Vulnerability to cocaine: role of stress hormones

Jong, I.E.M. de

1

General introduction

OUTLINE

1. Cocaine
   1.1 Historical perspective
   1.2 Neurochemistry and actions
2. The brain dopamine system
   2.1 Biochemical aspects
   2.2 Dopaminergic pathways
      2.2.1 Mesocorticolimbic dopamine
      2.2.2 Nigrostriatal dopamine
   2.3 Dopamine receptors
3. The stress response
   3.1 The sympathetic nervous system
   3.2 The HPA-axis
4. Neurobiology of reward
   4.1 Dopamine
   4.2 Glutamate
   4.3 Neurocircuitry
   4.4 Other neurotransmitters
5. Individual differences in cocaine sensitivity
   5.1 Human studies
   5.2 Animal studies
   5.3 Genetics
   5.4 Early life events
   5.5 Later life stressors
      5.5.1 HPA-axis
      5.5.2 CRH
6. Selected inbred mouse strains
7. Incentive sensitisation: from theory to animal model
8. Scope and outline of the thesis

Cocaine, together with amphetamine, is one of the most well-characterised and widely abused psychostimulant drugs. In humans, psychostimulants increase alertness and induce a subjective sense of well being. However, with repeated exposure, these drugs produce changes in the brain that, within a vulnerable individual, may promote continued drug taking behaviour that becomes compulsive in nature and increasingly more difficult to control. Despite the powerful psychostimulant properties of cocaine, not every individual who experiments with the drug will acquire compulsive drug use. In fact, the risk of becoming cocaine dependent after occasional use of cocaine is estimated at 15-20%. Similarly, there is large variation in behavioural responsiveness of laboratory animals to psychostimulant drugs. Comparable individual differences in vulnerability exist for all known drugs of abuse and these depend on complex interactions between genes and life experiences, acting together with contextual factors such as the environment in which the drug is taken and drug availability. Especially stress, and the neuroendocrine response it evokes, has gained increasing attention as it has been demonstrated to enhance vulnerability to drugs of abuse in both humans and laboratory animals.

The research in this thesis focuses on a further analysis of factors that increase vulnerability to cocaine, with special emphasis on stress hormones. It is hypothesised that stress hormones increase vulnerability to cocaine, but that their actions are dependent on the *genetic background* of the individual and the *context* in which these hormones operate. The focus is on glucocorticoid hormones that are secreted from the adrenal glands as final step in the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoid concentrations are pharmacologically manipulated in two inbred mouse strains in order to study the interplay between *genetic background* and glucocorticoids. In addition, the *context* required for the glucocorticoid actions will be investigated in one of the two mouse strains that proves to be most susceptible to the impact of glucocorticoids on cocaine sensitivity.

In the following chapters, a summary is presented on the actions of cocaine and the neurobiology of the brain reward circuit, with emphasis on dopamine. Furthermore the neuroendocrinology of the stress response and the HPA-axis are described. Finally, individual differences in psychostimulant sensitivity are discussed with special attention for the two inbred strains used in these studies.

1. COCAINE

1.1 Historical perspective

Cocaine is a psychoactive alkaloid that is obtained from the leaves of the coca plant (*Erythroxylum coca*) indigenous to Peru, Colombia and Bolivia. From archaeological findings and studies on the presence of cocaine metabolites in mummies it has become evident that coca use dates back to as far as 2500 – 1800 BC [94,208,551]. Chewing of coca leaves was an integral part of many pre-Hispanic cultures where it was first used in religious ceremonies and celebrations by priests and members of the upper social classes. Only from the time of the Inca empire (1450-1530), the whole of the population could access coca and its psychostimulant properties started to outweigh its symbolic meaning [15,208].

When the Spanish colonised South America, physicians recognised the beneficial effects of coca on mood and energy status of the indigenous population. However, it was not until the 19th century, when cocaine was first isolated by the German scientist Friedrich Gaedcke (1855) that the drug came into focus of western medicine. Rapidly after the discovery of this new alkaloid, two important events occurred that changed cocaine use: Von Anrep recognised cocaine's analgesic properties and Sigmund Freud described its euphoric and psychomotor effects [377,669,747]. By the end of the 19th century (between 1880 and 1930) the drug was readily prescribed as remedy for all kinds of indications such as asthma, mountain- and sea sickness, pregnancy vomiting and cramping pains and it was sold in various forms including cigarettes, powder, wine and even in coca cola™ [15,669].

In contrast to Freud's earlier observation that 'Absolutely no craving for the further use of cocaine appears after the first, or even after repeated taking of the drug...' it became evident by the turn of the 20th century that cocaine does possess addictive properties and several waves of cocaine abuse were reported throughout this century, peaking in the 1980's when, in America alone, 1.6 million new cases of cocaine use were reported (SAMHSA, Office of Applied Studies, National Survey of Drug Use and Health, 2002 and 2003). The recognition of scientists that drug dependence is a chronic relapsing brain disease, characterised by lasting changes in brain chemistry and function, rather than a 'weakness of character', has paved the way for the ongoing scientific research into the neurobiology of addiction that has started only around 30 years ago (reviewed in: [441]).

1.2 Neurochemistry and actions

Psychostimulant drugs such as cocaine and amphetamine act as indirect agonists of the monoaminergic systems, including the dopamine system (described in detail in section 2). Cocaine blocks the dopamine-, norepinephrine- and serotonin re-uptake transporters (DAT, NET and SERT respectively) thereby prolonging the availability of the monoamines in the synaptic cleft [549,550]. Amphetamine not only reduces monoamine re-uptake (primarily via the vesicular monoamine transporter), but also inhibits metabolism and stimulates release of these neurotransmitters (reviewed in: [642]).

As the dopamine system in the brain is considered to play a crucial role in reward [735], the predominant hypothesis has been that the addictive properties of cocaine are related to its ability to block the DAT [372]. This 'dopamine hypothesis' has been challenged by the observation that mice lacking the DAT still experience the reinforcing effects of cocaine [567,632] and display cocaine-induced increases in extracellular dopamine in the nucleus accumbens (NAc) [62]. The complete DAT knockout may however have resulted in compensatory adaptations that alter normal functioning of the reward pathways. In a very recent report, Chen *et al*. provide compelling evidence for the role of the DAT in cocaine reward. The authors show that transgenics expressing a functional DAT that is insensitive to cocaine, do not display drug-induced increases in locomotion and NAc dopamine release or drug reinforcement [102].

Via its actions on the NET, cocaine also has profound effects on the autonomic sympathetic nervous system (described in section 3.1). The sympathomimetic effects of cocaine include increases in heart rate, blood pressure, respiration and body temperature, vasoconstriction and pupil dilation [142,524]. Cocaine use is therefore associated with a high risk of death due to cardiovascular collapse, respiratory failure, stroke and cerebral haemorrhage. Furthermore, cocaine suppresses appetite, which can lead to malnourishment.

In addition to its effects on monoaminergic transmission, cocaine is known to block sodium channels which, together with its vasoconstrictive properties, is considered to mediate the anaesthetic effects of the drug [427]. Furthermore, a recent study has demonstrated that the local anaesthetic actions of cocaine are related to increases in intracellular Ca^{2+} concentrations that may, in addition to drug-induced vasoconstriction, contribute to the neurotoxic effects of cocaine [185].

2. THE BRAIN DOPAMINE SYSTEM

2.1 Biochemical aspects

Dopamine is a monoaminergic neurotransmitter belonging to the class of catecholamines that also includes norepinephrine and epinephrine. All catecholamines are synthesised from phenylalanine via a cascade of enzymatic reactions, the end product being determined by the number of steps (figure 1). The rate-limiting step in the synthesis of dopamine is conversion of tyrosine to dihydroxyphenylalanine (DOPA) by the tyrosine hydroxylase (TH) enzyme. Catecholamines are stored in

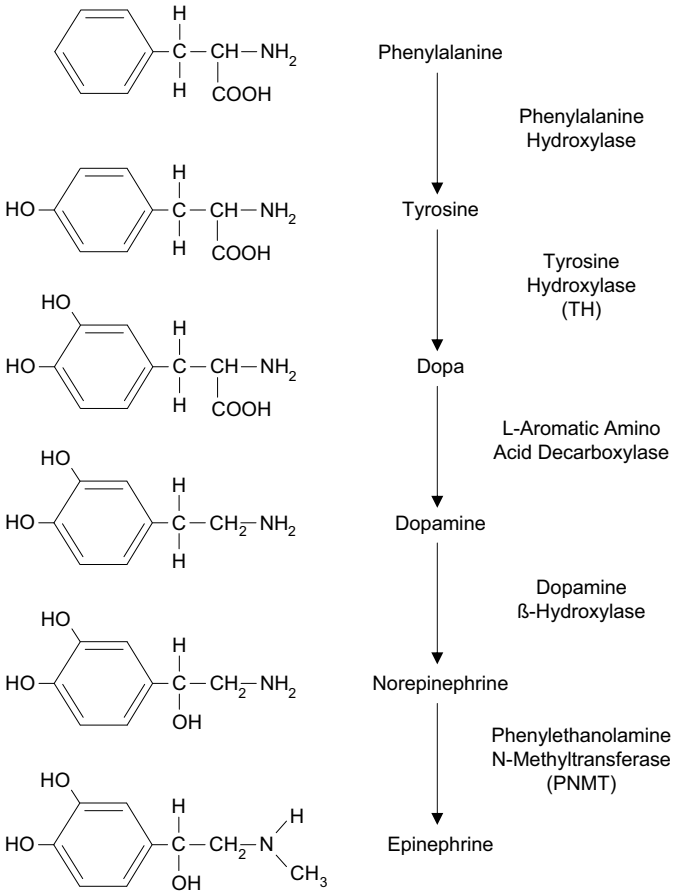

Figure 1: *Biosynthesis cascade of the catecholamines dopamine, norepinephrine and epinephrine.*
Arrows indicate an enzymatic conversion. The rate-limiting enzyme in the cascade is tyrosine hydroxylase (TH).

vesicles in the presynaptic terminals and are, upon neuronal depolarisation, released by exocytosis into the synaptic cleft where they can bind to receptors on post-synaptic nerve terminals. Within the intra- and extracellular space, catecholamines are subject to enzymatic degradation by catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO) resulting in formation of the two principal metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC). In addition, catecholamine transporters such as the dopamine transporter (DAT) and the norepinephrine transporter (NET) reabsorb the catecholamine into the presynaptic terminal where it can either be stored in vesicles or degraded.

2.2 Dopaminergic pathways

Dopaminergic neurons are widely distributed throughout the brain, the three major circuits being the nigrostriatal, mesocorticolimbic and tuberohypophysial pathways [138].

Dopaminergic neurons of the *tuberohypophysial* pathway are located in the arcuate nucleus of the hypothalamus and suppress prolactin and α-melanocyte-stimulating hormone (αMSH) secretion in the pituitary [224]. These actions are outside the scope of this thesis and will not be further discussed.

