CHAPTER 6

IMMUNOLOGICAL INFLUENCE ON THE NERVOUS SYSTEM AND BEHAVIOUR: THE CENTRAL ROLE OF CYTOKINES

In Chapter 2 numerous examples of the psychoimmunological interaction were presented without exploring the underlying mechanisms. Chapter 4 and the second section of Chapter 5 dealt with the mechanisms through which psychologically induced activation of the two major stress axes can influence the immune system. What remain to be discussed are the pathways and mechanisms through which immunological activity can influence the brain and behaviour. Chapter 6 a) deals with the pathways through which immunological activity can influence the neurobehavioural functions, b) shows that immune-derived cytokines can act as stressors and initiate the stress response, c) overviews the types of influences of cytokines on behaviour, d) supplies evidence that immunological activity may predispose to future behavioural abnormalities and, e) argues that the CRH/HPA system is a primary target of immunologically derived cytokines.

Introduction

In previous chapters we have seen how the immune system can be controlled by behaviour. This behavioural control of the immune system, be it through classical conditioning, psychological `stress, deliberate psychological intervention or the effect of environmental impact, was shown to be mediated through neuroendocrine pathways. The effects of the two major stress axes on the immune system were discussed and CRH was shown to play a central regulatory role. This chapter deals with the influence of the immune system on the brain and on behaviour. The purpose of this chapter is to demonstrate how the brain can, consciously or subconsciously, become aware of the activity of the immune system, as well as the subsequent effects of this communication on the neurobehavioural functions.

The immune system, in addition to its defense against infectious agents and its surveillance functions, is in the process of being perceived as yet another sensory organ, informing the brain about potential dangerous events such as infections. The organs traditionally seen as sensory organs generally inform the brain about peripheral occurrences through impulses send via neural pathways. Such information about potentially harmful situations usually results in the conscious perception of events. Identification of infectious conditions by the immune system differs from that of the other senses in that information seldom leads to direct conscious perception of the condition and that the information can be transferred to the brain by mechanisms other than direct neural pathways. The information about infectious complications is said to reach the brain via the production and circulatory transport of immune cell-derived cytokines that signals the brain about the immunological activities. This could then, in the acute situation, alter central nervous system neurohormonal secretory activities that could induce behavioural adaptations such as sickness behaviour and other mental and cognitive changes. It is further said that chronic overstimulation of the immune system, especially during neurological vulnerable periods, may lead to psychopathology. Cytokines have been established as the major immunobehavioural substances.

To establish whether the above described immunological influences on the brain, and thus on behaviour, are scientific facts or merely folklore, the following questions should be asked

- Does the neural system have the necessary receptors for cytokines in order to exert an effect on the functions of the brain, and by implication on behaviour?
- How do these peripherally produced cytokines reach the neural structures involved in higher brain functions and behaviour? It is known that most of these structures are situated inside the blood-brain barrier – an organ not generally permeable to most circulating substances.
- Can cytokines influence the neural structures by, for instance, acting as stressors that initiate a stress response similar to that caused by psychological stress? How would

this then interact with non-immunological-induced stress, including psychological activation of the neurohormonal stress reaction?

- If so, are the effects merely of a transient nature or can cytokines lead to more permanent structural and functional changes that will influence future behaviour and perhaps predispose to mental disorders?
- Are the proposed cerebral cytokine receptors merely influenced by peripheral immune cell-derived cytokines or does the brain have a cytokine network that partakes in normal cerebral homeostasis?
- Are scientifically verified examples available to support the functionality of a cytokine-brain interaction, and perhaps of adverse conditions that may result from such an interaction?
- The CRH/HPA-axis, and more specifically CRH, has previously been shown to be central to psychoneuroimmunology in terms of the two major stress axes. Is this system to any significant extent influenced by cytokines?
- By integrating the information gained from all the chapters this far – can the brain and behaviour, in psychoneuroimmunological context, be programmed to be more stress resistant?

The aim of this chapter is to briefly review the answers to these questions. In this way it is hoped to show how the immune system functions as sense organ, informing the brain about immunological events in order to procure relevant neural and behavioural adaptations. This represents part of the bidirectional communication where immune products signal the brain and the brain signalling immune function, and hence coordinate the host defense response with other aspects of the stress response.

6.1 Cytokine receptors on neural structures

For cytokines to act as immunological messengers to the brain, the brain must have the appropriate cytokine receptors. Cytokine receptors have been shown on many cerebral structures. An idea of the distribution of cytokine receptors can be obtained from Figure 6.1, as adapted from Haas and Schauenstein, 1997 (1). The illustration in Figure 6.1 is

