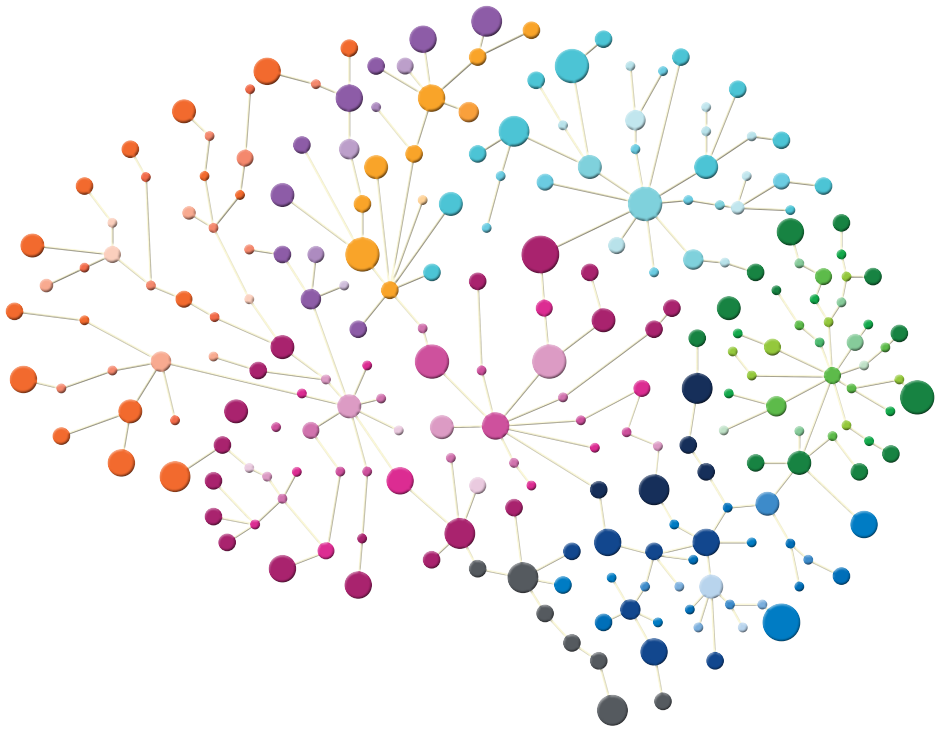

CHAPTER 4

DEVELOPMENT, DEGENERATION AND RECOVERY IN THE NERVOUS SYSTEM

CHAPTER BREAKDOWN

- Development, degeneration and recovery in the nervous system as fundamental processes underlying psychological and neurological disorders.
- An overview of the processes that convert the fertilised single cell into a multicellular embryo.
- Increasing cell number (proliferation), getting neurons to the correct locations (migration) and differentiation of potential neurons.
- Synaptic modelling: from initial formation of synapses, through loss of synapses (pruning) to life-long plasticity.
- The consequence of loss and the potential for the renewal of neurons in neurodegenerative disease.

ROADMAP

We have already seen (in Chapter 2) that the adult nervous system is composed of billions of cells of different types. Yet all humans start out life as a single cell. Therefore, there is a complex and highly regulated process of development. However, neurons can also die, leading to degeneration of the nervous system. This has led to a need to understand the processes that might allow for neuronal regeneration to restore lost function.

The building of a functioning brain requires two fundamental processes: first, the cells of the brain have to be produced and organised to be in the right place within the brain structure as a whole. However, this alone is not enough to produce the brain that underpins the complex human behaviours explored in this book. So, second, the connections between the cells have to form to allow the neurons of the brain to communicate with each other and form functional networks. The function of the brain is dependent not just on the formation of new neurons and connections, but also on the refinement of networks to make them functional. This actually involves the useful death of some of the neurons. However, neuronal death can also be disadvantageous, and is associated with both natural ageing and pathological disease. Unfortunately, there is little natural capacity for repair to damaged neurons or recovery of lost neurons within the central nervous system. However, contrary to popular belief, some areas of the brain produce new neurons well into adulthood, raising the prospect that therapeutic regeneration of the brain may not be impossible.

Our understanding of the processes involved in brain development and the factors that control them may help us to explain both disorders that can have a behavioural component and become apparent during early childhood, such as autism or dyslexia, and disorders that do not emerge until later in life, even in adulthood, such as schizophrenia, which is discussed in more depth in Chapter 10.

DEVELOPMENT

Much of our understanding of brain development has come from studying non-human organisms. Many of these studies use organisms that we would think of as considerably simpler than a human, such as fruit flies (*Drosophila melanogaster*) and microscopic nematode worms (*Caenorhabditis elegans*). However, what is now clear is that the underpinning principles of early brain development are conserved across species and are evolutionarily ancient (Arendt and Nübler-Jung, 1996). In the later stages of brain development some of the processes are almost unique to humans and, as our understanding improves, so does our ability to propose new interventions or therapies that may help not only to prevent maldevelopment of the brain, but also to encourage it to effectively rebuild itself after insult in adulthood.

Our current understanding of the development of the human brain, while not complete, is extensive, and a detailed account of the molecules and processes involved is beyond the scope of this book. However, an appreciation of the basic principles and how they may impact on the adult brain will provide a useful underpinning to understand how behaviour may be influenced by brain structure.

The fertilised egg undergoes a period of massive cell proliferation where repeated cell division results in a rapid increase in cell numbers within the embryo. Initially these cells appear to be virtually identical, but in humans, by about week 3 after fertilisation, the uniform ball of cells has begun to take on shape and a layer of cells that are destined to produce the nervous system, called the ectoderm, can be identified – a process known as gastrulation. During the gastrulation phase, the combination of differential gene expression and cell-to-cell interactions results in a process known as neural induction, whereby a region of ectoderm transforms into a structure known as the neural plate. As the name suggests, this is the first sign that a nervous system will ultimately be formed. The embryo then enters the **neurulation** phase, shown in Figure 4.1, which begins with the embryo elongating and the neural plate becoming a groove along the longitudinal axis of the embryo. As the cell numbers of the groove rapidly expand and the embryo elongates, the groove deepens until the top edges come together to fuse and form a hollow tube-like structure running the length of the embryo; the **neural tube**. Even at this stage it is known that cells along the longitudinal axis express particular genes that mark them as belonging to one end of the embryo or another, such that the fate of cells destined to produce the brain is already determined. Genetic manipulation of primitive species suggests that the factors that determine cell fate are not just determined by the activity within the cell itself, but also include environmental influences on the cell such that cell position can determine which genes are expressed. Hence, by about week 4, at the end of neurulation, the embryonic regions which will form the central nervous system have been established; the **posterior** part of the neural tube will form the spinal cord and the **anterior** region will go on to form the brain.

Neurulation The formation of the embryonic neural plate and its transformation into the neural tube.

Neural tube A hollow tube-like structure running the length of the vertebrate embryo that eventually forms the brain and spinal cord.

Posterior From the Latin word *posterus*, meaning 'coming after', in biology posterior refers to the back of something (e.g. the brain).

Anterior From the Latin word *ante*, meaning 'before', in biology anterior refers to the front of something (e.g. the brain).

Clearly, the processes that have been described so far are entirely reliant on the right genes and subsequent proteins being expressed in the right place and at the right time within the rapidly developing embryo. Any error in this process is therefore likely to have profound effects on the structure and function of the embryo as a whole. Congenital defects in neural tube formation

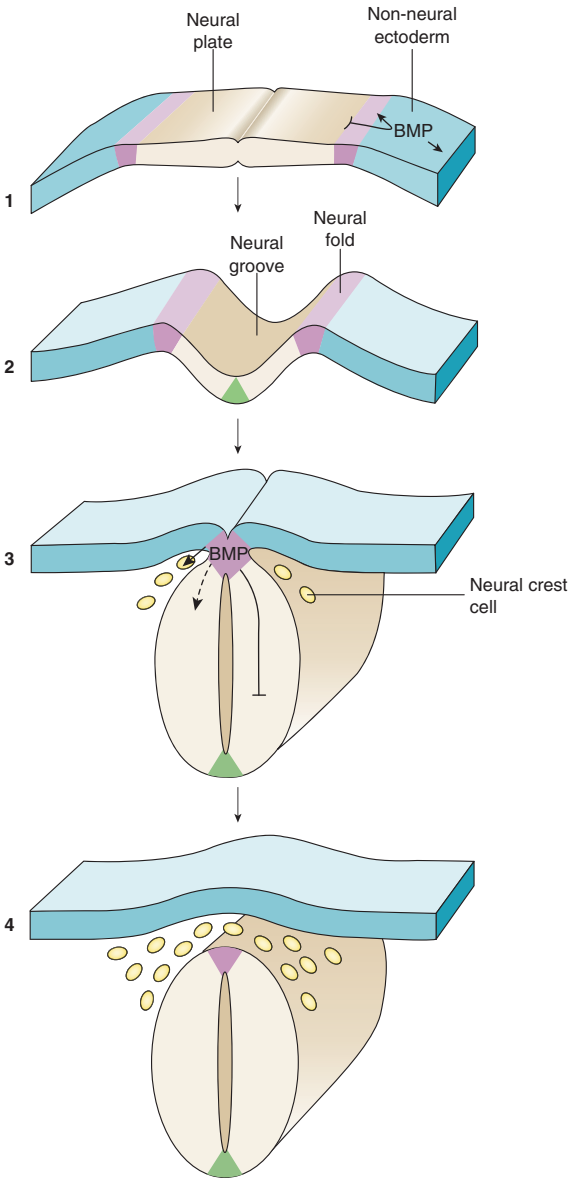

Figure 4.1 The CNS arises from a specialised epithelium, the neural plate (1). This process relies on the inhibition of bone morphogenetic protein (BMP) signalling. Folding of the neural plate to produce the neural groove is triggered by the formation of a distinct hinge point in the **ventral** region (the floor plate; 2). At the end of neurulation, the lateral edges of the neural plate fuse (3) and segregate from the non-neural epithelium to form a neural tube (4). The roof plate and floor plate form at the **dorsal** and ventral midline of the neural tube, respectively. The roof plate becomes a new organising centre that produces BMPs, which provide dorsal patterning information. Neural crest cells derive from the dorsal neural tube and migrate out to form the PNS, as well as melanocytes and cartilage in the head. Neural crest cells have been shown to form at an intermediate level of BMP signalling

Source: Reprinted by permission from Springer Nature: Liu, A., & Niswander, L. A. (2005). Bone morphogenetic protein signalling and vertebrate nervous system development. *Nature Reviews Neuroscience, 6*(12), 945–54

vary from those which are compatible with a good quality of life, such as mild forms of spina bifida, to anencephaly, where the foetus fails to develop a substantial part, if not all, of the cerebral cortex and may be stillborn or may die within a few days of birth (Sadler, 1998).

Clearly, the differences, which are sometimes subtle, we may see in adult behaviour are more likely to arise from events subsequent to neurulation, whereby the highly organised structure of the brain is established.

Ventral From the Latin *venter* (meaning 'belly'), in biology ventral refers to the front or lower side of something (e.g. bottom of the brain).

Dorsal From the Latin *dorsum*, meaning 'back', dorsal refers to the back, or upper side, of something (e.g. top of the brain).

Forming the foetal brain

At the end of neurulation, the anterior end of the embryo is not just a tube but has swellings known as vesicles. Each of these five vesicles will go on to form a predictable region of the brain. Continuing proliferation of stem cells coupled with specific protein expression and cell-to-cell interactions continues, with the cells gradually differentiating to form ever more specialised and neuron-like cells, and can ultimately dictate what type of neuron is produced, including the neurotransmitters it will produce.

Migration and differentiation

Within the forming brain, neurons that can differentiate to form one or more kinds of cells (known as progenitor cells) are produced at the same location, a region that borders the fluid-filled interior space known as the ventricular zone, but must then travel to their ultimate destination to group together with other neurons of the same type. The evidence suggests that once cells move away from the ventricular zone they lose the capability to divide, thus preventing inappropriate proliferation, and can be said to be entering a phase where they are committed to become neurons.

The processes involved in controlling this migration have been studied most in the cerebral cortex, since this region contains a vast number of neurons in the adult and these can be observed histologically to show very precise organisation throughout the depth of the cortex, indicating that a highly precise process has occurred during development.

