I) and symmetric (Gray type II) synapses. Asymmetric synapses are 1–2 μm long with a 30 nm (300 Å) wide cleft and very pronounced postsynaptic density. Presynaptic vesicles are round (30 nm diameter). Symmetric synapses are shorter (1 μm) with a narrower cleft (10–20 nm, 200 Å) and although the postsynaptic density is less marked it is matched by a similar presynaptic one. The presynaptic vesicles are more disk-like (10–30 nm diameter)

the shape of the presynaptic vesicle is of particular interest because even if the net result of activating this synapse is inhibition, the initial event is depolarisation (excitation) of the axonal membrane. This might suggest that the vesicles should be spherical but since the NT is GABA, normally an inhibitory transmitter, the vesicles could be flattened. Thus, does the type of synapse or the NT and its function determine the shape of the vesicle? Generally the vesicles at these axo-axonic synapses are flattened (or disk-like) but some have spherical vesicles and so while the situation is not resolved vesicle shape tends to be linked with the NT they house.

In the lateral superior olive, antibody studies have shown four types of axon terminal with characteristic vesicles (Helfert *et al.* 1992). Those with round vesicles contain glutamate, those with flattened vesicles have glycine, while large plemorphic vesicles contain glycine and GABA and small plemorphic ones only GABA. Interestingly when GABA and glycine were found in the same terminals in the spinal cord, the post-synaptic membrane had receptors to both NTs.

Dendro-dendritic synapses have also been described which show characteristic synaptic connections and we need to abandon the belief that one neuron can only influence another through its axon terminals. Dendro-dendritic synapses can also be reciprocal, i.e. one dendrite can make synaptic contact with another and apparently be both pre- and postsynaptic to it.

If NTs can have distal non-synaptic effects then nerve terminals that do not make definite synaptic connections could be apparent. In smooth muscle the noradrenergic fibres ramify among and along the muscle fibres apparently releasing noradrenaline from swellings (varicosities) along their length rather than just at distinct terminals. These fibres are termed *en passant* axons (see Fig. 1.2). In the brain many aminergic terminals also originate from *en passant* fibres but it seems that not all of them form classical synaptic junctions.

Monoamines can also be found in terminals at both symmetric and asymmetric synapses, but this may be partly because they co-exist with the classical transmitters glutamate and GABA. The fact that vesicular and neuronal uptake transporters for the monoamines can be detected outside a synapse along with appropriate postsynaptic receptors does suggest, however, that some monoamine effects can occur distant from the synaptic junction (see Pickel, Nirenberg and Milner 1996, and Chapter 6).

For further details on the concept of synaptic transmission and the morphology of synapses see Shepherd and Erulkar (1997) and Peters and Palay (1996) respectively.

NEUROTRANSMITTER ORGANISATION AND UTILISATION

In the periphery at the mammalian neuromuscular junction each muscle fibre is generally influenced by only one nerve terminal and the one NT acts on one type of receptor localised to a specific (end-plate) area of the muscle. The system is fitted for the induction of the rapid short postsynaptic event of skeletal muscle fibre contraction and while the study of this synapse has been of immense value in elucidating some basic concepts of neurochemical transmission it would be unwise to use it as a universal template of synaptic transmission since it is atypical in many respects.

In smooth muscle, by contrast, one sympathetic nerve fibre can influence a number of muscle fibres by releasing noradrenaline from varicosities along its length without there being any defined 'end-plate' junctions. The result of receptor activation is a slow change in potential and inactivation of the NT is initially by uptake and then metabolism. In other words, the NT function is geared to the slower phasic changes in tone characteristic of smooth muscle.

In the CNS there are many forms of neuronal organisation. One neuron can have many synaptic inputs and a multiplicity of NTs and NT effects are utilised within a complex interrelationship of neurons. There are also positive and negative feedback circuits as well as presynaptic influences all designed to effect changes in excitability and frequency of neuronal firing, i.e. patterns of neuronal discharge.

