

# 1

## Principles of Brain Function and Structure: 1 Genetics, Physiology and Chemistry

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### INTRODUCTION

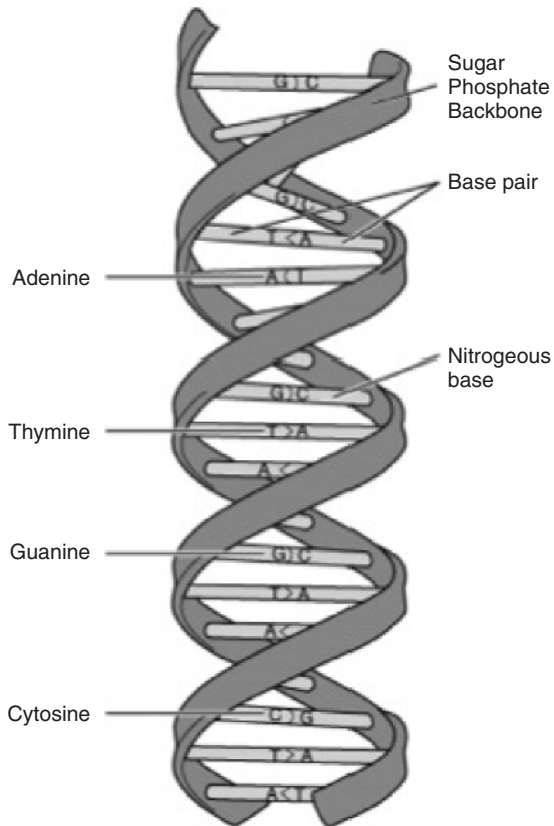
It has become fashionable periodically to ascribe much psychopathology to the evils of modern society, and the resurgence of this notion from time to time reflects the popularity of the simple. Often imbued with political overtones, and rarely aspiring to scientific insights, such a view of the pathogenesis of psychiatric illness ignores the long tradition of both the recognition of patterns of psychopathology and successful treatment by somatic therapies. Further, it does not take into account the obvious fact that humanity's biological heritage extends back many millions of years.

In this and the next chapter, consideration is given to those aspects of the neuroanatomy and neurochemistry of the brain that are important to those studying biological psychiatry. Most emphasis is given to the limbic system and closely connected structures, since the understanding of these regions of the brain has been of fundamental importance in the development of biological psychiatry. Not only has a neurological underpinning for 'emotional disorders' been established, but much research at the present time relates to the exploration of limbic system function and dysfunction in psychopathology.

### GENETICS

Every cell in the human body contains the nuclear material needed to make any other cell. However, cells differentiate into a specific cell by expressing only partially the full genetic information for that individual. While it is beyond the scope of this book to fully explain all of modern genetics, it is important to grasp several basic aspects that are involved in building and maintaining the nervous system, and which may impact on psychiatric diseases.

Modern genetic theories are based on knowledge of the deoxyribonucleic acid (DNA) molecule, its spontaneous and random mutations and the recombination of its segments. DNA is composed of two intertwined strands (the double helix) of sugar-phosphate chains held together by covalent bonds linked to each other by hydrogen bonds between pairs of bases. There is always complementary pairing between the bases, such that the guanine (G) pairs with cytosine (C), and the adenine (A) with thymine (T). This pairing is the basis of replication, and each strand of the DNA molecule thus forms the template for the generation of another. Mammalian DNA is



**Figure 1.1** DNA replication and transcription. During transcription, the DNA strands separate, and one is transcribed. The primary mRNA transcript is a copy of the DNA strand, except that a U has been substituted for every T (reproduced with permission from Professor Nick Wood)

supercoiled around proteins called histones (Figure 1.1). Recently it has been discovered that histones can be modified by life experiences. The actual protein folding of the genetic materials changes as a function of histones or methylation on the DNA. Early life experiences can actually cause large strands of the DNA to become silent and not expressed (McGowan *et al.*, 2008 ; Parent and Meaney, 2008).

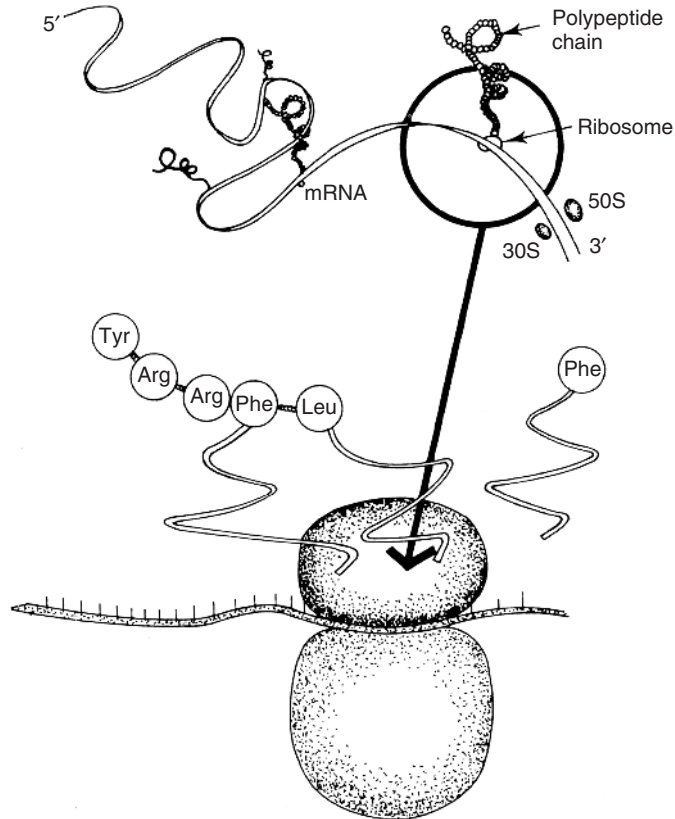
On the DNA strand are many specific base sequences that encode for protein construction. Thus, proteins are chains of amino acids, and one amino acid is coded by a triplet sequence of bases (the codon). For example, the codon TTC codes for phenylalanine.

DNA separates its double helix in a reaction catalysed by DNA polymerase. In the synthesis of protein, ribonucleic acid (RNA) is an intermediary. RNA is almost identical to DNA, except that uracil (U) replaces thymine, the sugar is ribose, and it is single-stranded. Thus, an RNA molecule is created with a complementary base sequence to the DNA, referred to as messenger RNA (mRNA). This enters the cytoplasm, attaches to ribosomes, and serves as a template for protein synthesis. Transfer RNA (tRNA) attaches the amino acids to mRNA, lining up the amino acids one at a time to form the protein. The tRNA achieves this by having an anticodon at one end attach to the mRNA and the amino acid at the other (see Figure 1.2). Coding occurs between the start and stop codons.

Much is now known regarding the various sequences of bases that form the genetic code. In total, chromosomal DNA in the human genome has approximately 3 billion base pairs. There are 20 amino acids that are universal constituents of proteins, and there are 64 ways of ordering the bases into codons (Wolpert, 1984). Most amino acids are represented by more than one triplet, and there are special techniques for starting and stopping the code. Although it was thought that the only direction of information flow was from DNA to RNA to protein, investigations of tumour cells have revealed retroviruses: RNA viruses that can be incorporated into host DNA.

The genetic programme is determined by DNA, and at various times in development, and in daily life, various genes will be turned on or off depending on the requirements of the organism. There is a constant interplay between the genetic apparatus and chemical constituents of the cell cytoplasm.

In the human cell there is one DNA molecule for each chromosome, and there are some 100 000 genes on 46 chromosomes. This constitutes only a small portion of the total genomic DNA, and more than 90% of the genome seems non-coding. About 50% of human DNA consists of short repetitive sequences that either encode small high-abundance proteins such as histones, or are not transcribed. A lot of these are repetitive sequences dispersed throughout the genome, or arranged as regions of tandem repeats, referred to as satellite DNA.



**Figure 1.2** RNA translation. The mRNA (top) acts as a template that specifies the sequential attachment of tRNAs: amino acid complexes to make a protein shown in the bottom of the figure. Coding occurs between the start codon (3') and ends at the stop codon (5') (reproduced with permission from Lowenstein *et al.*, 1994; *Biol Psych*, 2 Ed, p. 44)

Such repeats are highly variable between individuals, but are inherited in a Mendelian fashion. These variations produce informative markers, and when they occur close to genes of interest are used in linkage analysis. Complimentary cDNA probes are produced using mRNA as a template along with the enzyme reverse transcriptase. The latter is present in RNA viruses; HIV is a well-known example. Reverse transcriptase permits these viruses to synthesize DNA from an RNA template. It is estimated that 30–50% of the human genome is expressed mainly in the brain.

Retroviruses enter host cells through interaction at the host cell surface, there being a specific receptor on the surface. Synthesis of viral DNA then occurs within the cytoplasm, the RNA being transcribed into DNA by reverse

transcriptase, and the viral DNA becoming incorporated into the host's genome.

Oncogenes are DNA sequences homologous to oncogenic nucleic acid sequences of mammalian retroviruses.

In the human cell the chromosomes are divided into 22 pairs of autosomes, plus the sex chromosomes: XX for females and XY for males. Individual genes have their own positions on chromosomes, and due to genetic variation different forms of a gene (alleles) may exist at a given locus. The genotype reflects the genetic endowment; the phenotype is the appearance and characteristics of the organism at any particular stage of development. If an individual has two identical genes at the same locus, one from each parent, this is referred to as being a homozygote; if they differ, a heterozygote. If a

heterozygote develops traits as a homozygote then the trait is called dominant. There are many diseases that are dominantly inherited. If the traits are recessive then they will only be expressed if the gene is inherited from both parents. Dominant traits with complete penetrance do not skip a generation, appearing in all offspring with the genotype.

If two heterozygotes for the same recessive gene combine, approximately one in four of any children will be affected; two will be carriers, and one unaffected. When there is a defective gene on the X chromosome, males are most severely affected, male-to-male transmission never occurs, but all female offspring of the affected male inherit the abnormal gene.

Many conditions seem to have a genetic component to their expression, but do not have these classic (Mendelian) modes of inheritance. In such cases polygenetic inheritance is suggested.

Mitochondrial chromosomes have been identified. They are densely packed with no introns and they represent around 1% of total cellular DNA. They are exclusively maternally transmitted. Unlike nuclear chromosomes, present normally in two copies per cell at the most, there are thousands of copies of the mitochondrial chromosomes per cell.

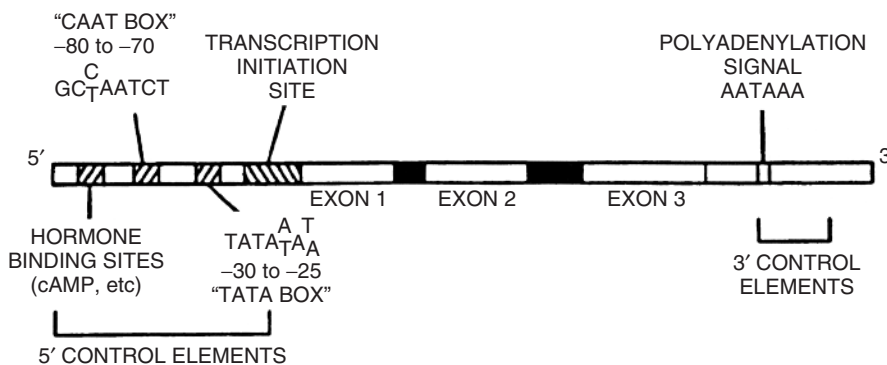
In the gene there are coding sequences, called exons, and intervening non-coding segments referred to as introns. Some sequences occur around a gene, regulating its function. It is not unusual for the genes of even small proteins

to be encoded in many small exons (under 200 bases) spread over the chromosome. Most genes have at least 1200 base pairs, but are longer because of introns. Further, important sequences precede the initiation site (or 59), and the end of the gene (39). A model of a generic gene is shown in Figure 1.3. The promoter region is at the 5' end, containing promoter elements and perhaps hormone binding sites. These activate or inhibit gene transcription. The coding region consists of sequences that will either appear in the mature mRNA (exons) or be deleted (introns).