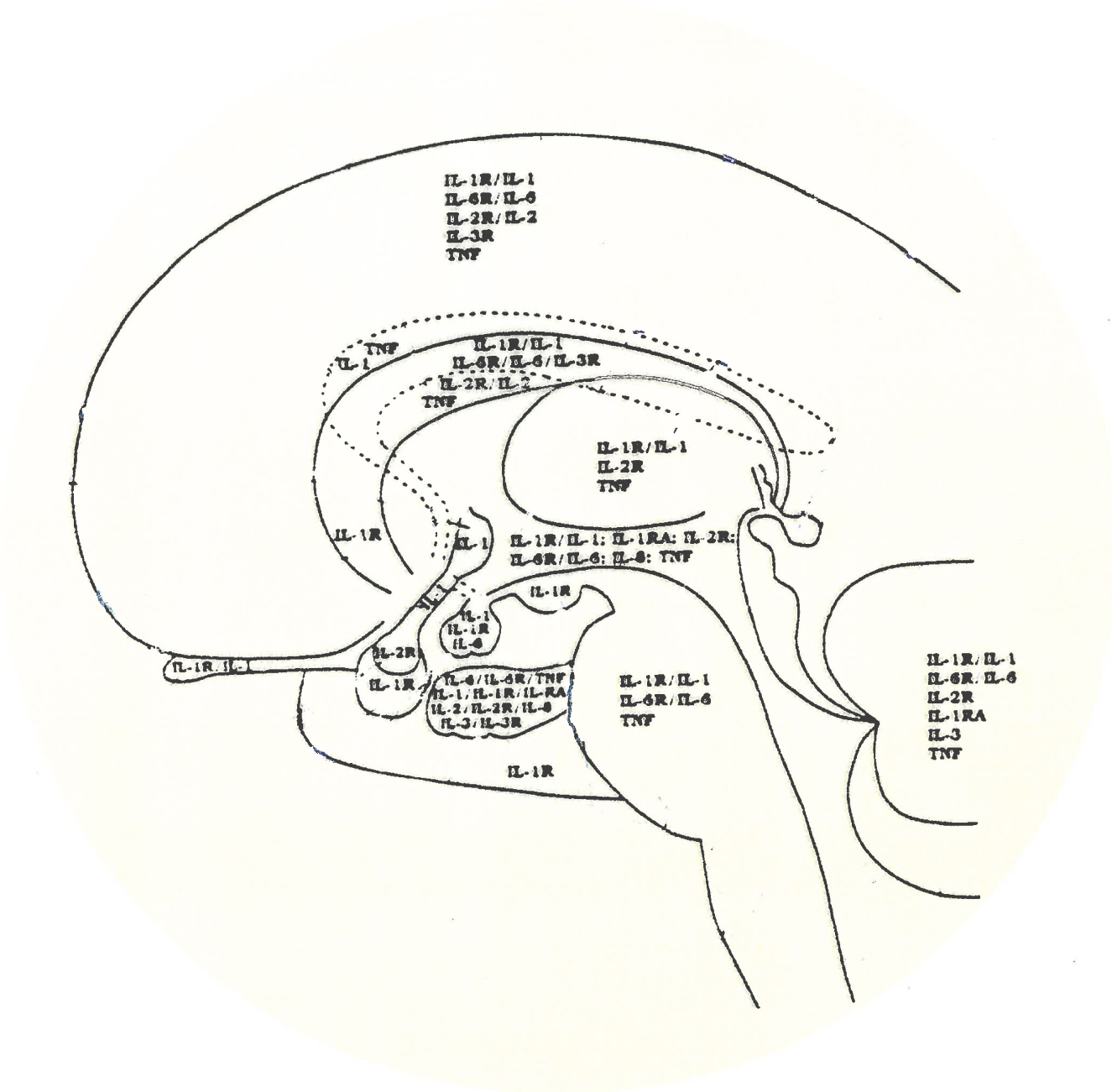

Figure 6.1: Cytokine receptors in the central nervous system
Adapted from (1)

still far from complete as new information is consistently being published. As would later be seen, stress hormones modulate the expression of some of the receptors. Apart from the expression of cytokine receptors in the brain, certain CNS cells can also produce cytokines, particularly microglial cells and astrocytes (2). The production of these cytokines can be constitutive or inducible – rendering their production open to psychoneuroimmunological modulation. Cerebral production of cytokines can be induced either by peripherally derived immune signals or by neuronal signals (3). More about cytokine production and function in the brain at a later stage of the chapter.

6.2 Pathways by which cytokines can influence the brain and behaviour

The presence of cytokine receptors in the brain offers the opportunity for immunological activity to influence the neurobehavioural functions – if the cytokines can get pass the blood-brain barrier. Various routes exist through which cytokines can influence neural function and it is postulated that different routes predominate under different conditions (3). Some of the pathways by which cytokines and other immunomodulators can influence the central noradrenergic system were referred to in Chapter 4 and would not be dealt with in this chapter.

Although we are primarily interested in the effects of peripherally derived cytokines on the brain and behaviour, it should be mentioned that immune-neural interactions also occur locally in peripheral areas. This is especially true with regard to the SAM-axis where locally produced IL-1 can control noradrenaline secretion from postganglionic sympathetic nerve fibres innervating primary and secondary lymphoid tissues (3). This local immune-neural interaction is referred to as the short-loop interaction between cytokines and neural elements. The second mode through which cytokines can influence neural tissue is by cerebral cytokines produced *in situ*, as previously mentioned (2,3). The third route involves the mechanisms through which peripherally produced, immune cell-derived, cytokines signal the central nervous system and behaviour.

Of all the cytokines, the way the interleukin-1 (IL-1) family of cytokines signals the brain is probably best understood. The reason why more work is done on IL-1 signalling is that we are for quite a while aware of the fact that IL-1β plays a role in fever-induction and that many workers were involved in finding the way in which IL-1 manages to reach the relevant neural structures. However, not one cytokine operates in isolation from other cytokines, they are all extremely pleotropic, and a great deal of functional and mechanistic overlap occur. The reason why the signalling of IL-1, and specifically IL-1β is of great importance for this writing is that it forms part of the non-specific or innate immune response. The cytokines of the IL-1 family are, in fact, members of the pro-inflammatory cascade of cytokines released by activated macrophages, that include, amongst others, IL-1α, IL-1β, TNFα and IL6. It is further known that IL-1β induces IL-2 production by T-helper (Th) cells, as well as the expression of IL-2 receptors (3,4). This becomes relevant when the effects of IL-2, a major immuno-neural communicator, are discussed in a later paragraph. The importance of the pathways for neural signalling via these pro-inflammatory cytokines turn out to be of even greater importance and relevance to the integration of the mind-body effects of cytokines when one recalls that (4) they

- Initiate and coordinate the inflammatory response – a response implicated in a number of mental disorders (refer to chapter 2).
- Initiate the acute phase response – a response that includes several adaptative mechanisms against injury and trauma, including physiological responses such as the hepatic shift to acute phase protein production, redistribution of iron stores and the induction of fever. These physiological alterations each have behavioural effects of their own. However, the acute phase response is also marked by a host of behavioural alterations grouped together under the rubric of "sickness behaviour". Sickness behaviour will be addressed in the next chapter as part of a model developed to illustrate the practical implications of the psychoneuroimmunological interaction.

The mechanisms of the third pathway through which cytokines signal the central nervous system and behaviour include blood-borne mechanisms where cytokines are transported

from the area of secretion to the brain, as well as neural mechanisms where cytokines influence peripheral nerves that in turn transfer the information to the cerebral structures.

Signalling through circulatory transport of cytokines, i.e., blood-borne mechanisms include

- Cytokine carrier mechanisms. Saturatable cytokine carrier-mediated mechanisms can transport cytokines across a concentration gradient to the brain and are responsible for at least a small part of the signalling process (4,5).
- Entry of cytokines at the circumventricular organs (CVOs) (4,6). These areas are devoid of a blood-brain barrier and include the area postrema (AP) and the organum vasculosum of the lamina terminalis (OVLT). Entry through these areas does, however, not provide entry to the rest of the brain, i.e., areas where the cytokine-behavioural interactions are known to take place. Cytokines bind to cytokines receptors on the CVOs – an event that stimulates prostaglandin synthesis. Prostaglandins, which are highly lipophilic and, in many instances, also neuroexcitatory, then cross the blood-brain barrier to cerebral areas not accessible to cytokines. Prostaglandins can now activate neurons that, in turn, project to appropriate areas such as the paraventricular nucleus and other structures of the HPA-axis, as well as to cerebral structures such as the amygdala and central noradrenergic neurons (6,7,8). The potential neuro-behavioural effects are self-evident.
- Binding of cytokines to cytokine receptors on cerebral blood vessels with subsequent signal transduction. The process is very similar to that of binding to receptors on the CVOs. Cytokine binding to these receptors on the vasculature can, as before, lead to prostaglandin synthesis through the COX-2 pathway, that will lead to neuronal activation and projection to the appropriate cerebral controlling areas – presumably with the same type of behavioural responses (4,9).