While we should try to exploit such differences between NT systems in developing drugs, rather than adopting a blanket concept of neurotransmission, it is still worth while trying to characterise different types of NT systems in the CNS in order to build up a functional framework and concept. The following patterns are suggested (see Fig. 1.8).

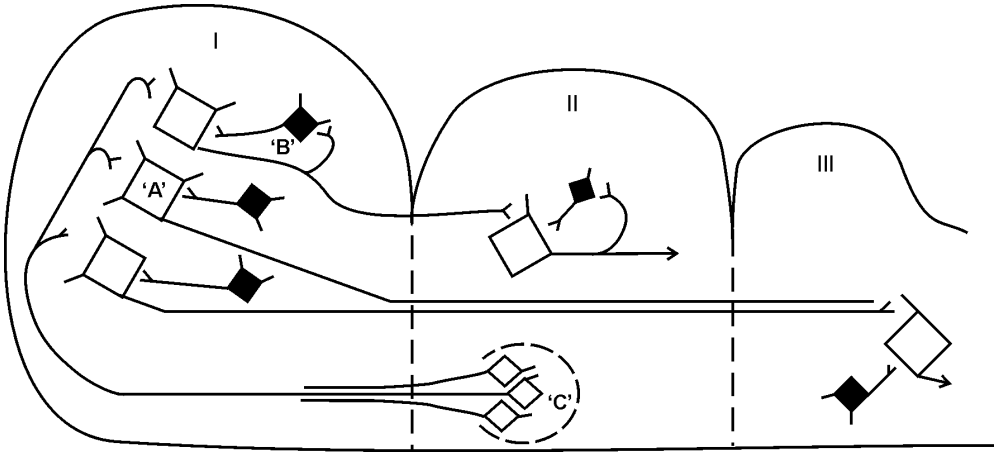


Figure 1.8 Some basic neuronal systems. The three different brain areas shown (I, II and III) are hypothetical but could correspond to cortex, brainstem and cord while the neurons and pathways are intended to represent broad generalisations rather than recognisable tracts. 'A' represents large neurons which have long axons that pass directly from one brain region to another, as in the cortico spinal or cortico striatal tracts. Such axons have a restricted influence often only synapsing on one or a few distal neurons. 'B' are smaller inter or intrinsic neurons that have their cell bodies, axons and terminals in the same brain area. They can occur in any region and control (depress or sensitise) adjacent neurons. 'C' are neurons that cluster in specific nuclei and although their axons can form distinct pathways their influence is a modulating one, often on numerous neurons rather than directly controlling activity, as with 'A'. Each type of neuron and system uses neurotransmitters with properties that facilitate their role

LONG-AXON (CLASSICAL) PATHWAYS (P1)

These include not only pathways with very long axons, such as the cortico-spinal and spino-thalamic tracts but also numerous shorter interconnecting systems, e.g. thalamus to cortex, etc. They may be regarded as the backbone of the CNS. The axons, especially the very long ones, show little divergence and have a relatively precise localisation, i.e. activation of particular motoneurons by stimulating a precise part of the motor cortex. Their influence on neurons is phasic and generally rapid with conventional EPSPs. Distinct axo-dendritic type I asymmetric synapses utilising glutamate acting on receptors (ionotropic) directly linked to the opening of N_a^+ channels are common and these systems form the basic framework for the precise control of movement and monitoring of sensation. Such pathways are well researched and understood by neuro-anatomists and physiologists, but their localised organisation makes them, perhaps fortunately, somewhat resistant to drug action.

INTRINSIC CONTROLLING SYSTEMS (P2)

These are basically neurons whose cell body and axon terminals are both found in the same part of the CNS (Fig. 1.2). They are not concerned with transmitting information from one part of the CNS to another but in controlling activity in their own area. They can be excitatory but are more often inhibitory. They may act postsynaptically through conventional IPSPs (or slower potential changes) or presynaptically by modifying NT

release. The former systems are generally thought to use amino acids as NTs, e.g. GABA or glycine acting on ionotropic receptors linked to Cl^- channels, while the latter systems may use GABA (presynaptic inhibition in cord) or peptides (enkephalin neurons). Excitatory interneurons may use ACh or an amino acid, like aspartate.