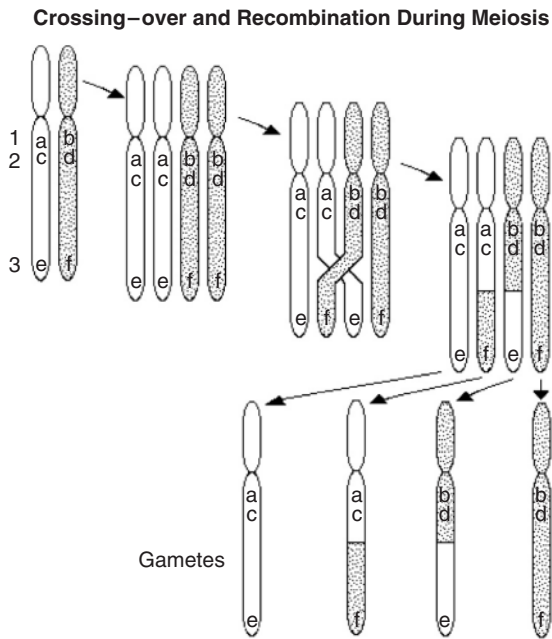
During meiosis, the strands from the two chromosomes become reattached to each other, but each chromosome carries a different allele. There is then a new combination of alleles in the next generation, this exchange being referred to as recombination. The frequency of a recombination between two loci is a function of the distance between them: the closer they are, the less is the likelihood that a recombination will occur between them (Figure 1.4).

Linkage analysis places the location of a particular gene on a chromosome; physical mapping defines the linear order among a series of loci. Genetic distance is measured in centimorgans, reflecting the amount of recombination of traits determined by genes at the two loci in successive generations.

In mutations, unstable mRNA is produced and cannot be translated into a functional polypeptide. Mutations may be referred to as point mutations (substitution of single



**Figure 1.3** A 'generic gene'. Three exons (white) and two introns (black) are shown (reproduced with permission from Ciaranello *et al.*, 1990; *Biol Psych*, 2 Ed, p. 46)



**Figure 1.4** The principle of crossover. If there are two genes A and C, the closer together they are, the more likely they are to remain together during meiosis (reproduced with permission from Professor Nick Wood)

incorrect nucleotide), deletions, insertions, rearrangements or duplications.

Molecular cloning techniques allow for the study of gene structure and function. Restriction endonucleases cut the DNA molecule at specific sites, allowing the fragments to be replicated on a large scale by transfecting other organisms, which produces multiple copies of an inserted DNA section. Foreign fragments of DNA are inserted into a plasmid, a cosmid or a bacteriophage vector capable of autonomous replication in a host cell: the process of cloning. Recombinant DNA molecules are amplified by growth in the host (e.g. bacteria) and then subsequently isolated and purified. Once isolated, complementary DNA (cDNA) can be chemically sequenced, introduced into a host cell to produce encoded protein, or hybridized to genomic DNA to examine the structure of the genes encoding for the target protein.

Complementary DNAs are made from mRNAs, prepared from the tissue of interest,

and then propagated *in vitro* to form cDNA libraries. A gene probe is a fragment of DNA that detects its complementary sequence. Often such cDNA probes are produced from animal protein, and for most diseases they are not specifically related to a disease gene, but may be linked to it genetically.

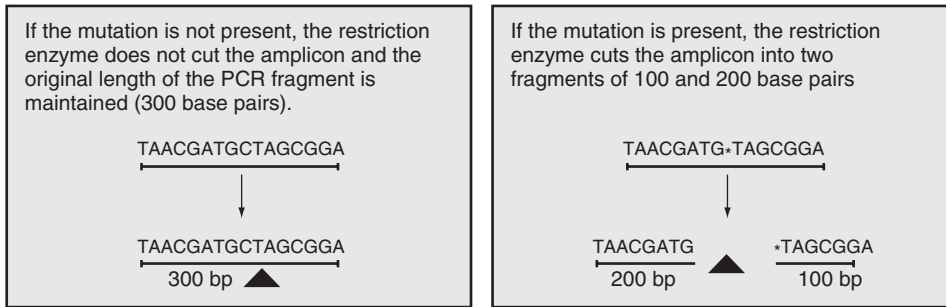
The 'lod' score refers to the 'log of the odds' and expresses the relative probability that two loci are linked as opposed to not linked. Thus, given a disease gene and a known DNA marker, if they co-segregate together more than by chance, they may be linked; the tighter the linkage, the greater the probability. The log value of the relative probability of linkage is the lod score, and a positive score of  $>3$  is usually taken as proof of linkage. This means that the odds are 1000 to 1 that the correlation is the result of gene linkage, rather than chance. A lod score of  $-2$  excludes linkage.

Lod scores from independent family observations are added together, overcoming the problem of the small size of the human families that are usually available for observation. Lod scores are calculated with available computer algorithms, and thus quantify probable linkage, but are most effective for conditions with Mendelian inheritance.

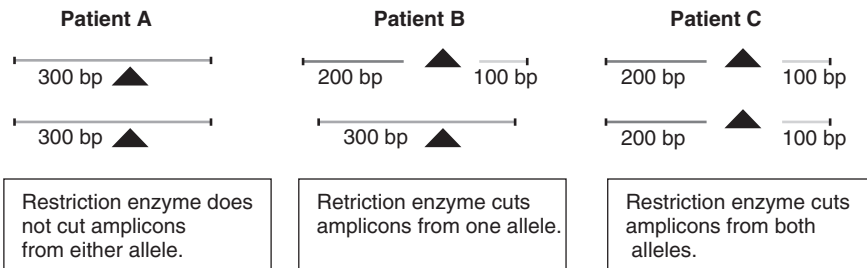
In modern genetics, restriction enzymes are used to split the DNA segments, which can then be recognized by gene probes. Restriction-fragment-length polymorphisms (RFLP) are DNA fragments that differ in length between individuals. Tandem repeated sequences vary between individuals, and smaller sequences of repeats are referred to as mini-satellites. Micro-satellites are very short sequences of repeated dinucleotides, usually GT, that are useful for mapping (Figure 1.5).

These satellite sequences and mutations are detected as RFLPs. The technique involves taking tissue – the origin is immaterial – and splitting the DNA into fragments. These are then displayed by a hybridization blot method (Southern blot), which depends on the ability of DNA to bind to nitrocellulose paper. The fixed DNA fragments are then hybridized with a radioactive DNA probe and detected by the subsequent band pattern dependent on

**Step 1: Digestion.** Amplicons (PCR-amplified DNA segments) are cut (▲) with a restriction enzyme wherever a specific DNA sequence occurs.

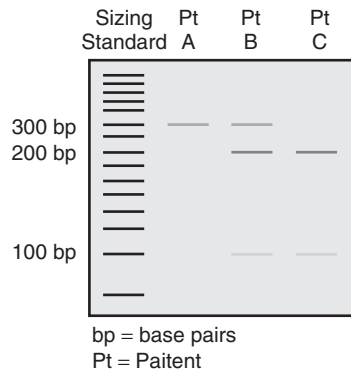


Three outcomes are possible:



**Step 2: Visualization of cut and uncut amplicons on an electrophoretic gel.**

The size of the DNA fragment determines the distance it migrates on the gel; short fragments travel farther than long fragments.



**Step 3: Interpretation.**

- Patient A:** No copies of mutation
- Patient B:** Heterozygous for mutation
- Patient C:** Homozygous for mutation

**Figure 1.5** Restriction fragment-length polymorphism method to test for genetic variants (reproduced with permission from [www.genetests.org](http://www.genetests.org) and the University of Washington, Seattle)

the speed of migration with electrophoresis. Libraries of DNA probes are available from across the spectrum of the human genome.

Recently, the discovery of the polymerase chain reaction has revolutionized the analysis of RFLPs. In this technique, sufficient high-quality

DNA is produced by biological amplification using DNA polymerase, increasing the speed and power of analysis.

The RFLPs are used as genetic markers for inherited diseases if they can be shown to be linked to a gene that is thought to be abnormal



and responsible for the condition. Families in which there are several affected members are investigated and RFLPs are probed to detect only those that are present in affected individuals. When found, the chromosome on which the abnormal gene resides can be identified, and the linked marker, which may be the gene itself, is used to detect genotypes likely to become phenotypes. There have been three generations of marker loci used for genetic linkage mapping. The first was the RFLPs described above, followed by the micro-satellites. More recently the single-nucleotide polymorphisms (SNPs, pronounced 'snips') have been used for the same purpose of tracking genetic variants in pedigrees. A SNP is a silent genetic variation in which one nucleotide (AGTC) is replaced by another, often not affecting the expression of the gene. These minor changes are useful in tracking genetic changes over time and in different populations.

Linkage studies are complicated by incomplete penetrance, age of onset of disease, variable expression of that disease and genetic heterogeneity (more than one gene leading to the phenotype).

The identity of a gene marker linked to Huntington's chorea in 1983 provided the first example of the locating of an autosomal dominant gene in neuropsychiatry. This is known to be on chromosome 4, and encodes a structurally unique protein of 348 kd (huntingtin). There is a specific trinucleotide repeat (CAG), which varies from 9 to 37 copies in normals, but can be massively expanded (>100 copies) in Huntington's chorea. It is also possible to pinpoint genes for specific enzymes and receptors; for example, the D<sub>2</sub> receptor at 11q22, and tyrosine hydrolase at 11p15.5.

Despite now having mapped the entire human genome, as well as the genome of many other plants and animals, there has been considerable difficulty in locating specific genes for psychiatric illnesses. The initial breakthroughs (see chapters on individual diseases) have not been replicated in many cases. This relates partly to difficulties of clinical diagnosis and partly to problems of genetic modelling. Thus, the same data set is used to generate the

lod score that is used to construct the model for inheritance in the first place.

Building on the world of genomics (study of genes), scientists are now investigating the world of proteomics (the study of the proteins an individual expresses, which change over time). This is immensely richer than even genetics, as the proteins that the RNA transcribes at any given moment are even more variable than the 'largely' static genetic information. In the example above, in Huntington's disease the abnormal protein made by the gene variation is huntingtin; the build-up of toxic proteins then goes on to impair intracellular dynamics and receptor and neuronal function, resulting in disease. A surprising finding of the Human Genome Project is that there are far fewer protein encoding genes than there are proteins in the human body. How can this be? Obviously there must be other factors – where the environment interacts with how genes make proteins – that explain this paradox. The whole study of how the micro-environment within the cell can impact back on which genes are expressed and when is called 'epigenetics'. Some of the more interesting new theories about stress and trauma involve the lifelong effect of the social environment (childhood stress) on gene expression (see Chapters 6 and 8).

It is important to remember that genes code for some facet of a disorder, and not a DSM category. Recently the concept of endophenotypes has emerged. This refers to a biomarker which is intermediate between the genotype (pure gene) and the phenotype (the individual with a disease). The endophenotype is closer to the genetic variation, and represents a risk factor or propensity for a behaviour that then leads to the development of a syndrome (Chapter 4). For example, someone might inherit a gene that codes for a particular cognitive profile (abnormal sensory gating or startle or problems with executive function) which predisposes someone to develop psychosis.

The genes that code for proteins make up about 1.5% of the human genome, and at least some of the rest regulates gene expression. The transcription factors turn on DNA sequences called enhancers, which determine that genes

are expressed only where they are wanted and at the time that they are wanted. The enhancers are often hundreds of base pairs in length, and may not be sited near the gene itself. They allow the same gene to be used over and over in different contexts, and from an evolutionary point of view may allow individual traits to be modified without changing the genes themselves. In understanding genetics, the study of the role of enhancers is in its infancy (Carroll *et al.*, 2008).

## BRAIN CHEMISTRY AND METABOLISM

In order to function adequately, the brain requires energy which is derived from the catabolism of the food we eat. The major nutrient for the brain is glucose, which, in the process of oxidization to carbon dioxide ( $\text{CO}_2$ ) and water gives up energy. This process results in the formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). In the course of energy use through cellular transport and biosynthesis the ATP is degraded to ADP (see Figure 1.6).

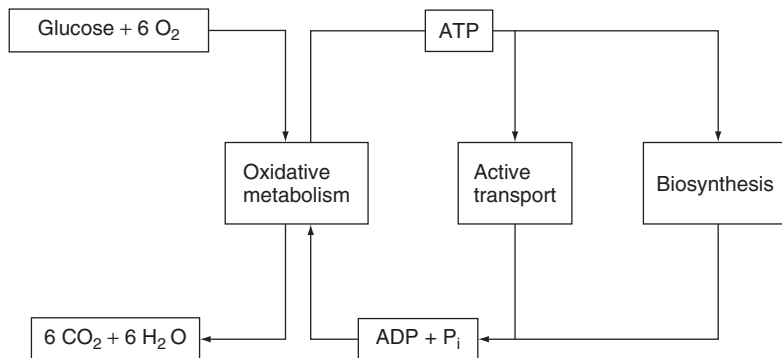
Glucose enters cells via transporters, and glucose utilization is higher in astrocytes than neurones. Glycogen in the brain is stored mainly in astrocytes, and the latter release energy to neurones via pyruvate and lactate. Glycogen turnover is rapid and coincides with synaptic activity. Several neurotransmitters (such as the monoamines) are glycogenolytic, releasing energy from the astrocytes. Another important

function of astrocytes is to remove glutamate from the synaptic space, which is recycled via glutamine back to neurones to replenish the glutamate pool. The metabolism of glucose in the brain is highly temporospatially specific and linked to neuronal-gial (the neutropil) activity, a property which has been used in the theories of glucose PET imaging (see Chapter 4) and is also important for understanding functional MRI.