Signalling through effects on peripheral nerves occurs, amongst others, through vagal afferents. Immune cell-derived cytokines, such as IL-1, act locally, in a paracrine fashion, to stimulate vagal afferents that would then signal the appropriate cerebral areas. The

syndrome of behavioural characteristics known as sickness behaviour is but one of the patterns of behavioural adaptations to be initiated in this way (4). The transduction process probably involves cytokine receptors on paraganglia that surrounds vagal afferents. These paraganglia, which consist of chemosensory glomus cells, provide afferent innervations to vagal afferents by means of catecholamine and indolamine neurotransmission (10). The induction of prostaglandins may also be involved in the immune-neural signal transduction (1). In addition to the vagus nerve, other afferent nerves, including most cutaneous as well as the sciatic nerve, can also be instrumental in the immune-neuronal signalling process.

It would at present appear as if the early cerebral responses may be through prostaglandins formed via constitutive COX-2 upon vagus-induced noradrenergic input from the nucleus tractus solitarius (12), while blood-borne mechanisms come into play later, when cytokine levels have reached certain minimum levels (4).

6.3 Cytokine effects on neural structures and neurobehavioural functions – immunological activity as stressor

Immune cell-derived cytokines, although not the only substances, represent the major mediating vehicles for information transfer from the immune to the neurobehavioural system. In the previous section it was shown that signal transduction can occur either in the central nervous system or in the periphery. Evidence for the influence of immune-derived cytokines on the brain and on behaviour is provided in the next couple of paragraphs.

In previous chapters it was seen that the CNA/SAM-axis and CRH/HPA-axis, as well as other neurohormonal systems, form part of the response to psychological stress. It was also shown that the interactions between the two major stress axes and, especially, the neuromodulatory systems of the brain stem, including the serotonergic, GABA, dopaminergic and acetyl cholinergic systems, are major determinants of the cerebral activation state as well as mood. Further shown were the influences of the two major

stress axes on peripheral immunocompetence and cytokine production. As we have seen, stress-induced neurohormonal activation can influence cytokine production, as well as the clinical course of infections. The first question that should thus be asked is whether infectious conditions and subsequent cytokine release can, in turn, influence the neurohormonal activity and by implication behaviour. In other words, can such events initiate or alter the neurobehavioural stress response? The answer is an unequivocal yes. There is, in fact, a marked similarity between the infection-induced stress response and the psychologically-induced stress response. Activation of, not only the CRH/HPA-axis and CNA/SAM-axis, but also of most of the other neuromodulatory systems, takes place, both during psychological stress and infections (13,14). Examples of the stress-inducing effects of infectious events on the main stress-involved neural structures will be presented in the last section of this chapter. Infections can thus indeed be seen as stressors with the ability to initiate the central nervous system stress response. Minor differences exist between the response initiated by psychological stress and that caused by infection – the major differences apparently that the infectious complications generally initiate larger noradrenaline responses in the hypothalamus than in other brain areas and that the dopamine response is virtually absent (13,14). An almost identical induction of the central stress response has repeatedly been reported with endotoxin (lipopolysaccharide or LPS) administration. However, the neuroendocrine/neurochemical response of LPS has, as could be expected, a much more rapid onset (13,14). A great number of studies showed that cytokines could exert a similar stimulatory pattern on the central neuroendocrine stress response. The main cytokines involved in initiating the central stress response would appear to be a) interleukin-1α and interleukin-1β, which stimulate the 2 major stress axes, as well as the tryptophan and serotonin, but not the dopamine system, b) interleukin-2 which stimulates, at least, the same systems, c) interleukin-6 that stimulates, at least, the CRH/HPA-axis and central serotonergic system, and d) tumour necrosis factor-α that stimulates the two main stress systems (13,14). Detailed descriptions of the effects of the various cytokines on the different cerebral regions involved in the stress response are available (13), but beyond the scope of this thesis.

The previous paragraphs discussed the ability of cytokines to initiate, like any psychological stressor, the central nervous system stress response. It speaks for itself that in activating a neurohormonal activation similar to that of psychological stressors, cytokines would also be able to cause behavioural changes similar to that of psychological stress.

Of interest to note is the fact that the two main stress axes would appear to contain the release of those cytokines that are known to have the most significant influences on the neurobehavioural processes. This is at least true for noradrenaline and the glucocorticosteroid, cortisol. As previously shown, these stress hormones differentially influence cytokine production by T helper (Th) cells. Cytokine production by Th1 cells, i.e., the pro-inflammatory cytokines such as IL-2, IFNγ, TNFα and IL-12 are inhibited both by the effects of catecholamines on β2-receptors and by cortisol. Catecholamines and cortisol will, in certain conditions, stimulate the production of Th2 cytokines such as IL-4, IL-10 and IL-13, i.e., the anti-inflammatory cytokines (15). It would thus, at first glance, appear as if stress has only anti-inflammatory effects. However, it should be remembered, as shown in chapter 5, that the effects of other hormones such as CRH can be almost completely pro-inflammatory. Also of major importance, as mentioned in Chapter 4 and 5 with regard to the influence of the neurohormonal systems on the immune system, is the fact that the effects vary, depending on the duration of the stress, the type of stressor, the subject pool, the intensity of the stress response, i.e., the levels of neurohormonal activity, previous conditioning of animal or man, and many other factors.

In view of the differential effects of the two main stress axes on Th1 and Th2 cell-related cytokines it is not surprising to find that most of the cytokine effects, reported on the brain and on behaviour, are those of the Th1 cells – the cytokines most strongly controlled by the stress axes. The reader is referred to an excellent analysis by Hanisch, 2001 (16) on the cerebral effects, pathways, interactions, receptors and mechanisms of action of two classes of these cytokines, i.e., interleukin-2 and the interferon family. Numerous examples of neurological and behavioural effects of these cytokines are given and discussed by Hanisch (16), including

- Decreased food intake and eating behaviour (IFN)
- Altered motor activity (IFN)
- Induction of sleep (IFN)
- Decreased electrical activity of neurons, including putative CRH secreting cells in the paraventricular nucleus (INF)
- Modifications of EEG-like activity in somatosensory, motor and limbic structures
- Increased discharge of cortical and hippocampal neurons (IFN)
- Suppressed overall behavioural activity (IL-2)
- Decreased responding to rewarding lateral hypothalmic stimulation in rats (IL-2)
- Induction of asymmetric body posture and ipsilateral turning in rats (IL-2)
- Decreases or increases in hypothalamus neuronal activity – depending on the area of the hypothalamus (IL-2, IFN)
- Proconvulsive effects (IL-2)
- Suppression of afferent sensory transmission to neurons of the primary somatosensory cortex (Il-2)
- Increased ADH release from the hypothalamus and amygdala (IL-2)
- Inhibition of GH, FSH and LH release with increases in the release of somatostatin, prolactin and thyroid stimulating hormone (IL-2)
- Increases in cortisol levels (IL-2), POMC, ACTH and other POMC-derived hormones

Most of the cytokine effects shown above are similar to that expressed during the psychologically-induced stress response, as well as to symptoms found with a number of mental disorders. This supports the possibility that cytokines can act as neuroregulatory substances.