Since these interneurons exert a background control of the level of excitability in a given area or system their manipulation by drugs is of great interest (e.g. attemps to increase GABA function in epilepsy), especially if this can be achieved without adversely affecting important primary activity in the area. Although intrinsic neurons can only have a localised action they may be influenced by long-axon inputs to them and so incorporated into long pathway effects (Fig. 1.3(d)), such as the cortical inhibition of motoneurons.

MODULATING SYSTEMS (P3)

These have relatively long axons that originate from neurons that are grouped together in subcortical nuclei of perhaps a few hundred cell bodies but spread to vast areas of the brain and cord. The NTs, generally the monoamines noradrenaline, dopamine and 5-HT, are released at various sites along considerable lengths of the axon and distinct synaptic contacts may not always be seen. They may act either postsynaptically or presynaptically to produce slow changes in activity or modify NT release generally through secondary messenger systems. The tonic background influence of these systems and their role in behaviour have instigated the development and study of many drugs to manipulate their function. It also seems that the cholinergic input into the cortex from subcortical nuclei can also be included in this category (see Chapter 5).

Of course, while the identification of these distinct systems may be useful there are many neural pathways that would not fit easily into one of them. Thus some inhibitory pathways, such as that from the caudate nucleus to substantia nigra, utilising GABA, are not intrinsic neurons. The dopamine pathway from the substantia nigra to striatum may start from a small nucleus but unlike other monoamine pathways it shows little ramification beyond its influence on the striatum. The object of the above classification is not to fit all neural pathways and mechanisms into a restricted number of functional categories but again to demonstrate that there are different forms of neurotransmission.

CO-EXISTENCE (P4)

Although it may be argued that this is not a pattern of NT organisation but merely a feature of some (or possibly all) neurotransmitter systems, it justifies separate consideration. Since there is already good evidence for the existence of a fairly large number of different NTs, which it is assumed are released from their own specific neurons, and as they can produce a diversity of postsynaptic events one might consider the release of more than one NT from one terminal a somewhat unnecessary complication. Nevertheless since co-existence is established, its significance must be evaluated in respect of NT function and drug action. This is considered in more detail later (Chapter 12) but it is important to know which NTs co-exist and whether there is a definite pattern, i.e. does neurotransmitter A always occur with B and never with C and is the ratio A:B always the same? Also what effects do the NTs produce, how do they interact

and are they both necessary for full synaptic transmission? The latter is a vital question for drug therapy based on NT replacement.

Thus it may be that a full understanding of how one NT works at a synapse will require knowledge of how that function depends on the actions of its co-released NT(s). It could unfold a whole new requirement and dimension to our understanding of synaptic physiology and pharmacology and the use of drugs. On the other hand, it may be of little significance in some cases for although cholinergic-mediated nicotinic and muscarinic responses as well as dopamine and peptide effects are observed in sympathetic ganglia, it is only nicotinic antagonists that actually reduce transmission, acutely anyway.

The brain could be likened to a television set in which the amino acids are providing the basic positive and negative power lines, while the other NTs (the multi-coloured wires) control the colour, contrast and brightness. All are required for a perfect picture but some are obviously more important than others.

FUNCTIONAL SYNAPTIC NEUROCHEMISTRY

To achieve their different effects NTs are not only released from different neurons to act on different receptors but their biochemistry is different. While the mechanism of their release may be similar (Chapter 4) their turnover varies. Most NTs are synthesised from precursors in the axon terminals, stored in vesicles and released by arriving action potentials. Some are subsequently broken down extracellularly, e.g. acetylcholine by cholinesterase, but many, like the amino acids, are taken back into the nerve where they are incorporated into biochemical pathways that may modify their structure initially but ultimately ensure a maintained NT level. Such processes are ideally suited to the fast transmission effected by the amino acids and acetylcholine in some cases (nicotinic), and complements the anatomical features of their neurons and the recepter mechanisms they activate. Further, to ensure the maintenance of function in vital pathways, glutamate and GABA are stored in very high concentrations (10 μmol/mg) just as ACh is at the neuromuscular junction.