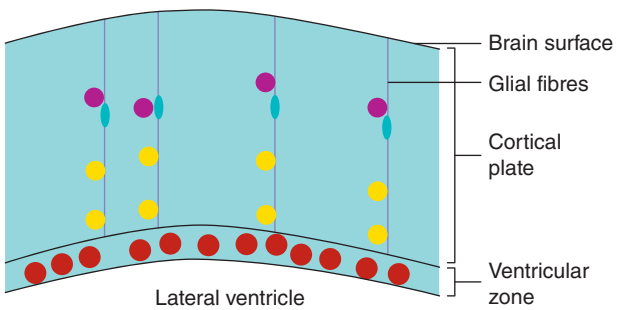

Figure 4.2 Inside-out layering. Post-mitotic immature neurons (neurons that do not exhibit mitosis and cell division) (red) migrate away from the ventricular zone along the radial glial cells (green). The earliest cells to migrate settle closest to the ventricular zone (yellow) while later cells migrate progressively further towards the surface of the brain (purple)

In brief, the newly produced neurons travel from their place of birth in the ventricular zone towards the outer surface and to their ultimate destination via a scaffolding-like network of specialised glial cells known as radial glia. This occurs in a very precise pattern whereby the first immature neurons migrate the shortest distance from the ventricular zone, and subsequently produced immature neurons migrate further, a process termed inside-out layering (Figure 4.2).

The human cerebral cortex is greatly enlarged when compared to other species and is therefore considered to underpin the enhanced behavioural repertoire of humans. Hence, it should come as no surprise that any disruption to this migratory process would result in serious disruption to behaviour. Studies suggest that a whole range of factors, from genetic mutations to exposure of the developing foetus to chemical substances, can have profound effects on the developing brain, including through disruption of migration (Liu, 2011). An environmental substance that disrupts normal development is known as a **teratogen**. Many teratogens are found by chance when the correlation between maternal exposure and foetal developmental abnormality is observed. Substances that have teratogenic effects on brain development include alcohol and even some prescription drugs (see Real world applications box). The observation that teratogens can produce brain abnormalities has been turned to scientific advantage to produce animal models of human disorders where developmental effects have been implicated. For example, if pregnant rats are exposed to methylazoxymethanol (MAM) at a particular point in their pups' gestation, then this agent interferes with the proliferation of neurons (Hradetzky et al., 2012). The pups are born appearing to be relatively normal but detailed analysis shows that they have some of the histological and behavioural characteristics that are seen in schizophrenia, making them a potential neurodevelopmental model of this condition (see Chapter 10).

Teratogen An agent that causes malformation of an embryo or foetus.

Real world applications

An example of a known potent teratogen is alcohol (ethanol), which, if consumed in large quantities, may result in a variety of developmental problems, including of the brain. As we have seen, the very early stages of brain development occur in the embryo within a matter of a few weeks post-fertilisation. At this time the mother may not even be aware that she is pregnant, and heavy alcohol consumption can result in irreparable damage being done to the developing embryo, known as Foetal Alcohol Syndrome (FAS). Remarkably, the embryo can survive and continue through subsequent foetal stages of development. However, if the damage was done in the early developmental stages (i.e. during neurulation), then the baby may be born with brain abnormalities such as a small forebrain. If the damage occurred later, for example during the migration phase, then problems such as learning impairments may result due to cortical malformation. A summary of the impact of alcohol across embryonic and foetal development can be seen in Figure 4.3. Ask yourself: what other substances cause developmental problems during pregnancy and what advice is given to pregnant women?

OVUM STAGE	EMBRYONIC STAGE	FOETAL STAGE – 2ND YEAR OF LIFE
• Reduced neural stem cells proliferation • Neural tube defects • FAS dismorphia • Increased neural crest cell death	• Abnormal radial glia: neuronal and astroglia deficits • Abnormal cell migration • Neural cell loss • Corpus callosum malformations	• Prominent microcephaly • Abnormal glial development • Increase in natural cell death and cell necrosis • Alterations in neural connections • Alterations in the cerebellum

Figure 4.3 The effect of alcohol at various stages in foetal development

Even if we take the situation where the potential neurons are all produced and migrate to their required final destinations, there is still some considerable activity required to produce a functioning brain. The cells can now start to differentiate and take on the physical and functional characteristics of neurons. This will require the development of processes such as dendrites and axons. These axons will need to travel through the surrounding tissue to find their intended targets and form synapses. To be functional, these synapses will need to be able to release a particular type of neurotransmitter and the postsynaptic cells will need to express the corresponding neurotransmitter receptors to ensure that communication between the pre- and postsynaptic cells can occur.

Real world applications

The clear importance of appropriate protein expression for cell production and migration means that any error in this process will have profound effects. One of the sources of these errors is in genetic mutation. Mutations that result in a lack of protein production at this early stage in development are unlikely to be compatible with life. However, mutations where a protein is produced, but either at lower or higher levels or in a slightly different form, are more likely to be compatible with life but with some disruption of function. An example of a gene that has been implicated in underlying psychiatric disorders is DISC-1 (disrupted in schizophrenia 1). Despite its name, this gene has been implicated in a number of human psychiatric conditions within a single family, including schizophrenia but also major depression, bipolar disorder and autistic spectrum disorders (Brandon et al., 2009). Experimental studies have demonstrated that the normal protein product produced by this gene is involved in supporting normal neuronal progenitor cell proliferation as well as migration of potential neurons (Mao et al., 2009). Ask yourself: what does the involvement of DISC-1 in several different disorders imply about what determines the behavioural profile of the affected individual? (See also Chapter 10.)

Connection formation – axon guidance

Prospective neurons being produced and making it to the appropriate location is not enough to produce a functioning brain. We have previously covered in Chapter 2 that the formation of circuits of interlinked neurons is what is required to produce a brain capable of determining behaviour. The formation of these circuits is not trivial, however, and requires the accurate execution of a number of processes. The first of these is that neurons that form a circuit may have their cell bodies some distance away from each other. At this point in developmental time, these prospective neurons are fairly featureless cells but, as we saw in Chapter 2, fully differentiated neurons are characterised by having long extensions from their cell bodies, known as axons, which can allow an individual cell to communicate with other cells some distance away via the use of regenerative action potentials. The question then arises as to how these axons are able to extend their processes in the right direction to find their intended target neurons.

Our knowledge of this process is incomplete but appears to involve the growing tip of the axon, known as a growth cone, 'tasting' the environment through which it is travelling, using receptor molecules which it expresses on its surface (see Figure 4.4). The environment contains molecules that may be fixed on other cells or floating free within the intracellular environment. It should be noted that the growth cones can be repelled as well as attracted by these interactions. Thus, axons are guided along a path by a combination of physical contact and concentration gradients of released molecules.

This process of axon guidance appears to be highly evolutionarily preserved, which is notable for two reasons. First, it suggests that solving the problem of axon targeting was key to the ability of organisms to evolve ever more complex brains to support complex behaviour. Furthermore, it is useful for our ability to study the process since it means we can use rather more primitive organisms than humans to dissect out the details of the process.

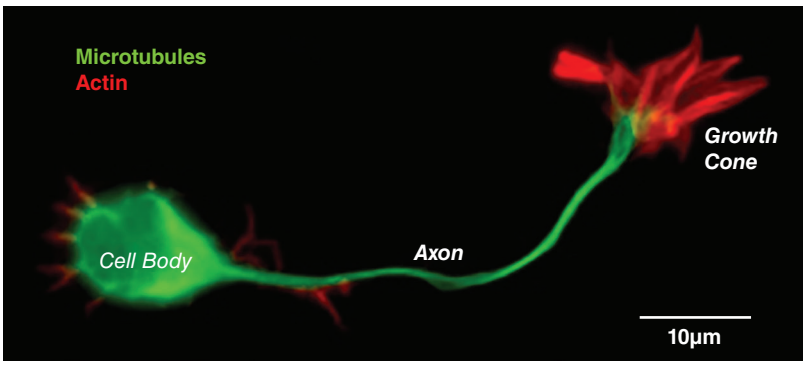

Figure 4.4 Axon guidance

Source: Simon Moore, Columbia University, MBINFO Defining Mechanobiology. Reprinted under CC-BY NC 4.0 license

Connection formation – synaptogenesis

Once axons reach their intended target, they will need to form synapses to allow neuron-to-neuron communication to occur. While clearly this must involve the correct type of target cell, there is also a requirement for the presynaptic cell to target the right part of the postsynaptic cell. As we saw in Chapter 2, these synapses tend to form on dendrites of the postsynaptic cell. However, the precise point on the dendrites that particular synapses form (e.g. close to or away

from the cell body) seems to be important. Furthermore, some synapses will need to form on the cell body of the postsynaptic cell rather than the dendrites, all of which suggest some sort of precise targeting mechanism must exist.

In Chapter 2 we saw that synapses consist of regions of the presynaptic cell that are specialised to release the neurotransmitter. The postsynaptic part of the synapse also has specialisations and the formation of these requires the exchange of reciprocal messages between the two neurons. The molecules that underpin these pre- and postsynaptic specialisations are mostly protein-based and are therefore subject to the influence of genetic mutations and other epigenetic factors that affect gene expression (discussed in more detail in Spotlight 4a). Clearly, minor changes in these proteins or their expression could still be compatible with synaptic activity but may change its character somewhat. The recognition that these changes may be important in disorders of brain function, including those where behaviour is changed, has led to the emergence of the term **synaptopathy**. Two recent candidates as synaptopathies are autism and attention deficit hyperactivity disorder (ADHD). Here individuals have a range of seemingly diverse functional impairments which emerge during the early years of life. Extensive study of the neurobiology of autism has long implicated a neurodevelopmental problem relating to basic developmental processes such as proliferation and migration. However, more recently, in-depth genetic studies have suggested that the major problem is centred around the formation and maintenance of synapses (Bourgeron, 2009). Similarly, dysfunction in synapse function has been implicated as a contributing factor in ADHD (Feng et al., 2005). Hence, the inability to associate disorders such as autism or ADHD, which appear to run in families, with any single gene may have come about because so many proteins are involved in synaptic formation. Since the synapse is the functional unit in neuronal communication, then any problem with any of the contributing proteins would potentially result in the disorder.

Synaptopathy Dysfunction in synapse function.

Neurochemistry

As we saw in Chapter 2, neurons produce and release a range of neurotransmitters. Clearly, precisely which neurotransmitters a neuron releases will be determined by the suite of proteins, including enzymes, necessary for synthesising the neurotransmitter that an individual neuron is producing. It seems that a multitude of factors determines the precise nature of this suite of proteins, and one of the more surprising findings is that it would appear that the cells that the neuron is just beginning to make contact with can influence this process through the production of releasable substances from the postsynaptic cell which impact upon the presynaptic cell.

Optimising brain function

The processes described so far have led us to the point of having made all the necessary components of the brain and to begin to connect them together. However, this is not the end of the developmental process for there needs to be refinement to optimise the functionality of this emerging new brain.

Neuronal death and synaptic modelling

Perhaps one of the more surprising findings to have emerged from studying development of the brain is how much resource is put into processes and elements that are not ultimately found

in the adult brain. For example, some estimates suggest that only approximately half of the neurons born make it into the adult brain. However, this loss appears not to be an accident or in any way random, but occurs in a highly targeted and organised way via a process known as programmed cell death or **apoptosis**. This process is distinct from that which occurs as a result of direct damage to cells, for example trauma, which is known as **necrosis**. The mechanisms that determine whether or not a neuron will undergo apoptosis are multifactorial but include such processes as whether the neuron receives appropriate messages from the target cells with which it is attempting to form synapses. Interestingly, it would appear that neurons maintain their ability to undergo apoptosis and that this process can be reactivated inappropriately in later life, resulting in neurodegenerative disease, as discussed below.

Apoptosis The process of programmed cell death in which cells self-destruct to remove unwanted cells.

Necrosis Cell death due to damage or trauma to cells.

Synaptic formation is clearly a critical process with resource implications. So critical is it that accurate connections are formed, that many of the ones formed in the initial phases of brain development are then subsequently lost: a process known as **refinement**. In short, this process involves ensuring that connections are functional by maintaining only those that are active and therefore forming useful circuits.

Refinement The process by which non-active synaptic connections are lost during development.

Clearly, circuits are most likely to be initiating activity postnatally, once the individual is beginning to interact with their environment. It would logically follow that a human infant would produce and maintain more synapses if the input into the brain in the form of experience were higher. Evidence for this is provided by studies in experimental animals whereby those which spent their early life in simple environments had fewer connections than those that had an enriched environment which provided opportunities for, for example, exploration and play. This work, started in the 1940s by Hebb (1947), has produced a vast literature (see Van Praag et al., 2000, for a review).

One of the findings to emerge from this work is that during postnatal development there are short stretches of time during which synaptic remodelling is at its peak. These are known as **critical periods**, so named because the high level of synaptic attrition during these periods appears to be critical for subsequent functioning throughout life. It is therefore clear that if the right signals and environment are absent during a critical period, the young human will have potentially life-long impairments in function. Most of the original work on critical periods is centred around sensory systems like the visual system, which is still today considered the model system for this process (Hensch, 2004). Much of this work is carried out in non-human mammals, such as the work on whisker stimulation indicating that the 'maps' of the body surface that arise in the somatosensory regions of the cerebral cortex do so according to the patterns of activity of the various inputs such that over time individual whiskers are associated with discrete regions of the cortex and, concurrently, inactive synapses retract (Fox and Wong, 2005).