Typical examples of cytokine effects on the neurobehavioural functions will be discussed in more detail in a later section of this chapter. However, it is at this stage necessary to say that it is by now well established that cytokines can lead to a wide spectrum of neurobehavioural effects. The initial effects shown in animals have been supported in humans over and over again. Although it would be unethical to experimentally subject humans to the administration of relatively high dosages of cytokines, the opportunity to

study such effects became available with the introduction of cytokines and cytokine antagonists as therapeutic agents. At this stage it is believed that neurological and neuropsychiatric symptoms are common in at least 50% of patients treated with IL-2 or with interferons. The neuropsychiatric alterations include symptoms like cognitive impairments and cognitive failure, hallucinations, abnormal mood states, psychotic behaviour, headaches, motor weakness, confusion, somnolence, anxiety, paranoid delusions and combative behaviour. Reviews can be found on such mental changes (17). It should, however be stressed that the effects are not limited to brain and behaviour, but that a wide shift in total mind-body homeostasis occurs. These changes include further neurohormonally-induced alterations in the immunological activity (18).

6.4 Possibility that infections, with the accompanying changes in cytokine profiles, could cause long-term effects on the brain and perhaps predispose to future psychopathology

Another point of major interest is whether cytokines can modulate cerebral structure and hence have a more permanent effect on the brain and on behaviour. If this is possible it would give credence to claims that infectious complications, be it *in utero* or during early life, could predispose to behavioural problems in later life.

There are, in fact, ample indications to support the hypothesis that infectious complications can presensitise to future behavioural problems. Correlational studies between maternal immunological afflictions and behavioural problems, such as schizophrenia in the offspring, were already mentioned in Chapter 2. In addition, mechanisms through which such presensitisation processes may occur are slowly becoming clear. Research this far is focussed on the effects of cytokines. Interleukin-2 has, for instance, been shown to support survival and growth of a variety of neuronal and glial cells, but also, under certain conditions, to be toxic to neurons. A whole cascade of effects are reported for IFNα, IFNβ and IFNγ, including the prevention of cell death after cerebral injury by acting as nerve growth factor, a role in proliferation and scar-forming activities of glial cells, and neuronal cell death by direct, as well as indirect mechanisms

(19). The effects of glial scar formation on brain and behaviour are well known and would not be discussed at this stage. There is also sufficient evidence to believe that IFNγ can be instrumental in demyelinisation, be it through T-cell or other immune cell infiltration, or through the activity of resident glial cells (20,21). It is tempting to extrapolate this to the behavioural problems seen in demyelinisation disorders such as multiple sclerosis. Reported long-term effects of Il-2 therapy, including memory and neuroendocrine disturbances, delayed as well as progressive brain injury and permanent brain damage, further support the experimental evidence that cytokines can have long-term influences on neurobehavioural processes (16,17, 22,23). The implications of such damage to brain structure for future neurobehavioural abnormalities speak for themselves. This major route through which infections can presensitise neuro-endocrine systems to future psychological disorders is often overlooked.

It can at this stage unequivocally be stated that infectious complications can influence behaviour in the short-, as well as in the long-term. Firstly, it can act as stressor and elicit a stress response very similar to that of the psychological stress response. This was discussed in earlier paragraphs. In addition, the effects of the psychological stress response on brain structure and function can presensitise to the development of abnormal behaviour in later life. This aspect will be returned to in Chapter 7. In summary, one could conclude that exposure of the developing brain to infections, be it *in utero*, in early childhood, or even later, may have serious consequences for later mental health. In view of sustained cerebral plasticity through a large part of the life cycle this would probably also apply to infectious complications occurring during adolescent and adult life.

6.5 The cerebral cytokine network: The relay system hypothesis

As mentioned earlier in this chapter, the brain contains cytokine receptors, can synthesise cytokines - constitutive, as well as inducible – and cytokine production can be induced by immune as well as by neural signals (2,24). Some cytokines produced by the brain have been reviewed by Hori, Katafuchi and Oka, 2001 (25), and include

- Members of the IL-1 family, secreted in response to inflammatory, as well as non-inflammatory stress.
- Tumour necrosis factor alpha (TNFα), secreted under basal and stimulated conditions, with high levels in the hypothalamic area.
- Interleukin-6 (IL-6) and leukemia inhibiting factor, expressed during fever and LPS injection with significant concentrations in the hypothalamus and limbic structures.
- Interferon alpha (INFα), expressed constitutively, as well as induced during infections by neurons and glial cells, shown to be involved in the CNS-mediated acute phase response and, in contrast to most other pro-inflammatory cytokines, known to inhibit the HPA-axis.
- Interleukin-10 (IL-10), - a cytokine known to inhibit the production of pro-inflammatory cytokines, found in glial cells of humans and animals during infectious, traumatic, neoplastic and neurodegenerative diseases.

Others were shown in Figure 1 and mentioned elsewhere in the text. Although the discussion this far centered, but for IL-10, around the pro-inflammatory cytokines, anti-inflammatory cytokines such IL-4, IL-10, IL-13 and TGF-β can also be produced in the brain. It is at present safe to say that they can, in cooperation with substances like the glucocorticoids and antidiuretic hormone, oppose the expression and actions of pro-inflammatory cytokines in the brain, i.e., it can offer some protection against pro-inflammatory cytokine-induced behavioural effects such as depression (26).

The induction of cerebral cytokine production by peripheral immunological activity leads to differential cytokine expression in very specific cerebral areas, especially the hypothalamus, hippocampus and thalamus-striatum. The cytokines thus produced are then, in turn, able to exert a controlling influence on the local neurohormonal structures. One can assume the purpose of this cytokine-induced response to be the coordination of those peripheral homeostatic processes that are under cerebral control (24). In view of the overall coordination between physiological and psychological events shown in chapter 5, the process would most probably also coordinate behaviour with the physiological homeostatic adjustments.