The *nigrostriatal* and *mesocorticolimbic* dopamine systems are both anatomically and functionally intertwined [271] (see figures 2 and 4). A central component of both dopaminergic circuits is the striatal complex, consisting of the caudate nucleus and putamen (together referred to as the caudate putamen: CP, or dorsal striatum) and the nucleus accumbens (NAc, together with portions of the olfactory tubercle referred to as ventral striatum). The NAc can be further divided into 'core' and 'shell' subregions [263,752], the former resembling more closely the CP while the latter is considered an integral part of the mesocorticolimbic tract.

The striatal complex receives input from the neocortex and relays information via the globus pallidus, subthalamic nucleus and substantia nigra pars reticulata (SNr) to the thalamus and ultimately the cerebral cortex, thereby completing the cortico-striato-thalamo-cortical loop (reviewed in: [7]). The cortex, thalamus and limbic structures such as the hippocampus and amygdala provide the striatum with cognitive, sensory and emotional input, via predominantly excitatory (glutamatergic) afferents. By contrast, the two major striatal output pathways consist of GABA-ergic projections to the globus pallidus and the substantia nigra pars reticulata (SNr). Furthermore, there are reciprocal GABA-ergic connections between the ventral tegmental area (VTA) and the NAc shell [325,680]. The striatal complex contains a large population of cholinergic interneurons and a high concentration of neuropeptides

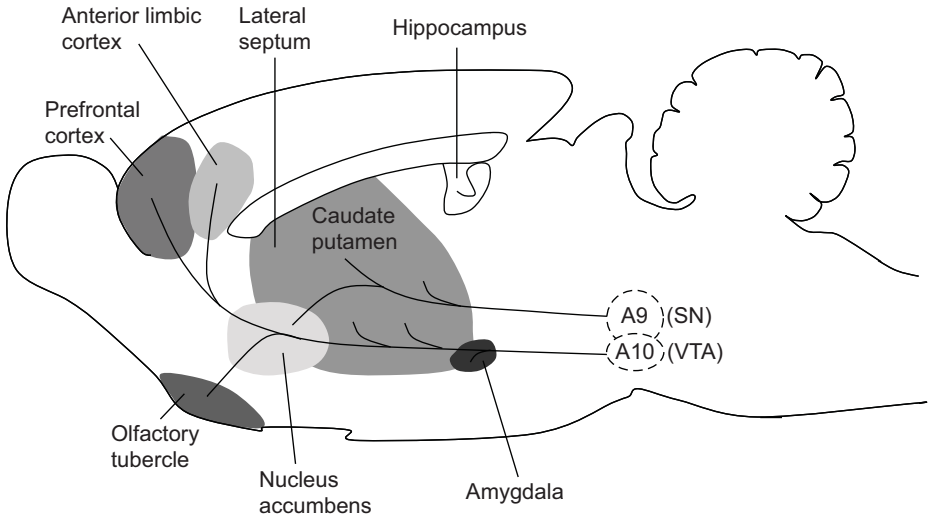

Figure 2: *Schematic representation of the mesocorticolimbic and nigrostriatal dopamine projections in the mouse brain.*
Midsagittal section showing the location of the A9 and A10 cell groups and the projection areas of the nigrostriatal and mesocorticolimbic neurons, respectively. SN: substantia nigra, VTA: ventral tegmental area.

such as enkephalin, dynorphin, substance P, somatostatin, neuropeptide Y and cholecystokinin (reviewed in: [270]). The dense dopaminergic innervation of the striatal complex originates from three nuclei in the ventral mesencephalon (A8-10). Based on the origin of this dopaminergic innervation and functional studies, a rough distinction has been made between the *nigrostriatal* and *mesocorticolimbic* dopaminergic pathways. It should be noted that this is an oversimplification since there are many reciprocal connections between the two systems.

2.2.1 Mesocorticolimbic dopamine

Cell bodies of the *mesocorticolimbic* dopamine pathway are localised in the ventral tegmental area (VTA, A10 cell group) and project to limbic regions including the NAc shell, limbic cortex (prefrontal- cingulate- and entorhinal cortices), amygdala, lateral septum, bed nucleus of the stria terminalis, ventral pallidum (VP, ventral analogue of the globus pallidus) and the olfactory tubercle (figure 2).

The mesocorticolimbic dopaminergic pathway plays an essential role in regulation of reward, motivation and goal-directed behaviours (reviewed in: [733]). The dopaminergic projection from the VTA to the NAc forms a neural substrate underlying the reinforcing properties of natural rewards such as food, water and sex [25,89,342], psychological rewards [42,356,457] and drugs of abuse (see section 4.1). Furthermore,

the mesocorticolimbic pathway has been implicated as the principal dopaminergic pathway involved in the aetiology of psychoses [239,331] and has, together with the nigrostriatal system, been associated with the pathology of attention-deficit hyperactivity disorder [631].

2.2.2 Nigrostriatal dopamine

The *nigrostriatal* system consists of dopaminergic neurons that originate in the substantia nigra (SN, A9 cell group) and project to the CP and the NAc core subregion (figure 2). The SN can be subdivided in a pars compacta (SNc) and a pars reticulata (SNr), the former containing the cell bodies of the nigrostriatal dopaminergic neurons, whereas the latter contains the GABA-ergic neurons that form one of the two major output pathways of the striatal complex to the motor thalamus (the other being via the globus pallidus).

The nigrostriatal dopaminergic pathway has traditionally been implicated in motor control, e.g. regulation of voluntary movement and stereotyped behaviours [259,452]. Loss of nigrostriatal dopaminergic neurons is the main pathological feature of Parkinson's disease and antipsychotic drugs that antagonise dopamine receptors have considerable extrapyramidal side effects (tardive dyskinesia) due to their actions within the nigrostriatal pathway [260,546]. Conversely, stereotyped behaviours produced by increasing doses of psychostimulants such as cocaine and amphetamine have been linked to the activating effects of these drugs on striatal dopamine [345]. More recent studies have indicated that the dorsal striatum plays a role in learning and memory and, more specifically, stimulus-response (habit) learning [489,749].

2.3 Dopamine receptors

Already in 1979, it was recognised that dopamine can bind to two types of G-protein coupled receptors that either inhibit or stimulate adenylate cyclase and can be distinguished on the basis of their pharmacological and biochemical properties [340]. Indeed, at the end of the 1980s, the D2 receptor was the first to be cloned [64] followed within two years by the D1 receptor [159,462,646,757]. The discovery of three additional receptors (D3, D4 and D5), two splice variants of the D2 receptor ('short' and 'long') and genetic polymorphisms in the D4 receptor has made the pharmacology of dopamine increasingly more complex, while at the same time providing new opportunities for more specific therapeutics [107,139,238,258,463,630,645,659,689,690,727]. Based on G-protein coupling, pharmacology, genomic organisation and central nervous system (CNS) distribution, the receptors were classified into two families: the D1 family, consisting of the D1 and D5 receptors, and the D2 family including the D2, D3 and D4 receptors (for a review see: [309]). Receptors of the D1 family are coupled

to Gs proteins and activate adenylate cyclase to produce cAMP, whereas the D2 receptors inhibit cAMP production via Gi proteins (reviewed in: [311]).

While the D1 receptor is the most abundant dopamine receptor in the CNS, D1 and D2 receptors are present in all dopaminoceptive brain regions, including the CP, NAc, olfactory tubercle and prefrontal cortex (PFC). Both receptors are detected in the septum, hippocampus, hypothalamus and thalamus, but expression levels vary considerably per receptor and per subregion [222,311,726]. Furthermore, the D1 receptor is extensively expressed in the amygdala [726]. In contrast to the D1 receptor, the D2 receptor is present on the dopaminergic neurons in the VTA and SNc and, upon activation, functions as autoreceptor that inhibits neuronal activity [442,726]. Some brain regions, including the SNr, have numerous binding sites for the D1 receptor but do not express D1 mRNA, suggesting that, in these areas, the D1 receptor is present in afferent projections only. Although co-localisation of D1 and D2 receptor mRNAs has been demonstrated in a considerable percentage of striatal neurons [385], there is also a clear segregation into two neuronal populations: D1 receptors are expressed predominantly on neurons that contain substance P and project to the dopaminergic cell bodies in the VTA and SNc, whereas D2 receptors are preferentially found on neurons that co-express enkephalin and project to the VP [233,375,407].

The D3, D4 and D5 receptors are much less abundantly expressed than the D1 and D2 receptors. The D3 receptor has an interesting distribution pattern as it is expressed in regions receiving dopaminergic innervation from the VTA such as the shell of the NAc, the bed nucleus of the stria terminalis, the olfactory tubercle and the islands of Calleja and also in limbic regions including the hippocampus and the amygdala. Low densities of the D3 receptor have also been found in the CP. The presence of D3 in the SNc, and to a lesser extent in the VTA, suggests that this receptor can function as dopaminergic autoreceptor [57,177,178]. The D4 receptor is also associated with the limbic regions such as the NAc shell, amygdala, frontal cortex and hypothalamus and low levels are detected in the CP [480,649,689] although it is considerably less abundant than the D2 and D3 receptors. By contrast, D5 receptor expression is restricted to few brain regions including the hippocampus, mammillary nuclei and the parafascicular nucleus of the thalamus [443,659]. More recently, the presence and functional importance of the D5 receptor in the NAc has been demonstrated [211].

3. THE STRESS RESPONSE

The term ‘stress’ is frequently used to describe the negative emotional state experienced when a person perceives that the demands (e.g. resulting from work or social

engagements) exceed the resources the individual is able to mobilise. Therefore, stress has a negative connotation, as it is associated with a reduced feeling of well being and, after prolonged periods, with the development of stress-related disease (e.g. anxiety and depression). In the present society most, if not all, individuals feel that they have experienced stress, however there is not a clear-cut definition for this phenomenon.

From the scientific point of view however, the stress response is part of an organisms natural defence mechanism to demanding situations. The internal environment of all living organisms is regulated in such a way that a dynamic equilibrium, called homeostasis, is maintained. Changes in the internal or external environment that threaten homeostasis (stressors) turn on a spectrum of physiological and behavioural responses aimed at restoring homeostatic balance [146]. This process of adaptation is also known as 'allostasis', meaning 'achieving stability through change'. However, when exposed to stressful situations repeatedly, or when the allostatic mechanisms remain activated when no longer needed, the price that has to be paid for maintaining stability (allostatic load) may become too high, resulting in the development of stress-related pathology [433]. The concept of allostasis is however a matter of debate as, in contrast to a physiological adaptive process as proposed by McEwen, it has also been suggested to represent a pathological maladaptive process (see e.g. Koob and Le Moal [361,433]). In 1936, Selye first defined the concept of stress as 'the non-specific response of the body to any demand' [615]. Non-specific indicates that the stress response is comprised of a fixed set of neuroendocrine adaptations, irrespective of the nature of the stressor. These include activation of the autonomic sympathetic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (figure 3), which are described in detail in the following sections. Nowadays, it is known that the degree to which the neuroendocrine cascades are activated depends on the severity of the stressor but can also show considerable individual variation due to genetic background and life history [146].

3.1 The sympathetic nervous system

Activation of the autonomic sympathetic nervous system (ANS), culminating in the release of the catecholamines norepinephrine and epinephrine into the general circulation, is the first and most rapid aspect of the stress response (figure 3). Norepinephrine is released from the post-ganglionic sympathetic nerve terminals throughout the body and exerts local control over autonomic effector organs, whereas epinephrine is secreted from the medulla of the adrenal glands and acts as a humoral messenger that can provide additional autonomic stimulation.