Critical periods Specific stages in development during which systems and behaviours are shaped and moulded for life.

However, what has become clear is that critical periods exist for even complex behaviours, such as emotional state and language acquisition and use. Indeed, it has been argued that the observation that disorders of adulthood, such as anxiety or schizophrenia, come about due to events and experiences (or the lack of them) that happen in the adolescent years could therefore imply that adolescence should be seen as another critical period in brain remodelling (Blakemore, 2008) (see Figure 4.5).

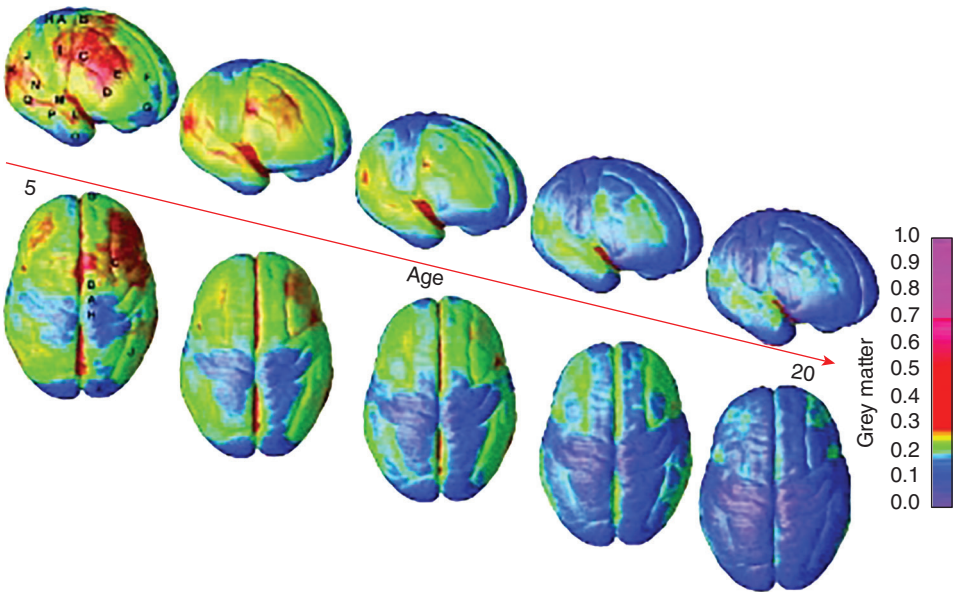

Figure 4.5 The volume of grey matter declines after birth until adolescence, suggesting that the brain may be particularly malleable in the early years of life

Source: From Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., Nugent, T. F. 3rd., Herman, D. H., Clasen, L. S., Toga, A. W., Rapoport, J. L., & Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. P*roceedings of the National Academy Sciences of the United States of America, 101*(21), 8174–9. Copyright (2004) National Academy of Sciences, U.S.A.

Myelination

In Chapter 2 we saw that neurons are reliant on interactions with glial cells for optimal functioning in a number of ways. One of these ways is in the determination of the speed with which axons can convey action potentials. The ensheathing of the axons by the spiral wrapping of the membranous processes of oligodendrocytes greatly enhances the speed of electrical signal conduction. This process does not occur until late on in brain development and is certainly not complete until axons have reached their targets. Indeed, it has been estimated that myelination is not fully complete in the human brain until the late teenage years.

Check your understanding

What would be the consequences for the developing foetal brain of damage at different points in time after fertilisation of the egg? Are all functional consequences going to be immediately apparent? If not, why not, and what are the potential consequences for later life? What public health messages could be used to reduce the chances of damage to the foetal brain?

Synaptic plasticity

It would be tempting to think that once the individual has reached late adolescence that the brain could be considered to have become optimally organised with all the right synapses in the right places, analogous to the wiring diagram of a computer or a map of bus routes in a city, and with complete myelination. However, it appears that the initial connectivity plan does not persist for the lifetime of the individual. As stated above, the adolescent phase appears to have a second wave of intense synaptic remodelling which is seemingly critical in determining adult brain function. Some degree of ongoing synaptic remodelling occurs throughout life and the importance of this fluid process cannot be underestimated. It is this process that allows us to form new memories and learn from our experience right up until old age, and this is explored in more depth in Chapter 5 on learning and memory.

However, a price has to be paid for this fluidity. First, it allows for inappropriate connections to be made, which may result in unwanted memories or behaviours emerging at any point throughout the life of the individual. Alternatively, the coincidence of intense experience may result in a reduced ability to form memories, which may be the mechanism that explains why soldiers who are seriously injured in battle may have little recollection of the time period around the time of their injury.

Sex differences in brain development

This chapter has given an overview of the complex processes that occur to produce a functioning brain where the genetic makeup of an individual will determine the fine detail of differences between individual members of the population. However, what has not been considered is whether there are any differences in these processes for the biological sexes and whether this may, at least in part, lead to predispositions in behavioural traits that may occur between the sexes. Although it would seem intuitive that sex differences in the brain structure of regions that are connected to reproductive behaviour will exist, it would appear that differences both in structure and impact on behaviour extend beyond reproduction. A number of human neuropsychiatric conditions have been linked to altered brain development and at least some of these show differences in prevalence between the sexes; for example, major depressive disorder is more frequently found in females while autistic spectrum disorder is more frequently seen in males (Bao and Swaab, 2010). It follows, then, that understanding how sex differences impact on brain development may be beneficial in understanding how and why adults exhibit neuropsychiatric disorders and whether this knowledge can be applied for therapeutic benefit.

It is only since the 1950s that there has been evidence that the development of the brain is influenced by the sex hormones that it is exposed to during development and that the structural consequences can then go on to influence the behaviour of the organism (Phoenix et al., 1959). Developmental studies indicate that the default pattern is to produce a female brain. Masculinisation requires exposure of the developing brain during a critical period to testosterone, which is produced in the male foetus when the testes develop which, itself, is determined by the presence of genes on the Y chromosome. In females, the absence of testes development ultimately results in the development of ovaries and the series of hormones associated with female reproductive behaviours, such as the oestrogens.

However, it is misleading to think that the presence of hormones is mutually exclusive between the sexes and only relevant to brain activity during development. What is now accepted

is that, at a population level, the average biologically male and female brain structure differs in many ways from the size of individual neurons, through the number of neurons in a brain nucleus right up to the size/weight of the whole brain, and that these differences are found in regions beyond those concerned with reproductive behaviour. It is also clear that the impact on sex hormones extends beyond the initial formation of the brain and into adulthood. The first piece of evidence that synaptic structure in the adult brain could be influenced by sex hormones was found in the hippocampus of female rats, where the changes covaried with the changing levels of female hormones through the oestrus cycle (Woolley et al., 1990). An unfortunate consequence of this realisation is that for many decades experimental studies have attempted to reduce variation by using male subjects. Only in recent years has the impact of this been recognised – much of what we can claim to know about the micro- and macro-level structure of the brain can only be said to hold true for the male brain.

Implications: looking ahead

While synaptic plasticity in the adult brain may be held responsible for a detrimental change in behaviour leading, for example, to symptoms of depression, it may also be exploited therapeutically. Many of the drug therapies used in psychological disorders, which we will meet in detail in Chapter 10, have effects which seem to defy simple molecular biological explanations, suggesting that other mechansims are involved. Also, for some patients, they are much more or much less effective than talking therapies. Evidence is gathering to suggest that for *any* therapeutic approach to be effective it requires that the brain undergoes plastic changes, almost certainly at the level of synaptic activity, which in effect reverses the processes that initiated the onset of the disorder in the first place (Castrén, 2005). Ask yourself: how and why do alterations in synaptic plasticity lead to mental health conditions and how can this information be used in the development of new therapies?

Key points

- The earliest signs of the nervous system are visible about three weeks after fertilisation when the embryo has undergone a period of massive cell proliferation.
- Progenitor neurons have to move from the position where they are produced to their final destination before they take on the characteristics of mature neurons.
- Brain development is determined by the switching on and off of the right genes at the right time and this process can be influenced by environmental agents.
- Synaptic plasticity is a life-long process enabling the changes in synaptic function that underpin behaviour.

DEGENERATION

Human brain degeneration is often thought of in the context of the mass loss of neurons following a traumatic insult, such as a car accident or stroke. However, somewhat alarmingly, what is clear is that brain material is lost fairly continuously from not long after we might consider the human brain to be complete! Brain size changes during life, getting larger until about 20 years of age before steadily declining such that an 80-year-old has a brain of approximately equivalent size to that of a 4-year-old (Courchesne et al., 2000).

So what is lost during this natural process of degeneration? This is a difficult question to answer since it is impossible to know the precise number of neurons at any one particular time in an adult brain and then to be able to track them over subsequent time. However, what is clear from the previous section on plasticity is that natural degeneration does not necessarily have to involve the loss of neurons but may involve the loss of synapses, such that a considerable amount of processing power can be lost without any significant reduction in the number of neuronal cells themselves. Accumulating evidence suggests, for example, that synaptic loss can account for the reduction in cognitive power seen in normal ageing. An attractive proposition, therefore, is to understand the processes by which synapses are lost, in order to develop means to slow or prevent this and thus maintain cognitive power with advancing years (Morrison and Baxter, 2012).

Pathological loss

The mass loss of neuronal populations through injury or pathological processes will clearly result in significant loss of function, whereby the precise functions that are lost will depend on which neurons are lost. In Chapter 2 we discussed the concept that some functions appear to be localised within predictable regions of the brain, so to some extent we should be able to predict the functional consequences of damage to particular areas and, in reverse, predict the area of damage according to the observed functional disorder following, for example, trauma.

The mechanisms that result in neuronal death are very variable. At one extreme, neuronal death may occur in otherwise healthy neurons if, for example, the basic requirements of the cells are no longer met. This is the situation seen in stroke patients, whereby disruption of the blood supply to neurons and the resultant reduction in oxygen and glucose supply and waste removal result in cell death. Neurons contain very little by way of reserves and so even temporary disruption for a few minutes can lead to cell death. If we look at the brain tissue of someone who has had a stroke at the cellular level, we see that there has been a mass breakdown of the cells in the affected area. It is difficult to discern individual neurons and there is a massive increase in glial numbers. In particular, large numbers of microglia will be seen and these will have a different appearance from the ones that can be found in the normal brain. Their job is to clean up the debris of the disintegrated neurons, presumably to stop the spilled intracellular contents affecting neighbouring neurons.

While the disruption to the blood supply of significant areas of the brain can lead to the extensive and diverse symptomatology of stroke, it is possible for smaller-scale neuronal death to cause problems. For example, it is now recognised that transient ischaemic attacks (TIAs), whereby a relatively small region may have diminished blood supply and for a very short period of time, can, with repeated episodes over time, result in a gradual decline in function related to the brain region affected. The most obvious situation is where an individual gradually loses

cognitive power, such that tasks that they may have previously done easily become more challenging or even impossible. However, sometimes more subtle changes in behaviour may occur, whereby the relatives of those in this situation may notice that the individual appears to be, for example, becoming more irritable or aggressive or even takes on a more negative view of the world where previously they had been generally optimistic.

Alternatively, neurons might die due to intracellular pathological processes, where normal cellular biochemistry is disrupted. This is clearly a different situation from that described above where mass necrosis is responsible for neuronal loss. Accumulating evidence suggests that in this situation the neuronal death that we can observe is very like the apoptosis that occurs as part of normal brain development, as described above. For some reason, which might be in response to a genetic mutation or an environmental insult or some combination of the two, the machinery of the cell switches into a degenerative mode that results in the death of the neurons. A striking feature of these neurodegenerative diseases is that it is often specific populations that are affected rather than some random, diffuse process throughout the brain. This suggests that for some reason the affected cells are more vulnerable to the environmental agent or are more reliant on the normal function of whichever gene(s) are dysfunctional. As we shall see in Spotlight 4a, most behavioural characteristics and disorders are complex and almost certainly involve both environmental as well as genetic influences. Furthermore, the evidence is accumulating that the number of genes involved is often extensive, such that we are not, for example, going to identify a single 'Parkinson's' gene which is mutated in all patients. However, an exception to this is Huntington's disease, which is monogenetic and appears to be influenced very little by environmental factors (see Figure 4.6).