By contrast, the peptides are not even synthesised in the terminal but are split from a larger precurser protein in the cell body or during transit down the axon. They are consequently only found in low concentrations (100 pmol/g) and after acting are broken down by peptidases into fragments that cannot be re-used. It is perhaps not surprising that they have a supporting rather than a primary role.

In between the above two extremes are the monoamines (1–10 nmol/g) which are preformed and stored in terminals but at much lower concentrations than the amino acids and when released are removed primarily by reuptake for re-use, or intraneuronal metabolism to inactive metabolites. Thus the appropriate synaptic organisation, biochemistry and receptor pharmacology of the NTs also varies in keeping with their function. It is often assumed, incorrectly, that the NTs found in the highest concentration are the most potent. In fact the opposite is true. Those like the amino acids while having high affinity for their receptors have low potency while the peptides found at much lower concentration have high potency but low affinity.

NEUROTRANSMITTER IDENTIFICATION

To achieve NT status a substance must fulfil three main criteria:

(1) *Presence*. It perhaps goes without saying that the proposed transmitter must be shown to be present in the CNS and preferably in the area and at the synapses where it is thought to act.
(2) *Release*. Stimulation of the appropriate nerves should evoke a measurable release of NT.
(3) *Identity of action*. The proposed NT must produce effects postsynaptically which are identical physiologically (appropriate membrane potential changes) and pharmacologically (sensitivity to antagonists) to that produced by neuronal stimulation and the relased endogenous NT.

These criteria should be regarded as guidelines rather than rules. As guidelines they provide a reasonable scientific framework of the type of investigations that must be undertaken to establish the synaptic role of a substance. As rigid rules they could preclude the discovery of more than one type of neurotransmitter or one form of neurotransmission. Nevertheless, the criteria have been widely employed and often expanded to include other features which will be considered as subdivisions of the main criteria.

PRESENCE

Distribution and concentration

It is generally felt that a substance is more likely to be a NT if it is unevenly distributed in the CNS although if it is widely used it will be widely distributed. Certainly the high concentration (5–10 μmol/g) of dopamine, compared with that of any other monoamine in the striatum or with dopamine in other brain areas, was indicative of its subsequently established role as a NT in that part of the CNS. This does not mean it cannot have an important function in other areas such as the mesolimbic system and parts of the cerebral cortex where it is present in much lower concentrations. In fact the concentration of the monoamines outside the striatum is very much lower than that of the amino acids but since the amino acids may have important biochemical functions that necessitate their widespread distribution, the NT component of any given level of amino acid is difficult to establish.

Nevertheless, useful information can be deduced from patterns of distribution. Glycine is concentrated more in the cord than cortex and in ventral rather than dorsal grey or white matter. This alone would be indicative of a NT role for glycine in the ventral horn, where it is now believed to be the inhibitory transmitter at motoneurons. GABA, on the other hand, is more concentrated in the brain than in the cord and in the latter it is predominantly in the dorsal grey so that although it is an inhibitory transmitter like glycine it must have a different pattern of activity.

Lesions in conjunction with concentration studies can also be useful. Section of dorsal roots and degeneration of afferent fibres produces a reduction in glutamate and substance P which can then be associated with sensory inputs. Temporary reduction of the blood supply to the cord causes preferential destruction of interneurons and a greater loss of asparate and glycine, compared with other amino acids and so links

those amino acids with interneurons. Intrinsic neurons can also be destroyed through overactivity caused by kainic acid injections.

Subcellular localisation

A NT might be expected to be concentrated in nerve terminals and this can be ascertained since when nervous tissue is appropriately homogenised the nerve endings break off from their axons and surrounding elements and then reseal. Such elements are known as synaptosomes. They have been widely used to study NT release *in vitro* (Chapter 4) and some NT should always be found in them, at least if it is released from vesicles.