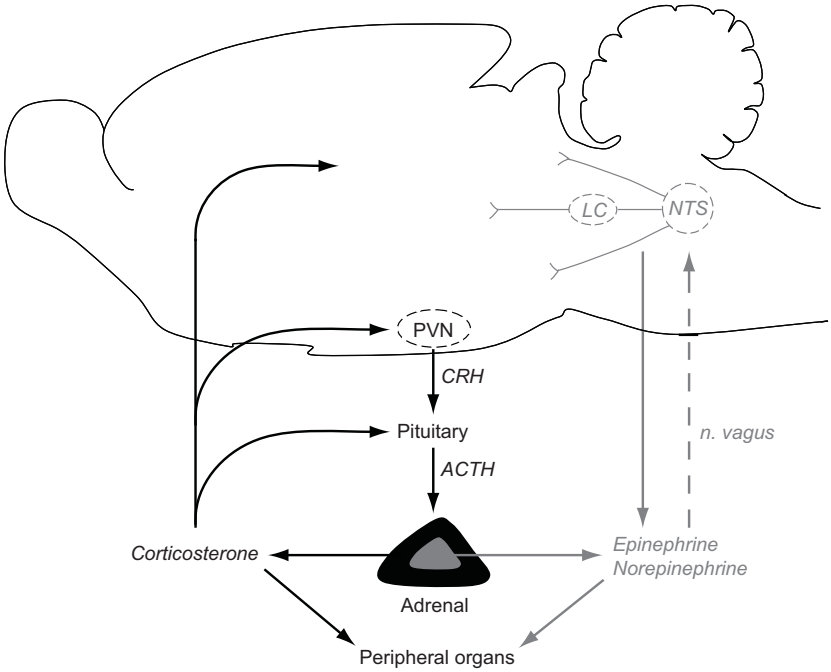

Figure 3: *Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic sympathetic nervous system (ANS).*
Black arrows indicate the HPA-axis, grey arrows the ANS. Exposure to stress leads to the release of corticotrophin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus. This, in turn, induces secretion of adrenocorticotrophic hormone (ACTH) from the pituitary, which results in the release of corticosterone from the adrenal cortex. Via GRs in the hypothalamus and pituitary, corticosteroids exert a negative feedback action, thereby reducing the enhanced HPA-activity. In addition, corticosteroids can affect brain function in many regions. Exposure to a stressor also results in rapid release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla and sympathetic nerve terminals that can, indirectly via the vagal nerve (n. vagus), solitary tract nucleus (NTS) and locus coeruleus (LC), lead to release of norepinephrine in the brain.

Together with the parasympathetic nervous system, the sympathetic nervous system forms the autonomic nervous system that innervates the skin and all visceral organs. Whereas the parasympathetic component is involved in maintaining the vegetative (resting) state of the body, the sympathetic component regulates processes related to a more active state of the body and increases energy expenditure. Furthermore, the sympathetic nervous system is involved in maintaining a constant internal environment regarding blood pressure, blood glucose and oxygen availability. Activation of the ANS enables an organism to respond to changes immediately. The famous

concept of 'fight or flight', proposed by Cannon in 1911, indicates that arousal in response to a perceived threat involves several elements which prepare the body physiologically either to take a stand and fight off an attacker, or to flee from the danger [86]. These 'elements' comprise increases in heart rate, blood pressure and respiration, more acute hearing and vision and transportation of blood from the extremities to the large muscles and the brain.

Epinephrine and norepinephrine exert their effects via two types of receptors belonging to the adrenoreceptor family: α and β, which can be further subdivided in α1, α2, β1 and β2. Depending on the receptors present and the interaction with the cholinergic system, smooth muscles are either contracted or relaxed and cellular secretion is stimulated or inhibited. Furthermore, the adrenoreceptors are present in the brain, where they mediate central noradrenergic neurotransmission [261,672-674]. Noradrenergic projections in the brain arise from the locus coeruleus (LC, A6 cell group) and the lateral tegmental group (consisting of the A1, A2 (nucleus of the solitary tract, NTS), A5 and A7 cell groups) and innervate many brain regions including the thalamus, hypothalamus, hippocampus, amygdala, cerebral cortex, and midbrain [324,482]. The CNS adrenergic receptors are however not a direct target for the peripheral catecholamines, as these are not likely to pass the blood-brain-barrier due to their hydrophilic structure [725].

3.2 The HPA-axis

Activation of the endocrine cascade between the hypothalamus, pituitary and adrenal glands (HPA-axis) comprises a second aspect of the stress response (figure 3) [146]. Exposure to a stressor rapidly induces the parvocellular neurons in the paraventricular nucleus of the hypothalamus (PVN) to secrete corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) into the portal vessel system, the vascular link between the hypothalamus and the anterior pituitary. CRH stimulates the corticotroph cells of the anterior pituitary to produce adrenocorticotrophic hormone (ACTH) from its precursor pro-opiomelanocortin (POMC) and to release the hormone into the general circulation. Whereas CRH is the primary trigger for ACTH production and release, AVP is believed to amplify the CRH effect. ACTH travels via the general circulation to the adrenals where it stimulates production of glucocorticoid hormones in the cortical layer of the gland. The principal glucocorticoid in humans is cortisol, whereas rodents including rats and mice have corticosterone.

In addition to stress-induced activation, the HPA-axis follows circadian rhythmicity that is controlled by the suprachiasmatic nucleus [63], resulting in peak concentrations of plasma glucocorticoids around the start of the active period. Furthermore, an

ultradian rhythm of corticosteroid release, with intervals of less than 24 hours, has been demonstrated in a variety of species [217,601,732]. The HPA-axis is also powerfully activated by psychostimulant drugs such as cocaine and amphetamine in both humans and laboratory rodents [31,281,355,448,461,590]. Both drugs stimulate secretion of hypothalamic CRH, which is mediated by multiple neurotransmitter systems, including catecholaminergic (dopaminergic, noradrenergic), glutamatergic, opiate, serotonergic and cholinergic systems [52,140,596].

Glucocorticoid hormones enable an organism to respond and adapt to a stressor and prepare for a subsequent event. Glucocorticoid actions can be considered indirect: depending on timing, context and endpoint these hormones either facilitate or attenuate physiological or behavioural outcomes. In the words of Robert Sapolsky: 'glucocorticoids can permit, suppress or stimulate an ongoing stress response and, in addition, prepare an organism for subsequent stressors' [591]. In short, glucocorticoids permit sympathoadrenal activity and cardiovascular activation, direct metabolism towards mobilisation of energy stores, have potent anti-inflammatory and immunosuppressive properties, inhibit reproduction and, as they can cross the blood-brain-barrier, have profound effects on brain function and behaviour.

The lipophilic glucocorticoid hormones readily pass the cell membrane and can bind to two types of intracellular receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) [147]. As the name indicates, the MR was originally identified as the receptor for a mineralocorticoid hormone aldosterone that is also produced in the adrenal cortex and primarily regulates salt and water balance in the kidney. Whereas in the kidney metabolic inactivation of glucocorticoids by 11β-hydroxysteroid dehydrogenase prevents these hormones from binding MR [189,223], in the brain activity of this enzyme is low and glucocorticoids can readily activate both MR and GR [116,374,541]. In fact, the MR has a 10-fold higher affinity for glucocorticoids than GR [147]. Therefore, brain MR is almost fully saturated at low circulating levels of glucocorticoids, during the circadian trough, whereas the GR becomes occupied only at increasing levels of the adrenal steroids, during stress or at the circadian peak [541]. Additional factors that determine uptake of glucocorticoids in the brain and other target tissues include the efflux transporter p-glycoprotein that is present at the blood-brain-barrier [336] and corticosteroid binding globulin, a plasma protein that binds circulating endogenous glucocorticoids [276].

Another striking difference between the two receptor types is their localisation in the brain. GR has a relatively widespread distribution pattern with highest concentrations in brain regions involved in HPA-axis regulation such as the PVN and the hippocampus. By contrast, MR expression is more restricted to the limbic regions

such as the hippocampus, amygdala, septum and low levels are also detectable in the CP [4,98,494,541,636,686].

The differences in receptor affinities and distribution have led to the hypothesis that the MR and GR mediate different aspects of glucocorticoid signalling. Whereas MR is suggested to regulate maintenance of HPA-axis activity and the threshold of the system to stress ('proactive mode'), GR is proposed to mediate steroid control of recovery from stress ('reactive mode') [148,149]. The most striking example of the reactive mode is the negative feedback exerted by glucocorticoids on HPA-axis activity itself, by binding to GR in the PVN and the pituitary [148,341]. In brain regions where both receptor types are co-localised, such as the hippocampus, the outcome of glucocorticoid action critically depends on the balance between MR and GR activation [317,481,685]. Furthermore, MR and GR may, depending on the context, act synergistically or antagonistically.

MR and GR belong to the superfamily of nuclear receptors that regulate gene transcription. Upon binding of a ligand in the cytosol, the receptor dissociates from a protein complex and translocates into the nucleus. The activated receptors form dimers and bind to specific glucocorticoid response elements (GREs) in the promotor areas of genes, where they recruit transcriptional machinery and activate transcription. The fact that the steroid receptors not only form homodimers (GR/GR) but also heterodimers (GR/MR) adds another level of functional diversity to corticosteroid action [668]. Furthermore, in addition to transactivation, glucocorticoids can also induce transrepression. This mechanism can involve direct protein-protein interactions of monomeric receptors with other transcription factors, the most well known being NFκB and AP-1 [603,723]. The existence of negative GREs, mediating transrepression rather than transactivation, has also been described for the promotors of the CRH and COMT genes [184,413]. Furthermore, there is considerable diversity in the stochiometry of the transcriptional co-regulator proteins that are thought to determine the magnitude and nature of the steroid response [446]. Interestingly, it has recently been demonstrated that glucocorticoids exert their actions not only via nuclear receptor-mediated transcriptional regulation, but also via a non-genomic mechanism involving membrane-bound receptors and requiring a considerably shorter time span [55,103,176,338]. Indeed, evidence is now accumulating that adrenal glucocorticoids regulate a wide range of behaviours via a rapid non-genomic mechanism (see e.g. [339,450,451,584,589]).

Taken together, the actions of glucocorticoids are highly context-dependent. Factors such as timing (allowing genomic vs. non-genomic actions), cellular context (e.g. target tissue, presence of receptor types, transcription factors and co-regulator

proteins), organismal context (e.g. genetics, current neuroendocrine status) and endpoint determine whether the glucocorticoids are stimulatory, inhibitory or even without effect.

4. NEUROBIOLOGY OF REWARD

4.1 Dopamine

The mesocorticolimbic dopamine system plays a critical role in the behavioural and reinforcing effects of all classes of abused drugs. In the following paragraphs, the focus is on psychostimulants. The evidence presented is based on studies using the self-administration paradigm that, to date, is the most representative animal model for aspects of human drug abuse. Some methodological considerations regarding this model are described in box 1.

