Vulnerability to cocaine: role of stress hormones

Jong, I.E.M. de

Vulnerability to cocaine: role of stress hormones

Inge E.M. de Jong

Vulnerability to cocaine: role of stress hormones

Inge Elisabeth Maria de Jong

Thesis, Leiden University
October 2007

ISBN: 978-90-8559-147-4

Cover: Inge de Jong
Printing: Optima Grafische Communicatie B.V., Rotterdam, The Netherlands

Vulnerability to cocaine: role of stress hormones

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 17 oktober 2007
klokke 15:00 uur

door

Inge Elisabeth Maria de Jong
geboren te Leidschendam
in 1978

PROMOTIECOMMISSIE:

Promotores:	Prof. Dr. E.R. de Kloet Prof. Dr. M.S. Oitzl
Referent:	Dr. L.J.M.J. Vanderschuren (Rudolf Magnus Instituut, Utrecht)
Overige leden:	Prof. Dr. F.G. Zitman Prof. Dr. J.M.A. van Gerven Prof. Dr. M. Danhof Prof. Dr. G.J. Mulder Dr. A.M. Pereira Arias Dr. O.C. Meijer

The studies described in this thesis have been performed at the division of Medical Pharmacology of the Leiden/Amsterdam Center for Drug Research (LACDR) and Leiden University Medical Center (LUMC), The Netherlands. This research was financially supported by The Dutch Organisation for Scientific Research (NWO/ZonMW) and the Royal Dutch Academy of Sciences (KNAW) and was part of a collaboration with Institut National de la Santé et de la Recherche Médicale (INSERM), Université de Bordeaux, France.

Financial support for the printing of this thesis was kindly provided by:
- Leiden/Amsterdam Center for Drug Research (LACDR)
- Noldus Information Technology B.V.
- J.E. Jurriaanse Stichting

Science may set limits to knowledge,
but should not set limits to imagination.

Bertrand Russel (1872-1970)

Voor mijn vader

Table of contents

Preface

Not every individual who experiments with cocaine will acquire compulsive drug use. The mechanism underlying this individual difference in susceptibility to drug addiction is still poorly understood. Recent studies have identified genes and adverse life events (stress) as risk factors. The objective of this thesis is to investigate the contribution of the adrenal stress hormones glucocorticoids and epinephrine to the psychostimulant effects of cocaine in the inbred DBA/2 and C57BL/6 mouse strains. Behavioural sensitisation, measured as an enhanced locomotor response to repeated cocaine exposure, was used as a model for the long-term neural adaptations underlying certain aspects of drug addiction.

The results demonstrate that adrenal hormones play a critical role in cocaine sensitivity, which depends on genetic background because surgical removal of the adrenals or 'adrenalectomy' fully prevented cocaine sensitisation in the DBA/2, but not the C57BL/6 strain. The impact of genetic background was further emphasised by strain-specific changes in the midbrain dopamine system that mediates the rewarding effects of drugs of abuse. The effects of adrenalectomy could only be fully reversed by co-administration of glucocorticoids and epinephrine. These findings show that, depending on genetic background, adrenal stress hormones are important risk factors for vulnerability to cocaine, suggesting that pharmacological intervention in stress hormone action may have therapeutic potential in drug addiction.

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

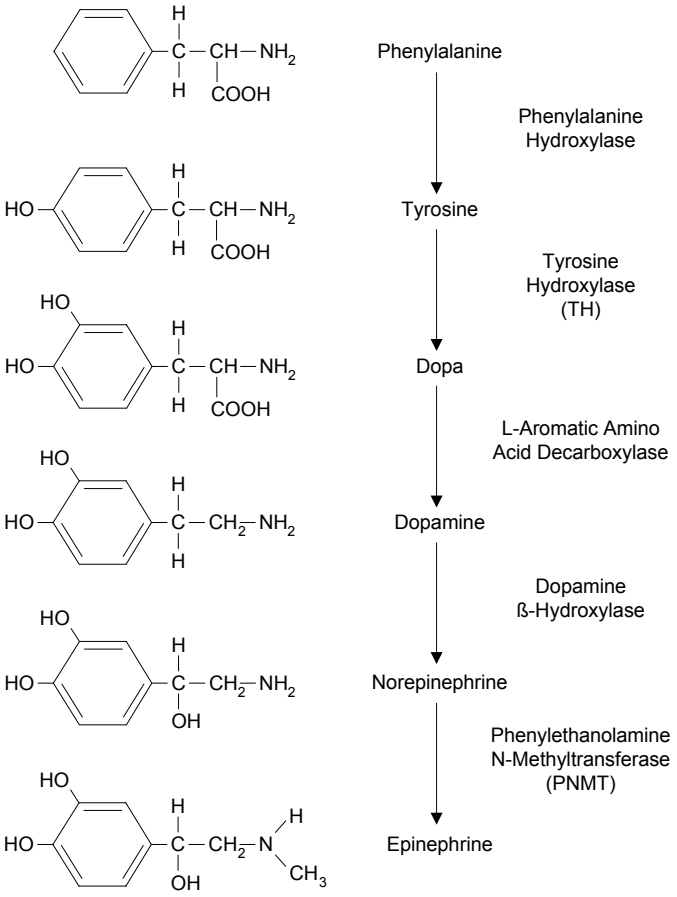

Figure 1: *Biosynthesis cascade of the catecholamines dopamine, norepinephrine and epinephrine.*
Arrows indicate an enzymatic conversion. The rate-limiting enzyme in the cascade is tyrosine hydroxylase (TH).

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

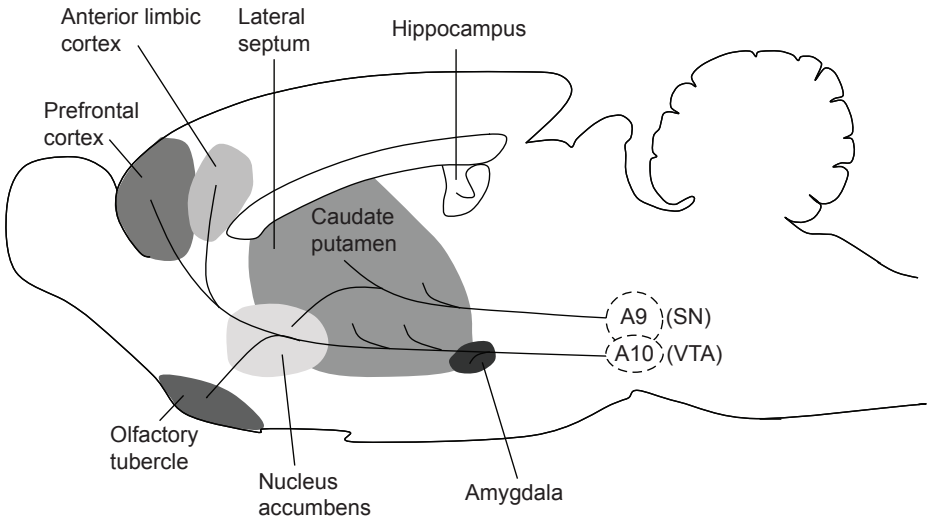

Figure 2: *Schematic representation of the mesocorticolimbic and nigrostriatal dopamine projections in the mouse brain.*
Midsagittal section showing the location of the A9 and A10 cell groups and the projection areas of the nigrostriatal and mesocorticolimbic neurons, respectively. SN: substantia nigra,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

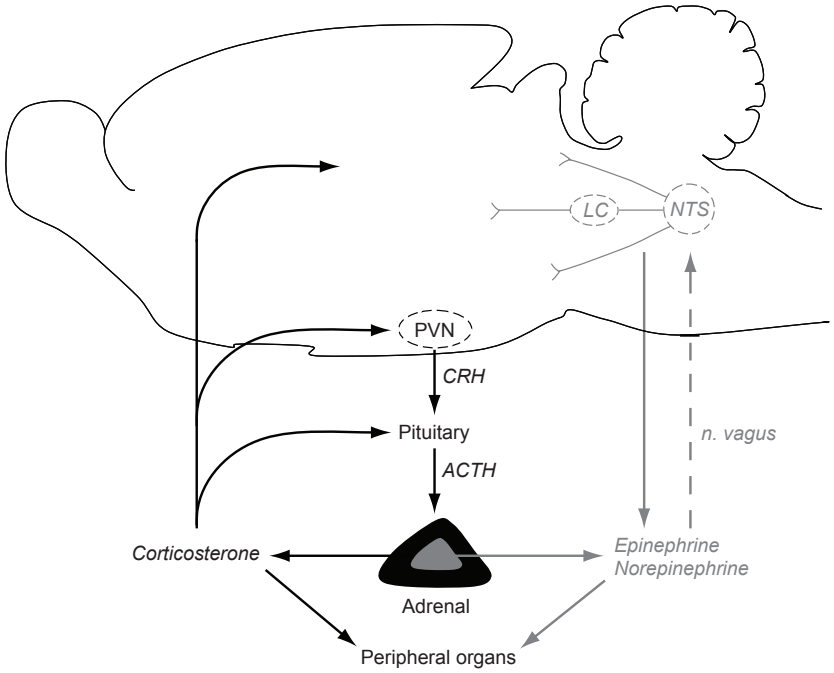

Figure 3: *Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic sympathetic nervous system (ANS).*
Black arrows indicate the HPA-axis, grey arrows the ANS. Exposure to stress leads to the release of corticotrophin-releasing hormone (CRH) from the paraventricular 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

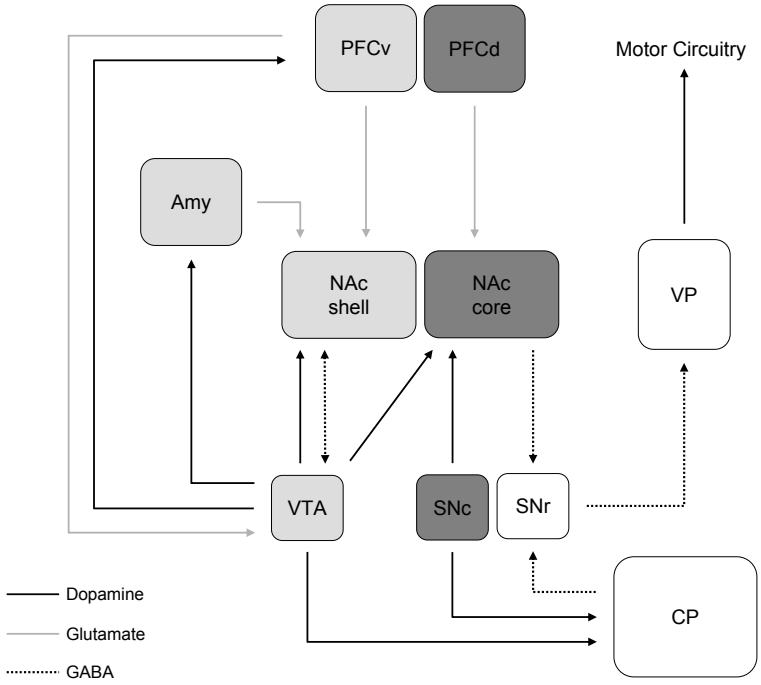

Figure 4: *Schematic drawing of the 'motive circuit'.*
Arrows indicate neuronal connections that are either dopaminergic (black), glutamatergic (grey) or GABA-ergic (dashed). Rectangles with similar colour indicate a sub-circuitry in the motive circuit. PFC v/d: prefrontal cortex ventral/dorsal subdivisions, 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**.

2

Adrenalectomy prevents behavioural sensitisation of mice to cocaine in a genotype-dependent manner

Inge E.M. de Jong, Melly S. Oitzl and E. Ronald de Kloet

Behavioural Brain Research 2007; 177(2): 329-339

ABSTRACT

The objective of the present study was to investigate the contribution of adrenal stress hormones to strain differences in cocaine sensitivity.

For this purpose, we have studied sensitisation to the locomotor stimulant effect of cocaine and, in parallel, cocaine-induced corticosterone secretion in two inbred mouse strains: C57BL/6 and DBA/2. Adrenalectomy ('ADX': surgical removal of the adrenal glands) was performed in a subset of animals to investigate the contribution of the adrenals. ADX and SHAM operated mice were subjected to repeated injections of cocaine (15.0 mg/kg) or saline for 9 consecutive days, followed by a 5 day withdrawal interval and a saline challenge on day 14. All animals were challenged with 7.5 mg/kg cocaine on day 15.

We report that repeated cocaine exposure induced locomotor sensitisation in both strains, while endocrine sensitisation was only observed in the DBA/2 strain. By contrast, cocaine attenuated corticosterone responses in C57BL/6 mice throughout the sensitisation paradigm. We have therefore identified one strain, the DBA/2 strain, that displays parallel sensitisation of cocaine-induced locomotion and -corticosterone secretion. Most interestingly, ADX prevented locomotor sensitisation only in DBA/2 mice, suggesting that behavioural sensitisation depends on the integrity of adrenal function and on secretion of adrenal glucocorticoids in this strain.

The present results demonstrate that adrenal stress hormones facilitate behavioural sensitisation to cocaine in a genotype-dependent manner and suggest that glucocorticoids contribute to strain differences in psychostimulant sensitivity.

INTRODUCTION

Behavioural responses to psychostimulant drugs are characterised by a large degree of individual variability, both in humans and laboratory animals [191,479,509]. Psychostimulants activate the mesocorticolimbic dopamine system and individual vulnerability to their effects may reflect a given predisposition to dopaminergic psychosis, such as observed in drug addiction, schizophrenia and psychotic depression. Knowledge of factors that enhance vulnerability to psychostimulants will therefore greatly increase our insight in the neurobiology of dopaminergic psychopathologies.

The existence of marked strain differences in responsiveness to drugs such as amphetamine and cocaine has demonstrated that genetic traits contribute to variations in psychostimulant vulnerability. Two inbred mouse strains that have been used frequently to study the psychopharmacology of dopamine are the C57BL/6 and DBA/2 strains. These strains display profound differences in the anatomy and functioning of the mesocorticolimbic dopamine system and in behavioural responsiveness to dopaminergic agonists and addictive drugs (reviewed in: [531]). Compared to DBA/2 mice, C57BL/6 mice are more sensitive to amphetamine-induced locomotion and reward and display higher drug-induced dopamine release in the nucleus accumbens [65,71,700,701,760]. Paradoxically, while C57BL/6 mice are also more vulnerable to the rewarding effects of cocaine, they appear less sensitive to cocaine-induced locomotion [483,665]. Robust differences between the two strains have also been reported for behavioural sensitisation to repeatedly administered psychostimulants, although the magnitude and direction thereof appears to be highly dependent on the design of the sensitisation paradigm [20,65,483,559].

Interestingly, the strain differences in dopaminergic transmission and sensitivity to the rewarding properties of psychostimulants are not stable, but can change under the influence of environmental challenges, pointing towards a role for the neuroendocrine stress system in psychostimulant vulnerability [70,71,533,704]. Indeed, a wealth of data suggests that stress modulates behavioural and neurochemical responses to psychostimulants and other addictive drugs [241,358,514,515,618].

Stressful stimuli, either physical or mental, induce concomitant activation of the hypothalamic-pituitary-adrenal axis (HPA-axis) and the sympathetic nervous system resulting in release of glucocorticoid hormones and epinephrine from the adrenal glands [146]. Glucocorticoids in particular, have been shown to modulate transmission in the mesocorticolimbic dopamine system and to facilitate behavioural responses to psychostimulants such as locomotor activity, behavioural sensitisation,

self-administration and relapse (reviewed in: [421]). Furthermore, corticosterone in the range of stress-induced levels has reinforcing potential and stress can, like drugs of abuse, increase strength of excitatory synapses on midbrain dopaminergic neurons [512,582]. Strong evidence indicates that the glucocorticoid-dopamine interactions are dependent on activation of the glucocorticoid receptor, that is widely distributed throughout the brain and is expressed by the majority of the midbrain dopaminergic neurons [144,153,168,279,308,579,582].

Taken together, these data suggest that variations in HPA-axis responsiveness to stress may contribute to individual differences in psychostimulant vulnerability, as was elegantly addressed by Piazza *et al.* [516]. In this respect, laboratory mouse or rat strains with differential stress responsivity provide a valuable tool to study the interaction between the neuroendocrine stress system and the mesocorticolimbic dopamine circuit. With respect to the C57BL/6 and DBA/2 inbred strains, few studies have addressed differences in HPA-axis activation and findings are contradictory. In one study, C57BL/6 mice displayed higher peak corticosterone levels in response to novelty which is in line with our findings (S. Dalm, personal communication), but contradictory to two reports using other stressors and experimental designs [66,321,622]. In addition, there may be differences between these strains in psychostimulant-induced HPA-axis activation, but this has to our knowledge not been reported yet. Differences in basal, stress- or psychostimulant-induced glucocorticoid release may however play a prominent role in the observed strain differences in psychostimulant sensitivity.

The present study was designed to test the hypothesis that adrenal stress hormones contribute to strain differences in cocaine sensitivity. The C57BL/6 and DBA/2 mouse strains were used as model for genetic differences in dopamine and HPA-axis function. We have measured behavioural sensitisation to the locomotor stimulant effect of cocaine and, in parallel, corticosterone responses to single and repeated cocaine exposure. In order to show involvement of the adrenal, we have tested whether strain differences persist when the adrenal is surgically removed (adrenalectomy: 'ADX') prior to the onset of drug treatment.

METHODS

Animals

Male C57BL/6 Rj (C57BL/6) and DBA/2 Rj (DBA/2) mice were obtained from Janvier (Le Genest Saint Isle, France) and received in the animal facility at the age of 8

weeks. Mice were housed in groups of four of the same strain in perspex cages (35x19x14cm) with food and water available *ad libitum* at a 12 hour light-dark cycle (lights on: 7 am) in a temperature (21±1ºC) and humidity (55±5%) controlled room. Surgery was performed 2 weeks after arrival in the animal facility. Animals were briefly handled in the week before surgery and otherwise left undisturbed. Animal experiments were approved by the local Committee for Animal Health, Ethics and Research of Leiden University. Animal care was conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC).

Experimental design

The study consisted of 8 experimental groups. Per mouse strain (C57BL/6 and DBA/2) animals were either SHAM operated (SHAM) or adrenalectomised (ADX). Each surgical group was subdivided into a cocaine (COC) and a saline (SAL) group, indicating the treatment given during the treatment period of the sensitisation paradigm. Each experimental group consisted of 9-12 animals.

Surgery

Animals were individually housed 1 day prior to surgery. The cages were transported to the operating room on the morning of the surgery where mice were allowed to recover from transportation for 2 hours. Inhalation anaesthesia consisted of a mixture of isoflurane (3 l/min), N_2O (0.8 l/min) and O_2 (0.4 l/min). During surgery mice were placed on a heat pad (37ºC). The skin on the back was shaved and disinfected and an incision of approximately 1 cm was made above and parallel to the spinal cord. Through a small opening in the muscle layer left and right of the spinal cord the adrenals were removed from the surrounding fat tissue. The skin was closed using a simple running suture. SHAM animals were treated similarly with the exception of the actual removal of the adrenals. Mice were kept individually housed for 24 hours following surgery after which they were housed two animals per cage of similar surgery and strain. After surgery all animals were given free access to 0.9% NaCl in addition to normal drinking water. The sensitisation paradigm was started following a recovery period of 1 week.

Drugs

Cocaine hydrochloride (BUFA Pharmaceuticals B.V., Uitgeest, The Netherlands) was dissolved in sterile saline, stored in aliquots at -20˚C and defrosted on the day of administration. Cocaine (room temperature) was administered intraperitoneally

(i.p.) in a volume of 200µl/25 grams bodyweight and a dosage of 7.5 or 15.0 mg/kg. Control groups received an equal volume of saline. From the start of the sensitisation paradigm, animals were weighed once every two days and the injection volumes were adjusted accordingly.

Sensitisation paradigm

One day prior to the first drug administration and thus the first behavioural test, animals were individually housed and kept single housed for the remainder of the experiment.

The sensitisation paradigm consisted of a treatment phase (days 1-9), a withdrawal interval (days 10-14), a saline challenge (day 14) and a cocaine challenge (day 15). The treatment phase consisted of i.p. injections of 15.0 mg/kg cocaine (COC) or saline (SAL) on 9 consecutive days and locomotion was measured on days 1 (first administration) and 9 (last administration). On days 2-8 animals received the injections in the home cage. The treatment period was followed by a withdrawal interval of 5 days (no treatment). On the last day of the withdrawal period (day 14), all animals received a saline challenge and on day 15, all animals received a challenge of 7.5 mg/kg cocaine. All injections were given 2 to 5 hours after lights on.

Measurement of locomotor activity

Behavioural tests were performed on days 1, 9, 14 and 15 in the room where animals were housed. Mice were placed in a test cage (same type and size (35x19x14cm) as the home cage) containing a standardised amount of sawdust. The cage was covered with a perspex lid. Following a 2 hour habituation period, animals were injected and activity was monitored on video for 30 minutes. At the end of this period, a blood sample was taken from the tail vein for endocrine measurements and the animals were returned to their home cage.

Analysis of locomotor activity

Video images were digitised and analysed using Ethovision Videotracking, Motion Analysis & Behavior Recognition System version 1.96 ('VTMAS', Noldus Information Technology B.V., Wageningen, The Netherlands). The position of the animal was sampled 5 times per second. Of each recording (30 minutes) 27 minutes were analysed since the animals were subjected to blood sampling at 30 minutes after injection. Data are represented in total distance moved over the entire 27 minute

treatment period and per 3 minute time blocks (cm). Locomotion was defined as movement with a minimal distance of 2 cm.

Corticosterone and adrenocorticotrophic hormone (ACTH)

Blood samples were taken from the tail vein by a small incision with a razorblade 30 minutes after injection at the test days 1, 9, 14 and 15. Blood was collected in small EDTA coated tubes (Microvette DB 200 K3E, Sarstedt, Nümbrecht, Germany). Mice were euthanised in the morning of day 16, 24 hours after the cocaine challenge, and trunk blood was collected following decapitation in large EDTA coated tubes (Tube 10 ml, 95x16.8 mm, K3E, Sarstedt, Nümbrecht, Germany). Plasma was obtained by centrifugation at 13000 rpm for 20 minutes at 4ºC and subsequently stored at -20ºC. Corticosterone and ACTH concentrations were determined by in-duplo measurement using radio-immuno-assay (RIA) kits from MP Biomedicals according to the protocol provided by the manufacturer (Corticosterone double antibody ^{125}I RIA kit and ACTH double antibody ^{125}I RIA kit, MP Biomedicals, Asse-Relegem, Belgium). All samples were analysed in one assay to exclude inter-assay variability. ADX effectively clamped plasma corticosterone to basal concentrations in both strains, and only animals with successful ADX were included in the study.

Statistics

Statistical analysis was performed using SPSS for Windows software (release 7.5, SPSS Benelux B.V., Gorinchem, The Netherlands). Locomotor activity, corticosterone and bodyweight data were subjected to repeated measures ANOVA with two between subject factors (surgery and treatment) and one within subject factor (test day). Subsequent analyses were performed per test day: two factor ANOVA for surgery and treatment. Locomotion represented per 3 minute time blocks was subjected to repeated measures ANOVA with two between subject factors (surgery and treatment) and one within subject factor (time block). ACTH data were analysed by two factor ANOVA for surgery and treatment. Correlations between locomotor activity data and corresponding corticosterone concentrations were analysed using Pearson's test (two-tailed). When statistical significance was revealed, post hoc tests were performed (Tukey HSD, or for within-subject comparison paired t-test). Differences were considered statistically significant when $p<0.05$.

RESULTS

Locomotor activity

The effects of ADX on locomotion of C57BL/6 and DBA/2 mice were studied during the different phases of the sensitisation paradigm. Figures 1A (C57BL/6) and 1B (DBA/2) depict total distance moved in the four behavioural tests (days 1, 9, 14 and 15) for the treatment groups SHAM/SAL, SHAM/COC, ADX/SAL and ADX/COC.

C57BL/6

The effects of ADX on cocaine-induced locomotion and sensitisation of C57BL/6 mice are shown in figure 1A. Surgery did not influence the response of C57BL/6 mice in any treatment group or at any time point ($F[surgery]_{1,35}=0.374$, $p=0.545$). Locomotion was significantly affected by treatment ($F_{1,35}=58.745$, $p<0.001$), day ($F_{3,105}=20.466$, $p<0.001$) and the interaction between both ($F_{3,105}=17.921$, $p<0.001$).

On days 1 and 9 of the treatment period, locomotion was increased three-fold by cocaine (15.0 mg/kg) in comparison with saline, irrespective of surgery (day 1: $F[treatment]_{1,39}=40.438$, $p<0.001$, $F[surgery]_{1,39}=0.508$, $p=0.418$, day 9: $F[treatment]_{1,40}=101.574$, $p<0.001$, $F[surgery]_{1,40}=0.569$, $p=0.455$, post hoc: $p\leq0.001$ compared to saline-treated for both surgical groups on both days). Remarkably, cocaine responses were not enhanced on the last compared to the first day of the treatment period. Similarly, saline responses on days 1 and 9 were comparable.

On day 14, *all* animals received a *saline* challenge in the test environment. Responses were higher in the cocaine-treated groups irrespective of surgery ($F[treatment]_{1,39}=9.290$, $p<0.01$, $F[surgery]_{1,39}=0.001$, $p=0.974$), although statistical significance was not reached when comparing individual groups. The treatment effect was however confirmed when locomotion was plotted per 3 minute time blocks ($F[treatment]_{1,36}=9.312$, $p<0.01$, $F[surgery]_{1,36}=0.001$, $p=0.975$, data not shown). These data indicate that cocaine treatment induced a distinct, yet small, conditioned hyperresponsiveness to the experimental conditions in both surgical groups.

On day 15, *all* animals received a challenge dose of 7.5 mg/kg *cocaine*. Responses are depicted in figure 1A (total distance moved) and figure 2A (distance moved per 3 minute time blocks). Subsequent statistics refer to total distance moved in figure 1A. Cocaine-treated mice displayed augmented locomotion in response to the cocaine challenge when compared to saline-treated mice, irrespective of surgery ($F[treatment]_{1,39}=18.773$, $p<0.001$, $F[surgery]_{1,39}=0.256$, $p=0.616$, post hoc:

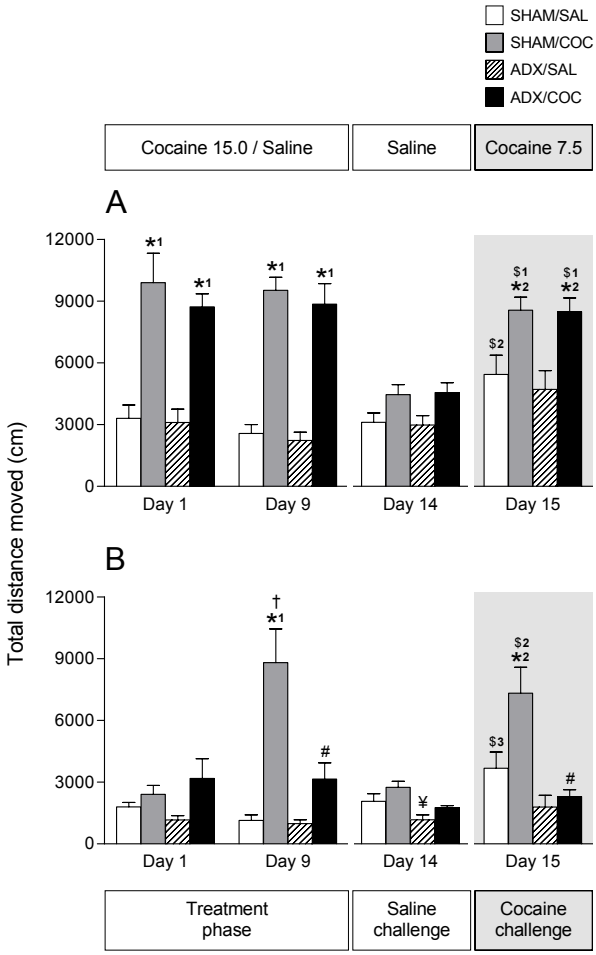

Figure 1: *Initiation and expression of sensitisation.*
Locomotion of C57BL/6 (A) and DBA/2 (B) mice in response to treatment on days 1, 9, 14 and 15 of the sensitisation paradigm. Adrenalectomised (ADX) or SHAM operated animals received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal interval, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented as mean total distance moved over the entire 27 minute treatment period (cm) ± SEM, n= 9-12 animals/group. *1 $p<0.001$, *2 $p<0.05$ vs. SAL (Tukey HSD), # $p<0.01$ vs. SHAM (Tukey HSD), ¥ $p<0.01$ vs. SHAM/COC (Tukey HSD), † $p<0.01$ vs. day 1 (paired t-test), $1 $p<0.001$, $2 $p<0.01$, $3 $p<0.05$ vs. saline challenge on day 14 (paired t-test).

$p<0.05$ for both SHAM and ADX). In addition, the cocaine-treated groups displayed twofold higher locomotion in response to the cocaine challenge when compared to the saline challenge (day 14), indicating that conditioned responsiveness cannot

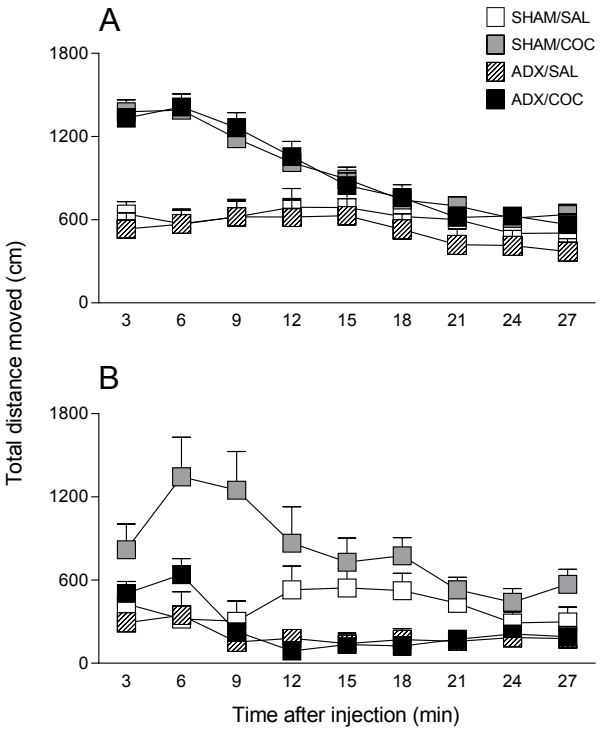

Figure 2: *Expression of sensitisation.*
Locomotion of C57BL/6 (A) and DBA/2 (B) mice in response to the 7.5 mg/kg cocaine challenge on day 15. Previously, adrenalectomised (ADX) or SHAM operated animals received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal interval and a saline challenge (day 14). Data are represented as mean distance moved per 3 minute time blocks (cm) ± SEM, n= 9-12 animals/group. C57BL/6 (A): $F[\text{surgery}]_{1,36}=0.258$, $p=0.615$, $F[\text{treatment}]_{1,36}=18.757$, $p<0.001$, $F[\text{time block}]_{8,288}=54.845$, $p<0.001$, $F[\text{time block x treatment}]_{8,288}=30.951$, $p<0.001$. DBA/2 (B): $F[\text{surgery}]_{1,38}=15.054$, $p<0.001$, $F[\text{treatment}]_{1,38}=5.449$, $p<0.05$, $F[\text{time block}]_{8,304}=5.098$, $p<0.001$, $F[\text{time block x surgery}]_{8,304}=2.223$, $p<0.05$, $F[\text{time block x treatment}]_{8,304}=4.220$, $p<0.001$.

have accounted for the full magnitude of the sensitised response ($p<0.001$, paired t-test for both surgical groups). The dose of 7.5 mg/kg was sufficient to enhance locomotion of drug-naïve SHAM mice above their saline response on day 14 and a similar effect was observed for ADX mice, although this just failed to reach statistical significance (SHAM/SAL: $p<0.01$, ADX/SAL: $p=0.06$, paired t-test).

Figure 3A depicts individual cocaine responses of SHAM operated C57BL/6 mice on days 1, 9 and 15. While responses to the first drug exposure were characterised by a considerable degree of inter-individual variability, variation became much less

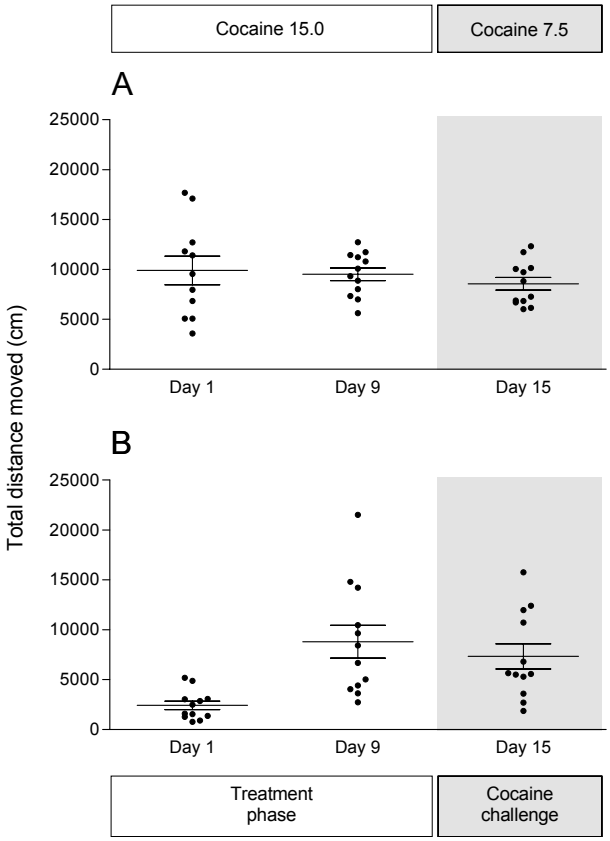

Figure 3: *Cocaine responses of individual animals.*
Locomotion of SHAM operated C57BL/6 (A) and DBA/2 (B) mice in response to cocaine on days 1, 9 (15.0 mg/kg) and 15 (7.5 mg/kg) of the sensitisation paradigm. Data are represented as mean total distance moved over the entire 27 minute treatment period (cm) ± SEM, n= 11-12. The black dots represent the data points for the individual animals.

during the course of the sensitisation paradigm and standard errors were very small (days 9 and 15). The behavioural responses or the degree of sensitisation thereof were not correlated with corticosterone concentrations on any day (Pearson's correlation coefficients: day 1: r=-0.059, p=0.864, day 9: r=0.023, p=0.942, day 15: r=0.496, p=0.101, day 9/day 1: r=-0.003, p=0.994, day 15/day 1: r=-0.241, p=0.476).

In summary, these results indicate that while C57BL/6 mice responded strongly to the first administration of cocaine (15.0 mg/kg), this locomotor response was not further enhanced by 8 drug administrations in this test setting. Despite the lack of increased responsiveness during the treatment phase, sensitisation was revealed when animals were challenged with 7.5 mg/kg cocaine following a 5 day withdrawal period. In addition, a small conditioned hyperactivity was observed in the

cocaine-treated C57BL/6 mice when challenged with saline. Remarkably, ADX did not affect any of the behavioural responses of the C57BL/6 mice measured in this study.

DBA/2

The effects of ADX on cocaine-induced locomotion and sensitisation of DBA/2 mice are shown in figure 1B. In this mouse strain, not only treatment ($F_{1,38}$=16.562, p<0.001) and test day ($F_{3,114}$=9.369, p<0.001) but also surgery ($F_{1,38}$=9.979, p<0.01) significantly affected locomotion. In addition, a surgery x treatment x day interaction was found ($F_{3,114}$=7.051, p<0.001).

Cocaine (15.0 mg/kg) increased locomotion of DBA/2 mice when compared to saline on both days 1 and 9 of the treatment period (day 1: $F[treatment]_{1,44}$=5.178, p<0.05, day 9: $F[treatment]_{1,41}$=22.684, p<0.001). On day 1, drug responses were very small, there were no surgery effects and post hoc analysis did not reveal significant differences between the treatment groups ($F[surgery]_{1,44}$=0.012, p=0.915). On day 9 however, SHAM animals were strongly activated by cocaine and locomotor responses were 8-fold higher compared to those of saline controls (p<0.001) and threefold enhanced compared to drug responses on day 1 (p<0.01, paired t-test). In ADX mice, on the contrary, cocaine did not increase activity above saline responses on day 9 (p=0.494) and cocaine responsiveness was not augmented when compared to day 1 (p=0.701, paired t-test). Furthermore, cocaine-induced locomotion on day 9 was significantly lower in ADX compared to SHAM mice ($F[surgery]_{1,41}$=7.910, p<0.01, $F[surgery \times treatment]_{1,41}$=7.072, p<0.05, post hoc: p<0.01).

On day 14, *all* animals received a *saline* challenge in the test environment. Behavioural responses were significantly affected by treatment and surgery ($F[treatment]_{1,41}$=4.988, p<0.05, $F[surgery]_{1,41}$=11.092, p<0.01), but both effects were mainly attributable to the less relevant group comparison between the SHAM/COC and ADX/SAL groups (p<0.01). No other significant differences were found between the treatment groups by means of post hoc analysis.

On day 15, following a 5 day withdrawal period, *all* animals were challenged with 7.5 mg/kg *cocaine*. Responses are depicted in figure 1B (total distance moved) and figure 2B (distance moved per 3 minute time blocks). Subsequent statistics refer to total distance moved in figure 2. In SHAM (p<0.05), but not in ADX animals (p=0.981), cocaine treatment resulted in augmented locomotor responsiveness to the cocaine challenge when compared to saline treatment ($F[treatment]_{1,41}$=5.459, p<0.05, $F[surgery]_{1,41}$=15.040, p<0.001, $F[surgery \times treatment]_{1,41}$=3.144, p=0.084). In addition, the locomotor responses of cocaine-treated ADX mice were significantly lower compared to those of cocaine-treated SHAM mice (p<0.01). Interestingly, the cocaine responses of the drug-naïve SHAM mice exceeded their saline responses

on day 14 ($p<0.05$, paired t-test) while this was not the case for the drug-naïve ADX mice ($p=0.385$, paired t-test). These data suggest that ADX may also reduce sensitivity of drug-naïve mice to the activating effects of low doses of cocaine, although statistical significance was not reached when comparing cocaine responses of the SHAM/SAL and ADX/SAL groups by post hoc analysis.

The DBA/2 strain was characterised by a relatively high degree of individual variability in cocaine responses after repeated drug exposure (days 9 and 15), reflecting the existence of considerable inter-individual differences in behavioural sensitisation (figure 3B). The variability within the DBA/2 strain was confirmed in subsequent experiments (data not shown). The behavioural responses or the degree of sensitisation thereof in the SHAM/COC group were not correlated with corticosterone concentrations on any day (Pearson's correlation coefficients: day 1: $r=0.024$, $p=0.939$, day 9: $r=-0.121$, $p=0.695$, day 15: $r=0.230$, $p=0.450$, day 9/day 1: $r=-0.097$, $p=0.764$, day 15/day 1: $r=0.318$, $p=0.314$).

In summary, these results indicate that while DBA/2 mice appeared relatively insensitive to the first administration of cocaine (15.0 mg/kg), sensitisation to the locomotor stimulant effects did develop in SHAM mice during the course of 9 drug treatments. Furthermore, sensitisation was expressed in SHAM animals in response to a challenge of 7.5 mg/kg cocaine after a 5 day withdrawal period. However, ADX abolished the ability of DBA/2 mice to develop and express sensitisation to the locomotor stimulant effects of cocaine.

Corticosterone

Plasma corticosterone concentrations were measured 30 minutes after treatment in the four behavioural tests on days 1, 9, 14 and 15 (figures 4A: C57BL/6 and 4B: DBA/2) and following decapitation in the morning of day 16 (figures 5A: C57BL/6 and 5B: DBA/2, left panels).

C57BL/6

The effects of ADX and drug treatment on plasma corticosterone concentrations of C57BL/6 mice are shown in figure 4A. In this strain, main effects were found for surgery ($F_{1,34}=224.014$, $p<0.001$), day ($F_{4,136}=63.347$, $p<0.001$) and the interaction between surgery, treatment and day ($F_{4,136}=2.438$, $p=0.05$). On all test days, corticosterone concentrations were significantly lower in the ADX groups (day 1: $F[\text{surgery}]_{1,40}=121.402$, $p<0.001$, day 9: $F[\text{surgery}]_{1,40}=126.309$, $p<0.001$, day 14: $F[\text{surgery}]_{1,38}=220.125$, $p<0.001$, day 15: $F[\text{surgery}]_{1,39}=115.274$, $p<0.001$, post hoc: $p<0.001$ compared to SHAM groups on all days). Endocrine responses of the SHAM animals are described in the following paragraphs.

On day 1, cocaine attenuated corticosterone secretion observed in response to saline treatment ($F[treatment]_{1,40}=4.890$, $p<0.05$, post hoc: $p<0.05$). A similar trend

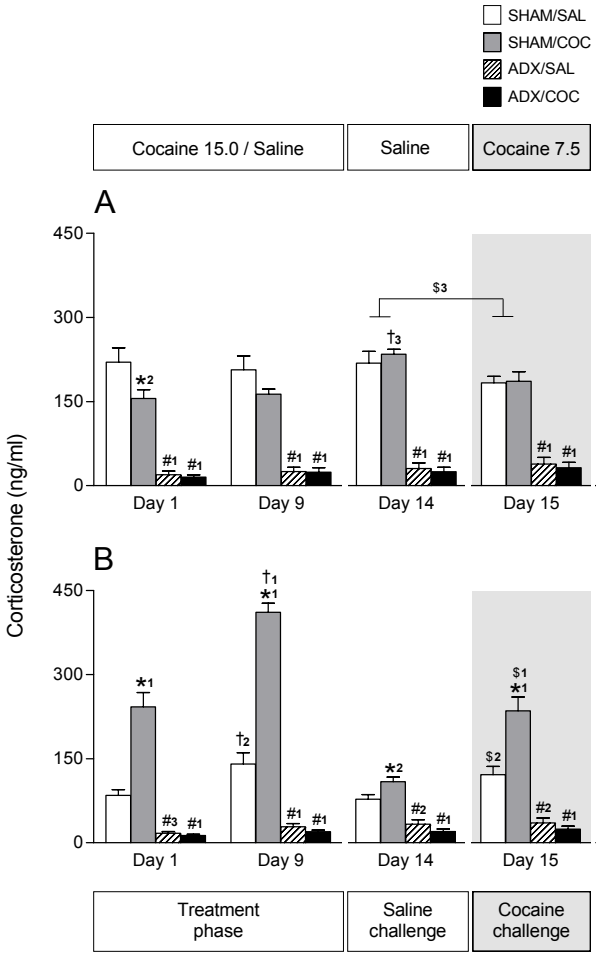

Figure 4: *Corticosterone concentrations.*
Plasma corticosterone concentrations of C57BL/6 (A) and DBA/2 (B) mice 30 minutes after treatment on days 1, 9, 14 and 15 of the sensitisation paradigm. Adrenalectomised (ADX) or SHAM operated animals received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal period, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented as mean plasma corticosterone concentration (ng/ml) ± SEM, n= 9-12 animals/group. *1 $p<0.001$, *2 $p<0.05$ vs. SAL (Tukey HSD), #1 $p<0.001$, #2 $p<0.01$, #3 $p<0.05$ vs. SHAM (Tukey HSD), †1 $p<0.001$, †2 $p<0.05$ vs. day 1 (paired t-test), †3 $p<0.001$ vs. days 1 and 9 (paired t-test), $1 $p<0.001$, $2 $p<0.05$ vs. saline challenge on day 14 (paired t-test), $3 $p<0.05$ vs. saline challenge on day 14, SHAM groups pooled (paired t-test).

was still observed on day 9 ($F[treatment]_{1,40}=2.417$, p=0.129, post hoc: p=0.136). Within the SAL and COC treatment groups, corticosterone responses were comparable on days 1 and 9, suggesting that neither sensitisation nor desensitisation occurred in response to either treatment.

When *all* animals were administered *saline* on day 14, the difference between the two SHAM groups disappeared due to a relative increase in the corticosterone response of the cocaine-treated mice. In this group, corticosterone concentrations were significantly enhanced compared to days 1 and 9 when animals were treated with cocaine (p<0.001, paired t-test for both days) and no longer different from the saline-treated group ($F[treatment]_{1,38}=0.151$, p=0.700). In addition, corticosterone responses of both SHAM groups were similar to those of saline-treated mice on days 1 and 9, indicating that responses to saline were stable throughout the paradigm.

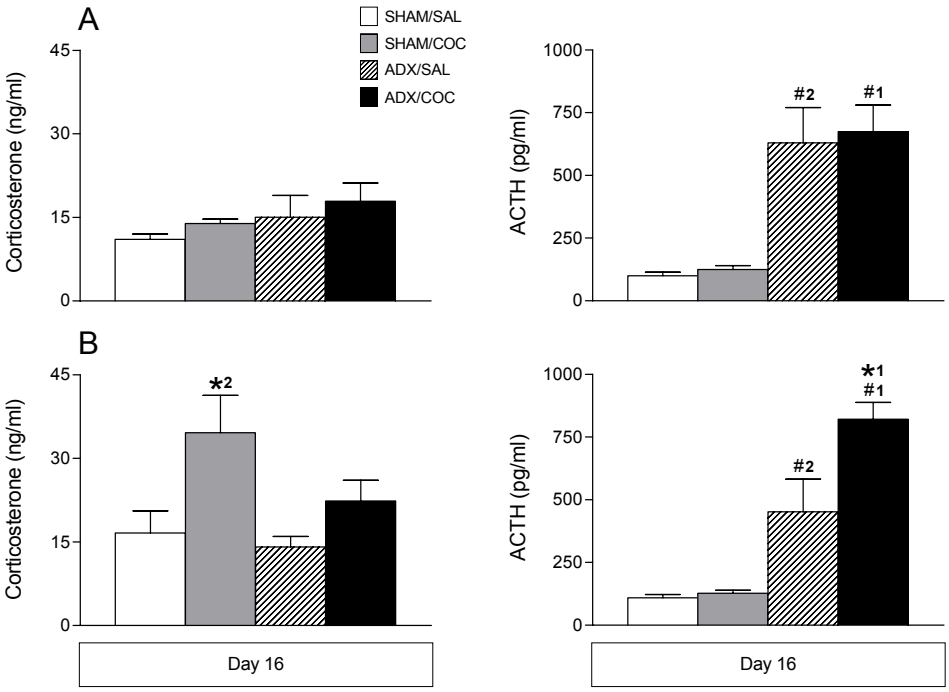

Figure 5: *Morning corticosterone and ACTH after sensitisation.*
Morning plasma corticosterone and ACTH concentrations of C57BL/6 (A) and DBA/2 (B) mice following decapitation on day 16. Previously, adrenalectomised (ADX) or SHAM operated animals received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal period, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented as mean plasma corticosterone (ng/ml) and ACTH (pg/ml) concentration ± SEM, n= 9-12 animals/group. #1 p<0.001, #2 p<0.01 vs. SHAM (Tukey HSD), *1 p<0.01, *2 p<0.05 vs. SAL (Tukey HSD).

The *cocaine* (7.5 mg/kg) challenge on day 15 again attenuated the corticosterone response in the two SHAM groups when compared to the saline challenge on day 14 ($p<0.05$, paired t-test). The effect of 7.5 mg/kg was less pronounced than that of 15.0 mg/kg, indicating that cocaine may dose-dependently attenuate HPA-axis activation in this strain.

Morning plasma corticosterone at the time of decapitation on day 16 when animals were taken directly from the home cage (figure 5A, left panel), was not affected by surgery or previous treatment ($F[surgery]_{1,38}=2.654$, $p=0.112$, $F[treatment]_{1,38}=1.362$, $p=0.251$).

In summary, ADX was effective in clamping plasma corticosterone concentrations to stable low levels. Compared to saline administration, cocaine treatment (15.0 mg/kg and to a lesser extent 7.5 mg/kg) attenuated corticosterone responses in SHAM operated animals, an effect that was observed throughout the sensitisation paradigm. No sensitisation or desensitisation was detected in response to either saline or cocaine treatment in the C57BL/6 strain.

DBA/2

The effects of surgery and drug treatment on corticosterone responses of DBA/2 mice are shown in figure 4B. Main effects were found for surgery ($F_{1,34}=224.104$, $p<0.001$), treatment ($F_{1,34}=51.631$, $p<0.001$), day ($F_{4,136}=78.390$, $p<0.001$) and the interaction between these factors ($F_{4,136}=25.179$, $p<0.001$). Also in the DBA/2 strain, corticosterone was significantly attenuated in the ADX groups on each test day (day 1: $F[surgery]_{1,43}=100.558$, $p<0.001$, day 9: $F[surgery]_{1,40}=315.116$, $p<0.001$, day 14: $F[surgery]_{1,39}=76.399$, $p<0.001$ and day 15: $F[surgery]_{1,40}=75.619$, $p<0.001$, post hoc comparison with SHAM groups: at least $p<0.05$ on all test days). The endocrine responses of the SHAM animals are described in the following paragraphs.

In contrast to C57BL/6 mice, DBA/2 mice responded to cocaine treatment (15.0 mg/kg) with an elevation of corticosterone when compared to saline treatment on both days 1 and 9 of the treatment period (day 1: $F[treatment]_{1,43}=27.059$, $p<0.001$, $F[surgery \times treatment]_{1,43}=29.911$, $p<0.001$, day 9: $F[treatment]_{1,40}=85.531$, $p<0.001$, $F[surgery \times treatment]_{1,40}=97.050$, $p<0.001$, post hoc: $p<0.001$ compared to saline on both days). In addition, cocaine-induced corticosterone secretion was markedly enhanced on day 9 compared to day 1 ($p<0.001$, paired t-test), an effect that was also observed for the response to saline treatment, although less pronounced ($p<0.05$, paired t-test).

When *all* animals received *saline* on day 14, cocaine-treated SHAM mice responded with a greater elevation in corticosterone compared to saline-treated mice ($F[treatment]_{1,39}=1.464$, $p=0.234$, $F[surgery \times treatment]_{1,39}=8.390$, $p<0.01$, post hoc: $p<0.05$).

On day 15, when *all* animals were challenged with 7.5 mg/kg *cocaine*, the corticosterone response of the cocaine-treated SHAM group was augmented compared to that of the saline-treated group ($F[\text{treatment}]_{1,40}=9.026$, $p<0.01$, $F[\text{surgery x treatment}]_{1,40}=13.452$, $p<0.01$, post hoc: $p<0.001$). Furthermore, the response to 7.5 mg/kg cocaine was not different from that on day 1 when animals were treated with 15.0 mg/kg. In both SHAM groups, the cocaine challenge enhanced corticosterone concentrations above those in response to the saline challenge on day 14 (at least $p<0.05$, paired t-test).

Interestingly, at the time of decapitation on day 16 when animals were taken directly from the home cage (figure 5B, left panel), morning corticosterone concentrations of the cocaine-treated SHAM mice were higher compared to those of saline-treated mice ($F[\text{treatment}]_{1,41}=7.452$, $p<0.05$, $F[\text{surgery}]_{1,41}=2.359$, $p=0.133$, post hoc: $p<0.05$).

In summary, ADX was effective in clamping plasma corticosterone concentrations to stable low levels. Cocaine increased corticosterone secretion in SHAM operated DBA/2 mice and repeated drug treatment resulted in sensitisation of HPA-axis activation that persisted until the cocaine challenge. Furthermore, cocaine-treated SHAM mice displayed higher corticosterone secretion in response to the saline challenge and elevated basal morning corticosterone on day 16.

ACTH

Basal plasma ACTH concentrations, measured following decapitation in the morning of day 16, are depicted in the right panels of figure 5A (C57BL/6) and 5B (DBA/2). In both strains, ACTH levels were significantly elevated in ADX mice (C57BL/6: $F[\text{surgery}]_{1,37}=40.779$, $p<0.001$, DBA/2: $F[\text{surgery}]_{1,39}=58.105$, $p<0.001$, post hoc: at least $p<0.01$ compared to SHAM for both strains). In the DBA/2 strain, the ACTH elevation was dependent on treatment, being higher in cocaine-treated ADX mice ($F[\text{treatment}]_{1,39}=8.150$, $p<0.01$, $F[\text{surgery x treatment}]_{1,39}=6.645$, $p<0.05$, post hoc: $p<0.01$ compared to ADX/SAL), whereas this was not the case in the C57BL/6 strain ($F[\text{treatment}]_{1,37}=0.165$, $p=0.688$). In both strains, ACTH levels of SHAM operated mice were around 100 pg/ml, irrespective of treatment.