The oxidation of one molecule of glucose generally gives 36–38 moles of ATP (Siesjo, 1978). It is obvious that ATP has a central role in cellular metabolism, being the product of oxidation and the substrate for further chemical reactions requiring energy. It is composed of a nitrogenous base (adenine), a five-carbon sugar and attached phosphate groups; it is one of several triphosphonucleotides in the cell that yield energy.

As noted, the main energy requirements are those of cellular transport mechanisms and biosynthesis. The former includes the movement of both charged and uncharged particles across cell membranes and the transport of molecules intracellularly. Biosynthesis involves the formation of simple and complex molecules required for cellular function, in addition to such energy storage molecules as glycogen.

A related molecule is adenosine 3,5-monophosphate (cyclic AMP), which has one phosphate molecule and forms a ring structure with links between the sugar and phosphate molecules. It is formed from ATP in a reaction



**Figure 1.6** Showing the generation of ATP by means of oxidative metabolism with glucose as substrate. Oxidation of 1 mole of glucose yields 36–38 moles of ATP (reproduced with permission from Siesjo, 1978; *Biol Psych*, 2 Ed, p. 50)



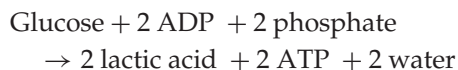
that utilizes adenyl cyclase as a catalyst. It is activated by adrenaline and is known to be involved, via the intermediary phosphorylase, in the activation of glycogen. Further, cyclic AMP appears to act as an intermediary in many other cellular reactions, including those stimulated by hormonal or neurotransmitter stimuli (see below).

Chemical reactions in the body are facilitated by enzymes, some of which themselves are activated by coenzymes which transfer atoms or groups of atoms from one molecule to another. Since the quantity of active enzyme present is the essential ingredient that determines the rate of a biochemical reaction in the presence of appropriate substrates, activation and inhibition of enzymes regulates the metabolic activity of cells. One method of inhibition of a particular metabolic reaction is by feedback from a resulting metabolite (feedback inhibition).

## THE METABOLISM OF GLUCOSE

Glucose and glycogen catabolism result in the production of ATP via the well-known tricarboxylic acid (Krebs) cycle (see Figure 1.7). Glucose is first phosphorylated by hexokinase

to yield glucose-6-phosphate. This is converted, via the several intermediary steps of glycolysis, to lactic acid. This yields two molecules of ATP thus:



Lactic acid is converted to acetyl-coenzyme A via pyruvic acid, and the former is oxidized by the tricarboxylic acid cycle to citrate and ultimately oxaloacetate, which itself is incorporated with acetyl-coenzyme A to yield citrate. Hydrogen atoms that are generated react with oxygen to form water, and further molecules of ATP are generated. Since other products of digestion undergoing catabolism also utilize the tricarboxylic acid cycle, it represents a final common path, and almost two-thirds of all energy released in the breakdown of food occurs during the reactions of this cycle.

## PROTEINS AND FATTY ACIDS

While glucose is the most important food involved in metabolism, proteins and fatty acids are also involved. Protein is broken down

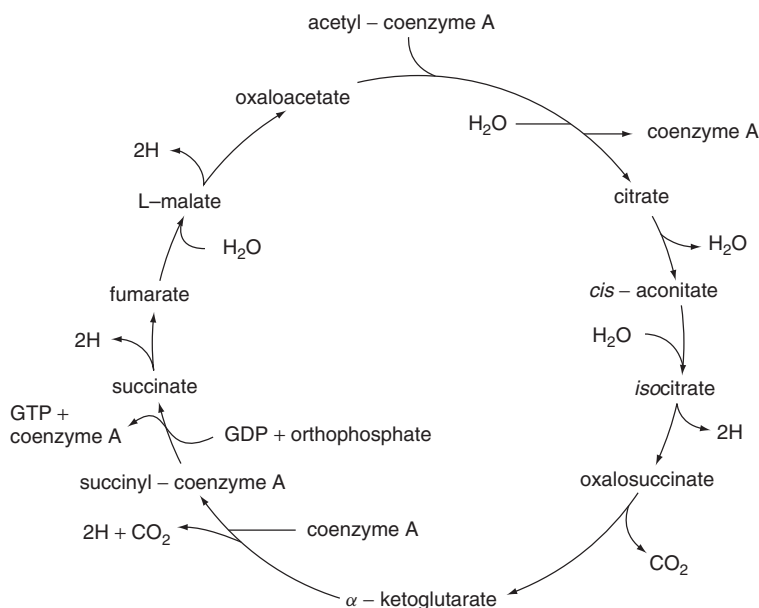


Figure 1.7 The tricarboxylic acid cycle (*Biol Psych*, 2 Ed, p. 52)

**Table 1.1** Some essential amino acids

Histidine
Arginine
Ornithine
Valine
Methionine
Lysine
Phenylalanine
Tyrosine
Tryptophan
Homocysteine
Cysteine
Glycine
Amino butyric acid

to amino acids, which can be oxidized through the tricarboxylic acid cycle. Degradation reactions common for several amino acids include deamination, in which an ammonia group is removed, transamination, in which there is exchange between amino and ketonic acids of amino and keto groups, and decarboxylation, in which a carbon dioxide radical is given up. Only some amino acids are broken down for energy, others being involved in the synthesis of neurotransmitters, and most in the building of proteins.

There are only a limited number of amino acids in living cells, some of the most important being shown in Table 1.1.

Fats are hydrolysed to free fatty acids and glycerol. The fatty acids are chains of carbon atoms terminated with a carboxylic acid ( $\text{—COOH}$ ) group. Glycerides are esters (compounds formed by the interaction of an acid and an alcohol) of fatty acids and glycerol, and phospholipids are esters of fatty acids and alcohol, the latter containing a phosphate group. Sphingolipids have the base sphingosine instead of glycerol, and sphingomyelins contain choline. The cerebrosides and gangliosides are sphingolipids with a hexose, and in the case of gangliosides, a polyhydroxy amino acid. All of these are found in abundance in nerve tissue.

## CELL MEMBRANES

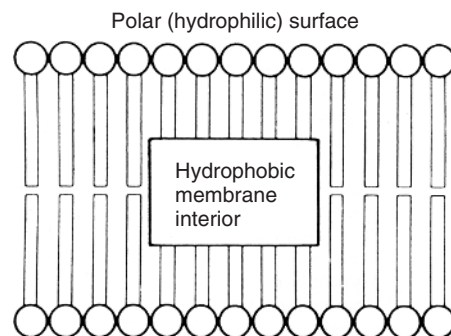
Neurons, in common with other cells, are bounded by a membrane. It is now known that

this is a continuous double structure with two electron-dense layers separated by an interzone. The thickness is approximately 100 Å, and similar membranes are seen in the cell, forming, for example, the endoplasmic reticulum or the mitochondria.

They are mainly formed from lipids (whose central components are fatty acids) and proteins. One end is hydrophilic and the other hydrophobic, and these align to form a structure suggested to look like that shown in Figure 1.8. The inside is hydrophobic, and proteins seem embedded in the structure, carrying out such functions as aiding active transport, forming the structure of receptors and enzyme activity. Channels in the membrane are thought to be protein aggregates, and they help regulate permeability. These are either active (which can be open or closed) or passive (which remain open all the time). Active channels may be influenced by various stimuli including electrical or chemical ones such as the neurotransmitters.

The primary structures of sodium, potassium and calcium channels have been revealed following cloning, and they are all similar, with four homologous transmembrane domains surrounding a central ion pore, which sits in the centre of the square array.

In the technique of patch clamping, a small micropipette with a polished end is pushed into the cell and then sucked. The membrane forms a tight seal on the pipette, and if it is then pulled the small patch of membrane remains intact



**Figure 1.8** A schematic representation of a cell membrane (reproduced from Shaw *et al.*, 1982, by permission of Butterworth & Co; *Biol Psych*, 2 Ed, p. 54)

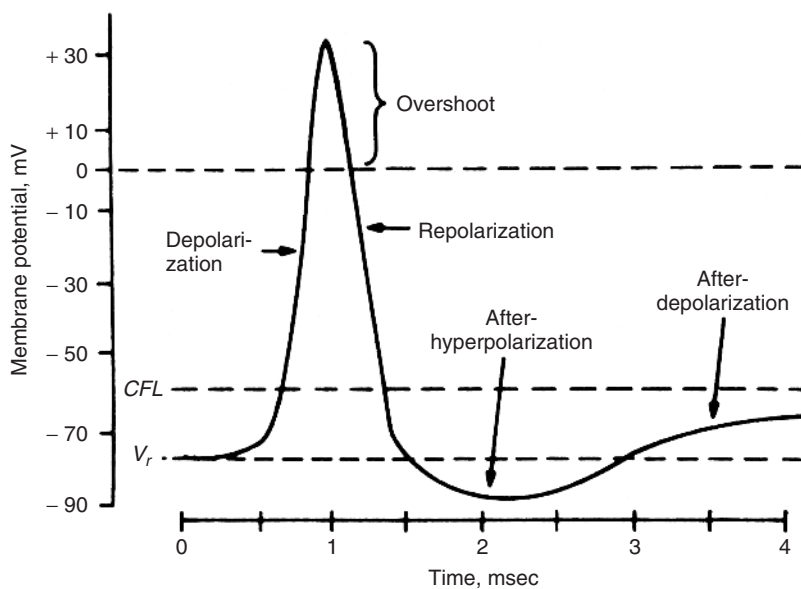
and is used to study receptor function in detail, including ion flow through channels.

In order that the cell is excitable, a potential difference across it must exist (polarization). This membrane potential is positive on the outside and negative on the inside. It is due to electrolyte differences on the two sides of the membrane, and in most neurones is in the region of 50–60 mV. This is referred to as the resting potential; to generate the action potential the membrane must be depolarized by alteration of the ion distribution on the two sides of the membrane.

There are four main ions in the cells: sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ) and organic anion ( $\text{A}^-$ ). Sodium and chloride are at lower concentrations on the inside of the cell; the other two have higher concentrations. At rest, the potential is maintained by the action of the sodium pump, which, at the expense of energy, forces the sodium out and draws the potassium in. The resting potential acts as the energy store of the cell, and neuronal action is dependent on it. The electrical signals of the nerve cells result from a change in the resting potential with alteration of the distribution of ions on either side of the membrane.

The action potential is generated by depolarization, during which sodium flows into the cell and, with slight delay, the potassium moves out. This process is initiated by the opening of the sodium channel, allowing sodium to flow in: a process that opens more sodium channels. Then, as sodium channels begin to close, the potassium voltage-regulated gated channels begin to open and repolarization occurs. The resulting phases of the action potential are shown in Figure 1.9.