Psychostimulants such as cocaine and amphetamine increase extracellular dopamine concentrations in the NAc to a greater extent than in the CP [170], an effect which is most pronounced in the NAc shell subregion [526]. It was demonstrated with selective lesions that drug-induced dopamine release in the NAc, but not the dorsal striatum, is critical for the locomotor stimulant effect of cocaine and amphetamine [344,345], which is considered to have predictive value for the reinforcing properties of these drugs [733]. During cocaine and amphetamine self-administration, extracellular dopamine levels in the NAc are tonically elevated [171,503] and fluctuate with phasic increases just after, and phasic decreases just before, infusions [234,537,734]. These observations have led to the hypothesis that animals self-administer the drugs to compensate for the falling concentrations of dopamine.

Cocaine and amphetamine are self-administered directly into the NAc [104,440,506,569], primarily in the shell subregion [569], and dopamine-selective lesions in the VTA or NAc attenuate maintenance of psychostimulant self-administration [79,409,502,556,557], whereas their effects on initiation of this behaviour are more controversial [235,409]. By contrast, destruction of noradrenergic or serotonergic neurons does not influence psychostimulant self-administration [213,556]. Paradoxically, whereas lesions attenuate self-administration, dopamine D1 and D2 receptor antagonists administered systemically or directly into the VTA, NAc or amygdala, enhance the rate of psychostimulant-maintained self-administration [8,78,299,301,411,439,506,538,750] while reducing the motivation to obtain the drug under a progressive ratio schedule [28,299,439,538]. The increase in drug intake is thought to reflect a decrease in the magnitude of the reinforcer, which is in agreement with the reduced motivation to obtain cocaine.

Box 1: *The self-administration model.*

To date, the most representative animal model for human substance abuse is the self-administration model, which has been developed for both laboratory rodents and non-human primates. In brief, animals are equipped with intravenous or intracerebral catheters and, upon *voluntary* performance of an instrumental response (e.g. to push a lever, or to poke the nose in a designated hole), earn a drug infusion. Different stages of the paradigm and variations therein allow for distinct features of drug addiction to be modelled.

In the acquisition phase, animals obtain a drug infusion by performing a fixed number of responses, the so-called 'fixed ratio schedule'. Acquisition of self-administration is therefore a measure of the reinforcing- or abuse potential of the drug (the extent to which it facilitates the acquisition of an instrumental response required to obtain it). Furthermore, during this phase, individual differences in sensitivity to the reinforcing effects of drugs can be distinguished.

Once self-administration is acquired, animals maintain stable responding that may be subject to secondary reinforcers: environmental stimuli that are associated with the drug (primary reinforcer). Different aspects of drug taking can be studied during the maintenance phase: i) Dose-response relationships: shifts in the typical inverted U-shaped dose-response curve provide another measure for individual differences in vulnerability to the drug reward [513], ii) The motivation to obtain the drug: under a progressive ratio schedule the effort (number of instrumental responses) required to obtain a drug infusion is progressively, and most often exponentially, increased. The 'break-point' at which an animal stops responding is an index of its motivation to work for a drug infusion, and iii) Continuance despite negative consequences: persistence of drug seeking, even when drug infusion is coupled to an adverse conditioned stimulus such as foot-shock [167,692].

During extinction, the drug-paired instrumental response is no longer reinforced by drug infusion. At this stage, the persistence of responding (thus fruitless drug seeking) provides a measure for the motivation to obtain the drug and may represent a certain degree of 'difficulty in limiting intake' [167]. Furthermore, during the extinction phase, there may be physical withdrawal symptoms, the nature of which varies across classes of abused drugs. Finally, reinstatement of drug seeking can be induced in abstinent animals by a priming injection of the drug itself, presentation of drug-associated cues that have acquired incentive motivational properties, or stressful events.

Thus, depending on the design, the self-administration model allows for several features of human drug abuse to be modelled, including i) extreme motivation to obtain the drug, ii) difficulty in limiting intake, iii) continuance despite negative consequences, iv) withdrawal, and v) high propensity for relapse. Deroche-Gamonet *et al.* recently scored outbred rats for multiple 'addiction-like' behaviours (i, ii and iii) in a cocaine self-administration paradigm and found that only a subset of rats (17%) shows a high score for all three characteristics, which is in good agreement with the risk of becoming cocaine dependent after single use of the drug in humans (15-20%) [167].

Cocaine, but not amphetamine, is also self-administered directly into the medial prefrontal cortex (mPFC) and this is critically dependent on the dopaminergic innervation of this brain region [242,247,248]. Ablation of dopamine in the mPFC enhances acquisition and maintenance of intravenous cocaine self-administration at low

doses of the drug and also the motivation for cocaine self-administration under a progressive ratio schedule [438,605]. Other studies have however not found effects of dopaminergic lesions in the mPFC on psychostimulant self-administration [379,425], which may be due to variations in the extent of the lesions.

Finally, dopamine also plays a prominent role in reinstatement of cocaine seeking, induced by re-exposure to the drug itself, drug-associated cues or stressors [9,11,12,38,88,115,152,227,351,352,434,435,604,612,613,644,740]. Whereas dopamine in the medial and dorsal PFC plays a role in cocaine- and stress-induced reinstatement [88,434,435,644], dopamine in the basolateral and central nuclei of the amygdala contributes to cue- and drug-induced reinstatement [8,38,612]. Interestingly, in addition to the D1 and D2 receptors, there appears to be a prominent role for the D3 receptor in all types of reinstatement of cocaine seeking [97,227,523,712,740,741].

In summary, there is convincing evidence for the role of the mesocorticolimbic dopamine system in psychostimulant reward. In addition, in accordance with the notion that the dorsal striatum plays a role in stimulus-response (habit) learning [489,749], it has been demonstrated that the nigrostriatal dopaminergic pathway is involved in established, or habitual, cocaine-seeking behaviour in both humans and laboratory rodents [307,697,711].

4.2 Glutamate

It has become increasingly evident that in addition to dopamine, glutamate plays an essential role in drug reward and reinforcement. Dopaminergic neurons in the NAc and VTA receive extensive glutamatergic input from the PFC, amygdala and hippocampus [48,112,215,256,264,343,654]. Cocaine and amphetamine both stimulate glutamate release in the PFC and NAc [540] which is potentiated with repeated exposure [539]. Glutamate enhances dopaminergic transmission by increasing activity of the dopaminergic neurons in the VTA, and facilitating dopamine release from the presynaptic terminals in the NAc [48,215,335,654]. Many of the actions of the excitatory transmitter rely on this stimulatory interaction with the dopamine system. For example, basal and psychostimulant-induced locomotion, which are critically dependent on dopamine, are stimulated and inhibited by glutamatergic agonists and antagonists, respectively [534,650]. However, as described below, some of the actions of the excitatory transmitter in psychostimulant reinforcement are independent of dopamine.

Glutamate acts via two classes of receptors: ionotropic (ligand-gated ion channels) and metabotropic (G-protein coupled) receptors, each consisting of multiple

subtypes that may, depending on localisation and function, have distinct roles in drug reinforcement. During maintenance of cocaine self-administration, stimulation of ionotropic glutamate receptors in the NAc causes a leftward shift in the dose-response curve, whereas antagonism of these receptors is ineffective [128]. The authors argue that signalling via ionotropic receptors in the NAc enhances cocaine reward whereas it is not required for maintenance of cocaine self-administration. Conversely, blockade of the metabotropic glutamate receptor 5 (mGluR5) reduces cocaine-maintained self-administration and the motivation to obtain the drug under a progressive ratio schedule, while elevating reward thresholds for intracranial self-stimulation [350,380,495,658]. Furthermore, mice lacking the mGluR5 do not self-administer cocaine and do not show increased locomotion after cocaine administration, despite the fact that dopamine function is comparable to that of wild-type mice [105]. These studies point to an important role for mGluR5 in cocaine reward, which may be independent of dopamine transmission.

Glutamatergic transmission via ionotropic and metabotropic receptors modulates drug- and cue-induced reinstatement of cocaine seeking which is in good agreement with the role of the PFC and amygdala in relapse to drug seeking [19,27,128,380]. In fact, the glutamatergic pathway from the PFC to the NAc, and in particular the core subregion, plays a critical role in cocaine-primed reinstatement of drug seeking [129,436,492,501] which may also involve the excitatory innervation of the VTA [643]. Furthermore, it was demonstrated by Cornish *et al.*, that glutamatergic, but not dopaminergic, transmission in the NAc is necessary for cocaine-induced reinstatement of drug seeking [129]. Excitatory transmission in the NAc also contributes to cue-controlled cocaine-seeking [172] and cocaine-associated cues increase glutamate release in the NAc [298]. Similarly, accumbal glutamate release is enhanced during cocaine- and stress-induced reinstatement [434,436].

Perhaps the most striking evidence for the importance of glutamate in addiction processes, comes form the observation that many of the enduring neuroplastic changes associated with repeated psychostimulant (self-)administration involve glutamatergic transmission [56,90,283,328,329,402,403,657,742,746]. Of particular interest is the synaptic plasticity that occurs in reward-related brain regions. It was demonstrated that a single *in vivo* cocaine exposure induces neuronal plasticity of AMPA-mediated currents at excitatory synapses onto dopamine cells in the VTA [676]. Furthermore, the structural plasticity in the NAc core and mPFC associated with cocaine-induced behavioural sensitisation, is localised to portions of the dendritic tree that might contain dopamine/glutamate synapses [392].

4.3 Neurocircuitry

When integrating the evidence described in the previous paragraphs, the neurocircuitry that mediates reward and translates biologically relevant stimuli into adaptive behavioural responses, also termed the 'motive circuit' can be envisaged as consisting of a network of several brain regions that communicate via multiple neurotransmitters. Similarly, neuroimaging studies in humans have indicated that cocaine craving is associated with the activation of several brain regions, including the PFC, amygdala, hippocampus and the striatal complex [106,229,353]. A simplified representation of the motive circuit is shown in figure 4 (for a review see: [328]).