Real world applications

Huntington's disease (HD) occurs approximately equally in males and females and usually only becomes apparent in adulthood. When the disorder was first being investigated it was notable that, while rare in any location worldwide, it was seen at the highest levels in the UK and regions settled during the exploration phases of the 17th and 18th centuries. This implied that the cause of HD may be some form of genetic mutation which had originated in the UK and had 'travelled' to each of the new settlements in an individual who then gave rise to a pedigree of affected individuals. Experimental work has now shown that HD is indeed monogenetic and the protein produced by the mutated gene, called huntingtin, appears to be critical for all normal cells, including brain neuronal activity, although its precise normal function is still debated. The nature of the mutation is that a region of the genetic sequence is repeated more frequently than is found in the normal version of the gene. This so-called trinucleotide repeat is translated into the mutant protein and as a consequence the protein is processed differently, in some cells at least, forming aggregates which interfere with normal neuronal activity. For some reason, the mutated huntingtin has devastating effects on a subset of neurons found in the caudate-putamen of the basal ganglia, although as the disease progresses it affects other neuronal populations too. Ask yourself: how do the signs and symptoms of HD such as problems with motor activity and cognitive ability relate to what is known about the brain area affected?

(Continued)

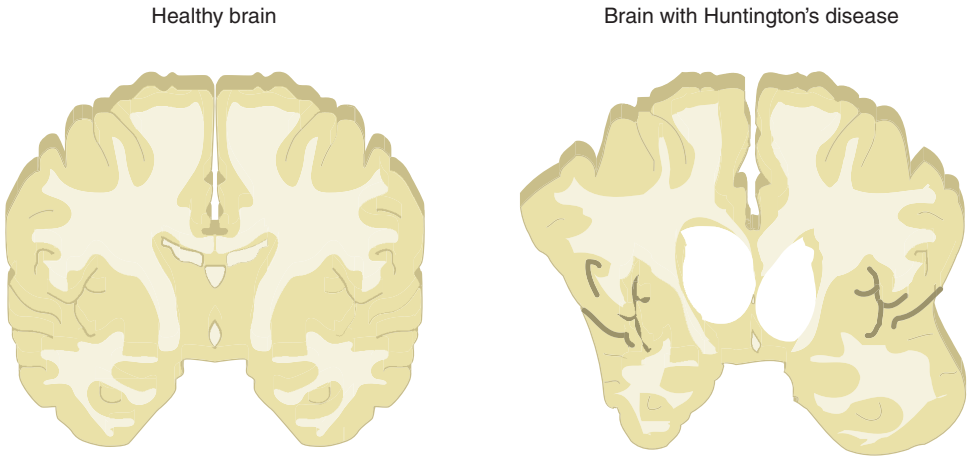

Figure 4.6 Healthy brain and brain with Huntington's disease

In many cases, our understanding of the cause of neurodegenerative disease remains rather poor, even though we may be able to characterise the signs and symptoms and the cellular changes that occur in the brains of individuals affected. For example, the prion diseases such as the human Creutzfeldt–Jacob disease and the form of dementia known as Alzheimer's disease result from the gradual accumulation of protein aggregates inside cells which eventually results in their death. However, we are still not certain, in either case, what starts this accumulation process or why the neurons finally die. It is likely that there won't be a single mechanism for all situations, but we will investigate in more detail the proposed causes of selective cell death seen in neurodegenerative disorders such as Alzheimer's and Parkinson's disease in Spotlight 4b.

Key points

- Neurons can die by two processes: necrosis and apoptosis.
- The cause of neuronal death may be genetic, environmental or a combination of the two.
- Functional effects of neuronal loss are related to the anatomical site of the loss.

REPAIR AND RECOVERY

From time to time we may all injure ourselves in some way but then, dependent upon the nature and severity of the injury, we would generally expect to recover, often leaving no trace of injury. For example, a skin wound or muscle tear will heal in a matter of weeks and even a broken bone can repair itself to give normal functionality within months. The human nervous system,

however, appears to behave differently, whereby some parts of the nervous system can show considerable recovery but other regions show almost none. Thus, if a peripheral nerve in the finger is severed, it may not necessarily result in the permanent loss of sensation but, as long as the cell bodies remain alive, sensation may gradually return as the cut end of the axons regrow to innervate the area. In contrast, the extent of capacity for recovery of the adult human brain appears to be limited, since otherwise we may expect to see at least some degree of recovery in degenerative disorders such as Parkinson's disease or following acute traumatic injury. Nonetheless, increasing our understanding of what potential the adult brain has for recovery and repair is attractive in the context of reversing human conditions with a behavioural component in which neuronal loss is a key feature, such as the personality changes observed alongside the decline seen in cognitive function in dementias (Emsley et al., 2005).

The rather limited capacity of the adult brain to regenerate seems rather at odds with its critical role in the survival of the individual. However, if we consider what would be required to regenerate damaged regions of the brain, it may be less surprising. In a case akin to the severed finger nerve described above, we would still need to be able to recreate the environment that was present in the developing foetal brain to enable appropriate axon extension, target finding and synaptogenesis. This is unlikely to be the case in the complex structure of the brain, and perhaps the prospect of regeneration resulting in inappropriate connections is a greater problem than the prospect of repairing damaged circuits. Study of the damaged adult brain indeed suggests that the lack of axonal regeneration when the cell bodies survive is due not to lack of capability of the neurons, but rather to the fact that there are mechanisms in place to actively inhibit the attempts of axonal regrowth within the CNS (reviewed in Yiu and He, 2006). These mechanisms include released factors and molecules expressed by glia, which often form a scar-like structure surrounding the injury site. Experimental studies, in which damaged neurons from the brain are exposed to a peripheral nervous system-like environment, have shown considerable axonal regrowth underlying the assertion that it is the nature of the environment, and not some intrinsic capacity of the cells, which determines the extent of regeneration.

Check your understanding

Why might mammals, including humans, have evolved to have limited potential for neuroregeneration in the adult brain? How might the use of stem cells in neurodegenerative diseases be able to overcome this? What challenges are there for stem cells that have been injected into the adult brain?

Neurogenesis

The requirements for full regeneration to replace dead neurons would require two main features. First, there would be a need to have a pool of undifferentiated stem cells that could undergo proliferation, and second, these newly formed cells would need to undertake migration before beginning the process of differentiating into neuronal cells and therefore would need to be able

to overcome the inhibitory nature of the environment described above. Up until fairly recently, the dogma was that new neurons could not be produced in the adult mammalian brain under any circumstances. However, experimental work, begun in the 1960s, has now shown this not to be true in a variety of species, including humans, and there appears to be ongoing neurogenesis in the normal brain, although it would appear that the capacity for neurogenesis is generally limited to a small number of regions and that this capacity declines with age (reviewed in Ming and Song, 2005). Although the extent of intrinsic neurogenesis appears to be limited, this does bring an unexpected benefit.

Focus on methods: immunohistochemistry

Identifying newly produced neurons in adult brain tissue is not easy because, to some degree, they will look identical to the old neurons. The fact that adult neurons have lost the capacity to divide is, however, very useful. Any newly produced neuron will contain newly formed DNA and so it follows that if we could find a way of labelling newly formed DNA we could distinguish newly born neurons from older ones. This is possible using bromodeoxyuridine (BrdU), which can be incorporated into DNA but which is not naturally found in the DNA. Hence, if we inject adult animals with BrdU and some short while later we examine their brains for the presence of the BrdU, we can conclude that any neurons containing BrdU must have been produced after the animal was given the BrdU injection. The presence of BrdU can be examined using immunohistochemistry, which is a technique for the visualisation of proteins in tissue. The tissue is incubated with an antibody that is capable of binding the protein of interest. The antibody is linked to a fluorescent dye which can be seen using a microscope.

Elsewhere in the body, the capacity of cells to reproduce themselves or for pools of stem cells to act as almost limitless sources of new cells is subject to dysregulation, which may result in the inappropriate production of high numbers of cells. This is the situation we find in cancers. Thus, tumours found within the adult brain are not produced from fully developed neurons but may be glial in origin or, more likely, they have spread from other parts of the body.

Although there is now strong evidence to suggest that neurogenesis occurs throughout life in the mammalian adult brain, there is considerable debate over the extent to which this occurs and whether it is important functionally in humans (Lazarini and Lledo, 2011). The neurogenic capacity appears to be largely restricted to certain populations of neurons – the best evidence existing for neurons of the olfactory (smell) system and a subset of neurons in a structure found in the temporal lobe called the hippocampus. Considerable research effort is going into understanding why this is limited to just a small number of locations and how these regions are able to continue undertaking neurogenesis throughout life. A detailed analysis of the many factors involved in this process is beyond the scope of this chapter, but a clear picture is emerging that glial cells – which, as described in Chapter 2, have long been considered as only having a simple role in physical support – are key as they produce factors, for example, which induce progenitor cells to differentiate into neurons.

Olfactory neurons are born continuously throughout life within a vestige of the developmentally important proliferative zone, which is found in the lining of the lateral ventricle, known as the subventricular zone, as described above. The neurons born here follow a defined path forwards to the olfactory bulb where they integrate into the existing circuitry. Studies of the molecular basis of the migratory process have indicated that many of these neurons mimic the processes observed during early development. In contrast, parts of the process of differentiation of the new neurons in the adult are different from that occurring in development and, while the functional consequence of this is unknown, it is possible that this reflects the fact that in the adult these new cells are integrating into already functioning circuitry as opposed to producing circuitry *de novo*. However, this is an interesting observation for any future attempt to use stem cells therapeutically for neurodegenerative disorders. In rodents, the loss of olfaction is linked with behavioural despair and, indeed, one of the early models for clinical depression involved removing the olfactory bulbs of rodents. Furthermore, there is evidence to suggest that olfactory processes are impaired in human psychological disorders, for example during episodes of clinical depression, and that this can be reversed by the use of antidepressant drugs. It is tempting therefore to conclude that antidepressants are somehow coupled to the restarting of the neurogenesis process and that interventions that maintain or enhance olfactory neurogenesis may be novel therapeutic targets (Perera et al., 2008).

The studies concerning neurogenesis in the dentate gyrus of the hippocampus indicate that a group of stem cells gives rise to immature neurons, which can migrate a short distance before extending dendrites and axons, the latter travelling some distance before forming functional synapses. The key role that the hippocampus has in mediating learning and memory (see Chapter 5) makes it tempting to speculate that neurogenesis is a key contributor to these functions. The evidence to support this is accumulating, with manipulations that increase neurogenesis in the dentate gyrus being linked to increases in specific aspects of learning, while those that decrease neurogenesis are linked to decreases in learning (reviewed in Ming and Song, 2005).

Despite our current acknowledgement that neurogenesis in the adult brain is possible, the role that neurogenesis might play in recovery from insult is still unknown. Two key questions are:

- whether the two stem cell populations described above can be induced to supply new progenitor neurons to the damaged areas even if they are distant from the sources of the new neurons; and
- whether other regions of the brain can be induced to begin neurogenesis in response to insult.

Either way, clearly an ability to manipulate the rate and extent of neurogenesis would potentially allow intervention to halt or even reverse neuronal loss following traumatic injury or, as seen in neurodegenerative conditions, where substantial numbers of neurons are lost (see Spotlight 4b).

Key points

- The brain retains some limited capacity to produce new neurons throughout life.
- Experimental studies in animals suggest that the effectiveness of pharmacological and non-pharmacological antidepressant therapies may require neurogenesis.

CHAPTER SUMMARY

The production of a fully and normally functioning brain requires intricate and precisely timed processes which are determined by the genetic makeup of the individual and which are influenced by environmental factors. The production of the basic structure of the brain is then further altered to allow early-life experiences to impact upon its structure and function. These may have immediate effects on the functioning of the individual or may, to some degree, lie dormant until later in life when they subsequently go on to have profound effects on the adult individual. The neurons of the brain remain relatively vulnerable to a variety of insults which may result in their death, although the likelihood of this increases with age. The brain appears to retain some capacity for the production of new neurons throughout life, although the frequency with which neurodegenerative disorders occur suggests that this is limited. Any approach to artificially increase the regenerative capacity of the brain will need to be able to do more than just increase cell numbers; enabling the correct environmental conditions for appropriate connections to form and restore circuit functions will be critical for success.

Key points summary

- The earliest signs of the nervous system are visible about three weeks after fertilisation when the embryo has undergone a period of massive cell proliferation.
- Progenitor neurons have to move from the position where they are produced to their final destination before they take on the characteristics of mature neurons.
- Brain development is determined by the switching on and off of the right genes at the right time and this process can be influenced by environmental agents.
- Synaptic plasticity is a life-long process enabling the changes in synaptic function that underpin behaviour.
- Neurons can die by way of two processes: necrosis and apoptosis.
- The cause of neuronal death may be genetic, environmental or a combination of the two.
- Functional effects of neuronal loss are related to the anatomical site of the loss.
- The brain retains some limited capacity to produce new neurons throughout life.
- Experimental studies in animals suggest that the effectiveness of pharmacological and non-pharmacological antidepressant therapies may require neurogenesis.