Synthesis and degradation

If a substance is to be a NT it should be possible to demonstrate appropriate enzymes for its synthesis from a precursor at its site of action, although peptides are transported to their sites of location and action after synthesis in the axon or distal neuronal cell body. The specificity of any enzyme system must also be established, especially if they are to be modified to manipulate the levels of a particular NT, or used as markers for it. Thus choline acetyltransferase (ChAT) may be taken as indicative of ACh and glutamic acid decarboxylase (GAD) of GABA but some of the synthesising enzymes for the monoamines lack such specificity.

After release there must be some way of terminating the action of a NT necessitating the presence of an appropriate enzyme and/or uptake mechanism. Such uptake processes can be quite specific chemically. Thus a high-affinity uptake (activated by low concentrations) can be found for glycine in the cord where it is believed to be a NT, but not in the cortex where is has no such action. This specific uptake can be utilised to map terminals for a particular NT, especially if it can be labelled, and also for loading nerves with labelled NT for release studies.

Of course, since CNS function depends on changes in the rate of neuronal firing, determined by a subtle balance between a number of different excitatory and inhibitory inputs, it may not always be necessary to destroy the NT rapidly. Excessive firing of a neuron may be controlled by activating a feedback inhibitory system or evoking presynaptic inhibition. There is also evidence for the release of the degrading enzyme together with NT at some purinergic (ATP) synapses (Kennedy *et al.* 1997) and possibly some cholinergic ones.

Pathways

If a substance (or its synthesising or degradative systems) can be demonstrated in particular neurons with a distinctive pattern of distribution, or bunched together into a well-defined nerve tract and/or nucleus, then this is not only good evidence for its role as a NT but it tells us something of its function. Indeed the distinct patterns of distribution of ascending monoamine pathways from brainstem nuclei could probably be considered as adequate evidence alone for their neurotransmitter role. In practical terms we can, of course, only study the release (and actions) of an endogenous NT if it can be evoked by stimulating an appropriate nerve pathway. Also the neurological and

behavioural consequences of lesioning such pathways can tell us much about the functions of the NT. It is therefore useful to try to map NT pathways.

Receptors

If a NT is to be effective, there must be receptors for it to act on. Thus demonstrating the presence of receptors for the proposed NT at sites where it is found is further proof of its NT role. This could be done by recording some effect of the NT, e.g. change in neuronal firing, by establishing specific binding sites for it using it in a labelled form, or showing the presence of its receptor mRNA. Unfortunately a substance can bind to sites other than a receptor (e.g. uptake sites) and not all receptors are innervated.

RELEASE

A substance cannot be considered as a NT unless it is released. Unfortunately, although it may be possible to show the presence of a substance and some effect when it is applied directly to neurons its release may not be measurable for technical reasons. This is even more true if one strives for the ideal of demonstrating the release of an endogenous substance by physiological stimuli.

In the CNS access to the site of release is a major problem and attempts to achieve it have led to the development of a wide range of techniques of varying complexity and ingenuity or to short-cuts of dubious value (see Chapter 4). The feasibility of release studies in the CNS is to some extent dependent on the type of NT being studied. If we are dealing with a straightforward neural pathway with a number of axons going from A to B then by stimulating A and perfusing B we should be able to collect the NT. Unfortunately such arrangements are rare in the CNS and where they exist (e.g. corticospinal tract) it is not easy to perfuse the receiving (collecting) area. Sometimes the origin of a pathway is clear and easy to stimulate, e.g. NA fibres in the locus coeruleus, but fibre distribution in the cortex is so widespread that collection of sufficient amounts for detection can be very difficult, although current methods are beginning to achieve it.

These approaches are, in any case, only suitable for classical neurotransmitters. Those with slow background effects will probably not be released in large amounts. For such substances we require a measure of their utilisation, or turnover, over a much longer period of time. With NTs released from short-axon interneurons there are no pathways to stimulate and it becomes necessary to activate the neurons intrinsically by field stimulation, which is of necessity not specific to the terminals of the interneurons.