In line with the classical 'dopamine hypothesis' of addiction, the mesocorticolimbic dopaminergic projection from the VTA to the NAc, PFC and amygdala forms the core of the motive circuit. The PFC (prelimbic, anterior cingulate and ventral orbital

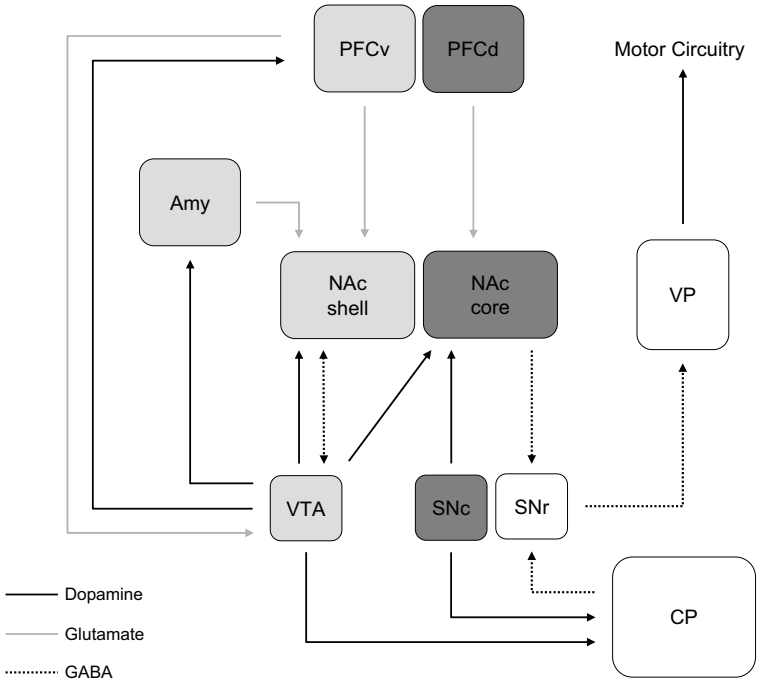

Figure 4: *Schematic drawing of the 'motive circuit'.*
Arrows indicate neuronal connections that are either dopaminergic (black), glutamatergic (grey) or GABA-ergic (dashed). Rectangles with similar colour indicate a sub-circuitry in the motive circuit. PFC v/d: prefrontal cortex ventral/dorsal subdivisions, NAc: nucleus accumbens, VTA: ventral tegmental area, SN c/r: substantia nigra pars compacta/reticulata, Amy: amygdala, CP: caudate putamen, VP: ventral pallidum.

regions) and amygdala in turn, send glutamatergic afferent projections to the VTA and the NAc. The PFC provides the subcortical dopamine systems with cognitive input and is involved in anticipation/predictability of reward [656,671], whereas the amygdala is involved in establishing learned associations between motivationally relevant events and otherwise neutral stimuli (cues) that become predictors of the event [201]. Two subcircuits can be distinguished, with the NAc shell being more closely connected with the VTA, the ventral PFC, the amygdala and the medial VP. By contrast the NAc core is more tightly associated with the dorsal PFC, the dorsolateral VP and the SNc [328,754]. Dopaminergic signalling within the NAc shell subcircuit is critically involved in the reinforcing properties of psychostimulants as well as in establishment of self-administration and behavioural sensitisation. However, it has been proposed that glutamatergic transmission, and in particular the subcircuitry involving the dorsal PFC and the NAc core, mediates expression of these behaviours when they have become more compulsive and habitual, such as during reinstatement of drug seeking [329]. Indeed, the PFC and the NAc mediate cocaine-, stress- and cue-induced reinstatement [88,434,435], whereas the basolateral and central nuclei of the amygdala play a prominent role in cue- and stress-induced relapse to cocaine seeking respectively [330,370,618]. In addition, it was recently demonstrated that the dorsal striatum is critical for cue-controlled cocaine seeking [697]. Despite the prominent role for glutamate in expression of addictive behaviours, the mesocorticolimbic dopaminergic projection remains compulsory, although in more advanced stages dopamine release in the PFC and amygdala, rather than in the NAc, may be required [88,434,612].

4.4 Other neurotransmitters

In addition to dopamine and glutamate, other neurotransmitters including gamma-aminobutyric acid (GABA) [61,554], norepinephrine [143,183,193,384], serotonin [209,467], acetylcholine [629], endogenous opioids [688] and endocannabinoids [412] are involved in reward processes. It is beyond the scope of this thesis to describe their involvement in detail. Of particular interest are GABA, because of its role in connectivity and output of the nuclei of the motive circuit (see section 2.2 and figure 4), and serotonin and norepinephrine because of the actions of cocaine on the SERT and NET respectively. Whereas GABA appears to have an overall inhibitory role in psychostimulant reinforcement [59,60,85,173,180,204,278,555,625], the roles of serotonin and norepinephrine are more controversial. Serotonin can facilitate or suppress psychostimulant reinforcement, depending on the receptor subtypes and brain regions involved (for reviews see: [209,467]). However, studies using amphetamine- and cocaine-like analogues with varying serotonin releasing potency, have demonstrated that the serotonergic

activity of these compounds is inversely related to their reinforcing potential [558,721]. In agreement with this, enhancement of serotonergic transmission attenuates cocaine self-administration [93,500]. Controversy exists regarding the role of the noradrenergic system. Several studies did not find support for the involvement of norepinephrine in psychostimulant reinforcement, whereas others point to a facilitatory role [143,183,548,556,602,722,739]. There is however strong evidence for the involvement of the noradrenergic system in stress-induced reinstatement of cocaine, ethanol and morphine seeking [193,376,384,620].

5. INDIVIDUAL DIFFERENCES IN COCAINE SENSITIVITY

5.1 Human studies

Despite the fact that cocaine is a highly addictive substance and large numbers of people experiment with it for variable periods of time, not every individual who tries the drug once will become an addict [478]. The same holds true for all other addictive drugs. Studies on the *incidence* of cocaine dependence, making use of large populations in America, have indicated that the risk of becoming dependent on cocaine within 1-2 years after the first use of the drug is between 5 and 6% [479,715]. The risk of cocaine dependence increases with time, being 15-16% after 10 years but reaches a plateau of around 20% after 20 years [715]. Table 1 shows the *prevalences* (during lifetime or recent: in the month prior to data collection) of the use of several addictive drugs in The Netherlands. The ratio between recent and lifetime use will give a crude indication of the percentage of problem users, although these data are confounded by first-time usage during the year prior to data collection. Despite the fact that the drugs differ considerably in prevalences and ratio, they share the common feature that only a relatively small percentage of lifetime users progresses

Table 1: *Drug use in the Dutch population (>12 years old) in 2001(%).*

	Lifetime	Recent*	Recent / Lifetime
Cocaine	2.9	0.4	13.8
Ecstacy	2.9	0.5	17.2
Amphetamine	2.6	0.2	7.7
Heroin	0.4	0.1	25.0
Cannabis	17.0	3.0	17.6

* During the month prior to data collection.

Source: National Drug Monitor 2005, Trimbos Institute, Utrecht, The Netherlands.

to problem users. Collectively, these data point to the existence of pronounced individual differences in sensitivity to addictive drugs, which has been confirmed in many studies (e.g.: [158,478]).

5.2 Animal studies

From animal studies, a similar picture has emerged. Within a large population of animals, phenotypes can be distinguished based on pre-existing traits, that are either vulnerable or resistant to the rewarding effects of psychostimulants such as cocaine and amphetamine [167,203,323,354,511,513,684] or display differential motivation to obtain the drugs [287]. In the work of Piazza *et al.* it was shown that 'novelty seekers' (rats that display higher locomotor responses and corticosterone secretion in response to a novel environment), also called 'high-responders' (HRs), were more prone to acquire amphetamine self-administration than rats with a low exploratory response [511]. Homberg *et al.* used a different criterion to pre-select animals and showed that rats being more vulnerable to stress-induced anxiety (as indexed by self-grooming) display higher motivation to self-administer cocaine [287]. These two animal models may represent two different motivational aspects of psychostimulant use: to engender a positive mood state ('high') or to alleviate negative affect [24] with the drug acting either as positive or as negative reinforcer respectively. Most interestingly, comparable pre-existing personality traits have been reported among human cocaine users [268,731]: Gunnarsdottir *et al.* showed that a distinction could be made between so-called 'self-medicators' and 'sensation seekers'. The former displayed higher anxiety scores whereas the latter were characterised by high novelty seeking scores [268].

It is of great importance to unravel the mechanisms behind individual differences in psychostimulant sensitivity. This will not only increase the understanding of the neurobiology of addiction but also open new perspectives for individualised prevention and treatment programs. As proposed by Ellenbroek *et al.*, addiction, like most other psychiatric diseases, can best be described by the so-called 'three hit model of psychopathology'. This model is based on the assumption that psychiatric diseases result from the interplay between three factors, being i) genetic factors, ii) early life events, and iii) later life stressors [190]. In addition, drug-induced neuroadaptations, contextual factors (such as drug availability, environment in which the drug is taken, social aspects) and pharmacokinetic properties of the drug itself (see e.g.: [588]) may contribute to individual differences. Evidence for the role of genetics, early and late environmental events in cocaine addiction is presented in the following paragraphs.

5.3 Genetics

There is considerable evidence that genetic susceptibility plays a role in the progression from substance use to dependence and ultimately addiction. Like for most diseases, the genetic contribution to addiction is highly complex, as heredity reflects both the variance attributable to genetic factors themselves and the variance resulting from interactions between genes and environment [132]. Thus, genetic factors not only determine individual differences in drug pharmacokinetics and vulnerability to the reinforcing properties of drugs, but also susceptibility to the effects of life events thereupon [710].

Twin studies have indicated that addictions are among the most heritable of psychiatric disorders [254]. Although most research has focused on alcohol and tobacco abuse, which have much higher prevalences that illicit drug use, several studies have indicated that there is a substantial genetic component in vulnerability to the reinforcing properties of cocaine [348,670], which is more pronounced for cocaine abuse than use [348]. Interestingly, it has been proposed that the genetic component in drug addiction is not substance-specific but rather extends to all classes of abused drugs [349]. In addition, there may be genes that are specific to a certain type of drug and its neurobiological and pharmacological profile.

Studies in humans have identified several genes that are associated with cocaine dependence, of which the D2 receptor gene has gained most attention. There is a strong association between cocaine dependence and certain alleles of the D2 receptor gene (A1 and B1) [475] and the A1 allele has also been associated with the occurrence of severe alcoholism [121], nicotine and opioid dependence, polysubstance abuse and obesity (for a review see: [473,476]). Conflicting data have, however, also been reported [231]. Carriers of the A1 allele have lower numbers of D2 receptors in the striatal complex and several additional metabolic and neurophysiological differences within dopamine rich brain regions (reviewed in: [474]). Interestingly, this allele has also been associated with the occurrence of post-traumatic stress disorder (PTSD) [123], which is intriguing in view of the high co-morbidity between PTSD and drug abuse (see section 5.5). This finding suggests that the D2 receptor gene might engage in gene-environment interactions, which has been supported by studies showing that cigarette craving [198] and cognitive function [41] were differentially affected by stress in carriers vs. non-carriers of the A1 allele.

Other genes that have been associated with either cocaine dependence or cocaine-induced paranoia in humans include the DAT [230,267], the D3 receptor [120], the serotonin transporter [312,426], dopamine-β-hydroxylase [133], the cannabinoid 1 receptor [122], prodynorphin [99], the mu-opioid receptor [761], myelin-related genes [6] and homer 1 [137]. However, the difficulty with association studies is that it cannot be

distinguished whether genes play a role in establishment of addiction, underlie aspects of withdrawal, or rather represent a compensatory homeostatic mechanism.