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FURTHER READING

Faust, T. E., Gunner, G., & Schafer, D. P. (2021). Mechanisms governing activity-dependent synaptic pruning in the developing mammalian CNS. ***Nature Reviews Neuroscience, 22***, 657–73.

This review considers the current view on the various factors that contribute to synaptic pruning during brain development and which might give us insight into how differential circuit development may lead to neurodevelopmental aspects of human brain disorders.

Lee, H. G., Wheeler, M. A., & Quintana, F. J. (2022). Function and therapeutic value of astrocytes in neurological diseases. ***Nature Reviews Drug Discovery, 21***, 339–58.

Our understanding of the importance of glial cells for neuron functioning is reviewed in this article, along with a consideration of how the subset of glia known as the astroglia may become a therapeutic target.

Blakemore, S. J. (2008). The social brain in adolescence. ***Nature Reviews Neuroscience, 9***, 267–77.

A review of the importance of understanding the plastic changes occurring in the brain during adolescence for determining both immediate and adult behaviour.

Castrén, E. (2005). Is mood chemistry? ***Nature Reviews Neuroscience, 6,*** 241–6.

A discussion, using depression as the example, of how the property of life-long synaptic plasticity might explain how behaviour can change over an individual's lifetime and whether interventions such as pharmacological agents and cognitive behavioural therapies might both rely on this property for effectiveness.

McCarthy, M. M. (2016). Sex differences in the developing brain as a source of inherent risk. ***Dialogues in Clinical Neuroscience, 18,*** 361–72.

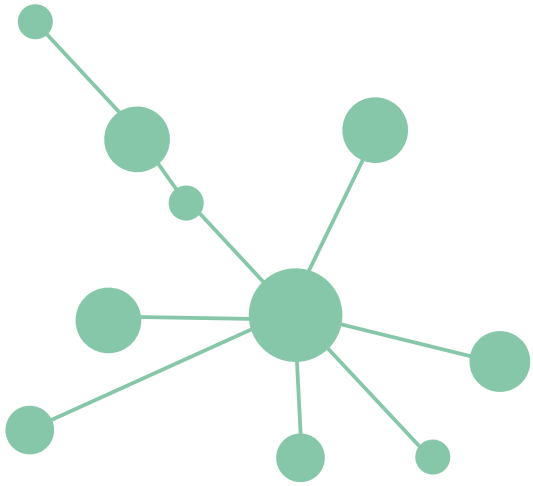
An overview of how sex differences arising from exposure to sex hormones during brain development can lead to changes in the adult brain and how this may lead to differential prevalence of neuropsychiatry disorders between the biological sexes.

Stiles, J., & Jernigan, T. L. (2010). The basics of brain development. ***Neuropsychology Review, 20***, 327–48.

A comprehensive survey of all of the major processes that occur during development, including a more detailed account of the role of specific genes in the processes.

CRITICAL THINKING QUESTIONS

1. What would you predict to be the effects on the brain of repeated heading of a ball, for example, when playing football? Should heading of footballs be banned in children's football?
2. How might our understanding of brain development during critical periods such as adolescence and older age be useful in informing education and healthcare and social policy?



SPOTLIGHT 4A

BEHAVIOURAL GENETICS

KEY ISSUES AND CONTROVERSIES

- How big an influence do genetics have in determining behaviour?
- How can we find genes that, individually, might only have a small impact on behaviour?
- How will an increased understanding of genetic influences impact on therapeutic options for behavioural disorders?
- How will the ethical considerations surrounding genetic testing impact on how society views mental illness?

INTRODUCTION

Like any individuals in a species, humans share a considerable number of anatomical, biochemical and behavioural features. Yet we can recognise individuals because of subtle differences in, for example, their appearance and behaviour. The reasons for these differences have been a matter of debate for millennia and form the basis of the 'nature versus nurture' question. The answer to this question is still far from being explained, although the consensus opinion is that, in broad terms, the differences can be attributed to differences in individuals' genetic makeup, differences in the environment and experiences that those individuals have been exposed to, and probably most commonly, a combination of the two. Hence, the response an individual makes to their experiences and environment may depend upon their genetic makeup, implying that it is not 'nature versus nurture' but nature *AND* nurture that form us as individuals, determining everything from our basic cell biology to our behavioural profile. One added complication is that differences in behavioural profiles can occur within a range; for example, mood is not in one state or another but occurs across a range. This means that we have to define what is the 'normal' range, with the implication that if an individual falls outside the normal range, we might then classify them as having a disorder.

The purpose of this spotlight is to examine the evidence for a role for genetic differences in determining behavioural profiles, using a few examples to illustrate the concept. We will also consider how scientific investigations have progressed to determine the extent to which genetics may be influencing behaviours and to then go on to find the genes and associated genetic control sequences within the individual's DNA.

THE GENETIC SEQUENCE

The genetic material, or **genome**, of all organisms is highly organised, and our understanding of the biology and chemistry of genetic material is now highly detailed but beyond the scope of this text. However, suffice to say that in humans the genome has two major layers of organisation. First, the whole genome is divided up into chromosomes: in most cells in the human body there are 23 individual chromosomes and we have two versions of each one – one inherited from our mother and the other from our father. Second, within each of these chromosomes are the functional units, which appear to be of two types: **genes**, each of which can be considered to act as the blueprint for the production of a particular protein; and the rest of the DNA, which was once considered 'junk' but is now acknowledged as containing units that regulate the expression of the genes. Consequently, we could describe the difference between species as reflecting the fact that any one species contains a unique set of genes and control regions that is different from the set of genes and control regions found in any other species. However, what this does not explain is how differences between individual members of a species arise. To explain this, we need to look at the detailed makeup of the individual genes and control regions. What becomes apparent is that each of these can exist in slightly different forms. For genes, these forms are known as **alleles**, whereby the same protein is produced in all individuals but the detailed nature of that protein can be subtly different between individuals. For example, if the protein were a receptor for a neurotransmitter, then two different alleles may produce receptors which bind the same neurotransmitter as an agonist but perhaps for different amounts of time, thereby resulting in subtle differences in the effect that the neurotransmitter

Genome The complete set of genes in an organism.

Gene The part of a chromosome that is the blueprint for a protein.

Allele An alternative form of a gene.

has on the neurons possessing the receptor; this in turn may affect the properties of the neural network that these neurons belong to and, ultimately, the behaviour of the organism. Darwinian principles imply that only the versions of genes which make the organism the most successful among its peers should dominate. So the observation of difference between individuals of a species suggests that it is possible for different alleles to confer no particular extreme advantage. A well-understood example in human biology is that of the different blood types seen in humans which are conferred by possession of different alleles, yet there is no evidence to suggest that, across the global population, possession of any one blood type is greatly advantageous over possession of the others. The coexistence of multiple types of allele or control region is known as polymorphism and the complete set of alleles an individual possesses is termed its **genotype**. In the context of behaviour, we can see that polymorphisms which influence neuronal function right from the development of the human brain to ongoing responses and activity in adulthood are likely to produce differences in behavioural expression, known as the **phenotype** of the individual. If these differences reach such a level that they fall outside the normal range seen in the human population, then we might expect to see a functional behavioural disorder of the type described in Chapter 10.

Genotype The genetic makeup of an organism which is a combination of alleles responsible for determining the characteristics and traits of an organism.

Phenotype The observable physical or biochemical characteristics of an organism, determined by both genetic makeup and environmental influences.

GENES AND HUMAN BEHAVIOUR

Even a basic understanding of genetics is sufficient to understand the potential implications of alterations in the genetic code, known as mutations. These mutations can range from a change in a single nucleotide (one of the four that make up the letter code of DNA) which produces a new allele, through to whole gene deletions or replications or even errors in chromosome number such as the trisomy seen in Down's syndrome. Here, the individual has three copies of one of the chromosomes (chromosome 21), rather than the usual two, in each of its cells. Clearly, the larger the scale of the mutation the larger the impact is likely to be, and it may even be sufficient, for example, to result in spontaneous abortion during development since, as we saw in Chapter 4, proteins play a critical role in normal brain development.

So to what extent might behavioural differences, either within the normal human range or that which can be classified as disordered, be due to genetic changes and/or differences? In theory, we might see situations whereby mutation in a single gene can dramatically alter behaviour or perhaps only alter the degree to which an individual exhibits a particular behaviour, for example by influencing their intelligence. In contrast, it might be that multiple genes influence a particular behaviour, and changes in any one can result in the variability we see across the normal population. In this case, behavioural disorders may require the combined effects of small-scale changes in multiple genes, any one of which may have low impact in isolation. In practice, accumulating evidence suggests that human behaviour is so complex that any one behaviour is influenced by multiple genes, so we generally see gradations in behaviour rather than the presence or absence of behaviour – although, having said that, there are some examples where single-gene mutation is responsible. For example, in Chapter 4 we looked at Huntington's disease, where mutation in the *HTT* gene that produces the huntingtin protein results in a diverse array of severe neurological and psychiatric disturbances in affected individuals which relate to neurodegeneration.

Epigenetics

We can see that there might be a link between the genes an individual possesses (its genotype) and how these enable the individual to interact with its environment and to assimilate experiences to produce its phenotype, including its behavioural profile. However, if this was all there was to it, we might expect individuals to be 'hard-wired' behaviourally with no opportunity for their behaviour to change. To extrapolate to the full, in the future, we might be able to predict how a newborn human will behave as an adult according to the alleles it possesses. Yet this is not and never will be the case, because what we have not taken into account is how the environment impacts upon the genes. What has become clear is that possession of a particular set of gene alleles is not sufficient to explain the phenotype of any one cell, let alone an individual. All cells in an individual's body contain the same genetic material, but we have a whole variety of cell types, including neurons, which are different from each other because of the particular complement of proteins that each expresses. To explain how this differential protein expression occurs, we have to take into account how and when the genes are activated to produce their related protein products. We have an ever-expanding understanding of these control processes and we now know a lot of detailed information about how the protein product of one gene may regulate the expression of the protein product of another. However, one of the most interesting findings in recent years has been that the control of expression of a particular gene can be influenced by factors that are independent of the actual genetic sequence itself. These factors are collectively known as epigenetic factors (Feinberg, 2007). We now have some understanding of the nature of these epigenetic factors and it is clear that there are a number of diverse processes involved, some of which control the permanent switching off of genes in early development, while others have the capacity to control gene activity in response to environmental conditions encountered throughout the life of the organism. This latter property is very attractive when thinking about behaviour because we know that human behaviour can change over time and in some cases with profound deleterious effects (see Chapter 10 for more detail on how epigenetics may explain some aspects of psychological disorders). So, the possibility that changes in epigenetic activity may be leading to psychiatric disorders is of obvious relevance for this text (Tsankova et al., 2007). A detailed consideration of all of these processes is beyond the scope of this spotlight, but by looking at a few examples that have been linked to behavioural traits and disorders we can get a sense of the potential impact epigenetics might have.

Genomic imprinting

One of the simplest ways of illustrating the impact of epigenetic factors is by considering the fact that all cells contain two versions of each chromosome, one from each parent. With the exception of the sex chromosomes, these two versions are considered to be equivalent such that each chromosome of the pair contains different alleles of the same genes. So, the first question we could ask is 'Are both alleles expressed and, if not, which of the two alleles is expressed and determines the protein complement of a cell?'. This would immediately lead us to a second question: 'Is the parental origin of the expressed allele random?' What has become clear is that for some proteins there is a particular pattern whereby usually either the maternal or the paternal version of the allele is silenced, allowing the other to dominate. This phenomenon, known as **imprinting**, requires a long-term modification of the DNA by methylation. Methylation is the addition of a methyl (CH3) group to DNA and it has the effect of affecting gene expression: it can silence genes (Figure 4a.2).

Imprinting Differential expression of a gene as a function of whether it was inherited from the male or the female parent. Either the maternal or the paternal version of the allele is silenced through the addition of methyl groups during egg or sperm formation.

Real world applications

An illustration of the effects of imprinting is shown by the example of Angelman and Prader–Willi syndromes. Individuals with Angelman or Prader–Willi syndrome exhibit rather different characteristics. Prader–Willi syndrome is characterised by, among other things, learning difficulties, repetitive behaviours, hypotonia and obesity. In contrast, individuals with Angelman syndrome develop late, communicate poorly and show a high propensity for epilepsy, although they are generally of a happy demeanour. Intense genetic studies have shown that individuals in both cases had the same part of chromosome 15 missing, meaning that protein expression could only be determined by the allele on the other chromosome without the deletion. However, what differs between the syndromes is whether the chromosome with the deletion was inherited from the mother or the father; if from the mother, then the child developed Angelman syndrome, and if from the father, then the child had Prader–Willi syndrome. The deleted region contains a number of genes and normally either the maternal or paternal allele is turned off by an epigenetic mechanism, a phenomenon known as imprinting. So, an individual develops Angelman syndrome because the paternal allele has been silenced and the maternal allele, which would normally be active, is missing due to the deletion, and the converse is the case for Prader–Willi syndrome. See Figure 4a.1.