These action potentials are generated by such stimuli as synaptic transmission, and propagation of the current along the membrane proceeds as adjacent portions of the membrane become depolarized by electronic conduction. Generally, the larger the diameter of the axon of the cell, the more rapid the current flow; however, in myelinated axons (see below), where the resistance at the nodes of Ranvier is low compared with that at the internodes, current travels along intracellular fluid from node to node, a process referred to as saltatory conduction. Following the action potential there is a brief refractory period during which the sodium channels return to their closed state. The entire neuron firing and refractory period take 5–6 milliseconds, allowing neurone to potentially



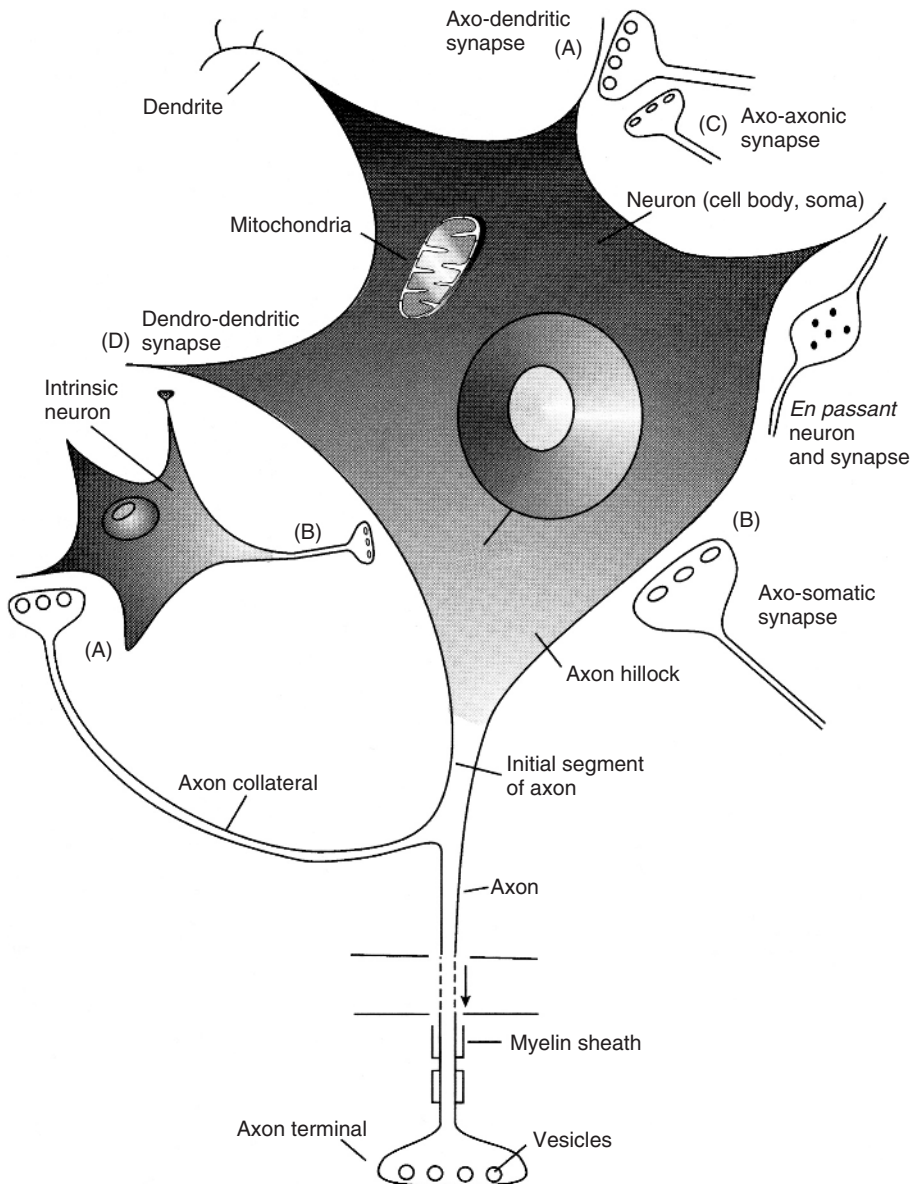
**Figure 1.9** The action potential and its components.  $V_r$ , resting membrane potential; CFL, critical firing level (*Biol Psych*, 2 Ed, p. 55)

refire or oscillate at very high frequencies (several hundred events per second). This is in contrast to the megahertz frequencies sometimes used in modern electronics or computers. However, neurone also have the ability to amplify their signal; that is, once the axon is triggered, it can spread to the full tree of the axon, and potentially interact with thousands of other neurone. Axonal information can travel at  $0.5\text{--}50\text{ m s}^{-1}$ .

Although the most important ions for the generation of the action potential are sodium and potassium, others exert influences, such as calcium and magnesium.

## SYNAPSES

The main link between one neurone and the next is the synapse, and in the human central



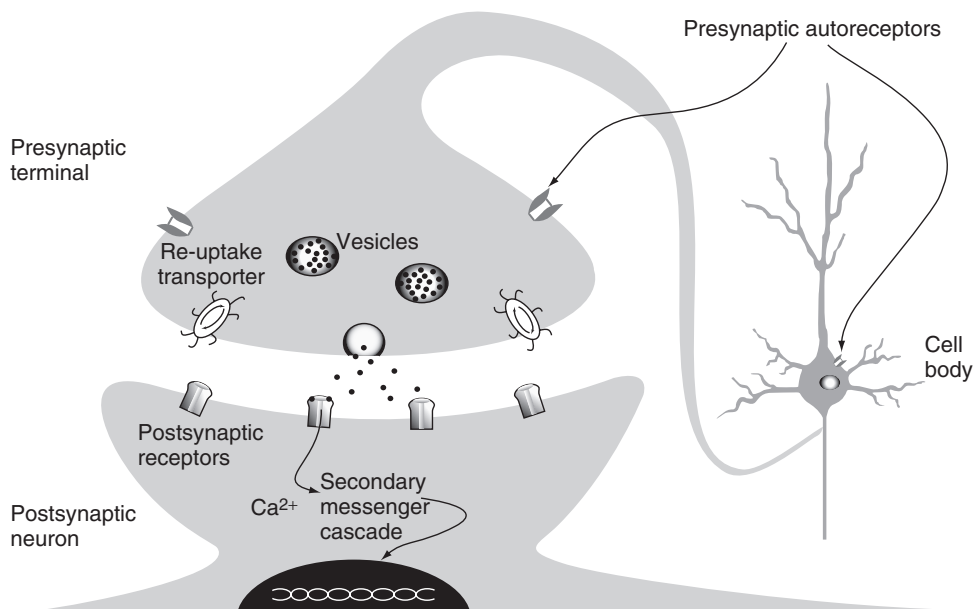
**Figure 1.10** Schematic representation of a neuron (Webster, 2002)

nervous system (CNS) transmission is mainly chemical. However, it is recognized that electrical synapses occur (bridged junctions) with only small gaps (around 20 Å) separating the presynaptic and postsynaptic membranes. In contrast, chemical synapses (unbridged junctions) have a larger gap (200 Å), the synaptic cleft, and specialized vesicles are identifiable in the presynaptic terminals. Information flow across the electrical synapse is more rapid than across chemical junctions, although the latter are more flexible.

Postsynaptic potentials are either inhibitory (IPSP) or excitatory (EPSP). The former prevent the initial area of the axon from reaching the threshold required to generate an action potential (hyperpolarization) by increasing the influx of potassium and chloride. These IPSPs can summate either temporally or spatially, and interact with EPSPs to determine the ultimate excitability of the postsynaptic cell. EPSPs result from an influx of sodium via chemically gated channels, and the spike discharge is generated not at the postsynaptic membrane, but at

the axon hillock, which has a low electrical threshold (see Figure 1.10).

In addition to these postsynaptic events, presynaptic factors which control transmitter release also affect postsynaptic activity. These include presynaptic inhibition or facilitation via activity in synapses on the presynaptic boutons (axo-axonic synapses) and ionic influences such as calcium ( $\text{Ca}^{2+}$ ) influx. The presynaptic terminals come from interneurons, and they provoke an EPSP or IPSP in the terminal of the afferent nerve fibre. In the case of inhibition, this partial depolarization reduces the amplitude of the oncoming afferent action potential. Since the transmitter release is proportional to the amplitude of the action potential, less transmitter is released and a smaller EPSP results in the postsynaptic cell. Although first discovered in relation to spinal cord afferents, this form of inhibition has been shown to exist in many CNS areas. Varieties of presynaptic connections are shown in Figure 1.11. Where the neurone has receptors to its own transmitter on these terminals they are called autoreceptors.



**Figure 1.11** Schematic representation of a generic excitatory synapse in the brain. This shows glutamate released by fusion of transmitter vesicles at the nerve terminal and diffusing across the synaptic cleft to activate AMPA and NMDA receptors. Glutamate also binds to metabotropic G-protein-coupled glutamate receptors, initiating secondary messenger intracellular signalling through the G-protein complex (Webster, 2002)

Calcium has been shown to be essential for transmitter release. During depolarization of the presynaptic terminal the calcium channels open and calcium moves into the cell. This allows the presynaptic vesicles that contain neurotransmitters to bind to releasing sites on the postsynaptic membrane. Control of calcium currents by presynaptic receptors is thought to be an important mechanism of their action.

The amount of transmitter released is related to the calcium levels and the presynaptic input. The response is thus plastic, and further varied by the postsynaptic variation in ion channels. Single vesicles can release up to 5000 molecules of a neurotransmitter.

A further consideration is the retrograde control of presynaptic activity by diffusible messengers from the postsynaptic cell. Nitrous oxide (NO) is a gaseous messenger involved in long-term potentiation (LTP). NO is synthesized in the postsynaptic cell following calcium influx at the N-methyl-D-aspartate (NMDA) receptor, and diffuses back to the presynaptic terminals, altering transmitter release. Other candidates are arachidonic acid and carbon monoxide. A possible target for these messengers is the presynaptic calcium influx.

There are two types of communication between cells: classical, with fast transmission, in which highly specialized regions of the presynaptic cell make contact with discrete areas of the postsynaptic cell; and more diffuse, with transmitter released over a broader area. The latter is less readily removed, and transmission is slower. Transmitters such as amines and opiates use this latter, slower method and set the level of a response.

## RECEPTORS

In recent years much attention has been paid to the structure and function of receptors. These are proteins to which transmitters bind, and are located on the outer surface of the cell membranes. Two types are defined, the transmitter-gated ion channel (called ionotropic) and the G-protein-coupled receptor (called metabotropic). The former act very

quickly, while the latter initiate a series of postsynaptic intracellular events which alter either intracellular proteins or the DNA of the nucleus, altering gene expression. The latter will lead to alteration of the configuration of the cell, for example by activating growth factors or receptor activity (see Figure 1.12).

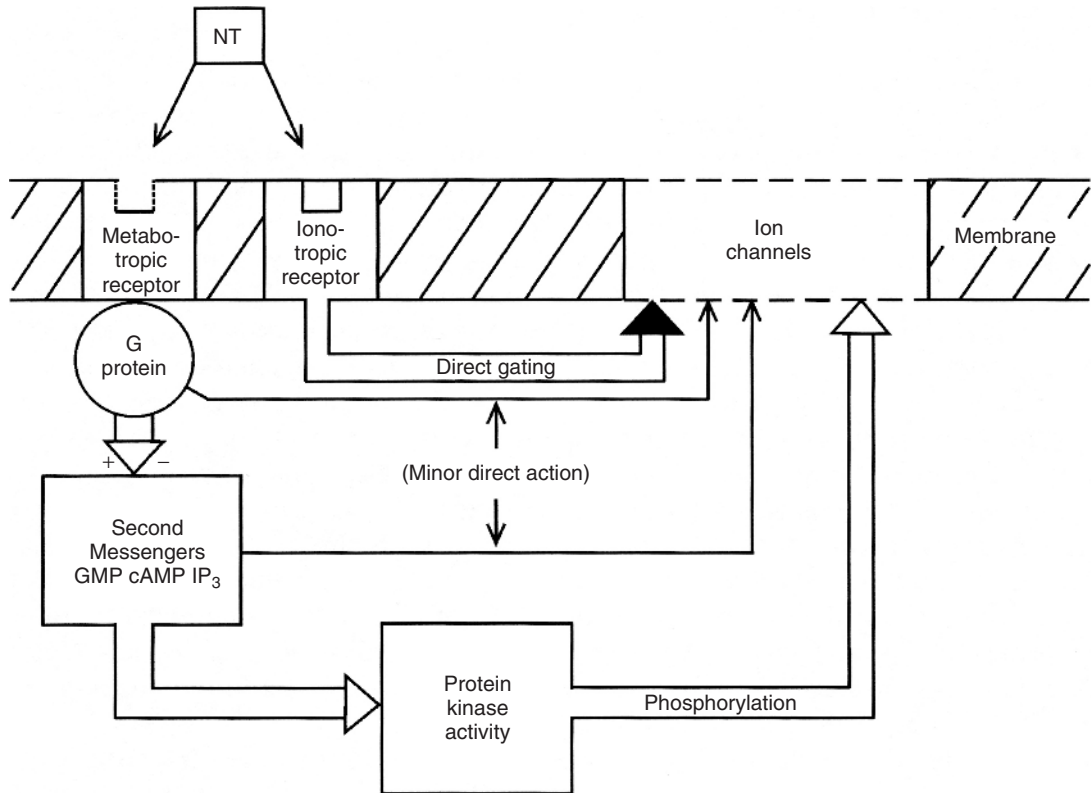
The genes for many receptors have been cloned and their polypeptide structures identified. Many have a similar conformation, perhaps suggesting a common evolutionary precursor. Since the sequence of the amino acids is known for the Dopamine D<sub>1</sub> receptor, it is possible to look at which parts of the sequence will interact with the lipid-rich membrane, and at the hydrophobic and hydrophilic parts identified. The general model is of five subunits, a channel, a gate and a receptor bound in a pocket. Some receptors have many subunits, and these are selected in different combinations to give different responses, the length of time the channel is open and the number of ions that flow through it (Sibley and Monsma, 1992).

G-protein-coupled receptors share a generic pattern of protein folding, with seven hydrophobic transmembrane helices joined by alternating intracellular and extracellular loops, an extracellular amino terminal and a cytoplasmic carboxyl terminal. The protein folding of several receptors is shown in Figure 1.13.