Body weight

Table 1 depicts bodyweights on the day prior to surgery and test days 1, 9 and 15 (7, 15 and 21 days after surgery respectively). Average pre-surgical bodyweight of each strain was set at 100%. In the C57BL/6 strain, bodyweight gradually increased and exceeded pre-surgical weight on all test days with no effects of

Table 1: *Bodyweight relative to pre-surgical weight (%).*

			Pre-surgical	Day 1	Day 9	Day 15
C57BL/6	**SHAM**	**SAL**	100.5 ± 0.7	104.3 ± 1.0 *1	107.6 ± 1.1 *1 #2	109.4 ± 1.2 *1 #2 †2
		COC	100.2 ± 1.2	104.6 ± 1.1 *1	107.0 ± 1.1 *1 #2	108.6 ± 1.3 *1 #1
	ADX	**SAL**	100.5 ± 1.7	103.8 ± 1.6 *3	108.9 ± 2.0 *1 #2	107.2 ± 1.8 *1 #3
		COC	98.6 ± 0.6	104.3 ± 1.3 *1	108.8 ± 1.5 *1 #2	106.5 ± 1.6 *2 #3 †2
DBA/2	**SHAM**	**SAL**	101.9 ± 1.5	98.7 ± 1.5 *2	102.2 ± 2.3 #2	102.6 ± 2.3 #3
		COC	99.2 ± 1.7	97.5 ± 1.9	98.8 ± 1.5	101.2 ± 1.7 #2 †1
	ADX	**SAL**	100.5 ± 1.4	97.2 ± 1.1	103.5 ± 2.0 #2	101.7 ± 2.0 #3
		COC	98.5 ± 1.5	95.5 ± 1.6 *3	96.2 ± 2.0	98.5 ± 2.3 †2

Relative bodyweights of C57BL/6 and DBA/2 mice, prior to surgery and on test days 1, 9 and 15 (7, 15 and 21 days after surgery respectively). For both strains, average pre-surgical bodyweight was set at 100%. Animals were adrenalectomised (ADX) or SHAM operated and received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal period, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented as percentage of mean pre-surgical bodyweight (%) ± SEM, n= 9-12 animals/group. *1 $p<0.001$, *2 $p<0.01$, *3 $p<0.05$ vs. pre-surgical (paired t-test), #1 $p<0.001$, #2 $p<0.01$, #3 $p<0.05$ vs. day 1 (paired t-test), †1 $p<0.01$, †2 $p<0.05$ vs. day 9 (paired t-test).

surgery or treatment ($F[day]_{3,108}=125.281$, $p<0.001$, $F[surgery]_{1,36}=0.150$, $p=0.701$, $F[treatment]_{1,36}=0.137$, $p=0.713$, post hoc: at least $p<0.05$ compared to pre-surgical on all test days, paired t-test). In the DBA/2 strain, bodyweight was reduced on day 1 compared to pre-surgical weight and recovery occurred faster in the saline-treated groups ($F[day]_{3,114}=12.099$, $p<0.001$, $F[day \times treatment]_{3,114}=3.252$, $p<0.05$). In contrast to the C57BL/6 strain, final bodyweight did not exceed pre-surgical weight. Also in this strain, there was no main effect of surgery or treatment on any day ($F[surgery]_{1,38}=0.648$, $p=0.426$, $F[treatment]_{1,38}=3.098$, $p=0.086$).

DISCUSSION

The present data show similarities, but also profound differences, between the C57BL/6 and DBA/2 strains in behavioural and endocrine sensitisation to cocaine. We have identified one strain, the DBA/2 strain, in which repeated cocaine exposure induces sensitisation of both drug-induced locomotion and -corticosterone secretion. Furthermore, only in this strain, behavioural sensitisation was prevented

by ADX, suggesting that adrenal hormones facilitate sensitisation to the locomotor stimulant effects of cocaine and do so in a genotype-dependent manner.

In both strains, cocaine-treated mice expressed behavioural sensitisation in response to a challenge dose of cocaine (7.5 mg/kg) after a 5 day withdrawal period. Occurrence of sensitisation in both strains was considered an important requirement, as the sensitisation paradigm was subsequently employed to investigate the contribution of adrenal stress hormones. Experimental parameters that may influence susceptibility of the two strains to psychostimulant sensitisation include i) repeated drug administration within or beyond the test context [65], ii) the duration of the withdrawal interval [20,559], and iii) habituation to the test setting [20].

It has been argued that C57BL/6 mice are more susceptible to context-dependent sensitisation, while DBA/2 mice are more likely to develop context-independent sensitisation [20,65,559]. The observed hyperactivity of cocaine-treated C57BL/6 mice following saline administration in the test setting supports the notion that the C57BL/6 strain is susceptible to the influence of contextual information. Therefore, a 'mixed' design was used in which animals received cocaine both in the test cage (days 1, 9 and 15) and in the home cage (days 2-8) on several occasions. This might allow the development of either type of sensitisation resulting in augmented cocaine responsiveness in both strains. In addition, a withdrawal interval was chosen comparable to that employed in studies demonstrating expression of amphetamine sensitisation in both strains [65,559]. Furthermore, animals were habituated to the test setting 2 hours prior to drug administration, in order to minimise the contribution of strain differences in the behavioural and endocrine response to novelty [66]. Finally, mice were single housed to avoid variable social influences that could play a role during drug exposure in the home cage.

Pronounced strain differences were observed for cocaine responsiveness in the treatment phase. In contrast to DBA/2 mice, C57BL/6 mice did not display increased drug-responsiveness on the last (day 9) compared to the first day. In view of the augmented response of cocaine-treated C57BL/6 mice to the cocaine challenge, this observation is not likely to reflect a complete lack of sensitisation. As discussed in the previous paragraph, a longer time interval may need to elapse before sensitisation becomes expressed in the C57BL/6 strain. It is generally accepted that expression of behavioural sensitisation intensifies with prolonged withdrawal and that associated neuroadaptations change over time [497,520]. In addition, it is conceivable that C57BL/6 mice reached a ceiling response already at the first drug administration, while behavioural sensitisation could only be unmasked by the lower challenge dose of 7.5 mg/kg cocaine. Indeed, an acute administration of cocaine (15.0 mg/kg) resulted in pronounced hyperactivity in the C57BL/6 strain

while being virtually ineffective in the DBA/2 strain. This finding is coherent with data obtained on amphetamine [65,700,760], but discrepant to two reports on cocaine [483,665]. The discrepancy may result from aforementioned differences in experimental methods.

Striking strain differences were also observed for HPA-axis responsiveness of SHAM operated animals throughout the sensitisation paradigm. In the DBA/2 strain, repeated cocaine treatment resulted in a persistent sensitisation of drug-induced corticosterone secretion, that was still evident in response to a cocaine challenge after a withdrawal period. By contrast, in the C57BL/6 strain, cocaine attenuated rather than enhanced corticosterone secretion in the context of a stress-induced response (e.g. induced by i.p. injection in the test setting) and there was no difference between the first and last drug exposure of the treatment phase.

The observed HPA-axis sensitisation in DBA/2 mice is in line with previous reports showing that repeated amphetamine treatment can induce hypersecretion of corticosterone to a subsequent drug challenge, even after as little as one exposure [29,694]. Furthermore, cocaine-treated DBA/2 mice displayed an augmented corticosterone response to a saline challenge, that may either reflect conditioned activation of the HPA-axis in response to drug-associated stimuli specifically [169], or enhanced sensitivity to challenging situations in general. In support of the latter, it has been shown repeatedly that the effects of psychostimulants and stress on behavioural and endocrine sensitisation are interchangeable (e.g. [13,29,493]). Furthermore, a small degree of sensitisation was also observed during saline treatment, indicating that the HPA-axis of DBA/2 mice can sensitise to repeated treatment procedures. It is conceivable that cocaine induced more pronounced and persistent endocrine sensitisation due to its higher potency in activating the HPA-axis. Interestingly, morning corticosterone concentrations of cocaine-treated mice were elevated one day after the drug challenge. This finding supports the notion of a deregulation of HPA-axis activity, although the contribution of either drug anticipation or withdrawal symptoms to this phenomenon cannot be excluded. Further studies are necessary to clarify whether and to what extent the observed HPA-axis sensitisation of DBA/2 mice is specific for cocaine or cocaine-associated stimuli.

In agreement with previous studies, our findings indicate that sensitisation takes place at the level of the pituitary or its regulatory areas, since elevated basal ACTH levels were revealed in cocaine- compared to saline-treated DBA/2 mice in which the negative glucocorticoid feedback was relieved by ADX [29,610]. In addition, the elevated morning corticosterone concentrations observed in SHAM operated DBA/2 mice on day 16 did not correspond with elevated ACTH levels. We interpret this observation as hypersensitivity of the adrenal to ACTH. From these data it cannot

be concluded at what time point during the sensitisation paradigm the apparent adrenal hypperresponsiveness develops and how it contributes to the enhanced corticosterone responsiveness.

The observation that, in C57BL/6 mice, cocaine attenuated rather than enhanced corticosterone responses in comparison with saline, contradicts many reports showing enhanced HPA-axis activation in response to psychostimulants, including the present results for the DBA/2 strain. In an additional study (data not shown), we have confirmed that, in C57BL/6 mice, cocaine attenuates corticosterone secretion in the context of a stress-induced response over the entire time span of the endocrine response. Strain differences in functioning or wiring of the HPA-axis itself and of the neurotransmitter systems that regulate the cocaine-induced corticotrophin releasing hormone and corticosterone release may underlie the opposite effects of cocaine on stress-induced corticosterone secretion in the two strains [52,66,285,321,622]. Alternatively, the difference in corticosterone responsiveness may reflect different strategies for coping with drug-induced arousal [454]. In addition, cocaine may alter either the perception of stressful events (e.g. test- and treatment procedures), or the kinetics of HPA-axis activation in response to such events, in a strain-dependent manner.

We have made the remarkable observation that ADX prevents behavioural sensitisation to cocaine in a genotype-dependent manner. ADX prevented development and expression of sensitisation to the locomotor stimulant effects of cocaine in the DBA/2 strain, while being ineffective in the C57BL/6 strain. Strain differences have also been reported for these two inbred strains regarding the impact of stress on dopaminergic transmission, dopamine receptor expression, amphetamine-induced conditioned place preference, -locomotion, and -sensitisation, and stereotyped behaviour [20,67,69-71,533,704]. With respect to psychostimulant-induced behaviour, the DBA/2 strain is consistently found to be stress-responsive, while C57BL/6 mice appear resistant to environmental manipulations [20,67,71]. In the present report, we show a similar strain-dependency for the effects of ADX. ADX did not influence behavioural responsiveness to cocaine in the C57BL/6 strain, in which cocaine attenuated corticosterone responses, while fully preventing behavioural sensitisation in the DBA/2 strain, that displayed cocaine-induced corticosterone secretion and sensitisation thereof.

These data suggest that adrenal glucocorticoids, and possibly sensitisation of their release, contribute to behavioural sensitisation of DBA/2 mice to cocaine. Many studies have shown that glucocorticoid hormones have a facilitatory role in behavioural responses to psychostimulant drugs, such as locomotor activity, self-administration and relapse (reviewed in: [421]). Furthermore, strong evidence exists

that activation of the glucocorticoid receptor is critically involved in the glucocorticoid effects on drug responses [168,308]. In mice, basal levels of corticosterone that are considered sufficient for occupation of the high-affinity mineralocorticoid receptor remain after ADX (the present study, [300]), also pointing towards a role for the relatively low-affinity glucocorticoid receptor in behavioural sensitisation of the DBA/2 strain to cocaine. There were however no strain differences in residual corticosterone following ADX, excluding this as explanation for the strain-dependent effects of ADX on behaviour.

The finding that locomotor sensitisation to cocaine depends on the integrity of adrenal function only in the DBA/2 strain, suggests that DBA/2 mice are more sensitive to the impact of adrenal stress hormones on cocaine sensitivity than C57BL/6 mice. In this respect, it is interesting to note that the DBA/2 strain was also characterised by a higher degree of individual variability in behavioural responses to repeatedly administered cocaine than the C57BL/6 strain, a phenomenon that has been reported previously [483]. As inbred strains are considered to be genetically homogenous, individual differences are assumed to arise from epigenetic changes induced by variations in life-experiences, either in the past or in the present, further strengthening the notion that the neuroendocrine stress system may play a regulatory role in drug sensitivity of DBA/2 mice. However, the behavioural cocaine responses or the degree of sensitisation thereof among the SHAM operated DBA/2 mice did not correlate with corticosterone levels at any day. Moreover, there was very little variation in corticosterone responsiveness and sensitisation. Therefore, behavioural sensitisation of DBA/2 mice to cocaine may depend on glucocorticoids, and possibly on sensitisation of their release, but these hormones do not account for the full extent of individual variability. Further research is therefore required to verify the involvement of corticosterone and to investigate whether other adrenal factors such as epinephrine also contribute to cocaine sensitivity of DBA/2 mice.

It is of great interest to unravel the mechanisms via which adrenal stress hormones facilitate cocaine responsiveness in a genotype-dependent manner. Compared to C57BL/6 mice, DBA/2 mice display lower amphetamine-induced dopamine release in the nucleus accumbens as a consequence of higher drug-induced dopamine release in the prefrontal cortex [700,701]. It would be interesting to investigate whether a similar mechanism controls the strain differences in cocaine sensitivity and whether adrenal hormones modulate the balance between drug-induced dopamine release in the prefrontal cortex and the nucleus accumbens. 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]. Alternatively, the strain difference in dopamine D2 autoreceptor-postsynaptic receptor ratio between the cell body and terminal regions of the mesocorticolimbic dopamine system, being higher in DBA/2 mice, may have contributed to the differences in cocaine sensitivity. Moreover, it has been demonstrated that dopamine D1 and D2 receptor expression can be regulated by stressful experiences in a genotype-dependent manner [69].

The present finding, that adrenal stress hormones play an essential role in behavioural sensitisation to cocaine only in certain strains of animals, may provide an explanation for some of the discrepancies in literature regarding the contribution of adrenal hormones to psychostimulant sensitisation. Strong evidence for the role of adrenal glucocorticoids in behavioural sensitisation has been presented most notably by Piazza and co-workers (e.g. [168,510]), although conflicting data have also been reported [21,609]. In the light of the current data, these different outcomes can be accounted for by different genetic, and perhaps environmental, backgrounds. In addition, some of the conflicting data obtained with the C57BL/6 and DBA/2 strains might be explained by environmental and experimental factors that influence adrenal stress hormones.

In summary, the present results demonstrate that the C57BL/6 and DBA/2 inbred mouse strains not only differ profoundly in behavioural, but also in endocrine responsiveness to cocaine. In both strains, intermittent cocaine administration resulted in locomotor hyperresponsiveness to the cocaine challenge, while only the DBA/2 strain displayed endocrine sensitisation. Remarkably, ADX prevented behavioural sensitisation only in the DBA/2 strain, suggesting that in this strain, glucocorticoids facilitate behavioural sensitisation to cocaine. All together, these results suggest that adrenal stress hormones modulate behavioural sensitisation to cocaine in a genotype-dependent manner and that genotypic differences in cocaine sensitivity may arise not only from differences in reward related signalling but also from differential HPA-axis responsiveness.

ACKNOWLEDGEMENTS

This research was supported by NWO/INSERM/ZON grants 985-10-014 and 985-10-504 and by the Royal Netherlands Academy of Arts and Sciences. We gratefully acknowledge drs. L. Enthoven and ing. M. van der Mark for technical support and dr. O.C. Meijer for critically reading the manuscript.

3

Strain differences in the effects of adrenalectomy on the midbrain dopamine system: implication for behavioural sensitisation to cocaine

Inge E.M. de Jong, Peter J. Steenbergen and E. Ronald de Kloet

ABSTRACT

Adrenalectomy (ADX) abolishes behavioural sensitisation to cocaine in DBA/2, but not C57BL/6 inbred mice. The present study therefore tests the hypothesis that this ADX effect on behavioural sensitisation in the susceptible DBA/2 strain involves changes in midbrain dopamine systems that do not occur in the resistant C57BL/6 strain.

For that purpose, we have measured tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA and D1- and D2-like receptor binding in C57BL/6 and DBA/2 mice that were i) unoperated, ii) ADX or SHAM operated, or iii) ADX or SHAM operated and subjected to a cocaine sensitisation regimen (15.0 mg/kg cocaine on 9 consecutive days, followed by a 7.5 mg/kg challenge after a 5 day withdrawal). ADX prevented behavioural sensitisation to cocaine in the DBA/2, but not the C57BL/6 strain. Mice were sacrificed under basal conditions, in the latter case 24 hours after the cocaine challenge.

ADX did not affect markers of the dopamine system in drug naïve mice. By contrast, strain-dependent neuroadaptations were found in the midbrain dopamine system of mice subjected to the sensitisation regimen. In the DBA/2 strain, the sensitisation-resistant ADX mice were characterised by reduced D2 binding in the nucleus accumbens core and rostral caudate putamen (CP). Furthermore, ADX prevented the increase in TH and DAT mRNA expression in the substantia nigra (SN), and the decrease in D2 binding in the dorsomedial subdivision of the caudal CP associated with sensitisation in SHAM mice. In the C57BL/6 strain ADX only marginally affected dopaminergic adaptations.

These data suggest that adrenal hormones modulate behavioural sensitisation to cocaine in a genotype-dependent fashion possibly through adaptations in pre- and post-synaptic components of the midbrain dopamine system. During cocaine sensitisation, the DBA/2, but not the C57BL/6 strain, was susceptible to ADX in the dopamine system with respect to somatic TH and DAT and terminal D2 receptor expression.

INTRODUCTION

There are marked individual differences in behavioural responsiveness to psychostimulant drugs that depend on the interplay between heritable traits and environmental factors [191]. The midbrain dopamine system, which plays a critical role in the behavioural and reinforcing properties of psychostimulant drugs [202], provides an important substrate for gene x environment interactions underlying dopaminergic psychopathologies. Studies using inbred mouse strains have indicated that there is prominent genetic control over anatomy and function of the midbrain dopamine system and related behavioural output. Furthermore, environmental factors and most notably stress, facilitate the neurochemical, behavioural and reinforcing effects of psychostimulant drugs [241,510,635] and there is ample evidence that this depends on the interaction between the midbrain dopamine system and adrenal glucocorticoid hormones, that are secreted upon activation of the hypothalamic-pituitary-adrenal (HPA) axis (for reviews see: [421,515]).

Inbred mouse strains provide a valuable tool to study gene x environment interactions in the workings of the midbrain dopamine system. The C57BL/6 and DBA/2 mouse strains have been widely exploited as a model for genetic differences in dopaminergic transmission and associated behavioural output (reviewed in: [531]). In addition, the two strains exhibit differences in the behavioural and endocrine adaptation to stress [66,67,321,622]. With respect to behavioural responsiveness to psychostimulants, the DBA/2 strain is susceptible to environmental manipulations, whereas C57BL/6 mice appear resistant to stress [20,67,71]. The difference in susceptibility to environmental influences may in part be explained by strain-dependent adaptations of the midbrain dopamine system to stress, involving genotype-specific changes in dopamine D1 and D2 receptor density and -function, and in dopamine release and -metabolism in distinct brain regions [69,70,704].

In a previous study, we have investigated in more detail the contribution of adrenal stress hormones to cocaine sensitivity of the C57BL/6 and DBA/2 strains [145]. C57BL/6 mice were found to be more responsive to the locomotor stimulant effects of an initial cocaine exposure, whereas surgical removal of the adrenals (adrenalectomy: 'ADX') did not influence the acute drug response in either strain. By contrast, both strains exhibited psychomotor sensitisation after repeated drug exposure, while this was prevented by ADX in the DBA/2, but not the C57BL/6 strain. These findings indicate that adrenal stress hormones play an essential role in behavioural sensitisation to cocaine, but only in the DBA/2 strain.

The present study was designed to investigate whether a neural correlate for the strain differences in behavioural responsiveness to cocaine and the contribution of adrenal stress hormones can be found in the midbrain dopamine system. The hypothesis is tested that DBA/2 and C57BL/6 strains are susceptible and resistant, respectively, to the effects of ADX on midbrain dopamine transmission. For this purpose, we have measured dopaminergic markers in the mesocorticolimbic and nigrostriatal dopamine systems of C57BL/6 and DBA/2 mice that were i) unoperated, ii) ADX or SHAM operated, or iii) ADX or SHAM operated and subjected to a cocaine sensitisation regimen. We have determined tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression and D1- and D2-like receptor binding using the radiolabelled ligands SCH23390 and iodosulpride respectively. Although these ligands cannot dissociate between the D1 (D1 and D5) and D2 (D2, D3 and D4) receptor subtypes, they allow for discrimination between the two dopamine receptor families.

METHODS

General methods

Animals

Male C57BL/6 Rj (C57BL/6) and DBA/2 Rj (DBA/2) mice were obtained from Janvier (Le Genest Saint Isle, France) and received in the animal facility at the age of 8 weeks. Mice were housed in groups of four of the same strain in perspex cages (35x19x14cm) with food and water available *ad libitum* at a 12 hour light-dark cycle (lights on: 7 am) in a temperature (21±1ºC) and humidity (55±5%) controlled room. Experiments started two weeks after arrival in the animal facility. Prior to this, animals were briefly handled and otherwise left undisturbed. Animal experiments were approved by the local Committee for Animal Health, Ethics and Research of Leiden University. Animal care was conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC).

Surgery

In experiments 2 and 3, animals were adrenalectomised (ADX) or SHAM operated. Mice were individually housed 1 day prior to surgery. The cages were transported to the operating room on the morning of the surgery where animals were allowed to recover from transportation for 2 hours. Inhalation anaesthesia consisted of a mixture of isoflurane (3 l/min), N_2O (0.8 l/min) and O_2 (0.4 l/min). Adrenalectomy was performed via the dorsal route as described previously [145]. SHAM animals were

treated similarly with the exception of the actual removal of the adrenals. ADX effectively clamped plasma corticosterone to basal concentrations and only animals with successful ADX were included in the study.

Tissue preparation
In all experiments, animals were sacrificed in the morning (3-5 hours after lights on) under basal conditions. Trunk blood was collected for endocrine measurements and brains were rapidly dissected, snap frozen in isopentane (cooled in ethanol placed on pulverised dry ice) and stored at -80 ºC until further use.

Sections of 16 µm were cut on a cryostat (Leica CM3050). Based on the brain atlas of Franklin and Paxinos [220] (figure 1), sections were taken at the level of the nucleus accumbens (NAc: core and shell subregions, bregma +1.44), rostral caudate putamen (rCP, bregma +1.44), caudal CP (cCP: lateral (Lat), dorsomedial (DM) and ventromedial (VM) subsections, bregma +0.14), ventral tegmental area (VTA,

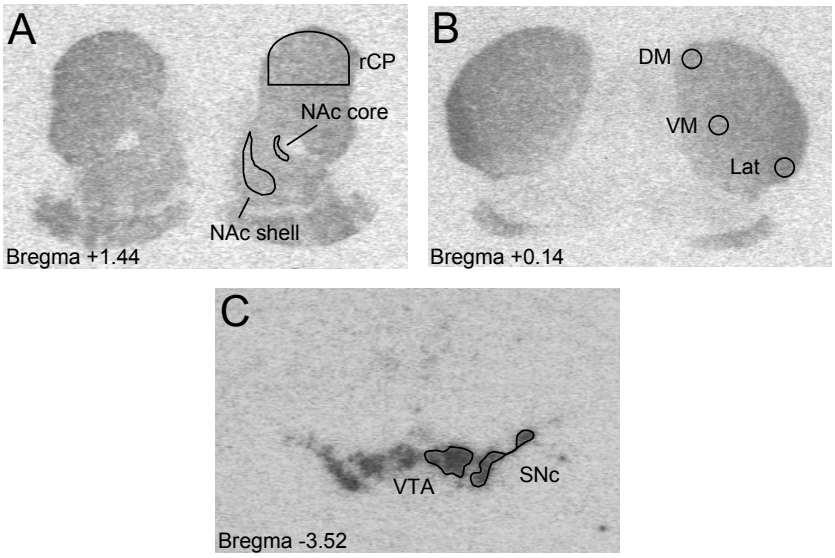

Figure 1: *Coronal sections of the mouse brain, indicating the areas sampled for optical density measurement.*
Autoradiographs at the level of A) the nucleus accumbens (NAc) and rostral caudate putamen (rCP), and B) caudal caudate putamen after binding of ^{125}I-iodosulpride, and C) the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) after hybridisation with the TH mRNA specific oligonucleotide probe. The line-shapes indicate the areas sampled for optical density measurement. DM: dorsomedial, VM: ventromedial, Lat: lateral.

bregma -3.52) and substantia nigra pars compacta (SNc, bregma -3.52). Sections were thaw-mounted on 0.001% poly-L-lysine (Sigma-Aldrich) coated slides and stored at -80 ºC until further use.

In situ hybridisation

Tyrosine hydroxylase (TH) and dopamine transporter (DAT)

In situ hybridisation was performed to investigate the mRNA expression of TH and DAT in the cell body regions of the midbrain dopamine systems. The oligonucleotides (table 1) were 3′ end-labelled with α [^{33}P]deoxyadenosine triphosphate (Perkin Elmer, Groningen, The Netherlands, 3000 Ci/mmol, 10 mCi/ml) using terminal deoxynucleotidyl transferase (GE Healthcare Europe Gmbh, Diegem, Belgium) according to the manufacturer's protocol.

Table 1: *The TH and DAT mRNA-specific oligonucleotides.*

TH	**Match**	5′-ATTTAGCTAATGGCACTCAGTGCTTGGGTCAGGGTGTGCAGCTCA-3′
	Mismatch	5′-ATTTAG**A**TAATGG**A**ACTCAG**G**GCTTGG**T**TCAGGGT**T**TGCAG**A**TCA-3′
DAT	**Match**	5′-CTTGTCCTCCGTGGCTCAGAACAGACCCTGCGTGTGTGTAATA-3′
	Mismatch	5′-CTTGT**A**TCCGTG**T**CTCAGA**C**CAGACC**A**TGCGTG**G**GTGTAA**G**A-3′

Sequences of the 45 and 43-mer oligonucleotide probes complementary to respectively tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA. To control for specificity, oligonucleotides were used that were identical, except for 6 mismatches (transversions, underlined) evenly spaced by 5-7 nucleotides.

For prehybridisation purposes, sections were fixed for 30 min. in 4% para-formaldehyde in PBS (pH 7.4), washed twice in PBS, acetylated in 0.1 M triethanolamine (pH 8.0) with 0.25% acetic anhydride for 10 min., rinsed for 10 min. in 2 x SSC (150 mM sodium chloride, 15 mM sodium citrate), dehydrated in an ethanol series, delipidated in chloroform for 1 min., air dried and stored at room temperature. To each slide, hybridisation mix (100 µl, containing 1 x 10^6 dpm. of labelled oligonucleotide) was applied. The hybridisation mix consisted of 50% formamide, 10% dextran sulfate, 4 x SSC, 25 mM sodium phosphate (pH 7.0), 1 mM Na pyrophosphate, 20 mM DTT, 5 x Denhardt's, 100 µg/ml poly-A and 100 µg/ml sheared herring sperm DNA. Sections were coverslipped and hybridised overnight in a moist chamber at 42 °C. The next morning, after removal of the coverslips, sections were rinsed in 1 x SSC at room temperature, washed twice for 30 min. in 1 x SSC at 50 °C, washed for 5 min. in 1 x SSC at room temperature, dehydrated in an ethanol series, air dried and

apposed to Kodak Biomax MR film (Kodak, Rochester, NY). After development of the films, sections were counter-stained with 0.5% cresyl violet.

Radioligand binding

D1 receptors

D1 receptor binding sites were analysed by in vitro autoradiography using the ^{3}H-labelled D1-like receptor antagonist SCH23390. Sections were pre-incubated in a 50 mM Tris buffer (containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.5) for 60 min. at room temperature. Subsequently, sections were incubated with 1 ml of the Tris buffer containing 2 nM ^{3}H-SCH23390 (GE Healthcare Europe Gmbh, Diegem, Belgium, 66 Ci/mmol, 1 mCi/ml) for 60 min. at room temperature. As SCH23390 has been reported to bind to serotonergic sites, 1 µM mianserin hydrochloride (Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands) was added to the incubation buffer. Non-specific binding was determined in the presence of 40 µM R(+)-SCH23390 hydrochloride (Sigma-Aldrich). After incubation, sections were drained, dipped in distilled water (4 ºC), washed two times in the Tris buffer (4 ºC) for 10 min., dipped in distilled water (4 ºC) and air-dried. Sections were apposed to a Kodak Biomax MS film (Kodak, Rochester, NY).

D2 receptors

Binding sites of the D2 receptor family were analysed by in vitro autoradiography using the ^{125}I-labelled D2-like antagonist iodosulpride. Sections were pre-incubated in a 50 mM Tris buffer (containing 5.7 mM ascorbic acid, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.0) for 60 min. at room temperature. Subsequently, sections were incubated with 0.4 ml of the Tris buffer containing 0.1 nM ^{125}I-iodosulpride (GE Healthcare Europe Gmbh, Diegem, Belgium, 2000 Ci/mmol, 100 µCi/ml) for 30 min. at room temperature. Non-specific binding was determined in the presence of 2 µM haloperidol (Sigma-Aldrich). After incubation, sections were drained, dipped in distilled water (4 ºC), washed two times in the Tris buffer (4 ºC) for 3 min., dipped in distilled water (4 ºC) and air-dried. Sections were apposed to a Kodak Biomax MS film (Kodak, Rochester, NY).

Analysis of mRNA expression and radioligand binding

Autoradiographs were digitised and relative expression levels or binding densities (grey values on film) were determined by computer-assisted optical densitometry using Analysis (analySIS 3.1, Soft Imaging System GmbH) and Scion Image software (a PC-version of the Macintosh program NIH Image: http://rsb.info.nih.gov/nih-image), available from Scion Corporation: http://www.scioncorp.com/). Standard

curves of ^{14}C (*in situ* hybridisation and D2 binding) and ^{3}H (D1 binding) were included to ensure that grey values were within the linear range between 0 and 255. Average optical density per region (in arbitrary units) was corrected for optical density in an adjacent region where no mRNA expression / receptor binding was observed (deep mesencephalic nucleus for VTA and SNc, corpus callosum for NAc, rCP and cCP).

Experimental design

Experiment 1: *The basal dopamine system*
Drug naïve C57BL/6 and DBA/2 mice were sacrificed under basal conditions two weeks after arrival in the animal facilities.

Experiment 2: *The effects of ADX on the dopamine system of drug naïve mice*
Mice of both strains were adrenalectomised (ADX) or SHAM operated and sacrificed after a post-surgery interval of 3 weeks, which is an identical protocol as used in experiment 3.

Experiment 3: *The effects of ADX and cocaine sensitisation on the dopamine system*
C57BL/6 and DBA/2 mice were ADX or SHAM operated and, after a recovery period of one week, subjected to a cocaine sensitisation regimen as described previously [145]. In brief, animals received i.p. injections of 15.0 mg/kg cocaine (COC, BUFA Pharmaceuticals B.V., Uitgeest, The Netherlands) or saline (SAL) on 9 consecutive days, followed by a withdrawal interval of 5 days, a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. On days 1, 9, 14 and 15, locomotor responses were recorded on video and analysed using Ethovision Videotracking, Motion Analysis & Behavior Recognition System version 1.96 ('VTMAS', Noldus Information Technology B.V., Wageningen, The Netherlands). Previously we discovered that ADX prevented initiation and expression of behavioural sensitisation to cocaine in the DBA/2, but not the C57BL/6 strain [145]. Mice were sacrificed in the morning of day 16, 24 hours after the cocaine challenge and 3 weeks after surgery.

Statistics

Statistical analysis was performed using SPSS for Windows software (release 7.5, SPSS Benelux B.V., Gorinchem, The Netherlands). In experiments 1 and 2, expression and binding data were subjected to ANOVA with strain (experiment 1) or strain and surgery (experiment 2) as factor(s). In experiment 3, analyses were performed per strain (two-factor ANOVA for surgery and treatment). When statistical

significance was revealed, post hoc tests were performed (Fisher's LSD). Differences were considered statistically significant when $p<0.05$.

RESULTS

The basal dopamine system

In drug naïve mice, either unoperated (experiment 1) or subjected to SHAM surgery (experiment 2), replicable strain differences were found for the dopaminergic markers, which are summarised in the following paragraphs.

SNc and VTA

Tables 2A and 3A depict the dopaminergic markers in somatic regions of unoperated or SHAM operated mice respectively. D1 receptor binding was undetectable and is therefore not shown. TH mRNA expression was higher in DBA/2 than in C57BL/6 mice, both in the SNc and the VTA. Similarly, DBA/2 mice displayed higher DAT mRNA expression in the SNc and a comparable, but non-significant, tendency was observed in the VTA. In agreement with previous reports, D2 receptor binding was higher in DBA/2 compared to C57BL/6 mice in the two dopaminergic cell groups.

Both TH and DAT mRNA are present within the dopaminergic neurons. Similarly, D2-like receptors in the cell body regions can function as autoreceptors and are localised on the dopaminergic neurons themselves. As all three markers were found to be higher in DBA/2 compared to C57BL/6 mice, we hypothesised that DBA/2 mice possess greater numbers of dopaminergic neurons. To test this, in experiment 1, the number of TH-positive cells was quantified in *in situ* hybridisation slides that were dipped in photographic emulsion and counterstained with Hoechst to visualise cell nuclei. Indeed, the number of TH-positive cells was higher in DBA/2 compared to C57BL/6 mice in the SNc (21%) and the VTA (32%) (table 2A).

NAc and CP

Strain differences were also observed in D1 and D2 receptor binding in the projection areas of the midbrain dopamine system (tables 2B and 3B), with a specific pattern for each receptor. DBA/2 mice displayed higher D1 binding in the NAc when compared to C57BL/6 mice, which was more pronounced in the shell compared to the core subdivision and a similar trend was observed in the rostral CP, which reached significance in experiment 2. Because this finding contradicts a previous report [69] we have in addition measured D1 receptor mRNA expression with a specific riboprobe and found this to correspond with receptor binding (DBA/2

Table 2: *The basal dopamine system.*

A

		C57BL/6	DBA/2	F
TH	**SNc**	47.8 ± 1.5	53.7 ± 1.5 *	$F_{1,19}$=8.149, p<0.05
	VTA	60.2 ± 3.1	72.3 ± 2.3 **	$F_{1,19}$=9.849, p<0.01
DAT	**SNc**	52.7 ± 1.4	58.7 ± 1.8 *	$F_{1,19}$=6.714, p<0.05
	VTA	50.5 ± 2.0	56.4 ± 2.3	$F_{1,18}$=3.811, p=0.068
D2	**SNc**	43.9 ± 0.7	48.2 ± 0.6 ***	$F_{1,18}$=19.672, p<0.001
	VTA	42.6 ± 0.3	48.0 ± 0.9 ***	$F_{1,19}$=30.891, p<0.001
TH+	**SNc**	66.2 ± 2.6	80.1 ± 5.8 *	$F_{1,19}$=4.755, p<0.05
	VTA	113.6 ± 8.6	149.9 ± 9.8 *	$F_{1,19}$=7.770, p<0.05

B

		C57BL/6	DBA/2	F
D1	**NAc core**	15.9 ± 0.7	18.2 ± 0.8 *	$F_{1,19}$=4.783, p<0.05
	NAc shell	13.3 ± 0.8	17.8 ± 0.6 ***	$F_{1,17}$=22.031, p<0.001
	rCP	22.4 ± 1.1	23.8 ± 0.9	$F_{1,19}$=0.945, p=0.344
	cCP Lat	20.5 ± 1.3	18.1 ± 1.0	$F_{1,19}$=2.086, p=0.166
	cCP DM	21.2 ± 1.3	20.8 ± 1.2	$F_{1,19}$=0.068, p=0.797
	cCP VM	17.7 ± 1.2	18.1 ± 1.0	$F_{1,19}$=0.081, p=0.780
D2	**NAc core**	30.7 ± 1.3 *	25.3 ± 2.0	$F_{1,18}$=5.020, p<0.05
	NAc shell	29.7 ± 1.5	27.7 ± 1.3	$F_{1,17}$=1.016, p=0.329
	rCP	42.8 ± 0.9 **	36.9 ± 1.1	$F_{1,15}$=16.969, p<0.01
	cCP Lat	49.3 ± 1.4	56.8 ± 1.8 **	$F_{1,17}$=10.524, p<0.01
	cCP DM	40.3 ± 0.9 *	35.9 ± 1.5	$F_{1,18}$=6.871, p<0.05
	cCP VM	34.8 ± 1.2	34.8 ± 1.2	$F_{1,18}$=0.001, p=0.976

Tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression, TH+ cell numbers and D1 and D2 receptor binding in the somatic (A) and dendritic (B) dopaminergic subfields of unoperated, drug naïve C57BL/6 and DBA/2 mice. Data represent optical density on film (arbitrary units) ± SEM. n=8-10 animals/group. *** p<0.001, ** p<0.01, * p<0.05 vs. the other strain (Fisher's LSD). SNc: substantia nigra pars compacta, VTA: ventral tegmental area, NAc: nucleus accumbens, (r/c) CP (Lat,DM,VM): (rostral/caudal) caudate putamen (lateral, dorsomedial, ventromedial regions).

Table 3: *The effects of ADX on the basal dopamine system.*

A

		C57BL/6		DBA/2	
		ADX	SHAM	SHAM	ADX
TH	SNc	55.4 ± 2.6	49.7 ± 2.0	57.9 ± 2.1 *	56.5 ± 2.1
	VTA	49.5 ± 2.5	47.1 ± 1.6	52.8 ± 1.4 *	57.2 ± 2.3 *
DAT	SNc	31.8 ± 1.2	33.9 ± 0.8	40.0 ± 1.1 **	36.8 ± 1.9 *
	VTA	32.2 ± 1.5	31.1 ± 1.1	32.7 ± 1.9	30.3 ± 0.7
D2	SNc	35.2 ± 0.6 #	37.1 ± 0.5	42.3 ± 0.2 ***	42.9 ± 0.6 ***
	VTA	33.2 ± 0.4	34.2 ± 0.4	36.1 ± 0.5 **	35.5 ± 0.3 **

B

		C57BL/6		DBA/2	
		ADX	SHAM	SHAM	ADX
D1	NAc core	28.4 ± 1.3	27.4 ± 1.1	31.5 ± 1.2 *	28.8 ± 1.2
	NAc shell	23.3 ± 1.5	20.1 ± 1.0	25.8 ± 1.5 *	24.9 ± 2.4
	rCP	33.9 ± 1.1	34.4 ± 1.0	37.9 ± 1.5 *	35.4 ± 1.2
	cCP Lat	34.3 ± 0.9	34.8 ± 0.9	33.6 ± 1.5	31.4 ± 1.2
	cCP DM	32.3 ± 0.8	32.9 ± 0.8	35.4 ± 2.1	33.5 ± 1.5
	cCP VM	30.7 ± 1.2	31.7 ± 0.9	33.3 ± 1.9	30.9 ± 1.3
D2	NAc core	23.6 ± 1.7 **	22.5 ± 0.8 *	16.6 ± 2.2	16.6 ± 1.3
	NAc shell	19.3 ± 2.5	17.6 ± 1.8	13.5 ± 1.1	14.9 ± 1.3
	rCP	33.3 ± 1.4 *	34.4 ± 0.7 *	30.7 ± 1.1	28.6 ± 1.6
	cCP Lat	41.7 ± 1.6	40.8 ± 1.5	47.0 ± 2.6 *	46.8 ± 2.7
	cCP DM	32.2 ± 2.2	32.4 ± 1.6	34.1 ± 2.4	31.8 ± 1.7
	cCP VM	29.6 ± 1.2	28.5 ± 1.9	31.0 ± 1.6	29.1 ± 1.9

Tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression and D1 and D2 receptor binding in the somatic (A) and dendritic (B) dopaminergic subfields of drug naïve C57BL/6 and DBA/2 mice 3 weeks after adrenalectomy (ADX) or SHAM surgery. Data represent optical density on film (arbitrary units) ± SEM. n=5-12 animals/group. *** $p<0.001$, ** $p<0.01$, * $p<0.05$ vs. mice of the other strain subjected to similar surgery. # $p<0.05$ vs. SHAM operated mice of the same strain (Fisher's LSD). SNc: substantia nigra pars compacta, VTA: ventral tegmental area, NAc: nucleus accumbens, (r/c) CP (Lat,DM,VM): (rostral/caudal) caudate putamen (lateral, dorsomedial, ventromedial regions).

vs. C57BL/6: NAc core 38.3 vs. 28.0, $p<0.05$, NAc shell 38.8 vs. 29.0, $p<0.01$, rCp: 39.5 vs. 34.8, ns.). There were no strain differences in D1 binding or mRNA expression in the three caudal subdivisions of the CP. By contrast, C57BL/6 mice exhibited greater D2 receptor binding in NAc core and the rCP. In experiment 1, a similar effect was observed in the DM subdivision of the cCP, but this could not be replicated in experiment 2. In the NAc shell, there was no significant strain difference in D2 binding, although there was a tendency towards higher densities in the C57BL/6 strain. In the Lat cCP by contrast, D2 binding was consistently found to be higher in DBA/2 when compared to C57BL/6 mice.

The effects of ADX on the dopamine system of drug naïve mice

Table 3 depicts the dopaminergic markers measured in drug naïve SHAM and ADX mice of both strains. D2 receptor binding was slightly reduced by ADX only in the SNc of the C57BL/6 strain ($F[strain]_{1,30}=134.245$, $p<0.001$, $F[surgery]_{1,30}=1.222$, $p=0.279$, $F[strain \times surgery]_{1,30}=5.080$, $p<0.05$, post hoc: $p<0.05$). The strain differences in D1 binding in the NAc and rCP were attenuated by ADX, but a significant surgery effect was not revealed in any of the subregions. No other effects of ADX on the dopaminergic markers were detected in either strain.

The effects of ADX and cocaine sensitisation on the dopamine system

In mice subjected to the sensitisation regimen, there were strain- and surgery-specific adaptations in D2 receptor binding (figures 2 and 3) and TH and DAT mRNA expression (table 4) in subfields of the nigrostriatal system. In the DBA/2 strain, D2 receptor binding was reduced in the NAc core and rCP of ADX mice resistant to behavioural sensitisation when compared to sensitised SHAMs, and a similar trend was observed in the NAc shell (core: $F[surgery \times treatment]_{1,37}=4.220$, $p<0.05$, shell: $F[treatment]_{1,37}=4.358$, $p<0.05$, rCP: $F[treatment]_{1,36}=5.048$, $p<0.05$, $F[surgery \times treatment]_{1,36}=4.138$, $p<0.05$, post hoc: core and rCP: $p<0.05$, shell: $p=0.155$ vs. SHAM/COC). Furthermore, in the DBA/2 strain, cocaine treatment was associated with an increase in TH and DAT mRNA expression in the SNc (TH: $F[treatment]_{1,36}=5.539$, $p<0.05$, DAT: $F[treatment]_{1,36}=4.873$, $p<0.05$, post hoc: $p<0.05$ vs. SHAM/SAL) and a reduction in D2 binding in the DM subdivision of the cCP ($F[treatment]_{1,38}=4.965$, $p<0.05$, post hoc: $p<0.05$ vs. SHAM/SAL), which were prevented by ADX. The effects of treatment and ADX on D2 binding in the DM cCP were also observed in the C57BL/6 strain ($F[treatment]_{1,30}=7.008$, $p<0.05$, post hoc: $p<0.05$ vs. SHAM/SAL). There were no other effects of treatment or surgery in either strain (data not shown).

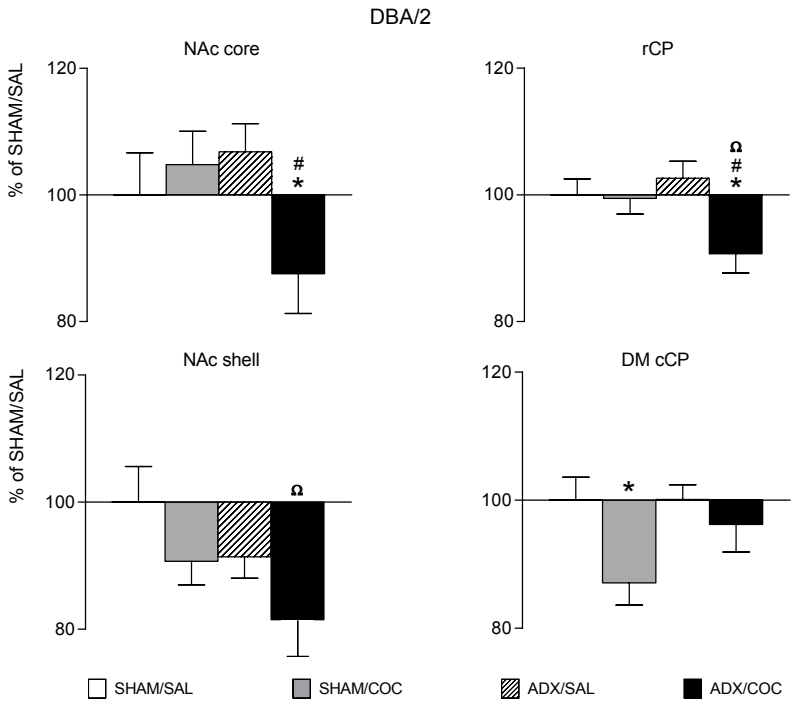

Figure 2: *D2 binding in DBA/2 mice subjected to the sensitisation regimen.*
D2 receptor binding in adrenalectomised (ADX) or SHAM operated DBA/2 mice subjected to the cocaine sensitisation regimen. Previously, animals received 15.0 mg/kg cocaine (COC) or saline (SAL) for 9 consecutive days, followed by a 5 day withdrawal interval and a 7.5 mg/kg cocaine challenge. Animals were sacrificed 24 hours after the drug challenge. Data are presented as percentage of SHAM/SAL (%) ± SEM. n=6-13 animals/group.
* $p<0.05$ vs. SAL-treated mice subjected to similar surgery, # $p<0.05$ vs. SHAM/COC, and Ω $p<0.05$ vs. SHAM/SAL (Fisher's LSD). NAc: nucleus accumbens, (r/c) CP (DM): (rostral/caudal) caudate putamen (dorsomedial region).

DISCUSSION

This study shows strain differences in the effects of ADX on neuroadaptations in the midbrain dopamine system in mice that were subjected to a 9 day cocaine sensitisation regimen. The DBA/2 strain was susceptible to adrenal hormones on measures for dopamine in the nigrostriatal system and the NAc core under these conditions of behavioural sensitisation, whereas ADX only marginally affected the dopamine system in the C57BL/6 strain. This confirms our hypothesis that the DBA/2 strain displays parallel susceptibility to the impact of adrenal hormones on behavioural and neurochemical responsiveness to cocaine. Therefore, these results suggest

Table 4: *TH and DAT mRNA expression in the SNc of mice subjected to the sensitisation regimen.*

			SHAM		ADX	
			SAL	COC	SAL	COC
TH	**SNc**	**C57BL/6**	100.0 ± 2.6	100.6 ± 2.4	94.6 ± 3.8	96.3 ± 1.7
		DBA/2	100.0 ± 2.3	106.1 ± 1.6 *	98.8 ± 1.8	101.6 ± 1.8
DAT	**SNc**	**C57BL/6**	100.0 ± 4.3	104.4 ± 3.2	103.0 ± 3.9	95.1 ± 3.4
		DBA/2	100.0 ± 2.0	111.9 ± 3.2 *	99.0 ± 4.6	103.2 ± 3.5

Tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression in the substantia nigra pars compacta (SNc) of adrenalectomised (ADX) or SHAM operated C57BL/6 and DBA/2 mice subjected to the cocaine sensitisation regimen. Previously, animals received 15.0 mg/kg cocaine (COC) or saline (SAL) for 9 consecutive days, followed by a 5 day withdrawal interval and a 7.5 mg/kg cocaine challenge. Animals were sacrificed 24 hours after the drug challenge. Data are presented as percentage of SHAM/SAL (%) ± SEM. n=7-11 animals/group. * $p<0.05$ vs. SAL-treated mice subjected to similar surgery.

that adrenal stress hormones influence psychostimulant sensitivity in a genotype-dependent manner through adaptations in the midbrain dopamine system.

Compared to the C57BL/6 strain, the DBA/2 strain is less sensitive to the neurochemical-, rewarding- and behavioural effects of amphetamine [65,71,124,447,611,700,701,760], and the locomotor-stimulant effect of cocaine [145]. This may result from strain differences in both pre- and/or post-synaptic transmission in the midbrain dopamine system, aspects of which were investigated using the dopaminergic markers.

The greater number of TH-positive neurons in the DBA/2 strain would imply greater dopaminergic input in the NAc and CP from the A9/A10 cell groups. By contrast, DBA/2 mice exhibit lower amphetamine-induced dopamine release in the NAc [701,760] whereas there are no strain differences in basal dopamine release in this brain region [760]. The apparent discrepancy between the midbrain dopaminergic cell numbers and the dopamine release indicates that there must be strain differences in presynaptic dopaminergic function. Indeed, the number of dopaminergic neurons can [212], but does not necessarily [753], correlate with mesencephalic TH activity. In the present study, TH mRNA expression was increased to a lesser extent (12-20%) than the number of dopaminergic cells (20-32%) in the DBA/2 compared to C57BL/6 strain, which is suggestive of a relatively lower TH function in the former strain. However, from mRNA expression it is not possible to draw a definitive conclusion regarding TH protein content and activity [373].

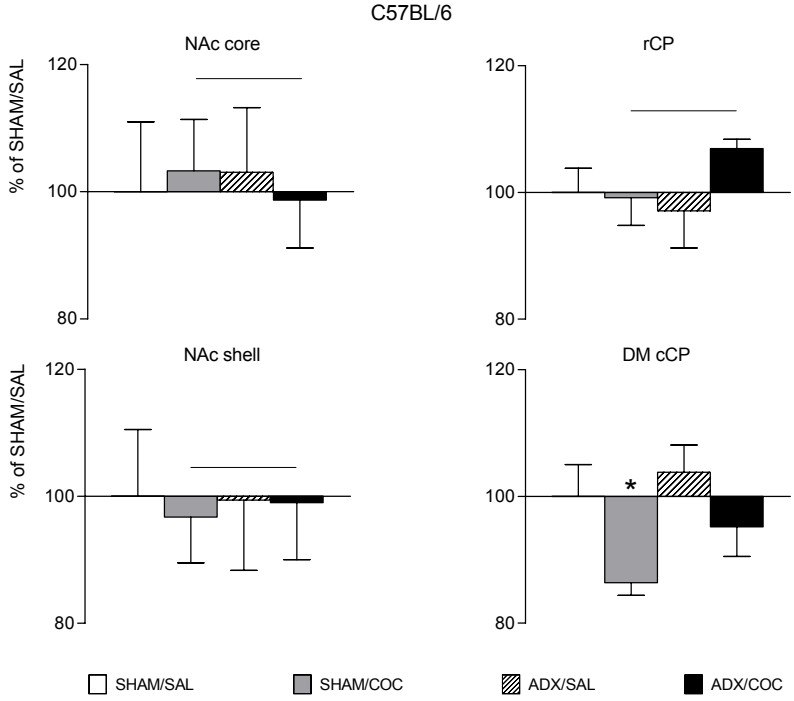

Figure 3: *D2 binding in C57BL/6 mice subjected to the sensitisation regimen.* D2 receptor binding in adrenalectomised (ADX) or SHAM operated C57BL/6 mice subjected to the cocaine sensitisation regimen. Previously, animals received 15.0 mg/kg cocaine (COC) or saline (SAL) for 9 consecutive days, followed by a 5 day withdrawal interval and a 7.5 mg/kg cocaine challenge. Animals were sacrificed 24 hours after the drug challenge. Data are presented as percentage of SHAM/SAL (%) ± SEM. n=6-8 animals/group. * $p<0.05$ vs. SAL-treated mice subjected to similar surgery (Fisher's LSD). NAc: nucleus accumbens, (r/c) CP (DM): (rostral/caudal) caudate putamen (dorsomedial region).