In laboratory studies, outbred strains can be used to identify subgroups of animals that are either vulnerable or resistant to psychostimulants, environmental manipulations and the interaction between these factors (see also section 5.2). Selective breeding of these subgroups can result in stable lines of susceptible and resistant animals that can be subsequently used for the identification of 'susceptibility genes'. The 'APO/SUS' and 'APO/UNSUS' rats are an example of this approach being successfully applied. These lines were generated by selective breeding of outbred rats that had been initially selected on the basis of SUSceptibility to APOmorphine-induced gnawing (gnawing score: APO/SUS: >500/45 min, -UNSUS: <10/45 min, in response to 1.5 mg/kg apomorphine). Whereas APO/UNSUS rats self-administer more cocaine under habituated conditions, the APO/SUS rats take more of the drug under stressful circumstances [684]. Furthermore these animals display several behavioural, neuroanatomical, neurochemical and endocrine differences related to the brain dopamine system, the HPA-axis and prolactin (for a review see: [191]). Gene-expression analysis revealed one gene that is differentially expressed in the hippocampus of APO/SUS vs. -UNSUS rats: the Aph-1b component of the gamma-secretase gene, which plays a role during (neuronal) development. Whereas APO/UNSUS rats have three copies of the gene, the APO/SUS rats possess only one or two [125]. The gene-dosage imbalance correlates with a number of behavioural phenotypes and it will be an interesting challenge to elucidate the mechanisms that underlie the contribution of this genetic variation alone, and in interaction with environmental factors.

Inbred mouse strains provide another valuable tool to study the impact of genes on behaviour. Differences between strains are attributable to genetic traits, whereas individual differences within a strain reflect the contribution of environmental factors. However, in both cases, gene-environment interactions are likely to play a significant role. An array of recombinant inbred mouse strains, generated from two parental inbred strains, can be used to identify quantitative trait loci (QTL): chromosomal markers that are associated with a certain phenotypic characteristic. In addition, broad-scale gene-expression profiling, and expression analysis of genes known to be involved in a certain type of behaviour, can be used to identify genes that underlie the phenotypic differences between inbred (and outbred) strains. Many inbred mouse strains have been tested for sensitivity to drugs of abuse and underlying genetic predisposition and it is outside the scope of this thesis to describe them

all. However, the C57BL/6 and DBA/2 strains will be discussed in detail in section 6, as these strains are used in the experiments presented in this thesis.

Studies in laboratory rodents have led to the identification of a considerable number of candidate genes that are associated with psychostimulant vulnerability and self-administration. Knockouts and transgenics can subsequently be used to further elucidate the putative role of these genes in addiction. Some examples of very promising genes include ΔFosB [101,117,294,346,432], cyclic AMP response element binding protein (CREB) [357,614,717] and their downstream targets, extracellularly regulated kinase (ERK) [39,679], cocaine- and amphetamine-regulated transcript (CART) [181,371], homer proteins [653] and glutamate receptors [221,403,662].

5.4 Early life events

Epidemiological studies have indicated that traumatic experiences in early life, such as physical or sexual abuse and neglect, are correlated with a higher risk of substance abuse in adolescence and adulthood (e.g.: [207,280,586]). Even more convincing evidence for the role of early environmental factors has come from twin studies. For instance, in twins discordant for childhood sexual abuse, the exposed twin is at higher risk for substance abuse disorders than the non-exposed twin [347].

Several groups have investigated the effects of early life manipulations on sensitivity to psychostimulants in laboratory rodents. Most frequently, these studies apply maternal separation as stressor. In this paradigm, pups are repeatedly separated from the mother, either alone or with the litter, during early postnatal days for time periods that can vary between several minutes to multiple hours. A wealth of data indicates that these manipulations have profound effects on emotional, stress and HPA-axis reactivity of animals in adulthood (see e.g. [80,81,219,525,528]). Maternal separation has consistently been reported to alter both acquisition and maintenance of cocaine self-administration, although controversy exists as to whether these processes are facilitated or inhibited by the neonatal manipulation [363,365,366,429,756]. Similarly, conflicting data have been reported regarding the effects of maternal separation on the exploratory response in a novel environment, psychostimulant-induced locomotion and drug- or stress-induced behavioural sensitisation to psychostimulants [58,83,393,428,728].

The discrepancies may in part be attributable to experimental differences in the neonatal manipulations, including individual or litter separation, frequency and duration of the separation, the nature of the control group, the circadian cycle and the temperature during maternal absence. Especially the duration of the manipulation

may be of great importance, as Moffett *et al*. have shown that daily 3 hours separations enhanced acquisition of cocaine self-administration when compared to non handled controls, whereas these behaviours were blunted in animals separated for 15 minutes per day [458]. In support of this, it has been proposed that short periods of maternal separation (also termed 'handling') result in reduced emotionality and HPA-axis responsiveness to stress in adulthood, whereas prolonged separations (also termed 'maternal deprivation') have reverse effects (reviewed in [386,445]). Conflicting results have also been obtained for the influence of isolation rearing during adolescence, a paradigm in which animals are housed separately from weaning for prolonged periods of time (multiple weeks), on cocaine self-administration. This procedure has been reported to either enhance or retard acquisition of intravenous cocaine self-administration [505,606,743] and to retard acquisition of intra NAc self-administration of amphetamine [504].

Despite considerable differences in experimental design and outcome, it is evident that early life stressors, such as maternal separation, have profound effects on psychostimulant sensitivity in adulthood. In line with this, early life manipulations have been shown to alter neuroanatomical and neurochemical parameters in multiple neurotransmitter systems in the brain, including the dopamine system (for a review see: [286,444]).

5.5 Later life stressors

Stressful life experiences are associated with a greater susceptibility to drug abuse and craving in humans [334,364,542,627]. Furthermore, there is high prevalence of PTSD pathology among drug addicts when compared to the general population [205,468,487] and the severity of PTSD symptoms has been positively correlated with the extent of cocaine use and craving [18,585]. In addition, stress-induced cocaine craving is associated with alterations in neuronal activity in reward-related regions in the brain [628].

In laboratory rodents, stress increases dopamine release in the NAc, CP and mPFC [1,303,305,532] and potentiates psychostimulant-induced dopamine release [635]. In line with the neurochemical effect, stress increases the locomotor response to psychostimulants, administered either systemically or into the NAc [164,272,284,383,389,635]. Acquisition of cocaine and amphetamine self-administration is enhanced by stress [244,277,510,536,660] the extent of which may depend on the nature of the stressor [536]. The few studies that have examined the effects of stress on cocaine maintained self-administration report enhanced rates of responding and higher cocaine intake after stress [131,417]. Furthermore, exposure to a stressor can reinstate extinguished cocaine seeking [194].

It is interesting to note that there are considerable individual differences in susceptibility to the impact of stress on psychostimulant-induced dopamine release, -locomotion and -self-administration [20,323,580].

5.5.1 The HPA-axis

Many studies have focused on the contribution of the HPA-axis in mediating the effects of stress on drug sensitivity. It has been demonstrated that acute cortisol administration triggers craving in individuals with cocaine dependence [192] and that cortisol levels are positively correlated with amphetamine-induced dopamine release in the ventral striatum and dorsal CP, and the positive subjective effects of the drug [486]. In addition, it was recently shown that the extent of stress-induced craving is positively correlated with the time to cocaine relapse, whereas stress-induced CRH and cortisol responses were predictive of the amount of cocaine taken per occasion [627]. By contrast, ketoconazole, a cortisol synthesis inhibitor, has been reported to increase cocaine and opioid use [367] while having no influence on the subjective effects of smoked cocaine [719]. Similarly, acute hydrocortisone was found not to affect subjective responses to d-amphetamine [714].

In animal models, corticosterone in the range of stress-induced levels increases basal and psychostimulant-induced dopamine release in the NAc and potentiates locomotor responses to the drugs [77,164,518,577]. Conversely, surgical removal of the adrenals (adrenalectomy: ADX) decreases basal and cocaine-induced dopamine release [508,580] in the shell subregion of the NAc [30] and reduces psychostimulant-induced locomotion [77,166,420,423]. The ADX effects can be restored by replacement of corticosterone in the range of circadian physiological concentrations [30,77,423,508]. Furthermore, ADX inhibits the effects of stress on dopamine release and the behavioural responsiveness to psychostimulants [166,420,577,580]. It has however also been argued that HPA-axis activation is not required for stress-induced dopamine release [304]. A series of experiments by the group of Piazza might provide an explanation for this discrepancy, as it was demonstrated that effects of glucocorticoids on dopamine release may be state-dependent, e.g. only occurring in the dark-period, during eating and in HR rats (see section 5.2) [518,580].

Acquisition of cocaine and amphetamine self-administration is facilitated by administration of corticosterone [418,516] and blocked by ADX [245]. Corticosterone dose-dependently reverses the effects of ADX and fully restores self-administration when hormone concentrations are within the range of those induced by stress [163]. Furthermore, the effects of stress on acquisition and maintenance of self-administration are

abolished when corticosterone secretion is prevented surgically or pharmacologically [84,577].

During the maintenance phase, metyrapone and ketoconazole, both corticosteroid synthesis inhibitors, decrease cocaine self-administration [245,246], although this effect was only observed for low doses of cocaine. By contrast, exogenous corticosterone did not affect maintenance of cocaine self-administration [243]. The authors argue that during ongoing self-administration, corticosterone concentrations are elevated to such extent that additional increases are without effect. By contrast, in the case of low dose cocaine self-administration, corticosteroid synthesis inhibitors might reduce corticosterone concentrations below the threshold critical for reward [241].

Spontaneous relapse to cocaine self-administration is dose-dependently facilitated by corticosterone and reduced by metyrapone [163,517]. However, ketoconazole has been demonstrated only to decrease stress-primed, but not cocaine-primed reinstatement of cocaine self-administration [415,416]. In these studies, metyrapone and ketoconazole were effective in a dose-range that reduced or blocked stress-induced, but not basal, corticosterone concentrations. By contrast, it has also been argued that stress-induced relapse to cocaine seeking does not require concentrations of corticosterone in the range of those induced by stress [195,621] but rather basal levels of the hormone [195].

Further evidence for the role of the HPA-axis in psychostimulant reinforcement has come from studies showing that the magnitude of the corticosterone response to stress can be predictive of the propensity of animals to self-administer psychostimulants [244,516]. In addition, administration of corticosterone either 10 minutes prior to a self-administration session or in the amphetamine solution, facilitates amphetamine self-administration in animals that do not spontaneously acquire this behaviour (low responder (LR) rats) while decreasing amphetamine intake in HR rats (see section 5.2) [516]. Interestingly, corticosterone in the range of stress-induced levels is self-administered by laboratory rodents, indicating that the adrenal glucocorticoid itself possesses reinforcing potential [512]. In support of this, it has been shown that glucocorticoids can trigger synaptic adaptations in dopaminergic neurons similar to those induced by psychostimulants [582]. There is substantial evidence that the effects of glucocorticoids on dopamine transmission and sensitivity to the behavioural and reinforcing effects of psychostimulants are mediated via the GR [153,168,308], that is expressed by the majority of midbrain dopamine neurons [279].

Taken together, the HPA-axis and adrenal glucocorticoids play an important role in the neurochemical-, behavioural- and reinforcing effects of psychostimulants.

Whereas basal and drug-induced dopamine release and the locomotor response to psychostimulants require corticosterone in the range of basal circadian concentrations, corticosterone in the range of stress-induced levels is required for cocaine self-administration. In addition, stress can further facilitate all these processes. Importantly, there are pronounced individual differences in psychostimulant sensitivity and the influence of stress thereupon, and this may be in part attributable to differences in susceptibility to adrenal glucocorticoids.