Apart from actually demonstrating release it is important to consider how NTs are released and whether they all need to be released in the same way, especially if they do different things. The variable time-courses of NT action referred to previously may require NTs to be released at different rates and in different ways, only some of which are achievable by, or require, vesicular mechanisms and exocytosis (see Chapter 4).

It should be remembered that with the possible exception of voltammetry when the monitoring electrode is sufficiently small to reach synapses, it is not the actual release of the NT that is being measured in perfusion studies. It is overflow. As discussed previously, most of any released NT is either physically restricted to the synapse or destroyed before it can diffuse away.

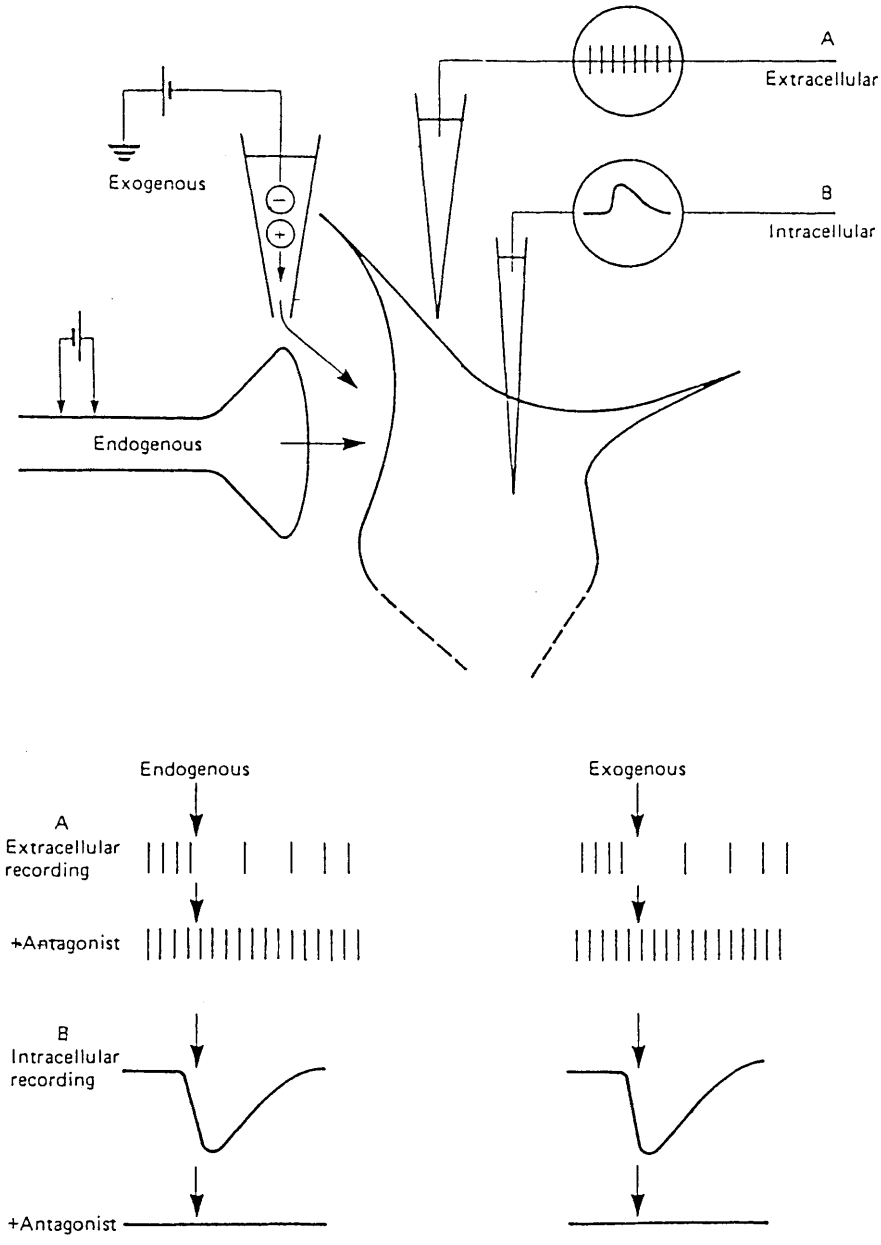


Figure 1.9 Comparison of the effects of an endogenously released and exogenously applied neurotransmitter on neuronal activity (identity of action). Recordings are made either of neuronal firing (extracellularly, A) or of membrane potential (intracellularly, B). The proposed transmitter is applied by iontophoresis, although in a brain slice preparation it can be added to the bathing medium. In this instance the applied neurotransmitter produces an inhibition, like that of nerve stimulation, as monitored by both recordings and both are affected similarly by the antagonist. The applied neurotransmitter thus behaves like and is probably identical to that released from the nerve

IDENTITY OF ACTION

Many people consider this to be the most important of all the criteria. Obviously a substance must have an effect of some kind if it is to be a NT but not all substances that have an effect on neurons need to be NTs. It may seem unnecessary to say this but the literature contains many accounts of the study of various substances on neuronal activity from which a NT role is predicted without any attempt to compare its effect with that of physiologically evoked (endogenous NT) effects. The importance of this safeguard is highlighted by the ease with which both smooth muscle and neurons will respond to a range of substances that are not released onto them as NTs. Thus the value of this criterion depends very much on the rigour with which it is applied and on its own is no more or less important than any other approach.

Ideally it should be shown that application of the proposed NT to a neuron, e.g. by iontopheresis (see Chapter 2), produces changes in membrane potential that are identical to and mediated by the same ionic mechanism as those produced by nerve stimulation and that the effects of both are equally overcome by an appropriate chemical antagonist. The basic system is outlined in Fig. 1.9. Clearly, changes in membrane potential can only be recorded if the neuron is large enough to take an intracellular electrode