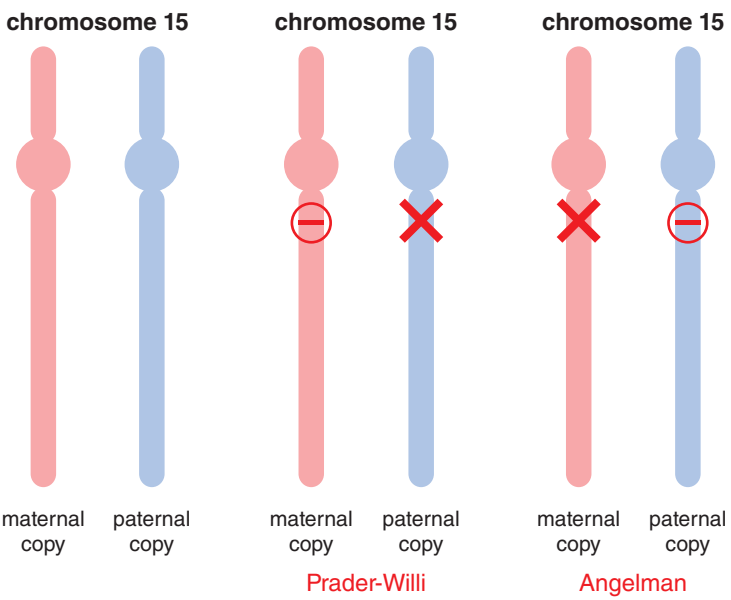


Figure 4a.1 Prader-Willi and Angelman syndromes

The Prader–Willi/Angelman situation is a rather extreme example of imprinting, and we might ask how this phenomenon might relate to complex behavioural profiles where multiple genes from a variety of locations in the genome have been implicated. In fact, where studies have been conducted, there is gathering evidence that epigenetic control of allelic expression extends beyond early development and does not even have to occur in a predictable pattern

for any one gene. Hence, some recent evidence has shown that which of the maternal/paternal alleles is expressed may switch during development as cells start to differentiate, including into neurons, suggesting that less permanent epigenetic control is possible (Lin et al., 2012).

Epigenetic effects and behaviour

The relevance of epigenetic factors in allowing the precise silencing of one allele, or switching on and off genes to produce the myriad cell types during development, is undeniable. However, the potential that epigenetics might be able to explain how the experience an individual has when young can influence behavioural expression later in life is very attractive and arguably

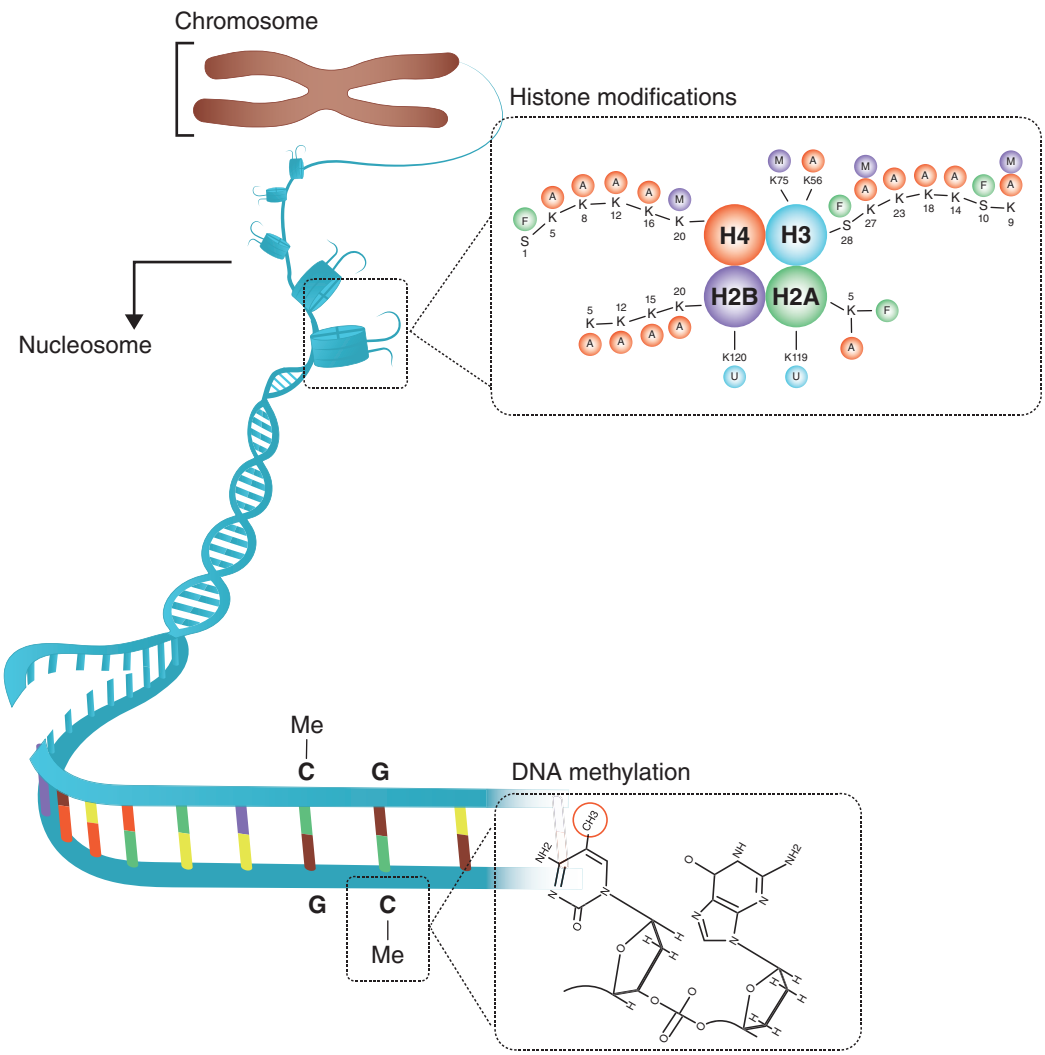


Figure 4a.2 Complex structure of chromatin. The DNA sequence wraps around histone proteins to be packaged into nucleosomes. Epigenetic change can involve modifying the histones or the DNA, for example by methylation

provides the link between nature and nurture. Furthermore, epigenetics may help to explain a number of observations that have been used as arguments against evidence for genetic involvement in certain traits. For example, how is it possible that **monozygotic** twins with identical genetic material may express differences in behavioural profiles? Or how may disorders not be manifest from birth but only emerge in later life, such as appears to be the case for schizophrenia (see also Chapter 10)? For these processes to occur, a more flexible means of turning genes on and off is needed, and again we have now got some way towards explaining these processes. In addition to the process of DNA methylation mentioned above, many of them seem to involve modification of a set of proteins known as histones, which associate with DNA to form **chromatin** and thus allow the DNA to have a highly ordered structure that allows it to pack into the relatively small volume of the cell nucleus (see Figure 4a.2).

Monozygotic From a single fertilised egg; relates to identical twins.

Chromatin Part of the nucleus of a cell (consisting of DNA and histone proteins) that makes up chromosomes.

Modification of these histones is required in order to allow the chromatin to open up to allow **transcription** of the genes, and so it can easily be imagined that, even in normally functioning neurons, the chromatin is undergoing constant change relating to which regions are transcriptionally active and therefore which proteins a cell can produce. Chromatin remodelling processes have been implicated in mediating changes of behaviour, for example those seen in response to drugs of abuse, such as cocaine (Kumar et al., 2008). Furthermore, chromatin remodelling at any point in the life of the organism seems to be possible and might well allow the experiences of the individual to influence their gene expression. This might benefit the individual if they are, for example, forming new memories and this might partly explain the findings of the impact of environmental enrichment on synapse formation that we discussed in Chapter 4. Conversely, epigenetic mechanisms might also explain how negative early experiences can lead to behavioural disorders such as clinical depression (as discussed in Chapter 10) in later life (Sweatt, 2009).

Transcription The process of making a copy of genetic information stored in a DNA strand into a complementary strand of RNA.

Real world applications

It has long been recognised that adult individuals with behavioural disorders such as those of mood or anxiety are more likely to have suffered adverse experiences when young than those who do not. This situation can be replicated in experimental animals of a wide range of species, suggesting a common cross-species mechanism. The considerable research effort, utilising a wide range of experimental approaches from molecular studies to human family pedigrees, has converged on the physiological system that controls production of the so-called stress hormone cortisol as being of critical importance. It would appear that these early-life experiences could somehow alter how this system works many years hence. In 2012, Tyrka et al. published results which indicate that the epigenetic state of the control regions of a gene that encodes a receptor for cortisol in human adults is different in those who experienced childhood adversity, suggesting a possible epigenetic mechanism by which experience ('nurture') can influence genetic activity ('nature'), which might then manifest behaviourally many years after the precipitating factor was encountered.

Studying the degree of genetic influence on behaviour in the general population

So, how do we go about investigating whether or not particular behaviours in the human population are influenced by genetics and, conversely, if we can identify genes in the laboratory, how can we work out whether they can influence behaviour?

For centuries it has been recognised that behavioural traits and disorders can run in families or have high prevalence in isolated communities. This implies that something can be passed on from one generation to the next and, of course, what we now know is that it is genetic material that is inherited by the offspring from the parent. Investigating the role that our genetic material has in influencing any particular trait requires a number of factors. First, we need to be able to define a particular behaviour that is not trivial. For example, what exactly is extrovert behaviour and how do we categorise degrees of it? Second, we need to be able to accurately and reproducibly measure the behaviour. This is not trivial either because we will be assessing the behaviour in individuals being studied under particular environmental circumstances, and if we accept that nature and nurture interact, then clearly changing the environment could influence their behaviour. Nevertheless, being able to derive an estimate of how much an individual's genetics influences their behaviour has been a long-standing goal.

The concept that human behaviour may be influenced by something other than the environment and experiences of the individual is not new. In the middle of the 19th century the Englishman Francis Galton was studying heredity in humans, particularly in relation to what we would now call cognitive function. He introduced some key statistical processes for analysing non-binary data sets where the likelihood of a factor having influence could be assessed – something critical for our ability to now analyse the degree of influence that genetic makeup can have on an individual's characteristics. However, Galton was also the founder of eugenics (the scientifically incorrect and immoral theory of 'racial improvement' and 'planned breeding') and so his legacy is now seen in this light.

Key debates: controversy surrounding behavioural genetics

Scientific research into the biological basis of behaviour can be seen as ranging from idle curiosity in attempting to explain normal variation in humans to a serious attempt to gain a better understanding of a behavioural disorder which may then form the basis for improved therapies. However, the subject is not without controversy since it raises a number of ethical issues. For example, if we can define desirable behavioural characteristics, should we only allow those possessing at least a subset of those characteristics to breed? This quickly gives rise to unpalatable thoughts about Nazism and eugenics. Even at a lower level, we might ask the question as to how far genetic testing of a foetus in utero or in embryos used for in vitro fertilisation should go. While it is currently acceptable to test for, for example, Down's syndrome, would it be acceptable to test for a particular set of alleles which has been shown to produce an increased likelihood of developing mental illness?

Where there is a suspicion that a trait can be inherited, insight into this can be gained by producing a pedigree or family tree which could indicate, for example, that a trait could be seen in a number of family members over several generations (an example is shown in Figure 4a.3). An obvious criticism is that it is impossible to disentangle genetic from environmental factors as members of the family are likely to share both genetic but also environmental factors.

So, how could we design human studies to disentangle the relative contribution of the genetic from the environmental factors? Twins are ideal study subjects in addressing the nature versus nurture question. Twins who are brought up together are interesting to study since there are two types: identical twins are known as monozygotic, as they arise due to the cleavage of a single fertilised egg and therefore have identical genetic makeup, while those who are fraternal are no different genetically from siblings born at different times and so are **dizygotic**. Hence, these studies assume equivalent environmental experience for each twin, but by collating data from many sets of twins and comparing the relative incidence of a behavioural trait for identical twins versus fraternal twins we can calculate how likely a particular trait is to arise due to genetics rather than the environment. So, if we observe that the likelihood of both identical twins having a behavioural trait is higher than both fraternal twins having the trait, then we can conclude that there is very likely to be a genetic component determining that trait.

Dizygotic Non-identical or fraternal twins where each twin is derived from two separately fertilised eggs.