Cerebral cytokine activity should, however, not be seen only as a phenomenon limited to periods of stress. The cerebral production of cytokines, induced by neuronal activity, forms part of normal cerebral function. A good example of the functionality of neuronal activity-induced cytokine production is the increase in IL-1β in the hippocampus during long-term potentiation (LTP) (27). LTP, part of the learning process and involved in memory formation, is marked by a sustained increase in synaptic transmission and post-synaptic neural activity. The cytokines produced in response to this increase in neuronal activity are, in turn, responsible for the maintenance of the LTP (24), and by implication necessary for normal learning and memory processes.

In view of the influence of peripheral cytokines on the immunological and neurological activities of the brain, a relay hypothesis, describing the dual immune and neural control of cerebral cytokine production, was proposed by Besedovsky and Del Rey, 2001 (24).

A relay system is postulated that integrates peripheral immune and neural/sensory signals, and induces a re-setting of neuro-endocrine functions. This relay system is based on interactions between cytokine-producing brain cells and neurons located in their close vicinity. When increased cytokine production is induced in areas such as the hypothalamus and the hippocampus as consequence of immune and/or neuronal signals, a resetting of homeostatic functions would occur (thermoregulation, glucose homeostasis, neuro-endocrine feed-back, etc). This resetting of homeostasis is expected to be specially relevant for neuro-endocrine adjustments needed during conditions in which primarily the immune system (e.g. infections) or the CNS (e.g. stress) is affected.

Besedovsky and Del Rey, 2001 (24).

Figure 6.2, based on the work presented in this chapter, including the relay hypothesis of Besedovsky and Del Rey, is a schematic representation of the psychoneuroimmunological interaction in which it is shown that

- The cerebral neuroendocrine systems responsible for behavioural characteristics can interact with the cerebral cytokine system under basal conditions. These interactions, as previously discussed, form part of normal processes such as learning and memory.

- During peripheral infectious or inflammatory conditions increased peripheral production of inflammatory cytokines will a) influence the cerebral neurohormonal systems and adaptive neurohormonal changes will occur, b) peripherally produced cytokines will induce increases in cerebral production of cytokines and c) inducible cerebral cytokine production will be further increased by the adaptational neuronal activity. In other words immune derived cytokines (non-cognitive stressors) signal neural structures via cytokine specific transport process, or via the induction of secondary signals such as prostaglandins through receptors on blood vessels or circumventricular organs, or through the influence of cytokines on peripheral afferent nerves. This signalling leads to adaptive changes in neurohormonal functioning which in themselves are stimuli for further cerebral induction of cytokine production. Cerebral cytokine expression is thus upgraded both through the effects of the peripherally derived cytokines on the glial, neuronal and other brain cells, and through the cytokine-induced altered neuronal activity.
- The upgraded cerebral cytokine expression, in cooperation with high levels of peripheral cytokines change the activity and most probably the set points of the central nervous system neurohormonal control systems to bring about adaptational changes which help to cope physically and mentally with the stressor. This fits in perfectly with the concept of the stress condition primarily intended as an adaptational state.
- This adaptational state involves physiological processes, such as altered neurohormonal functioning, that lead to adaptive behavioural processes, as well as adaptive metabolic, motor, sensory and immunological responses. These adaptive responses result in a new mind-body homeostasis that enables the individual to cope mentally and physically with, in this case, the non-cognitive stressor.