and even if it can be shown that the applied and released NT produce similar changes in membrane potential and share a common reversal potential and ionic mechanism this would not be so surprising, since the number of available ionic mechanisms is limited (i.e. both GABA and glycine produce hyperpolarisation by increasing chloride influx). Now that the properties of single ion channels can be recorded using modern patch-clamp techniques it will be necessary to show that application of the presumed NT produces identical changes in the frequency (n), degree (y, amount of current conducted) and duration (r) of channel opening to that achieved by synaptic activation. Unfortunately such a detailed analysis is presently only applicable to relatively simple systems with restricted innervations.

The use of antagonists is absolutely vital but even they can give false positives. Thus GABA, B-alanine and glycine all produce hyperpolarisation of cord motoneurons by increasing chloride influx but only GABA is unaffected by strychnine. Since strychnine abolishes inhibition in the cord, GABA cannot be the inhibitory NT but other features (distribution, release) had to be satisfied before glycine rather than B-alanine was shown to have that role.

It must be remembered that a substance can only be shown to be identical in its action with that of a particular endogenous NT if the latter's precise mode of action is clearly established and easily studied. Thus it may be relatively easy to consider those NTs mediating classical postsynaptic excitation through distinct potentical change but more difficult for NTs which function over a much longer time-course and possible without producing recordable potentical changes. Nevertheless they are still NTs. Or are they?

WHAT IS A NT?

The question is obviously an important one. Substances released from neurons are not always called neurotransmitters. Some of them are referred to as neuromodulators, neurohormones, neurotrophic factors or neurotoxins but since they all produce some effect on a neuron they could be said to have a transmitter role and justify the term

neurotransmitter. On that basis every substance mentioned already and to be discussed further could be called a neurotransmitter. Even if we did try to distinguish between, say, a fast neurotransmitter and a slow neuromodulater effect, we have to realise that one substance can easily have both actions either at different synapses (ACh) or the same one (glutamate — ionotropic and metabotropic effects) and so could be both a neurotransmitter and a neuromodulater. If, however, the response they set up (transmit) has no reasonably quick and recordable effect, i.e. takes hours or days to develop (e.g. growth factors), or can actually kill the neuron (nitric oxide) it is difficult to conceive of them as neurotransmitters, however interesting they may be.