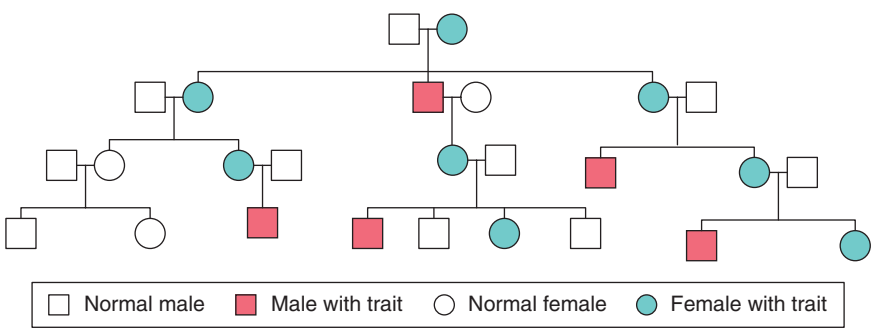


Figure 4a.3 Example of a genetic pedigree. Females are represented by circles and males by squares. Normal individuals are represented by open symbols and those with a particular trait or disease with filled-in symbols

Implications: looking ahead

For many years, the role of genetic factors in autism has been debated. In 1977, Folstein and Rutter provided the first evidence for a genetic influence using a twin study. Since then, there have been a large number of studies from different parts of the world which have looked at ever more detailed aspects of autism and related conditions as our knowledge of the disorder has

(Continued)

increased (Ronald and Hoekstra, 2011), and most have reinforced this idea. However, despite this weight of evidence, other studies have provided evidence to suggest that the genetic component may have been over-estimated (Hallmayer, 2011). The reason for this discrepancy in the literature is not clear. It is possible that the intricate details of study design may differ or maybe this is just a reflection of the problem highlighted earlier; that the results will depend on who is recruited into a study and how autism is defined. Research in this area is ongoing and brings with it ethical debates around genetic testing for heritable conditions such as autism. There are concerns that genetic testing would heighten intolerance of diversity and could be used for the purpose of eugenics. Therefore, it is important that people with autism and their families are involved in such research.

Clearly, we could estimate the environmental influence indirectly as everything that is not due to genetic influence, but is it possible to *directly* estimate environmental influence? Theoretically, the ideal study design would be the opposite of that just described; that is, we need to have a constant genetic situation but a varying environmental one. This can be achieved where monozygotic twins are adopted into different families, but, as you might imagine, this situation is relatively rare and it is difficult to get enough sets of twins for a big enough data set for meaningful analysis. A less perfect, but more achievable, study design would be to analyse differences between adopted children and their biological parents (so some shared genetics but different environments) with their adopted parents (different genetics but shared environment). However, these studies are not without difficulty either; for example, a full history of the biological parents may not be available. Also, now that we understand something about epigenetics, it is clear that the age of adoption will be important, since the later that occurs the more the chance that an environmental influence has already impacted on the adopted child's behaviour or may do so at some point in the future.

Although these types of studies can tell us the extent of influence genetics might have on a behavioural trait, they can't tell us anything about the nature of the genes involved; for this we will need to look closely at the differences in genetic material at the molecular level.

Finding genes linked to human behaviour

A good place to start to find the genes implicated by the kind of studies described above may be to ask the question as to whether we know the genetic makeup of a 'standard' human being, since this may allow comparison with someone with a different behavioural profile, even if that difference is not so great that we would consider the individual to have a disorder.

In 2003, the Human Genome Project produced the first full sequencing of the human genome. We became aware that humans contain approximately 25–30,000 genes and it would appear that we are more than 99% identical to each other. One theoretical possibility, therefore, would be to sequence the entire genome of each of a group of individuals with a particular diagnosis and compare the sequences to look for this minuscule difference when compared to so-called normal individuals. This, however, is generally impractical as this would involve an enormous amount of work, taking a very long time. A more efficient approach is required.

Implications: looking ahead

The Human Genome Project was an initiative to determine the sequence of the nucleotide base pairs in the DNA in a number of people. This has produced a reference map of all of the genes, enabling us to localise genes to regions of particular chromosomes. It also allows us to compare the sequence of a gene from individuals with a trait or disorder with the reference genome to seek evidence that the gene may have a role in the trait/disorder. However, it is acknowledged that the data in genome databases comes mainly from people of European descent who live in high-income nations, even though they make up only 16% of the global population. More diverse representation is required to avoid biases in sampling because applying knowledge from European-based studies to non-Europeans may result in inaccurate assessment of genetic risk. Efforts are now underway to create a reference map that is more complete and more representative: The Human Pangenome Project. Researchers are also working with under-represented groups to try and ensure that past mistakes involving misuse of data from Indigenous peoples that lead to mistrust in biomedical research are not repeated.

A sensible approach would be to start by trying to find which of our 23 pairs of chromosomes carries the gene(s) we are looking for. For the most part our chromosomes are indistinguishable, but one pair, the so-called sex chromosomes that are either X or Y, are considerably different from the others. This means that if the gene that is associated with a trait/disorder that we are looking for resides on one of these, we would expect to see a predictable pattern of inheritance in individuals of a particular gender in family pedigrees. For non-sex-linked disorders, though, we will need to adopt a different approach.

Over the years, a number of methods have been used to try to locate a gene for a disease or trait on the chromosomes. The original methods were described as **linkage analyses** as they usually relied on comparing the frequency with which an identifiable stretch of DNA (the location of which is known and is termed a marker) occurs in related affected individuals compared to non-affected individuals. These identifiable parts of DNA may be stretches of DNA that are highly variable in the general population, known as **single nucleotide polymorphisms** (SNPs or 'snips') or regions of tandem repeats, which are notable because they are composed of very many repeats of the same sequence. Statistically, the higher the incidence that one of these marker regions is found in affected individuals compared to unaffected individuals, the more likely it is that the gene being looked for occurs close to the location of the marker. Although this approach does not allow the precise location of the gene linked to a particular diagnosis, it does allow us to narrow down the location to a relatively restricted region of DNA.

Linkage analysis A method that investigates whether there is an association between easily identifiable pieces of DNA (genetic markers) and a trait of interest or disease. If the marker is present in all affected individuals, then this suggests that a gene close to the marker region is involved in the trait/disorder.

Single nucleotide polymorphisms (SNPs) A variation in DNA sequence that occurs when a single nucleotide is altered.

The linkage analysis approach is very successful where a disease/trait is monogenetic, such as for Huntington's disease, which we discussed in Chapter 4. However, as we have already established, behaviour is most often attributed to the activity of multiple genes in different locations

Quantitative trait locus (QTL) analysis The statistical analysis and identification of stretches of DNA that are linked to the genes that underlie a particular trait.

Genome-wide association studies (GWAS) An examination of genetic variants to see whether any variant is associated with a trait. The method involves assessing whether gene variations, called single nucleotide polymorphisms or SNPs, occur more frequently in people with a particular disease than in people without the disease.

(loci) which might each have a minor role to play, so this approach would only be successful if we can track multiple genes and markers simultaneously. The advancement of technology has meant that we can do just that due to an approach known as **quantitative trait locus (QTL) analysis** (Miles and Wayne, 2008). However, these studies only tell us information about the trait loci in the pedigrees that have been studied and it may not be the case that these data can be extrapolated to the whole population of individuals with a particular trait or disorder. However, technological advances have also helped here because we now have the ability to look for subtle differences in the genomes of large numbers of non-related individuals with or without a particular trait/disorder using **genome-wide association studies (GWAS)** (Stranger et al., 2011). These highly computerised studies systematically assess the variation in SNPs in affected and unaffected individuals on a chromosome-by-chromosome basis. The results of these studies can then highlight which locations show an association between particular SNP variants and the disease in question. Genome-wide association studies are a technological advancement of linkage analysis to enable potential genes linked to traits/disorders to be identified. The technological improvements mean that whereas linkage analyses use related individuals and focus on a limited number of markers, a genome-wide association study can compare multiple markers and in a large number of unrelated individuals. As a consequence, these studies are more likely to identify genes that only have a minor contribution to a trait/disorder.

The results are usually presented as a Manhattan plot, an example of which is shown in Figure 4a.4, where the strength of the association is represented on the *y* axis and the location of the SNP according to chromosome is shown on the *x* axis. The name is derived from the similarity of the plot appearance to the Manhattan skyline. However, it is still important for us to recognise that at this stage all we have been able to do is to associate small regions of a chromosome with a trait; identifying the actual gene or control region involved still requires further investigation.

The large-scale nature of these studies, with many thousands of individuals' DNA to be tested, means that they are expensive to organise and run and so many of them can only be conducted by well-funded organisations and with very many research collaborators. Frustratingly, the relatively few large studies that have been done so far have sometimes produced equivocal or differing results for specific disorders. Early indications suggest that this may be due to a number of factors, including the numbers of individuals that are included and also precisely how they are categorised as 'affected' or 'unaffected', further reinforcing the idea that behavioural traits may be a common endpoint for a number of unique sets of genetic variabilities. Nevertheless, an ever-advancing understanding of the key factors and statistical processing required in designing such studies will doubtless lead to more productive studies in the future.

Of course, finding genes that might have some involvement in determining behaviour is one thing, but it is the protein products of these genes which actually underpin the function. So, once candidate genes have been found, we need to find out something about their activity by looking at the profile of their expression. Again, new technology has massively speeded up this process, such that we can realistically assess the relative activity of all 20,000 or so human genes in individuals simultaneously using a DNA or protein microarray and comparing the patterns of activity in affected versus non-affected individuals. This will allow us to gain insights into the role these proteins play in behavioural traits and disorders.

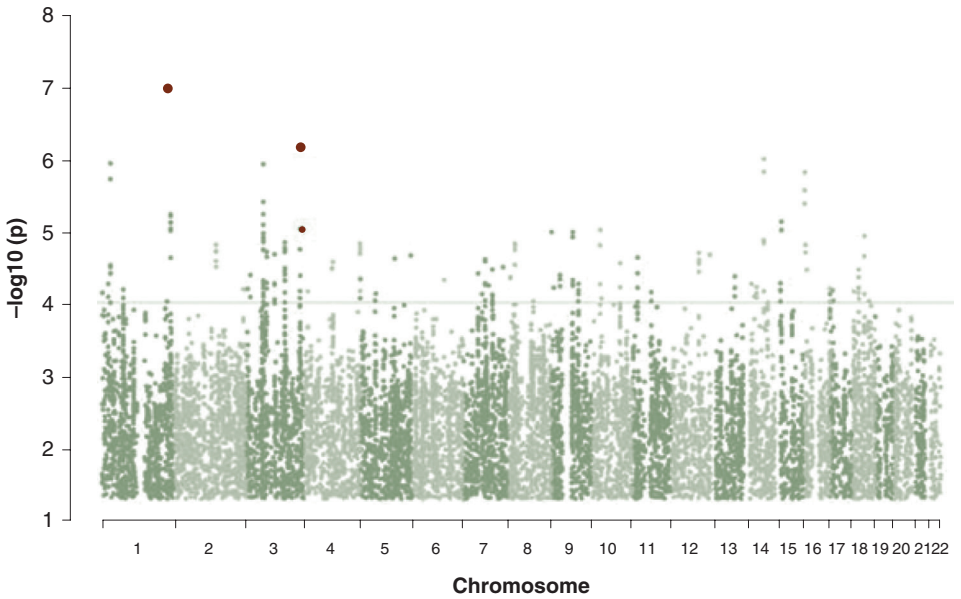


Figure 4a.4 Manhattan plot for a GWAS study examining genetic involvement in major depression (Wray et al., 2012). The data show that a large number of SNPs on many chromosomes appear to have some association with depression

Source: Reprinted by permission from Springer Nature: Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, Ripke, S., Wray, N. R., Lewis, C. M., Hamilton, S. P., Weissman, M. M., Breen, G., Byrne, E. M., Blackwood, D. H., Boomsma, D. I., Cichon, S., Heath, A. C., Holsboer, F., Lucae, S., Madden, P. A., Martin, N. G., McGuffin, P., Muglia, P., Noethen, M. M., ... Sullivan PF. (2013). A mega-analysis of genome-wide association studies for major depressive disorder. *Molecular Psychiatry, 18*(4), 497–511

One of the criticisms of association studies like QTL studies and GWAS has been that only a region of a chromosome linked to a trait is identified and not the actual gene itself. However, an interesting finding to emerge from QTL studies is that some regions implicated in being responsible for behavioural traits or disorders do not contain genes and so might previously have been classified as 'junk' DNA. However, an emerging picture is that these regions may encode small pieces of ribonucleic acid (RNA), which do not themselves encode proteins but which can interfere with protein production and thus alter the level of expression of proteins which are themselves perfectly normal. Our understanding of the role of these RNAs is still very primitive, but these regions and the RNA that they produce could one day be of interest as future therapeutic targets.