Factors that control dopaminergic transmission, possibly in a genotype-dependent manner, include regulation by afferent projections and by autoreceptors of the D2 receptor family. Indeed, when compared to the C57BL/6 strain, the DBA/2 strain displays lower drug-induced dopamine release in the NAc as a consequence of higher dopamine release in the prefrontal cortex [700,701]. Furthermore, DBA/2 mice have been proposed to exhibit greater autoreceptor-mediated inhibition of dopaminergic transmission, due to the higher D2 receptor availability in the VTA and SN [69,470]. However, the observed difference in cell numbers suggests that the higher D2 receptor binding in the somatic regions of the DBA/2 strain reflects a greater number of neurons, rather than a higher density of receptors per cell. Further studies linking dopaminergic cell counts to D2 receptor numbers and function will be

necessary to evaluate the capacity for autoinhibition of dopaminergic transmission in the two strains. Collectively, these findings suggest that presynaptic regulation of dopaminergic neurotransmission may be a crucial factor in restraining the higher dopaminergic output that would be expected to arise from the greater numbers of TH positive neurons in the DBA/2 strain.

Pronounced strain differences were also found for dopamine receptor binding in the terminal fields, which are in agreement with most previous findings [69,199,470]. Most remarkable is the opposite strain difference in D1 and D2 binding in the NAc and rCP. How the regionally differentiated dopamine receptor binding contributes to the strain differences in behavioural responsiveness to psychostimulants remains to be investigated. One avenue of further research could involve the implication of the various dopamine receptor subtypes of the D1 and D2 families, that have partially overlapping but also distinct functional characteristics.

We have demonstrated previously, that ADX does not alter the locomotor response of C57BL/6 and DBA/2 mice to a single administration of cocaine [145]. In parallel, no major changes were observed in the basal dopamine system following ADX. By contrast, studies in several strains of rats have indicated that depletion of adrenal glucocorticoids attenuates basal and cocaine-induced dopamine release and psychostimulant-induced locomotion [77,166,420,423,508,580] and substantially reduces D1 and D2 receptor binding in subdivisions of the dopamine system [47,136]. The discrepancy between the present findings in mice and the observations in rats may be related to the residual corticosterone after ADX that circulates in mice, but not in rats [300]. Indeed, it has been demonstrated in rats that corticosterone in the range of diurnal concentrations is sufficient to restore the effects of ADX on basal dopamine release and on neurochemical and behavioural responsiveness to an acute psychostimulant exposure [30,77,423,508].

ADX prevented behavioural sensitisation in the DBA/2, but not the C57BL/6 strain [145]. Strain- and surgery-specific adaptations were also observed in the midbrain dopamine system of mice subjected to the sensitisation regimen. Given that a single psychostimulant exposure can induce long-term behavioural, neuroendocrine, and neurochemical sensitisation [694], it should be noted that none of the groups were drug naïve (all animals were exposed to the cocaine challenge one day prior to sacrifice).

In the DBA/2 strain, TH and DAT mRNA expression were increased in the SNc of sensitised SHAM mice, suggesting an enhanced tone of the DA system, which is in agreement with previous studies employing comparable withdrawal durations (<1 week) [33,406,623,633,713]. The enhanced expression of the presynaptic markers, which we interpret as increased capacity of nigrostriatal dopaminergic neurons under basal

conditions, was fully prevented by ADX in the DBA/2 strain, thus correlating with behavioural responsiveness to the drug challenge. Although it cannot be concluded whether the increases in TH and DAT mRNA are critical for expression of sensitisation, it does reflect the plasticity of the presynaptic component of the dopamine system in the DBA/2 strain. In the C57BL/6 strain, by contrast, sensitisation did not correlate with enhanced expression of presynaptic markers, which might indicate that this neural adaptation is not required for behavioural sensitisation in the C57BL/6 background. Alternatively, given the marked time-dependency in neuroadaptations associated with psychostimulant sensitisation, the presynaptic adaptations could occur after a different withdrawal duration in the C57BL/6 strain [497,520].

The finding that D2 family receptors are subject to regulation by adrenal hormones in a genotype-dependent manner, is in line with previous observations that D2 receptors engage in gene x environment interactions, both in humans and in laboratory rodents [41,69,198]. The sensitisation-resistant ADX mice of the DBA/2 strain exhibited reduced D2 receptor binding in the NAc core and rCP, with a similar trend in the NAc shell. As discussed previously, D2-like receptors within the terminal regions may represent both pre- and postsynaptic receptors, the former functioning as inhibitory autoreceptors. Given the lack of behavioural sensitisation in ADX mice of the DBA/2 strain, it is tempting to speculate that the decrease in D2 binding reflects a deficit in postsynaptic signalling. Previous studies have demonstrated that supersensitivity of postsynaptic D2 receptors may play a role in long-term expression of behavioural sensitisation to amphetamine [306,675,695]. Furthermore, concurrent activation of D1 and D2 receptors is required for expression of neural- and behavioural phenotypes of cocaine sensitisation [87]. Therefore, the reduction in D2 binding in the NAc and CP might precipitate the sensitisation deficit of DBA/2 mice in which the adrenals were surgically removed.

By contrast, in sensitised SHAM mice of both strains, D2 binding was reduced in the DM subdivision of the cCP. Previous studies have demonstrated subsensitivity of axonal and somatodendritic D2 dopamine autoreceptors during the first few days of withdrawal from repeated psychostimulant administration [3,736,744,748]. The decrease in D2 binding associated with a history of cocaine treatment may therefore represent such an autoreceptor subsensitivity. The observation that ADX prevented the reduction in D2 binding in the DM cCP of *both* strains, suggests that the adaptation in this brain region is not critical for the impact of adrenal hormones on behavioural sensitisation. Clearly, the role of the regional changes in D2 receptor binding in behavioural sensitisation requires further investigations. Furthermore, it will be necessary to distinguish between the D2 and D3 receptors, given that activation of D3 receptors is thought to suppress, rather than enhance, locomotion and behavioural sensitisation [543] (but see also [23]).

Collectively, these findings indicate that in the DBA/2 strain, adrenal hormones modulate dopaminergic transmission, presumably at the level of both pre- and post-synaptic neurotransmission. Further studies are required to investigate the importance of the individual neural adaptations in psychostimulant sensitisation, e.g. by regional overexpression of TH and DAT or by downregulation of D2 receptors in the NAc and CP. Interestingly, the neural adaptations were most evident in the nigrostriatal dopamine system and NAc core. This contradicts the notion that the mesocorticolimbic pathway, and particularly the NAc shell, plays a critical role in initiation and expression of psychostimulant sensitisation [210,519]. However, it has become increasingly evident that the mesocorticolimbic and nigrostriatal projections are both anatomically and functionally intertwined, and evidence is emerging for a role of the latter in behavioural sensitisation. Indeed, expression of psychostimulant sensitisation has been associated with structural changes in the NAc core, but not the shell, subregion [390] and one study reported sensitisation of dopamine transmission specifically in the NAc core [74]. Furthermore, alterations in cellular activity associated with expression of amphetamine sensitisation have been reported to occur in the CP, but not the NAc [696]. Finally, the dorsal PFC-NAc core connection (via glutamatergic efferents) plays an increasingly more prominent role in expression of drug-induced adaptations, under more habitual circumstances [329].

In conclusion, the present data suggest that adrenal hormones modulate behavioural sensitisation to cocaine through adaptations in the nigrostriatal dopamine system and the NAc core, in a genotype-dependent manner. The DBA/2, but not the C57BL/6 strain, appeared vulnerable to the impact of adrenal stress hormones on cocaine sensitivity at the level of both dopaminergic neurotransmission and, as previously described, behavioural responsiveness [145]. The 'susceptible' DBA/2 phenotype therefore provides a good model for further research into the interaction between stress, the brain dopamine system and vulnerability to psychostimulant drugs.

ACKNOWLEDGEMENTS

This research was supported by NWO/INSERM/ZON grants 985-10-014 and 985-10-504 and by the Royal Netherlands Academy of Arts and Sciences. We gratefully acknowledge dr. S. Smits for kindly providing the D1 receptor riboprobe, drs. J.C. de Greef for technical support and dr. O.C. Meijer for critically reading the manuscript.

4

Critical time-window for the actions of adrenal glucocorticoids in behavioural sensitisation to cocaine

Inge E.M. de Jong and E. Ronald de Kloet

ABSTRACT

Glucocorticoids, secreted by the adrenals in response to stress, have profound effects on behavioural responsiveness to psychostimulant drugs. We have studied the critical time-window for the influence of corticosterone on behavioural sensitisation to cocaine in relation to i) the stage of behavioural sensitisation, and ii) the time of drug exposure.

Previously, we have identified a mouse strain (DBA/2) in which surgical removal of the adrenals (adrenalectomy: 'ADX') fully prevented locomotor sensitisation to cocaine. To investigate the role of corticosterone in expression of behavioural sensitisation, the glucocorticoid receptor (GR) antagonist mifepristone (RU38486) was administered to previously sensitised mice prior to a cocaine challenge. Furthermore, ADX mice were given corticosterone replacement at different intervals prior to each drug administration, to investigate the role of the glucocorticoid in initiation of behavioural sensitisation, and in relation to the time of drug exposure.

Administration of mifepristone to previously sensitised animals failed to block expression of cocaine-induced behavioural sensitisation. In ADX mice, intermittent replacement of corticosterone (1 mg/kg i.p., either 2 hours or 5 minutes prior to each cocaine administration), did not reverse the sensitisation deficit. By contrast, chronic corticosterone replacement (20% pellet) partially restored initiation of behavioural sensitisation. These data indicate that the presence of corticosterone facilitates the initiation rather than the expression of behavioural sensitisation to cocaine. However, because high corticosterone concentrations only partially reversed the sensitisation deficit of ADX mice, the adrenal glucocorticoid seems necessary, but not sufficient, for full behavioural sensitisation to cocaine in the DBA/2 strain.

INTRODUCTION

Adverse life experiences, and the neuroendocrine response they evoke, have a profound impact on vulnerability to the behavioural and reinforcing effects of psychostimulant drugs (reviewed in: [405,514]). The contribution of the hypothalamic-pituitary-adrenal (HPA) axis and adrenal glucocorticoids (cortisol in humans, corticosterone in rodents) to psychostimulant sensitivity has been extensively studied [241,421]. In laboratory rodents, glucocorticoids facilitate acquisition, maintenance and relapse of psychostimulant self-administration as well as drug-induced locomotion and behavioural sensitisation (reviewed in: [144,240,421,515]). Furthermore, it has been shown that the propensity of animals to acquire psychostimulant self-administration positively correlates with their plasma corticosterone concentrations [244,516]. The observation that glucocorticoids in the range of stress-induced levels are readily self-administered by laboratory rodents, suggests that these hormones themselves also possess 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 convincing evidence that the actions of glucocorticoids on dopaminergic transmission and psychostimulant responsiveness are dependent on activation of the glucocorticoid receptor (GR) in the brain [153,168,308,579,582].

Despite the wealth of data showing that glucocorticoids mediate the effects of stress on psychostimulant sensitivity, the context- and time-dependency of their actions is poorly understood. Glucocorticoids can either facilitate or attenuate physiological or behavioural outcomes depending on timing, context and endpoint. For instance, the beneficial effects of glucocorticoids on memory consolidation and retrieval may only occur when stress is experienced closely linked in time to, and within the context of, the information to be learned (reviewed in: [318]). Furthermore, glucocorticoids can 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 (e.g. [339,450,451,584,589]).

In the present study, we have investigated the critical time-window for the actions of corticosterone in sensitisation to the locomotor stimulant effects of cocaine. The DBA/2 inbred mouse strain was used, as it displays parallel sensitisation of cocaine-induced locomotion and corticosterone secretion, whereas surgical removal of the adrenals (adrenalectomy: 'ADX') prevents behavioural sensitisation [145]. We have

investigated the time-window for the actions of corticosterone i) in relation to the stage of behavioural sensitisation (initiation vs. expression) and ii) in relation to the time of drug exposure. First, the glucocorticoid receptor (GR) antagonist mifepristone (RU38486) was administered to previously sensitised animals to investigate the contribution of corticosterone to expression of sensitisation. Second, ADX mice were given corticosterone replacement (5 minutes or 2 hours prior to *each* drug exposure or chronically), to study the role of the hormone during initiation of sensitisation and in relation to the time of drug exposure.

METHODS

General methods

Animals
Male DBA/2 Rj mice were obtained from Janvier (Le Genest Saint Isle, France) at the age of 8 weeks. Mice were housed in groups of four in perspex cages (35x19x14cm) with food and water available *ad libitum* at a 12 hour light-dark cycle (lights on: 7 am) in a temperature (21±1ºC) and humidity (55±5%) controlled room. Surgery was performed 2 weeks after arrival in the animal facility. Animals were briefly handled in the week before surgery and otherwise left undisturbed. Animal experiments were approved by the local Committee for Animal Health, Ethics and Research of Leiden University. Animal care was conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC).

Surgery
Animals were individually housed 1 day prior to surgery. The cages were transported to the operating room on the morning of the surgery where mice were allowed to recover from transportation for 2 hours. Inhalation anaesthesia consisted of a mixture of isoflurane (3 l/min), N_2O (0.8 l/min) and O_2 (0.4 l/min). Adrenalectomy was performed via the dorsal route as described previously [145]. SHAM animals were treated similarly with the exception of the actual removal of the adrenals. Mice were kept individually housed for 24 hours following surgery after which they were housed two animals per cage of similar surgery. After surgery, all animals were given free access to 0.9% NaCl in addition to normal drinking water. The sensitisation paradigm was started following a recovery period of 1 week.

Drugs

Cocaine hydrochloride (BUFA Pharmaceuticals B.V., Uitgeest, The Netherlands) was dissolved in sterile saline and administered intraperitoneally (i.p.) in a dosage of 7.5 or 15.0 mg/kg. Mifepristone (RU38486, Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands) was dissolved to a concentration of 100 mg/ml in sterile saline containing 2.5 mg carboxymethylcellulose and 2 µl Tween-20 per ml. Animals received 50 µl of this solution applied on oats resulting in a dose of 200 mg/kg bodyweight. Corticosterone (corticosterone:HBC complex, Sigma-Aldrich) was dissolved in sterile saline and administered i.p. in a dosage of 1 mg/kg of the free base. Corticosterone pellets (100 mg, w x h: 9 x 2 mm) consisted of 20% corticosterone (ICN Biomedicals Inc, Aurora, Ohio, USA) in cholesterol vehicle (cholesterol 95% stabilised, Acros Organics, Geel, Belgium) and were implanted subcutaneously in the flank of the animal. Control groups received equal volumes of saline, 100% cholesterol pellets in the chronic corticosterone substitution study, and oats containing vehicle in the mifepristone study. From the start of the sensitisation paradigm, animals were weighed once every two days and the injection volumes were adjusted accordingly.

Sensitisation paradigm

One day prior to the first drug administration and thus the first behavioural test, animals were individually housed and kept single housed for the remainder of the experiment. The sensitisation paradigm consisted of a treatment phase (days 1-9), a withdrawal interval (days 10-14), a saline challenge (day 14), and a cocaine challenge (day 15). The treatment phase consisted of i.p. injections of 15.0 mg/kg cocaine (COC) or saline (SAL) on 9 consecutive days and locomotion was measured on days 1 (first administration) and 9 (last administration). On these days, animals received the treatment in the test setting as described in the following paragraph. On days 2-8 animals received the injections in the home cage. The treatment period was followed by a withdrawal interval of 5 days (no treatment). On the last day of the withdrawal interval (day 14), all animals received a saline challenge and on day 15, all animals received a 7.5 mg/kg cocaine challenge. In experiments 2-4, cocaine-treated mice were re-challenged with 7.5 mg/kg cocaine on day 21. For this day, only data of cocaine-treated mice are presented, as the saline-treated groups received cocaine for the second time and can therefore no longer be considered proper controls. All injections were given 2 to 5 hours after lights on.

Measurement of locomotor activity

All behavioural tests (days 1, 9, 14, 15 and 21) were performed in the room where animals were housed. Mice were placed in a test cage (same type and size

(35x19x14cm) as the home cage) containing a standardised amount of sawdust, covered with a perspex lid. Following a 2 hour habituation period, animals were injected and activity was monitored on video for 30 minutes. At the end of this period, a blood sample was taken from the tail vein for endocrine measurements and the animals were returned to their home cage.

Analysis of locomotor activity
Video images were digitised and analysed using Ethovision Videotracking, Motion Analysis & Behavior Recognition System version 1.96 ('VTMAS', Noldus Information Technology B.V., Wageningen, The Netherlands). The position of the animal was sampled 5 times per second. Of each recording (30 minutes) 27 minutes were analysed since the animals were subjected to blood sampling at 30 minutes after injection. Data are represented in total distance moved (cm) over the entire 27 minute treatment period. Locomotion was defined as movement with a minimal distance of 2 cm.

Corticosterone assay
Blood samples were taken from the tail vein by a small incision with a razorblade 30 minutes after treatment on the test days 1, 9, 14, 15 and 21. Blood was collected in small EDTA coated tubes (Microvette DB 200 K3E, Sarstedt, Nümbrecht, Germany). Plasma was obtained by centrifugation at 13000 rpm for 20 minutes at 4ºC and subsequently stored at -20ºC. Corticosterone concentrations were determined by in-duplo measurement using a radio-immuno-assay kit according to the protocol provided by the manufacturer (Corticosterone double antibody ^{125}I RIA kit, MP Biomedicals, Asse-Relegem, Belgium). All samples were analysed in one assay to exclude inter-assay variability. ADX effectively clamped plasma corticosterone to basal concentrations and only animals with successful ADX were included in the study.

Experimental design

Experiment 1: Effect of mifepristone (RU38486) on expression of behavioural sensitisation to cocaine in intact mice
This experiment was designed to investigate whether corticosterone plays a critical role in expression of previously established behavioural sensitisation to cocaine. For this purpose, animals were administered the glucocorticoid receptor antagonist mifepristone (200 mg/kg, orally via oats) 2.5 hours prior to the cocaine challenge on day 15. Unoperated DBA/2 mice were used for this part of the study. In the week before start of the sensitisation paradigm, animals were familiarised with the smell

and taste of plain oats. A feeding cup was placed permanently in the home cage for oat administration. Initially, animals were divided over 2 experimental groups: SAL and COC (n=20, 34). Based on behavioural responses on the last day of the treatment period (day 9), animals were divided over vehicle and mifepristone ('RU') treatment groups such that a homogenous distribution of relatively low- and high-responders was created (SAL/VEH, SAL/RU, COC/VEH, COC/RU n=10-18). On day 15, the oats containing mifepristone or vehicle were given 2.5 hours prior to the cocaine challenge, thus 30 minutes prior to the onset of the habituation phase. When animals were transferred to the test cages, the home cages were checked to verify whether all oats had been eaten.

Experiments 2-4 were designed to investigate the role of corticosterone during initiation of behavioural sensitisation to cocaine and the time-window for the actions of the hormone in relation to drug exposure.

Experiments 2 and 3: Effect of corticosterone substituted 5 minutes or 2 hours prior to cocaine on behavioural sensitisation in ADX mice

This part of the study consisted of 6 experimental groups (n=8-12). Animals were either adrenalectomised (ADX) or SHAM operated. ADX animals were assigned to the corticosterone substitution or control groups (ADXcort, ADX). All groups were subdivided in saline (SAL) and cocaine (COC) groups, indicating the treatment given during the treatment phase of the sensitisation paradigm. Corticosterone (1 mg/kg, free base) or saline was injected i.p. 5 minutes (experiment 2) or 2 hours (experiment 3) prior to each treatment (days 1-9, 14 and 15). To investigate drug responsiveness in absence of corticosterone substitution, animals were re-challenged with 7.5 mg/kg cocaine one week after the initial cocaine challenge (day 21). On this day, all animals received saline 2 hours prior to drug treatment.

Experiment 4: Effect of chronic corticosterone substitution on behavioural sensitisation in ADX mice

Again, 6 treatment groups were used: SHAM (SAL/COC), ADX (SAL/COC) and ADXcort (SAL/COC) (n=8-12). Corticosterone (20%) and cholesterol pellets were implanted one day prior to the onset of the sensitisation paradigm. To this aim, animals were briefly anaesthetised with an isoflurane mixture (see surgical procedures) and the pellet was placed subcutaneously in the flank of the animal. Following surgery, animals were housed individually and the sensitisation paradigm was started the following day. Also in this study, animals were re-challenged on day 21 with 7.5 mg/kg cocaine.

Statistics

Statistical analysis was performed using SPSS for Windows software (release 7.5, SPSS Benelux B.V., Gorinchem, The Netherlands). For the mifepristone study, analyses were performed per test day: one factor ANOVA for treatment (days 1, 9 and 14) and two factor ANOVA for treatment and mifepristone (day 15). For the corticosterone substitution studies, overall locomotor activity and corticosterone data were subjected to repeated measures ANOVA with three between subject factors (surgery, substitution and treatment) and one within subject factor (test day). Subsequent analyses were performed per test day: three factor ANOVA for surgery, substitution and treatment. When statistical significance was revealed, post hoc tests were performed (LSD, or for within subject comparison paired t-test). Differences were considered statistically significant when $p<0.05$.

RESULTS

Experiment 1: Effect of mifepristone (RU38486) on expression of behavioural sensitisation to cocaine in intact mice

The GR antagonist mifepristone (RU38486, 'RU') was administered orally to intact animals 2.5 hours prior to the 7.5 mg/kg cocaine challenge on day 15. Figure 1A depicts total distance moved for the treatment groups SAL and COC (days 1, 9 and 14) and SAL/VEH, SAL/RU, COC/VEH and COC/RU (day 15).

Cocaine stimulated locomotion in the treatment period (day 1: $F[treatment]_{1,53}=18.412$, $p<0.001$, day 9: $F[treatment]_{1,52}=49.246$, $p<0.001$, post hoc: $p<0.001$) and drug responses were enhanced on day 9 compared to day 1 ($p<0.01$, paired t-test). Furthermore, cocaine-treated mice displayed elevated locomotor responses to the saline challenge on day 14 ($F[treatment]_{1,52}=16.192$, $p<0.001$, post hoc: $p<0.001$). Based on responsiveness on day 9, cocaine-treated mice were distributed over the RU and VEH groups such that relatively low- and high-responders were divided equally. The cocaine challenge on day 15 resulted in augmented locomotion in cocaine- compared to saline-treated mice ($F[treatment]_{1,48}=9.623$, $p<0.01$, post hoc: $p<0.05$), while there was no effect of mifepristone on drug responses of either cocaine-treated or drug-naïve animals ($F[RU]_{1,48}=0.000$, $p=1.000$).

Figure 1B depicts plasma corticosterone concentrations 30 minutes after drug treatment, thus 3 hours after RU or VEH administration. Plasma corticosterone concentrations were elevated in RU compared to VEH-treated groups ($F[RU]_{1,51}=126.566$,

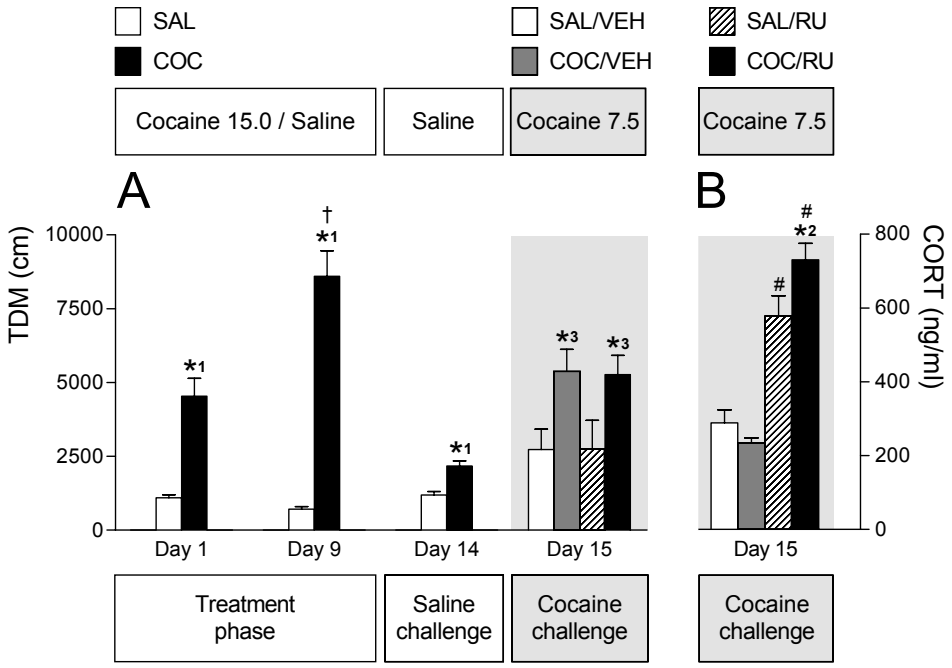

Figure 1: *The effect of mifepristone on expression of locomotor sensitisation in intact DBA/2 mice.*

A) Locomotion in response to treatment on days 1, 9, 14 and 15 of the sensitisation paradigm. Animals were treated during 9 consecutive days with cocaine (COC, n=33) 15.0 mg/kg or saline (SAL, n=20), followed by a 5 day withdrawal interval and a saline challenge (day 14). Mice received 200 mg/kg mifepristone ('RU', n=14-17) or vehicle (VEH, n=9) on oats 2.5 hours prior to the 7.5 mg/kg cocaine challenge on day 15. Data are represented in average total distance moved (TDM, cm) ± SEM.

B) Plasma corticosterone concentrations 30 minutes after the 7.5 mg/kg cocaine challenge and 3 hours after mifepristone or vehicle administration on day 15. Data are represented in average corticosterone (CORT, ng/ml) ± SEM. n=9-17 animals/group. *1 $p<0.001$, *2 $p<0.01$, *3 $p<0.05$ vs. SAL (LSD), # $p<0.001$ vs. VEH (LSD), † $p<0.001$ vs. the previous test day (paired t-test).

$p<0.001$, post hoc: $p<0.001$), with the most pronounced elevation in the COC/RU group ($F[\text{treatment}]_{1,51}=126.566$, $p=0.210$, $F[\text{treatment x RU}]_{1,51}=9.672$, $p<0.01$, post hoc: $p<0.01$ vs. SAL/RU).

The results of experiment 1 did not support a critical role for glucocorticoid signalling via GR in expression of locomotor sensitisation in DBA/2 mice. We therefore hypothesised that corticosterone may contribute to induction of behavioural sensitisation. Experiments 2-4 were designed to investigate whether the deficit of

ADX mice in locomotor sensitisation to cocaine can be reversed by replacement of corticosterone from the start of the sensitisation paradigm, at different time intervals in relation to drug exposure.

Plasma corticosterone concentrations

Table 1 shows the effects of ADX and the different hormone replacement regimens on plasma corticosterone concentrations 30 minutes after treatment on days 1, 9, 14, and 15.

For SHAM and ADX mice, data were replicated across experiments and therefore pooled. Main effects were found for treatment, surgery, day and the interaction between these factors ($F[treatment]_{1,89}=19.897$, $p<0.001$, $F[surgery]_{1,89}=314.423$,

Table 1: *Effect of ADX and hormone replacement on plasma corticosterone concentrations.*

		Day 1	Day 9	Day 14 saline challenge	Day 15 cocaine challenge 1
SHAM	**SAL**	108.9 ± 12.3	140.4 ± 17.3 †	138.8 ± 13.6	216.2 ± 15.7
	COC	220.5 ± 13.4 *	364.0 ± 16.4 *†	167.7 ± 18.0	261.1 ± 17.6 *
ADX	**SAL**	18.6 ± 2.9 #	30.0 ± 4.6 #	35.7 ± 5.3 #	40.4 ± 6.6 #
	COC	20.8 ± 3.0 #	30.9 ± 4.2 #	35.6 ± 4.0 #	38.7 ± 4.8 #
CORT 5 min.	**SAL**	422.9 ± 50.3 # Ω	530.1 ± 31.6 # Ω	577.2 ± 17.1 # Ω	418.5 ± 64.0 # Ω
	COC	557.7 ± 31.3 # Ω	633.0 ± 43.3 # Ω	521.6 ± 67.5 # Ω	469.5 ± 63.8 # Ω
CORT 2 hrs.	**SAL**	71.7 ± 13.4 Ω	72.2 ± 6.8 #	95.3 ± 10.8 Ω	102.0 ± 15.0 # Ω
	COC	86.7 ± 13.9 # Ω	84.3 ± 10.3 # Ω	56.8 ± 11.0 #	65.0 ± 9.9 #
CORT pellet	**SAL**	474.1 ± 22.8 # Ω	129.5 ± 6.2 Ω	118.6 ± 6.5 Ω	135.6 ± 5.9 # Ω
	COC	527.5 ± 19.5 # Ω	130.8 ± 4.9 # Ω	110.2 ± 4.6 # Ω	120.5 ± 5.5 # Ω

Plasma corticosterone concentrations 30 minutes after treatment on days 1, 9, 14 and 15. Animals were SHAM operated, adrenalectomised (ADX), or ADX and given corticosterone (CORT) replacement: 5 minutes or 2 hours prior to drug administration (1 mg/kg, i.p.) or chronically (20% pellet). Mice were treated during 9 consecutive days with cocaine (COC) 15.0 mg/kg or saline (SAL), followed by a 5 day withdrawal interval. All animals received a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. Data are represented in average plasma corticosterone concentration (ng/ml) ± SEM, n=7-29 animals/group. * $p<0.05$ vs. SAL (LSD), # $p<0.05$ vs. SHAM (LSD), Ω $p<0.05$ vs. ADX (LSD), † $p<0.05$ vs. day 1 (paired t-test).

$p<0.001$, $F[day]_{4,356}=22.342$, $p<0.001$, $F[day \times surgery \times treatment]_{4,356}=16.922$, $p<0.001$). Corticosterone concentrations were attenuated in ADX compared to SHAM mice on all test days ($p<0.001$). In agreement with previous observations (chapter 2, [145]), cocaine-induced corticosterone secretion in SHAM mice was enhanced on day 9 compared to day 1 ($p<0.001$, paired t-test) and a similar effect was observed for the response to saline treatment, though less pronounced ($p<0.05$, paired t-test). In addition, cocaine-treated SHAM mice showed greater corticosterone secretion in response to the drug challenge on day 15, when compared to saline-treated mice ($p<0.05$).

Corticosterone substitution 5 minutes prior to drug treatment resulted in a pronounced elevation of the hormone to concentrations even higher than in the SHAM groups ($F[substitution]_{1,103}=805.511$, $p<0.001$, post hoc: $p<0.001$ vs. SHAM and ADX on all days). By contrast, when corticosterone was administered 2 hours prior to drug administration, hormone concentrations were lower than of SHAM mice, though not fully normalised to ADX levels 30 minutes after drug treatment ($F[substitution]_{1,102}=9.839$, $p<0.01$). On day 21, corticosterone substitutions were omitted and hormone concentrations were similar to those of ADX mice (data not shown).

In animals with corticosterone pellets ($F[substitution]_{1,105}=171.434$, $p<0.001$), hormone concentrations were elevated above SHAM levels on day 1 ($p<0.001$) and thereafter declined to on average 125 ng/ml on days 9, 14 and 15, being higher than in the ADX groups (at least $p<0.01$), but in the case of cocaine treatment, lower than in the SHAM groups (at least $p<0.05$). A further decline was observed from day 15 to 21, when corticosterone concentrations in the substituted groups dropped below 100 ng/ml (ADXcort/SAL: 92 ± 8, ADXcort/COC: 81 ± 6). These data indicate that the subcutaneous corticosterone pellet generated plasma corticosterone concentrations that were very high on the first day of the treatment phase and thereafter declined to concentrations in the range of stress-induced levels that persisted until the first cocaine challenge on day 15.

Experiment 2: Effects of corticosterone substituted 5 minutes prior to cocaine on behavioural sensitisation in ADX mice

Corticosterone (1 mg/kg, free base) or saline was administered 5 minutes prior to drug treatment on days 1-9, 14 and 15, but not 21. Main effects were found for treatment, day and the interaction between day, surgery and treatment ($F[treatment]_{1,43}=37.622$, $p<0.001$, $F[day]_{4,172}=12.413$, $p<0.001$, $F[day \times surgery \times treatment]_{4,172}=3.444$, $p<0.05$). Responses to saline treatment were not affected by either surgery or substitution in any of the tests.

Figure 2 depicts total distance moved for the treatment groups SHAM (SAL/COC), ADX (SAL/COC) and ADXcort (SAL/COC) on days 1, 9 and 15. On the first day of the treatment period, there was no effect of surgery or substitution on cocaine-induced locomotion ($F[treatment]_{1,54}=4.532$, $p<0.05$, $F[surgery]_{1,54}=0.292$, $p=0.591$, $F[substitution]_{1,54}=0.235$, $p=0.630$). Consistent with previous observations, drug responses of SHAM ($p<0.05$, paired t-test), but not of ADX mice ($p=0.735$, paired t-test), were enhanced on day 9 compared to day 1. The ADXcort group also displayed increased cocaine responsiveness ($p<0.01$, paired t-test), although this effect appeared to be mainly attributable to the slightly lower drug response

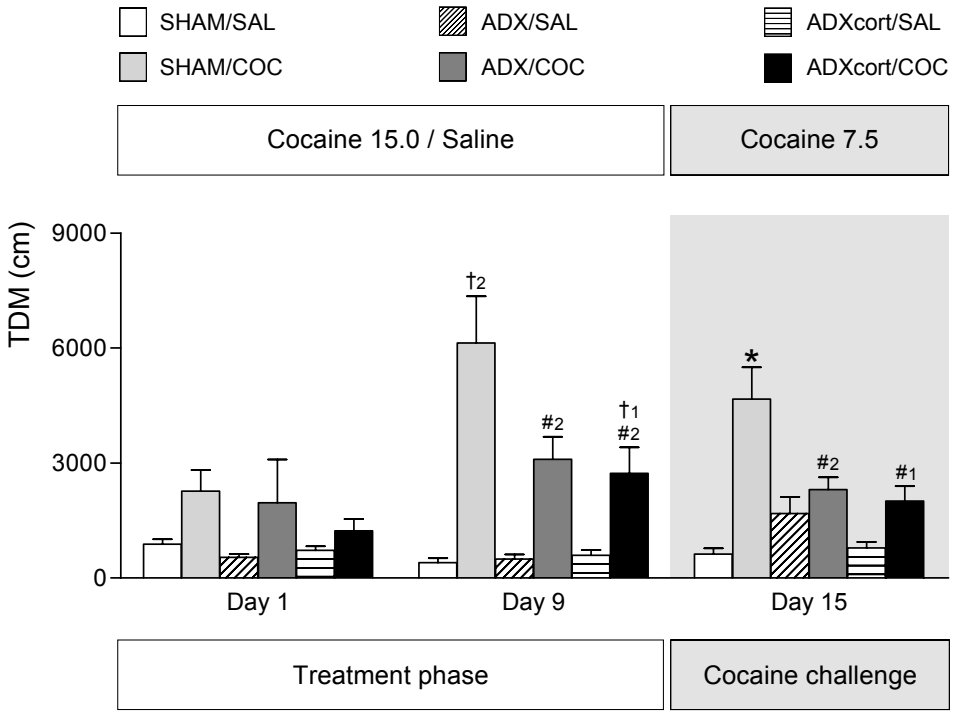

Figure 2: *The effect of corticosterone substituted 5 minutes prior to drug administration on behavioural sensitisation in ADX mice.*

Locomotion in response to treatment on days 1, 9 and 15. Animals were SHAM operated, adrenalectomised (ADX), or ADX and given corticosterone replacement 5 minutes prior to drug administration (ADXcort). Mice were treated during 9 consecutive days with cocaine (COC) 15.0 mg/kg or saline (SAL), followed by a 5 day withdrawal interval. All animals received a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. Data are represented in average total distance moved (TDM, cm) ± SEM, n=7-12 animals/group. * $p<0.001$ vs. SAL (LSD), #1 $p<0.001$, #2 $p<0.01$ vs. SHAM (LSD), †1 $p<0.01$, †2 $p<0.05$ vs. day 1 (paired t-test).

on day 1, rather than to true sensitisation. In support of this, cocaine responses of ADX mice were lower than of SHAM mice on day 9 ($p<0.01$), irrespective of substitution ($F[\text{treatment}]_{1,54}=39.504$, $p<0.001$, $F[\text{surgery}]_{1,54}=4.339$, $p<0.05$, $F[\text{substitution}]_{1,54}=0.040$, $p=0.843$, $F[\text{surgery x treatment}]_{1,54}=4.886$, $p<0.05$). Furthermore, in contrast to SHAM/COC mice ($p<0.001$), neither ADX/COC nor ADXcort/COC mice displayed augmented responsiveness to the 7.5 mg/kg cocaine challenge on day 15 ($F[\text{treatment}]_{1,53}=29.765$, $p<0.001$, $F[\text{surgery}]_{1,53}=1.786$, $p=0.188$, $F[\text{substitution}]_{1,53}=1.571$, $p=0.216$, $F[\text{surgery x treatment}]_{1,53}=12.160$, $p<0.01$). Moreover, locomotor responses of cocaine-treated ADX and ADXcort mice were lower than of SHAM mice on day 15 ($p<0.01$) and on day 21 (ADXcort: $p<0.05$, ADX: $p=0.068$), when animals were re-challenged with 7.5 mg/kg cocaine without prior corticosterone substitution (day 21: SHAM: 5603 ± 1038, ADX: 3771 ± 619, ADXcort: 3017 ± 650, $F[\text{treatment}]_{1,51}=10.536$, $p<0.01$, $F[\text{surgery}]_{1,51}=0.195$, $p=0.661$, $F[\text{substitution}]_{1,51}=1.965$, $p=0.168$, $F[\text{surgery x treatment}]_{1,51}=8.059$, $p<0.01$).

Experiment 3: Effects of corticosterone substituted 2 hours prior to cocaine on behavioural sensitisation in ADX mice

Corticosterone (1 mg/kg, free base) or saline was administered 2 hours prior to drug treatment on days 1-9, 14 and 15, but not 21. Locomotion was influenced by surgery, treatment and day and interactions between these factors ($F[\text{surgery}]_{1,41}5.566$, $p<0.05$, $F[\text{treatment}]_{1,41}=16.192$, $p<0.001$, $F[\text{day}]_{4,164}=29.030$, $p<0.001$, $F[\text{day x surgery}]_{4,164}=4.500$, $p<0.01$, $F[\text{day x treatment}]_{4,164}=2.504$, $p<0.05$). Responses to saline treatment were not affected by either surgery or substitution in any of the tests.

Locomotor responses of the SHAM (SAL/COC), ADX (SAL/COC) and ADXcort (SAL/COC) groups on days 1, 9 and 15 are depicted in figure 3. On the first day of the treatment period, there was no effect of surgery or substitution on cocaine-induced locomotion ($F[\text{treatment}]_{1,57}=9.719$, $p<0.01$, $F[\text{surgery}]_{1,57}=1.529$, $p=0.222$, $F[\text{substitution}]_{1,57}=0.232$, $p=0.632$). Similar to experiment 2, only the SHAM group displayed enhanced drug responsiveness on day 9 compared to day 1 ($p<0.05$, paired t-test). Furthermore, cocaine responses of ADX mice were lower than of SHAM mice on day 9, irrespective of substitution, although this just failed to reach significance for the ADX group ($F[\text{treatment}]_{1,50}=26.694$, $p<0.001$, $F[\text{surgery}]_{1,50}=2.157$, $p=0.149$, $F[\text{substitution}]_{1,50}=0.566$, $p=0.456$, post hoc: ADX: $p=0.055$, ADXcort: $p<0.01$ vs. SHAM). Also in this study, only SHAM/COC mice ($p<0.05$) displayed enhanced responsiveness to the 7.5 mg/kg cocaine challenge on day 15 ($F[\text{treatment}]_{1,49}=7.188$, $p<0.05$, $F[\text{surgery}]_{1,49}=1.572$, $p=0.216$,

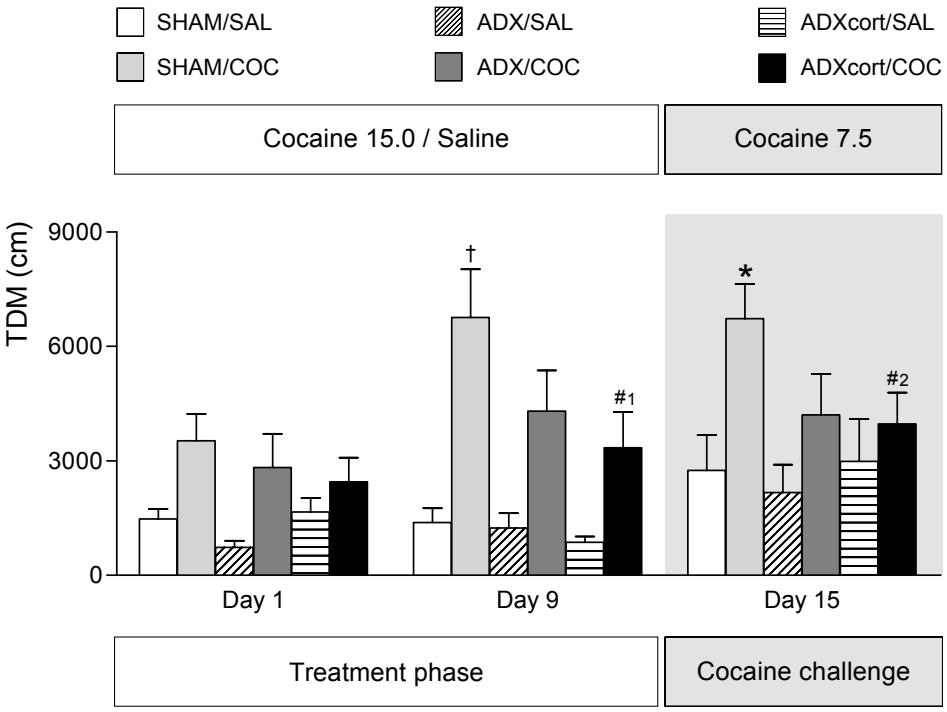

Figure 3: *The effect of corticosterone substituted 2 hours prior to drug administration on behavioural sensitisation in ADX mice.*
Locomotion in response to treatment on days 1, 9 and 15. Animals were SHAM operated, adrenalectomised (ADX), or ADX and given corticosterone replacement 2 hours prior to drug administration (ADXcort). Mice were treated during 9 consecutive days with cocaine (COC) 15.0 mg/kg or saline (SAL), followed by a 5 day withdrawal interval. All animals received a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. Data are represented in average total distance moved (TDM, cm) ± SEM, n=7-12 animals/group. * $p<0.05$ vs. SAL (LSD), #1 $p<0.01$, #2 $p<0.05$ vs. SHAM (LSD), † $p<0.05$ vs. day 1 (paired t-test).

$F[substitution]_{1,49}=0.086$, $p=0.770$). Furthermore, locomotor responses of cocaine-treated ADX ($p=0.066$) and ADXcort ($p<0.05$) mice were lower than of SHAM mice on day 15 and this was even more pronounced on day 21 ($p<0.05$ for both groups), when animals were re-challenged with 7.5 mg/kg cocaine without prior corticosterone substitution (SHAM: 9859 ± 1481, ADX: 4290 ± 1182, ADXcort: 5960 ± 1338, $F[treatment]_{1,47}=3.920$, $p=0.054$, $F[surgery]_{1,47}=9.646$, $p<0.01$, $F[substitution]_{1,47}=0.483$, $p=0.491$).

Experiment 4: Effect of chronic corticosterone substitution on behavioural sensitisation in ADX mice

Corticosterone (20% corticosterone in cholesterol) or vehicle (100% cholesterol) pellets were implanted subcutaneously (s.c.) one day prior to the first drug administration. Main effects were found for surgery, treatment, day and the interaction between these factors ($F[surgery]_{1,41}=10.640$, $p<0.01$, $F[treatment]_{1,41}=37.136$, $p<0.001$, $F[day]_{4,164}=35.683$, $p<0.001$, $F[day \times surgery \times treatment]_{4,164}=2.800$, $p<0.05$). Responses to saline treatment were not affected by either surgery or substitution in any of the tests.

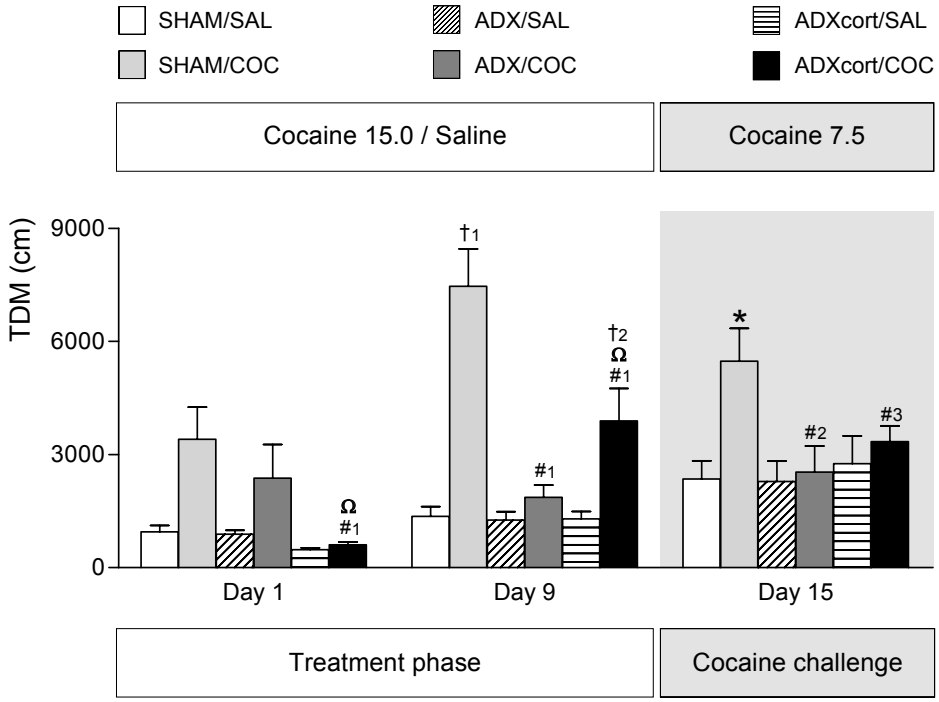

Figure 4: *The effect of chronic corticosterone substitution on behavioural sensitisation in ADX mice.*

Locomotion in response to treatment on days 1, 9 and 15. Animals were SHAM operated, adrenalectomised (ADX), or ADX and implanted with a 20% corticosterone pellet (ADXcort). Mice were treated during 9 consecutive days with cocaine (COC) 15.0 mg/kg or saline (SAL), followed by a 5 day withdrawal interval. All animals received a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. Data are represented in average total distance moved (TDM, cm) ± SEM, n=6-10 animals/group. * $p<0.01$ vs. SAL, #1 $p<0.001$, #2 $p<0.01$, #3 $p<0.05$ vs. SHAM (LSD), Ω $p<0.05$ vs. ADX (LSD), †1 $p<0.01$, †2 $p<0.05$ vs. day 1 (paired t-test).

Figure 4 depicts locomotor responses of SHAM (SAL/COC), ADX (SAL/COC) and ADXcort (SAL/COC) groups on days 1, 9 and 15. On the first day of the treatment period, corticosterone substitution reduced cocaine responses ($F[treatment]_{1,53}=9.398$, $p<0.01$, $F[substitution]_{1,53}=4.659$, $p<0.05$, $F[surgery]_{1,53}=1.099$, $p=0.300$, post hoc: $p<0.05$ vs. SHAM/COC and ADX/COC). In this experiment, not only SHAM, but also ADXcort mice, displayed enhanced cocaine-induced locomotion on day 9 compared to day 1 ($p<0.05$, paired t-test). The apparent sensitisation in the ADXcort group was not solely due to the reduced drug response on day 1, as cocaine responses of these mice were higher than of ADX mice on day 9 ($p<0.05$), although not fully normalised to SHAM levels ($p<0.001$) ($F[treatment]_{1,49}=46.014$, $p<0.001$, $F[surgery]_{1,49}=18.168$, $p<0.001$, $F[substitution]_{1,49}=2.481$, $p=0.122$, $F[surgery \times treatment]_{1,49}=16.909$, $p<0.001$). However, only SHAM/COC animals ($p<0.01$) displayed enhanced drug responsiveness to the 7.5 mg/kg cocaine challenge on day 15 ($F[surgery]_{1,50}=5.106$, $p<0.05$, $F[treatment]_{1,50}=8.135$, $p<0.01$, $F[substitution]_{1,50}=0.979$, $p=0.328$, $F[surgery \times treatment]_{1,50}=4.645$, $p<0.05$). Furthermore, locomotor responses of cocaine-treated ADX and ADXcort mice were lower than of SHAM mice on day 15 ($p<0.05$). On day 21 however, when animals were re-challenged with 7.5 mg/kg cocaine, only cocaine responses of ADX mice were significantly lower than of SHAM mice (SHAM: 7101 ± 1102, ADX: 4651 ± 761, ADXcort: 5311 ± 572, $F[treatment]_{1,49}=3.558$, $p=0.066$, $F[surgery]_{1,49}=1.506$, $p=0.226$, $F[substitution]_{1,49}=1.333$, $p=0.255$, post hoc: ADX: $p<0.05$, ADXcort: $p=0.112$).

DISCUSSION

The present study shows that adrenal glucocorticoids play a role during initiation rather than expression of cocaine-induced behavioural sensitisation in DBA/2 mice. The time window for the actions of corticosterone during initiation of sensitisation was investigated in ADX mice. It was demonstrated that whereas intermittent corticosterone replacement, either 5 minutes or 2 hours prior to each drug administration, was ineffective in reversing the sensitisation deficit of ADX mice, initiation of behavioural sensitisation was partially restored by chronic corticosterone substitution.

The observation that ADX prevents both initiation and expression of locomotor sensitisation to cocaine in DBA/2 mice, suggests that adrenal stress hormones contribute to the developmental stage of behavioural sensitisation (the present study, [145]).

This hypothesis was strengthened by the present finding that the GR antagonist mifepristone (RU38486) administered 2.5 hours prior to the cocaine challenge (day 15) was ineffective in reversing expression of previously established behavioural sensitisation of intact mice (figure 1A). In agreement with this, it has been demonstrated that ADX performed in already sensitised animals does not prevent expression of the behavioural hyperresponsiveness [527,529]. The present finding does however contradict two reports in which the motivation to self-administer cocaine, and amphetamine-induced behavioural sensitisation, were blocked by pretreatment with mifepristone in animals that had previously acquired these behaviours [153,168]. This discrepancy could result from the use of different species and genetic backgrounds. In support of this, Deroche-Gamonet *et al*. show that mifepristone only reduces the motivation to obtain cocaine in a subset of outbred rats that display an exceptionally high drug response, and we have demonstrated previously that the effects of ADX on behavioural sensitisation to cocaine are highly strain-dependent [145,168].

The dramatic increase in plasma corticosterone observed in mifepristone-pretreated mice (as measured 30 minutes after drug treatment, figure 1B), indicates that the antagonist was effective in preventing negative feedback on the HPA-axis and thus in blocking GR. From these data it can however not be concluded whether sufficient concentrations of mifepristone reached the brain, but this is likely to have occurred since other studies using lower oral doses have shown effects of the antagonist on brain function and behaviour [160,431].

The observation that the 20% corticosterone pellet facilitated initiation of sensitisation in ADX mice, is in agreement with reports showing that chronic administration of the adrenal glucocorticoid in concentrations similar to those induced by stress, enhances behavioural responsiveness to single and repeated psychostimulant exposure [77,485,498]. However, mice implanted with the 20% pellets did not show behavioural hyperresponsiveness to the cocaine challenge on day 15. It is conceivable that a certain degree of behavioural sensitisation did develop in mice receiving chronic corticosterone, and we hypothesise that a longer withdrawal duration may be required for this behaviour to become expressed. Indeed, psychostimulant sensitisation is characterised by a time-dependent cascade of cellular changes that differs between short- and long-term withdrawal periods [520,693]. The observation that locomotor responses of ADXcort and SHAM mice were no longer different by the time of the second cocaine challenge (day 21), supports this hypothesis. This finding was replicated in a subsequent experiment in which drug responses of ADXcort mice were even equal to those of SHAM mice on day 21 (see chapter 5). Further

research is required to investigate the time course of behavioural sensitisation in mice receiving chronic corticosterone.

Several explanations can be proposed for the inefficacy of corticosterone in fully reversing the effects of ADX on behavioural sensitisation to cocaine.