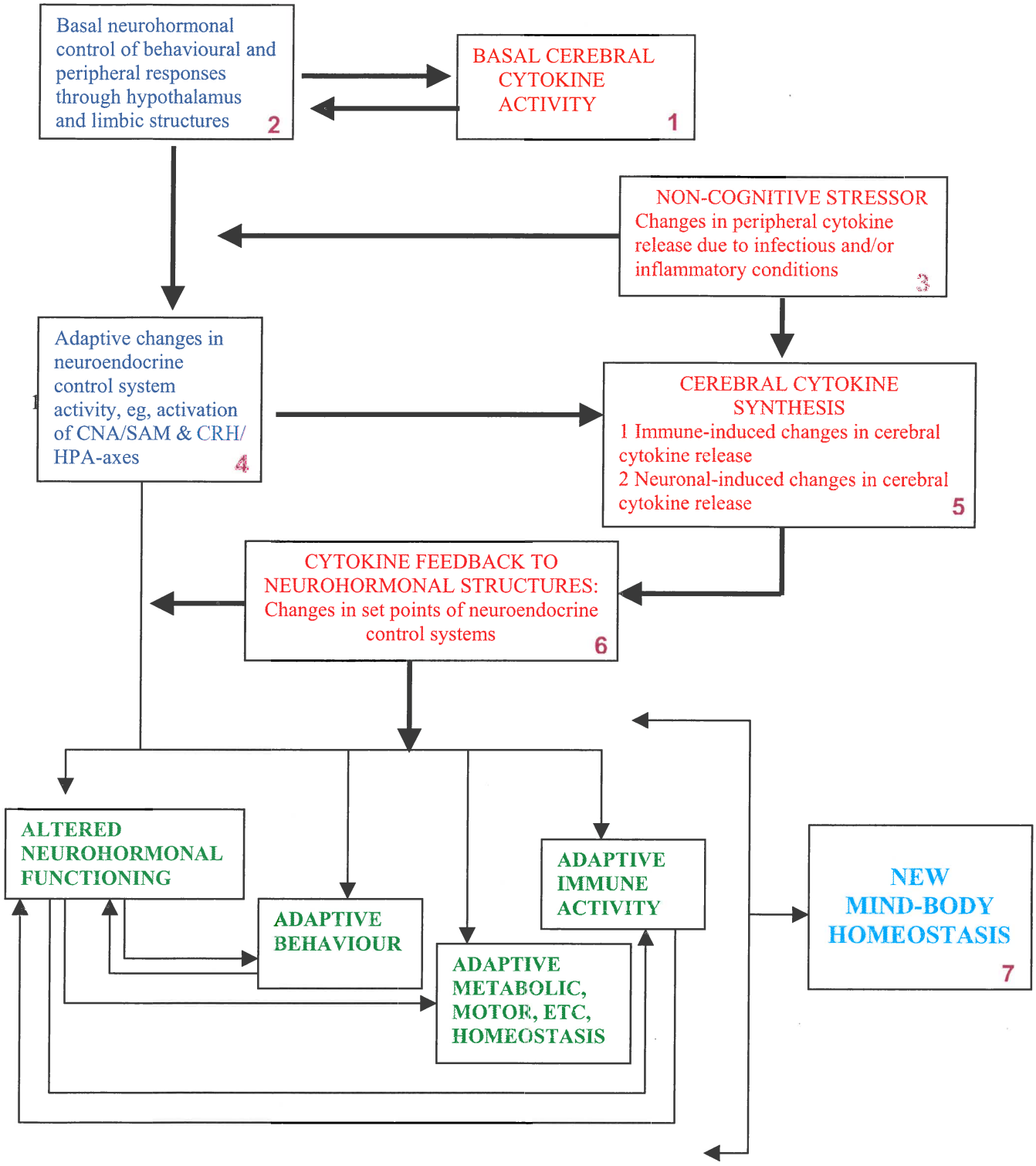

Figure 6.2: Relay hypothesis of the dual control of cerebral cytokine synthesis

[Figure 6.2 shows that basal cerebral cytokine production (1) plays a role in neuroendocrine activity (2) and *vice versa*. In the face of immunological activity, as non-cognitive stressor (3), selective neurohormonal activity (4) is increased by the effects of peripheral immune signals (cytokines) on the brain. Cerebral cytokine production is then increased by the effects of the peripherally-derived cytokines on the brain, as well as by the increased neurohormonal activity (5). This increased cerebral cytokine production can alter the neurohormonal set points (6) with subsequent development of a new mind-body homeostasis (7) characterized by alterations in behaviour and in the peripheral stress response]

The discussion above focussed on the changes that occur as a result of a non-cognitive stressor such as infection or a chronic inflammatory condition. This was intentionally done in order to show the psychoneuroimmunological interaction from an immunological point of intervention into the basic neuro-behavioural processes. This same schematic presentation will, however, apply should the primary stressor be of a psychological or environmental nature. The similarities between induction of the stress response by psychological and by immunological stressors were described earlier in this chapter.

What then is the relevance to the discussions on the CNA/SAM-axis and CRH/HPA-axis of previous chapters? This, based on discussions in previous chapters, speaks for itself, and has already been incorporated into Figure 6.2. The effects of the two major stress axes on peripheral immune responses are largely to curb excessive immune responses and have been discussed in Chapter 4 and Chapter 5 – before demonstrating that the central neurohormonal activity involved in the immunological control can actually be stimulated by the very same immunological activity. The neurohormonal activities of the two major stress axes are, in fact, amongst the most important neurohormonal immune regulatory mechanisms in the body. It is, as just mentioned, known that the cytokines centrally produced in response to peripheral immune activity also influence the two stress axes (28,29,30). This aspect will be further addressed in the last section of this chapter. It is thus possible for the immune system to, via the two main stress axes, indirectly feed back on its own activity and hence control its own activity in an integrated fashion with that of the neurohormonal and behavioural functions.

To summarise these effects of central cytokines, mediated via the related neurohormonal systems, on the peripheral immune response (25), it can be said that:

- Intracerebral IL-1β causes immune suppression through activation of the SAM-axis and HPA-axis (25,28,29,30).
- Cerebral IFN-α can suppress peripheral immunity through processes involving the sympathetic system, the opioids and CRH (25).

- IL-2 probably exerts its effects, if any, via activation of other hormonal responses (25).
- Opioids such as beta-endorphin, a component of the HPA-axis inducible by cerebral cytokines, probably cause immunosuppression in a process involving the SAM-axis (30,31).
- Corticotropin-releasing factor (CRH), as shown in Chapter 5, can be pro-inflammatory. However, it would appear to exert immunosuppressive effects through its control of the SAM-axis (25,28,32).
- Prostaglandin E_2 (PGE2), represents part of the pathway through which pro-inflammatory cytokines can induce the acute phase response. Here immune suppression would once again be dependent on activation of the two main stress axes (25,32) and thus on CRH activity.
- The cerebral cytokines may also be involved in the stress axes immunomodulation during non-inflammatory stress (see 25 for original references).
- Peripheral inflammation can also be controlled by cerebral cytokines. Peripheral inflammation can influence the CRH/HPA-axis through neural afferents or blood-borne cytokines and in turn be controlled by the axis (25,33). However, central pro-inflammatory cytokines may, under certain conditions, further enhance peripheral inflammatory conditions (34).
- Central cytokines can, in addition, modulate peripheral inflammation-associated nociception, with IL-1β, IL-6 and TNFα leading to hyperalgesia and IL-1α, IL-2, IFNα, TNFα contributing to analgesia. It would appear that cytokine-induced changes in CRH are involved, e.g., CRH can stimulate the local peripheral release of endorphins that will increase the pain threshold, while activation of the central noradrenergic and CRH systems may be involved in the central perception of the pain (25). The central cytokines may lead to hyperalgesia in the early phases of the disease, to act as warning system, and to sickness behaviour, an adaptational measure, in the more advanced stages.

It is perhaps, at this stage, necessary to return for a moment to the stress-induced neurohormonal effects of stress – be it psychologically-induced or immunologically-

induced. In previous chapters chronic stress was seen as predominantly immunosuppressive. Acute activation of the HPA-axis can, however, be immune enhancing (Chapter 5). Recently it was suggested that the psychological disposition before stressor application is a major determinant of the kind of immunological response elicited by psychological stressors. The work of Maes (18), showed that there are two kinds of stress-induced immunological profiles, i.e., a predominant suppressive immune regulatory response, and a predominant pro-inflammatory response. The pro-inflammatory response is associated with significant stress-induced anxiety and depression and the stress-induced cytokine production is sensitive to graded differences in the perception of stressor severity (18).

In this section it was shown that basal levels of cerebral cytokines are produced constitutively or are functionally induced. However, when peripheral immunological activity increases, the production of cerebral cytokines is upgraded. This increase in cerebral cytokine activity contributes to the integration of physiological and behavioural functions in a new adaptive homeostasis.

6.6 Practical examples of the immunological influence on neurobehavioural functions

Some examples were already given during discussions of the various mechanisms and pathways. This section will therefore present a wide overview of the effects the immune system exerts on the neurobehavioural functions in order to see the pervasiveness of the influence in perspective. Cytokines will again be shown to be the predominant primary mediators.