The neurotransmitter binds with the receptor to form a transmitter-receptor complex, which changes the conformation of the latter. With the transmitter-gated ion channels, opening up the ionophore allows ion exchange to occur with consequent changes of the membrane potential. An influx of negative ions such as Cl<sup>-</sup> leads to IPSPs, while an influx of positive ions such as Na<sup>+</sup> will encourage EPSPs. Some ionophores are specific for chloride or sodium, others are less selective. With the G-protein-coupled receptors, interaction of the transmitter and receptor provokes alteration of intracellular metabolism by the stimulation of second messengers.

In addition to neurotransmitter receptors, there are receptors for other chemicals, some of which help with the early development of neuronal systems, such as for nerve growth factors (NGF) and steroid receptors. The latter





**Figure 1.12** The difference between ionotropic and metabotropic mechanisms. With the former, the neurotransmitter combines with the receptor, leading to opening of the ion channel. With the latter, G-protein is activated, leading to a secondary messenger cascade. The image also shows how the intracellular activations can feed back, affecting ion channels (Webster, 2002)

are intracellular molecules which carry the steroid to the nucleus.

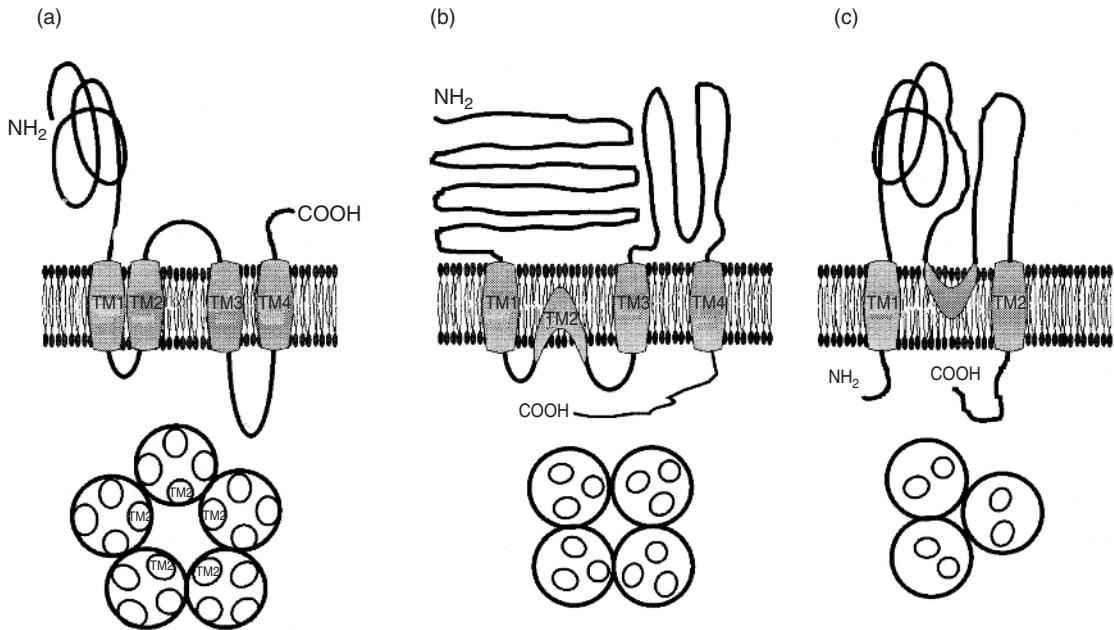
Ligands (transmitters or synthesized chemicals that act on receptors), which are agonists, antagonists, partial agonists (exhibiting less than a maximal response) and inverse agonists (leading to the opposite response to a full agonist) have been identified, depending on the receptor type.

There are several second messenger systems. Following contact between the receptor and a neurotransmitter, a G-protein is activated (a GTP-binding protein), which in turn activates further proteins, leading to the conversion of ATP to cAMP.

One secondary messenger system is via adenylate cyclase to cyclic AMP. There is an intermediary G-protein which binds with the

receptor-transmitter complex and alters its configuration; it then links with adenylate cyclase and activates it, leading to cyclic AMP formation. An important feature of this system is amplification, whereby each activated receptor protein stimulates many molecules of G-protein, which in turn activate many molecules of adenylate cyclase, each further generating many cyclic AMP molecules. Protein kinases are enzymes which phosphorylate (add phosphorous to) proteins and help physiologically activate them (Figure 1.12).

Another second messenger system is via the hydrolysis of a membrane phospholipid (phosphatidylinositol biphosphate), which leads to the production of diacylglycerol and, via phospholipase C, inositol triphosphate (IP<sub>3</sub>). Diacylglycerol activates protein kinase C and



**Figure 1.13** How transmembrane subunits are conceived. The subunits are embedded in the cell membrane. The example in (a) reflects the structure, for example, of a GABA<sub>a</sub> receptor. Below is shown pentameric stoichiometry of the domain receptors lining the central ion channel. (b) shows the structure of ionotropic glutamate receptors. (c) shows a subunit of an ATP receptor (Webster, 2002)

IP<sub>3</sub> causes release of calcium from the endoplasmic reticulum, which activates a protein kinase called calmodulin-dependent protein kinase. G-proteins can also elevate intracellular calcium, thus indirectly activating kinases. Calcium is intimately related to these secondary messenger systems, and a calcium-binding protein, calmodulin, mediates these events and release of neurotransmitters from synaptic vesicles.

Thus, phosphorylation modifies the action of intracellular enzymes and cell regulatory proteins; signals mediated through second messengers influence protein kinases and protein phosphatases, which in turn modify the action of genes via transcription factors in the cytoplasm that bind to DNA. Genes encoding transcription factors have been referred to as third messengers. Genes such as *c-fos* are actively switched on quickly, having binding sites for the transcription factor CREB. The receptors for steroid hormones are also transcription factors, acting in the cell cytoplasm.

## Calcium Channels

Calcium channels are essential because they allow the influx of calcium into the synapse, calcium being involved in the attachments of vesicles to the synaptic membrane, which is essential for neurotransmitter release. Thus, calcium channels, and their proper functioning, can affect many different neurotransmitter systems. Several different types of calcium channel have been identified, some of which, such as the T-type, or the subcomponent alpha-2-delta, have been implicated in the action of some anti-convulsant medications. There is evidence that calcium-channel blockers may be effective in stabilizing mood in bipolar patients, particularly those who are treatment-resistant or are rapid-cycling.

## Third Messengers and Other Proteins

Recent research has unravelled many other intracellular proteins involved in either

**Table 1.2** Types of dopamine receptors

	DA <sub>1</sub>	DA <sub>2</sub>	DA <sub>3</sub>	DA <sub>4</sub>	DA <sub>5</sub>
Coupled	adenylate cyclase	<	phospholipase C	>	adenylate cyclase
Chromosome	5	11	3	11	4
Highest CNS Density	<neostriatum> nucleus accumbens olfactory tubercle		paleostriatum archicerebellum hypothalamus	frontal cortex	hypothalamus hippocampus
Adenylate cyclase	activate	inhibits	?	inhibits	activates
Pituitary	no	yes	no	yes	no
Affinity for DA	micromol	micromol	nanomol	submicromolar	submicromolar

the biology of psychiatric disorders or the action of psychotropic drugs, for example DARPP (dopamine- and cyclic-AMP-regulated phosphoprotein) and CREB (cAMP-response-element binding) proteins. These form part of a complex intracellular machinery, leading to longer-term genetic and synaptic changes in cells. It is now known that receptors under the influence of these factors can actually move in and out of synapses. Genes encoding transcription factors have been referred to as third messengers. The receptors for steroid hormones are also transcription factors, acting in the cell cytoplasm. New research is focusing on whether these systems are abnormal in psychiatric disorders, and if so, whether they might be targets for novel therapies.

A single receptor in a homogenous cell population may couple differently to different effector systems, allowing for a range of activity from near antagonism to full agonism.

At the present time many different receptors have been classified and subclassified, although there is not universal agreement. It should be noted that, when receptor binding studies are presented, usually either receptor agonist affinity (K<sub>d</sub>) or receptor number (B<sub>Max</sub>) is referred to. Receptor reactivity, a third but crucial variable, is poorly investigated owing to the limitations of *in vivo* techniques. Some of the more relevant receptors for psychiatry are now presented.

## Dopamine

It is now accepted that there are five types of dopamine receptor (DA<sub>1-5</sub>). They are all linked with a binding protein, and all have been cloned.

There are two subfamilies: DA<sub>1</sub> and DA<sub>5</sub>, and DA<sub>2</sub>, DA<sub>3</sub> and DA<sub>4</sub> (see Table 1.2). The first subfamily of receptors couples with adenylate cyclase, and the other subfamily couples with phospholipase C, which then inhibits adenylate cyclase.

The distribution of the D<sub>1</sub> and the D<sub>2</sub> receptors is wide compared with the others, but this does not relate to functional significance. D<sub>3</sub> has a limited distribution in the limbic striatum, especially in the olfactory tubercle and nucleus accumbens, while D<sub>4</sub> is found in the prefrontal cortex.

D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> are also autoreceptors. They may inhibit DA cell firing, DA release or synthesis.

Individual cells have been shown to contain both D<sub>1</sub> and D<sub>2</sub> receptors. D<sub>2</sub> receptors have high affinity for traditional antipsychotic drugs, D<sub>1</sub> less so. Interestingly, there are less abundant dopamine transporters in the frontal cortex, which suggests that dopamine may act differently there than in the basal ganglia.

## Receptors for Excitatory Amino Acids (EAA)

Glutamate and aspartate are the main EAAs. The glutamate receptors are of two main types, AMPA and NMDA. The former have fast kinetics and are coupled to a sodium channel; the latter are slower, having high calcium permeability, which activates intracellular processes. Others include the kainate receptor and the trans-ACPD receptor. Excessive NMDA receptor activity can lead to excess calcium influx and neuronal death.

**Table 1.3** Agonists and antagonists of adrenoceptors

	$\alpha$ -1	$\alpha$ -2	$\beta$ -1	$\beta$ -2
Agonists	Adrenaline Noradrenaline Methoxamine Phenylephrine	Adrenaline Clonidine	Adrenaline Isoprenaline Noradrenaline	Adrenaline Isoprenaline Salbutamol Terbutaline
Antagonists	Phenoxybenzamine Phentolamine Prazosin	Yohimbine	Practolol Atenolol Metoprolol Propranolol	Propranolol

The molecular structure of these receptors is known, and subtypes have been defined. It is thought that NMDA receptors are involved in synaptic plasticity, and probably with LTP and memory storage.

The NMDA receptor has within it a glycine binding site, and occupancy of this is necessary to allow opening of the channel by glutamate. Phencyclidine, some anaesthetics and MK-801 have high-affinity binding for sites in the ionic channel. The channel is normally blocked by magnesium, which is removed on depolarization.

Many glutamate synapses have two receptor sites – NMDA and non-NMDA – with an interaction between them. If the non-NMDA receptor fires once, because of the presence of the magnesium, the NMDA receptor ion channel remains closed. The latter is opened only by the cell firing in bursts, with sufficient voltage change in the post-synaptic neurone to remove the magnesium ion and allow an influx of sodium and calcium.

Glutamate receptors are widely distributed in the cortex, and are linked to activity in the CA<sub>1</sub> cells in the hippocampus.

## Adrenoceptors

In the same way that dopamine receptors can be subdivided, so can those for several other neurotransmitters. The adrenergic receptors are divided into three main subtypes:  $\alpha$ <sub>1</sub>,  $\alpha$ <sub>2</sub> and  $\beta$ . Each of these has three subtypes:  $\alpha$ <sub>1a</sub>,  $\alpha$ <sub>1b</sub>,  $\alpha$ <sub>1d</sub>,  $\alpha$ <sub>2a</sub>, etc.  $\alpha$ <sub>1</sub> receptors are postsynaptic and excitatory, and  $\alpha$ <sub>2</sub> are both post- and presynaptic and inhibitory. At the presynaptic

location they are involved in noradrenaline release, and their postsynaptic role is undetermined. Relatively selective agonists and antagonists have been defined for all subtypes (see Table 1.3). The  $\alpha$ <sub>1</sub> receptor is believed to play a role in smooth muscle contraction and has been implicated in effecting blood pressure, nasal congestion and prostate function. Although widely expressed in the CNS, the central role of the  $\alpha$ <sub>1</sub> receptor remains to be determined. Locomotor activation and arousal have been suggested by some studies. Stimulation of the  $\alpha$ <sub>1</sub> receptor may synergistically increase the activity of the 5-HT neurone in the raphe nucleus, although stimulation of the  $\alpha$ <sub>2</sub> receptor may have the opposite effect.  $\alpha$ <sub>1</sub> receptors stimulate the IP<sub>3</sub> system;  $\alpha$ <sub>2</sub> inhibit adenylate cyclase.