First, it is conceivable that the hormone was not replaced in sufficient amounts. This is however not likely, since the dose of 1 mg/kg used in the intermittent replacement studies resulted in extraphysiological peak plasma concentrations of 1200 ng/ml (5 minutes after i.p. administration, data not shown). Furthermore, corticosterone in the same dose range has been shown to sensitise the locomotor response to amphetamine in rats [165]. However, it has also been demonstrated that administration of 10 mg/kg, but not 5 mg/kg, corticosterone prior to cocaine enhances behavioural sensitisation to the drug in intact rats, indicating that even in the extra-physiological dose range, there may be dose-dependency for the effects of corticosterone [529]. In the case of the 20% corticosterone pellet, mice were exposed to high glucocorticoid levels especially during the initiation of behavioural sensitisation, to which the hormone was hypothesised to contribute. Furthermore, concentrations in the range of stress-induced levels were still present at the time of the first cocaine challenge. Previously, a 20% corticosterone pellet has been demonstrated to fully restore amphetamine-induced locomotion in ADX mice [681].

Second, sensitisation of HPA-axis responsiveness to cocaine may be a requirement for behavioural sensitisation in this strain. Indeed, DBA/2 mice display parallel sensitisation of locomotion and corticosterone secretion during the treatment phase and in response to the cocaine challenge on day 15 (the present study, [145]). However, it has been shown previously that sensitisation of corticosterone secretion may not be a prerequisite for long-term expression of behavioural sensitisation [609]. Further studies are therefore required to investigate the potential contribution of endocrine sensitisation to behavioural responsiveness in this mouse strain.

Third, it may be necessary to restore the circadian and/or ultradian rhythmicity in corticosterone secretion. In contrast to rats, mice maintain basal levels of corticosterone that are considered sufficient for occupation of the high-affinity mineralocorticoid receptor after ADX (the present study, [300]). However, ADX mice do lack the rise in plasma corticosterone that precedes the nocturnal period. Although diurnal concentrations of corticosterone are sufficient for the locomotor response to a single cocaine exposure in rats [422,423], a full circadian cycle might be required for behavioural sensitisation to repeated drug exposure.

Assuming that sufficient amounts of corticosterone were replaced, the observation that only chronic high concentrations of the hormone partially restored initiation of

sensitisation, suggests that glucocorticoids may not be the only adrenal factors that contribute to behavioural sensitisation in the DBA/2 strain. This conclusion is supported by several observations. It is well accepted that stress-induced behavioural sensitisation to psychostimulants depends on corticosterone secretion [164,166]. However, studies using ADX, genetic- or pharmacological inactivation of the GR have generated conflicting results regarding the necessity of adrenal glucocorticoids in psychostimulant sensitisation [21,168,527,529,552,609]. Furthermore, two recent reports have indicated that corticosterone may be necessary, but not sufficient for the effects of stress on escalation of cocaine self-administration [417] and on the neurochemical and rewarding effects of morphine [161]. Mantsch *et al.* elegantly showed that restoration of corticosterone levels in the range of those induced by footshock stress is necessary to reinstate stress-induced escalation of cocaine self-administration in ADX animals. However, corticosterone alone was not sufficient to reproduce the effects of the stressor in animals that were not exposed to footshock [417]. The requirement of the stressor suggests that stress-induced factors other than corticosterone play an additional role. In view of the present data with ADX, it is tempting to speculate a role for the adrenal catecholamine epinephrine. Indeed, the HPA-axis and the sympathetic nervous system have been demonstrated to interact in regulation of behaviour, most notably in memory processing [53,571]. Further research is required to investigate the potential role of other adrenal factors such as epinephrine, in addition to that of corticosterone, in behavioural sensitisation to cocaine.

The present study showed that, in a mouse strain in which behavioural sensitisation to cocaine is critically dependent on adrenal hormones, the glucocorticoid corticosterone facilitated the initiation and retention of the behavioural sensitisation to repeated drug exposure, provided it was continuously circulating. To full blown recovery of the impaired behavioural sensitisation to psychostimulants in ADX mice other adrenal factors, such as epinephrine, may contribute.

ACKNOWLEDGEMENTS

This research was supported by NWO/INSERM/ZON grants 985-10-014 and 985-10-504 and by the Royal Netherlands Academy of Arts and Sciences. We gratefully acknowledge ing. M. van der Mark and drs. L. Enthoven for technical support and dr. O.C. Meijer for critically reading the manuscript.

5

Behavioural sensitisation to cocaine: cooperation between glucocorticoids and epinephrine

Inge E.M. de Jong, Peter J. Steenbergen and E. Ronald de Kloet

ABSTRACT

Stressful life experiences facilitate behavioural responsiveness to psychostimulant drugs. While there is ample evidence that adrenal glucocorticoids mediate the effects of stress, the role of the sympathoadrenal system is poorly understood.

The present study was designed to investigate the role of the two adrenal stress hormones, corticosterone and epinephrine, in sensitisation to the locomotor stimulant effects of cocaine. The DBA/2 mouse strain was used, as behavioural sensitisation in this strain is critically dependent on adrenal hormones. Animals were subjected to adrenalectomy ('ADX', surgical removal of the adrenals) or SHAM surgery and ADX mice were given replacement therapies of epinephrine ($5x10^{-3}$ mg/kg, s.c. just prior to each drug administration), corticosterone (20% s.c. pellet) or both. We have measured sensitisation to the locomotor stimulant effects of repeated cocaine exposure and, in addition, cocaine-induced c-fos mRNA expression in a number of brain regions to identify a neural substrate for the actions of the adrenal hormones.

In agreement with our previous observations, ADX prevented initiation and expression of cocaine-induced locomotor sensitisation. Whereas neither corticosterone nor epinephrine alone were sufficient to reverse the ADX effect, both adrenal stress hormones were necessary to fully restore the initiation and retention of locomotor sensitisation to levels observed in SHAM animals. In addition, we show that epinephrine potentiated cocaine-induced c-fos mRNA expression in the hypothalamic PVN, indicating that the catecholamine may also contribute to sensitisation of the hypothalamic-pituitary-adrenal (HPA) axis to cocaine.

The present findings indicate that corticosterone and epinephrine cooperate to facilitate behavioural responsiveness to cocaine. These data emphasise that, in addition to the HPA-axis, the autonomic sympathetic nervous system (ANS) plays a critical role in psychostimulant sensitivity.

INTRODUCTION

It is well known that stress, resulting in activation of the hypothalamic-pituitary-adrenal axis (HPA-axis) and the autonomic sympathetic nervous system (ANS) [146], can increase sensitivity to the behavioural and reinforcing effects of psychostimulant drugs, a phenomenon known as behavioural sensitisation [249,514]. Studies in laboratory rodents have demonstrated that stress facilitates acquisition and relapse of psychostimulant self-administration [244,277,510,660] and enhances vulnerability to the locomotor stimulant properties of these drugs [164,272,284,383,635].

A wealth of data indicates that adrenal glucocorticoids, the output hormones of the HPA-axis, mediate the effects of stress on psychostimulant responsiveness [144,168,240,421], primarily via their actions on the mesencephalic dopamine system [515]. By contrast, the role of the ANS in psychostimulant sensitivity has received little attention. This is surprising since catecholamines (epinephrine, norepinephrine) are rapidly released into the general circulation during stress and in response to psychostimulant administration [109]. Although the catecholamines are not likely to cross the blood-brain-barrier due to their polar structure [725], substantial evidence indicates that peripheral epinephrine can alter brain function and behaviour indirectly by activating vagal afferents to the central nervous system [54,252,456,641]. Indeed, it has been shown that, in addition to their independent actions, the HPA- and ANS components of the stress response can interact to regulate behaviour [53,571].

We have previously identified a mouse strain, the DBA/2 strain, in which sensitisation to the locomotor stimulant effects of cocaine is critically dependent on adrenal hormones [145]. Interestingly, whereas surgical removal of the adrenals (adrenalectomy, 'ADX') prevented initiation and expression of psychomotor sensitisation, replacement of corticosterone to concentrations similar or even higher than those induced by stress was not sufficient to fully reverse the effects of ADX (chapter 4). Similarly, it has recently been reported that glucocorticoids are necessary, but not sufficient, to restore the effects of stress on escalation of cocaine self-administration [417] and on morphine-induced conditioned place preference [161] in ADX rats. In addition, there is considerable controversy in literature regarding the necessity of adrenal glucocorticoids in psychostimulant-induced behavioural sensitisation [21,527,529,552]. Collectively, these findings suggest that adrenal glucocorticoids may not be 'the sole players on the scene'. In view of our findings with ADX, we propose an additional role for the adrenal catecholamine epinephrine [145].

In the present study, we have therefore investigated the role of adrenal glucocorticoids and epinephrine in sensitisation to the locomotor stimulant effects of cocaine in the DBA/2 strain. Animals were adrenalectomised or SHAM operated and ADX mice were given replacement of corticosterone (20% pellet, see chapter 4), epinephrine ($5x10^{-3}$ mg/kg, s.c. prior to each drug administration) or both. We have measured sensitisation to the locomotor stimulant effects of cocaine and, in addition, cocaine-induced c-fos mRNA expression in a number of brain regions to identify a neural substrate for the actions of the adrenal hormones.

METHODS

Animals

Male DBA/2 Rj mice were obtained from Janvier (Le Genest Saint Isle, France) at the age of 8 weeks. Mice were housed in groups of four in perspex cages (35x19x14cm) with food and water available *ad libitum* at a 12 hour light-dark cycle (lights on: 7 am) in a temperature (21±1ºC) and humidity (55±5%) controlled room. Surgery was performed 2 weeks after arrival in the animal facility. Animals were briefly handled in the week before surgery and otherwise left undisturbed. Animal experiments were approved by the local Committee for Animal Health, Ethics and Research of Leiden University. Animal care was conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC).

Experimental design

Animals were either adrenalectomised (ADX) or SHAM operated. Replacement therapies consisted of corticosterone (ADXcort), epinephrine (ADXepi), or both (ADXcort+epi). Mice were administered saline (SAL) or cocaine (COC) during the treatment phase of the sensitisation paradigm, resulting in 10 treatment groups: SHAM (SAL/COC), ADX (SAL/COC), ADXcort (SAL/COC), ADXepi (SAL/COC) and ADXcort+epi (SAL/COC).

Surgery

Animals were individually housed 1 day prior to surgery. The cages were transported to the operating room on the morning of the surgery where mice were allowed to recover from transportation for 2 hours. Inhalation anaesthesia consisted of a mixture of isoflurane (3 l/min), N_2O (0.8 l/min) and O_2 (0.4 l/min). Adrenalectomy

was performed via the dorsal route as described previously [145]. SHAM animals were treated similarly with the exception of the actual removal of the adrenals. Mice were kept individually housed for 24 hours following surgery after which they were housed two animals per cage of similar surgery. After surgery, all animals were given free access to 0.9% NaCl in addition to normal drinking water. The sensitisation paradigm was started following a recovery period of 1 week.

Drugs

Cocaine hydrochloride (BUFA Pharmaceuticals B.V., Uitgeest, The Netherlands) was dissolved in sterile saline and administered intraperitoneally (i.p.) in a dosage of 7.5 or 15.0 mg/kg. Corticosterone pellets (100 mg, w x h: 9 x 2 mm) consisted of 20% corticosterone (ICN Biomedicals Inc, Aurora, Ohio, USA) in cholesterol vehicle (cholesterol 95% stabilised, Acros Organics, Geel, Belgium) and were implanted subcutaneously in the flank of the animal. Epinephrine ((-)-epinephrine(+)-bitartrate salt, Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands) was dissolved in sterile saline and administered subcutaneously (s.c.) in a dosage of $5x10^{-3}$ mg/kg. The epinephrine solution was made freshly on the day of use, adjusted to pH 5 and protected from light. Control groups were implanted with 100% cholesterol pellets and received equal volumes of saline instead of cocaine or epinephrine. From the start of the sensitisation paradigm, animals were weighed once every two days and the injection volumes were adjusted accordingly.

Sensitisation paradigm

One day prior to the first drug administration and thus the first behavioural test, animals were individually housed and kept single housed for the remainder of the experiment. The sensitisation paradigm consisted of a treatment phase (days 1-9), a withdrawal interval (days 10-14), a saline challenge (day 14), and two cocaine challenges (days 15 and 21). The treatment phase consisted of i.p. injections of 15.0 mg/kg cocaine (COC) or saline (SAL) on 9 consecutive days and locomotion was measured on days 1, 5 and 9. On these days, animals received the treatment in the test setting as described in the following paragraph. On the remaining days, animals received the injections in the home cage. The treatment period was followed by a withdrawal interval of 5 days (no treatment). On the last day of the withdrawal interval (day 14), all animals received a saline challenge and on day 15, all animals received a 7.5 mg/kg cocaine challenge. To investigate drug responsiveness in absence of epinephrine, animals were re-challenged with 7.5 mg/kg cocaine one week after the initial cocaine challenge (day 21) without prior epinephrine

substitution. For this day, only data of cocaine-treated mice are presented, as the saline-treated groups received cocaine for the second time and can therefore no longer be considered proper controls. All injections were given 2 to 5 hours after lights on.

Measurement of locomotor activity

All behavioural tests (days 1, 5, 9, 14, 15 and 21) were performed in the room where animals were housed. Mice were placed in a test cage (same type and size (35x19x14cm) as the home cage) containing a standardised amount of sawdust, covered with a perspex lid. Following a 2 hour habituation period, animals were injected and activity was monitored on video for 30 minutes. At the end of this period, a blood sample was taken from the tail vein for endocrine measurements and the animals were returned to their home cage.

Analysis of locomotor activity

Video images were digitised and analysed using Ethovision Videotracking, Motion Analysis & Behavior Recognition System version 1.96 ('VTMAS', Noldus Information Technology B.V., Wageningen, The Netherlands). The position of the animal was sampled 5 times per second. Of each recording (30 minutes) 27 minutes were analysed since the animals were subjected to blood sampling at 30 minutes after injection. Data are represented in total distance moved (cm) over the entire 27 minute treatment period. Locomotion was defined as movement with a minimal distance of 2 cm.

Corticosterone assay

Blood samples were taken from the tail vein by a small incision with a razorblade 30 minutes after drug treatment on the test days 1, 5, 9, 14 and 15 and collected in small EDTA coated tubes (Microvette DB 200 K3E, Sarstedt, Nümbrecht, Germany). On day 21, animals were sacrificed 30 minutes after treatment and trunk blood was collected in large EDTA coated tubes (Tube 10 ml, 95x16.8 mm, K3E, Sarstedt, Nümbrecht, Germany). Plasma was obtained by centrifugation at 13000 rpm for 20 minutes at 4ºC and subsequently stored at -20ºC. Corticosterone concentrations were determined by in-duplo measurement using a radio-immuno-assay (RIA) kit from MP Biomedicals according to the protocol provided by the manufacturer (Corticosterone double antibody ^{125}I RIA kit, MP Biomedicals, Asse-Relegem, Belgium). All samples were analysed in one assay to exclude inter-assay variability.

ADX effectively clamped plasma corticosterone to basal concentrations and only animals with successful ADX were included in the study.

c-fos mRNA *in situ* hybridisation

Brains were dissected, snap frozen in isopentane (cooled in ethanol placed in pulverised dry ice) and stored at -80 ºC until further use. Sections of 16 µm were cut on a cryostat (Leica CM3050). Based on the brain atlas of Franklin and Paxinos [220], sections were taken at the level of the prefrontal cortex (PFC: medial orbital (MO, bregma: +2.34), infralimbic (IL, bregma: +1.54) and prelimbic (PrL, bregma: +1.54) regions), nucleus accumbens (Nac: core and shell regions, bregma: +1.44), rostral caudate putamen (rCP, bregma: +1.44), caudal CP (cCP: lateral (Lat), dorsomedial (DM) and ventromedial (VM) regions, bregma: +0.32, see chapter 3), paraventricular nucleus of the hypothalamus (PVN, bregma: -0.82), hippocampus (Hipp: CA1 and dentate gyrus (DG) subdivisions, bregma: -1.58), amygdala (Amy: basolateral (BLA) and central (CE) nuclei, bregma: -0.94), ventral tegmental area (VTA, bregma: -3.52), substantia nigra pars compacta (SNc, bregma: -3.52), locus coeruleus (LC, bregma: -5.52) and nucleus of the solitary tract (NTS, bregma: -7.20). Sections were thaw-mounted on 0.001% poly-L-lysine (Sigma-Aldrich) coated slides and stored at -80 ºC until further use.

For prehybridisation purposes, sections were fixed for 30 min. in 4% paraformaldehyde in PBS (pH 7.4), washed twice in PBS, treated with 1% HCL for 10 min., treated with 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min., rinsed for 10 min. in 2 x SSC (150 mM sodium chloride, 15 mM sodium citrate), dehydrated in an ethanol series, delipidated in chloroform for 1 min., air dried and stored at room temperature until the hybridisation.

A 45-mer oligonucleotide probe encoding mouse c-fos mRNA was used: 5′-TGCTGGAGAAGGAGTCGGCTGGGGAATGGTAGTAGGAAAGGCTG-3′. To control for specificity we used an oligonucleotide that was identical except for 6 mismatches (transversions) evenly spaced by 6 nucleotides: 5′-TGCGGGAGAATGAGTCGTCTGGGGCATGGTATTAGGAACGGCTG-3′. The oligonucleotides were 3′ end-labelled with α [^{35}S]deoxyadenosine triphosphate (GE Healthcare Europe Gmbh, Diegem, Belgium) using terminal deoxynucleotidyl transferase (Promega Benelux, Leiden, The Netherlands).

To each slide, 160 µl of hybridisation mix containing 2 x 10^6 dpm of labelled oligonucleotide were applied. The hybridisation mix consisted of 50% formamide, 10% dextran sulfate, 2 x SSC, 20 mM sodium phosphate, 200 mM DTT, 1 x Denhardt's, 0.5 mg/ml sheared herring sperm DNA, 0.25 mg/ml tRNA and 5% sarcosyl. Sections were coverslipped and hybridised overnight in a moist chamber at 42 °C.

The next morning, after removal of the coverslips, sections were rinsed in 1 x SSC at room temperature, washed twice for 30 min. in 1 x SSC at 50 °C, washed for 5 min. in 1 x SSC at room temperature, dehydrated in an ethanol series, air dried and apposed to Kodak Biomax MR film for 5-15 days (Kodak, Rochester, NY). After development of the films, sections were counter-stained with 0.5% cresyl violet.

Analysis of expression was performed by measuring grey levels from film. A ^{14}C-standard curve was included to ensure that grey values were within the linear range between 0 and 255. There was no signal in tissue hybridised with the mismatch oligonucleotide. Average optical density per region (in arbitrary units) was corrected for film background.

Statistics

Statistical analysis was performed using SPSS for Windows software (release 7.5, SPSS Benelux B.V., Gorinchem, The Netherlands). Overall locomotor activity and corticosterone data were subjected to repeated measures ANOVA with three between subject factors (surgery, substitution and treatment) and one within subject factor (test day). Subsequent analyses were performed per test day: three factor ANOVA for surgery, substitution and treatment. c-Fos data (grey values) were subjected to two factor ANOVA for surgery and substitution. When statistical significance was revealed, post hoc tests were performed (LSD, or for within subject comparison paired t-test). Differences were considered statistically significant when $p<0.05$.

RESULTS

Effect of corticosterone and epinephrine on behavioural sensitisation in ADX mice

Main effects were found for treatment, surgery, substitution, day and interactions between these factors ($F[treatment]_{1,126}=39.878$, $p<0.001$, $F[surgery]_{1,126}=6.667$, $p<0.05$, $F[substitution]_{3,126}=3.006$, $p<0.05$, $F[day]_{5,630}=28.002$, $p<0.001$, $F[day \times substitution]_{15,630}=1.726$, $p<0.05$ and $F[day \times treatment]_{5,630}=14.038$, $p<0.001$).

Figure 1 depicts locomotor responses of the SHAM (SAL/COC), ADX (SAL/COC), ADXcort (SAL/COC), ADXepi (SAL/COC) and ADXcort+epi (SAL/COC) groups on days 1 and 5 of the treatment period. In agreement with our previous observations (chapter 4), corticosterone replacement via the 20% pellet reduced the initial cocaine response ($p<0.001$ vs. SHAM, $p<0.05$ vs. ADX and ADXepi), while there was a strong tendency for epinephrine to reverse this effect (ADXcort vs. ADXcort+epi: $p=0.060$). In addition, cocaine responses on day 1 were highest in the

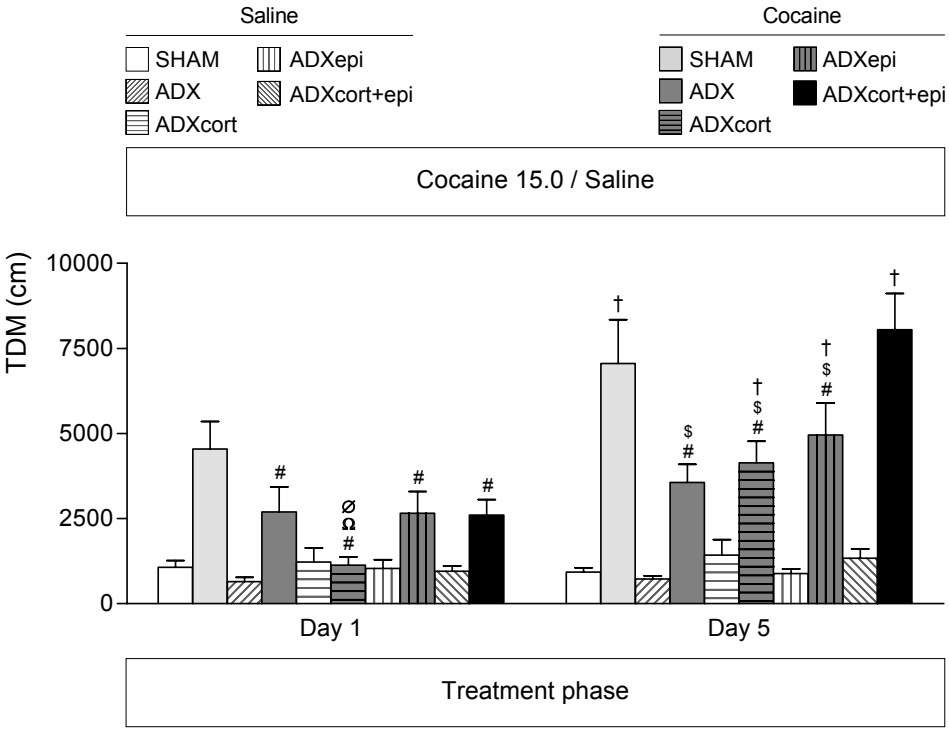

Figure 1: *Initiation of behavioural sensitisation.*
Locomotion in response to treatment on days 1 and 5 of the treatment period, in which mice received daily administration of cocaine (15.0 mg/kg) or saline. Animals were SHAM operated or adrenalectomised (ADX) and ADX mice received either no substitution or replacement with corticosterone (ADXcort), epinephrine (ADXepi), or both (ADXcort+epi). Data are represented in average total distance moved (TDM, cm) ± SEM, n=7-9 (saline) or 17-24 (cocaine) animals/group. # at least $p<0.05$ vs. SHAM, Ω $p<0.05$ vs. ADX, ∅ $p<0.05$ vs. ADXepi, $ at least $p<0.01$ vs. ADXcort+epi (LSD), † at least $p<0.05$ vs. day 1 (paired t-test).

SHAM group ($F[treatment]_{1,150}=13.131$, $p<0.001$, $F[surgery]_{1,150}=2.342$, $p=0.128$, $F[substitution]_{3,150}=0.317$, $p=0.813$, post hoc: at least $p<0.05$ vs. all other treatment groups).

As described previously (chapters 2 and 4, [145]), SHAM mice displayed an increase in drug responsiveness during the treatment period (day 5 vs. day 1: $p<0.05$, paired t-test), whereas this was prevented by ADX ($p=0.191$, paired t-test). Furthermore, cocaine responses on day 5 were reduced in ADX when compared to SHAM mice ($p<0.01$). Co-replacement of corticosterone and epinephrine fully restored initiation of sensitisation (day 5 vs. day 1: $p<0.001$, paired t-test), and drug responses on day 5, to the level observed in SHAM mice. The ADXcort and ADXepi groups

also displayed an increase in cocaine responsiveness from day 1 to day 5 ($p<0.01$, paired t-test) but drug responses on day 5 were not different from those of ADX mice and lower than in the SHAM and ADXcort+epi groups ($F[treatment]_{1,142}=41.974$, $p<0.001$, $F[surgery]_{1,142}=2.870$, $p=0.093$, $F[substitution]_{3,142}=2.373$, $p=0.073$, post hoc: at least $p<0.05$ vs. SHAM and ADXcort+epi).

Drug responses on day 9 of the treatment period showed a similar group distribution and were not further enhanced when compared to day 5, indicating that behavioural sensitisation develops during the first half of the treatment period (SHAM: 7508 ± 1009, ADX: 4193 ± 725, ADXcort: 5641 ± 747, ADXepi: 5718 ± 898, ADXcort+epi: 6252 ± 1030). Similar to previous observations (chapter 4),

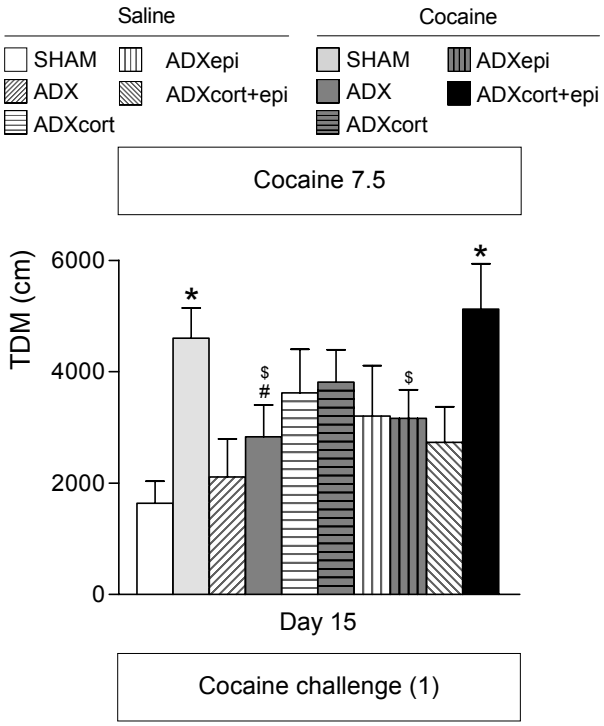

Figure 2: *Expression of behavioural sensitisation (day 15).*
Locomotion in response to the 7.5 mg/kg cocaine challenge on day 15. Animals were SHAM operated or adrenalectomised (ADX) and ADX mice received either no substitution or replacement with corticosterone (ADXcort), epinephrine (ADXepi), or both (ADXcort+epi). Previously, mice received daily administrations of 15.0 mg/kg cocaine or saline (days 1-9), followed by a 5 day withdrawal interval and a saline challenge (day 14). Data are represented in average total distance moved (TDM, cm) ± SEM, n=7-11 (saline) or 17-22 (cocaine) animals/group. * at least $p<0.05$ vs. SAL, # $p<0.05$ vs. SHAM, $ at least $p<0.05$ vs. ADXcort+epi (LSD).

drug responses of ADXcort mice exceeded those of ADX mice on day 9, but this did not reach statistical significance (p=0.208). A similar trend was observed in the ADXepi group (p=0.195) ($F[treatment]_{1,139}$=43.477, p<0.001, $F[surgery]_{1,139}$=2.390, p=0.125, $F[substitution]_{3,139}$=0.425, p=0.735).

Figure 2 depicts locomotor responses to the first cocaine challenge on day 15. Cocaine-treated SHAM mice displayed hyperresponsiveness to the 7.5 mg/kg cocaine challenge (p<0.01 vs. SHAM/SAL), whereas this was not observed in ADX mice receiving no substitution or either corticosterone or epinephrine alone. By contrast, sensitisation was restored in ADX mice receiving co-replacement of corticosterone

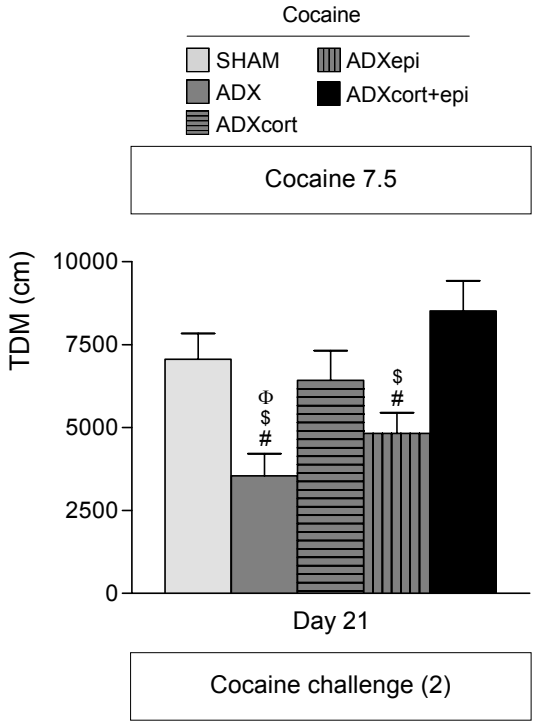

Figure 3: *Expression of behavioural sensitisation (day 21).*
Locomotion of *cocaine-treated* mice in response to the second 7.5 mg/kg cocaine challenge on day 21. On this day, epinephrine replacement was omitted. Previously, SHAM operated and adrenalectomised (ADX) mice receiving either no substitution or replacement with corticosterone (ADXcort), epinephrine (ADXepi) or both (ADXcort+epi), were given daily administrations of 15.0 mg/kg cocaine or saline (days 1-9), followed by a 5 day withdrawal interval, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented in average total distance moved (TDM, cm) ± SEM, n=7-12 (saline) or 17-22 (cocaine) animals/group. # p<0.01 vs. SHAM, ϕ p<0.01 vs. ADXcort, $ p<0.001 vs. ADXcort+epi (LSD).

and epinephrine (ADXcort+epi COC vs. SAL: $p<0.05$). Furthermore, drug responses of cocaine-treated SHAM and ADXcort+epi mice were higher than of ADX and ADXepi mice (at least $p<0.05$, except SHAM/COC vs. ADXepi/COC: $p=0.078$), but not different from ADXcort mice ($F[\text{treatment}]_{1,138}=8.182$, $p<0.01$, $F[\text{surgery}]_{1,138}=0.644$, $p=0.424$, $F[\text{substitution}]_{3,138}=1.401$, $p=0.245$). Similar group differences were observed on day 21, when cocaine-treated mice were re-challenged with 7.5 mg/kg cocaine without prior epinephrine substitution (figure 3). SHAM and ADXcort+epi mice displayed cocaine responses higher than of ADX and ADXepi mice (at least $p<0.05$) but not different from those of ADXcort mice ($F[\text{treatment}]_{1,139}=6.156$, $p<0.05$, $F[\text{surgery}]_{1,139}=10.867$, $p<0.01$, $F[\text{substitution}]_{3,139}=6.186$, $p<0.01$).

Remarkably, responses to the saline challenge on day 14 showed a similar distribution as to the cocaine challenges on days 15 and 21, being higher for cocaine-treated SHAM, ADXcort and ADXcort+epi mice than for ADX and ADXepi mice (SHAM: 2496 ± 259, ADX: 1429 ± 114, ADXcort: 2597 ± 208, ADXepi: 1477 ± 154, ADXcort+epi: 2582 ± 239). A significant effect of treatment was only found in the SHAM group ($F[\text{treatment}]_{1,139}=3.130$, $p=0.079$, $F[\text{surgery}]_{1,139}=2.969$, $p=0.087$, $F[\text{substitution}]_{3,139}=4.062$, $p<0.01$, post hoc: SHAM COC vs. SAL: $p<0.05$).

Effect of ADX and hormone replacement on plasma corticosterone

Table 1 shows the effects of ADX and hormone replacement on plasma corticosterone concentrations 30 minutes after treatment on days 1, 5, 9, 14 and 15. Main effects were found for treatment, surgery, substitution, day and interactions between these factors ($F[\text{treatment}]_{1,108}=30.233$, $p<0.001$, $F[\text{surgery}]_{1,108}=94.276$, $p<0.001$, $F[\text{substitution}]_{3,108}=129.940$, $p<0.001$, $F[\text{day}]_{5,540}=141.546$, $p<0.001$, $F[\text{day x substitution}]_{15,540}=105.773$, $p<0.001$ and $F[\text{day x surgery x treatment}]_{5,540}=4.936$, $p<0.001$).

Corticosterone concentrations were attenuated in the ADX and ADXepi groups compared to the SHAM group on all test days (at least $p<0.01$, except ADX/SAL day 1: $p=0.058$, day 14: $p=0.073$, ADXepi/SAL day 1: $p=0.055$, day 14: $p=0.070$ vs. SHAM/SAL). Cocaine-treated SHAM mice exhibited enhanced corticosterone secretion on day 5 compared to day 1 of the treatment period ($p<0.001$, paired t-test), with no further increase on day 9, indicating that also endocrine sensitisation develops during the first half of the treatment period. In addition, corticosterone secretion in response to the saline challenge on day 14 was augmented in SHAM/COC mice ($p<0.001$ vs. SHAM/SAL). A similar effect was observed in response to the cocaine challenge on day 15, but this did not reach statistical significance ($p=0.248$).

Table 1: *Effect of ADX and hormone replacement on plasma corticosterone concentrations.*

		Day 1	Day 5	Day 9	Day 14 saline challenge	Day 15 cocaine challenge 1
SHAM	**SAL**	87.5 ± 13.7	91.7 ± 19.9	125.2 ± 29.9	66.2 ± 11.1	182.2 ± 12.8
	COC	161.6 ± 12.0 *	313.1 ± 14.7 *†	307.3 ± 20.1 *	140.3 ± 15.8 *	208.9 ± 21.6
ADX	**SAL**	14.2 ± 3.5	17.4 ± 6.8 #	28.2 ± 6.6 #	31.2 ± 7.5	32.7 ± 8.8 #
	COC	15.0 ± 2.6 #	25.9 ± 4.5 #	35.9 ± 7.1 #	31.7 ± 5.4 #	33.4 ± 6.0 #
CORT	**SAL**	538.0 ± 32.0 # Ω	169.9 ± 6.2 # Ω	111.7 ± 8.7 Ω	75.5 ± 2.9 Ω	83.9 ± 8.8 # Ω
	COC	500.1 ± 23.8 # Ω	179.1 ± 5.8 # Ω	129.6 ± 8.2 # Ω	85.6 ± 4.8 # Ω	92.0 ± 8.7 # Ω
EPI	**SAL**	10.9 ± 2.3	19.5 ± 5.6 #	21.7 ± 6.3 #	29.6 ± 8.6	30.4 ± 9.5 #
	COC	15.3 ± 2.3 #	28.8 ± 4.2 #	39.0 ± 5.9 #	36.2 ± 5.5 #	38.2 ± 6.1 #
CORT+EPI	**SAL**	514.4 ± 27.3 # Ω	150.4 ± 9.6 # Ω	91.4 ± 6.6 Ω	69.9 ± 4.7 Ω	78.8 ± 6.1 # Ω
	COC	459.8 ± 26.0 # Ω	173.8 ± 7.8 # Ω	131.1 ± 9.0 # Ω	82.6 ± 6.4 # Ω	88.2 ± 7.8 # Ω

Plasma corticosterone concentrations 30 minutes after treatment on days 1, 5, 9, 14, and 15. Animals were SHAM operated or adrenalectomised (ADX) and ADX mice received either no substitution or replacement with corticosterone (cort), epinephrine (epi), or both (cort+epi). Mice received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal interval, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented in average plasma corticosterone concentration (ng/ml) ± SEM, n=6-11 (saline) or 17-21 (cocaine) animals/group. * $p<0.001$ vs. SAL, # at least $p<0.01$ vs. SHAM, Ω at least $p<0.05$ vs. ADX *and* ADXepi (LSD), † $p<0.001$ vs. day 1 (paired t-test).

In animals with corticosterone pellets (ADXcort and ADXcort+epi), hormone concentrations were elevated above SHAM levels on day 1 ($p<0.001$) and thereafter gradually declined to on average 90 ng/ml on day 15, being higher than in ADX and ADXepi mice ($p<0.05$), but in the case of cocaine treatment, lower than in SHAM mice ($p<0.01$). On day 21, there was no longer a difference between ADX animals substituted with or without corticosterone (data not shown). These data indicate that the subcutaneous corticosterone pellet generated plasma corticosterone concentrations that were very high on the first day of the treatment phase and thereafter declined to concentrations in the range of those induced by stress, that remained until the first cocaine challenge on day 15.

c-Fos mRNA expression

On day 21, animals were sacrificed 30 minutes after the second cocaine challenge and c-fos mRNA expression was measured in a number of brain regions of cocaine-

Table 2: *c-Fos mRNA expression.*

		Cocaine-treated			
	SHAM	**ADX**	**ADXcort**	**ADXepi**	**ADXcort+epi**
PFC MO	24.4 ± 5.3	32.6 ± 7.0	28.7 ± 4.1	28.8 ± 4.4	32.6 ± 4.8
PFC IL	41.0 ± 5.6	40.9 ± 6.5	44.4 ± 5.0	41.6 ± 3.8	43.8 ± 5.0
PFC PrL	47.7 ± 5.4	50.8 ± 6.3	54.4 ± 5.3	45.1 ± 2.2	50.8 ± 4.0
NAc shell	18.6 ± 4.7	24.2 ± 4.9	20.9 ± 4.5	19.9 ± 3.5	22.7 ± 4.1
NAc core	23.6 ± 5.3	27.2 ± 5.6	24.9 ± 4.9	23.6 ± 3.9	29.5 ± 5.7
rCP	22.3 ± 4.8	25.2 ± 5.1	26.7 ± 5.0	23.7 ± 3.6	28.4 ± 5.1
cCP Lat	17.9 ± 4.9	19.9 ± 5.5	15.4 ± 3.3	16.1 ± 3.9	18.6 ± 4.2
cCP DM	30.1 ± 5.7	27.4 ± 5.8	33.3 ± 5.2	30.5 ± 2.8	36.2 ± 4.8
cCP VM	15.9 ± 5.0	18.1 ± 4.7	14.6 ± 3.1	14.9 ± 3.5	20.4 ± 4.1
Hipp CA1	59.7 ± 8.6	65.2 ± 10.3	61.5 ± 8.0	56.3 ± 7.7	73.7 ± 9.3
Hipp DG	58.5 ± 11.6	72.5 ± 12.9	66.0 ± 10.3	59.3 ± 12.5	77.6 ± 12.5
Amy BLA	17.0 ± 3.4	17.7 ± 3.7	19.5 ± 3.7	21.1 ± 3.6	19.8 ± 4.1
Amy CE	12.3 ± 2.9	14.9 ± 3.7	12.5 ± 3.5	14.3 ± 3.1	14.2 ± 3.6
VTA	20.1± 6.0	29.2 ± 7.5	31.2 ± 5.9	25.2 ± 5.6	29.2 ± 4.9
SNc	24.2 ± 7.6	31.7 ± 8.0	29.7 ± 5.5	25.4 ± 4.8	28.2 ± 4.0
LC	31.7 ± 6.3	28.6 ± 7.5	31.3 ± 6.3	22.4 ± 3.9	27.5 ± 2.7
NTS	26.9 ± 5.8	24.8 ± 5.6	23.8 ± 5.5	26.8 ± 5.1	18.2 ± 4.1

c-Fos mRNA expression in *cocaine-treated* mice, 30 minutes after the 7.5 mg/kg cocaine challenge on day 21. Previously, SHAM operated and adrenalectomised (ADX) mice receiving either no substitution or replacement with corticosterone (ADXcort), epinephrine (ADXepi) or both (ADXcort+epi), were given daily administrations of 15.0 mg/kg cocaine or saline (days 1-9), followed by a 5 day withdrawal interval, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented in optical density in arbitrary units (au) ± SEM. n=10-12 animals/group. PFC: prefrontal cortex, MO: medial orbital, IL: infralimbic, PrL: prelimbic, NAc: nucleus accumbens, rCP: rostral caudate putamen, cCP: caudal CP, Lat: lateral, DM: dorsomedial, VM: ventromedial, Hipp: hippocampus, DG: dentate gyrus, Amy: amygdala, BLA: basolateral, CE: central, VTA: ventral tegmental area, SNc: substantia nigra pars compacta, LC: locus coeruleus, NTS: nucleus of the solitary tract.

treated mice (table 2). This time interval was chosen as cocaine-induced c-fos mRNA expression can be readily observed 30 minutes after drug administration [40]. c-Fos mRNA was expressed in all brain regions examined and, with the exception of the hypothalamic PVN, no group differences in expression were observed. Figure 4 depicts c-fos mRNA expression in the hypothalamic PVN. A main effect was found for substitution ($F[substitution]_{3,52}=9.350$, $p<0.001$, $F[surgery]_{1,52}=0.273$, $p=0.604$). In animals previously receiving epinephrine replacement, cocaine-induced c-fos mRNA expression was higher than in all other groups (at least $p<0.05$), which just failed to reach significance for the SHAM group ($p=0.073$). By contrast, c-fos mRNA

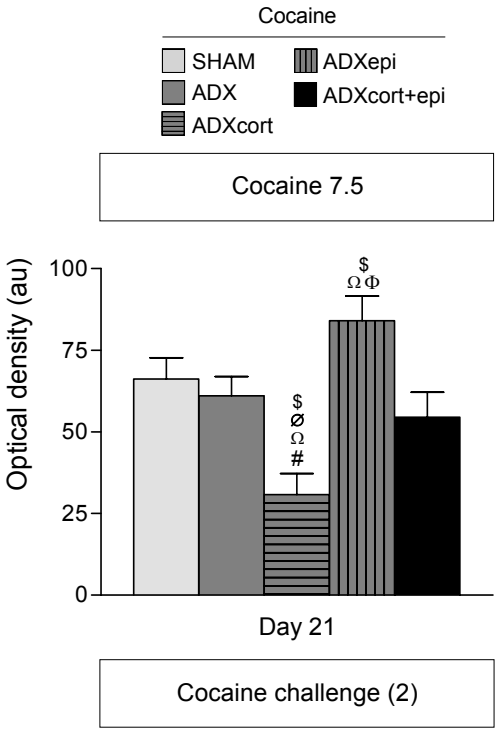

Figure 4: *c-Fos mRNA expression in the PVN.*
c-Fos mRNA expression in the hypothalamic PVN of *cocaine-treated* mice, 30 minutes after the 7.5 mg/kg cocaine challenge on day 21. Previously, SHAM operated and adrenalectomised (ADX) mice receiving either no substitution or replacement with corticosterone (ADXcort), epinephrine (ADXepi) or both (ADXcort+epi), were given daily administrations of 15.0 mg/kg cocaine or saline (days 1-9), followed by a 5 day withdrawal interval, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented in optical density in arbitrary units (au) ± SEM. n=10-12 animals/group. # $p<0.01$ vs. SHAM, Ω at least $p<0.05$ vs. ADX, ∅ $p<0.001$ vs. ADXepi, ϕ $p<0.001$ vs. ADXcort, $ at least $p<0.05$ vs. ADXcort+epi (LSD).

expression was reduced in ADXcort mice when compared to all other groups (at least $p<0.05$). In animals previously receiving epinephrine co-substitution, this reduction was reversed ($p<0.05$ vs. ADXcort), to the level of SHAM and ADX, but not of ADXepi mice ($p<0.01$).

DISCUSSION

The major finding emerging from the present experiments is that, in addition to corticosterone, epinephrine is necessary for sensitisation to the locomotor stimulant effects of cocaine in DBA/2 mice. By contrast, neither corticosterone nor epinephrine alone were sufficient to reverse the deficit of ADX mice in psychomotor sensitisation. In addition, we find that cocaine-induced c-fos mRNA expression in the hypothalamic PVN is potentiated in epinephrine-pretreated mice, indicating that the adrenal catecholamine may also play a role in endocrine sensitisation to the psychostimulant.

The present findings indicate that there is a cross-talk between the HPA-axis and the ANS in regulation of the neuronal mechanism underlying behavioural sensitisation to cocaine. The notion that adrenal glucocorticoids and catecholamines can interact to regulate behaviour is supported by previous studies that describe both synergistic and antagonistic interactions, most notably in memory processing [53,571]. We show that, in the case of behavioural sensitisation to cocaine, corticosterone and epinephrine cooperate. Furthermore, the results suggest that the cooperation between the two adrenal stress hormones occurs during initiation rather than expression of cocaine-induced behavioural sensitisation in DBA/2 mice. In a previous study, we have shown that expression of sensitisation is independent of glucocorticoids as the glucocorticoid receptor (GR) antagonist mifepristone failed to block hyperresponsiveness to a drug challenge in animals that had previously acquired this behaviour (chapter 4). The same holds true for epinephrine, as drug responses of mice receiving combined corticosterone and epinephrine replacement were maintained when epinephrine was omitted prior to the second cocaine challenge (day 21).

The observation that by the time of the second cocaine challenge (day 21), drug responses of mice receiving chronic corticosterone mimicked those of SHAM mice is in agreement with our previous observations (chapter 4), and suggests that while a certain degree of sensitisation develops in mice receiving corticosterone, a longer withdrawal duration may be required for this behaviour to become expressed. By contrast, mice substituted with only epinephrine did not show drug responses higher

than of ADX mice on day 21. Collectively, these findings suggest that, of the two adrenal stress hormones, corticosterone plays the most critical role in behavioural sensitisation to cocaine, whereas the primary effect of epinephrine is to facilitate the actions of the glucocorticoid.

Behavioural sensitisation to psychostimulant drugs is mediated by the mesocorticolimbic dopamine system and the neuronal circuitry it is embedded in (reviewed in: [520,693]). Corticosterone readily passes the blood-brain-barrier and the GR is highly expressed throughout the motive circuit and the limbic system [225,279,541]. Studies using brain-specific GR knockouts have shown that the impact of glucocorticoids on psychostimulant sensitivity and behavioural sensitisation is mediated via the GR in the brain [168,308]. Epinephrine, by contrast, is a polar substance that cannot cross the blood-brain-barrier [725]. Exogenous epinephrine does however modulate a wide variety of brain functions (e.g. memory storage, cortical information processing, arousal and attention), suggesting that the catecholamine influences the central nervous system indirectly. The most probable route is via activation of vagal afferents to the nucleus of the solitary tract resulting in activation of central noradrenergic signalling [251,456,570,730]. In addition, it has been proposed that epinephrine may influence brain function by increasing glucose concentrations in the blood [253], although this view has been challenged [228].

To investigate the possible involvement of glucose, we have established a dose-response curve for the effects of a wide dose-range of epinephrine ($5x\ 10^{-1}$, 10^{-2},10^{-3} and 10^{-5} mg/kg) on plasma glucose (data not shown). Whereas the two highest doses increased plasma glucose in a dose-dependent manner, this was not the case for the two lowest doses, including the one used in the present study (5×10^{-3} mg/kg). In addition, we have measured plasma glucose 30 minutes after drug treatment on the test days 1, 9, 14 and 15 of the sensitisation paradigm and found no difference between epinephrine- and saline-substituted mice on any test day (data not shown). These observations indicate that the dose of epinephrine that influences behavioural sensitisation is devoid of a metabolic effect and therefore excludes the possibility that a change in glucose metabolism has contributed to the effects of epinephrine on behavioural sensitisation.

We therefore hypothesise that norepinephrine mediates the effects of epinephrine on behavioural sensitisation to cocaine. Noradrenergic signalling would then be expected to play a critical role in behavioural sensitisation to psychostimulants as epinephrine is rapidly secreted in response to these drugs [109,388]. In support of this hypothesis, it has been shown that α-adrenergic receptors play a role in

cocaine- and amphetamine-induced locomotion, -behavioural sensitisation and -reward [182,183], although one study reported no effects of α- and β-adrenergic receptor antagonists on behavioural sensitisation to amphetamine or cocaine [691]. The discrepancy between these studies is likely to have originated from differences in experimental procedures, such as e.g. the doses of the psychostimulants (being considerably lower in the studies of Drouin *et al.*), the design of the sensitisation paradigm and the species and strain of animals used. Additional evidence for the role of norepinephrine in psychostimulant sensitisation has come from a recent study by Salomon *et al.,* suggesting that behavioural sensitisation to amphetamine results from an uncoupling between noradrenergic and serotonergic signalling [587]. Taken together, these studies point to a role for norepinephrine in psychostimulant sensitisation, although it can of course not be concluded whether this represents an indirect effect of epinephrine, direct actions of cocaine on the norepinephrine transporter, or both. In further support of the present findings, it has been demonstrated that the adrenergic system plays a role in the interaction between stress and drug responsiveness as α2-adrenoreceptor agonists block stress-induced reinstatement of cocaine, heroin and alcohol seeking [193,376,620].

Several brain regions are likely to be involved in the interaction between norepinephrine and the mesocorticolimbic dopamine system, and the presence of GRs throughout the motive circuit suggests that glucocorticoids can modulate this interaction at multiple levels. Evidence exists that direct noradrenergic innervation of the ventral tegmental area regulates dopamine release in the terminal regions of these neurons (nucleus accumbens, prefrontal cortex) [302,491]. The amygdala is an area of interest as norepinephrine in this region is of critical importance for the beneficial effects of both epinephrine and corticosterone on memory consolidation [395,396,535,574] and the amygdala provides excitatory input to the mesocorticolimbic dopamine system [566]. Furthermore, norepinephrine in the prefrontal cortex (PFC) regulates amphetamine-induced reward, -locomotion, and -mesoaccumbens dopamine release [49,141,639,702] and the excitatory corticofugal projections from the PFC to the dopamine system are under the control of adrenergic innervation [368,419]. Finally, the hippocampus, receiving prominent noradrenergic innervation [401], plays a role in the acute reinforcing effects of drugs and in relapse to drug taking, and also provides excitatory input to the midbrain dopaminergic neurons [216].

In the present study, we have measured c-fos mRNA expression 30 minutes after the second cocaine challenge (day 21) to identify a neuronal substrate for the differences in behavioural sensitisation resulting from adrenalectomy and hormone replacement. The immediate-early-gene c-fos is considered a marker for neuronal

activation by cocaine and may contribute to downstream changes in gene expression that ultimately play a role in drug addiction [469]. The observation that c-fos mRNA was expressed in the midbrain dopamine system, amygdala, prefrontal cortex, hippocampus and the noradrenergic cell bodies, indicates that all these brain regions were activated in the context of the complex stimulus (cocaine administration in the test context). The specificity of the c-fos signal for the effects of cocaine merits further investigation, as there was no group receiving saline administration. Interestingly, the pronounced differences in behavioural responsiveness to the cocaine challenge did not correlate with group differences in c-fos mRNA expression in the abovementioned brain regions. This indicates that expression of the immediate-early-gene c-fos might not be a suitable marker to distinguish neuronal responsiveness to cocaine at this stage of sensitisation. Rather, there might be downstream alterations in gene- or protein expression and modification [46,100,648]. It is also conceivable that a different withdrawal duration [168,663], or time lapse after the cocaine challenge [168], would allow for group differences in c-fos mRNA expression to be detected. Further studies are required to investigate which brain regions mediate the effects of corticosterone and epinephrine on initiation and expression of behavioural sensitisation to cocaine.

In the hypothalamic PVN by contrast, c-fos mRNA expression was potentiated in epinephrine pretreated mice. This neuronal sensitisation, like behavioural sensitisation, has become independent of the catecholamine by the time of the drug challenge, as epinephrine was not administered on day 21. The observation that mice pretreated with only epinephrine displayed higher c-fos mRNA expression than ADX animals, indicates that this effect of the catecholamine, in contrast to its influence on behavioural sensitisation, does not require corticosterone. In fact, epinephrine and corticosterone appear to counteract, as the reduction in c-fos mRNA expression observed in corticosterone-substituted mice, which is in agreement with the negative feedback action of corticosterone, was reversed by epinephrine pretreatment to the level observed in SHAM and ADX, but not in ADXcort+epi mice.