Studying the role of a known gene in human behaviour

Candidate proteins, and hence genes, can be proposed on the basis of observation of the effect of exogenous substances on animal behaviour. For example, observing the effect that an abused drug may have, or the serendipitous observation that a drug developed for one purpose may influence – perhaps entirely unexpectedly – a particular behaviour, can lead us to suggest that particular forms of genes encoding proteins responsible for mediating the pharmacology of these agents may have a role in determining a particular behaviour. If we know the approximate location of such a gene, then we can do an association study whereby a number of individuals, not necessarily related to each other, can be

screened to see whether there is a correlation between exhibition of a trait/disorder and possession of a particular gene allele that is different from that in the general population (Bird et al., 2001).

From decades of research on a variety of non-human species, we have acquired considerable knowledge of their behaviours, which appear to have some correlation with human behaviour. There are, of course, a large number of assumptions to be made here about how comparable animal and human behaviours are. Once we track the gene down in the animal's genome, we can do two things. First, we can look to see whether humans with a behavioural disorder that seems to correlate with the change in animal behaviour possess equivalent genes. Second, we can undertake genetic manipulation studies where we alter or even delete the gene in, for example, mice, and observe the effect this has on the animals. One of the problems with this type of approach is that the mice may not be able to develop properly and so we can learn nothing about the role this gene may have after birth in the young or adult mouse when it can be subject to epigenetic influences. This problem has been solved by the creation of conditional **transgenic** animals where we have the ability to control the turning on and off of the expression of a gene at will, thus producing an animal model of the disorder.

Transgenic An organism that has a segment of foreign DNA incorporated into their genome.

What have behavioural genetics studies told us?

Over time our ability to rigorously study to what degree genes might influence our behaviour has greatly improved and we have seen that current technology allows us to study even genes that may individually have a very minor influence. So what is the current view on how much our behaviour, disordered or otherwise, is influenced by our genetics? In order to give some sense of how this huge body of work can be coherently brought together, we will take schizophrenia as our example. As covered in detail in Chapter 10, schizophrenia is an example of a human disorder with behavioural features which have been studied extensively in order to better understand the underlying biology, and in the hopes of finding therapeutic interventions to either prevent it or negate its effects should they emerge. We can see that if one goal may be to explain the cause of schizophrenia, then we may have to return to the nature versus nurture debate and ask the question about what behavioural genetic studies have to tell us about the potential extent of a genetic influence. Decades of research have led to the conclusion that there is a significant genetic component to schizophrenia. So how might these various genes influence brain function to result in schizophrenia symptoms? Clearly, if genes are dysfunctional at the point of fertilisation, then initial brain development in early life may be disrupted. However, the notable feature of schizophrenia is that it isn't obviously manifest in the very young but most often emerges during the early adult years. This suggests that either the dysfunctional genes are relatively unimportant during early brain development or that some other factor impacts upon the brain during adolescence which allows the dysfunction to then become apparent. Of course, as we saw earlier in this spotlight, we cannot discount the possibility that the dysfunction of genes themselves may not be the issue but rather some epigenetic factor relating to the experiences of the individual which may influence gene function. Given all of these possibilities, what have the various studies actually provided evidence for? A detailed analysis of the many thousands of studies is impractical here and reviews in this field are published frequently (e.g. see Tiwari et al., 2010), but some examples to illustrate how the various approaches to studying the role of genes in schizophrenia will be covered.

The traditional types of population studies, as described above, have suggested that genetic factors may account for a variable likelihood that an individual would develop schizophrenia; for example, Lichtenstein et al. (2009) calculated a figure of 64%, although others have suggested a figure as high as 81% (Sullivan et al., 2003). However, it has also become clear that this genetic influence does not follow the simple rules of genetics that were first described by Mendel following his studies of the inheritance of individual characteristics of peas. Instead, exactly what trait is inherited and how it is seen in successive generations is complex and almost certainly involves multiple genes that may well not be entirely independent of each other. As described above, there are a number of methodological strategies that have been employed and improved upon over the decades to identify candidate genes. The simplest studies involve looking for relatively large-scale chromosomal abnormalities and a number have been reproducibly identified. For example, a deletion from chromosome 22 containing very many genes has been linked with a syndrome that includes schizophrenia-like manifestations (Murphy, 2002). A less dramatic effect on chromosomal material involving chromosomes 1 and 11 has also been reported. Here, material is not lost but a portion of one chromosome is moved to the other, a phenomenon known as a translocation. The consequence of the translocation is that gene function is affected, either directly or because the gene has been separated from its control regions. Two genes, appropriately named 'disrupted in schizophrenia' 1 and 2 (DISC1 and DISC2), have thus far been identified as being affected by the translocation (Millar et al., 2004). Functional studies in animal models have linked normal DISC1 function to glutamatergic neurotransmission (Maher and LoTurco, 2012) and neuronal proliferation during development (Mao et al., 2009). As discussed in depth in Spotlight 10a, both of these processes have been implicated in the pathogenesis of schizophrenia.

Translocation Transfer of a chromosomal segment to a new position.

A variety of linkage and, more recently, GWAS of the types described above have resulted in the proposal of a number of candidate genes implicated in schizophrenia. The proteins produced by these genes are involved in a very diverse range of processes, from brain development to neurotransmission. Some of these results for candidate genes have proven to be non-reproducible in subsequent studies or are linked to a number of psychiatric conditions, suggesting common pathological mechanisms for aspects of these conditions (e.g. see Fatemi et al., 2000). Candidate genes have been linked to critical processes involved in brain development (see Chapter 4), such as proliferation (Mao et al., 2009), migration (Kähler et al., 2008), and dendrite maturation reliant on the protein reelin (Förster et al., 2010), thus adding weight to the idea that schizophrenia is a multi-factorial neurodevelopmental disorder.

Multiple studies have implicated genes whose protein products are involved in some way with neurotransmission, such as those for neurotransmitter receptors, for example serotonergic and dopaminergic receptors, or those that influence neurotransmission via poorly understood mechanisms, such as dysbindin, which is postulated to influence glutamate release (Voisey et al., 2010) or GABAergic transmission (Bullock et al., 2008). Synaptic neurotransmitter levels are also influenced by the rate of their breakdown, and the gene encoding the enzyme COMT (catechol-O-methyltransferase), a catecholamine degradation enzyme, has been implicated in such studies (Williams et al., 2007). However, some association studies have implicated genes involved in less neurotransmitter-specific processes, for example activity of myelinating oligodendrocytes, which would have the potential to have widespread effects in the brain (Tkachev et al., 2003). A summary of some of the genes with neurotransmitter-related functions implicated in schizophrenia is given in Figure 4a.5.

Although there is now evidence for individual genes being directly linked to schizophrenia, the differences between individuals even within families and the sometimes irreproducible results of separate studies suggest that there may be highly variable 'nurture' factors to take into account. As we saw above, epigenetic factors can have profound influences on the expression of even normal genes. In the context of schizophrenia, there is now evidence for epigenetic effects at various points during the life of affected individuals. For example, in utero viral infection may affect activity of enzymes responsible for the methylation, and consequent inactivation, of control regions for genes involved in GABAergic neurotransmission (Costa et al., 2007). There is also accumulating evidence to suggest that hypoxia occurring during birth can also initiate epigenetic-mediated effects on the expression of a number of genes (Schmidt-Kastner et al., 2006). Evidence of epigenetic effects but with unknown cause can also be seen in post-mortem brains of people with schizophrenia versus those without schizophrenia, whereby the degree of chromatin modification of some candidate genes is altered in people with schizophrenia, implying altered levels of protein expression (Tang et al., 2011). Epigenetic mechanisms of this kind may explain the results from a plethora of studies conducted on post-mortem brains where differences in the levels of mRNA or proteins for neurotransmission-related processes have been compared in the brains of people with schizophrenia (untreated and treated) and without schizophrenia; for example, levels of dopamine D2 receptors.

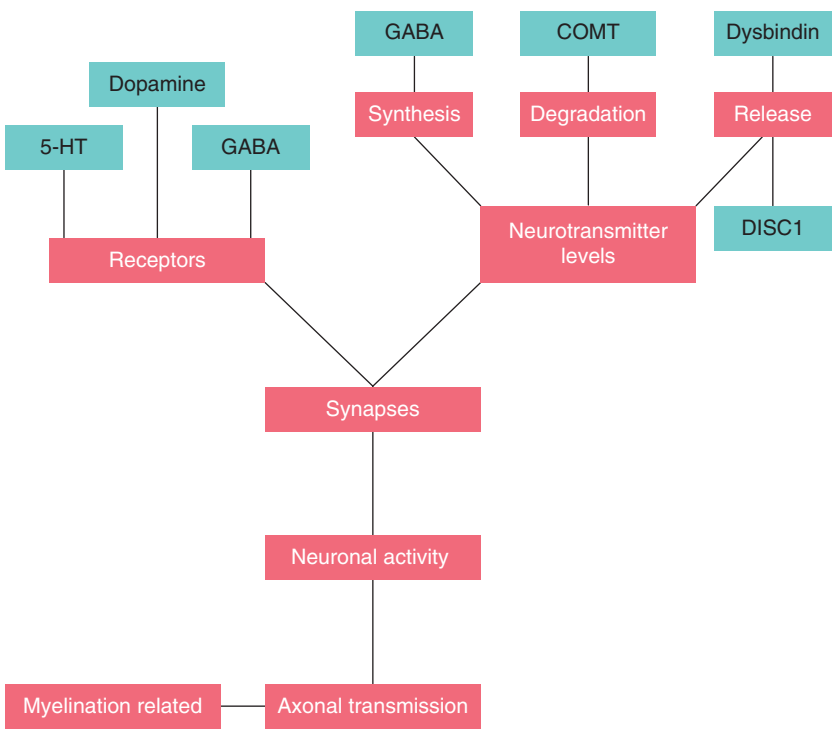


Figure 4a.5 Examples of some genes with neurotransmission-related functions implicated in schizophrenia

Real world applications

So where has all of this experimental work got us? We may not yet have the fruits of these research approaches in terms of effective therapeutic interventions, but for some behavioural traits and disorders we are developing a long list of potentially implicated genes or chromosomal regions and an understanding of the factors that determine whether these bits of DNA are expressed as proteins. Clearly, this knowledge has the potential to allow us to develop a detailed understanding of the underlying biology of the disorder and therefore how to prevent manifestation of a disorder through altering protein expression.

A long-held criticism of a molecular genetic approach to research into behavioural disorders is that knowing that a single or set of genes can, if mutated, produce the disorder might be interesting but is unlikely to result in simple therapeutic strategies. For example, we might want to think about the impracticalities of gene therapy as an attempt to replace defective genes in neurons in the brain. However, with the recognition of epigenetics as a key player in the control of protein production, there is a potential new therapeutic angle. Taking as an example the case of genetic imprinting, we can see that if we could turn on a 'normal' but silenced allele in an individual carrying a mutation/deletion of the expressed allele, then we should in theory be able to restore normal protein production. Early forays into this area using experimental animals have produced some exciting results where an agent was able to reactivate the silenced allele in a mouse model of Angelman syndrome (Huang et al., 2011).

FURTHER READING

Charney, E. (2022). The 'golden age' of behavior genetics? ***Perspectives on Psychological Science***. https://doi.org/10.1177/17456916211041602

This review evaluates the complexities of linking genes and behaviour and whether our current methodological approaches fully acknowledge their limitations.

Van den Bergh, B. R. H., van den Heuvel, M. I., Lahti, M., Braeken, M., de Rooij, S. R., Entringer, S., Hoyer, D., Roseboom, T., Räikkönen, K., King, S., & Schwab, M. (2020). Prenatal developmental origins of behaviour and mental health: The influence of maternal stress in pregnancy. ***Neuroscience & Biobehavioural Reviews, 117***, 26–64.

A review considering the diverse range of evidence that is accumulating to indicate that there are many factors that impact on the psychological function of an individual, including many that are not related to the genes of the individual but to factors that influence how the proteins produced from genes function.

Bazzett, T. J. (2008). ***An Introduction to Behavior Genetics***. Sunderland, MA: Sinauer Associates.

A comprehensive text discussing the issues and practicalities of behavioural genetics in much more detail.

Aristizabal, M. J., Anreiter, I., Halldorsdottir, T., Odgers, C. L., McDade, T. W., Goldenberg, A., … & O'Donnell, K. J. (2020). Biological embedding of experience: A primer on epigenetics. ***Proceedings of the National Academy of Sciences***, ***117***(38), 23261–9.

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A review of the role that epigenetic mechanisms may play in behaviour, especially disorders such as addiction and schizophrenia.