The  $\alpha$ <sub>2</sub> receptor subtypes in the CNS inhibit the firing of the noradrenaline neurone through autoreceptors. This mechanism of action is believed to mediate the sedative and hypotensive effects of the  $\alpha$ <sub>2</sub> receptors agonist clonidine. Additionally, stimulation of the  $\alpha$ <sub>2</sub> receptors decreases sympathetic activity, which may explain the therapeutic utility of clonidine for suppressing the heightened sympathetic state of patients in opiate withdrawal.

The  $\beta$  receptor subtypes are more famous for their part in slowing cardiac rhythm and lowering blood pressure. The functions of the  $\beta$  receptors in the CNS, although widely distributed, are not well understood. It is not uncommon to use a  $\beta$  blocker, such as propranolol, to treat performance anxiety or antipsychotic-induced akathisia. Whether these benefits come from a central or peripheral blockage (or both) of the  $\beta$  receptor is not known. All  $\beta$  subtypes interact with adenylate cyclase.

## Histamine Receptors

There are now four histamine receptors, although H<sub>4</sub> is predominately in the periphery and was only recently discovered. The H<sub>1</sub> receptor is the target for the classic antihistamines, which highlights its role in sedation and, conversely, arousal. Of great interest to psychiatrists is the role of H<sub>1</sub> in weight gain. Recent analysis has shown that the potential to gain weight with antipsychotic agents correlates with their antagonism for the H<sub>1</sub> receptor; examples include clozapine and olanzapine (Han *et al.*, 2008; Nasrallah, 2008).

The H<sub>2</sub> receptor is more traditionally associated with the gut. Blockade of the H<sub>2</sub> receptor has been a widely used treatment for peptic ulcer disease. The H<sub>3</sub> receptor functions as an inhibitory receptor on the histamine neurone as well as on other non-histamine nerve terminals. The role of this receptor is not clearly understood, but may be involved in appetite, arousal and cognition.

## 5-HT Receptors

The original discovery of the 5-HT receptor led to two subtypes: 5-HT<sub>1</sub> and 5-HT<sub>2</sub>. Further discoveries, especially the application of molecular

cloning techniques, have resulted in multiple subdivisions of these two receptors and the addition of several new ones, for a total of 14. While the prospect of activating or blocking the various receptors for further refinement of psychopharmacological treatment is enticing, the clinical results, with a few exceptions, have been limited (see Table 1.4).

The 5-HT<sub>1</sub> receptors make up the largest subtype, with 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub> and 5-HT<sub>1F</sub>. 5-HT<sub>1A</sub> has received the most interest and seems to play a prominent role in depression and anxiety. It is an autoreceptor on the cell body. Stimulation of this receptor reduces cell firing and curtails the release of 5-HT. How this would improve mood is unclear, but blocking this receptor has decreased the effectiveness of tricyclic antidepressants in rat models of depression. The anxiolytic buspirone is a partial 5-HT<sub>1A</sub> agonist, which suggests that 5-HT<sub>1A</sub> has some role in anxiety. The development and distribution of buspirone is an example of specific 5-HT-receptor targeting, which has had only a marginal effect on clinical practice.

The 5-HT<sub>1D</sub> receptor is also an autoreceptor but is located on the nerve terminal at the synapse. There it appears to function to sense the 5-HT in the synaptic cleft and turn off release of more 5-HT when stimulated. The 5-HT<sub>1D</sub>

**Table 1.4** 5-HT receptor subtypes

Main type	Sub-main site type	Second messenger	Agonist (AG)* antagonist (ANT)
5-HT <sub>1</sub>	a raphe; hippocampus	ac	buspirone (a) propranolol (ant)
	b substantia nigra; globus pallidus d basal ganglia	ac	propranolol (ant)
		ac	metergoline (ant) sumatriptan (ag)
5-HT <sub>2</sub>	a cerebral cortex; caudate; limbic system	IP <sub>3</sub>	ritanserin (ant) ketanserin (ant) spiperone (ant) cyproheptadine (ant) mianserin (ant) methysergide (ant) metergoline (ant)
5-HT <sub>3</sub>	c choroid plexus entorhinal cortex	IP <sub>3</sub> ion channel	m-CPP (ag) quizapine (ant) ondansetron
5-HT <sub>4</sub>	limbic system; basal ganglia; frontal cortex	ac	

\*Only limited ones given. Note that drugs like quizapine (ag), L-tryptophan (ag), fenfluramine (ag), p-chloroamphetamine (ag), methysergide (ant) and metergoline (ant) act at multiple 5-HT receptors. Ac is adenylate cyclase, IP<sub>3</sub> is phosphatidyl inositol.



receptor is stimulated by the antimigraine drug sumatriptan, although the importance of this effect in the overall efficacy of the medication is unclear. Some researchers are exploring the effectiveness of a 5-HT<sub>1D</sub> receptor antagonist for the treatment of depression. The goal is to block the negative feedback mediated through the 5-HT<sub>1D</sub> receptor so that more 5-HT is released into the synapse.

Other important 5-HT receptors for the psychiatrist are 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub>. The 5-HT<sub>2A</sub> receptor has been identified as playing an important role in the 'atypical-ness' of the second-generation antipsychotic agents (clozapine, risperidone, olanzapine, etc). These newer agents have a greater capacity to block 5-HT<sub>2A</sub> than the traditional agents like haloperidol and it is speculated that this results in the observed decrease in extrapyramidal side effects (EPS) and greater cognitive improvements.

5-HT receptors are involved in the regulation of dopamine and acetylcholine release. 5-HT<sub>1A</sub> is an autoreceptor on the cell body and reduces 5-HT release; 5-HT<sub>3</sub> activation stimulates striatal and mesolimbic dopamine release, and it is in mesolimbic areas that many of the 5-HT<sub>3</sub> binding sites are to be found. In contrast, this receptor decreases the release of acetylcholine. 5-HT<sub>1D</sub> is also an autoreceptor, but at the nerve terminal, and also inhibits 5-HT release. There has been much interest in the 5-HT<sub>2A</sub> receptor, because it is involved in the action of the atypical antipsychotics (Chapter 12).

## GABA and Benzodiazepine Receptors

Multiple types of the GABA receptor exist. This receptor is composed of three subunits, alpha, beta and gamma, each with its own isoforms. There are at least six for the alpha, four for the beta, and two for the gamma. There seem to be around 48 possible structures of the receptor, although it has to be noted that these have been identified with the techniques of molecular biology, not pharmacology. These subunit types differ with respect to their sensitivity for GABA and recognition sites for other molecules such as the benzodiazepines. For the latter, binding occurs at the alpha subunit, although it requires the presence of the gamma subunit

for stabilization. Two types of benzodiazepine receptors are described, BZ<sub>1</sub> and BZ<sub>2</sub>, depending on the alpha subunits from which they are composed (Doble and Martin, 1992). Other important modulators of the GABA receptor include neurosteroids and alcohol.

GABA<sub>a</sub> receptors are ligand-gated ion channels composed of five subunits, the recognition site for GABA being present on the beta unit. Picrotoxin and bicuculline are antagonists, and they contain a benzodiazepine and a barbiturate binding site. They are linked to chloride channels.

GABA<sub>b</sub> receptors are coupled to G-protein and activated by baclofen; they may decrease the flow of calcium and are not linked to the benzodiazepine recognition site. The distribution of the subunits in the brain reveals GABA receptors to be widespread; indeed, GABA is one of the most common neurotransmitters, occurring in over 30% of all synapses (Leonard, 1992). Ethanol enhances the function of the GABA receptor. Long-term use of ethanol decreases the expression of the GABA receptor, which may explain the tolerance that develops with alcoholism. Whether the receptor alterations contribute to the propensity for seizures when the alcohol is withdrawn remains unclear. The steroid hormones can also modulate GABA receptors (sometimes called neurosteroids when they have effects on neurone). The distribution of GABA receptors with different subunits varies widely in brain; alpha subunits localize especially with 5-HT and catecholamine neurone.

More recently, extra-synaptic GABA receptors have been identified, which are thought to act by using the overspill of GABA from the synapse, and provide tonic background inhibition. This tonic conduction is activated by low ambient levels of GABA. Research is underway to find selective agents that act extra-synaptically. These compounds (e.g. gaboxadol) would increase inhibition and perhaps be useful as a hypnotic or anticonvulsant.

## Acetylcholine

Acetylcholine has four protein subunits (alpha, beta, gamma and delta). Muscarinic and



nicotinic subtypes are recognized, and five molecularly distinct muscarinic proteins (M<sub>1</sub>–M<sub>5</sub>) have been cloned. They transduce their intracellular signals by coupling with G-proteins.

It was with the cholinergic receptor that scientists first realized that one neurotransmitter (ACh) could have different receptors. The initial subtypes were identified and named after the drug that distinguished their effects. For example, nicotine will stimulate cholinergic receptors in skeletal muscle but not the heart. Conversely, muscarine will stimulate the heart but has no effect on skeletal muscle. Thus the two receptors can be identified by the actions of different drugs and the receptors were named after those drugs: nicotinic and muscarinic. Unfortunately, it has been hard to find a drug with unique action on each receptor subtype, so we have designations such as 1A, 2B and so on.

Many more subtypes of the nicotinic and muscarinic receptors have been identified since the early days of receptor delineation, but the significance of these various subtypes remains obscure. Clearly, ACh is important in cognition and memory, as shown by the benefits of inhibiting acetylcholinesterase as a treatment for Alzheimer's disease (Chapter 12). Likewise the blockage of the muscarinic receptor by tricyclic antidepressants and antipsychotic medications results in troublesome dry mouth, constipation and urinary hesitancy (which are generically referred to as the anticholinergic side effects). However, the importance of one receptor subtype over another has not been shown.

### **Other Receptors**

Many other receptors and their subtypes have been identified in the CNS, although often their functional significance is unknown.

Peptide and opiate receptors have been identified. There are at least three classes of opiate receptor (Reisine and Bell, 1993): delta, kappa and mu. The delta and kappa bind enkephalins and endorphin, and the kappa receptors bind dynorphins. Mu receptors are selectively sensitive to morphine. All three types couple to adenylate cyclase, inhibiting cAMP.

Receptors for substance P, enkephalins, prolactin, steroids, adenosine (A<sub>1</sub>–A<sub>3</sub>) and even

some drugs such as imipramine, have also been described. In the latter instance, it is the case that the imipramine, or another drug such as paroxetine, binds to the 5-HT transporter site. Sigma receptors, once considered to be opiate receptors, are well represented in limbic structures, and have a high affinity for some neuroleptics.

The body and brain produce endogenous endocannabinoid substances. The cannabinoid receptors are of two types: CB<sub>1</sub> is found in the brain and the heart, CB<sub>2</sub> in immune and hematopoietic cells. They work through G-protein receptor mechanisms.

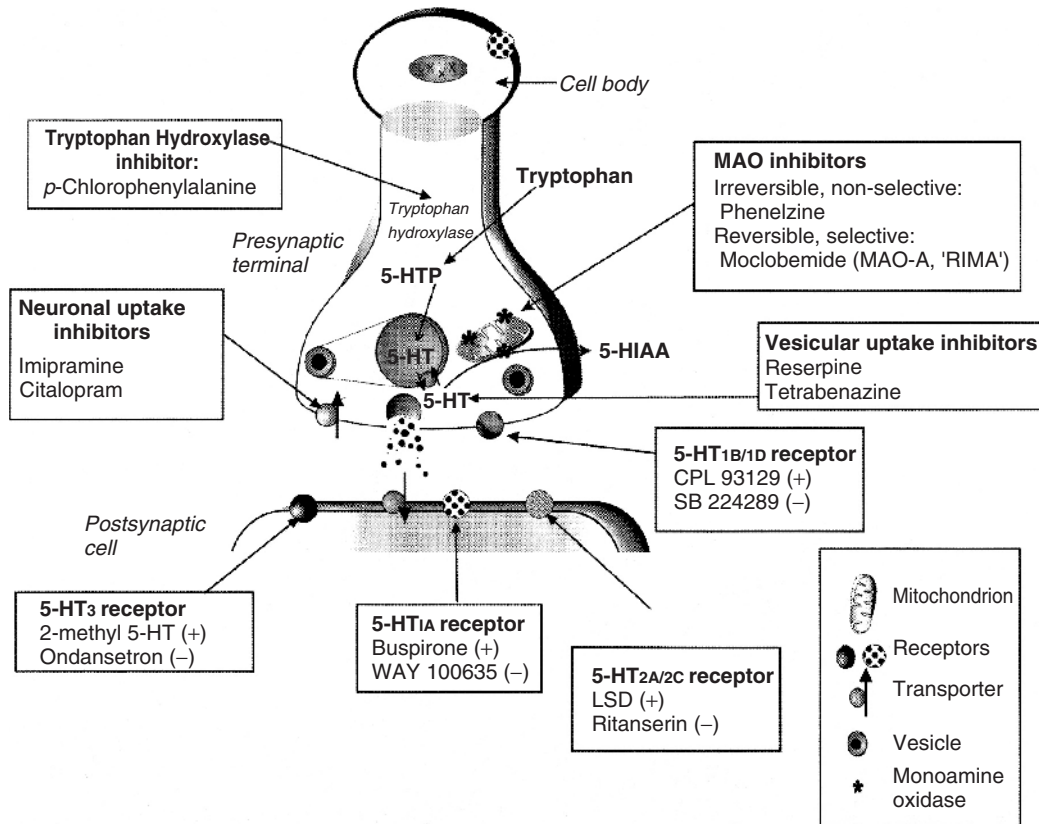
### **Transporters**

Neurotransmitter transporters collect released transmitters from the synaptic space, bringing them back to the presynaptic terminals. Transport is sodium-dependent, and their regional distributions are consistent with those of their released neurotransmitters. Many drugs, for example antidepressants, cocaine and amphetamine, act at such sites. The DNA structures of many transporter sites have now been identified, and while most seem to be situated on neurones, glial localization has also been shown for some transmitters. Reversal of flow through the transporter from the cell back to the synaptic space is known to occur.