The finding that epinephrine sensitises PVN neurons to the excitatory effects of cocaine is very interesting in several respects. First, it indicates that a history of epinephrine exposure can indeed (indirectly) modulate neurotransmission in the brain. Second, by rendering PVN neurons hypersensitive to cocaine, epinephrine may contribute to the sensitisation of the HPA-axis that occurs with repeated exposure to the psychostimulant in the DBA/2 strain (the present study, [145]). This effect of the catecholamine may also involve the noradrenergic system, as the PVN receives prominent direct and indirect noradrenergic input from the brain stem catecholaminergic

nuclei [26,134,135,206,218,724]. Furthermore, norepinephrine in the PVN regulates HPA-axis activation [113,226,382] and may also be involved in sensitisation of the HPA-axis to psychostimulants or stressors [315,608]. Finally, it is interesting to note that electrical stimulation of the afferent vagal nerve has also been shown to increase CRH mRNA in the PVN and plasma adrenocorticotrophic hormone and corticosterone concentrations [297]. Collectively, these findings suggest that epinephrine may, via activation of the afferent vagal nerve and direct or indirect noradrenergic projections to the PVN, facilitate HPA-axis sensitisation to cocaine, provided that the c-fos activation indeed signals activation of CRH neurons. In support of this, it has recently been demonstrated that repeated cocaine administration leads to accumulation of fosB and the stable isoform delta fosB in the PVN, indicating that long term changes in expression of fos family transcription factors may constitute a molecular mechanism via which cocaine induces adaptive changes in the HPA-axis [111].

In summary, the present data show that both the HPA-axis and the ANS play a crucial role in long-term behavioural sensitisation to cocaine, and that epinephrine may in addition facilitate HPA-axis sensitisation to the psychostimulant. This is an important finding since numerous investigations have been dedicated to the role of the HPA-axis in psychostimulant sensitisation and reward, whereas the ANS has hardly gained any attention. Further characterisation of the relationship between glucocorticoids, epinephrine and psychostimulant responsiveness will lead to a better understanding of the mechanisms that underlie the effects of stress on drug addiction.

ACKNOWLEDGEMENTS

This research was supported by NWO/INSERM/ZON grants 985-10-014 and 985-10-504 and by the Royal Netherlands Academy of Arts and Sciences. We gratefully acknowledge S. Lachize, ing. M. van der Mark and drs. L. Enthoven for technical support and dr. O.C. Meijer for critically reading the manuscript.

6

General discussion

OUTLINE

1. Strain differences in the response to cocaine
   1.1 Dopamine
   1.2 HPA-axis
   1.3 Role of adrenal hormones
2. Other models for individual differences in psychostimulant sensitivity
3. The context of stress hormone action
   3.1 Stage of behavioural sensitisation
   3.2 Time dependence: mode of corticosterone replacement
   3.3 The role of the sympathetic nervous system
4. Perspectives
5. Conclusions

Individuals may vary widely in susceptibility to acquire cocaine abuse after recreational use of the drug. This individual difference has led to the distinction of phenotypes that are either 'vulnerable' or 'resistant' to the behavioural and reinforcing effects of psychostimulant drugs. Recent studies have identified genes and adverse life events as factors that contribute to psychostimulant sensitivity but how the genetic and environmental inputs (inter)act is still poorly understood.

The objective of this thesis was to investigate the contribution of adrenal stress hormones to individual differences in vulnerability to the psychostimulant effects of cocaine. It was hypothesised that adrenal glucocorticoids contribute to cocaine sensitivity, while their actions are dependent on the *genetic background* of the individual and on the *context* in which these hormones operate. For this purpose, studies were designed to investigate the impact of adrenal hormones on cocaine sensitivity in mice of two genetic backgrounds (C57BL/6 and DBA/2). Only the DBA/2 strain was found to be susceptible to the influence of adrenal hormones on cocaine sensitivity.

The main focus was on the context in which glucocorticoid hormones operate, with emphasis on the timing of the corticosteroid action and the role of the sympathetic nervous system. To that aim, adrenal hormones were surgically and pharmacologically manipulated in the DBA/2 strain by adrenalectomy (surgical removal of the adrenals, 'ADX') and hormone replacement respectively. Behavioural sensitisation, the progressive enhancement of the motor stimulant effects of cocaine with repeated exposure, was used as a read-out parameter as this model reflects long-lasting adaptations in neural circuits involved in motivation and reward. The results show that corticosterone facilitates initiation and retention of the behavioural sensitisation to repeated drug exposure, provided it is continuously circulating. However, in addition to corticosterone, epinephrine is required for full behavioural sensitisation to cocaine in the DBA/2 strain.

In the following paragraphs, the major findings of this thesis are discussed, starting with the importance of genetic background, followed by the critical time-window for the glucocorticoid effects and finally the coordinate actions of corticosterone and epinephrine. Methodological considerations regarding the sensitisation paradigm are discussed in box 1.

1. STRAIN DIFFERENCES IN THE RESPONSE TO COCAINE

*The C57BL/6 and DBA/2 inbred strains represent a model for genetic differences in behavioural responsiveness to cocaine (**chapter 2,** summarised in table 1).* Whereas C57BL/6 mice showed greater locomotor responses to the first cocaine exposure,

Table 1 : *Overview of the locomotor responses of cocaine-treated mice during the sensitisation paradigm.*

Strain	Day 1	Day 9	Increase days 1-9	Day 15*
C57BL/6	↑	↑	no	↑
DBA/2		↑	yes	↑

Arrows indicate a significant difference compared to saline-treated mice of the same strain. No arrow indicates no significant difference. On days 1-9, mice received 15.0 mg/kg cocaine. * On day 15, *all* animals, including the saline-treated control group, received a 7.5 mg/kg cocaine challenge (data from **chapter 2**).

only DBA/2 mice exhibited an increase in drug responsiveness during repeated drug administration. However, both strains expressed sensitisation if challenged with cocaine after a withdrawal interval. The observed strain differences are in line with previous studies showing that C57BL/6 and DBA/2 mice differ in sensitivity to the behavioural, rewarding and reinforcing properties of virtually all classes of abused drugs [43,65,257,447,455]. The present findings are however in disagreement with reports that C57BL/6 mice are less sensitive to the locomotor activating effects of cocaine [483,568,665], whereas one report claimed that neither strain develops behavioural sensitisation during the course of repeated cocaine treatment [483]. Differences in the design of the sensitisation paradigm (box 1) and, in view of the present data, experimental conditions that influence the secretion of adrenal stress hormones, may contribute to variable outcomes across laboratories.

Under the given experimental conditions, three factors are discussed that may underlie the strain differences in behavioural responsiveness to cocaine. These are the pharmacokinetics of cocaine, the properties of the midbrain dopamine system and the stress hormones as modulators of cocaine responsiveness.

First, the psychostimulant may have a different pharmacokinetic profile in the C57BL/6 and DBA/2 strains. However, it has been demonstrated that comparable concentrations of cocaine reach the brain in both strains following i.p. [664,737] or i.v. administration (R. van der Veen, personal communication). This has been contradicted by one report, showing earlier peak concentrations and a longer half-life of cocaine in the brains of C57BL/6 mice after i.p. administration [17]. Given the positive correlation between the rate of psychostimulant bio-availability and the ability to produce behavioural sensitisation [588], the latter finding would imply that cocaine has greater potency to induce sensitisation in the C57BL/6 strain. This is not supported by the present data. However, it could explain the high initial drug response

Box 1: *Behavioural sensitisation: methodological considerations.*

Behavioural sensitisation, the progressive enhancement of the motor stimulant effects of cocaine with repeated exposure, is thought to reflect long-lasting adaptations in neural circuits involved in motivation and reward. Robinson and Berridge first proposed the possible link between behavioural sensitisation and drug addiction. The 'incentive sensitisation' theory implies that drugs of abuse render the neural systems that regulate their motivational aspects hypersensitive, making the drugs increasingly more attractive or 'wanted' [561]. The view that sensitisation is associated with certain aspects of addiction is supported by the observations that: i) sensitisation facilitates subsequent drug taking as measured in self-administration paradigms [295,296,510,607], ii) it reflects adaptations in a common neural substrate, most notably the midbrain dopamine system [708], iii) the neural and behavioural adaptations are very stable and persist until months after the discontinuation of repeated drug treatment [497], iv) the degree of sensitisation may be positively correlated with vulnerability to relapse [152] and, comparable to drug addiction, v) there are considerable individual differences in the propensity to develop behavioural sensitisation [291,559], and vi) sensitisation is influenced by contextual and environmental influences [22,65,510]. However, it is still a matter of debate whether behavioural sensitisation represents initial changes in the addiction process, rather than more advanced stages [36,154,694]. Furthermore, it should be noted that in the present studies, behavioural sensitisation was induced by experimenter-delivered drug administrations. Consequently, this model does not measure voluntary drug seeking or the motivational aspects thereof. It will be essential to extrapolate the present findings to animal models that do, such as the self-administration paradigm. Finally, two aspects of behavioural sensitisation, the time- and context- dependency, require further consideration and are discussed in the following paragraphs.

Behavioural sensitisation is not a unitary phenomenon. First, it represents a time-dependent cascade of cellular and behavioural adaptations [497,520]. The kinetics and direction thereof could be influenced by genetics. Indeed, despite considerable differences in locomotor responsiveness during repeated drug administration, both strains expressed sensitisation in response to a cocaine challenge after a withdrawal interval (**chapter 2**). Furthermore, there were distinct but also partially overlapping neural adaptations in the midbrain dopamine system of C57BL/6 and DBA/2 mice with a history of cocaine exposure (**chapter 3**). Thus, genotype controls both the nature and/or the time course of the neural and behavioural adaptations associated with repeated cocaine exposure. Therefore, the observed strain differences in behaviour and neural correlates are representative for the time point at which they were measured, whereas further research is required to investigate their significance after e.g. longer episodes of withdrawal.

Second, at least two types of behavioural sensitisation can be distinguished: context-independent and -dependent sensitisation. Whereas the former relies primarily on the pharmacological actions of the drug, the latter involves learned associations between the psychostimulant effect of the drug and otherwise neutral stimuli. Both phenomena may co-exist, however the extent to which they contribute to the behavioural hyperresponsiveness can be influenced by genetics. Indeed, the C57BL/6 strain is more sensitive to context-dependent sensitisation [20,65,559] and exhibits greater spatial and contextual memory [10,499,581,677], when compared to the DBA/2 strain. In the present studies, animals received cocaine both in the test cage and in the home cage on several occasions, a setting which may promote the development of either type of sensitisation. An important challenge is to unravel which neuronal substrates contribute to context dependent vs. -independent sensitisation.

of C57BL/6 mice [729], which may have masked subsequent behavioural sensitisation (**chapter 2**).

Second, the strain difference in the midbrain dopamine system that mediates the behavioural and reinforcing effects of psychostimulant drugs, may play a role in the differential cocaine responsiveness (**chapter 3**, [69,199,470,737]). The few studies that have investigated neurotransmitter systems other than dopamine, point to strain differences in GABA-ergic-, glutamatergic- and endogenous opioid-signalling [114,156,157,313,453,471,718]. However, their contribution to the strain difference in cocaine responsiveness remains to be established and would be an interesting line of further research. In section 1.1 further aspects of the role of dopamine are discussed.

Third, the adrenal glucocorticoids may modulate behavioural responsiveness to cocaine. This argument is based on the wealth of data showing that the glucocorticoids contribute to psychostimulant sensitivity [168,240,418,421,424,516], the notion that psychostimulant drugs activate the HPA-axis [31,281,355,448,461,590], and the observed strain differences in HPA-axis function [66,321,622]. Therefore, an essential aspect of the strain comparison in this thesis was to characterise HPA-axis responsiveness to cocaine and to investigate the role of adrenal hormones in behavioural sensitisation (see sections 1.2 and 1.3, respectively).

1.1 Dopamine

*The two strains are characterised by pronounced differences in the mesocorticolimbic and nigrostriatal dopamine systems (**chapter 3**, see table 2).* The most direct measure of dopaminergic transmission is dopamine release. Compared to DBA/2 mice, C57BL/6 mice display greater amphetamine-induced dopamine release in the NAc [700,760], which is a critical determinant of the behavioural response to the drug [700]. The observed differences in initial cocaine responsiveness (**chapter 2**), suggest that a comparable phenomenon applies to cocaine. This appears to be contradicted by the higher TH and DAT mRNA expression in cell body regions of the DBA/2 strain (**chapter 3**), which is suggestive of a higher tone in the DBA/2 dopamine system. However, the increased expression of all presynaptic markers appeared to be fully attributable to the higher number of dopaminergic (TH^+) neurons in this strain. In fact, the latter observation, together with the notion that basal dopamine output is comparable in the two strains [760], suggests that there must be greater inhibition of dopaminergic transmission in the DBA/2 strain. The PFC may play an important role therein, as dopamine in the mPFC controls subcortical dopamine release in a strain-dependent manner [700,701]. Further studies applying microdialysis to measure dopamine release and turnover in response to cocaine in the NAc and PFC are required to investigate this issue.

Table 2 : *Overview of the strain differences in the basal dopamine system of DBA/2 relative to C57BL/6 mice.*

		DBA/2 vs. C57BL/6
TH	**SNc**	↑
	VTA	↑
DAT	**SNc**	↑
	VTA	
D2	**SNc**	↑
	VTA	↑
TH⁺ cells	**SNc**	↑
	VTA	↑
D1	**NAc core**	↑
	NAc shell	↑
	rCP	
	cCP Lat	
	cCP DM	
	cCP VM	
D2	**NAc core**	↓
	NAc shell	
	rCP	↓
	cCP Lat	↑
	cCP DM	
	cCP VM	

Arrows indicate a significant difference compared to C57BL/6 mice. No arrow indicates that there is no significant difference. TH: tyrosine hydroxylase, DAT: dopamine transporter, SNc: substantia nigra pars compacta, VTA: ventral tegmental area, NAc: nucleus accumbens, (r/c) CP (Lat,DM,VM): (rostral/caudal) caudate putamen (lateral, dorsomedial, ventromedial regions) (data from **chapter 3**).

Whereas several studies have demonstrated strain differences in dopamine receptor densities or binding, their role in behavioural responsiveness to psychostimulants remains to be established. A consistent finding across laboratories is the strain difference in D2 receptor density in distinct terminal fields (higher in C57BL/6) and the cell body regions (higher in DBA/2) of the mesocorticolimbic and nigrostriatal dopamine system (**chapter 3**, [69,199,470]). The latter finding has led to the hypothesis

that DBA/2 mice have greater auto-inhibition of dopaminergic transmission [69,531]. However, in view of the abovementioned strain difference in neuron number, it remains to be investigated how much of the lower D2 binding is attributable to the fewer number of dopaminergic neurons in this strain. The higher D2 binding in the CP of C57BL/6 mice could be a marker of greater cocaine responsiveness, given that apomorphine-susceptible rats (APO/SUS, see section 2), that are more responsive to psychostimulant-induced locomotion than APO/UNSUS rats [127], also display greater D2 binding in the CP when compared to their counterparts [576]. However, in the C57BL/6 and DBA/2 mouse strains this difference is subregion-dependent, as DBA/2 mice displayed greater D2 binding in the lateral subdivision of the caudal CP. With respect to D1 receptor binding in the terminal fields, conflicting results have been obtained and this is an issue that requires further investigation (**chapter 3**, [69,199,470]). One approach to investigate the functionality of the strain differences in D1 and D2 receptor density in the different subfields could involve local administration of receptor agonists and antagonists. Furthermore, there are now ligands with relative selectivity for the 5 individual dopamine receptor subtypes, the contribution of which could be another interesting line for further research.

In the present studies, strain differences in behavioural responsiveness to cocaine were related to 'candidate genes' in the midbrain dopamine system. It should be noted that these markers do not prove a causal relationship and further research is required to evaluate their role in cocaine responsiveness. In addition, it would be interesting to search for novel genes, e.g. by microarray screening. This technique may allow the identification of novel pathways that underlie the strain differences in cocaine responsiveness. Furthermore, recombinant inbred (RI) strains (crossings between C57BL/6 and DBA/2 strains) can be used to determine quantitative trait loci (QTL), sites on a chromosome that contain genes that influence a given behaviour. This approach has already been applied for cocaine-induced seizures [273], acute and sensitised locomotion [507,664]. Interestingly, one study found a QTL in the region of chromosome 9 that contains the drd2 gene which encodes the D2 receptor [273]. Finally, there are two different splice variants of the D2 receptor ('short' and 'long') [107,139,238,463] and evidence is emerging that there are polymorphisms and single-nucleotide-polymorphisms (SNPs) in the dopamine receptors. Especially the D4 receptor is unique in having an exceptionally high number of polymorphic sites [96,477,651,690], some of which have been associated with psychosis [96], novelty seeking personality traits [37,188] and heroin abuse [652] in humans. It would be interesting to investigate whether there are such polymorphisms in the D2 and D4 receptors in the C57BL/6 and DBA/2 strains.

In conclusion, there are profound differences in the midbrain dopamine system of C57BL/6 and DBA/2 mice that may underlie the strain differences in behavioural responsiveness to psychostimulants. However, the nature of the crucial event(s) in dopamine signalling requires further investigation.

1.2 HPA-axis

The C57BL/6 and DBA/2 strains displayed very different endocrine responses to cocaine. In contrast to the HPA-axis activation and endocrine sensitisation in DBA/2 mice, cocaine attenuated HPA-axis reactivity in C57BL/6 mice throughout the sensitisation paradigm (**chapter 2,** summarised in table 3).

The endocrine sensitisation observed with repeated cocaine exposure in the DBA/2 strain is in agreement with most earlier observations in rats [29,51,387,610,694]. The data from **chapters 2** and **5** suggest that sensitisation takes place at the level of the PVN and may in addition involve adrenal hyperresponsiveness to ACTH. In support of this, HPA-axis sensitisation to amphetamine is associated with neuroplastic changes in the PVN [315]. Also, chronic cocaine treatment increases the sensitivity of adrenocortical cells to the stimulatory effects of ACTH on steroidogenesis [369]. Furthermore, the data in **chapter 5** point to a role for the adrenal epinephrine in HPA-axis sensitisation at the level of the PVN. Since epinephrine itself cannot cross the blood-brain-barrier, the catecholamine most likely influences brain function via β-adrenoreceptors on vagal afferents to the CNS. The contribution of epinephrine to HPA-axis sensitisation could be tested by administration of a peripheral β-blocker prior to cocaine administration.

The attenuation of HPA-axis activation by cocaine in the C57BL/6 strain was unexpected and this phenomenon has, to our knowledge, not been reported previously. In line with the present findings, C57BL/6 mice display lower, though not

Table 3 : *Overview of corticosterone secretion of cocaine-treated mice during the sensitisation paradigm.*

Strain	Day 1	Day 9	Increase days 1-9	Day 15*
C57BL/6	↓	(trend ↓)	no	
DBA/2	↑	↑↑	yes	↑

Arrows indicate a significant difference compared to saline-treated mice of the same strain. No arrow indicates no significant difference. On days 1-9, mice received 15.0 mg/kg cocaine. * On day 15, *all* animals, including the saline-treated control group, received a 7.5 mg/kg cocaine challenge (data from **chapter 2**).

reduced, corticosterone secretion in response to ethanol when compared to DBA/2 mice [553]. Opposite strain differences exist for endocrine responsiveness to mild stressors, such as novelty (S. Dalm, personal communication, [66]) or saline administration in the test setting (**chapter 2**), in response to which C57BL/6 mice show a greater increase in corticosterone secretion than DBA/2 mice. By contrast, more severe stressors (electrical shock, restraint) induce greater HPA-axis activation in the DBA/2 strain [68,321,622]. Therefore, the degree of endocrine responsiveness to cocaine and various stressors is not necessarily correlated. Depending on their nature and severity, these 'stimuli' appear to recruit different neuronal pathways that regulate activity of the HPA-axis.

Several mechanisms could contribute to the differential HPA-axis responsiveness of C57BL/6 and DBA/2 mice to cocaine.

First, pre-existing differences or drug-induced alterations in functioning or wiring of the HPA-axis may play a role. Cabib *et al*. demonstrated higher GR and MR binding capacity *in vitro* in hippocampal cytosol of DBA/2 mice [68]. Whether this plays a role in the strain differences in HPA-axis activity remains to be established, and additional investigation of HPA-axis parameters at the level of the PVN and pituitary is required. Furthermore, given the strain-difference in endocrine adaptation to repeated cocaine exposure, it would be interesting to investigate neural and functional aspects of the HPA-axis in sensitised mice. In humans, there is limited evidence that drugs of abuse influence negative feedback regulation of the HPA-axis [14,174]. Could it be that cocaine alters glucocorticoid negative feedback in a strain-dependent manner? This question could be addressed by performing a dexamethasone suppression test in addition to measuring GR densities in the pituitary and PVN.

We found that DBA/2 mice have considerably higher AVP mRNA expression (on average 25%) in the magnocellular and parvocellular neurons of the PVN (data not shown). AVP expression and release are under monoaminergic control [381,745], suggesting that they can be regulated by cocaine. Interestingly, AVP may not only play a role in endocrine-, but also in behavioural responsiveness to cocaine. There is considerable evidence that AVP reduces sensitivity to the behavioural (locomotion, sensitisation) and reinforcing effects of drugs of abuse [108,155,362,600,687]. Recently, it was demonstrated that environmental manipulations that increase cocaine self-administration in the DBA/2, but not the C57BL/6 strain, are associated with a decrease in AVP mRNA expression in the extended amygdala of the DBA/2 strain only (R. van der Veen *et al.*, personal communication). This suggests that extrahypothalamic AVP engages in gene x environment interactions that correlate with sensitivity to the reinforcing effects of cocaine. The role of hypothalamic

and extrahypothalamic AVP in the observed strain differences in endocrine and behavioural responsiveness to cocaine could be further investigated by e.g. targeted knock-down or overexpression of AVP.

Second, there could be strain differences in the neurotransmitter systems that regulate HPA-axis activity. Given i) the monoaminergic actions of cocaine, ii) the strain differences in the dopamine system (**chapter 3**), and iii) the role of dopamine and norepinephrine in HPA-axis activation [52,113,226,382,398], it is tempting to hypothesise a role for the catecholaminergic innervation to the PVN. Indeed, as proposed in **chapter 5**, norepinephrine may play a role in HPA-axis sensitisation in DBA/2 mice. Further characterisation of the monoaminergic innervation of the PVN in the two strains is required to address this issue.

Third, the differences in HPA-axis responsiveness to cocaine may reflect strain differences in coping with drug-induced arousal [454] or in the perception of stressful events (e.g. test- and treatment procedures). In these cases, higher brain centers involved in perception, appraisal, cognitive processes and emotional arousal exert indirect control over HPA-axis activity.

In view of the working hypothesis that adrenal glucocorticoids contribute to the strain differences in behavioural responsiveness to cocaine, it is of interest to compare locomotor- and corticosterone responses to the psychostimulant. In the C57BL/6 strain, there was a clear dissociation between the effect of cocaine on locomotion (increased) and corticosterone secretion (attenuated) on all test days. Furthermore, C57BL/6 mice displayed behavioural, but not endocrine, sensitisation in response to the cocaine challenge. In the DBA/2 strain, by contrast, cocaine stimulated locomotion and corticosterone secretion, and both responses sensitised with repeated drug exposure.

In summary, the C57BL/6 and DBA/2 inbred strains differ not only in behavioural, but also in HPA-axis responsiveness to cocaine. Furthermore, there was a striking parallel between behavioural and endocrine sensitisation in the DBA/2 strain. This raises the question whether the HPA-axis is involved in behavioural sensitisation, and whether it contributes to the differences in cocaine responsiveness between the strains.

1.3 Role of adrenal hormones

*The DBA/2, but not the C57BL/6 strain, is susceptible to the impact of adrenal hormones on behavioural sensitisation to cocaine (**chapter 2**)*. ADX prevented initiation and retention of behavioural sensitisation in the DBA/2 strain only. However, the

strain differences in behavioural responsiveness to cocaine were not diminished, but rather enhanced by ADX. By contrast, a period of food shortage abolished the strain differences in amphetamine-induced place preference and locomotion by increasing responsiveness selectively in the DBA/2 strain [71]. This is likely to depend on stress-induced corticosterone secretion, since it could be blocked by a glucocorticoid antagonist [420]. These data point to an important facilitatory role for corticosteroid hormones in behavioural responsiveness to psychostimulants in the DBA/2, but not the C57BL/6 strain.

Is it a general phenomenon that the C57BL/6 strain is relatively resistant to glucocorticoids, or does this depend on the experimental design or the read-out parameter? Among experimental parameters that could influence susceptibility to adrenal hormones are the dosage of the psychostimulant and the design of the sensitisation paradigm, such as e.g. the context in which the drug was administered. The dose of 15.0 mg/kg cocaine resulted in pronounced locomotor activation in the C57BL/6, but not the DBA/2 strain (**chapter 2**). It is conceivable that for C57BL/6 mice, this dosage was high enough to override the influence of other factors, including adrenal hormones. In this respect, it would be interesting to establish a dose-response curve for cocaine-induced behavioural sensitisation in SHAM and ADX mice.

Alternatively, different types of behavioural tasks might reveal sensitivity to adrenal hormones in the C57BL/6 strain. If compared to DBA/2 mice, C57BL/6 mice exhibit greater spatial and contextual memory [10,499,581,677], which has been related to strain differences in hippocampal function [575]. In support of this, cocaine-treated C57BL/6 mice displayed greater locomotor responses when exposed to the test context in the absence of cocaine (**chapter 2**). The C57BL/6 strain could be susceptible to the influence of adrenal hormones in a sensitisation paradigm designed to exclusively reveal context-dependent sensitisation (see also box 1), or in behavioural tasks that strongly depend on hippocampal function (e.g. radial arm maze, fear conditioning, Morris water maze). Indeed, in C57BL/6 mice, exogenous corticosterone and stress modulate spatial learning in water maze and circular hole board tasks [265,266] and memory consolidation in tests for passive avoidance [68,95]. Therefore, the relative resistance of C57BL/6 mice to adrenal hormones or environmental manipulations is not a general phenomenon for this strain, although it has been consistently reported for behavioural responsiveness to psychostimulant drugs. This could indicate that neural mechanisms that mediate psychostimulant responsiveness are relatively resistant to glucocorticoid hormones in the C57BL/6 strain. *Indeed, the DBA/2, but not the C57BL/6 strain, was susceptible to the impact of adrenal hormones on neuroadaptations associated with repeated cocaine exposure (**chapter 3,** summarised in table 4).*

Table 4: *Overview of the adaptations in the dopamine system of mice subjected to the sensitisation regimen.*

		DBA/2		C57BL/6	
		SHAM	**ADX**	**SHAM**	**ADX**
TH	**SNc**	↑			
DAT	**SNc**	↑			
D2	**NAc core**		↓*		
	rCP		↓*		
	cCP DM	↓		↓	

SHAM operated and adrenalectomised (ADX) mice were subjected to the sensitisation regimen and sacrificed 24 hours after the cocaine challenge under basal conditions. Arrows indicate a significant difference in cocaine- compared to saline-treated mice of similar surgery and strain. No arrow indicates that there is no significant difference. * Significantly different from cocaine-treated SHAM mice. TH: tyrosine hydroxylase, DAT: dopamine transporter, SNc: substantia nigra pars compacta, NAc: nucleus accumbens, (r/c) CP (DM): (rostral/caudal) caudate putamen (dorsomedial region) (data from **chapter 3**).

The data point to a role for the nigrostriatal dopamine system and the NAc core in mediating the effects of adrenal hormones in a strain dependent manner. In the DBA/2 strain, sensitised SHAM mice displayed increased expression of presynaptic markers, whereas sensitisation-resistant ADX mice were characterised by reduced D2 binding in the terminal fields of the NAc core and rostral CP. These changes were not observed in C57BL/6 mice that developed sensitisation independent of the adrenals. Although speculative, these data point to i) greater reactivity of the nigrostriatal neurons in SHAM mice, and ii) reduced potency for postsynaptic signalling via the D2-like receptors in ADX mice, of the DBA/2 strain. Further research is required to test these hypotheses, and to determine which of the changes are critical for the behavioural response to cocaine and the influence of adrenal hormones. One approach could involve overexpression of TH and DAT in ADX mice, or conversely, knock down of D2 receptors in SHAM mice. Furthermore, it would be of interest to investigate these markers after longer episodes of withdrawal, given that neural adaptations associated with behavioural sensitisation are characterised by marked time-dependency [497,520]. In addition, it will be necessary to investigate the contribution of the D2 and D3 receptors individually, in view of their presumed opposite effects on psychostimulant-induced locomotion.

With respect to the D2 receptor, there is considerable evidence that D2-like dopamine receptors engage in gene x environment interactions. In humans, variations in D2 receptor alleles have been associated with drug addiction [121,473,475,476]

and stress differentially affects cigarette craving or performance in a cognitive task between carriers and non-carriers of the A1 allele [41,198]. Furthermore, stress induces opposite alterations in D2 receptor binding in the C57BL/6 and DBA/2 strains [69]. In view of the affinity of the D2-like receptor ligand iodosulpride for both D2 and D3 receptors, it is interesting to note that the D3 receptor has been suggested to play a role in gene x environment interactions in schizophrenia and other psychotic disorders [490]. In addition, studies in laboratory rodents have demonstrated that the D3 receptor contributes to stress-induced reinstatement of cocaine seeking [740]. Thus, signalling via D2-like receptors may be subject to regulation by adrenal hormones in a genotype-dependent manner.

Finally, the lack of major changes in the dopamine system of sensitised C57BL/6 mice suggests that other neurotransmitter systems play a more prominent role in sensitisation of this strain. A likely candidate is glutamate, given that the C57BL/6 strain is relatively more susceptible to contextual information, and glutamatergic transmission has been proposed to play a prominent role in context-dependent sensitisation [10,35,499,581,677]. In addition, glutamate may contribute to the effects of glucocorticoids on behavioural sensitisation in the DBA/2 strain. There is considerable evidence that adrenal glucocorticoids modulate glutamatergic transmission in the limbic system, e.g. in the midbrain dopamine system, hippocampus and prefrontal cortex [2,275,400,414,460,638,720]. These effects of corticosterone have been most extensively characterised for the hippocampus and involve regulation of glutamate receptor expression [275,414], glutamate release [400,460,638] and transmission via NMDA and AMPA receptors [337,655]. It has been demonstrated both *in vitro* and *in vivo* that corticosterone potentiates responses of VTA dopaminergic neurons to excitatory amino acids, although there is controversy regarding the roles of GR and MR [110,488]. The time course of the effects of corticosterone in the study of Cho *et al.* suggests that this may involve non-genomic actions of the hormone [110], which is in good agreement with a growing body of evidence that glucocorticoids can modulate excitatory transmission via a rapid, non-genomic mode of action involving membrane-bound receptors [175,338,466,655,699,759]. By contrast, other effects of glucocorticoids on glutamatergic transmission require genomic mechanisms [337]. Taken together, the direct facilitatory effect of glucocorticoids on responsiveness of midbrain dopamine neurons to excitatory amino acids, in combination with the actions on glutamatergic transmission in other limbic regions such as the hippocampus, may facilitate responsiveness to psychostimulant drugs. It would be of great interest to further investigate the interplay between glucocorticoids and glutamate in behavioural sensitisation to cocaine with special emphasis on genomic- vs. non-genomic mechanisms. Especially given the present observation that a modest degree of sensitisation was observed in DBA/2 mice receiving corticosterone 5 min. prior to

cocaine administration (**chapter 4**, see section 3.2 for discussion). In addition, two recent studies have demonstrated that the rapid effects of glucocorticoids on glutamate release in the hypothalamus are mediated via endocannabinoid release and subsequent CB1 receptor activation [175,176]. Given the role of endocannabinoids in drug addiction, it would be interesting to investigate whether a similar mechanism applies to the contribution of corticosterone to behavioural sensitisation. Finally, in view of the present data, an important line of further research involves comparing the C57BL/6 and DBA/2 mouse strains with respect to the interaction between corticosteroids and glutamate in behavioural sensitisation to psychostimulants.

*In summary, the evidence presented in **chapters 2** and **3** shows that the two inbred strains provide a model for genetic differences in neural, behavioural and endocrine responsiveness to cocaine. Furthermore the DBA/2, but not the resistant C57BL/6 strain, is susceptible to the impact of adrenal stress hormones on cocaine sensitivity at the level of both dopaminergic neurotransmission and behavioural responsiveness.*

2. OTHER MODELS FOR INDIVIDUAL DIFFERENCES IN PSYCHOSTIMULANT SENSITIVITY

Individual differences in psychostimulant sensitivity have gained increasing attention during the past decades. A number of models have been developed in which animals were selected based on genetics (inbred rat or mouse strains) [214,485,626], or pre-existing traits [44,203,237,287,290,354,378,509,684]. These models may reflect different motivational aspects of drug-taking that have also been distinguished in humans [268]: to 'seek novelty (sensation)' [509] or to 'alleviate negative affect' [287]. In the following paragraphs, the C57BL/6 and DBA/2 strains are compared to other selected animals such as e.g. the so-called high- (HR) and low responder (LR) rats of Piazza *et al.*, to which they bear striking resemblance. It should be noted that inbred strains represent one particular set of genes and it is fraught with difficulty to draw a direct parallel to outbred strains.

The HR and LR rats are distinguished from an *outbred* population, based on behavioural reactivity in a novel environment (above or below the mean, respectively) [509]. These sub-populations of animals have certain phenotypic characteristics that have been observed in various animal models. A positive correlation may exist between behavioural reactivity in a novel environment [200,232,511] and i) the locomotor response to acute administration of psychostimulants [82,203,290,378,509], ii) psychostimulant-induced dopamine release in the NAc [82,236,290,378,578] and iii) the propensity to self-administer psychostimulants [232,354,509]. In addition, these parameters have

been positively correlated with the endocrine response to stress, although this is an issue of debate [255,511,716]. These characteristics are however not consistent across all models [287,684]. The APO/SUS and -UNSUS rat lines, bred from rats that were selected for apomorphine-induced gnawing (gnawing score: APO/SUS: >500/45 min, -UNSUS: <10/45 min in response to 1.5 mg/kg apomorphine) are an example. Whereas APO/SUS rats are more responsive to novelty and psychostimulant-induced locomotion, they self-administer less cocaine under habituated conditions [126,127,684]. However, these animals were selected and bred for a dopaminergic phenotype, which makes them essentially different from the HR/LR rats.

The notion that a cluster of phenotypic parameters is observed across laboratories in animals with different genetic backgrounds, indicates that these are representative for at least a sub-population of individuals. The C57BL/6 and DBA/2 strains display differences in behavioural and endocrine responsiveness to novelty comparable to those of the HR and LR rats, respectively (personal observations, [66]). The HR rats show greater amphetamine-induced locomotion when compared to LR rats, and these differences are abolished with repeated drug exposure by a progressive increase in the LR, but not the HR group [509], although this may depend on experimental parameters [179,291]. Similarly, whereas C57BL/6 mice were more responsive to the first cocaine exposure, only DBA/2 showed an increase in drug responsiveness during the treatment period (**chapter 2)**. Furthermore, C57BL/6 mice and HR rats show greater psychostimulant-induced dopamine release in the NAc and propensity for amphetamine self-administration, when compared to DBA/2 mice and LR rats respectively [290,354,447,509,701,760].

The parallel between the HR/LR rats and the two mouse strains also extends to susceptibility to the effects of corticosterone and stressors. Administration of corticosterone (prior to a self-administration session or in the amphetamine solution) and social defeat stress facilitate psychostimulant self-administration in LR rats, while decreasing it in HR rats [323,516]. Similarly, adrenal hormones (**chapter 2**) and environmental manipulations [20,71] facilitate behavioural responsiveness to cocaine and amphetamine in the DBA/2, but not the C57BL/6 strain. A comparable phenomenon has been observed for the Fischer 344 and Lewis inbred rat strains. Fischer 344 rats (comparable to LR rats in terms of behavioural responsiveness to novelty and psychostimulants), were susceptible to the influence of corticosterone on cocaine sensitisation, whereas Lewis rats (HR-like) were not [485]. However, with respect to novelty-induced corticosterone secretion, these rat strains are distinct from the HR/LR rats, with Fischer 344 rats having higher corticosterone secretion than Lewis rats [255]. There are also differences between the HR/LR rats and the two inbred mouse strains. Whereas exposure to similar concentrations of corticosterone abolishes the difference in amphetamine-induced locomotion between HR and LR rats [516], the C57BL/6

Table 5: *Overview of parameters of the dopamine system in various animal models for individual differences in sensitivity to psychostimulants.*

		HR/LR	C57/DBA	SUS/UNSUS	LEW/FIS	Reference
D2	**NAc**	↓ (p)	↑ (p)	↔ (p)	↓ (p)	69,214,292,576*
	CP	↓ (p)	↔ ↓ ↑ (p)	↑ (p)	↓ (p)	69,199,214,292,470,576*
	VTA		↓ (p)			69*
	SNc		↓ (p)	↔ (p)		69,576*
D1	**NAc**	↑ (p)	↔ ↓ (p)	↔ (p)	↔ (p)	69,214,292,576*
	CP	↔ (p)	↔ ↑ (p)	↔ (p)	↔ (p)	69,199,214,292,470,576*
TH	**NAc**	↔ (p)		↑ (p)	↓ (p)	32,408,682
	CP	↔ (p)			↔ (p)	32,408
	VTA	↔ (m) ↓ (p)	↓ (m)	↔ (m)	↑ (p)	32,293,408,576 *
	SNc	↔ (m) ↔ (p)	↓ (m)	↑ (m)	↔ (p)	32,293,408,576*
DAT	**NAc**	↔ (p)			↓ (p)	214,292
	CP	↔ (p)			↓ (p)	214,292
	VTA		↓ (m)			*
	SNc		↓ (m)			*

Arrows indicate significant difference compared to LR, DBA/2, APO/UNSUS or Fischer 344. p: protein, results obtained with radioligand binding or immunostaining, m: mRNA, measured by *in situ* hybridisation, TH: tyrosine hydroxylase, DAT: dopamine transporter, NAc: nucleus accumbens, CP: caudate putamen, VTA: ventral tegmental area, SNc: substantia nigra pars compacta, * data from the present studies. It should be noted that the binding studies are not fully comparable due to the use of different ligands with different affinities for the dopamine receptor subtypes.

and DBA/2 strains by nature show opposite strain differences in cocaine-induced locomotion and corticosterone secretion (**chapter 2**). Therefore, it is not possible to draw a general conclusion regarding the relationship between the HPA-axis and psychostimulant responsiveness across models. Further analysis of corticosterone concentrations *prior* to drug administration and, given the present data, *in response to* the psychostimulant, would be an interesting line of further research.

At the level of dopaminergic transmission, there appears to be little consistency between the models (see table 5). For example, the dopaminergic markers in drug naïve animals of the different 'high-low' responder pairs (HR-LR, C57BL/6-DBA/2, Lewis-Fischer 344) do not show a consistent pattern. Furthermore, stress- or corticosterone-induced dopamine release in the NAc is higher in HR rats, but lower in C57BL/6 mice [73,518,580,683,703], when compared to their counterparts. In addition,

whereas the DBA/2 strain is most susceptible to the impact of ADX on drug-induced adaptations in the dopamine system (**chapter 3**), ADX selectively reduces stress-induced dopamine release in the HR rat [580]. This indicates that, except for dopamine release in response to psychostimulants, other markers of the dopamine system do not seem to be predictive for psychostimulant-induced behaviour *across* models. This does, however, not exclude that in any particular setting or strain dopamine is the key determinant for psychostimulant sensitivity.

In summary, the C57BL/6 and DBA/2 mice show resemblance to animals that are selected on the basis of high and low 'novelty seeking'. Interestingly, it appears that stressors or exogenous corticosterone facilitate behavioural responsiveness to psychostimulants in less sensitive individuals, such as the LR rats, Fischer 344 rats and DBA/2 mice. By contrast, 'high-responders' appear less susceptible to the influence of corticosterone. This interpretation should be regarded with caution, given that the 'HR/LR' phenotype in psychostimulant responsiveness might depend on the dose of the psychostimulant (see section 1.3), as well as on the experimental design, a good example being the susceptibility to context dependent (higher in C57BL/6) versus -independent (higher in DBA/2) sensitisation of the two inbred strains (see box 1) [65,559]. Furthermore, it would be interesting to characterise the C57BL/6 and DBA/2 strains for behavioural parameters that reflect coping with negative affect, e.g. stress-induced self-grooming, which has been demonstrated to predict cocaine self-administration [287].

In summary, the genetic differences in behavioural responsiveness to novelty and psychostimulant drugs are not unique to the C57BL/6 and DBA/2 strains. The HPA-axis plays a prominent role in psychostimulant responsiveness, which is especially evident in animals that are less sensitive to the drugs under the given experimental conditions. However the exact contribution of adrenal hormones may vary with the genetic background, which is consistent with the view that there are considerable individual differences in susceptibility to the impact of glucocorticoids on psychostimulant sensitivity.

3. THE CONTEXT OF STRESS HORMONE ACTION

The actions of adrenal glucocorticoid hormones are highly context-dependent [318]. However, despite convincing evidence that glucocorticoids mediate the effects of stress on psychostimulant sensitivity, the context- and time-dependency of their actions is poorly understood. The studies presented in **chapters 4** and **5** were therefore designed to investigate the role of adrenal glucocorticoids in behavioural

sensitisation to cocaine in relation to i) the stage of behavioural sensitisation, ii) the time of drug exposure, and iii) the activity of the sympathetic nervous system. The DBA/2 strain was used because of its susceptibility to the impact of adrenal hormones.

3.1 Stage of behavioural sensitisation

Glucocorticoids play a role during the initiation, rather than the expression of cocaine sensitisation in DBA/2 mice. Administration of the GR antagonist mifepristone to previously sensitised animals failed to block expression of locomotor sensitisation to cocaine (**chapter 4**), whereas substitution of ADX mice from the start of the sensitisation paradigm with chronic corticosterone alone (**chapter 4**), or in combination with epinephrine (**chapter 5**), respectively partially and fully restored the deficit in behavioural sensitisation.

The role of glucocorticoids in initiation of sensitisation is supported by previous studies [498,527] and points to a coordinate mechanism of action of glucocorticoids and psychostimulant drugs in facilitating neurochemical and behavioural adaptations. In support of this, glucocorticoids can increase extracellular concentrations of dopamine by i) increasing the levels of TH [485,637], ii) decreasing dopamine catabolism [397], and iii) decreasing catecholamine re-uptake [598], but see also [186,397]. Furthermore, glucocorticoids may control dopaminergic transmission through other neurotransmitter systems that modulate the activity of DA neurons [459,488]. Stressors and exogenous glucocorticoids increase psychostimulant-induced dopamine release in the NAc [77,164,518,577], induce (cross-) sensitisation to the neurochemical-, behavioural- and reinforcing effects of psychostimulants [165,166,472,493,510], and facilitate psychostimulant self-administration already during the acquisition phase [244,418,516]. Furthermore, adrenal glucocorticoids can induce synaptic alterations on dopaminergic neurons similar to those induced by drugs of abuse [582] and are readily self-administered by laboratory rodents [512]. Finally, in humans, high glucocorticoid concentrations can induce 'steroid psychoses' [274] and adverse life experiences may precipitate drug use and abuse.

Whereas there is a considerable body of evidence that glucocorticoids contribute to initiation and acquisition of hyperresponsiveness to the behavioural and reinforcing effects of psychostimulants (in animals with certain genetic backgrounds), controversy exists regarding the contribution of corticosteroids to sustained expression of these behaviours once they have been established. Indeed, the present data (**chapter 4**) do not support a role for glucocorticoids in expression of behavioural sensitisation to cocaine, which is in agreement with observations that ADX performed in already sensitised animals does not prevent expression of the behavioural

hyperresponsiveness [527,529]. However, other studies have shown that mifepristone can reduce the motivation to self-administer cocaine, and block amphetamine-induced behavioural sensitisation, in rats that had previously acquired these behaviours [153,168]. Furthermore, stress and glucocorticoids can play a role during maintenance [92,131,245,246,417] and reinstatement [163,194,517] of psychostimulant self-administration. Therefore, it appears that glucocorticoids can contribute to expression of these behaviours, whereas the experimental parameters and possibly genetic influences determine whether this is revealed. Indeed, Deroche-Gamonet *et al*. demonstrated that mifepristone only reduces motivation for cocaine self-administration in a subset of outbred rats that display an exceptionally high drug response [168]. Furthermore, the length of the withdrawal duration and the extent to which behavioural sensitisation has developed, may determine whether its expression is susceptible to adrenal hormones or environmental manipulations at a given time point.

In conclusion, adrenal glucocorticoids contribute to initiation of cocaine-induced behavioural sensitisation in DBA/2 mice. A next step towards understanding the context in which glucocorticoids operate, was to investigate the time-window for the actions of corticosterone within the initiation phase. To achieve this, ADX mice were given corticosterone replacement either 5 min. or 2 hrs. prior to each drug exposure, or continuously via release from a s.c. implanted pellet.

3.2 Time dependence: mode of corticosterone replacement

*Neither of the three corticosterone replacement regimens was effective in fully restoring behavioural sensitisation in ADX mice (**chapter 4**, see table 6)*. This is intriguing, given the vast body of evidence that glucocorticoid hormones contribute to the neurochemical and behavioural effects of psychostimulants, as outlined in the previous section. In agreement with reports that repeated stress and chronic administration of high doses of corticosterone can enhance locomotor responsiveness and behavioural sensitisation to psychostimulants [77,284,383,485,498], the continuous hormone replacement via the s.c. pellet was effective in (partially) restoring behavioural sensitisation to cocaine in ADX mice. However, the data suggest that the time-kinetics of the sensitisation in mice receiving chronic corticosterone is different from that observed in SHAM-operated animals and may require a longer withdrawal duration (**chapters 4** and **5**).

The continuous and stable corticosterone level achieved through a slow release pellet does not represent a physiological condition, because it does not mimic the ultradian and circadian rhythms in circulating corticosterone concentrations [751]. Therefore, the time-window for the action of glucocorticoids requires further

Table 6: *Overview of the locomotor responses of cocaine-treated DBA/2 mice receiving various regimens of corticosterone replacement during the sensitisation paradigm.*

Method	Day 1	Day 9	Increase days 1-9	Expression day 15*	Day 21
5 mins.			yes (minor)	no	
2 hrs.			no	no	
chronic	↓	↑	yes	no	↑

Arrows indicate a significant difference compared to adrenalectomised (ADX) mice receiving cholesterol replacement. No arrow indicates no significant difference. On days 1-9 mice received 15.0 mg/kg cocaine and on days 15 and 21 animals were given a 7.5 mg/kg cocaine challenge. * Enhanced response to the cocaine challenge when compared to saline-treated controls receiving similar substitution (data from **chapter 4**).

investigation. Piazza *et al.* demonstrated that administration of corticosterone 10 minutes prior to a session facilitates self-administration in LR rats [516]. In the present study, a slight degree of sensitisation was observed in mice receiving corticosterone 5 minutes prior to cocaine administration, although locomotor responses did not exceed those of ADX mice on day 9. In view of the findings of Piazza *et al.*, this suggests that there might be a non-genomic mode of action of the hormone, since the involvement of genomic effects that develop at 2 hours after corticosterone administration when the hormone concentrations are declining, is less likely. The rapid non-genomic effects of glucocorticoids can be mediated via both MR or GR putatively located in the cell membrane, that have considerably lower affinity for glucocorticoids than their 'classical' intracellular form [319,338,755]. Administration of MR or GR antagonists shortly prior to drug administration could be an interesting approach to investigate the involvement of non-genomic glucocorticoid actions. Furthermore, given the critical role for the ANS (**chapter 5**, see section 3.3 for discussion), it would be interesting to repeat the 2 hrs. and 5 mins. replacement studies in combination with epinephrine substitution.

An obvious line of reasoning to explain the insufficiency of corticosterone in fully reversing the ADX effect, would be to focus on experimental parameters such as the hormone dosage, the necessity of endocrine sensitisation and the possible requirement of ultradian and circadian rhythmicity. These aspects could very well play a role and are discussed in detail in **chapter 4**. However, the discrepancy in literature regarding the role of corticosterone in psychostimulant responsiveness, even when similar strains of animals are used, suggests that glucocorticoids are not the only factors of importance (e.g. [21,510]). Furthermore, two recent publications have proposed

that an elevation of glucocorticoids is necessary, but not sufficient, for the effects of stress on escalation of cocaine self-administration [417] and on morphine-induced conditioned place preference and dopamine release in the NAc shell [161]. For instance, in the study by Mantsch *et al.*, it was shown that stress-induced escalation of cocaine self-administration was prevented in ADX animals with diurnal corticosterone replacement. Administration of additional corticosterone (in the range of that induced by the stressor) by itself did not restore the ADX effect, while it was only effective in animals that were in addition exposed to the stressor [417]. This suggests that factors other than corticosterone may play an additional role in the effects of stress on the behavioural and reinforcing effects of psychostimulants.

In conclusion, corticosterone facilitates initiation and retention of sensitisation to the locomotor stimulant effects of cocaine in DBA/2 mice. However, a full restoration of the ADX effect was not achieved with various regimens of corticosterone replacement. Because stressors and psychostimulants activate the autonomic sympathetic nervous system (ANS) even more rapidly than the HPA-axis, studies were designed to investigate the actions of corticosterone in the context of epinephrine administration.

3.3 The role of the sympathetic nervous system

*In **chapter 5** it was demonstrated that the ANS and the HPA-axis act in a coordinate fashion to facilitate behavioural sensitisation of DBA/2 mice to cocaine.* Whereas neither corticosterone nor epinephrine alone were sufficient, both adrenal stress hormones were necessary to fully restore locomotor sensitisation of ADX mice to cocaine. Previous studies have demonstrated that the HPA-axis and the ANS are intimately linked during processes of learning and memory [53,571], however little attention has been dedicated to the role of the ANS in sensitivity to drugs of abuse.

The polar substance epinephrine does not cross the blood-brain-barrier. The catecholamine therefore most likely modulates brain function by activating β-adrenoreceptors on vagal afferents to the noradrenergic cell bodies in the solitary tract nucleus (NTS) [251,456,570,730]. Interestingly, cocaine itself increases availability of synaptic norepinephrine by blocking the NET [549]. The present data therefore suggest that additional noradrenergic stimulation (by peripheral epinephrine) may be required for full behavioural sensitisation in DBA/2 mice. Studies showing that adrenoreceptor agonists block stress-induced reinstatement of cocaine, heroin and alcohol seeking provide further evidence for the role of the noradrenergic system in mediating the effects of stress on drug responsiveness [193,376,620]. This would imply an important role for norepinephrine in psychostimulant sensitisation, a proposal

that is supported by most [182,183,587], but not all [691] studies. In the light of the present data (**chapter 2**), this discrepancy could be explained by the use of different strains and species. Furthermore, the considerable number of reports that glucocorticoids alone *are* sufficient to restore the effects of ADX, suggest that differences in sympathetic tone and, consequently, release of norepinephrine from sympathetic nerve terminals, may determine whether additional adrenergic stimulation is required.

The mechanism underlying the coordinate actions of corticosterone and epinephrine in cocaine sensitisation forms an interesting topic for further research. First, it would be of interest to investigate which of the hormones is rate-limiting. The observation that chronic corticosterone, but not repeated epinephrine administrations, partially reverses the sensitisation deficit in ADX mice (**chapters 4** and **5**), suggests that the glucocorticoid (if continuously present) is the primary regulator, whereas the role of epinephrine would be to facilitate the actions of corticosterone. However, this is highly speculative as these experiments involved 'artificial' conditions such as ADX, which by itself is likely to change the set point of the sympathetic nervous system, and hormone replacement in a non-physiological manner. Furthermore, especially during arousing tasks, there may be substantial norepinephrine release from sympathetic nerve terminals, which may have facilitated the actions of corticosterone. The independent contribution of adrenal glucocorticoids and epinephrine could be investigated by administration of MR and GR antagonists, or peripheral β-blockers.

Second, it is of great interest to investigate which brain regions are a substrate for the (inter)action of corticosterone and epinephrine, e.g. by microinjections of agonists and antagonists of glucocorticoid- (MR and GR) and adrenoreceptors. This may involve both direct [302,491] and indirect (via e.g. amygdala, hippocampus or PFC) [368,395,396,419,535,574] noradrenergic projections to the midbrain dopamine system. Of particular interest are the basolateral amygdala (BLA) and the NAc, as these brain regions are critical for the effects of glucocorticoids and epinephrine on memory consolidation [437,572-574,616].

Taken together, behavioural sensitisation of DBA/2 mice to cocaine depends on adrenal glucocorticoids. These hormones play a role during initiation, rather than expression, of sensitisation. Furthermore, the glucocorticoid action appears to require activation of the sympathetic nervous system. Regarding the time frame of action, a continuous presence of corticosterone is most effective in facilitating behavioural sensitisation, whereas the effects of the intermittent hormone substitutions (2 hrs. and 5 mins. prior to cocaine) need to be revisited in the light of the potential necessity of ANS activation.