6.6.1 Cytokines, diseases of the central nervous system and psychiatric disorders

Cytokine involvement is known to contribute to the neurobehaviour abnormalities of CNS pathologies where inflammatory or autoimmune processes, or hyperactivation of glial cells are present. Examples include multiple sclerosis (MS), Alzheimer's disease (AD), stroke, post-traumatic lesions, as well as various other forms of neurodegeneration

and several psychiatric disorders (35,36,37). A good review of the role and mechanisms of cytokines in MS and AD, as well as the types of cytokines (IL-1β, TNFα, TFGβ, IL-6), the receptors and receptor complexes, the adhesion molecules, free radicals, and the redox sensitive transcription factors involved in CNS inflammation can be found in a writing by Merrill, 2001 (35). MS and AD are good examples of the influence of the inflammatory cytokines on behavioural functions in diseases of the adult central nervous system. In MS, an inflammatory, autoimmune, demyelinating disease, pro-inflammatory cytokine-induced damage to the myelin and myelin-producing oligodendrocytes lead to disturbed impulse conduction accompanied by abnormalities of motor and behavioural functions. AD, a neurodegenerative disease with marked cognitive and emotional changes, is now said to be primarily initiated by activated cytokine-producing microglia with subsequent inflammation and neuronal damage (35).

Abnormal cytokine production is further implicated in a large number of stress-related psychiatric disorders (38), including

- Obsessive-compulsive disorder (OCD). An increase in cytokine activity is reported in individuals with Sydenham's chorea, an infection-triggered autoimmune subtype of pediatric OCD and in Tourette's syndrome. The most prevalent cytokines in OCD would appear to be those associated with cell mediated immunity. (For further information on the immune activity in these two disorders, please return to Chapter 2.)
- Panic disorder where the most consistent cytokine deviation is a significantly higher IL-1β.
- Generalised anxiety disorder where the cytokine-related disturbances would appear to correlate with that seen in severe stress.
- Post-traumatic stress disorder where abnormal levels of IL-1β is once again reported as the most significant cytokine disturbance.
- Anorexia nervosa, a disorder generally associated with overactivity of the HPA-system, in which decreased IL-2 production and increases in IL-6 are reported.

- Many other stress-related psychiatric disorders in which the cytokine involvement where already referred to in Chapter 2.

The mood disorders are probably the psychiatric disorders best investigated in terms of immunological involvement, especially with regard to cytokines. There are some excellent overviews available on the immunological involvement in depression (39,40,41,42,43) and a short discussion here would be of little help. It is, however, necessary to mention that major depression is almost without exception accompanied by a moderate inflammatory response and by an increase in the secretion of pro-inflammatory cytokines. Depression and its associated cytokine profile would once again be returned to in the penultimate chapter of the thesis.

6.6.2 Neurobehavioural effects of cytokine therapy

A golden opportunity to study the effects of cytokines on human behaviour presented with the advent of cytokine therapy. Reported effects of cytokine therapy on the brain vary from mild headaches, to psychomotor retardation to psychiatric disorders to electroencephalographic changes – in a dose-dependent manner. In fact, two distinct patterns of cerebral toxicity are known to occur with cytokine therapy (44), i.e.,

- An acute phase where constitutional symptoms (fever, chills, headache and fatigue), now referred to as the flue-like syndrome, develop. It may last 1 to 3 weeks.
- A chronic phase, often referred to as neurasthenia or the chronic fatigue syndrome of cytokine therapy, with symptoms such as asthenia, malaise, lethargy, somnolence, headaches, low-grade fevers and anorexia. Other side effects such as psychomotor, cognitive and psychiatric abnormalities, including delirium and coma, may develop (44).

Typical neurobehavioural symptoms associated with specific cytokines, as summarised from an overview by Turowski and Triozzi, 1999 (44) include

- Interferon-α: At high dosages severe, chronic fatigue, psychomotor retardation, social withdrawal, gesticulation, articulation, anorexia and cognitive changes such as a decreased attention span, the inability to concentrate, suppressed verbal learning, impaired short term memory, loss of decisiveness and mental clouding, are common. Hallucinations, expressive dysphasia and gait difficulties may occur with chronic administration of high dosages. The psychiatric toxicities of interferon-α are sometimes classified into a) an organic personality syndrome (uncontrollable overreaction to minor frustrations, marked irritability, short temper), b) an organic affective syndrome (feelings of depression and hopelessness, tearfulness and crying), and c) a delirium category (clouding of consciousness, disorientation, the inability to perform simple calculations, memory problems, irritation and mood changes). The latter often not returning to normal after cessation of the treatment.
- Interferon-β: Symptoms are not as frequent and not as severe and, as before, depend on the dosage, administrative route and duration of therapy. Symptoms range from mild, acute constitutional symptoms, to chronic neurasthenia, to confusion, somnolence and emotional instability, to agitation, disorientation, dementia and personality changes.
- Interferon-γ: Symptoms, in a dose dependent fashion, range form mild constitutional problems such as headaches and chills and, infrequently, to dizziness, slowing of thought processes, confusion, crying episodes and Parkinson's-like symptoms – that are resolved upon cessation of therapy.
- IL-2: CNS toxicities occur frequently, ranging from constitutional problems to the less frequent appearance of somnolence, coma, disorientation and delirium at higher dosages during chronic administration.
- IL-4: Moderate grade fever, fatigue, anorexia, and headaches are common and there are indications that it may cause transient partial blindness, photophobia and visual hallucination at higher dosages.
- IL-12: Not properly investigated yet, but indications are that it causes fever, chills, headache and perhaps gastrointestinal and other problems.
- IL-1: This pro-inflammatory cytokine is at present not approved for clinical use due to its toxicity. Constitutional symptoms at low dosages with somnolence, confusion,

agitation, delusional ideation, photophobia, blurred vision and seizures at higher dosages are some of the symptoms reported when still approved.

- TNF: This is another pro-inflammatory cytokine at present not approved for clinical administration due to toxicity. Constitutional symptoms at low dosages, with amnesia, aphasia, hallucinations and diplopia at higher dosages were previously reported.
- IL-6: Yet another pro-inflammatory cytokine at present not approved for clinical use, despite reports that the toxicity is not as pronounced as that of IL-1 and TNF.
- Hematopoietins: Hematopoietins, including erythropoietin, granulocyte colony-stimulating factor, macrophage colony-stimulating factor, stem cell factor, interleukin-3, IL-11 and thrombopoietin all have a degree of neurotoxicity but are fairly well tolerated. Although the underlying mechanisms are said to be largely unknown, work in our laboratory showed that erythropoietin can dramatically increase intracellular calcium concentration – a cellular disturbance that is generally known to influence neuronal conductivity (45).

Cytokine therapy affects the behavioural functions through alteration of various cerebral activities. It can for instance

- induce secondary production of cytokines that can change the activity levels of specific neurohormonal circuits in the brain (46),
- alter the release of those stress hormones involved in cognitive function and emotion (47),
- alter behaviour through the development of auto-immune thyroid disease (48),
- change the circuitry of the frontal-subcortical circuit by influencing the neurotransmitters that control it (49) and
- influence the cerebral endothelium (47).