5.5.2 CRH

In addition to adrenal glucocorticoids, CRH has gained increasing attention for its role in drug addiction. CRH belongs to a family of neuropeptides that also includes urocortin 2 and 3 and can bind to two classes of receptors: CRH1 and CRH2, the latter consisting of multiple subtypes. Hypothalamic CRH is the critical regulator of HPA-axis activity via CRH1 receptors in the pituitary. However, during the last decades, evidence has accumulated that CRH, its family members and receptors have a broad extrahypothalamic distribution in the CNS and act as neurotransmitters and neuromodulators that regulate the response to stress at multiple levels [150]. Therefore, the CRH system has been implicated in stress-related neuropsychiatric disorders including depression, anxiety disorders [547], PTSD [282], and Alzheimer's disease [16]. It is thus not surprising that CRH plays a role in drug addiction. For a comprehensive overview on the effects of CRH and related peptides in drug addiction, the reader is advised to consult these excellent reviews [358,592,599]. The following paragraph focuses on the role of CRH in responsiveness to psychostimulants.

Administration of cocaine releases CRH not only from the hypothalamus [599], but also from extrahypothalamic sites [545,594]. CRH mediates HPA-axis activation in response to cocaine [595] and contributes to psychostimulant-induced locomotor hyperactivity and stereotypy [118,404,530,597], cocaine-induced dopamine release in the NAc and VTA [404], conditioned place-preference for cocaine [404], behavioural sensitisation produced by repeated exposure to psychostimulants and stress [76,119,196,359,530] and may contribute to maintenance of cocaine self-administration ([250], but also see a contradictory report [530]). These actions of CRH appear to be mainly attributable to the CRH1 receptor [530] and are parallel to the effects of stress and adrenal glucocorticoids on psychostimulant sensitivity. It is therefore likely that hypothalamic CRH, via activation of the HPA-axis, contributes to the abovementioned behavioural and reinforcing effects of psychostimulants [599].

However, the CRH system has also been demonstrated to play a prominent role in drug withdrawal and reinstatement of extinguished drug seeking. CRH contributes to the anxiogenic behaviour which is manifested during early withdrawal

from cocaine [593] and the amygdala has been proposed to mediate this effect [544,758]. Furthermore, a series of studies by Erb, Shaham and colleagues have indicated that the CRH system mediates footshock stress-induced relapse to cocaine and heroin seeking, while only minimally influencing drug-induced reinstatement [195,617]. More recently, the CRH1 receptor has also been proposed to play a role in cue-induced cocaine craving [269]. The bed nucleus of the stria terminalis (BNST), but not the amygdala, mediates the effects of the CRH system on stress-induced reinstatement of drug seeking [197] under conditions where stress-induced corticosterone secretion is not required [195,619].

These findings indicate that, whereas extra-hypothalamic CRH peptides contribute to the anxiogenic effects associated with drug withdrawal and stress-induced reinstatement of drug seeking, the majority of the effects of CRH on the neurochemical, behavioural and reinforcing properties of psychostimulant drugs depend on its role in HPA-axis activation and corticosterone secretion.

6. SELECTED INBRED MOUSE STRAINS

The C57BL/6 and DBA/2 inbred mouse strains have been used extensively to investigate the contribution of genetics and life events to the psychopharmacology of dopamine. These strains display profound differences in the functional and anatomical characteristics of the brain dopamine systems and in behavioural responsiveness to dopaminergic agonists and addictive drugs. In addition, the C57BL/6 and DBA/2 strains differ considerably in susceptibility to the impact of life events on psychostimulant sensitivity (reviewed in: [531]).

Characteristics of the midbrain dopamine system of the two strains are depicted in table 2. Remarkable is the strain difference in D2 receptor density in the terminal fields (higher in C57BL/6) and the cell body regions (higher in DBA/2) of the mesocorticolimbic and nigrostriatal dopamine system [69,199,470]. D2 receptors in the VTA and SNc can function as autoreceptors, which suggests that DBA/2 mice have greater autoinhibitory control over dopaminergic signalling. In support of this, the DBA/2 strain is more sensitive to apomorphine-induced inhibition of behaviour and dopamine metabolism when the dopaminergic agonist is administered in a dose-range that is likely to selectively activate the high-affinity D2 autoreceptors (for review see: [531]). Furthermore, DBA/2 mice are characterised by low basal levels of HVA in the nucleus accumbens [72,760]. However, there are no strain differences in basal dopamine release in the NAc [760].

Table 2: *Characteristics of the midbrain dopamine system of DBA/2 relative to C57BL/6 mice.*

		DBA/2 vs. C57BL/6	Reference
D2	**NAc**	↓	69
	CP	↔ ↓	69,199,470
	VTA	↑	69
	SN	↑	69
D1	**NAc**	↔	69
	CP	↔ / ↓	69,199,470
	VTA	↔	69
	SN	↔	69
DAT	**CP**	↔ / ↑	199,314,737
DA	**NAc**	↔	760,320,704
DA	**CP**	↔	320
DA	**PFC**	↔ ↑	320,704
HVA	**NAc**	↓	704,760

Arrows indicate a significant difference compared to the C57BL/6 strain. Dopamine receptor (D1 and D2) and dopamine transporter (DAT) densities were measured by radioligand binding. Dopamine (DA) and homovanillic acid (HVA) concentrations were determined by microdialysis or high-performance liquid chromatography (HPLC) on tissue samples. NAc: nucleus accumbens, CP: caudate putamen, VTA: ventral tegmental area, SN: substantia nigra, PFC: prefrontal cortex.

Table 3 summarises behavioural and neurochemical responses of the two strains to psychostimulant drugs. C57BL/6 mice are more active in a novel environment, which may have predictive value for the susceptibility to amphetamine self-administration [484,511]. Indeed, C57BL/6 mice are more sensitive to amphetamine-induced locomotion and -reward, whereas controversy exists regarding susceptibility to the rewarding effects of cocaine. With respect to behavioural sensitisation to repeated psychostimulant exposure, robust strain differences have been reported, although the nature and direction thereof varies considerably across laboratories.

Some of the discrepancies obtained with the behavioural paradigms may have resulted from the strain differences in susceptibility to environmental stimuli, such as contextual information and stress. Whereas C57BL/6 mice have greater contextual- and spatial memory and are more susceptible to context-dependent sensitisation, the DBA/2 strain is more responsive to the impact of stress or environmental manipulations on psychostimulant sensitivity [10,20,65,499,559,575,581,677]. Indeed, a period

Table 3: *Neurochemical and behavioural responses to psychostimulant drugs of DBA/2 relative to C57BL/6 mice.*

	DBA/2 vs. C57BL/6	Reference
Amphetamine		
DA NAc	↓	700,760
DA PFC	↑	700
Locomotion	↓	65,700,760
CPP	↓	71
Acquisition SA	↓	447
Sensitisation	↓ / ↑	20,65,484,559
Cocaine		
DA NAc	NI	-
DA PFC	NI	-
Locomotion	↑	483,568,665
CPP	↓	483
Acquisition SA	↑ /↓	91,257,568
Maintenance SA	↓	257,568
Sensitisation	only DBA/2 / neither strain	483,666

Arrows indicate a significant difference compared to the C57BL/6 strain. Dopamine (DA) concentrations were determined by microdialysis. DA: dopamine, NAc: nucleus accumbens, PFC: prefrontal cortex, CPP: conditioned place preference paradigm, SA: self-administration paradigm, Sensitisation: of drug-induced locomotion, NI: not investigated.

of food shortage abolishes the strain differences in amphetamine-induced place preference and locomotion by altering responsiveness selectively in the DBA/2 strain [71]. Furthermore, chronic stress induces sensitisation to the locomotor stimulant effects of amphetamine and promotes stereotypy in drug-free mice only in the DBA/2 strain [20,67]. Interestingly, it has recently been shown in DBA/2 mice that food restriction can increase action potential-dependent dopamine release in the nucleus accumbens, the component of dopamine release that is controlled by the prefrontal cortex and most likely mediates behavioural sensitisation [705]. Furthermore, repeated stress induces strain-dependent alterations in dopamine receptor densities, which are summarised in table 4. Of particular interest are the adaptations in D2 receptor density that appear to counteract the initial strain differences.

In view of the strain differences in susceptibility to environmental manipulations, it is interesting to note that the C57BL/6 and DBA/2 strains also display differences in HPA-axis function. Whereas C57BL/6 mice show a greater increase in corticosterone secretion in response to mild stressors such as exposure to a novel environment

Table 4: *Stress-induced changes in the dopamine system of DBA/2 and C57BL/6 mice.*

		DBA/2	C57BL/6	Reference
D2[1]	**NAc**	↑	↔	69
	CP	↔	↔	69
	VTA	↓	↑	69
	SN	↓	↔	69
D1[1]	**NAc**	↑	↔	69
	CP	↔	↓	69
	VTA	↔	↔	69
	SN	↔	↔	69
DA[2]	**NAc**	↓ (trend)	↑ (trend)	704
DA[2]	**PFC**	↑ (trend)	↓	704

Arrows indicate a significant difference compared to control mice of the same strain. Dopamine receptor (D1 and D2) densities were measured by radioligand binding. Dopamine (DA) concentrations were determined by high-performance liquid chromatography (HPLC) on tissue samples. [1] Mice were exposed to restraint stress on 9 consecutive days and sacrificed 24 hours after the last stress experience under basal conditions. [2] Measured 2.5 minutes after onset of a single session of restraint stress. NAc: nucleus accumbens, CP: caudate putamen, VTA: ventral tegmental area, SN: substantia nigra, PFC: prefrontal cortex.

(S. Dalm, personal communication, [66]), more severe stressors (e.g. electrical shock, restraint) induce greater HPA-axis activation in the DBA/2 strain [68,321,622].

Because of the profound differences in the function and anatomy of the brain dopamine systems and the differential susceptibility to environmental challenges, the C57BL/6 and DBA/2 inbred strains provide an interesting animal model to investigate the contribution of genotype and life events to psychostimulant sensitivity.

7. INCENTIVE SENSITISATION: FROM THEORY TO ANIMAL MODEL

The current view that drug addiction is a chronic relapsing brain disease implies that it must be associated with persistent neuroadaptations in the brain. This concept forms the core of the 'incentive-sensitisation' theory first proposed by Robinson and Berridge in 1993 [560,561]. According to this theory, repeated exposure to drugs of abuse induces long-lasting neuronal adaptations in the brain systems that regulate

the motivational effects of these drugs, rendering them hypersensitive to drugs and drug-associated stimuli. A prominent role is proposed for the mesocorticolimbic dopamine system, which is involved in the perception of the 'incentive value', or the attractiveness, of stimuli. Hypersensitivity of this system would thus make drugs increasingly more attractive or 'wanted', thereby enhancing motivation to obtain the drugs. This excessive wanting may represent (some aspects of) drug craving and the persistent nature of the sensitised state has been proposed to be a determinant of the high vulnerability of addicts to relapse, which remains even after prolonged periods of drug abstinence [152,154,561].