4. PERSPECTIVES

Clinical and experimental studies point to a role for adverse life-events and glucocorticoid hormones in vulnerability to the reinforcing effects of drugs of abuse. This thesis revealed that susceptibility to the psychostimulant effects of cocaine results from complex interactions between genetic factors, the HPA-axis and the sympathetic nervous system. Our studies in mice of two genetic backgrounds show that certain individuals may acquire long-term neural and behavioural adaptations to cocaine independent of stress hormones, whereas in others adrenal glucocorticoids *and* catecholamines constitute important risk factors.

Figure 1 depicts the interplay between adrenal corticosteroids and catecholamines in the process of behavioural sensitisation to cocaine. In agreement with observations in other laboratories, it was demonstrated that glucocorticoids facilitate initiation of sensitisation [240,418,514,516], but only in certain genotypes (the DBA/2 strain). However, the present studies point to an additional role for the sympathetic nervous system. Adrenal glucocorticoids and catecholamines act in a coordinate fashion to enhance behavioural sensitisation to cocaine, which may involve facilitated dopaminergic transmission in the nigrostriatal circuit and regulation of D2 receptor densities in the projection areas of the dopamine system. Furthermore, whereas glucocorticoids have a permissive role in adrenal epinephrine synthesis [583,738], the current data suggest that epinephrine in turn facilitates HPA-axis activation by cocaine at the level of the PVN. Therefore, under conditions of repeated cocaine exposure, the two adrenal hormones may mutually facilitate each other's synthesis and release in a feed-forward fashion, which could further enhance the contribution of these hormones to psychomotor sensitisation.

The present data open up a window of opportunities for further research.

First, the DBA/2 strain provides an interesting animal model to investigate the neural, endocrine and genetic mechanisms underlying vulnerability to psychostimulant drugs that may precipitate in response to adverse events. This inbred strain is susceptible to the influence of adrenal hormones and environmental manipulations and is characterised by considerable inter-individual variability in cocaine responsiveness (**chapter 2**). Identification of population extremes, so-called 'responders' and 'non-responders', and subsequent phenotyping constitutes one approach to investigate precipitation of drug responsiveness by stress hormones. Alternatively, manipulations in early life (e.g. lack of maternal care) or in adulthood (e.g. exposure to various chronic stressors) can be applied to further discriminate phenotypes that are relatively resistant or hypersensitive to psychostimulants. Combining these

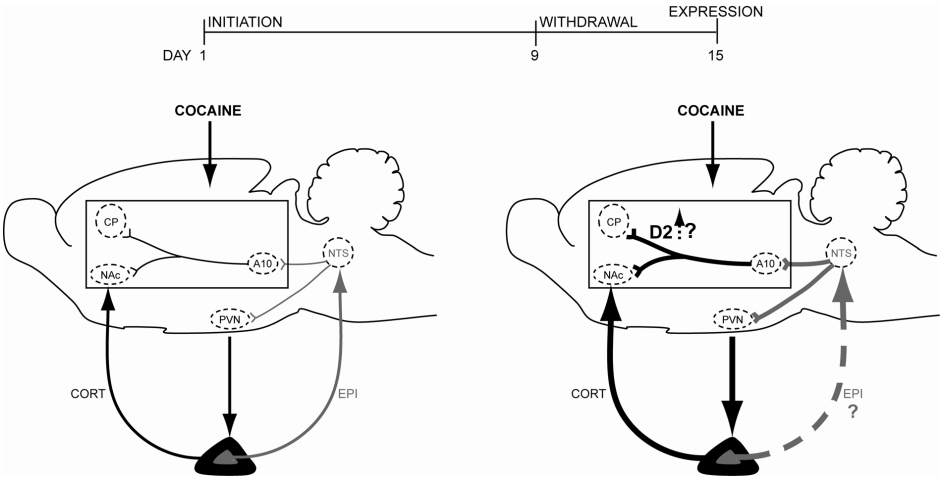

Figure 1: *Proposed model for the interaction between adrenal glucocorticoids and epinephrine in behavioural sensitisation to cocaine.*
The model represents the DBA/2 strain that is 'susceptible' to the impact of adrenal hormones on initiation of behavioural sensitisation to cocaine. Corticosterone (cort) and epinephrine (epi) act or interact in a coordinate fashion to facilitate initiation of sensitisation via adaptations in the nigrostriatal dopamine system and the NAc core that may include i) greater reactivity of nigrostriatal neurons, and ii) alterations in D2 receptor signalling in the terminal fields, possibly towards supersensitivity as reported elsewhere [306,675,695]. Corticosterone can enter the reward-related brain areas directly, whereas epinephrine activates the noradrenergic system in the nucleus of the solitary tract (NTS). With repeated drug exposure, there is enhanced secretion of corticosterone, which may be further facilitated by epinephrine, and possibly also increased epinephrine release from the adrenals. The resulting feed-forward cascade involving epinephrine and corticosterone interactions may further facilitate behavioural sensitisation to cocaine. PVN: hypothalamic paraventricular nucleus, NAc: nucleus accumbens, CP: caudate putamen.

approaches with genome-wide microarray gene expression profiling on reward-related brain regions may lead to the identification of new pathways and molecular targets that underlie susceptibility to psychostimulant drugs and dopaminergic psychoses in general.

Second, an important next step involves the extrapolation of the present results to other models for drug addiction in laboratory animals, such as the self-administration paradigm. It would be of great interest to investigate the coordinate action of corticosteroids and catecholamines in acquisition, maintenance and relapse of self-administration. The present data, which show a critical role for adrenal hormones in the initiation of behavioural sensitisation, suggest that these hormones play a role in acquisition of drug self-administration. This has indeed been demonstrated

for corticosteroids [418,516], however the role of epinephrine is unknown. Furthermore, given that glucocorticoid hormones act in a context-dependent fashion, it would be interesting to investigate the role of adrenal hormones in cue-controlled cocaine seeking and relapse, e.g. under a second-order schedule of reinforcement.

Third, the mechanism and brain circuitry via which the ANS and the HPA-axis modulate psychostimulant responsiveness form an important focus for further research. With respect to the ANS, this issue can be addressed by local or systemic administration of α- and β-adrenoreceptor agonists and antagonists prior to an experimenter- or self-administered drug infusion. This could be combined with telemetry or continuous blood sampling to monitor the activity of the ANS. Agonists/antagonists that are incapable of crossing the blood-brain-barrier would provide a useful tool to distinguish between the central and peripheral actions of catecholamines. Glucocorticoid actions can be manipulated systemically or centrally via the 'classical' endocrine and pharmacological methods as applied in this thesis, as well as by the use of transgenic animals or the application of small-interference (si)RNA-mediated knock-down of GR and MR (and their targets) in distinct brain regions. The initial focus could be on the dopaminergic and noradrenergic nuclei, the primary substrates for cocaine and epinephrine in the brain, thereafter being extended to brain regions such as the amygdala, prefrontal cortex and hippocampus that provide the midbrain dopamine system with excitatory or inhibitory input.

Fourth, whereas there is a wealth of data on the influence of stress and glucocorticoids on psychostimulant sensitivity, an interesting line of further research would be to study the role of adrenal hormones in the behavioural and reinforcing effects of different classes of drugs of abuse, such as opioids, nicotine, ethanol and cannabinoids. Indeed, there is limited evidence that corticosteroids differentially facilitate dopamine-mediated behavioural responses to cocaine and morphine [422]. Furthermore, whereas direct or indirect activation of the midbrain dopamine system is a commonality shared by all drugs of abuse, distinct classes of drugs have diverging pharmacological actions that may be differentially influenced by adrenal hormones.

Finally, the implications of the present findings for human drug addiction remain to be investigated. In individuals with cocaine dependence, acute cortisol can trigger craving [192] and plasma cortisol concentrations are positively correlated with the subjective effects of the drug and drug-induced dopamine release in the midbrain [486]. Despite the controversy regarding the role of glucocorticoids in established drug responsiveness (the present data, [153,168]), this suggests that GR antagonists may have therapeutic potential in reducing some of the subjective and neurochemical effects of cocaine. Interestingly, recent clinical trials have demonstrated that the GR antagonist mifepristone may have efficacy in the treatment of psychotic major depression

Box 2: *Stereotypy.*

In the present studies, locomotion was measured as an index of behavioural responsiveness to cocaine. However, the effects of psychostimulants in rodents are dose-dependent and can initiate a spectrum of behavioural responses. Whereas lower doses induce horizontal locomotor hyperactivity, higher doses produce stereotyped behaviours; repetitions of movements that are part of the normal behavioural repertoire including head shaking, rearing, sniffing, gnawing, licking and circling. In humans, stereotyped behaviours are symptoms of a number of psychiatric disorders [262,640,667]. Stereotypy is an issue that deserves attention in the light of gene x environment interaction in psychostimulant responsiveness.

It has been proposed that stereotypy serves a coping function that reduces drug-induced arousal and stress [410,454]. Indeed, an amphetamine sensitisation regimen that significantly increases stereotyped behaviours, has been shown to attenuate the normal amphetamine-induced elevation in corticosterone [454]. Conversely, a decrease in amphetamine stereotypy induced by mesostriatal dopamine depletion prolonged the amphetamine-induced elevation in corticosterone secretion [322]. Furthermore, stressful conditions can enhance amphetamine- and cocaine-induced stereotypy, and this has been proposed to occur only when the stress is uncontrollable [410]. Thus, coping is an important variable in the interaction between the HPA-axis and psychostimulant drugs, which may be reflected by stereotyped behaviour [410].

Strain differences may exist in the susceptibility of laboratory rodents to psychostimulant-induced stereotypies. This has also been demonstrated for the C57BL/6 and DBA/2 strains, the latter but not the former, developing stereotyped behaviours during the course of repeated cocaine (32 mg/kg) treatment [666] or in response to food restriction [67]. In the present studies, the test setting did not allow for the detailed behavioural analysis required to distinguish subtle stereotyped movements. It is therefore conceivable that a difference in stereotyped responding may have contributed to the observed strain differences in behavioural sensitisation to cocaine. However, the doses of cocaine used in the present study (15.0 mg/kg during the treatment phase and 7.5 mg/kg for the drug challenge) were relatively low and, especially the challenge dose is not likely to have induced a high degree of stereotypy [45]. Furthermore, the observation that corticosterone secretion was attenuated by cocaine in the C57BL/6 strain that displayed high horizontal locomotion throughout the sensitisation paradigm (**chapter 2**), contradicts the notion that stereotypy might function to attenuate HPA-axis responsiveness in this strain.

The possibility of stereotypy in ADX animals should also be considered. Activation of the HPA-axis and the ANS enable an organism to respond and adapt to stressful situations and are therefore part of their coping mechanism. ADX mice are however not capable of releasing adrenal corticosterone or catecholamines in response to any stimulus. It is therefore conceivable that ADX animals develop stereotyped behaviours to cope with cocaine-induced arousal. In this line of reasoning, stereotypy would be part of the coping response not only in animals that are exposed to too high, but also too low levels of stress hormones. Further studies are therefore required to investigate whether stereotypy plays a significant role in the behavioural response of ADX mice to cocaine, especially given the seemingly high susceptibility of DBA/2 mice to develop such behavioural responses.

[34,160]. Moreover, mifepristone selectively reduced psychotic rather than depressive symptoms, which suggests that the antagonist modulates dopaminergic transmission [160]. Therefore, it would be interesting to test the effects of this compound on drug craving in human cocaine addicts. Clearly, more research is required to investigate the involvement of the sympathetic nervous system alone, and in interaction with various components of the HPA-axis, in human drug addiction. The present data suggest that β-blockers may have therapeutic potential. One first approach could be to investigate whether, in addition to cortisol secretion, the activity of the ANS (e.g. heart frequency) is a parameter that correlates with the extent of drug craving, and whether craving can be reduced by administration of β-blockers.

The data presented in this thesis indicate that, depending on genetic background, stress hormones can provide important risk factors for vulnerability to the psychostimulant effects of cocaine. Furthermore, these data add a new dimension to the concept of stress in addiction research, extending the focus from being solely on glucocorticoids to a wider scope involving the HPA-axis and the sympathoadrenal system with their central targets.

5. CONCLUSIONS

i) *Genetic background* determines sensitivity to the psychostimulant effects of cocaine and the contribution of *adrenal hormones*. One strain was identified, the DBA/2 strain, that is susceptible to the influence of adrenal stress hormones on behavioural sensitisation to cocaine.

ii) The interaction between *genetic background* and *adrenal stress hormones* in cocaine sensitivity can be monitored by measuring markers in the midbrain dopamine system. Most notably the D2 receptor in the terminal fields of the nigrostriatal dopamine system and the NAc core is an interesting candidate to mediate this interaction.

iii) The initiation phase of behavioural sensitisation is a critical *time-window* for the action of adrenal glucocorticoids. During this phase, continuous presence of high glucocorticoid concentrations facilitates sensitisation, whereas it remains to be established whether this involves non-genomic and/or genomic mechanisms.

iv) The sympathetic nervous system may signal aspects of the *physiological context* in which glucocorticoids operate. Adrenal epinephrine and corticosterone act in a coordinate fashion to facilitate behavioural sensitisation to cocaine.

7

Summary

Despite the powerful psychostimulant properties of cocaine, not every individual will acquire cocaine abuse after occasional use of the drug. A fundamental question in the neurobiology of addiction is therefore how these individual differences in susceptibility to drugs of abuse emerge. Evidence is accumulating that genes and adverse life experience can constitute risk factors. Especially the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system responses to stress have gained increasing attention for their role in vulnerability to psychostimulants in both humans and laboratory animals. The **objective** of this thesis was to assess the role of the end products of both stress response systems -the adrenal glucocorticoid hormones and epinephrine- in the susceptibility to the psychostimulant effects of cocaine, taking into account the *midbrain dopamine circuits*, the *genetic background* of the individual and the *context* in which the hormones operate.

Studies were designed with two inbred mouse strains (C57BL/6 and DBA/2) that can be considered a model for genetic differences in the midbrain dopamine system, the HPA-axis and susceptibility to the behavioural and reinforcing properties of psychostimulant drugs. The *context* required for the action of adrenal hormones was investigated in the DBA/2 strain that proved most susceptible to adrenal hormones in cocaine sensitivity. Adrenal hormones were manipulated by surgical removal of the adrenals (adrenalectomy: 'ADX') and subsequent hormone replacement. The sensitisation measured as an enhanced locomotor response to repeated drug exposure is thought to reflect long term neuronal adaptations underlying certain aspects of drug addiction. In addition, endocrine parameters were measured to investigate HPA-axis responsiveness to cocaine. Since the dopamine system plays a critical role in the rewarding effects of drugs of abuse, several markers of dopaminergic transmission were measured.

In **chapter 2** the C57BL/6 and DBA/2 inbred mouse strains were characterised for behavioural and endocrine responsiveness to cocaine. Whereas C57BL/6 mice showed greater locomotor responses to the first cocaine exposure, only DBA/2 mice exhibited an increase in drug responsiveness during repeated drug treatment. However, when exposed to a challenge dose of cocaine after a 5 day withdrawal interval, both strains expressed behavioural sensitisation. Therefore, the sensitisation paradigm was suitable to investigate the contribution of adrenal hormones in both strains. ADX completely prevented initiation and expression of behavioural sensitisation in the DBA/2, but not the C57BL/6 strain. Furthermore, only the DBA/2 strain displayed sensitisation of corticosterone secretion with repeated cocaine exposure, whereas the psychostimulant attenuated this endocrine measure in the C57BL/6 strain. These data indicate that the C57BL/6 and DBA/2 strains represent a model for genetic differences in behavioural and endocrine responsiveness to

cocaine. Moreover, the adrenals play an essential role in cocaine-induced behavioural sensitisation, but only in the DBA/2 strain.

In **chapter 3** it was investigated whether a neural correlate for the strain differences in behavioural responsiveness to cocaine, and susceptibility to the impact of adrenal stress hormones, can be found in the midbrain dopamine system. Whereas ADX did not affect strain differences in the basal dopamine system or the locomotor response to the first cocaine exposure, strain-dependent adaptations were observed in behavioural and neural responsiveness to cocaine. In the DBA/2 strain, sensitisation resistant ADX mice were characterised by reduced D2 binding in the nucleus accumbens core and rostral caudate putamen. Conversely, ADX prevented the cocaine-induced increase in TH and DAT mRNA expression in the substantia nigra and the decrease in D2 binding in a subdivision of the dorsal caudate putamen observed in SHAM mice. In the C57BL/6 strain, by contrast, behavioural sensitisation was independent of the adrenals and ADX only marginally affected drug-induced neuroadaptations. These results demonstrate that adrenal stress hormones affect psychostimulant sensitivity in a strain-dependent fashion, possibly through adaptations in the midbrain dopamine system. Furthermore, the DBA/2, but not the C57BL/6 strain, is vulnerable to the impact of adrenal stress hormones on cocaine sensitivity at the level of dopaminergic neurotransmission and behavioural responsiveness. This strain was therefore chosen to investigate the context-dependency of the glucocorticoid actions (**chapters 4** and **5**).

In **chapter 4**, the critical time-window for the actions of adrenal glucocorticoids was investigated in DBA/2 mice in relation to i) the stage of behavioural sensitisation to cocaine (induction vs. expression) and ii) the time of drug exposure. Administration of the glucocorticoid receptor antagonist mifepristone to sensitised animals failed to block expression of previously established behavioural sensitisation. This suggests that corticosterone plays a role during initiation of sensitisation, as appeared from the observations in the ADX mice. Intermittent corticosterone replacement 2 hours or 5 minutes prior to cocaine was ineffective in reversing the effect of ADX on behavioural sensitisation. By contrast, initiation of behavioural sensitisation was partially restored if the glucocorticoid was continuously substituted through release from a s.c. implanted pellet. These data indicate that corticosterone facilitates initiation rather than expression of behavioural sensitisation to cocaine, provided that the hormone is continuously circulating. However, because only chronic high concentrations of the hormone partially restored behavioural sensitisation, it appeared that the adrenal glucocorticoid is necessary, though not sufficient, for full sensitisation in the DBA/2 strain.

Therefore, the possible involvement of other adrenal factors was investigated in **chapter 5**. The focus was on epinephrine, the catecholamine that is rapidly released in response to both stress and psychostimulant administration. Whereas replacement with neither epinephrine nor continuous corticosterone alone was sufficient to reverse the ADX effect, co-substitution of the two adrenal hormones was sufficient to fully restore initiation and retention of behavioural sensitisation to the level observed in SHAM-operated mice. In addition, the catecholamine may play a permissive or facilitatory role in the HPA-axis sensitisation in the DBA/2 strain, since cocaine-induced c-fos mRNA expression in the hypothalamic PVN was potentiated in mice with a history of epinephrine replacement. Given the critical role for glucocorticoids in cocaine sensitivity of DBA/2 mice, this might be one of the mechanisms via which epinephrine facilitates behavioural sensitisation. These findings indicate that corticosterone and epinephrine act in a coordinate fashion to facilitate behavioural responsiveness to cocaine in the DBA/2 genotype.

Overall, the data presented in this thesis show that complex interactions between genes, the HPA-axis and the sympathetic nervous system contribute to the existence of individual differences in susceptibility to the psychostimulant effects of cocaine. It was demonstrated that *genetic background* determines the contribution of adrenal hormones to cocaine sensitivity. The C57BL/6 and DBA/2 strains represent a model for respectively resistance and susceptibility to the impact of adrenal hormones on cocaine-induced behavioural sensitisation and associated neuroadaptations in the brain dopamine system. Regarding the *context* required for the hormone actions, the initiation phase of sensitisation constitutes the critical *time-window*, whereas the sympathetic nervous system may signal aspects of the *physiological context*. Therefore, in susceptible individuals, not only adrenocortical but also adrenomedullary hormones, may contribute to initiation of long-term neuronal and behavioural adaptations to cocaine.

8

Samenvatting

INTRODUCTIE

Cocaïne behoort tot de stimulerende middelen; stoffen die energie en alertheid verhogen en een kortstondig goed gevoel of zelfs euforie geven. Herhaaldelijk gebruik van cocaïne kan echter leiden tot een oncontroleerbare drang om te gebruiken, oftewel verslaving. Verslaving is een groot probleem in de maatschappij, gezien de gevolgen hiervan voor gezondheid, sociaal functioneren en criminaliteit. Ondanks het feit dat cocaïne een zeer verslavende stof is, wordt het risico op het ontstaan van verslaving na recreatief gebruik geschat op 15-20%. Dit geeft aan dat niet elk persoon even gevoelig is voor de verslavende werking van de stof. Dergelijke individuele verschillen bestaan voor alle verslavende drugs, ondanks het feit dat zij tot zeer verschillende farmacologische klassen kunnen behoren (bijv. psychostimulantia, alcohol, opiaten, cannabis). Een belangrijke vraag in het onderzoek naar het ontstaan van verslaving is hoe deze grote individuele verschillen in gevoeligheid tot stand komen. Het is bekend dat enerzijds genetische factoren en anderzijds (negatieve) levenservaringen een belangrijke rol spelen. Echter, de hersenmechanismen die hieraan ten grondslag liggen, zijn veelal onbekend.

STRESSHORMONEN

Recent onderzoek heeft aangetoond dat *stress*, en de hormonen die tijdens stress worden uitgescheiden door de bijnier, belangrijke risicofactoren vormen voor verslaving. Wanneer een individu een situatie of gebeurtenis als stressvol ervaart, of wanneer er veranderingen optreden in de omgeving of in het lichaam, worden hormonale en neuronale processen geactiveerd die de balans in het lichaam herstellen en die de persoon in staat stellen adequaat op de situatie te reageren. Twee verschillende systemen kunnen worden onderscheiden: het sympathische zenuwstelsel en de hypofyse-bijnier-as (in het Engels: Hypothalamic-Pituitary-Adrenal (HPA) as). Activering van het sympathische zenuwstelsel, resulterend in afgifte van adrenaline door de bijnieren en noradrenaline door bepaalde zenuwen, zorgt o.a. voor verhogingen in hartfrequentie, bloeddruk en bloedtoevoer naar de spieren. Dit is een zeer snel proces en stelt de persoon in staat vrijwel direct op de situatie te reageren. Activering van de HPA-as leidt tot afgifte van glucocorticoïd hormonen door de bijnieren (cortisol bij de mens en corticosteron bij knaagdieren). Zoals uit de naam valt af te leiden, zorgen deze hormonen o.a. voor het vrijmaken van glucose waardoor zij de beschikbare energie verhogen. Daarnaast beïnvloeden glucocorticoïden processen in de hersenen die te maken hebben met cognitie, perceptie, stemming en beloning. Kortstondige afgifte van glucocorticoïden is daarom

van essentieel belang voor een adequate reactie op veranderingen in de omgeving. Echter, langdurige of juist onvoldoende afgifte van deze hormonen kan het ontstaan van bepaalde ziekten, waaronder verslaving, in de hand werken. De mechanismen die hieraan ten grondslag liggen zijn echter nog veelal onbekend.

Het onderzoek in dit proefschrift was gericht op de rol van beide *stress*systemen in gevoeligheid voor cocaïne, met speciale aandacht voor de *genetische achtergrond* van het individu. De interactie tussen deze factoren werd in een muismodel onderzocht.

MUISMODEL

Pyschostimulantia zoals cocaïne en amfetamine verhogen de afgifte van de signaalstof dopamine in hersenkernen die betrokken zijn bij motivatie en beloning. Bij knaagdieren uit de stimulerende werking van deze drugs zich in een verhoging van de beweeglijkheid (locomotoriek), wat direct gerelateerd is aan de dopamine afgifte in de hersenen. Eén van de processen die een rol spelen bij verslaving is sensitisatie: de toename van de respons op de drug, of hiermee geassocieerde stimuli, bij herhaaldelijke blootstelling. Sensitisatie gaat gepaard met blijvende veranderingen in het dopaminesysteem en wordt geacht bij te dragen aan de, voor verslaving kenmerkende, buitensporige motivatie om de drug te verkrijgen. Ook de locomotorische respons van knaagdieren kan toenemen bij herhaalde toediening van psychostimulantia, wat een diermodel vormt voor sensitisatie. In het huidige onderzoek werd dit principe toegepast door aan muizen gedurende 9 opeenvolgende dagen cocaïne toe te dienen en te bepalen of, en zo ja in welke mate, de motorische respons toeneemt ('initiatie' van sensitisatie). Tevens werden dieren na een ontwenningsperiode van enkele dagen opnieuw aan cocaïne blootgesteld om te onderzoeken of de sensitisatie van langdurige aard is ('expressie' van sensitisatie). Hierbij werd de respons van met cocaïne voorbehandelde dieren vergeleken met die van dieren die niet eerder aan de drug waren blootgesteld.

Om de invloed van *genetische achtergrond* te onderzoeken, werd gebruik gemaakt van twee verschillende inteelt muizenstammen, C57BL/6 en DBA/2, die gekenmerkt worden door verschillen in gevoeligheid voor verslavende drugs en stress. De bijdrage van beide *stress*systemen aan cocaïne sensitisatie werd onderzocht door de bijnieren, bron van zowel corticosteroïden als adrenaline, chirurgisch te verwijderen (adrenalectomie 'ADX'). Door aan ADX dieren vervolgens corticosteron en/of adrenaline toe te dienen, werd de afzonderlijke en/of gezamenlijke werking van deze hormonen onderzocht. Aangezien het *dopamine*systeem in de hersenen

een cruciale rol speelt bij de verslavende effecten van drugs, werden verschillende markers in het dopaminesysteem gemeten.

In **hoofdstuk 2** worden de onderzochte cocaïnegevoeligheid van beide muizenstammen, en de rol van bijnierhormonen hierin, beschreven. C57BL/6 muizen waren gevoeliger voor de activerende effecten van de eerste cocaïne injectie, terwijl DBA/2 muizen een sterke toename in de locomotorische respons vertoonden bij herhaalde blootstelling. Ondanks deze verschillen werd bij beide stammen sensitisatie waargenomen bij hernieuwde blootstelling aan cocaïne na een ontwenningsperiode van vijf dagen. Daarentegen vertoonde alleen de DBA/2 stam een toename in corticosteron secretie bij herhaalde cocaïne toediening. ADX voorkwam de gedragssensitisatie in de DBA/2, maar niet de C57BL/6 stam. Uit deze bevindingen kan worden geconcludeerd dat de C57BL/6 en DBA/2 muizenstammen een model vormen voor genetische verschillen in gevoeligheid voor cocaïne, zowel wat betreft de gedragsmatige als de hormonale respons. De bijnieren lijken alleen in de DBA/2 stam een essentiële rol te spelen in sensitisatie van de locomotorische respons op cocaïne.

In **hoofdstuk 3** worden verschillende markers in het dopaminesysteem beschreven die gemeten zijn met als doel een neuronaal correlaat te vinden voor de stamverschillen in gevoeligheid voor cocaïne en de rol van bijnierhormonen hierin. De markers reflecteren verschillende aspecten van dopaminerge transmissie: tyrosine hydroxylase (TH, een cruciaal enzym in de synthese van dopamine), dopamine transporter (DAT, bewerkstelligt heropname van dopamine in het neuron, wat de transmissie 'beëindigt') en de twee meest voorkomende klassen van dopaminereceptoren (D1 en D2). Expressie van deze markers werd onderzocht in twee dopaminerge circuits, die beide een rol spelen bij verslaving: i) het mesolimbisch systeem, met neuronen die van het ventraal tegmentaal gebied (VTA) naar o.a. de nucleus accumbens (NAc) projecteren, en ii) het nigrostriataal systeem dat de substantia nigra (SN) met het striatum (caudate putamen, CP) verbindt. ADX had geen invloed op de stamverschillen in het dopaminesysteem van onbehandelde dieren en op de locomotorische respons bij eerste cocaïne toediening (**hoofdstuk 2**). Daarentegen werden stamafhankelijke veranderingen waargenomen in de locomotorische en neuronale responsen bij herhaalde blootstelling aan de drug. 'Sensitisatie-resistente' ADX muizen van de DBA/2 stam werden gekarakteriseerd door i) verminderde D2 binding in subdivisies van de NAc en het CP (de NAc core en het rostrale CP respectievelijk), en ii) een gebrek aan toename in TH en DAT mRNA expressie in de SN geassocieerd met sensitisatie in SHAM muizen. Echter, in de C57BL/6 stam was sensitisatie van locomotoriek onafhankelijk van de

bijnieren en waren er slechts marginale veranderingen in dopaminerge markers ten gevolge van ADX. Deze resultaten geven aan dat bijnierhormonen cocaïne gevoeligheid beïnvloeden op een stamafhankelijke wijze, wellicht door adaptaties in het dopaminesysteem. De DBA/2, maar niet de C57BL/6 stam was gevoelig voor de effecten van ADX op cocaïne gevoeligheid zowel op het niveau van dopaminerge neurotransmissie als op dat van locomotorische responsen. Om deze reden werd de DBA/2 stam gekozen voor verdere studies naar de rol van bijnierhormonen in cocaïne gevoeligheid (**hoofdstukken 4 en 5**).

In **hoofdstuk 4** wordt de 'timing' van de effecten van glucocorticoïden onderzocht met betrekking tot i) het stadium van de sensitisatie (initiatie vs. expressie), en ii) de tijd waarop cocaïne blootstelling plaats vindt. Blokkering van de receptor voor glucocorticoïden (GR) in eerder gesensitiseerde dieren had geen effect op de expressie van de sensitisatie. Dit suggereert dat corticosteron een rol speelt tijdens initiatie van sensitisatie, wat overeenkomt met de observaties in ADX muizen. Echter, toediening van corticosteron 2 uur of 5 minuten voor elke cocaïne injectie was niet voldoende om het effect van ADX op gedragssensitisatie te herstellen. Continue blootstelling aan corticosteron door afgifte vanuit een onderhuidse pellet resulteerde in gedeeltelijk herstel van sensitisatie. Deze bevindingen geven aan dat corticosteron de initiatie, maar niet de expressie, van sensitisatie faciliteert. Echter, de observatie dat alleen hoge concentraties van het hormoon de effecten van ADX deels herstellen, suggereert dat het glucocorticoïd noodzakelijk, maar niet voldoende, is voor volledige sensitisatie in de DBA/2 stam.

Daarom wordt in **hoofdstuk 5** de mogelijke betrokkenheid van andere bijnierhormonen onderzocht. Hierbij was de focus op adrenaline, gezien het feit dat dit 'catecholamine' zeer snel wordt uitgescheiden door de bijnieren in respons op zowel stress als blootstelling aan psychostimulantia. Terwijl substitutie met adrenaline of met corticosteron afzonderlijk onvoldoende was om de effecten van ADX terug te draaien, kon met co-substitutie van beide hormonen zowel de initiatie als de expressie van cocaïne sensitisatie volledig worden hersteld. Daarnaast speelt adrenaline wellicht een rol bij de sensitisatie van de HPA-as die optreedt in de DBA/2 stam bij herhaalde cocaïne toediening. In met adrenaline behandelde dieren werd in respons op cocaïne een verhoogde expressie waargenomen van c-fos, een marker voor neuronale activiteit, in de nucleus paraventricularis, de kern van de hypothalamus die een cruciale rol speelt bij de regulatie van de HPA-as en dus de corticosteron afgifte. Gezien de belangrijke rol van corticosteron in cocaïne gevoeligheid van de DBA/2 stam, is dit wellicht één van de mechanismen via welke adrenaline gedragssensitisatie faciliteert. Uit deze resultaten kan worden

geconcludeerd dat de gezamenlijke werking van corticosteron én adrenaline bijdraagt aan het tot stand komen van sensitisatie van de locomotorische respons op cocaïne in de DBA/2 stam.

Uit het onderzoek beschreven in dit proefschrift kan worden geconcludeerd dat complexe interacties tussen genen, de HPA-as en het sympathische zenuwstelsel bijdragen aan individuele verschillen in gevoeligheid voor cocaïne. Het werd aangetoond dat de bijdrage van bijnierhormonen aan cocaïne gevoeligheid wordt bepaald door de genetische achtergrond van het individu. De C57BL/6 en DBA/2 muizenstammen vormen een model voor respectievelijk resistentie en gevoeligheid voor de impact van bijnierhormonen op sensitisatie van de locomotorische respons en geassocieerde veranderingen in het dopaminesysteem. Wat betreft de context waarin de glucocorticoïden werken, kan worden geconcludeerd dat de initiatie van sensitisatie de kritieke periode vormt, waarin activatie van het sympathische zenuwstelsel een onderdeel van de fysiologische context vormt. In hiervoor gevoelige individuen, kunnen dan ook zowel glucocorticoïden als catecholamines uit de bijnieren bijdragen aan de langdurige neuronale en gedragsmatige effecten van cocaïne.

9

References

[1] Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J Neurochem* 1989; 52(5): 1655-1658.

[2] Abraham I, Juhasz G, Kekesi KA, Kovacs KJ. Corticosterone peak is responsible for stress-induced elevation of glutamate in the hippocampus. *Stress* 1998; 2(3): 171-181.

[3] Ackerman JM, White FJ. A10 somatodendritic dopamine autoreceptor sensitivity following withdrawal from repeated cocaine treatment. *Neurosci Lett* 1990; 117(1-2): 181-187.

[4] Ahima R, Krozowski Z, Harlan R. Type I corticosteroid receptor-like immunoreactivity in the rat CNS: distribution and regulation by corticosteroids. *J Comp Neurol* 1991; 313(3): 522-538.

[5] Akimoto K, Hamamura T, Kazahaya Y, Akiyama K, Otsuki S. Enhanced extracellular dopamine level may be the fundamental neuropharmacological basis of cross-behavioral sensitization between methamphetamine and cocaine - an in vivo dialysis study in freely moving rats. *Brain Res* 1990; 507(2): 344-346.

[6] Albertson DN, Schmidt CJ, Kapatos G, Bannon MJ. Distinctive profiles of gene expression in the human nucleus accumbens associated with cocaine and heroin abuse. *Neuropsychopharmacology* 2006; 31(10): 2304-2312.

[7] Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci* 1990; 13(7): 266-271.

[8] Alleweireldt AT, Hobbs RJ, Taylor AR, Neisewander JL. Effects of SCH-23390 infused into the amygdala or adjacent cortex and basal ganglia on cocaine seeking and self-administration in rats. *Neuropsychopharmacology* 2006; 31(2): 363-374.

[9] Alleweireldt AT, Weber SM, Kirschner KF, Bullock BL, Neisewander JL. Blockade or stimulation of D1 dopamine receptors attenuates cue reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacology (Berl)* 2002; 159(3): 284-293.

[10] Ammassari-Teule M, Passino E, Restivo L, de Marsanich B. Fear conditioning in C57BL/6 and DBA/2 mice: variability in nucleus accumbens function according to the strain predisposition to show contextual- or cue-based responding. *Eur J Neurosci* 2000; 12(12): 4467-4474.

[11] Anderson SM, Bari AA, Pierce RC. Administration of the D1-like dopamine receptor antagonist SCH-23390 into the medial nucleus accumbens shell attenuates cocaine priming-induced reinstatement of drug-seeking behavior in rats. *Psychopharmacology (Berl)* 2003; 168(1-2): 132-138.

[12] Anderson SM, Schmidt HD, Pierce RC. Administration of the D2 dopamine receptor antagonist sulpiride into the shell, but not the core, of the nucleus accumbens attenuates cocaine priming-induced reinstatement of drug seeking. *Neuropsychopharmacology* 2006; 31(7): 1452-1461.

[13] Antelman SM, Eichler AJ, Black CA, Kocan D. Interchangeability of stress and amphetamine in sensitization. *Science* 1980; 207(4428): 329-331.

[14] Aouizerate B, Ho A, Schluger JH, Perret G, Borg L, Le Moal M, Piazza PV, Kreek MJ. Glucocorticoid negative feedback in methadone-maintained former heroin addicts with ongoing cocaine dependence: dose-response to dexamethasone suppression. *Addict Biol* 2006; 11(1): 84-96.

[15] Appelboom T. Consumption of coca in history. *Verh K Acad Geneeskd Belg* 1991; 53(5): 497-505.

[16] Auchus AP, Green RC, Nemeroff CB. Cortical and subcortical neuropeptides in Alzheimer's disease. *Neurobiol Aging* 1994; 15(4): 589-595.

[17] Azar MR, Acar N, Erwin VG, Barbato GF, Morse AC, Heist CL, Jones BC. Distribution and clearance of cocaine in brain is influenced by genetics. *Pharmacol Biochem Behav* 1998; 59(3): 637-640.

[18] Back SE, Brady KT, Jaanimagi U, Jackson JL. Cocaine dependence and PTSD: a pilot study of symptom interplay and treatment preferences. *Addict Behav* 2006; 31(2): 351-354.

[19] Backstrom P, Hyytia P. Ionotropic and metabotropic glutamate receptor antagonism attenuates cue-induced cocaine seeking. *Neuropsychopharmacology* 2006; 31(4): 778-786.

[20] Badiani A, Cabib S, Puglisi-Allegra S. Chronic stress induces strain-dependent sensitization to the behavioral effects of amphetamine in the mouse. *Pharmacol Biochem Behav* 1992; 43(1): 53-60.

[21] Badiani A, Morano MI, Akil H, Robinson TE. Circulating adrenal hormones are not necessary for the development of sensitization to the psychomotor activating effects of amphetamine. *Brain Res* 1995; 673(1): 13-24.

[22] Badiani A, Robinson TE. Drug-induced neurobehavioral plasticity: the role of environmental context. *Behav Pharmacol* 2004; 15(5-6): 327-339.

[23] Bahi A, Boyer F, Bussard G, Dreyer JL. Silencing dopamine D3-receptors in the nucleus accumbens shell in vivo induces changes in cocaine-induced hyperlocomotion. *Eur J Neurosci* 2005; 21(12): 3415-3426.

[24] Baker TB, Morse E, Sherman JE. The motivation to use drugs: a psychobiological analysis of urges. *Nebr Symp Motiv* 1986; 34: 257-323.

[25] Balfour ME, Yu L, Coolen LM. Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. *Neuropsychopharmacology* 2004; 29(4): 718-730.

[26] Banihashemi L, Rinaman L. Noradrenergic inputs to the bed nucleus of the stria terminalis and paraventricular nucleus of the hypothalamus underlie hypothalamic-pituitary-adrenal axis but not hypophagic or conditioned avoidance responses to systemic yohimbine. *J Neurosci* 2006; 26(44): 11442-11453.

[27] Baptista MA, Martin-Fardon R, Weiss F. Preferential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on conditioned reinstatement versus primary reinforcement: comparison between cocaine and a potent conventional reinforcer. *J Neurosci* 2004; 24(20): 4723-4727.

[28] Bari AA, Pierce RC. D1-like and D2 dopamine receptor antagonists administered into the shell subregion of the rat nucleus accumbens decrease cocaine, but not food, reinforcement. *Neuroscience* 2005; 135(3): 959-968.

[29] Barr AM, Hofmann CE, Weinberg J, Phillips AG. Exposure to repeated, intermittent d-amphetamine induces sensitization of HPA axis to a subsequent stressor. *Neuropsychopharmacology* 2002; 26(3): 286-294.

[30] Barrot M, Marinelli M, Abrous DN, Rouge-Pont F, Le Moal M, Piazza PV. The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. *Eur J Neurosci* 2000; 12(3): 973-979.

[31] Baumann MH, Gendron TM, Becketts KM, Henningfield JE, Gorelick DA, Rothman RB. Effects of intravenous cocaine on plasma cortisol and prolactin in human cocaine abusers. *Biol Psychiatry* 1995; 38(11): 751-755.

[32] Beitner-Johnson D, Guitart X, Nestler EJ. Dopaminergic brain reward regions of Lewis and Fischer rats display different levels of tyrosine hydroxylase and other morphine- and cocaine-regulated phosphoproteins. *Brain Res* 1991; 561(1): 147-150.

[33] Beitner-Johnson D, Nestler EJ. Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. *J Neurochem* 1991; 57(1): 344-347.

[34] Belanoff JK, Rothschild AJ, Cassidy F, DeBattista C, Baulieu EE, Schold C, Schatzberg AF. An open label trial of C-1073 (mifepristone) for psychotic major depression. *Biol Psychiatry* 2002; 52(5): 386-392.

[35] Bell K, Kalivas PW. Context-specific cross-sensitization between systemic cocaine and intra-accumbens AMPA infusion in the rat. *Psychopharmacology (Berl)* 1996; 127(4): 377-383.

[36] Ben Shahar O, Ahmed SH, Koob GF, Ettenberg A. The transition from controlled to compulsive drug use is associated with a loss of sensitization. *Brain Res* 2004; 995(1): 46-54.

[37] Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. *Nat Genet* 1996; 12(1): 81-84.

[38] Berglind WJ, Case JM, Parker MP, Fuchs RA, See RE. Dopamine D1 or D2 receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. *Neuroscience* 2006; 137(2): 699-706.

[39] Berhow MT, Hiroi N, Nestler EJ. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. *J Neurosci* 1996; 16(15): 4707-4715.

[40] Berke JD, Paletzki RF, Aronson GJ, Hyman SE, Gerfen CR. A complex program of striatal gene expression induced by dopaminergic stimulation. *J Neurosci* 1998; 18(14): 5301-5310.

[41] Berman SM, Noble EP. The D2 dopamine receptor (DRD2) gene and family stress; interactive effects on cognitive functions in children. *Behav Genet* 1997; 27(1): 33-43.

[42] Berns GS. Something funny happened to reward. *Trends Cogn Sci* 2004; 8(5): 193-194.

[43] Berrettini WH, Alexander R, Ferraro TN, Vogel WH. A study of oral morphine preference in inbred mouse strains. *Psychiatr Genet* 1994; 4(2): 81-86.

[44] Bevins RA, Klebaur JE, Bardo MT. Individual differences in response to novelty, amphetamine-induced activity and drug discrimination in rats. *Behav Pharmacol* 1997; 8(2-3): 113-123.

[45] Bhattacharyya AK, Pradhan SN. Interactions between motor activity and stereotypy in cocaine-treated rats. *Psychopharmacology (Berl)* 1979; 63(3): 311-312.

[46] Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, Greengard P. Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* 2001; 410(6826): 376-380.

[47] Biron D, Dauphin C, Di Paolo T. Effects of adrenalectomy and glucocorticoids on rat brain dopamine receptors. *Neuroendocrinology* 1992; 55(4): 468-476.

[48] Blaha CD, Yang CR, Floresco SB, Barr AM, Phillips AG. Stimulation of the ventral subiculum of the hippocampus evokes glutamate receptor-mediated changes in dopamine efflux in the rat nucleus accumbens. *Eur J Neurosci* 1997; 9(5): 902-911.

[49] Blanc G, Trovero F, Vezina P, Herve D, Godeheu AM, Glowinski J, Tassin JP. Blockade of prefrontocortical alpha 1-adrenergic receptors prevents locomotor hyperactivity induced by subcortical D-amphetamine injection. *Eur J Neurosci* 1994; 6(3): 293-298.

[50] Bonci A, Williams JT. A common mechanism mediates long-term changes in synaptic transmission after chronic cocaine and morphine. *Neuron* 1996; 16(3): 631-639.

[51] Borowsky B, Kuhn CM. Chronic cocaine administration sensitizes behavioral but not neuroendocrine responses. *Brain Res* 1991; 543(2): 301-306.

[52] Borowsky B, Kuhn CM. Monoamine mediation of cocaine-induced hypothalamo-pituitary-adrenal activation. *J Pharmacol Exp Ther* 1991; 256(1): 204-210.

[53] Borrell J, De Kloet ER, Bohus B. Corticosterone decreases the efficacy of adrenaline to affect passive avoidance retention of adrenalectomized rats. *Life Sci* 1984; 34(1): 99-104.

[54] Borrell J, De Kloet ER, Versteeg DH, Bohus B. Inhibitory avoidance deficit following short-term adrenalectomy in the rat: the role of adrenal catecholamines. *Behav Neural Biol* 1983; 39(2): 241-258.

[55] Borski RJ. Nongenomic membrane actions of glucocorticoids in vertebrates. *Trends Endocrinol Metab* 2000; 11(10): 427-436.

[56] Boudreau AC, Wolf ME. Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J Neurosci* 2005; 25(40): 9144-9151.

[57] Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC. Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. *Brain Res* 1991; 564(2): 203-219.

[58] Brake WG, Zhang TY, Diorio J, Meaney MJ, Gratton A. Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur J Neurosci* 2004; 19(7): 1863-1874.

[59] Brebner K, Ahn S, Phillips AG. Attenuation of d-amphetamine self-administration by baclofen in the rat: behavioral and neurochemical correlates. *Psychopharmacology (Berl)* 2005; 177(4): 409-417.

[60] Brebner K, Phelan R, Roberts DC. Effect of baclofen on cocaine self-administration in rats reinforced under fixed-ratio 1 and progressive-ratio schedules. *Psychopharmacologia* 2000; 148(3): 314-321.

[61] Brebner K, Childress AR, Roberts DCS. A potential role for GABAB agonists in the treatment of psychostimulant addiction. *Alcohol Alcoholism* 2002; 37(5): 478-484.

[62] Budygin EA, John CE, Mateo Y, Jones SR. Lack of cocaine effect on dopamine clearance in the core and shell of the nucleus accumbens of dopamine transporter knock-out mice. *J Neurosci* 2002; 22(10): RC222.

[63] Buijs RM, Kalsbeek A, van der Woude TP, van Heerikhuize JJ, Shinn S. Suprachiasmatic nucleus lesion increases corticosterone secretion. *AJP - Legacy* 1993; 264(6 Pt 2): R1186-R1192.

[64] Bunzow JR, Van Tol HH, Grandy DK, Albert P, Salon J, Christie M, Machida CA, Neve KA, Civelli O. Cloning and expression of a rat D2 dopamine receptor cDNA. *Nature* 1988; 336(6201): 783-787.

[65] Cabib S. Strain-dependent behavioural sensitization to amphetamine: role of environmental influences. *Behav Pharmacol* 1993; 4(4): 367-374.

[66] Cabib S, Algeri S, Perego C, Puglisi-Allegra S. Behavioral and biochemical changes monitored in two inbred strains of mice during exploration of an unfamiliar environment. *Physiol Behav* 1990; 47(4): 749-753.

[67] Cabib S, Bonaventura N. Parallel strain-dependent susceptibility to environmentally-induced stereotypies and stress-induced behavioral sensitization in mice. *Physiol Behav* 1997; 61(4): 499-506.

[68] Cabib S, Castellano C, Patacchioli FR, Cigliana G, Angelucci L, Puglisi-Allegra S. Opposite strain-dependent effects of post-training corticosterone in a passive avoidance task in mice: role of dopamine. *Brain Res* 1996; 729(1): 110-118.

[69] Cabib S, Giardino L, Calza L, Zanni M, Mele A, Puglisi-Allegra S. Stress promotes major changes in dopamine receptor densities within the mesoaccumbens and nigrostriatal systems. *Neuroscience* 1998; 84(1): 193-200.

[70] Cabib S, Kempf E, Schleef C, Oliverio A, Puglisi-Allegra S. Effects of immobilization stress on dopamine and its metabolites in different brain areas of the mouse: role of genotype and stress duration. *Brain Res* 1988; 441(1-2): 153-160.

[71] Cabib S, Orsini C, Le Moal M, Piazza PV. Abolition and reversal of strain differences in behavioral responses to drugs of abuse after a brief experience. *Science* 2000; 289(5478): 463-465.

[72] Cabib S, Puglisi-Allegra S. Genotype-dependent effects of chronic stress on apomorphine-induced alterations of striatal and mesolimbic dopamine metabolism. *Brain Res* 1991; 542(1): 91-96.

[73] Cabib S, Puglisi-Allegra S, Ventura R. The contribution of comparative studies in inbred strains of mice to the understanding of the hyperactive phenotype. *Behav Brain Res* 2002; 130(1-2): 103-109.

[74] Cadoni C, Solinas M, Di Chiara G. Psychostimulant sensitization: differential changes in accumbal shell and core dopamine. *Eur J Pharmacol* 2000; 388(1): 69-76.

[75] Cador M, Bjijou Y, Cailhol S, Stinus L. Amphetamine-induced behavioral sensitization: implication of a glutamatergic medial prefrontal cortex-ventral tegmental area innervation. *Neuroscience* 1999; 94(3): 705-721.

[76] Cador M, Cole BJ, Koob GF, Stinus L, Le Moal M. Central administration of corticotropin releasing factor induces long-term sensitization to D-amphetamine. *Brain Res* 1993; 606(2): 181-186.

[77] Cador M, Dulluc J, Mormede P. Modulation of the locomotor response to amphetamine by corticosterone. *Neuroscience* 1993; 56(4): 981-988.

[78] Caine SB, Heinrichs SC, Coffin VL, Koob GF. Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Res* 1995; 692(1-2): 47-56.

[79] Caine SB, Koob GF. Effects of mesolimbic dopamine depletion on responding maintained by cocaine and food. *J Exp Anal Behav* 1994; 61(2): 213-221.

[80] Caldji C, Diorio J, Meaney MJ. Variations in maternal care in infancy regulate the development of stress reactivity. *Biol Psychiatry* 2000; 48(12): 1164-1174.

[81] Caldji C, Francis D, Sharma S, Plotsky PM, Meaney MJ. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. *Neuropsychopharmacology* 2000; 22(3): 219-229.

[82] Camp DM, Browman KE, Robinson TE. The effects of methamphetamine and cocaine on motor behavior and extracellular dopamine in the ventral striatum of Lewis versus Fischer 344 rats. *Brain Res* 1994; 668(1-2): 180-193.

[83] Campbell J, Spear LP. Effects of early handling on amphetamine-induced locomotor activation and conditioned place preference in the adult rat. *Psychopharmacology (Berl)* 1999; 143(2): 183-189.

[84] Campbell UC, Carroll ME. Effects of ketoconazole on the acquisition of intravenous cocaine self-administration under different feeding conditions in rats. *Psychopharmacology (Berl)* 2001; 154(3): 311-318.

[85] Campbell UC, Lac ST, Carroll ME. Effects of baclofen on maintenance and reinstatement of intravenous cocaine self-administration in rats. *Psychopharmacologia* 1999; 143(2): 209-214.

[86] Cannon WB, de la Paz D. Emotional stimulation of adrenal secretion. *AJP - Legacy* 1911; 28(1): 64-70.

[87] Capper-Loup C, Canales JJ, Kadaba N, Graybiel AM. Concurrent activation of dopamine D1 and D2 receptors is required to evoke neural and behavioral phenotypes of cocaine sensitization. *J Neurosci* 2002; 22(14): 6218-6227.

[88] Capriles N, Rodaros D, Sorge RE, Stewart J. A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)* 2003; 168(1-2): 66-74.

[89] Carelli RM. Nucleus accumbens cell firing and rapid dopamine signaling during goal-directed behaviors in rats. *Neuropharmacology* 2004; 47(suppl 1): 180-189.

[90] Carlezon WA, Jr., Nestler EJ. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci* 2002; 25(12): 610-615.

[91] Carney JM, Landrum RW, Cheng MS, Seale TW. Establishment of chronic intravenous drug self-administration in the C57BL/6J mouse. *Neuroreport* 1991; 2(8): 477-480.

[92] Carroll ME. The role of food deprivation in the maintenance and reinstatement of cocaine-seeking behavior in rats. *Drug Alcohol Depend* 1985; 16(2): 95-109.

[93] Carroll ME, Lac ST, Asencio M, Kragh R. Intravenous cocaine self-administration in rats is reduced by dietary L-tryptophan. *Psychopharmacology (Berl)* 1990; 100(3): 293-300.

[94] Cartmell LW, Aufderhide A, Weems C. Cocaine metabolites in pre-Columbian mummy hair. *J Okla State Med Assoc* 1991; 84(1): 11-12.

[95] Castellano C, Puglisi-Allegra S. Strain-dependent modulation of memory by stress in mice. *Behav Neural Biol* 1983; 38(1): 133-138.

[96] Catalano M, Nobile M, Novelli E, Nothen MM, Smeraldi E. Distribution of a novel mutation in the first exon of the human dopamine D4 receptor gene in psychotic patients. *Biol Psychiatry* 1993; 34(7): 459-464.

[97] Cervo L, Cocco A, Petrella C, Heidbreder CA. Selective antagonism at dopamine D3 receptors attenuates cocaine-seeking behaviour in the rat. *Int J Neuropsychopharmacol* 2007; 10(2): 167-181.