Many other examples of the influence of cytokines on the neurobehavioural functions are available, including fever, sleep and mood disturbances. However, a discussion on such aspects will be deferred to the next chapter where the cytokine-induced behavioural changes of sickness behaviour are to be discussed.

In line with the aim of this thesis it is, at this stage, necessary to show that the structures of the two main stress axes constitute major targets for cytokines.

6.7 The effects of cytokines on the CRH/HPA-axis – a regulatory role for CRH

In Chapter 5 the effects of the CRH/HPA-system on the immune system and cytokine production were described. The conclusion was that CRH is in fact the main neuroendocrine modulator of the immunological reactivity – be it direct or indirect. Chapter 5 further showed that CRH can coordinate all aspects of the psychological stress response, including the neurobehavioural, endocrine, autonomic and motor responses. It was, in fact, shown that "psychoneuroimmunology in terms of the two main stress axes" could perhaps be seen as "psychoneuroimmunology in terms of CRH".

In previous paragraphs of this chapter it was seen that the immune system has both short- and long-term effects on the brain and behaviour. To be able to equate "psychoneuroimmunology in terms of the two main stress axes" with "psychoneuroimmunology in terms of CRH" it is essential that one should be able to show that CRH neurons are major targets of the immune system derived cytokines. The next section will therefore deal, firstly with the effects of cytokines on the CRH/HPA axis, and secondly with that on the CRH system *per se*.

6.7.1 Effects of cytokines on the HPA-axis

The three parts of the HPA-axis that could theoretically be influenced by peripherally derived cytokines are the hypothalamus, the anterior pituitary and the adrenal cortex. Changes in the hormonal release from all three parts do indeed take place upon cytokine administration. An increase in CRH (and sometimes ADH) from the hypothalamus, ACTH from the anterior pituitary, and glucocorticosteroids from the adrenal cortex, have been shown in animals and in man upon administration of the IL-1 cytokine family (IL-1α, IL-1β), IL-2, IL-6, TNFα, IFNα and IFNγ. Indications are that some of the processes might be mediated through prostaglandin synthesis. The idea that cytokines can influence

the HPA-axis has over the last couple of years become an established fact (50,51,52,53,54,55,56,57,58,59,60), and would not be discussed in further detail.

6.7.2 Effects of cytokines on the corticotropin-releasing hormone (CRH) secreting neurons

The previous section showed the CRH/HPA-axis to be a major target of cytokines. However, there are strong indications that ACTH and glucocorticosteroids are released secondary to activation of the CRH and CRH/ADH neurons in the paraventricular nucleus (PVN) of the hypothalamus. In other words, that the PVN CRH neurons are the primary targets of the pro-inflammatory cytokines (61,62,63). Blockade of the CRH neurons with CRH-specific antibodies not only suppresses CRH secretion, in response to cytokine administration, but also the release of ACTH and glucocorticoids. It is also obvious that ADH, as in the case of the stress response to psychological stressors (Chapter 5), acts synergistically to enhance the effect of the cytokine-induced CRH on ACTH- and on subsequent cortisol-release. It is highly feasible to expect that ADH, as in the case of psychological stressors, will further augment the CRH effects on the behavioural functions. Depending on the route of administration the pro-inflammatory cytokines would appear to stimulate CRH secretion directly, or via prostaglandins and nitric oxide (NO) or carbon monoxide (CO) as secondary signals, or even via vagal afferents (62,63). Support for the earlier statement that the pro-inflammatory cytokines exert their cerebral functions largely by their actions on the CRH and CRH/ADH producing neurons of the paraventricular nucleus can be derived from research results which show

- That antibodies against CRH and ADH, as well as blockade of pituitary CRH receptors, prevent the HPA response to pro-inflammatory cytokines (64).
- The inability of TNF-α and IL-1β to stimulate the HPA-axis in PVN-lesioned rats (65).
- The PVN up-regulation of cNOS during endotoxaemia (66).
- Other similar experimental results as reviewed by Rivier, 2001 (61), as well as examples given earlier in this chapter.

Although the CRH neurons would appear to be the primary HPA-axis targets of peripherally derived cytokines, there can be no doubt that cytokines also do some fine-tuning of the system through an influence on other structures of the HPA-axis – and indirectly through effects on the noradrenergic neurons (61).

Directly relevant to the work discussed in chapter 5, and perhaps irrefutable support for CRH as major cytokine target, is the fact that a model has been developed - mapping the pathway of interleukin-2-induced release of CRH (67,68). The model involves muscarinic cholinergic receptors, nitric oxide (NO), cyclo-oxygenase activation, prostaglandin E synthesis, adenylate cyclase activation, cAMP generation and protein kinase A activation.

There seems, at least at this stage, to be very little difference between the final effect of cognitive and non-cognitive stressors such as cytokines on the hypothalamus. Other non-cognitive immune-related phenomena such as tissue injury (which, depending on the offending agent, may lead to preferential increases in some of the cytokines) and chronic inflammatory conditions such as arthritis, are also known to stimulate the PVN CRH neurons (61). In addition, some, at present considered non-immunological, non-cognitive stimuli such as haemorrhage (69), physical stressors such as restraint (70), as well as psychological stressors (71) have, however, also been shown to induce the production of pro-inflammatory cytokines. The role of cytokines in stress-induced stimulation of the PVN CRH neurons may thus be much more pervasive than suspected.

The PVN CRH neurons, as shown in Chapter 5, are the major regulators of the peripheral expression of the stress response. Also shown in Chapter 5 was the central role of the amygdalar CRH neurons in the behavioural aspects of the stress response. As in the case of the PVN CRH neurons, the amygdalar CRH neurons would appear to be important targets of cytokines with at least part of the cytokine-induced signalling of the amygdalar CRH neurons being NO-mediated (68). The importance of cytokine signalling of amygdalar activity can, with regard to the behavioural function, not be overestimated.

In view of the central role of CRH neurons as targets for pro-inflammatory cytokines, it can indeed be said that psychoneuroimmunology, in terms of the two major stress axes, can be equated with psychoneuroimmunology in terms of the CRH system with cytokines as a major interface between non-cognitive, and perhaps even cognitive stressors, and the neurobehavioural functions.

This chapter showed the pathways through which peripheral immunological events can influence neuroendocrine and behavioural functions and by acting as stressors, induce a new adaptive mind-body homeostasis intended to facilitate the necessary physical and psychological coping responses. Examples of the immunological influence on neurobehavioural functions were discussed, as well as the cytokine associations with a number of psychiatric disorders. The major neurobehavioural effects of cytokines were described and the CRH/HPA-axis, as well as CRH neurons of the amygdala, shown to be major targets of cytokine action. The next chapter will demonstrate the practical implications of the cytokine influence on the brain and behaviour.

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