In laboratory rodents, sensitisation to repeated drug exposure has been studied in different paradigms and typically manifests itself as an increased behavioural or neurochemical response upon re-exposure to a drug. For instance, pre-exposure to psychostimulants or a history of drug self-administration increase drug-induced dopamine overflow in the NAc [399,709], the predisposition to acquire drug self-administration [509,522,678], the incentive motivational properties of drugs [162,399,449,464,647,709], drug-induced place preference [624] and the locomotor stimulant effects of drugs [130,520]. As reviewed by Vezina, the increases in psychostimulant-induced dopamine release and -locomotion and in the pursuit and self-administration of drugs all reflect adaptations in a common neural substrate, most notably the mesocorticolimbic dopamine system [708]. Furthermore, psychostimulant sensitisation results from complex interactions between the neuropharmacological effects of the drugs and environmental factors associated with drug exposure [22,562]. Interestingly, also pre-exposure to stress, which is known to increase dopamine release in the NAc, can induce cross-sensitisation to the neurochemical-, behavioural- and reinforcing properties of psychostimulants [20]. Finally, there are considerable individual differences in the propensity to develop behavioural sensitisation, which depend on the interplay between genetic- and environmental factors [20,65].

Psychomotor sensitisation, the gradual augmentation of the motor stimulant effects of drugs that occurs with repeated intermittent exposure, is a model that has been widely exploited to investigate the neuronal mechanisms underlying behavioural sensitisation. In line with the 'incentive sensitisation' theory, psychomotor sensitisation in rodents is a long-lasting phenomenon [392,497] that is characterised by time-dependent neuroadaptations in the motive circuit involving multiple neurotransmitters, most notably dopamine and glutamate [520,693]. Two different time domains can be distinguished in behavioural sensitisation: the initiation- and the expression phase. During initiation, animals are exposed to the psychostimulant, most often repeatedly, and the neuronal adaptations that underlie the augmented behavioural

responsiveness are initiated. Expression of sensitisation, reflecting the existence of enduring neuroadaptations, is tested by re-exposing animals to the drug following a withdrawal interval in which no drug is administered and that may vary in duration. Furthermore, expression of sensitisation is not a unitary phenomenon, as the behavioural hypersensitivity intensifies with prolonged withdrawal and the associated neuroadaptations change over time [497,520].

As reviewed by Vanderschuren and Kalivas [693], initiation and expression of psychomotor sensitisation involve distinct neuronal mechanisms which are not fully identical for cocaine and amphetamine. It applies to both psychostimulants that initiation of psychomotor sensitisation is dependent on the VTA and involves glutamatergic transmission [75,130,187,289,391], whereas the NAc plays an indispensable role in expression of previously established sensitisation which is associated with facilitated dopaminergic transmission [327,694]. Discrepancies between the two psychostimulants include a role for the NAc in initiation of cocaine-, but not amphetamine sensitisation [151,288,707], and for the PFC in both stages of cocaine sensitisation [391,521], whereas this has been less well established for amphetamine [394]. In addition, dopamine appears to be necessary only for initiation of amphetamine sensitisation [430,706], whereas a role for glutamate in expression of sensitisation has been more convincingly demonstrated for cocaine than amphetamine [310,332,333]. Taken together, psychomotor sensitisation involves dopamine-glutamate cross talk between different nuclei of the motive circuit, with a more distinct role for the corticofugal glutamatergic projection in cocaine than in amphetamine sensitisation. In addition, as described in section 4.4, other neurotransmitter systems modulate the motive circuit and thus psychomotor sensitisation.

Behavioural sensitisation to psychostimulants is associated with many cellular and structural neuroadaptations within the motive circuit, a comprehensive summary of which is outside the scope of this thesis. As reviewed by Pierce and Kalivas [520], these neuroadaptations can be collectively viewed upon as 'increasing the gain of the motive circuit'. In the case of cocaine sensitisation, the following neuroadaptations have been described. Within the NAc, dopaminergic and glutamatergic neurotransmission (drug-induced transmitter release and receptor transduction) are enhanced [5,327,496,539], both of which appear critical for expression of sensitisation. In the VTA, glutamatergic transmission is facilitated whereas GABA-ergic transduction is reduced, both via a D1 receptor-dependent mechanism [50,326]. These changes would permit enhanced activity of the mesocorticolimbic dopaminergic neurons. Furthermore, drug-induced dopamine release is reduced in the PFC [634], thereby disinhibiting corticofugal glutamatergic projections to the subcortical dopamine

system. Finally, GABA-ergic transmission in the VP is augmented in cocaine-sensitised rats [520].

In addition to neurochemical changes, psychostimulant sensitisation is associated with persistent morphological alterations within the mesocorticolimbic and nigrostriatal dopamine systems. These include increases in the number of dendritic branches and the density of dendritic spines on the medium spiny neurons of the NAc shell, core and striatum, and on the pyramidal cells in the PFC [392,564,565]. Most interestingly, induction of psychomotor sensitisation to cocaine has been associated with structural changes in the NAc core, but not shell, subregion [390]. Furthermore, these changes in connectivity may occur preferentially at the site of dopamine-glutamate synapses [392]. Finally, comparable structural changes have been demonstrated in animals with a history of cocaine self-administration [563]. These structural changes represent by far the most convincing evidence for the enduring nature of psychostimulant sensitisation and provide additional justification for the sensitisation phenomenon being interpreted as underlying certain aspects of addiction.

It must be noted, that the 'incentive sensitisation' theory is only one of the theoretical constructs for a mechanism underlying compulsive drug seeking. Several more 'exposure theories', implying that addiction results from changes induced by the drug itself, have emerged (for a review see: [698]). For instance, compulsive drug use has been postulated i) to result from a gradual progression from goal-directed behaviour to an automated process or habit, that is driven by drug-associated stimuli rather than drug reward [661], ii) to be in part attributable to drug-induced dysfunction of the PFC, resulting in reduced frontostriatal control over impulsive behaviour, especially related to the drug and associated stimuli [316], and iii) to result from a functional downregulation of the brain reward systems, resulting in an allostatic state in which the hedonic set point is shifted [360]. Evidence has accumulated in favour of all these theories and they are therefore likely to represent mechanisms that contribute to different, or partially overlapping, stages of the development of compulsive drug use [698]. Most likely, 'incentive sensitisation' represents initial changes in the addiction process as it can be induced by a single administration of a psychostimulant [694] and transition from controlled to compulsive drug use has been associated with a loss of behavioural and neural sensitisation [36]. However, as discussed in the previous paragraphs, the enduring nature of the structural and neurochemical changes as well as the association between sensitisation and relapse [152,154], suggest that 'incentive sensitisation' might also apply to more advanced stages of addiction.

8. SCOPE AND OUTLINE OF THE THESIS

Objective

As outlined in the previous paragraphs, there are considerable individual differences in susceptibility to the behavioural and reinforcing effects of psychostimulant drugs. To explain these individual differences, it is evident that both genes and life experiences influence psychostimulant sensitivity, although the mechanisms via which they act and interact are still poorly understood. This thesis focuses on a further analysis of the contribution of the HPA-axis to individual differences in cocaine sensitivity in a mouse model. The **objective** is to assess the role of adrenal glucocorticoid hormones in the susceptibility to the psychostimulant effects of cocaine, as well as to examine the dependence of their action on the *genetic background* of the individual and the *context* in which these hormones operate.

The specific **aims** pursued in the thesis are:

i) To test the hypothesis that the contribution of adrenal glucocorticoids to cocaine sensitivity depends on the *genetic background* of the individual.

ii) To determine whether the interaction between glucocorticoids and genetic background is accompanied by basal and/or cocaine-induced adaptations in the midbrain dopamine system.

iii) To investigate the *time-window* for the actions of glucocorticoids in relation to cocaine exposure.

iv) To explore the possibility that the sympathetic nervous system is implicated in the action of corticosterone.

Experimental approach

The interaction between glucocorticoid hormones and *genetic background* is studied in two inbred mouse strains: C57BL/6 and DBA/2. As outlined in section 6, these strains can be considered a model for genetic differences in i) the midbrain dopamine system, ii) susceptibility to the behavioural and reinforcing properties of psychostimulants, and iii) the effects of environmental manipulations thereupon. The strain that proves most susceptible to the impact of adrenal hormones on cocaine sensitivity will thereafter be used to investigate the *context* in which glucocorticoids

operate, with emphasis on the timing of the corticosteroid action and the role of the sympathetic nervous system. Behavioural sensitisation, the progressive augmentation of the locomotor response with repeated cocaine exposure, is used as a read-out parameter as this is a well-characterised model thought to underlie certain aspects of drug addiction. Adrenal hormones are surgically and pharmacologically manipulated by adrenalectomy (surgical removal of the adrenals, 'ADX') and hormone replacement respectively. Endocrine parameters (corticosterone and ACTH plasma concentrations) will be measured to determine potential strain differences in HPA-axis responsiveness to cocaine.

Outline

In **chapter 2** the hypotheses are tested that i) adrenal stress hormones contribute to cocaine sensitivity and ii) this may depend on the genetic make-up of the individual. For this purpose mice of the C57BL/6 and DBA/2 strains are ADX or SHAM operated and subjected to a cocaine sensitisation regimen. Locomotor and endocrine (corticosterone) responses are measured at different stages of the sensitisation paradigm. The strain that is most susceptible to the impact of ADX on cocaine-induced behavioural sensitisation (DBA/2) is selected for further research (**chapters 4-5**).

In **chapter 3** it is investigated whether the strain differences in behavioural responsiveness to cocaine and the effects of ADX thereupon, as described in **chapter 2**, are associated with changes in the midbrain dopamine system. Tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression and D1- and D2-like receptor binding are measured under basal conditions in the somatic and dendritic regions of the mesocorticolimbic and nigrostriatal dopamine systems. Comparisons are made between C57BL/6 and DBA/2 mice that are i) unoperated, ii) ADX or SHAM operated, or iii) ADX or SHAM operated and subjected to the sensitisation regimen.

Chapter 4 describes studies designed to investigate the critical time-window for the glucocorticoid effects with respect to i) the stage of behavioural sensitisation, and ii) the time of drug exposure, in the DBA/2 strain. To investigate the role of corticosterone in expression of behavioural sensitisation, the GR antagonist mifepristone is administered to sensitised mice prior to a cocaine challenge. ADX mice are given corticosterone replacement from the start of the sensitisation paradigm to investigate the role of the glucocorticoid in initiation of sensitisation. Different replacement schedules (intermittent administration 2 hrs. or 5 mins. prior to each drug exposure,

or continuous substitution via release from a s.c. pellet) are compared to investigate the time-dependency of the glucocorticoid effects.

In **chapter 5** it is investigated whether the sympathetic nervous system plays an additional role in behavioural sensitisation of DBA/2 mice to cocaine. For this purpose, ADX mice are given replacement of epinephrine, corticosterone (the most effective replacement regimen as determined in chapter 4) or both, and are subjected to the cocaine sensitisation regimen. In addition, c-fos mRNA expression in response to a cocaine challenge is measured in a number of brain regions of sensitised mice to identify a neuronal substrate for the actions of the adrenal hormones.

A general discussion of the data is presented in **chapter 6** and the major findings of this thesis are summarised in **chapter 7**.