[98] Chao HM, Choo PH, McEwen BS. Glucocorticoid and mineralocorticoid receptor mRNA expression in rat brain. *Neuroendocrinology* 1989; 50(4): 365-371.

[99] Chen AC, LaForge KS, Ho A, McHugh PF, Kellogg S, Bell K, Schluger RP, Leal SM, Kreek MJ. Potentially functional polymorphism in the promoter region of prodynorphin gene may be associated with protection against cocaine dependence or abuse. *Am J Med Genet* 2002; 114(4): 429-435.

[100] Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ. Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. *J Neurosci* 1997; 17(13): 4933-4941.

[101] Chen J, Nye HE, Kelz MB, Hiroi N, Nakabeppu Y, Hope BT, Nestler EJ. Regulation of delta FosB and FosB-like proteins by electroconvulsive seizure and cocaine treatments. *Mol Pharmacol* 1995; 48(5): 880-889.

[102] Chen R, Tilley MR, Wei H, Zhou F, Zhou FM, Ching S, Quan N, Stephens RL, Hill ER, Nottoli T, Han DD, Gu HH. Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. *Proc Natl Acad Sci U S A* 2006; 103(24): 9333-9338.

[103] Chen YZ, Hua SY, Wang CA, Wu LG, Gu Q, Xing BR. An electrophysiological study on the membrane receptor-mediated action of glucocorticoids in mammalian neurons. *Neuroendocrinology* 1991; 53(suppl 1): 25-30.

[104] Chevrette J, Stellar JR, Hesse GW, Markou A. Both the shell of the nucleus accumbens and the central nucleus of the amygdala support amphetamine self-administration in rats. *Pharmacol Biochem Behav* 2002; 71(3): 501-507.

[105] Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S, Corsi M, Orzi F, Conquet F. Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nat Neurosci* 2001; 4(9): 873-874.

[106] Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP. Limbic activation during cue-induced cocaine craving. *Am J Psychiatry* 1999; 156(1): 11-18.

[107] Chio CL, Hess GF, Graham RS, Huff RM. A second molecular form of D2 dopamine receptor in rat and bovine caudate nucleus. *Nature* 1990; 343(6255): 266-269.

[108] Chiu J, Kalant H, Le DA. Vasopressin opposes locomotor stimulation by ethanol, cocaine and amphetamine in mice. *Eur J Pharmacol* 1998; 355(1): 11-17.

[109] Chiueh CC, Kopin IJ. Centrally mediated release by cocaine of endogenous epinephrine and norepinephrine from the sympathoadrenal medullary system of unanesthetized rats. *J Pharmacol Exp Ther* 1978; 205(1): 148-154.

[110] Cho K, Little HJ. Effects of corticosterone on excitatory amino acid responses in dopamine-sensitive neurons in the ventral tegmental area. *Neuroscience* 1999; 88(3): 837-845.

[111] Chocyk A, Czyrak A, Wedzony K. Acute and repeated cocaine induces alterations in FosB/delta FosB expression in the paraventricular nucleus of the hypothalamus. *Brain Res* 2006; 1090(1): 58-68.

[112] Christie MJ, Summers RJ, Stephenson JA, Cook CJ, Beart PM. Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[3H]aspartate and [3H]GABA. *Neuroscience* 1987; 22(2): 425-439.

[113] Chuluyan HE, Saphier D, Rohn WM, Dunn AJ. Noradrenergic innervation of the hypothalamus participates in adrenocortical responses to interleukin-1. *Neuroendocrinology* 1992; 56(1): 106-111.

[114] Ciamei A, Cestari V, Castellano C. Strain-dependent interactions between MK-801 and cocaine on retention of C57BL/6 and DBA/2 mice tested in a one-trial inhibitory avoidance task: involvement of dopaminergic mechanisms. *Neurobiol Learn Mem* 2000; 73(2): 188-194.

[115] Ciccocioppo R, Sanna PP, Weiss F. Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D(1) antagonists. *Proc Natl Acad Sci U S A* 2001; 98(4): 1976-1981.

[116] Coirini H, Magarinos AM, De Nicola AF, Rainbow TC, McEwen BS. Further studies of brain aldosterone binding sites employing new mineralocorticoid and glucocorticoid receptor markers in vitro. *Brain Res* 1985; 361(1-2): 212-216.

[117] Colby CR, Whisler K, Steffen C, Nestler EJ, Self DW. Striatal cell type-specific overexpression of delta FosB enhances incentive for cocaine. *J Neurosci* 2003; 23(6): 2488-2493.

[118] Cole BJ, Koob GF. Low doses of corticotropin-releasing factor potentiate amphetamine-induced stereotyped behavior. *Psychopharmacology (Berl)* 1989; 99(1): 27-33.

[119] Cole BJ, Cador M, Stinus L, Rivier J, Vale W, Koob GF, Le Moal M. Central administration of a CRF antagonist blocks the development of stress-induced behavioral sensitization. *Brain Res* 1990; 512(2): 343-346.

[120] Comings DE, Gonzalez N, Wu S, Saucier G, Johnson P, Verde R, MacMurray JP. Homozygosity at the dopamine DRD3 receptor gene in cocaine dependence. *Mol Psychiatry* 1999; 4(5): 484-487.

[121] Comings DE, Muhleman D, Ahn C, Gysin R, Flanagan SD. The dopamine D2 receptor gene: a genetic risk factor in substance abuse. *Drug Alcohol Depend* 1994; 34(3): 175-180.

[122] Comings DE, Muhleman D, Gade R, Johnson P, Verde R, Saucier G, MacMurray J. Cannabinoid receptor gene (CNR1): association with i.v. drug use. *Mol Psychiatry* 1997; 2(2): 161-168.

[123] Comings DE, Muhleman D, Gysin R. Dopamine D2 receptor (DRD2) gene and susceptibility to posttraumatic stress disorder: a study and replication. *Biol Psychiatry* 1996; 40(5): 368-372.

[124] Conversi D, Orsini C, Cabib S. Distinct patterns of Fos expression induced by systemic amphetamine in the striatal complex of C57BL/6JICo and DBA/2JICo inbred strains of mice. *Brain Res* 2004; 1025(1-2): 59-66.

[125] Coolen MW, van Loo KM, van Bakel NN, Pulford DJ, Serneels L, De Strooper B, Ellenbroek BA, Cools AR, Martens GJ. Gene dosage effect on gamma-secretase component Aph-1b in a rat model for neurodevelopmental disorders. *Neuron* 2005; 45(4): 497-503.

[126] Cools AR, Brachten R, Heeren D, Willemen A, Ellenbroek B. Search after neurobiological profile of individual-specific features of wistar rats. *Brain Res Bull* 1990; 24(1): 49-69.

[127] Cools AR, Ellenbroek BA, Gingras MA, Engbersen A, Heeren D. Differences in vulnerability and susceptibility to dexamphetamine in Nijmegen high and low responders to novelty: a dose-effect analysis of spatio-temporal programming of behaviour. *Psychopharmacology (Berl)* 1997; 132(2): 181-187.

[128] Cornish JL, Duffy P, Kalivas PW. A role for nucleus accumbens glutamate transmission in the relapse to cocaine-seeking behavior. *Neuroscience* 1999; 93(4): 1359-1367.

[129] Cornish JL, Kalivas PW. Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. *J Neurosci* 2000; 20(15): 89RC.

[130] Cornish JL, Kalivas PW. Repeated cocaine administration into the rat ventral tegmental area produces behavioral sensitization to a systemic cocaine challenge. *Behav Brain Res* 2001; 126(1-2): 205-209.

[131] Covington HE, III, Miczek KA. Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". *Psychopharmacology (Berl)* 2001; 158(4): 388-398.

[132] Crabbe JC. Genetic contributions to addiction. *Annu Rev Psychol* 2002; 53(1): 435-462.

[133] Cubells JF, Kranzler HR, McCance-Katz E, Anderson GM, Malison RT, Price LH, Gelernter J. A haplotype at the DBH locus, associated with low plasma dopamine beta-hydroxylase activity, also associates with cocaine-induced paranoia. *Mol Psychiatry* 2000; 5(1): 56-63.

[134] Cunningham ET, Jr., Bohn MC, Sawchenko PE. Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J Comp Neurol* 1990; 292(4): 651-667.

[135] Cunningham ET, Jr., Sawchenko PE. Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J Comp Neurol* 1988; 274(1): 60-76.

[136] Czyrak A, Wedzony K, Michalska B, Fijal K, Dziedzicka-Wasylewska M, Mackowiak M. The corticosterone synthesis inhibitor metyrapone decreases dopamine D1 receptors in the rat brain. *Neuroscience* 1997; 79(2): 489-495.

[137] Dahl JP, Kampman KM, Oslin DW, Weller AE, Lohoff FW, Ferraro TN, O'Brien CP, Berrettini WH. Association of a polymorphism in the Homer1 gene with cocaine dependence in an African American population. *Psychiat Genet* 2005; 15(4): 277-283.

[138] Dahlstroem A, Fuxe K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand* 1964; 62(suppl 232): 1-55.

[139] Dal Toso R, Sommer B, Ewert M, Herb A, Pritchett DB, Bach A, Shivers BD, Seeburg PH. The dopamine D2 receptor: two molecular forms generated by alternative splicing. *EMBO J* 1989; 8(13): 4025-4034.

[140] Damianopoulos EN, Carey RJ. Evidence for N-methyl-aspartate receptor mediation of cocaine induced corticosterone release and cocaine conditioned stimulant effects. *Behav Brain Res* 1995; 68(2): 219-228.

[141] Darracq L, Blanc G, Glowinski J, Tassin JP. Importance of the noradrenaline-dopamine coupling in the locomotor activating effects of D-amphetamine. *J Neurosci* 1998; 18(7): 2729-2739.

[142] Das G. Cocaine and the cardiovascular system. *Can J Cardiol* 1990; 6(9): 411-415.

[143] Davis WM, Smith SG, Khalsa JH. Noradrenergic role in the self-administration of morphine or amphetamine. *Pharmacol Biochem Behav* 1975; 3(3): 477-484.

[144] De Jong IEM, De Kloet ER. Glucocorticoids and vulnerability to psychostimulant drugs: toward substrate and mechanism. *Ann N Y Acad Sci* 2004; 1018: 192-198.

[145] de Jong IEM, Oitzl MS, de Kloet ER. Adrenalectomy prevents behavioural sensitisation of mice to cocaine in a genotype-dependent manner. *Behav Brain Res* 2007; 177(2): 329-339.

[146] De Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 2005; 6(6): 463-475.

[147] De Kloet ER, Oitzl MS, Joels M. Functional implications of brain corticosteroid receptor diversity. *Cell Mol Neurobiol* 1993; 13(4): 433-455.

[148] De Kloet ER, Reul JM. Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems. *Psychoneuroendocrinology* 1987; 12(2): 83-105.

[149] De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 1998; 19(3): 269-301.

[150] De Souza EB. Corticotropin-releasing factor receptors: physiology, pharmacology, biochemistry and role in central nervous system and immune disorders. *Psychoneuroendocrinology* 1995; 20(8): 789-819.

[151] De Vries TJ, Cools AR, Shippenberg TS. Infusion of a D-1 receptor agonist into the nucleus accumbens enhances cocaine-induced behavioural sensitization. *Neuroreport* 1998; 9(8): 1763-1768.

[152] De Vries TJ, Schoffelmeer AN, Binnekade R, Raaso H, Vanderschuren LJ. Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. *Neuropsychopharmacology* 2002; 26(1): 18-26.

[153] De Vries TJ, Schoffelmeer AN, Tjon GH, Nestby P, Mulder AH, Vanderschuren LJ. Mifepristone prevents the expression of long-term behavioural sensitization to amphetamine. *Eur J Pharmacol* 1996; 307(2): R3-R4.

[154] de Vries TJ, Schoffelmeer ANM, Binnekade R, Mulder AH, Vanderschuren LJMJ. Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. *Eur J Neurosci* 1998; 10(11): 3565-3571.

[155] de Vry J, Donselaar I, Van Ree JM. Effects of desglycinamide9, (Arg8) vasopressin and vasopressin antiserum on the acquisition of intravenous cocaine self-administration in the rat. *Life Sci* 1988; 42(26): 2709-2715.

[156] de Waele JP, Gianoulakis C. Enhanced activity of the brain beta-endorphin system by free-choice ethanol drinking in C57BL/6 but not DBA/2 mice. *Eur J Pharmacol* 1994; 258(1-2): 119-129.

[157] De Waele JP, Gianoulakis C. Characterization of the mu and delta opioid receptors in the brain

of the C57BL/6 and DBA/2 mice, selected for their differences in voluntary ethanol consumption. *Alcohol Clin Exp Res* 1997; 21(4): 754-762.

[158] De Wit H, Uhlenhuth EH, Johanson CE. Individual differences in the reinforcing and subjective effects of amphetamine and diazepam. *Drug Alcohol Depend* 1986; 16(4): 341-360.

[159] Dearry A, Gingrich JA, Falardeau P, Fremeau RT, Jr., Bates MD, Caron MG. Molecular cloning and expression of the gene for a human D1 dopamine receptor. *Nature* 1990; 347(6288): 72-76.

[160] DeBattista C, Belanoff J, Glass S, Khan A, Horne RL, Blasey C, Carpenter LL, Alva G. Mifepristone versus placebo in the treatment of psychosis in patients with psychotic major depression. *Biol Psychiatry* 2006; 60(12): 1343-1349.

[161] Der-Avakian A, Bland ST, Schmid MJ, Watkins LR, Spencer RL, Maier SF. The role of glucocorticoids in the uncontrollable stress-induced potentiation of nucleus accumbens shell dopamine and conditioned place preference responses to morphine. *Psychoneuroendocrinology* 2006; 31(5): 653-663.

[162] Deroche V, Le Moal M, Piazza PV. Cocaine self-administration increases the incentive motivational properties of the drug in rats. *Eur J Neurosci* 1999; 11(8): 2731-2736.

[163] Deroche V, Marinelli M, Le Moal M, Piazza PV. Glucocorticoids and behavioral effects of psychostimulants. II: cocaine intravenous self-administration and reinstatement depend on glucocorticoid levels. *J Pharmacol Exp Ther* 1997; 281(3): 1401-1407.

[164] Deroche V, Marinelli M, Maccari S, Le Moal M, Simon H, Piazza PV. Stress-induced sensitization and glucocorticoids. I. Sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. *J Neurosci* 1995; 15(11): 7181-7188.

[165] Deroche V, Piazza PV, Maccari S, Le Moal M, Simon H. Repeated corticosterone administration sensitizes the locomotor response to amphetamine. *Brain Res* 1992; 584(1-2): 309-313.

[166] Deroche V, Piazza PV, Casolini P, Le Moal M, Simon H. Sensitization to the psychomotor effects of amphetamine and morphine induced by food restriction depends on corticosterone secretion. *Brain Res* 1993; 611(2): 352-356.

[167] Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. *Science* 2004; 305(5686): 1014-1017.

[168] Deroche-Gamonet V, Sillaber I, Aouizerate B, Izawa R, Jaber M, Ghozland S, Kellendonk C, Le Moal M, Spanagel R, Schutz G, Tronche F, Piazza PV. The glucocorticoid receptor as a potential target to reduce cocaine abuse. *J Neurosci* 2003; 23(11): 4785-4790.

[169] DeVries AC, Taymans SE, Sundstrom JM, Pert A. Conditioned release of corticosterone by contextual stimuli associated with cocaine is mediated by corticotropin-releasing factor. *Brain Res* 1998; 786(1-2): 39-46.

[170] Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A* 1988; 85(14): 5274-5278.

[171] Di Ciano P, Coury A, Depoortere RY, Egilmez Y, Lane JD, Emmett-Oglesby MW, Lepiane FG, Phillips AG, Blaha CD. Comparison of changes in extracellular dopamine concentrations in the

nucleus accumbens during intravenous self-administration of cocaine or d-amphetamine. *Behav Pharmacol* 1995; 6(4): 311-322.

[172] Di Ciano P, Everitt BJ. Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. *Neuropsychopharmacology* 2001; 25(3): 341-360.

[173] Di Ciano P, Everitt BJ. The GABA(B) receptor agonist baclofen attenuates cocaine- and heroin-seeking behavior by rats. *Neuropsychopharmacology* 2003; 28(3): 510-518.

[174] Di Paolo T, Rouillard C, Morissette M, Levesque D, Bedard PJ. Endocrine and neurochemical actions of cocaine. *Can J Physiol Pharmacol* 1989; 67(9): 1177-1181.

[175] Di S, Malcher-Lopes R, Marcheselli VL, Bazan NG, Tasker JG. Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and gamma-aminobutyric acid inputs to hypothalamic magnocellular neurons. *Endocrinology* 2005; 146(10): 4292-4301.

[176] Di S, Malcher-Lopes R, Halmos KC, Tasker JG. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *J Neurosci* 2003; 23(12): 4850-4857.

[177] Diaz J, Levesque D, Griffon N, Lammers CH, Martres MP, Sokoloff P, Schwartz JC. Opposing roles for dopamine D2 and D3 receptors on neurotensin mRNA expression in nucleus accumbens. *Eur J Neurosci* 1994; 6(8): 1384-1387.

[178] Diaz J, Levesque D, Lammers CH, Griffon N, Martres M-P, Schwartz J-C, Sokoloff P. Phenotypical characterization of neurons expressing the dopamine D3 receptor in the rat brain. *Neuroscience* 1995; 65(3): 731-745.

[179] Dietz DM, Tapocik J, Gaval-Cruz M, Kabbaj M. Dopamine transporter, but not tyrosine hydroxylase, may be implicated in determining individual differences in behavioral sensitization to amphetamine. *Physiol Behav* 2005; 86(3): 347-355.

[180] Doherty MD, Gratton A. Effects of medial prefrontal cortical injections of GABA receptor agonists and antagonists on the local and nucleus accumbens dopamine responses to stress. *Synapse* 1999; 32(4): 288-300.

[181] Douglass J, McKinzie AA, Couceyro P. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. *J Neurosci* 1995; 15(3 Pt 2): 2471-2481.

[182] Drouin C, Blanc G, Villegier AS, Glowinski J, Tassin JP. Critical role of alpha1-adrenergic receptors in acute and sensitized locomotor effects of D-amphetamine, cocaine, and GBR 12783: influence of preexposure conditions and pharmacological characteristics. *Synapse* 2002; 43(1): 51-61.

[183] Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S, Tassin JP. Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. *J Neurosci* 2002; 22(7): 2873-2884.

[184] Drouin J, Sun YL, Chamberland M, Gauthier Y, De Lean A, Nemer M, Schmidt TJ. Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. *The EMBO Journal* 1993; 12(1): 145-156.

[185] Du C, Yu M, Volkow ND, Koretsky AP, Fowler JS, Benveniste H. Cocaine increases the intracellular calcium concentration in brain independently of its cerebrovascular effects. *J Neurosci* 2006; 26(45): 11522-11531.

[186] Dunn AJ. Stress-related changes in cerebral catecholamine and indoleamine metabolism: lack of effect of adrenalectomy and corticosterone. *J Neurochem* 1988; 51(2): 406-412.

[187] Dunn JM, Inderwies BR, Licata SC, Pierce RC. Repeated administration of AMPA or a metabotropic glutamate receptor agonist into the rat ventral tegmental area augments the subsequent behavioral hyperactivity induced by cocaine. *Psychopharmacology (Berl)* 2005; 179(1): 172-180.

[188] Ebstein RP, Novick O, Umansky R, Priel B, Osher Y, Blaine D, Bennett ER, Nemanov L, Katz M, Belmaker RH. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. *Nat Genet* 1996; 12(1): 78-80.

[189] Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, De Kloet ER, Monder C. Localisation of 11 beta-hydroxysteroid dehydrogenase - tissue specific protector of the mineralocorticoid receptor. *Lancet* 1988; 2(8618): 986-989.

[190] Ellenbroek BA. Animal models in the genomic era: possibilities and limitations with special emphasis on schizophrenia. *Behav Pharmacol* 2003; 14(5-6): 409-417.

[191] Ellenbroek BA, van der Kam EL, van der Elst MCJ, Cools AR. Individual differences in drug dependence in rats: the role of genetic factors and life events. *Eur J Pharmacol* 2005; 526(1-3): 251-258.

[192] Elman I, Lukas SE, Karlsgodt KH, Gasic GP, Breiter HC. Acute cortisol administration triggers craving in individuals with cocaine dependence. *Psychopharmacol Bull* 2003; 37(3): 84-89.

[193] Erb S, Hitchcott PK, Rajabi H, Mueller D, Shaham Y, Stewart J. Alpha-2 adrenergic receptor agonists block stress-induced reinstatement of cocaine seeking. *Neuropsychopharmacology* 2000; 23(2): 138-150.

[194] Erb S, Shaham Y, Stewart J. Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. *Psychopharmacology (Berl)* 1996; 128(4): 408-412.

[195] Erb S, Shaham Y, Stewart J. The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. *J Neurosci* 1998; 18(14): 5529-5536.

[196] Erb S, Brown ZJ. A role for corticotropin-releasing factor in the long-term expression of behavioral sensitization to cocaine. *Behav Brain Res* 2006; 172(2): 360-364.

[197] Erb S, Stewart J. A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. *J Neurosci* 1999; 19(20): 35RC.

[198] Erblich J, Lerman C, Self DW, Diaz GA, Bovbjerg DH. Stress-induced cigarette craving: effects of the DRD2 TaqI RFLP and SLC6A3 VNTR polymorphisms. *Pharmacogenomics J* 2004; 4(2): 102-109.

[199] Erwin VG, Womer DE, Campbell AD, Jones BC. Pharmacogenetics of cocaine: II. Mesocorticolimbic and striatal dopamine and cocaine receptors in C57BL and DBA mice. *Pharmacogenetics* 1993; 3(4): 189-196.

[200] Escorihuela RM, Fernandez-Teruel A, Gil L, Aguilar R, Tobena A, Driscoll P. Inbred Roman

high- and low-avoidance rats: differences in anxiety, novelty-seeking, and shuttlebox behaviors. *Physiol Behav* 1999; 67(1): 19-26.

[201] Everitt BJ, Cardinal RN, Parkinson JA, Robbins TW. Appetitive behavior: impact of amygdala-dependent mechanisms of emotional learning. *Ann N Y Acad Sci* 2003; 985(1): 233-250.

[202] Everitt BJ, Wolf ME. Psychomotor stimulant addiction: a neural systems perspective. *J Neurosci* 2002; 22(9): 3312-3320.

[203] Exner M, Clark D. Behaviour in the novel environment predicts responsiveness to d-amphetamine in the rat: a multivariate approach. *Behav Pharmacol* 1993; 4(1): 47-56.

[204] Fadda P, Scherma M, Fresu A, Collu M, Fratta W. Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. *Synapse* 2003; 50(1): 1-6.

[205] Falck RS, Wang J, Siegal HA, Carlson RG. The prevalence of psychiatric disorder among a community sample of crack cocaine users: an exploratory study with practical implications. *J Nerv Ment Dis* 2004; 192(7): 503-507.

[206] Feldman S, Weidenfeld J. Involvement of amygdalar alpha adrenoceptors in hypothalamo-pituitary-adrenocortical responses. *Neuroreport* 1996; 7(18): 3055-3057.

[207] Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, Koss MP, Marks JS. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: the adverse childhood experiences (ACE) study. *Am J Prev Med* 1998; 14(4): 245-258.

[208] Fierens E. Archeological and artistic sources of coca consumption in pre-hispanic America. *Verh K Acad Geneeskd Belg* 1991; 53(5): 463-485.

[209] Filip M, Frankowska M, Zaniewska M, Golda A, Przegalinski E. The serotonergic system and its role in cocaine addiction. *Pharmacol Rep* 2005; 57(6): 685-700.

[210] Filip M, Siwanowicz J. Implication of the nucleus accumbens shell, but not core, in the acute and sensitizing effects of cocaine in rats. *Pol J Pharmacol* 2001; 53(5): 459-466.

[211] Filip M, Thomas ML, Cunningham KA. Dopamine D5 receptors in nucleus accumbens contribute to the detection of cocaine in rats. *J Neurosci* 2000; 20(19): RC98.

[212] Fink JS, Reis DJ. Genetic variations in midbrain dopamine cell number: parallel with differences in responses to dopaminergic agonists and in naturalistic behaviors mediated by central dopaminergic systems. *Brain Res* 1981; 222(2): 335-349.

[213] Fletcher PJ, Korth KM, Chambers JW. Depletion of brain serotonin following intra-raphe injections of 5,7-dihydroxytryptamine does not alter d-amphetamine self-administration across different schedule and access conditions. *Psychopharmacology (Berl)* 1999; 146(2): 185-193.

[214] Flores G, Wood GK, Barbeau D, Quirion R, Srivastava LK. Lewis and Fischer rats: a comparison of dopamine transporter and receptors levels. *Brain Res* 1998; 814(1-2): 34-40.

[215] Floresco SB, Yang CR, Phillips AG, Blaha CD. Basolateral amygdala stimulation evokes glutamate receptor-dependent dopamine efflux in the nucleus accumbens of the anaesthetized rat. *Eur J Neurosci* 1998; 10(4): 1241-1251.

[216] Floresco SB, Todd CL, Grace AA. Glutamatergic afferents from the hippocampus to the nucleus

accumbens regulate activity of ventral tegmental area dopamine neurons. *J Neurosci* 2001; 21(13): 4915-4922.

[217] Follenius M, Simon C, Brandenberger G, Lenzi P. Ultradian plasma corticotropin and cortisol rhythms: time-series analyses. *J Endocrinol Invest* 1987; 10(3): 261-266.

[218] Forray MI, Gysling K. Role of noradrenergic projections to the bed nucleus of the stria terminalis in the regulation of the hypothalamic-pituitary-adrenal axis. *Brain Res Brain Res Rev* 2004; 47(1-3): 145-160.

[219] Francis DD, Meaney MJ. Maternal care and the development of stress responses. *Curr Opin Neurobiol* 1999; 9(1): 128-134.

[220] Franklin KB, Paxinos G. The mouse brain in stereotaxic coordinates. Academic Press Inc, San Diego: 1997.

[221] Freeman WM, Brebner K, Lynch WJ, Robertson DJ, Roberts DC, Vrana KE. Cocaine-responsive gene expression changes in rat hippocampus. *Neuroscience* 2001; 108(3): 371-380.

[222] Fremeau RT, Jr., Duncan GE, Fornaretto M, Dearry A, Gingrich JA. Localization of D1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. *Proc Natl Acad Sci U S A* 1991; 88(9): 3772-3776.

[223] Funder JW, Pearce PT, Smith R, Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 1988; 242(4878): 583-585.

[224] Fuxe K, Hokfelt T. Further evidence for the existence of tubero-infundibular dopamine neurons. *Acta Physiol Scand* 1966; 66(1): 245-246.

[225] Fuxe K, Wikstrom AC, Okret S, Agnati LF, Harfstrand A, Yu ZY, Granholm L, Zoli M, Vale W, Gustafsson. Mapping of glucocorticoid receptor immunoreactive neurons in the rat tel- and diencephalon using a monoclonal antibody against rat liver glucocorticoid receptor. *Endocrinology* 1985; 117(5): 1803-1812.

[226] Gaillet S, Alonso G, Le Borgne R, Barbanel G, Malaval F, Assenmacher I, Szafarczyk A. Effects of discrete lesions in the ventral noradrenergic ascending bundle on the corticotropic stress response depend on the site of the lesion and on the plasma levels of adrenal steroids. *Neuroendocrinology* 1993; 58(4): 408-419.

[227] Gal K, Gyertyan I. Dopamine D3 as well as D2 receptor ligands attenuate the cue-induced cocaine-seeking in a relapse model in rats. *Drug Alcohol Depend* 2006; 81(1): 63-70.

[228] Gamaro GD, Denardin JD, Jr., Michalowski MB, Catelli D, Correa JB, Xavier MH, Dalmaz C. Epinephrine effects on memory are not dependent on hepatic glucose release. *Neurobiol Learn Mem* 1997; 68(3): 221-229.

[229] Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA. Cue-Induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. *Am J Psychiat* 2000; 157(11): 1789-1798.

[230] Gelernter J, Kranzler HR, Satel SL, Rao PA. Genetic association between dopamine transporter protein alleles and cocaine-induced paranoia. *Neuropsychopharmacology* 1994; 11(3): 195-200.

[231] Gelernter J, Kranzler H, Satel SL. No association between D2 dopamine receptor (DRD2) alleles

or haplotypes and cocaine dependence or severity of cocaine dependence in European- and African-Americans. *Biol Psychiatry* 1999; 45(3): 340-345.

[232] George FR, Goldberg SR. Genetic approaches to the analysis of addiction processes. *Trends Pharmacol Sci* 1989; 10(2): 78-83.

[233] Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Jr., Sibley DR. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 1990; 250(4986): 1429-1432.

[234] Gerrits MA, Petromilli P, Westenberg HG, Di Chiara G, Van Ree JM. Decrease in basal dopamine levels in the nucleus accumbens shell during daily drug-seeking behaviour in rats. *Brain Res* 2002; 924(2): 141-150.

[235] Gerrits MAFM, Van Ree JM. Effect of nucleus accumbens dopamine depletion on motivational aspects involved in initiation of cocaine and heroin self-administration in rats. *Brain Res* 1996; 713(1-2): 114-124.

[236] Giorgi O, Corda MG, Carboni G, Frau V, Valentini V, Di Chiara G. Effects of cocaine and morphine in rats from two psychogenetically selected lines: a behavioral and brain dialysis study. *Behav Genet* 1997; 27(6): 537-546.

[237] Giorgi O, Piras G, Lecca D, Corda MG. Behavioural effects of acute and repeated cocaine treatments: a comparative study in sensitisation-prone RHA rats and their sensitisation-resistant RLA counterparts. *Psychopharmacology (Berl)* 2005; 180(3): 530-538.

[238] Giros B, Sokoloff P, Martres MP, Riou JF, Emorine LJ, Schwartz JC. Alternative splicing directs the expression of two D2 dopamine receptor isoforms. *Nature* 1989; 342(6252): 923-926.

[239] Glenthoj BY, Hemmingsen R. Dopaminergic sensitization: implications for the pathogenesis of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 1997; 21(1): 23-46.

[240] Goeders NE. The HPA axis and cocaine reinforcement. *Psychoneuroendocrinology* 2002; 27(1-2): 13-33.

[241] Goeders NE. Stress and cocaine addiction. *J Pharmacol Exp Ther* 2002; 301(3): 785-789.

[242] Goeders NE, Dworkin SI, Smith JE. Neuropharmacological assessment of cocaine self-administration into the medial prefrontal cortex. *Pharmacol Biochem Behav* 1986; 24(5): 1429-1440.

[243] Goeders NE, Guerin GF. Pretreatment with corticosterone or dexamethasone fails to affect ongoing cocaine self-administration in rats. *Neuroscience abstracts* 2006; 25: 1872.

[244] Goeders NE, Guerin GF. Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. *Psychopharmacology (Berl)* 1994; 114(1): 63-70.

[245] Goeders NE, Guerin GF. Effects of surgical and pharmacological adrenalectomy on the initiation and maintenance of intravenous cocaine self-administration in rats. *Brain Res* 1996; 722(1-2): 145-152.

[246] Goeders NE, Peltier RL, Guerin GF. Ketoconazole reduces low dose cocaine self-administration in rats. *Drug Alcohol Depend* 1998; 53(1): 67-77.

[247] Goeders NE, Smith JE. Cortical dopaminergic involvement in cocaine reinforcement. *Science* 1983; 221(4612): 773-775.

[248] Goeders NE, Smith JE. Reinforcing properties of cocaine in the medical prefrontal cortex: primary

action on presynaptic dopaminergic terminals. *Pharmacol Biochem Behav* 1986; 25(1): 191-199.

[249] Goeders NE. The impact of stress on addiction. *Eur Neuropsychopharmacol* 2003; 13(6): 435-441.

[250] Goeders NE, Guerin GF. Effects of the CRH receptor antagonist CP-154,526 on intravenous cocaine self-administration in rats. *Neuropsychopharmacology* 2000; 23(5): 577-586.

[251] Gold PE, van Buskirk R. Posttraining brain norepinephrine concentrations: correlation with retention performance of avoidance training and with peripheral epinephrine modulation of memory processing. *Behav Biol* 1978; 23(4): 509-520.

[252] Gold PE, Van Buskirk RB. Facilitation of time-dependent memory processes with posttrial epinephrine injections. *Behav Biol* 1975; 13(2): 145-153.

[253] Gold PE, Vogt J, Hall JL. Glucose effects on memory: behavioral and pharmacological characteristics. *Behav Neural Biol* 1986; 46(2): 145-155.

[254] Goldman D, Oroszi G, Ducci F. The genetics of addictions: uncovering the genes. *Nat Rev Genet* 2005; 6(7): 521-532.

[255] Gomez F, de Kloet ER, Armario A. Glucocorticoid negative feedback on the HPA axis in five inbred rat strains. *AJP - Reg Integr Comp Physiol* 1998; 274(2): R420-R427.

[256] Gorelova N, Yang CR. The course of neural projection from the prefrontal cortex to the nucleus accumbens in the rat. *Neuroscience* 1997; 76(3): 689-706.

[257] Grahame NJ, Cunningham CL. Genetic differences in intravenous cocaine self-administration between C57BL/6J and DBA/2J mice. *Psychopharmacology (Berl)* 1995; 122(3): 281-291.

[258] Grandy DK, Zhang Y, Bouvier C, Zhou Q, Johnson RA, Allen L, Buck K, Bunzow JR, Salon J, Civelli O. Multiple human D5 dopamine receptor genes: a functional receptor and two pseudogenes. *Proc Natl Acad Sci U S A* 1991; 88(20): 9175-9179.

[259] Graybiel AM, Aosaki T, Flaherty AW, Kimura M. The basal ganglia and adaptive motor control. *Science* 1994; 265(5180): 1826-1831.

[260] Graybiel AM, Hirsch EC, Agid Y. The nigrostriatal system in Parkinson's disease. *Adv Neurol* 1990; 53: 17-29.

[261] Greenberg DA, Prichard DC, Snyder SH. Alpha-noradrenergic receptor binding in mammalian brain: differential labeling of agonist and antagonist states. *Life Sci* 1976; 19(1): 69-76.

[262] Gritti A, Bove D, Di Sarno AM, D'Addio AA, Chiapparo S, Bove RM. Stereotyped movements in a group of autistic children. *Funct Neurol* 2003; 18(2): 89-94.

[263] Groenewegen HJ, Russchen FT. Organization of the efferent projections of the nucleus accumbens to pallidal, hypothalamic, and mesencephalic structures: a tracing and immunohistochemical study in the cat. *J Comp Neurol* 1984; 223(3): 347-367.

[264] Groenewegen HJ, Vermeulen-Van der Zee E, te KA, Witter MP. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. *Neuroscience* 1987; 23(1): 103-120.

[265] Grootendorst J, De Kloet ER, Vossen C, Dalm S, Oitzl MS. Repeated exposure to rats has persis-

tent genotype-dependent effects on learning and locomotor activity of apolipoprotein E knockout and C57Bl/6 mice. *Behav Brain Res* 2001; 125(1-2): 249-259.

[266] Grootendorst J, Kempes MM, Lucassen PJ, Dalm S, De Kloet ER, Oitzl MS. Differential effect of corticosterone on spatial learning abilities in apolipoprotein E knockout and C57BL/6J mice. *Brain Res* 2002; 953(1-2): 281-285.

[267] Guindalini C, Howard M, Haddley K, Laranjeira R, Collier D, Ammar N, Craig I, O'Gara C, Bubb VJ, Greenwood T, Kelsoe J, Asherson P, Murray RM, Castelo A, Quinn JP, Vallada H, Breen G. A dopamine transporter gene functional variant associated with cocaine abuse in a Brazilian sample. *Proc Natl Acad Sci U S A* 2006; 103(12): 4552-4557.

[268] Gunnarsdottir ED, Pingitore RA, Spring BJ, Konopka LM, Crayton JW, Milo T, Shirazi P. Individual differences among cocaine users. *Addict Behav* 2000; 25(5): 641-652.

[269] Gurkovskaya O, Goeders NE. Effects of CP-154,526 on responding during extinction from cocaine self-administration in rats. *Eur J Pharmacol* 2001; 432(1): 53-56.

[270] Haber SN. Neurotransmitters in the human and nonhuman primate basal ganglia. *Hum Neurobiol* 1986; 5(3): 159-168.

[271] Haber SN, Fudge JL, McFarland NR. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J Neurosci* 2000; 20(6): 2369-2382.

[272] Haile CN, GrandPre T, Kosten TA. Chronic unpredictable stress, but not chronic predictable stress, enhances the sensitivity to the behavioral effects of cocaine in rats. *Psychopharmacology (Berl)* 2001; 154(2): 213-220.

[273] Hain HS, Crabbe JC, Bergeson SE, Belknap JK. Cocaine-induced seizure thresholds: quantitative trait loci detection and mapping in two populations derived from the C57BL/6 and DBA/2 mouse strains. *J Pharmacol Exp Ther* 2000; 293(1): 180-187.

[274] Hall RC, Popkin MK, Stickney SK, Gardner ER. Presentation of the steroid psychoses. *J Nerv Ment Dis* 1979; 167(4): 229-236.

[275] Halpain S, McEwen BS. Corticosterone decreases 3H-glutamate binding in rat hippocampal formation. *Neuroendocrinology* 1988; 48(3): 235-241.

[276] Hammond GL. Molecular properties of corticosteroid binding globulin and the sex-steroid binding proteins. *Endocr Rev* 1990; 11(1): 65-79.

[277] Haney M, Maccari S, Le Moal M, Simon H, Piazza PV. Social stress increases the acquisition of cocaine self-administration in male and female rats. *Brain Res* 1995; 698(1-2): 46-52.

[278] Haney M, Hart CL, Foltin RW. Effects of baclofen on cocaine self-administration: opioid- and nonopioid-dependent volunteers. *Neuropsychopharmacology* 2006; 31(8): 1814-1821.

[279] Harfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikstrom AC, Okret S, Yu ZY, Goldstein M, Steinbusch H. Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. *Proc Natl Acad Sci U S A* 1986; 83(24): 9779-9783.

[280] Harrison PA, Fulkerson JA, Beebe TJ. Multiple substance use among adolescent physical and sexual abuse victims. *Child Abuse Neglect* 1997; 21(6): 529-539.

[281] Heesch CM, Negus BH, Keffer JH, Snyder RW, Risser RC, Eichhorn EJ. Effects of cocaine on cortisol secretion in humans. *Am J Med Sci* 1995; 310(2): 61-64.

[282] Heim C, Owens MJ, Plotsky PM, Nemeroff CB. Persistent changes in corticotropin-releasing factor systems due to early life stress: relationship to the pathophysiology of major depression and post-traumatic stress disorder. *Psychopharmacol Bull* 1997; 33(2): 185-192.

[283] Hemby SE, Horman B, Tang W. Differential regulation of ionotropic glutamate receptor subunits following cocaine self-administration. *Brain Res* 2005; 1064(1-2): 75-82.

[284] Herman JP, Stinus L, Le Moal M. Repeated stress increases locomotor response to amphetamine. *Psychopharmacology (Berl)* 1984; 84(3): 431-435.

[285] Hermann G, Tovar CA, Beck FM, Sheridan JF. Kinetics of glucocorticoid response to restraint stress and/or experimental influenza viral infection in two inbred strains of mice. *J Neuroimmunol* 1994; 49(1-2): 25-33.

[286] Holmes A, le Guisquet AM, Vogel E, Millstein RA, Leman S, Belzung C. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. *Neurosci Biobehav Rev* 2005; 29(8): 1335-1346.

[287] Homberg JR, van den AM, Raaso HS, Wardeh G, Binnekade R, Schoffelmeer AN, De Vries TJ. Enhanced motivation to self-administer cocaine is predicted by self- grooming behaviour and relates to dopamine release in the rat medial prefrontal cortex and amygdala. *Eur J Neurosci* 2002; 15(9): 1542-1550.

[288] Hooks MS, Jones GH, Hemby SE, Justice JB, Jr. Environmental and pharmacological sensitization: effects of repeated administration of systemic or intra-nucleus accumbens cocaine. *Psychopharmacology (Berl)* 1993; 111(1): 109-116.

[289] Hooks MS, Jones GH, Liem BJ, Justice J. Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intracranial amphetamine infusions. *Pharmacol Biochem Behav* 1992; 43(3): 815-823.

[290] Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB, Jr. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. *Synapse* 1991; 9(2): 121-128.

[291] Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB, Jr. Individual differences in locomotor activity and sensitization. *Pharmacol Biochem Behav* 1991; 38(2): 467-470.

[292] Hooks MS, Juncos JL, Justice JB, Jr., Meiergerd SM, Povlock SL, Schenk JO, Kalivas PW. Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. *J Neurosci* 1994; 14(10): 6144-6152.

[293] Hooks MS, Sorg BA, Kalivas PW. The relationship between mRNA levels and the locomotor response to novelty. *Brain Res* 1994; 663(2): 312-316.

[294] Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS, Nestler EJ. Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* 1994; 13(5): 1235-1244.

[295] Horger BA, Giles MK, Schenk S. Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. *Psychopharmacologia* 1992; 107(2-3): 271-276.

[296] Horger BA, Shelton K, Schenk S. Preexposure sensitizes rats to the rewarding effects of cocaine. *Pharmacol Biochem Behav* 1990; 37(4): 707-711.

[297] Hosoi T, Okuma Y, Nomura Y. Electrical stimulation of afferent vagus nerve induces IL-1beta

expression in the brain and activates HPA axis. *AJP - Reg Integr Comp Physiol* 2000; 279(1): R141-R147.

[298] Hotsenpiller G, Giorgetti M, Wolf ME. Alterations in behaviour and glutamate transmission following presentation of stimuli previously associated with cocaine exposure. *Eur J Neurosci* 2001; 14(11): 1843-1855.

[299] Hubner CB, Moreton JE. Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. *Psychopharmacologia* 1991; 105(2): 151-156.

[300] Hummel KP. Accessory adrenal cortical nodules in the mouse. *Anat Rec* 1958; 132(3): 281-295.

[301] Hurd YL, McGregor A, Ponten M. In vivo amygdala dopamine levels modulate cocaine self-administration behaviour in the rat: D1 dopamine receptor involvement. *Eur J Neurosci* 1997; 9(12): 2541-2548.

[302] Ihalainen JA, Tanila H. In vivo regulation of dopamine and noradrenaline release by alpha2A-adrenoceptors in the mouse nucleus accumbens. *J Neurochem* 2004; 91(1): 49-56.

[303] Imperato A, Angelucci L, Casolini P, Zocchi A, Puglisi-Allegra S. Repeated stressful experiences differently affect limbic dopamine release during and following stress. *Brain Res* 1992; 577(2): 194-199.

[304] Imperato A, Puglisi-Allegra S, Grazia SM, Casolini P, Bacchi S, Angelucci L. Cortical and limbic dopamine and acetylcholine release as neurochemical correlates of emotional arousal in both aversive and non-aversive environmental changes. *Neurochem Int* 1992; 20(suppl): 265S-270S.

[305] Imperato A, Cabib S, Puglisi-Allegra S. Repeated stressful experiences differently affect the time-dependent responses of the mesolimbic dopamine system to the stressor. *Brain Res* 1993; 601(1-2): 333-336.

[306] Ishihara T, Akiyama K, Kashihara K. Enhanced AP-1 binding in brain induced by D1 and D2 agonists in methamphetamine-sensitized rats. *Neuroreport* 1998; 9(17): 3913-3917.

[307] Ito R, Dalley JW, Robbins TW, Everitt BJ. Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. *J Neurosci* 2002; 22(14): 6247-6253.

[308] Izawa R, Jaber M, Deroche-Gamonet V, Sillaber I, Kellendonk C, Le Moal M, Tronche F, Piazza PV. Gene expression regulation following behavioral sensitization to cocaine in transgenic mice lacking the glucocorticoid receptor in the brain. *Neuroscience* 2006; 137(3): 915-924.

[309] Jaber M, Robinson SW, Missale C, Caron MG. Dopamine receptors and brain function. *Neuropharmacology* 1997; 35(11): 1503-1519.

[310] Jackson A, Mead AN, Rocha BA, Stephens DN. AMPA receptors and motivation for drug: effect of the selective antagonist NBQX on behavioural sensitization and on self-administration in mice. *Behav Pharmacol* 1998; 9(5-6): 457-467.

[311] Jackson DM, Westlind-Danielsson A. Dopamine receptors: molecular biology, biochemistry and behavioural aspects. *Pharmacol Ther* 1994; 64(2): 291-370.

[312] Jacobsen LK, Staley JK, Malison RT, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB. Elevated central

serotonin transporter binding availability in acutely abstinent cocaine-dependent patients. *Am J Psychiatry* 2000; 157(7): 1134-1140.

[313] Jamensky NT, Gianoulakis C. Content of dynorphins and kappa-opioid receptors in distinct brain regions of C57BL/6 and DBA/2 mice. *Alcohol Clin Exp Res* 1997; 21(8): 1455-1464.

[314] Janowsky A, Mah C, Johnson RA, Cunningham CL, Phillips TJ, Crabbe JC, Eshleman AJ, Belknap JK. Mapping genes that regulate density of dopamine transporters and correlated behaviors in recombinant inbred mice. *J Pharmacol Exp Ther* 2001; 298(2): 634-643.

[315] Jansen ASP, Schmidt ED, Voorn P, Tilders FJH. Substance induced plasticity in noradrenergic innervation of the paraventricular hypothalamic nucleus. *Eur J Neurosci* 2003; 17(2): 298-306.

[316] Jentsch JD, Taylor JR. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl)* 1999; 146(4): 373-390.

[317] Joels M, De Kloet ER. Control of neuronal excitability by corticosteroid hormones. *Trends Neurosci* 1992; 15(1): 25-30.

[318] Joels M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ. Learning under stress: how does it work? *Trends Cogn Sci* 2006; 10(4): 152-158.

[319] Johnson LR, Farb C, Morrison JH, McEwen BS, LeDoux JE. Localization of glucocorticoid receptors at postsynaptic membranes in the lateral amygdala. *Neuroscience* 2005; 136(1): 289-299.

[320] Jones BC, Hou X, Cook MN. Effect of exposure to novelty on brain monoamines in C57BL/6 and DBA/2 mice. *Physiol Behav* 1996; 59(2): 361-367.

[321] Jones BC, Sarrieau A, Reed CL, Azar MR, Mormede P. Contribution of sex and genetics to neuroendocrine adaptation to stress in mice. *Psychoneuroendocrinology* 1998; 23(5): 505-517.

[322] Jones GH, Mittleman G, Robbins TW. Attenuation of amphetamine-stereotypy by mesostriatal dopamine depletion enhances plasma corticosterone: implications for stereotypy as a coping response. *Behav Neural Biol* 1989; 51(1): 80-91.

[323] Kabbaj M, Norton CS, Kollack-Walker S, Watson SJ, Robinson TE, Akil H. Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine-taking behavior. *Psychopharmacology (Berl)* 2001; 158(4): 382-387.

[324] Kalia M, Woodward DJ, Smith WK, Fuxe K. Rat medulla oblongata. IV. Topographical distribution of catecholaminergic neurons with quantitative three-dimensional computer reconstruction. *J Comp Neurol* 1985; 233(3): 350-364.

[325] Kalivas PW, Churchill L, Klitenick MA. GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. *Neuroscience* 1993; 57(4): 1047-1060.

[326] Kalivas PW, Duffy P. Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. *J Neurochem* 1998; 70(4): 1497-1502.

[327] Kalivas PW, Duffy P. Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. *J Neurosci* 1993; 13(1): 266-275.

[328] Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry* 2005; 162(8): 1403-1413.

[329] Kalivas PW. Glutamate systems in cocaine addiction. *Curr Opin Pharmacol* 2004; 4(1): 23-29.

[330] Kantak KM, Black Y, Valencia E, Green-Jordan K, Eichenbaum HB. Dissociable effects of lidocaine inactivation of the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. *J Neurosci* 2002; 22(3): 1126-1136.

[331] Kapur S, Mamo D. Half a century of antipsychotics and still a central role for dopamine D2 receptors. *Prog Neuropsychopharmacol Biol Psychiatry* 2003; 27(7): 1081-1090.

[332] Karler R, Calder LD, Bedingfield JB. Cocaine behavioral sensitization and the excitatory amino acids. *Psychopharmacology (Berl)* 1994; 115(3): 305-310.

[333] Karler R, Chaudhry IA, Calder LD, Turkanis SA. Amphetamine behavioral sensitization and the excitatory amino acids. *Brain Res* 1990; 537(1-2): 76-82.

[334] Karlsgodt KH, Lukas SE, Elman I. Psychosocial stress and the duration of cocaine use in non-treatment seeking individuals with cocaine dependence. *Am J Drug Alcohol Abuse* 2003; 29(3): 539-551.

[335] Karreman M, Westerink BHC, Moghaddam B. Excitatory amino acid receptors in the ventral tegmental area regulate dopamine release in the ventral striatum. *J Neurochem* 1996; 67(2): 601-607.

[336] Karssen AM, Meijer OC, van dS, I, Lucassen PJ, de Lange EC, de Boer AG, De Kloet ER. Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. *Endocrinology* 2001; 142(6): 2686-2694.

[337] Karst H, Joels M. Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal cells. *J Neurophysiol* 2005; 94(5): 3479-3486.

[338] Karst H, Berger S, Turiault M, Tronche F, Schutz G, Joels M. Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proc Natl Acad Sci U S A* 2005; 102(52): 19204-19207.

[339] Kavaliers M, Ossenkopp KP. Corticosterone rapidly reduces male odor preferences in female mice. *Neuroreport* 2001; 12(13): 2999-3002.

[340] Kebabian JW, Calne DB. Multiple receptors for dopamine. *Nature* 1979; 277(5692): 93-96.

[341] Keller-Wood ME, Dallman MF. Corticosteroid inhibition of ACTH secretion. *Endocr Rev* 1984; 5(1): 1-24.

[342] Kelley AE. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neurosci Biobehav Rev* 2004; 27(8): 765-776.

[343] Kelley AE, Domesick VB, Nauta WJ. The amygdalostriatal projection in the rat - an anatomical study by anterograde and retrograde tracing methods. *Neuroscience* 1982; 7(3): 615-630.

[344] Kelly PH, Iversen SD. Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. *Eur J Pharmacol* 1976; 40(1): 45-56.

[345] Kelly PH, Seviour PW, Iversen SD. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res* 1975; 94(3): 507-522.

[346] Kelz MB, Chen J, Carlezon WA, Jr., Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ,

Marotti L, Self DW, Tkatch T, Baranauskas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR, Nestler EJ. Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* 1999; 401(6750): 272-276.

[347] Kendler KS, Bulik CM, Silberg J, Hettema JM, Myers J, Prescott CA. Childhood sexual abuse and adult psychiatric and substance use disorders in women: an epidemiological and cotwin control analysis. *Arch Gen Psychiatry* 2000; 57(10): 953-959.

[348] Kendler KS, Prescott CA. Cocaine use, abuse and dependence in a population-based sample of female twins. *Br J Psychiatry* 1998; 173: 345-350.

[349] Kendler KS, Jacobson KC, Prescott CA, Neale MC. Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. *Am J Psychiatry* 2003; 160(4): 687-695.

[350] Kenny PJ, Boutrel B, Gasparini F, Koob GF, Markou A. Metabotropic glutamate 5 receptor blockade may attenuate cocaine self-administration by decreasing brain reward function in rats. *Psychopharmacology (Berl)* 2005; 179(1): 247-254.

[351] Khroyan TV, Barrett-Larimore RL, Rowlett JK, Spealman RD. Dopamine D1- and D2-like receptor mechanisms in relapse to cocaine-seeking behavior: effects of selective antagonists and agonists. *J Pharmacol Exp Ther* 2000; 294(2): 680-687.

[352] Khroyan TV, Platt DM, Rowlett JK, Spealman RD. Attenuation of relapse to cocaine seeking by dopamine D1 receptor agonists and antagonists in non-human primates. *Psychopharmacology (Berl)* 2003; 168(1-2): 124-131.

[353] Kilts CD, Schweitzer JB, Quinn CK, Gross RE, Faber TL, Muhammad F, Ely TD, Hoffman JM, Drexler KPG. Neural activity related to drug craving in cocaine addiction. *Arch Gen Psychiatry* 2001; 58(4): 334-341.

[354] Klebaur JE, Bevins RA, Segar TM, Bardo MT. Individual differences in behavioral responses to novelty and amphetamine self-administration in male and female rats. *Behav Pharmacol* 2001; 12(4): 267-275.