What this discussion does highlight, however, is that some modification is required to the standard dictionary definition of a neurotransmitter given in the introduction to this chapter, which sees a NT as a substance that transmits the impulse from one neuron to another neuron (or excitable cell). A more comprehensive definition of a NT might be

> A substance preformed, stored and then released from a neuron by a calcium dependent exocytotic mechanism activated by invading action potentials which induces a change in excitability and function of an adjacent neuron without entering the bloodstream.

This description would cover the classical NTs such as glutamate, GABA, ACh, DA, NA, and 5-HT as well as some peptides and ATP. That is irrespective of whether the effect produced by them is basic to the actual process of transmitting an impulse from one neuron to another, as with glutamate and ACh, rapidly inducing inhibition (GABA) or just making the neuron more or less responsive to other inputs (monoamines, peptides).

There is no room within the definition for nitric oxide, the prostaglandins and steroids mainly because they are not released in a controlled manner by neuronal activity and only the last are preformed. Thus if they are to be classified as NTs then the definition must be simplified so that a NT becomes:

> A substance produced in and released from a neuron to affect some aspect of neuronal function without being transported in the blood.

This would encompass those steroids synthesised in the CNS but not those entering it from the circulation (see Chapter 13).

While I feel that substances which only meet the abbreviated definition do not justify being called neurotransmitters, they will be treated as such in this text because this is the accepted practice and they are substances released from neurons to affect CNS function. Clearly, it is more important to distinguish between the different effects that a substance can produce when released from a nerve than to worry about what it is called. Nevertheless, it is unfortunate that the word neurotransmitter will inevitably be associated with the actual transmission of activity from one neuron to another and yet most of the substances we will be discussing do not actually do that.

REFERENCES

Barbour, B and Hausser, M (1997) Intersynaptic diffusion of neurotransmitter. *Trends Neurosci.* **20**: 377–384.

Brezina, V and Weiss, KR (1997) Analysing the functional consequences of transmitter complexity. *Trends Neurosci.* **11**: 538–543.

Dale, HH (1935) Pharmacology and nerve endings. *Proc. Roy. Soc. Med.* **28**: 319–332.

Gray, EG (1959) Axo-somatic and axo-dendritic synapses of the cerebral cortex. An electron-microscope study. *J. Anat*. **93**: 420–433. See also Gray, EG (1976) Problems of understanding the substructure of synapses. *Progr. Brain Res*. **45**: 208–234.

Helfert, RH, Juiz, JM, Bledsoe, SC, Bonneau, JM, Wenthold, RJ and Altschuler, RA (1992) Patterns of glutamate, glycine and GABA immunolabelling in four synaptic terminal classes in the lateral superior olive of the guinea pig. *J. Comp. Neurol*. **323**: 305–325.

Kennedy, C, Todorov, LD, Mihaylova-Todorova, S and Sneddon, P (1997) Release of soluble nucleotidases; a novel mechanism for neurotransmitter inactivation. *Trends Pharmacol. Sci*. **18**: 263–266.

Langer, SZ (1981) Presynaptic regulation of the release of catecholamines. *Pharmac. Rev*. **32**: 337–362.

Langer, SZ (1997) 25 years since the discovery of presynaptic receptors; present knowledge and future perspectives. *Trends Pharmacol. Sci*. **18**: 95–99.

Peters, A and Palay, SL (1996) The morphology of synapses. *J. Neurocytol*. **25**: 687–700.

Pickel, VM, Nirenberg, MJ and Milner, TA (1996) Ultrastructural view of central catecholaminergic transmission; immunocytochemical localization of synthesising enzymes, transporters and receptors. *J. Neurocytol*. **25**: 843–856.

Shepherd, GM and Erulkar, SD (1997) Centenary of the synapses from Sherrington to the molecular biology of the synapse and beyond. *Trends Neurosci*. **20**: 385–392.