The genes coding for the 5-HT transporter (5-HTT), which removes 5-HT from the synaptic cleft, have been identified. A polymorphism 5-HTTLPR in the promoter region has 22 base pair repeats, consisting of a short (s) and a long (l) version, which influences the function of the transporter, the s variant leading to less 5-HTT mRNA and thus higher concentrations of 5-HT in the synaptic cleft.

### **NEURONES**

The main types of cell in the CNS are the neurones and the glial cells. The neurone has dendrites, an axon, the soma or cell body, and sites for synapses (see Figures 1.10 and 1.14). Essential metabolic molecules are synthesized in the soma and transported to other regions of the neurone. The difficult process of moving products down the long axon is accomplished



**Figure 1.14** Example of the manufacture and distribution of 5-HT and various 5-HT receptors and sites of drug action (Webster, 2002)

by a microtubular system composed of a protein called tubulin. The larger axons are surrounded by a myelin sheath, which aids the speed of electrical conduction.

The glial cells vastly outnumber the neurones, and while they play a structural supportive role, they are also involved in metabolic processes and the manufacture of myelin. Five types are identified: astrocytes, oligodendrocytes, microglia, Schwann cells and ependyma cells, the latter of which line the inner surface of the brain.

The role of glial cells in cerebral activity is still largely unknown, but astrocytes, which are closely enmeshed with synapses, also have receptors for some neurotransmitters, especially glutamate and GABA. Astrocytes have several functions within the CNS in addition to transmitter uptake and release. These include metabolic support for the neurone, modulation

of synaptic transmission, phagocytosis (microglia) and gap-junction syncytiation for  $\text{Ca}^{++}$  wave propagation for astrocytic glutamate release. By regulating glutamate metabolism the astrocytes are central for glutamate homeostasis.

The synaptic region of the neurone is highly specialized for the storage and release of neurotransmitters. The latter are contained in storage vesicles along with ATP and proteins, and are there protected from breakdown by intracytoplasmic degradation enzymes. Release of the transmitter into the synaptic cleft is by exocytosis, and quanta of transmitter are shed into the synaptic cleft. This process requires calcium, which interacts with presynaptic release areas, facilitating fusion between the vesicle and cell membranes. Calcium enters the cell terminals through voltage-dependent calcium channels. The interaction of transmitter with receptors then takes place, and IPSPs or EPSPs

are generated. The synaptic contacts can be to an opposing soma (axo-somatic), dendrite (axo-dendritic) or axon (axo-axonic), and the position of contact has relevance for the postsynaptic effect: the nearer to the axon hillock, the greater the effect.

In contrast to the rather rigid anatomical structure shown in cartoons of the neurone, the neurone – especially the dendrites and the synapses – must be seen as part of a dynamic system (Smythies, 2002). The electronic structure of the dendrites is forever shifting: signals spread from dendrites to soma and vice versa, and the electronic properties of the latter alter with the resting membrane potential. Synaptic responses are often nonlinear, and related to prior activity.

The synapses themselves should be viewed as quite plastic; they are continually pruned and replaced. Their receptors are modulated in number and location, endocytosis- and exocytosis-redistributing membrane components, and the highly regulated intracellular processes directing cell growth, including synaptic modification (Smythies, 2002).

## Neurodevelopment and Neurogenesis

It used to be believed that an individual was born with a fixed number of neurone and lost them as they aged. New research has established that the brain is not such a fixed structure: although the rate of change and development is especially prominent early in life, alteration of the structure of the brain in response to environmental factors remains a feature across the entire life span.

A single fertilized egg develops into 100 billion neurone with 100 trillion connections in a short amount of time. *In utero*, this process is largely activity-independent and genetically driven. After birth, interactions with the environment begin to modify development and play a greater role. Unequivocally newly created neurone have been identified in the hippocampus of elderly subjects whose brains were examined shortly after death.

Undifferentiated neural stem cells remain in the CNS and continue to divide throughout life. They divide into more neural stem cells as well

as neural precursors that grow into neurone or glial cells. But they must migrate away from the influence of the stem cell before they can differentiate and only about half the cells successfully move and transform.

Neurotrophic factors are chemicals that are essential for neuron survival and differentiation. Nerve growth factor (NGF) is a prototypical neurotrophin, and NGF receptors are found on cells which when activated lead to rapid intracellular phosphorylation events. Programmed cell death seems to be a process that occurs across vertebrates and invertebrates alike, and the two main mechanisms are necrosis and apoptosis.

Clear evidence for adult neurogenesis has been limited to the granule cells of the dentate gyrus and olfactory bulb. Newly formed cells are identified by tagging them with a molecule such as bromodeoxyuridine (BrdU), a thymidine analogue that can be incorporated into newly synthesized DNA. A fluorescent antibody specific for BrdU is then used to detect the incorporated molecule and thus indicate DNA replication.

The rate of neurogenesis is modulated by various factors. It is known that enriched environments and exercise will increase neurogenesis. Additional research suggests that gonadal steroid hormones may also enhance new cell production. There is more research showing the positive effects of oestrogen on mammal brains, but testosterone will stimulate nerve-cell production in songbirds (Duman, 2005). Stress, on the other hand, has an inhibitory effect on neurogenesis. Extended maternal separation is a well-characterized model of early-life stress for a rodent. As adults, such rodents will show protracted elevations of CRF, ACTH and corticosterone (cortisol in a rat), as well as behavioural inhibition in response to stress. Rats exposed to prolonged maternal separation will have a long-lasting blunting of neurogenesis (Fabricius *et al.*, 2008).

## Stem Cells

One potential way of rebuilding neurone is to implant human embryonic stem cells that have been isolated from a very immature embryo,

placenta or bone marrow (only 100–200 cells). So far it has been difficult to get these immature cells to differentiate into functioning neurones outside of the olfactory bulb and hippocampus. The problem may be the absence of biochemical signals that normally prompt the developing stem cell to migrate and differentiate.

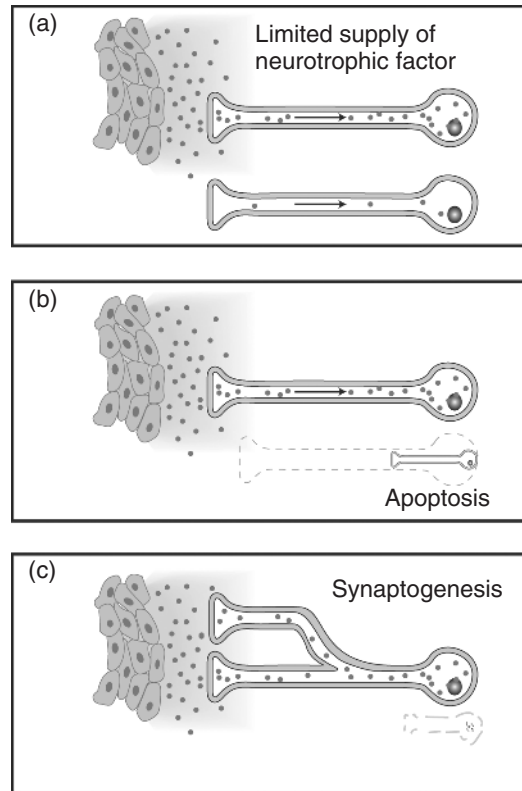
As noted above, it used to be considered that the synapse was fixed and static. Indeed, many cartoons of synaptic function falsely reinforced this view. In fact, however, the synapse is a constantly changing and dynamic concept and structure. Synaptogenesis describes the extensive growth of axons and dendrites to make synaptic connections – a process that primarily occurs early in life but continues even in adults. During development, the tips of axons and dendrites have growth cones that appear to reach out with finger-like structures, filopodia, and literally pull the growth cone to its destination. The axon is guided in a specific direction by chemical signals that attract and repel the growth cone; that is, chemoattractants and chemorepellents.

Another important phase of neuronal cell development is pruning or synaptic elimination, which entails retraction and elimination of excessive connections. Some refer to this constant struggle of neurones to connect as ‘neural Darwinism’. The evolution of cortical connectivity reflects an adaptation to sensory inputs in a constantly competitive fashion, with winners and losers depending on the environmental surroundings of importance to the organism and input to the brain (Edeleman, 1989). Disordered pruning may be the pathological basis for such conditions as autism and schizophrenia.

Apoptosis is referred to as programmed cell death, which reflects the fact that the cells actually carry genetic instructions to self-destruct, leaving no scars or damaged tissue.

## Neurotrophic Factors

Neurotrophic or nerve-growth factors (NGFs) are best defined as any molecule that affects the nervous system by influencing the growth or differentiation of neurones or glia. Neurotrophic factors are the stimulus behind neurogenesis and synaptogenesis (see Figure 1.15). NGFs



**Figure 1.15** Neurotrophic factors mediate cell proliferation and elimination by promoting cell growth, stimulating synaptogenesis and preventing apoptosis (reproduced with permission from Higgins and George, 2007, Figures 7–8, p. 82)

mediate cell survival, and cells that do not receive enough NGFs die. Target organs produce NGFs and specific NGF receptors are present on nerve terminals which attract afferent input.

Several other growth factors have been discovered and are being studied, including neurotrophin-3 (NT-3), glial cell-line-derived neurotrophin factor (GDNF) and insulin-like growth factor (IGF) to name a few, but the one of most interest is brain-derived neurotrophic factor (BDNF).

## NEUROTRANSMITTERS

A neurotransmitter is a substance that is manufactured by a cell, released into the synaptic

cleft in response to stimulation and has a specific effect on another cell. In the CNS this cell is a neurone, but peripherally it may be a secretory cell. Further, to qualify, the substance should, when applied experimentally, mimic the effect of the natural release, and some mechanism should be available to remove it from the synaptic cleft (Schwartz, 1981).

Although it was at first thought that a single neurone used only one transmitter, it is now known that two or more transmitters can be identified in many neurones, especially with the coexistence of a peptide and an amine (see below). In fact, different neurotransmitters sometimes share the same vesicle. Further, while the criteria given for neurotransmitter status have been identified, the situation is not so clearly defined. Thus it is possible to see all levels of chemical communication between cells, from direct neurone–neurone contact, through to neurosecretory cells that release neurohormones either into the hypophyseal portal system, which influences pituitary cell output, or directly into the circulation via the posterior pituitary gland (neurohypophysis). Receptors for some neurohormones have been identified in the brain, and the possibility of feedback from release into the peripheral circulation exists. Finally, as in the autonomic system, neurones make contact with adrenal medulla cells, directly influencing hormonal output.

The situation is further complicated by the concept of neuromodulators. Thus, the action of neurotransmitters is considered to be brief and to operate over a short distance. However, some neurotransmitter candidates, especially the peptides, lead to longer alterations of synaptic tone, modulating the environment of other ongoing neurotransmitter events.

Of the many potential transmitters, the synthesis of a few key ones is described.

### **Acetylcholine**

This is synthesized from choline and acetyl coenzyme A, the reaction being catalysed by choline acetyltransferase. Following release into the synaptic cleft, it is broken down by acetylcholinesterase. It is the main transmitter used

by motor neurones in the spinal cord and is the transmitter for all preganglionic autonomic neurones and for postganglionic parasympathetic neurones. In the CNS it is found in high concentration in the caudate nucleus and hippocampus, and an ascending cholinergic system has been defined innervating the thalamus, the striatum, the cerebellum, the limbic system and the cerebral cortex. The basal nucleus of Meynert is an important CNS location of acetylcholine.

### **GABA**

GABA is synthesized from L-glutamate, utilizing the enzyme glutamate decarboxylase (GAD). It is metabolized by GABA transaminase to glutamic acid and succinic semialdehyde, which, following oxidation, enters the citric acid cycle. It is involved in the activity of some 30–50% of CNS neurones, especially of interneurones. The highest concentrations of GABA are in the substantia nigra, globus pallidus, hippocampus, hypothalamus and cortex. In the spinal column it is in the spinal grey matter. It is an inhibitory transmitter, and antagonists such as bicuculline provoke convulsions. It is one of a group of amino acid transmitters that have a ubiquitous distribution, some of which serve as substrates in metabolic cycles. These include glycine, beta-alanine, glutamate and aspartate.

### **Glycine**

Glycine is another inhibitory transmitter, especially in the spinal cord and brainstem. It inhibits neuronal firing by gating chloride channels. It can modulate the action of glutamate at the NMDA receptor.

### **Serotonin (5-HT)**

This is one of the amine neurotransmitters; others include dopamine and noradrenaline. It is synthesized from tryptophan under the influence of the enzyme tryptophan hydroxylase, which converts it to 5-hydroxy-tryptophan. This is decarboxylated to 5-HT. It is metabolized to



5-hydroxyindole acetic acid (5-HIAA) by the enzyme monoamine oxidase.

The main nuclei containing 5-HT are the raphe nuclei of the brainstem, from which fibres ascend and descend to influence many areas of the brain, especially the neocortex, limbic system, thalamus and hypothalamus. In the pineal gland, 5-HT is converted to melatonin.

## Catecholamines

These are metabolized from tyrosine. Conversion to DOPA occurs under the influence of tyrosine hydrolase. DOPA is then decarboxylated to dopamine. In the presence of dopamine-beta hydrolase this is converted to noradrenaline. In a few areas, *N*-methylation of the latter results in adrenaline.

Breakdown involves two main enzyme systems: monoamine oxidase and catechol-*O*-methyl transferase. The former acts mainly intraneuronally, the latter in the synaptic cleft. The main metabolites of dopamine are homovanillic acid (HVA) and DOPAC, while noradrenaline (NA) breaks down to vanillomandelic acid (VMA) and methoxyhydroxy-phenylglycol (MHPG).

Noradrenaline is the transmitter at post-ganglionic sympathetic neurones, but in the brain the main synthesizing neurones are in the brainstem, the locus coeruleus and related nuclei. The ascending neurones terminate widely to influence the cerebral cortex, limbic system and hypothalamus. Catechol *O*-methyl transferase, responsible for breaking down the catecholamines, is regulated by the COMT gene, which has polymorphisms, and of importance is the Met (methionine)–Val (valine) substitution at codon 158 of chromosome 22q. The Met allele is associated with low enzyme activity, and the Val with high activity.

Dopamine derives from nuclei in the brainstem (notably the substantia nigra and the ventral tegmental area), but its output is more restricted than noradrenaline or 5-HT. In particular, it involves the striatum and the limbic system. It is of interest that cortical dopamine projections in primates suggest a functional specialization. The major influences are motor rather than sensory:

sensory association areas have more dopamine than primary sensory regions, and auditory association cortex has more than visual association areas (Lewis *et al.*, 1986). A further location of dopamine is in the tuberoinfundibular system of the hypothalamus, which is involved in the regulation of prolactin release, with stimulation leading to inhibition.

## Peptides

Many peptides that may have a central role, either as neurotransmitters or as neuromodulators, have been recognized. In contrast to classic neurotransmitters, they are large molecules and are composed of chains of aminoacids. A list of these is given in Table 1.5. At present the evidence that many are actually transmitters awaits confirmation. Moreover, attempts at developing treatments based on peptides have been disappointing to date. Most peptides are formed by

**Table 1.5** Potential peptide neurotransmitters

<i>ACTH</i>
Angiotensin
Bombesin
Bradykinin
Calcitonin
Carnosine
CCK (cholecystokinin)
CRF (corticotrophin releasing factor)
Dynorphin
Beta-endorphin
Met-enkephalin
Leu-enkephalin
Gastrin
Glucagon
Growth hormone
Lipotropin
LHRH
Alpha-MSH
Motilin
Neuropeptide Y
Neurotensin
Oxytocin
Prolactin
Secretin
Somatostatin
Substance P
TRH
Vasopressin
VIP



cleavage of larger precursors and understanding of the functional significance of the fragments is quite incomplete.

An interesting feature of the peptides is their wide distribution throughout the body, identical ones being found, for example, in the gut and the brain. These include cholecystokinin, vasoactive intestinal peptide (VIP) and gastrin. It can be seen that several are hormones.

Peptide receptors are coupled mainly with secondary messenger systems.

### ***Enkephalins***

These were the first morphine-like (endorphin) substances to be discovered in the brain, being penta peptides. They were shown to possess opiate-like activity, along with other peptides such as beta-endorphin and dynorphin. Enkephalin neurones and opiate receptors have been identified in the limbic system and striatum, and receptors in such areas as the hippocampus, nucleus accumbens, thalamus and amygdala. They occur in several areas of the spinal cord, including the substantia gelatinosa. Beta-endorphin distribution is more restricted, the highest concentration being in the pituitary, which structure is almost devoid of enkephalins. Mu receptors are more related to sensory events, being found in the cerebral cortex, whereas the limbic system has an abundance of delta receptors.

### ***Neurotensin***

This is found in high concentrations in the hypothalamus, basal ganglia and amygdala. It inhibits neuronal firing in the locus coeruleus, where neurotensin is abundant. Receptor sites in the brain are widespread.

### ***Substance P***

This occurs in dorsal root ganglia, with terminals in the substantia gelatinosa, a region of the spinal cord thought to be involved in the pain pathways. It is found in high concentrations in the striatal–nigral system, the habenula,

the amygdala and the bed nucleus of the stria terminalis.

### ***Cholecystokinin***

This is found in high concentrations in the cortex, it and VIP being brain peptides well represented within cells in the cortex. It is found in the hypothalamus, and terminals are in the amygdala. It also is seen in the periaqueductal grey region and, like substance P, in the dorsal root ganglia cells.

### ***Vasoactive Intestinal Peptide***

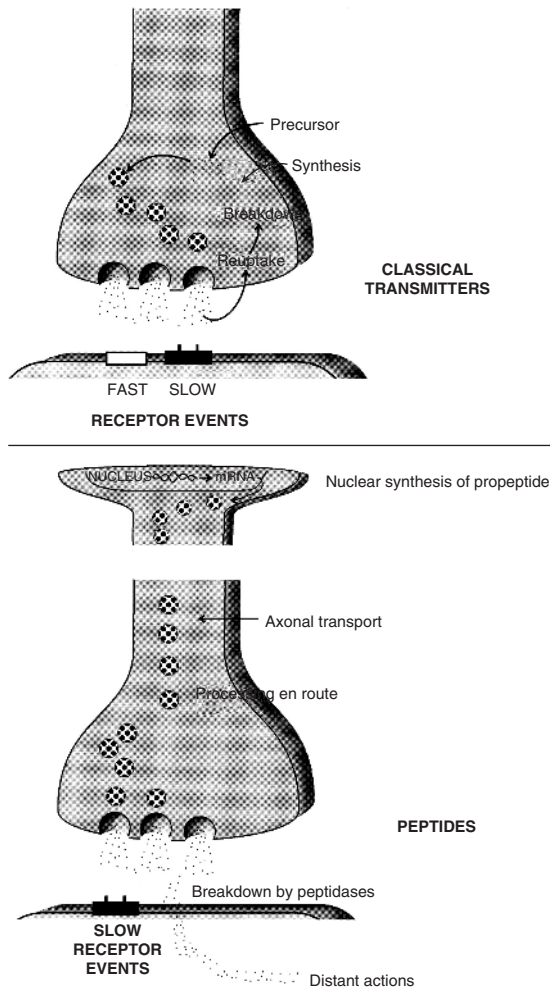
Highest levels are found in the cerebral cortex, and terminals containing it are identified in the amygdala and the hypothalamus. In the body it has several functions, including vasodilation and enhancing lipolysis and pancreatic secretion.

### ***Angiotensin***

This has for some time been known to be involved in vasoconstriction and sodium regulation by the kidney. It is found centrally in several regions, including the hypothalamus, and many angiotensin receptor sites have been identified. Complementing its peripheral action, it is involved in the central regulation of drinking.

### ***Releasing Factors***

These are found in the median eminence of the hypothalamus, and pass through the portal capillaries to influence hormonal release from the anterior pituitary. They include thyrotropin-releasing hormone (TRH), a tripeptide, the majority of which is found outside the hypothalamus. Somatostatin inhibits growth hormone release and is found in the amygdala, hippocampus and cortex, with terminals in these sites and in the striatum. Luteinizing hormone-releasing factor (LHRH) stimulates LH and FSH release, and is found primarily in



**Figure 1.16** Comparison of the manufacture and release of classical neurotransmitters and peptides (Webster, 2002)

the hypothalamus. Corticotropin-releasing factor (CRF) releases ACTH. Various medications modifying CRF were once considered promising candidates as anxiolytics or anti-depressants but were disappointing in clinical trials.

### Other Central Peptides

The posterior pituitary hormones oxytocin and vasopressin seem to have a central role, with pathways projecting to some brainstem and limbic-system structures. These two peptides appear to act selectively in some animals in terms of promoting or inhibiting interpersonal bonding (Stein and Ythilingum, 2009; Young *et al.*, 2008). Active investigation involves administering synthetic oxytocin and observing changes in interpersonal behaviour, and in disorders of bonding such as autism. Adrenocorticotrophic hormone (ACTH) is found throughout the brain, especially in the hypothalamus, thalamus, periaqueductal grey and reticular formation.

Some of the differences between the peptide transmitters and the more classical ones are summarized by Hokfelt *et al.* (1980) and are shown in Figure 1.16. In particular, peptides are produced in the cell soma and not synthesized at synaptosomes, unlike other transmitters, and there are no re-uptake mechanisms from the synaptic cleft. This may be compensated for by their effective action at much lower

**Table 1.6** The coexistence of classical and peptide transmitters

<i>Transmitter</i>	<i>Peptide</i>	<i>Location</i>
Dopamine	Enkephalin	Carotid body
Dopamine	CCK	Ventral tegmental area
Noradrenaline	Somatostatin	Adrenal medulla
Noradrenaline	Enkephalin	Adrenal medulla
Noradrenaline	Neurotensin	Adrenal medulla
5-HT	Substance P	Medulla oblongata
5-HT	TRH	Medulla oblongata
Adrenaline	Enkephalin	Adrenal medulla
Acetylcholine	VIP	Autonomic ganglia, sweat glands

concentrations, their more prolonged action and their intermittent rather than tonic release.

## **INTERRELATIONSHIPS AMONG TRANSMITTERS**

Dale's principle, namely that one neurone synthesizes and releases only one neurotransmitter, has had to be modified in the light of recent data. Although it has been known for some time that certain neurones probably released both acetylcholine and noradrenaline, the coexistence of peptides and other neurotransmitters has been reported for several neurone groups. A list is shown in Table 1.6. However, not all neurones possessing one classical transmitter contain the same peptide, and vice versa. The functional significance of such arrangements has been a matter of speculation. For example, in one group of neurones from sympathetic ganglia involved in sweat gland secretion, both acetylcholine and VIP are present (Hokfelt *et al.*, 1980). It is suggested that the VIP may be the mediator of vasodilation aiding the acetylcholine-primed secretion. A further example is the coexistence of CCK in a subpopulation of dopamine neurones. These are mainly in the substantia nigra and related ventral tegmental area (VTA), and project to the limbic forebrain (Hokfelt *et al.*, 1980).

Other examples of interaction include the influence of monoamine release by peptides, such as the association between substance P and dopamine, the former acting as an

excitatory transmitter for some dopamine neurones (Iversen and Iversen, 1981), or peptides interacting with peptides as in the case of opiate receptors located on substance P terminals.

## **TRANSMITTER DISPERSAL**

Following interaction with the postsynaptic cell, transmitters are either broken down by enzyme systems in the synaptic cleft or taken back up by the neurone for degradation for re-use. Some are lost by simple diffusion away from the cell. The intracellular enzymes, monoamine oxidase and catechol-*O*-methyl transferase, are of importance to the amine transmitters, and the major extracellular mechanism for the degradation of acetylcholine is acetylcholinesterase. Peptides are degraded by peptidases.

## **CNS INFLAMMATION**

Cytokines are produced by activated macrophages, and are either proinflammatory (interferon, INF, and tumour necrosis factor, TNF) or anti-inflammatory (interleukin 4 or 10, IL4/IL10). A cascade of events leads to T-helper lymphocyte activation, and these release cytokines. There are receptors for these cytokines in the brain, and they have been shown to activate the CRF system. The ratio between pro-inflammatory and anti-inflammatory cytokines can be measured, and has been shown to relate to psychopathology.