[355] Knych ET, Eisenberg RM. Effect of amphetamine on plasma corticosterone in the conscious rat. *Neuroendocrinology* 1979; 29(2): 110-118.

[356] Koepp MJ, Gunn RN, Lawrence AD, Cunningham VJ, Dagher A, Jones T, Brooks DJ, Bench CJ, Grasby PM. Evidence for striatal dopamine release during a video game. *Nature* 1998; 393(6682): 266-268.

[357] Konradi C, Cole RL, Heckers S, Hyman SE. Amphetamine regulates gene expression in rat striatum via transcription factor CREB. *J Neurosci* 1994; 14(9): 5623-5634.

[358] Koob GF. Stress, corticotropin-releasing factor, and drug addiction. *Ann N Y Acad Sci* 1999; 897: 27-45.

[359] Koob GF, Cador M. Psychomotor stimulant sensitization: the corticotropin-releasing factor-steroid connection. Commentary on Wise and Leeb "Psychomotor-stimulant sensitization: a unitary phenomenon?". *Behav Pharmacol* 1993; 4(4): 351-354.

[360] Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. *Science* 1997; 278(5335): 52-58.

[361] Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 2001; 24(2): 97-129.

[362] Kornet M, Goosen C, Thyssen JH, Van Ree JM. Endocrine profile during acquisition of free-choice alcohol drinking in rhesus monkeys; treatment with desglycinamide-(Arg8)-vasopressin. *Alcohol Alcoholism* 1992; 27(4): 403-410.

[363] Kosten TA, Miserendino MJ, Kehoe P. Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. *Brain Res* 2000; 875(1-2): 44-50.

[364] Kosten TR, Rounsaville BJ, Kleber HD. A 2.5-year follow-up of depression, life crises, and treatment effects on abstinence among opioid addicts. *Arch Gen Psychiatry* 1986; 43(8): 733-738.

[365] Kosten TA, Sanchez H, Zhang XY, Kehoe P. Neonatal isolation enhances acquisition of cocaine self-administration and food responding in female rats. *Behav Brain Res* 2004; 151(1-2): 137-149.

[366] Kosten TA, Zhang XY, Kehoe P. Heightened cocaine and food self-administration in female rats with neonatal isolation experience. *Neuropsychopharmacology* 2005; 31(1): 70-76.

[367] Kosten TR, Oliveto A, Sevarino KA, Gonsai K, Feingold A. Ketoconazole increases cocaine and opioid use in methadone maintained patients. *Drug Alcohol Depend* 2002; 66(2): 173-180.

[368] Kovacs P, Hernadi I. Alpha2 antagonist yohimbine suppresses maintained firing of rat prefrontal neurons in vivo. *Neuroreport* 2003; 14(6): 833-836.

[369] Krueger RJ, Lin CM, Fung YK. Effect of chronic administration of nicotine or cocaine on steroidogenesis in rat adrenocortical cells. *J Pharm Pharmacol* 1991; 43(3): 200-203.

[370] Kruzich PJ, See RE. Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. *J Neurosci* 2001; 21(14): RC155.

[371] Kuhar MJ, Jaworski JN, Hubert GW, Philpot KB, Dominguez G. Cocaine- and amphetamine-regulated transcript peptides play a role in drug abuse and are potential therapeutic targets. *AAPS J* 2005; 7(1): E259-E265.

[372] Kuhar MJ, Ritz MC, Boja JW. The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci* 1991; 14(7): 299-302.

[373] Kumer SC, Vrana KE. Intricate regulation of tyrosine hydroxylase activity and gene expression. *J Neurochem* 1996; 67(2): 443-462.

[374] Lakshmi V, Sakai RR, McEwen BS, Monder C. Regional distribution of 11 beta-hydroxysteroid dehydrogenase in rat brain. *Endocrinology* 1991; 128(4): 1741-1748.

[375] Le Moine C, Bloch B. D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *J Comp Neurol* 1995; 355(3): 418-426.

[376] Le AD, Harding S, Juzytsch W, Funk D, Shaham Y. Role of alpha-2 adrenoceptors in stress-induced reinstatement of alcohol seeking and alcohol self-administration in rats. *Psychopharmacology (Berl)* 2005; 179(2): 366-373.

[377] Lebzeltern G. Sigmund Freud and cocaine. *Wien Klin Wochenschr* 1983; 95(21): 765-769.

[378] Lecca D, Piras G, Driscoll P, Giorgi O, Corda MG. A differential activation of dopamine output in

the shell and core of the nucleus accumbens is associated with the motor responses to addictive drugs: a brain dialysis study in Roman high- and low-avoidance rats. *Neuropharmacology* 2004; 46(5): 688-699.

[379] Leccese AP, Lyness WH. Lesions of dopamine neurons in the medial prefrontal cortex: effects on self-administration of amphetamine and dopamine synthesis in the brain of the rat. *Neuropharmacology* 1987; 26(9): 1303-1308.

[380] Lee B, Platt DM, Rowlett JK, Adewale AS, Spealman RD. Attenuation of behavioral effects of cocaine by the Metabotropic Glutamate Receptor 5 Antagonist 2-Methyl-6-(phenylethynyl)-pyridine in squirrel monkeys: comparison with dizocilpine. *J Pharmacol Exp Ther* 2005; 312(3): 1232-1240.

[381] Leibowitz SF, Eidelman D, Suh JS, Diaz S, Sladek CD. Mapping study of noradrenergic stimulation of vasopressin release. *Exp Neurol* 1990; 110(3): 298-305.

[382] Leibowitz SF, Diaz S, Tempel D. Norepinephrine in the paraventricular nucleus stimulates corticosterone release. *Brain Res* 1989; 496(1-2): 219-227.

[383] Lepsch LB, Gonzalo LA, Magro FJ, Delucia R, Scavone C, Planeta CS. Exposure to chronic stress increases the locomotor response to cocaine and the basal levels of corticosterone in adolescent rats. *Addict Biol* 2005; 10(3): 251-256.

[384] Leri F, Flores J, Rodaros D, Stewart J. Blockade of stress-induced but not cocaine-induced reinstatement by infusion of noradrenergic antagonists into the bed nucleus of the stria terminalis or the central nucleus of the amygdala. *J Neurosci* 2002; 22(13): 5713-5718.

[385] Lester J, Fink S, Aronin N, DiFiglia M. Colocalization of D1 and D2 dopamine receptor mRNAs in striatal neurons. *Brain Res* 1993; 621(1): 106-110.

[386] Levine S. Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology* 2005; 30(10): 939-946.

[387] Levy AD, Li Q, Alvarez Sanz MC, Rittenhouse PA, Kerr JE, Van de Kar LD. Neuroendocrine responses to cocaine do not exhibit sensitization following repeated cocaine exposure. *Life Sci* 1992; 51(12): 887-897.

[388] Lewander T. Effects of amphetamine on urinary and tissue catecholamines in rats after inhibition of its metabolism with desmethylimipramine. *Eur J Pharmacol* 1968; 5(1): 1-9.

[389] Leyton M, Stewart J. Preexposure to foot-shock sensitizes the locomotor response to subsequent systemic morphine and intra-nucleus accumbens amphetamine. *Pharmacol Biochem Behav* 1990; 37(2): 303-310.

[390] Li Y, Acerbo MJ, Robinson TE. The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. *Eur J Neurosci* 2004; 20(6): 1647-1654.

[391] Li Y, Hu XT, Berney TG, Vartanian AJ, Stine CD, Wolf ME, White FJ. Both glutamate receptor antagonists and prefrontal cortex lesions prevent induction of cocaine sensitization and associated neuroadaptations. *Synapse* 1999; 34(3): 169-180.

[392] Li Y, Kolb B, Robinson TE. The location of persistent amphetamine-induced changes in the density

of dendritic spines on medium spiny neurons in the nucleus accumbens and caudate-putamen. *Neuropsychopharmacology* 2003; 28(6): 1082-1085.

[393] Li Y, Robinson TE, Bhatnagar S. Effects of maternal separation on behavioural sensitization produced by repeated cocaine administration in adulthood. *Brain Res* 2003; 960(1-2): 42-47.

[394] Li Y, Wolf ME. Ibotenic acid lesions of prefrontal cortex do not prevent expression of behavioral sensitization to amphetamine. *Behav Brain Res* 1997; 84(1-2): 285-289.

[395] Liang KC, Chen LL, Huang TE. The role of amygdala norepinephrine in memory formation: involvement in the memory enhancing effect of peripheral epinephrine. *Chin J Physiol* 1995; 38(2): 81-91.

[396] Liang KC, Juler RG, McGaugh JL. Modulating effects of posttraining epinephrine on memory: involvement of the amygdala noradrenergic system. *Brain Res* 1986; 368(1): 125-133.

[397] Lindley SE, Bengoechea TG, Schatzberg AF, Wong DL. Glucocorticoid effects on mesotelencephalic dopamine neurotransmission. *Neuropsychopharmacology* 1999; 21(3): 399-407.

[398] Liposits Z, Paull WK. Association of dopaminergic fibers with corticotropin releasing hormone (CRH)-synthesizing neurons in the paraventricular nucleus of the rat hypothalamus. *Histochemistry* 1989; 93(2): 119-127.

[399] Lorrain DS, Arnold GM, Vezina P. Previous exposure to amphetamine increases incentive to obtain the drug: long-lasting effects revealed by the progressive ratio schedule. *Behav Brain Res* 2000; 107(1-2): 9-19.

[400] Lowy MT, Gault L, Yamamoto BK. Adrenalectomy attenuates stress-induced elevations in extracellular glutamate concentrations in the hippocampus. *J Neurochem* 1993; 61(5): 1957-1960.

[401] Loy R, Koziell DA, Lindsey JD, Moore RY. Noradrenergic innervation of the adult rat hippocampal formation. *J Comp Neurol* 1980; 189(4): 699-710.

[402] Lu L, Dempsey J, Shaham Y, Hope BT. Differential long-term neuroadaptations of glutamate receptors in the basolateral and central amygdala after withdrawal from cocaine self-administration in rats. *J Neurochem* 2005; 94(1): 161-168.

[403] Lu L, Grimm JW, Shaham Y, Hope BT. Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. *J Neurochem* 2003; 85(6): 1604-1613.

[404] Lu L, Liu Z, Huang M, Zhang Z. Dopamine-dependent responses to cocaine depend on corticotropin-releasing factor receptor subtypes. *J Neurochem* 2003; 84(6): 1378-1386.

[405] Lu L, Shepard JD, Scott Hall F, Shaham Y. Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. *Neurosci Biobehav Rev* 2003; 27(5): 457-491.

[406] Lu W, Wolf ME. Expression of dopamine transporter and vesicular monoamine transporter 2 mRNAs in rat midbrain after repeated amphetamine administration. *Brain Res Mol Brain Res* 1997; 49(1-2): 137-148.

[407] Lu X-Y, Behnam Ghasemzadeh M, Kalivas PW. Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience* 1997; 82(3): 767-780.

[408] Lucas LR, Angulo JA, Le Moal M, McEwen BS, Piazza PV. Neurochemical characterization of individual vulnerability to addictive drugs in rats. *Eur J Neurosci* 1998; 10(10): 3153-3163.

[409] Lyness WH, Friedle NM, Moore KE. Destruction of dopaminergic nerve terminals in nucleus accumbens: effect on d-amphetamine self-administration. *Pharmacol Biochem Behav* 1979; 11(5): 553-556.

[410] MacLennan AJ, Maier SF. Coping and the stress-induced potentiation of stimulant stereotypy in the rat. *Science* 1983; 219(4588): 1091-1093.

[411] Maldonado R, Robledo P, Chover AJ, Caine SB, Koob GF. D1 dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. *Pharmacol Biochem Behav* 1993; 45(1): 239-242.

[412] Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* 2006; 29(4): 225-232.

[413] Malkoski SP, Dorin RI. Composite glucocorticoid regulation at a functionally defined negative glucocorticoid response element of the human corticotropin-releasing hormone gene. *Mol Endocrinol* 1999; 13(10): 1629-1644.

[414] Mangat HS, Islam A, Heigenskold C, Mustafa A, Winblad B, Adem A. Long-term adrenalectomy decreases NMDA receptors in rat hippocampus. *Neuroreport* 1998; 9(9): 2011-2014.

[415] Mantsch JR, Goeders NE. Ketoconazole does not block cocaine discrimination or the cocaine-induced reinstatement of cocaine-seeking behavior. *Pharmacol Biochem Behav* 1999; 64(1): 65-73.

[416] Mantsch JR, Goeders NE. Ketoconazole blocks the stress-induced reinstatement of cocaine-seeking behavior in rats: relationship to the discriminative stimulus effects of cocaine. *Psychopharmacology (Berl)* 1999; 142(4): 399-407.

[417] Mantsch JR, Katz ES. Elevation of glucocorticoids is necessary but not sufficient for the escalation of cocaine self-administration by chronic electric footshock stress in rats. *Neuropsychopharmacology* 2007; 32(2): 367-376.

[418] Mantsch JR, Saphier D, Goeders NE. Corticosterone facilitates the acquisition of cocaine self-administration in rats: opposite effects of the type II glucocorticoid receptor agonist dexamethasone. *J Pharmacol Exp Ther* 1998; 287(1): 72-80.

[419] Marek GJ, Aghajanian GK. 5-HT2A receptor or alpha1-adrenoceptor activation induces excitatory postsynaptic currents in layer V pyramidal cells of the medial prefrontal cortex. *Eur J Pharmacol* 1999; 367(2-3): 197-206.

[420] Marinelli M, Le Moal M, Piazza PV. Acute pharmacological blockade of corticosterone secretion reverses food restriction-induced sensitization of the locomotor response to cocaine. *Brain Res* 1996; 724(2): 251-255.

[421] Marinelli M, Piazza PV. Interaction between glucocorticoid hormones, stress and psychostimulant drugs. *Eur J Neurosci* 2002; 16(3): 387-394.

[422] Marinelli M, Piazza PV, Deroche V, Maccari S, Le Moal M, Simon H. Corticosterone circadian secretion differentially facilitates dopamine-mediated psychomotor effect of cocaine and morphine. *J Neurosci* 1994; 14(5 Pt 1): 2724-2731.

[423] Marinelli M, Rouge-Pont F, Deroche V, Barrot M, Jesus-Oliveira C, Le Moal M, Piazza PV. Glucocorticoids and behavioral effects of psychostimulants. I: locomotor response to cocaine depends on basal levels of glucocorticoids. *J Pharmacol Exp Ther* 1997; 281(3): 1392-1400.

[424] Marinelli M, Rouge-Pont F, Jesus-Oliveira C, Le Moal M, Piazza PV. Acute blockade of corticosterone secretion decreases the psychomotor stimulant effects of cocaine. *Neuropsychopharmacology* 1997; 16(2): 156-161.

[425] Martin-Iverson MT, Szostak C, Fibiger HC. 6-Hydroxydopamine lesions of the medial prefrontal cortex fail to influence intravenous self-administration of cocaine. *Psychopharmacology (Berl)* 1986; 88(3): 310-314.

[426] Mash DC, Staley JK, Izenwasser S, Basile M, Ruttenber AJ. Serotonin transporters upregulate with chronic cocaine use. *J Chem Neuroanat* 2000; 20(3-4): 271-280.

[427] Matthews JC, Collins A. Interactions of cocaine and cocaine congeners with sodium channels. *Biochem Pharmacol* 1983; 32(3): 455-460.

[428] Matthews K, Hall FS, Wilkinson LS, Robbins TW. Retarded acquisition and reduced expression of conditioned locomotor activity in adult rats following repeated early maternal separation: effects of prefeeding, d-amphetamine, dopamine antagonists and clonidine. *Psychopharmacologia* 1996; 126(1): 75-84.

[429] Matthews K, Robbins TW, Everitt BJ, Caine SB. Repeated neonatal maternal separation alters intravenous cocaine self-administration in adult rats. *Psychopharmacology (Berl)* 1999; 141(2): 123-134.

[430] Mattingly BA, Hart TC, Lim K, Perkins C. Selective antagonism of dopamine D1 and D2 receptors does not block the development of behavioral sensitization to cocaine. *Psychopharmacology (Berl)* 1994; 114(2): 239-242.

[431] Mayer JL, Klumpers L, Maslam S, De Kloet ER, Joels M, Lucassen PJ. Brief treatment with the glucocorticoid receptor antagonist mifepristone normalises the corticosterone-induced reduction of adult hippocampal neurogenesis. *J Neuroendocrinol* 2006; 18(8): 629-631.

[432] McClung CA, Nestler EJ. Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nat Neurosci* 2003; 6(11): 1208-1215.

[433] McEwen BS. Allostasis, allostatic load, and the aging nervous system: role of excitatory amino acids and excitotoxicity. *Neurochem Res* 2000; 25(9-10): 1219-1231.

[434] McFarland K, Davidge SB, Lapish CC, Kalivas PW. Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. *J Neurosci* 2004; 24(7): 1551-1560.

[435] McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci* 2001; 21(21): 8655-8663.

[436] McFarland K, Lapish CC, Kalivas PW. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci* 2003; 23(8): 3531-3537.

[437] McGaugh JL, Cahill L, Roozendaal B. Involvement of the amygdala in memory storage: interaction with other brain systems. *Proc Natl Acad Sci U S A* 1996; 93(24): 13508-13514.

[438] McGregor A, Baker G, Roberts DC. Effect of 6-hydroxydopamine lesions of the medial prefrontal

cortex on intravenous cocaine self-administration under a progressive ratio schedule of reinforcement. *Pharmacol Biochem Behav* 1996; 53(1): 5-9.

[439] McGregor A, Roberts DCS. Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. *Brain Res* 1993; 624(1-2): 245-252.

[440] McKinzie DL, Rodd-Henricks ZA, Dagon CT, Murphy JM, McBride WJ. Cocaine is self-administered into the shell region of the nucleus accumbens in Wistar rats. *Ann N Y Acad Sci* 1999; 877: 788-791.

[441] McLellan AT, Lewis DC, O'Brien CP, Kleber HD. Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. *JAMA* 2000; 284(13): 1689-1695.

[442] Meador-Woodruff JH, Mansour A, Bunzow JR, Tol HHMV, Watson SJ, Civelli O. Distribution of D2 dopamine receptor mRNA in rat brain. *Proc Natl Acad Sci U S A* 1989; 86(19): 7625-7628.

[443] Meador-Woodruff JH, Mansour A, Grandy DK, Damask SP, Civelli O, Watson J. Distribution of D5 dopamine receptor mRNA in rat brain. *Neurosci Lett* 1992; 145(2): 209-212.

[444] Meaney MJ, Brake W, Gratton A. Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology* 2002; 27(1-2): 127-138.

[445] Meaney MJ, Diorio J, Francis D, Widdowson J, LaPlante P, Caldji C, Sharma S, Seckl JR, Plotsky PM. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. *Dev Neurosci* 1996; 18(1-2): 49-72.

[446] Meijer OC, van der Laan S, Lachize S, Steenbergen PJ, De Kloet ER. Steroid receptor coregulator diversity: what can it mean for the stressed brain? *Neuroscience* 2006; 138(3): 891-899.

[447] Meliska CJ, Bartke A, McGlacken G, Jensen RA. Ethanol, nicotine, amphetamine, and aspartame consumption and preferences in C57BL/6 and DBA/2 mice. *Pharmacol Biochem Behav* 1995; 50(4): 619-626.

[448] Mendelson JH, Teoh SK, Mello NK, Ellingboe J, Rhoades E. Acute effects of cocaine on plasma adrenocorticotropic hormone, luteinizing hormone and prolactin levels in cocaine-dependent men. *J Pharmacol Exp Ther* 1992; 263(2): 505-509.

[449] Mendrek A, Blaha CD, Phillips AG. Pre-exposure of rats to amphetamine sensitizes self-administration of this drug under a progressive ratio schedule. *Psychopharmacology (Berl)* 1998; 135(4): 416-422.

[450] Mikics E, Barsy B, Barsvari B, Haller J. Behavioral specificity of non-genomic glucocorticoid effects in rats: Effects on risk assessment in the elevated plus-maze and the open-field. *Horm Behav* 2005; 48(2): 152-162.

[451] Mikics E, Kruk MR, Haller J. Genomic and non-genomic effects of glucocorticoids on aggressive behavior in male rats. *Psychoneuroendocrinology* 2004; 29(5): 618-635.

[452] Mink JW, Thach WT. Basal ganglia intrinsic circuits and their role in behavior. *Curr Opin Neurobiol* 1993; 3(6): 950-957.

[453] Misra K, Pandey SC. Differences in basal levels of CREB and NPY in nucleus accumbens regions

between C57BL/6 and DBA/2 mice differing in inborn alcohol drinking behavior. *J Neurosci Res* 2003; 74(6): 967-975.

[454] Mittleman G, Jones GH, Robbins TW. Sensitization of amphetamine-stereotypy reduces plasma corticosterone: implications for stereotypy as a coping response. *Behav Neural Biol* 1991; 56(2): 170-182.

[455] Mittleman G, Van Brunt CL, Matthews DB. Schedule-induced ethanol self-administration in DBA/2J and C57BL/6J mice. *Alcohol Clin Exp Res* 2003; 27(6): 918-925.

[456] Miyashita T, Williams CL. Epinephrine administration increases neural impulses propagated along the vagus nerve: role of peripheral beta-adrenergic receptors. *Neurobiol Learn Mem* 2006; 85(2): 116-124.

[457] Mobbs D, Greicius MD, Abdel-Azim E, Menon V, Reiss AL. Humor modulates the mesolimbic reward centers. *Neuron* 2003; 40(5): 1041-1048.

[458] Moffett MC, Harley J, Francis D, Sanghani SP, Davis WI, Kuhar MJ. Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. *J Pharmacol Exp Ther* 2006; 317(3): 1210-1218.

[459] Moghaddam B. Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. *Biol Psychiatry* 2002; 51(10): 775-787.

[460] Moghaddam B, Bolinao ML, Stein-Behrens B, Sapolsky R. Glucocorticoids mediate the stress-induced extracellular accumulation of glutamate. *Brain Res* 1994; 655(1-2): 251-254.

[461] Moldow RL, Fischman AJ. Cocaine induced secretion of ACTH, beta-endorphin, and corticosterone. *Peptides* 1987; 8(5): 819-822.

[462] Monsma FJ, Jr., Mahan LC, McVittie LD, Gerfen CR, Sibley DR. Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. *Proc Natl Acad Sci U S A* 1990; 87(17): 6723-6727.

[463] Monsma FJ, McVittie LD, Gerfen CR, Mahan LC, Sibley DR. Multiple D2 dopamine receptors produced by alternative RNA splicing. *Nature* 1989; 342(6252): 926-929.

[464] Morgan D, Smith MA, Roberts DCS. Binge self-administration and deprivation produces sensitization to the reinforcing effects of cocaine in rats. *Psychopharmacologia* 2005; 178(2-3): 309-316.

[465] Morse AC, Erwin VG, Jones BC. Strain and housing affect cocaine self-selection and open-field locomotor activity in mice. *Pharmacol Biochem Behav* 1993; 45(4): 905-912.

[466] Mulholland PJ, Self RL, Hensley AK, Little HJ, Littleton JM, Prendergast MA. A 24 h corticosterone exposure exacerbates excitotoxic insult in rat hippocampal slice cultures independently of glucocorticoid receptor activation or protein synthesis. *Brain Res* 2006; 1082(1): 165-172.

[467] Muller CP, Huston JP. Determining the region-specific contributions of 5-HT receptors to the psychostimulant effects of cocaine. *Trends Pharmacol Sci* 2006; 27(2): 105-112.

[468] Najavits LM, Runkel R, Neuner C, Frank AF, Thase ME, Crits-Christoph P, Blaine J. Rates and symptoms of PTSD among cocaine-dependent patients. *J Stud Alcohol* 2003; 64(5): 601-606.

[469] Nestler EJ. Cellular responses to chronic treatment with drugs of abuse. *Crit Rev Neurobiol* 1993; 7(1): 23-39.

[470] Ng GY, O'Dowd BF, George SR. Genotypic differences in brain dopamine receptor function in the DBA/2J and C57BL/6J inbred mouse strains. *Eur J Pharmacol* 1994; 269(3): 349-364.

[471] Ng GY, O'Dowd BF, George SR. Genotypic differences in mesolimbic enkephalin gene expression in DBA/2J and C57BL/6J inbred mice. *Eur J Pharmacol* 1996; 311(1): 45-52.

[472] Nikulina EM, Covington HE, III, Ganschow L, Hammer RP, Jr., Miczek KA. Long-term behavioral and neuronal cross-sensitization to amphetamine induced by repeated brief social defeat stress: Fos in the ventral tegmental area and amygdala. *Neuroscience* 2004; 123(4): 857-865.

[473] Noble EP. Polymorphisms of the D2 dopamine receptor gene and alcoholism and other substance use disorders. *Alcohol Alcoholism* 1994; 2(suppl): 35-43.

[474] Noble EP. Addiction and its reward process through polymorphisms of the D2 dopamine receptor gene: a review. *Eur Psychiatry* 2000; 15(2): 79-89.

[475] Noble EP, Blum K, Khalsa ME, Ritchie T, Montgomery A, Wood RC, Fitch RJ, Ozkaragoz T, Sheridan PJ, Anglin MD. Allelic association of the D2 dopamine receptor gene with cocaine dependence. *Drug Alcohol Depend* 1993; 33(3): 271-285.

[476] Noble EP. The D2 dopamine receptor gene: a review of association studies in alcoholism and phenotypes. *Alcohol* 1998; 16(1): 33-45.

[477] Nothen MM, Cichon S, Hemmer S, Hebebrand J, Remschmidt H, Lehmkuhl G, Poustka F, Schmidt M, Catalano M, Fimmers R. Human dopamine D4 receptor gene: frequent occurrence of a null allele and observation of homozygosity. *Hum Mol Genet* 1994; 3(12): 2207-2212.

[478] O'Brien CP, Ehrman RN, Ternes JW. Classical conditioning in human opioid dependence. In: Goldberg S, Stolerman I (eds). *Behavioral analysis of drug dependence*. Academic Press, New York: 1986; 329-356.

[479] O'Brien MS, Anthony JC. Risk of becoming cocaine dependent: epidemiological estimates for the United States, 2000-2001. *Neuropsychopharmacology* 2005; 30(5): 1006-1018.

[480] O'Malley KL, Harmon S, Tang L, Todd RD. The rat dopamine D4 receptor: sequence, gene structure, and demonstration of expression in the cardiovascular system. *New Biol* 1992; 4(2): 137-146.

[481] Oitzl MS, van Haarst AD, De Kloet ER. Behavioral and neuroendocrine responses controlled by the concerted action of central mineralocorticoid (MRS) and glucocorticoid receptors (GRS). *Psychoneuroendocrinology* 1997; 22(suppl 1): S87-S93.

[482] Olson L, Fuxe K. Further mapping out of central noradrenaline neuron systems: projections of the 'subcoeruleus' area. *Brain Res* 1972; 43(1): 289-295.

[483] Orsini C, Bonito-Oliva A, Conversi D, Cabib S. Susceptibility to conditioned place preference induced by addictive drugs in mice of the C57BL/6 and DBA/2 inbred strains. *Psychopharmacology (Berl)* 2005; 181(2): 327-336.

[484] Orsini C, Buchini F, Piazza PV, Puglisi-Allegra S, Cabib S. Susceptibility to amphetamine-induced place preference is predicted by locomotor response to novelty and amphetamine in the mouse. *Psychopharmacology (Berl)* 2004; 172(3): 264-270.

[485] Ortiz J, DeCaprio JL, Kosten TA, Nestler EJ. Strain-selective effects of corticosterone on locomotor

sensitization to cocaine and on levels of tyrosine hydroxylase and glucocorticoid receptor in the ventral tegmental area. *Neuroscience* 1995; 67(2): 383-397.

[486] Oswald LM, Wong DF, McCaul M, Zhou Y, Kuwabara H, Choi L, Brasic J, Wand GS. Relationships among ventral striatal dopamine release, cortisol secretion, and subjective responses to amphetamine. *Neuropsychopharmacology* 2005; 30(4): 821-832.

[487] Ouimette PC, Brown PJ, Najavits LM. Course and treatment of patients with both substance use and posttraumatic stress disorders. *Addict Behav* 1998; 23(6): 785-795.

[488] Overton PG, Tong ZY, Brain PF, Clark D. Preferential occupation of mineralocorticoid receptors by corticosterone enhances glutamate-induced burst firing in rat midbrain dopaminergic neurons. *Brain Res* 1996; 737(1-2): 146-154.

[489] Packard MG, Knowlton BJ. Learning and memory functions of the basal ganglia. *Annu Rev Neurosci* 2002; 25(1): 563-593.

[490] Palomo T, Archer T, Kostrzewa RM, Beninger RJ. Gene-environment interplay in schizopsychotic disorders. *Neurotox Res* 2004; 6(1): 1-9.

[491] Pan WH, Sung JC, Fuh SM. Local application of amphetamine into the ventral tegmental area enhances dopamine release in the nucleus accumbens and the medial prefrontal cortex through noradrenergic neurotransmission. *J Pharmacol Exp Ther* 1996; 278(2): 725-731.

[492] Park WK, Bari AA, Jey AR, Anderson SM, Spealman RD, Rowlett JK, Pierce RC. Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *J Neurosci* 2002; 22(7): 2916-2925.

[493] Patacchioli FR, Pontieri FE, Orzi F, Di Grezia R, Angelucci L. Increased motor response to cocaine administration following recovery from chronic corticosterone treatment in the rat. *Eur Neuropsychopharmacol* 1998; 8(1): 43-46.

[494] Patel PD, Lopez JF, Lyons DM, Burke S, Wallace M, Schatzberg AF. Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. *J Psychiat Res* 2000; 34(6): 383-392.

[495] Paterson NE, Markou A. The metabotropic glutamate receptor 5 antagonist MPEP decreased break points for nicotine, cocaine and food in rats. *Psychopharmacology (Berl)* 2005; 179(1): 255-261.

[496] Patrick SL, Thompson TL, Walker JM, Patrick RL. Concomitant sensitization of amphetamine-induced behavioral stimulation and in vivo dopamine release from rat caudate nucleus. *Brain Res* 1991; 538(2): 343-346.

[497] Paulson PE, Camp DM, Robinson TE. Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. *Psychopharmacology (Berl)* 1991; 103(4): 480-492.

[498] Pauly JR, Robinson SF, Collins AC. Chronic corticosterone administration enhances behavioral sensitization to amphetamine in mice. *Brain Res* 1993; 620(2): 195-202.

[499] Paylor R, Tracy R, Wehner J, Rudy JW. DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning. *Behav Neurosci* 1994; 108(4): 810-817.

[500] Peltier R, Schenk S. Effects of serotonergic manipulations on cocaine self-administration in rats. *Psychopharmacology (Berl)* 1993; 110(4): 390-394.

[501] Peters J, Kalivas PW. The group II metabotropic glutamate receptor agonist, LY379268, inhibits both cocaine- and food-seeking behavior in rats. *Psychopharmacology (Berl)* 2006; 186(2): 143-149.

[502] Pettit HO, Ettenberg A, Bloom FE, Koob GF. Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology (Berl)* 1984; 84(2): 167-173.

[503] Pettit HO, Justice J. Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. *Pharmacol Biochem Behav* 1989; 34(4): 899-904.

[504] Phillips GD, Howes SR, Whitelaw RB, Robbins TW, Everitt BJ. Isolation rearing impairs the reinforcing efficacy of intravenous cocaine or intra-accumbens d-amphetamine: impaired response to intra-accumbens D1 and D2/D3 dopamine receptor antagonists. *Psychopharmacology (Berl)* 1994; 115(3): 419-429.

[505] Phillips GD, Howes SR, Whitelaw RB, Wilkinson LS, Robbins TW, Everitt BJ. Isolation rearing enhances the locomotor response to cocaine and a novel environment, but impairs the intravenous self-administration of cocaine. *Psychopharmacology (Berl)* 1994; 115(3): 407-418.

[506] Phillips GD, Robbins TW, Everitt BJ. Bilateral intra-accumbens self-administration of d-amphetamine: antagonism with intra-accumbens SCH-23390 and sulpiride. *Psychopharmacology (Berl)* 1994; 114(3): 477-485.

[507] Phillips TJ, Huson MG, McKinnon CS. Localization of genes mediating acute and sensitized locomotor responses to cocaine in BXD/Ty recombinant inbred mice. *J Neurosci* 1998; 18(8): 3023-3034.

[508] Piazza PV, Barrot M, Rouge-Pont F, Marinelli M, Maccari S, Abrous DN, Simon H, Le Moal M. Suppression of glucocorticoid secretion and antipsychotic drugs have similar effects on the mesolimbic dopaminergic transmission. *Proc Natl Acad Sci U S A* 1996; 93(26): 15445-15450.

[509] Piazza PV, Deminiere JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self- administration. *Science* 1989; 245(4925): 1511-1513.

[510] Piazza PV, Deminiere JM, Le Moal M, Simon H. Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. *Brain Res* 1990; 514(1): 22-26.

[511] Piazza PV, Deminiere JM, Maccari S, Mormede P, Le Moal M, Simon H. Individual reactivity to novelty predicts probability of amphetamine self-administration. *Behav Pharmacol* 1990; 1(4): 339-345.

[512] Piazza PV, Deroche V, Deminiere JM, Maccari S, Le Moal M, Simon H. Corticosterone in the range of stress-induced levels possesses reinforcing properties: implications for sensation-seeking behaviors. *Proc Natl Acad Sci U S A* 1993; 90(24): 11738-11742.

[513] Piazza PV, Deroche-Gamonent V, Rouge-Pont F, Le Moal M. Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. *J Neurosci* 2000; 20(11): 4226-4232.

[514] Piazza PV, Le Moal M. The role of stress in drug self-administration. *Trends Pharmacol Sci* 1998; 19(2): 67-74.

[515] Piazza PV, Le Moal ML. Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu Rev Pharmacol Toxicol* 1996; 36: 359-378.

[516] Piazza PV, Maccari S, Deminiere JM, Le Moal M, Mormede P, Simon H. Corticosterone levels determine individual vulnerability to amphetamine self-administration. *Proc Natl Acad Sci U S A* 1991; 88(6): 2088-2092.

[517] Piazza PV, Marinelli M, Jodogne C, Deroche V, Rouge-Pont F, Maccari S, Le Moal M, Simon H. Inhibition of corticosterone synthesis by Metyrapone decreases cocaine- induced locomotion and relapse of cocaine self-administration. *Brain Res* 1994; 658(1-2): 259-264.

[518] Piazza PV, Rouge-Pont F, Deroche V, Maccari S, Simon H, Le Moal M. Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. *Proc Natl Acad Sci U S A* 1996; 93(16): 8716-8720.

[519] Pierce RC, Kalivas PW. Amphetamine produces sensitized increases in locomotion and extracellular dopamine preferentially in the nucleus accumbens shell of rats administered repeated cocaine. *J Pharmacol Exp Ther* 1995; 275(2): 1019-1029.

[520] Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res Brain Res Rev* 1997; 25(2): 192-216.

[521] Pierce RC, Reeder DC, Hicks J, Morgan ZR, Kalivas PW. Ibotenic acid lesions of the dorsal prefrontal cortex disrupt the expression of behavioral sensitization to cocaine. *Neuroscience* 1997; 82(4): 1103-1114.

[522] Pierre PJ, Vezina P. Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. *Psychopharmacology (Berl)* 1997; 129(3): 277-284.

[523] Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz JC, Everitt BJ, Sokoloff P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* 1999; 400(6742): 371-375.

[524] Pitts DK, Marwah J. Autonomic actions of cocaine. *Can J Physiol Pharmacol* 1989; 67(9): 1168-1176.

[525] Plotsky PM, Meaney MJ. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Brain Res Mol Brain Res* 1993; 18(3): 195-200.

[526] Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. *Proc Natl Acad Sci U S A* 1995; 92(26): 12304-12308.

[527] Prasad BM, Ulibarri C, Kalivas PW, Sorg BA. Effect of adrenalectomy on the initiation and expression of cocaine-induced sensitization. *Psychopharmacology (Berl)* 1996; 125(3): 265-273.

[528] Pryce CR, Ruedi-Bettschen D, Dettling AC, Feldon J. Early life stress: long-term physiological impact in rodents and primates. *News Physiol Sci* 2002; 17(4): 150-155.

[529] Przegalinski E, Filip M, Siwanowicz J, Nowak E. Effect of adrenalectomy and corticosterone on cocaine-induced sensitization in rats. *J Physiol Pharmacol* 2000; 51(2): 193-204.

[530] Przegalinski E, Filip M, Frankowska M, Zaniewska M, Papla I. Effects of CP 154,526, a CRF1 receptor antagonist, on behavioral responses to cocaine in rats. *Neuropeptides* 2005; 39(5): 525-533.

[531] Puglisi-Allegra S, Cabib S. Psychopharmacology of dopamine: the contribution of comparative studies in inbred strains of mice. *Prog Neurobiol* 1997; 51(6): 637-661.

[532] Puglisi-Allegra S, Imperato A, Angelucci L, Cabib S. Acute stress induces time-dependent responses in dopamine mesolimbic system. *Brain Res* 1991; 554(1-2): 217-222.

[533] Puglisi-Allegra S, Kempf E, Cabib S. Role of genotype in the adaptation of the brain dopamine system to stress. *Neurosci Biobehav Rev* 1990; 14(4): 523-528.

[534] Pulvirenti L, Swerdlow NR, Koob GF. Microinjection of a glutamate antagonist into the nucleus accumbens reduces psychostimulant locomotion in rats. *Neurosci Lett* 1989; 103(2): 213-218.

[535] Quirarte GL, Roozendaal B, McGaugh JL. Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. *Proc Natl Acad Sci U S A* 1997; 94(25): 14048-14053.

[536] Ramsey NF, Van Ree JM. Emotional but not physical stress enhances intravenous cocaine self-administration in drug-naive rats. *Brain Res* 1993; 608(2): 216-222.

[537] Ranaldi R, Pocock D, Zereik R, Wise RA. Dopamine fluctuations in the nucleus accumbens during maintenance, extinction, and reinstatement of intravenous d-Amphetamine self-administration. *J Neurosci* 1999; 19(10): 4102-4109.

[538] Ranaldi R, Wise RA. Blockade of D1 dopamine receptors in the ventral tegmental area decreases cocaine reward: possible role for dendritically released dopamine. *J Neurosci* 2001; 21(15): 5841-5846.

[539] Reid MS, Berger SP. Evidence for sensitization of cocaine-induced nucleus accumbens glutamate release. *Neuroreport* 1996; 7(7): 1325-1329.

[540] Reid MS, Hsu K, Jr., Berger SP. Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. *Synapse* 1997; 27(2): 95-105.

[541] Reul JM, De Kloet ER. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology* 1985; 117(6): 2505-2511.

[542] Rhoads DL. A longitudinal study of life stress and social support among drug abusers. *Int J Addict* 1983; 18(2): 195-222.

[543] Richtand NM, Woods SC, Berger SP, Strakowski SM. D3 dopamine receptor, behavioral sensitization, and psychosis. *Neurosci Biobehav Rev* 2001; 25(5): 427-443.

[544] Richter RM, Weiss F. In vivo CRF release in rat amygdala is increased during cocaine withdrawal in self-administering rats. *Synapse* 1999; 32(4): 254-261.

[545] Richter RM, Pich EM, Koob GF, Weiss F. Sensitization of cocaine-stimulated increase in extracellular levels of corticotropin-releasing factor from the rat amygdala after repeated administration as determined by intracranial microdialysis. *Neurosci Lett* 1995; 187(3): 169-172.

[546] Rifkin A. Extrapyramidal side effects: a historical perspective. *J Clin Psychiatry* 1987; 48(suppl): 3-6.

[547] Risbrough VB, Stein MB. Role of corticotropin releasing factor in anxiety disorders: a translational research perspective. *Horm Behav* 2006; 50(4): 550-561.

[548] Risner M, Jones BE. Role of noradrenergic and dopaminergic processes in amphetamine self-administration. *Pharmacol Biochem Behav* 1976; 5(4): 477-482.

[549] Ritz MC, Cone EJ, Kuhar MJ. Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study. *Life Sci* 1990; 46(9): 635-645.

[550] Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self- administration of cocaine. *Science* 1987; 237(4819): 1219-1223.

[551] Rivera MA, Aufderheide AC, Cartmell LW, Torres CM, Langsjoen O. Antiquity of coca-leaf chewing in the south central Andes: a 3,000 year archaeological record of coca-leaf chewing from northern Chile. *J Psychoactive Drugs* 2005; 37(4): 455-458.

[552] Rivet JM, Stinus L, LeMoal M, Mormede P. Behavioral sensitization to amphetamine is dependent on corticosteroid receptor activation. *Brain Res* 1989; 498(1): 149-153.

[553] Roberts AJ, Crabbe JC, Keith LD. Genetic differences in hypothalamic-pituitary-adrenal axis responsiveness to acute ethanol and acute ethanol withdrawal. *Brain Res* 1992; 579(2): 296-302.

[554] Roberts DC. Preclinical evidence for GABAB agonists as a pharmacotherapy for cocaine addiction. *Physiol Behav* 2005; 86(1-2): 18-20.

[555] Roberts DC, Andrews MM, Vickers GJ. Baclofen attenuates the reinforcing effects of cocaine in rats. *Neuropsychopharmacology* 1996; 15(4): 417-423.

[556] Roberts DC, Corcoran ME, Fibiger HC. On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav* 1977; 6(6): 615-620.

[557] Roberts DC, Koob GF. Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav* 1982; 17(5): 901-904.

[558] Roberts DC, Phelan R, Hodges LM, Hodges MM, Bennett B, Childers S, Davies H. Self-administration of cocaine analogs by rats. *Psychopharmacology (Berl)* 1999; 144(4): 389-397.

[559] Robinson TE. Stimulant drugs and stress: factors influencing individual differences in the susceptibility to sensitization. In: Kalivas PW, Barnes C (eds). *Sensitization of the Nervous System*. Teldford Press, Caldwell: 1988; 145-173.

[560] Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* 1993; 18(3): 247-291.

[561] Robinson TE, Berridge KC. Incentive-sensitization and addiction. *Addiction* 2001; 96(1): 103-114.

[562] Robinson TE, Browman KE, Crombag HS, Badiani A. Modulation of the induction or expression of psychostimulant sensitization by the circumstances surrounding drug administration. *Neurosci Biobehav Rev* 1998; 22(2): 347-354.

[563] Robinson TE, Gorny G, Mitton E, Kolb B. Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse* 2001; 39(3): 257-266.

[564] Robinson TE, Kolb B. Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur J Neurosci* 1999; 11(5): 1598-1604.

[565] Robinson TE, Kolb B. Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. *J Neurosci* 1997; 17(21): 8491-8497.

[566] Robinson TG, Beart PM. Excitant amino acid projections from rat amygdala and thalamus to nucleus accumbens. *Brain Res Bull* 1988; 20(4): 467-471.

[567] Rocha BA, Fumagalli F, Gainetdinov RR, Jones SR, Ator R, Giros B, Miller GW, Caron MG. Cocaine self-administration in dopamine-transporter knockout mice. *Nat Neurosci* 1998; 1(2): 132-137.

[568] Rocha BA, Odom LA, Barron BA, Ator R, Wild SA, Forster MJ. Differential responsiveness to cocaine in C57BL/6J and DBA/2J mice. *Psychopharmacology (Berl)* 1998; 138(1): 82-88.

[569] Rodd-Henricks ZA, McKinzie DL, Li TK, Murphy JM, McBride WJ. Cocaine is self-administered into the shell but not the core of the nucleus accumbens of Wistar rats. *J Pharmacol Exp Ther* 2002; 303(3): 1216-1226.

[570] Roozendaal B. Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. *Neurobiol Learn Mem* 2002; 78(3): 578-595.

[571] Roozendaal B, Carmi O, McGaugh JL. Adrenocortical suppression blocks the memory-enhancing effects of amphetamine and epinephrine. *Proc Natl Acad Sci U S A* 1996; 93(4): 1429-1433.

[572] Roozendaal B, de Quervain DJF, Ferry B, Setlow B, McGaugh JL. Basolateral amygdala-nucleus accumbens interactions in mediating glucocorticoid enhancement of memory consolidation. *J Neurosci* 2001; 21(7): 2518-2525.

[573] Roozendaal B, McGaugh JL. The memory-modulatory effects of glucocorticoids depend on an intact stria terminalis. *Brain Res* 1996; 709(2): 243-250.

[574] Roozendaal B, Okuda S, Van der Zee EA, McGaugh JL. Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proc Natl Acad Sci U S A* 2006; 103(17): 6741-6746.

[575] Rossi-Arnaud C, Ammassari-Teule M. What do comparative studies of inbred mice add to current investigations on the neural basis of spatial behaviors? *Exp Brain Res* 1998; 123(1-2): 36-44.

[576] Rots NY, Cools AR, Berod A, Voorn P, Rostene W, De Kloet ER. Rats bred for enhanced apomorphine susceptibility have elevated tyrosine hydroxylase mRNA and dopamine D2-receptor binding sites in nigrostriatal and tuberoinfundibular dopamine systems. *Brain Res* 1996; 710(1-2): 189-196.

[577] Rouge-Pont F, Marinelli M, Le Moal M, Simon H, Piazza PV. Stress-induced sensitization and glucocorticoids. II. Sensitization of the increase in extracellular dopamine induced by cocaine depends on stress-induced corticosterone secretion. *J Neurosci* 1995; 15(11): 7189-7195.

[578] Rouge-Pont F, Piazza PV, Kharouby M, Le Moal M, Simon H. Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self- administration. A microdialysis study. *Brain Res* 1993; 602(1): 169-174.

[579] Rouge-Pont F, Abrous DN, Le Moal M, Piazza PV. Release of endogenous dopamine in cultured mesencephalic neurons: influence of dopaminergic agonists and glucocorticoid antagonists. *Eur J Neurosci* 1999; 11(7): 2343-2350.

[580] Rouge-Pont F, Deroche V, Moal ML, Piazza PV. Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. *Eur J Neurosci* 1998; 10(12): 3903-3907.

[581] Roullet P, Lassalle JM. Genetic variation, hippocampal mossy fibres distribution, novelty reactions and spatial representation in mice. *Behav Brain Res* 1990; 41(1): 61-70.

[582] Saal D, Dong Y, Bonci A, Malenka RC. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* 2003; 37(4): 577-582.

[583] Sabban EL, Kvetnansky R. Stress-triggered activation of gene expression in catecholaminergic systems: dynamics of transcriptional events. *Trends Neurosci* 2001; 24(2): 91-98.

[584] Sajadi AA, Samaei SA, Rashidy-Pour A. Intra-hippocampal microinjections of anisomycin did not block glucocorticoid-induced impairment of memory retrieval in rats: an evidence for non-genomic effects of glucocorticoids. *Behav Brain Res* 2006; 173(1): 158-162.

[585] Saladin ME, Drobes DJ, Coffey SF, Dansky BS, Brady KT, Kilpatrick DG. PTSD symptom severity as a predictor of cue-elicited drug craving in victims of violent crime. *Addict Behav* 2003; 28(9): 1611-1629.

[586] Saleptsi E, Bichescu D, Rockstroh B, Neuner F, Schauer M, Studer K, Hoffmann K, Elbert T. Negative and positive childhood experiences across developmental periods in psychiatric patients with different diagnoses - an explorative study. *BMC Psychiatry* 2004; 4(1): 40.

[587] Salomon L, Lanteri C, Glowinski J, Tassin JP. Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons. *Proc Natl Acad Sci U S A* 2006; 103(19): 7476-7481.

[588] Samaha AN, Robinson TE. Why does the rapid delivery of drugs to the brain promote addiction? *Trends Pharmacol Sci* 2005; 26(2): 82-87.

[589] Sandi C, Venero C, Guaza C. Novelty-related rapid locomotor effects of corticosterone in rats. *Eur J Neurosci* 1996; 8(4): 794-800.

[590] Saphier D, Welch JE, Farrar GE, Goeders NE. Effects of intracerebroventricular and intrahypothalamic cocaine administration on adrenocortical secretion. *Neuroendocrinology* 1993; 57(1): 54-62.

[591] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 2000; 21(1): 55-89.

[592] Sarnyai Z. Neurobiology of stress and cocaine addiction. Studies on corticotropin- releasing factor in rats, monkeys, and humans. *Ann N Y Acad Sci* 1998; 851: 371-387.

[593] Sarnyai Z, Biro E, Gardi J, Vecsernyes M, Julesz J, Telegdy G. Brain corticotropin-releasing factor mediates 'anxiety-like' behavior induced by cocaine withdrawal in rats. *Brain Res* 1995; 675(1-2): 89-97.

[594] Sarnyai Z, Biro E, Gardi J, Vecsernyes M, Julesz J, Telegdy G. Alterations of corticotropin-releasing

factor-like immunoreactivity in different brain regions after acute cocaine administration in rats. *Brain Res* 1993; 616(1-2): 315-319.

[595] Sarnyai Z, Biro E, Penke B, Telegdy G. The cocaine-induced elevation of plasma corticosterone is mediated by endogenous corticotropin-releasing factor (CRF) in rats. *Brain Res* 1992; 589(1): 154-156.

[596] Sarnyai Z, Biro E, Telegdy G. Cocaine-induced elevation of plasma corticosterone is mediated by different neurotransmitter systems in rats. *Pharmacol Biochem Behav* 1993; 45(1): 209-214.

[597] Sarnyai Z, Hohn J, Szabo G, Penke B. Critical role of endogenous corticotropin-releasing factor (CRF) in the mediation of the behavioral action of cocaine in rats. *Life Sci* 1992; 51(26): 2019-2024.

[598] Sarnyai Z, McKittrick CR, McEwen BS, Kreek MJ. Selective regulation of dopamine transporter binding in the shell of the nucleus accumbens by adrenalectomy and corticosterone-replacement. *Synapse* 1998; 30(3): 334-337.

[599] Sarnyai Z, Shaham Y, Heinrichs SC. The role of corticotropin-releasing factor in drug addiction. *Pharmacol Rev* 2001; 53(2): 209-243.

[600] Sarnyai Z, Szabo G, Kovacs GL, Telegdy G. Opposite actions of oxytocin and vasopressin in the development of cocaine-induced behavioral sensitization in mice. *Pharmacol Biochem Behav* 1992; 43(2): 491-494.

[601] Sarnyai Z, Veldhuis JD, Mello NK, Mendelson JH, Eros-Sarnyai M, Mercer G, Gelles H, Kelly M. The concordance of pulsatile ultradian release of adrenocorticotropin and cortisol in male rhesus monkeys. *J Clin Endocrinol Metab* 1995; 80(1): 54-59.

[602] Schank JR, Ventura R, Puglisi-Allegra S, Alcaro A, Cole CD, Liles LC, Seeman P, Weinshenker D. Dopamine beta-hydroxylase knockout mice have alterations in dopamine signaling and are hypersensitive to cocaine. *Neuropsychopharmacology* 2006; 31: 2221-2230.

[603] Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin AS, Jr. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol* 1995; 15(2): 943-953.

[604] Schenk S, Gittings D. Effects of SCH 23390 and eticlopride on cocaine-seeking produced by cocaine and WIN 35,428 in rats. *Psychopharmacology (Berl)* 2003; 168(1-2): 118-123.

[605] Schenk S, Horger BA, Peltier R, Shelton K. Supersensitivity to the reinforcing effects of cocaine following 6-hydroxydopamine lesions to the medial prefrontal cortex in rats. *Brain Res* 1991; 543(2): 227-235.

[606] Schenk S, Lacelle G, Gorman K, Amit Z. Cocaine self-administration in rats influenced by environmental conditions: implications for the etiology of drug abuse. *Neurosci Lett* 1987; 81(1-2): 227-231.

[607] Schenk S, Partridge B. Sensitization to cocaine's reinforcing effects produced by various cocaine pretreatment regimens in rats. *Pharmacol Biochem Behav* 2000; 66(4): 765-770.

[608] Schmidt ED, Schoffelmeer ANM, De Vries TJ, Wardeh G, Dogterom G, Bol JGJM, Binnekade R, Tilders FJH. A single administration of interleukin-1 or amphetamine induces long-lasting

increases in evoked noradrenaline release in the hypothalamus and sensitization of ACTH and corticosterone responses in rats. *Eur J Neurosci* 2001; 13(10): 1923-1930.

[609] Schmidt ED, Tilders FJ, Binnekade R, Schoffelmeer AN, De Vries TJ. Stressor- or drug-induced sensitization of the corticosterone response is not critically involved in the long-term expression of behavioural sensitization to amphetamine. *Neuroscience* 1999; 92(1): 343-352.

[610] Schmidt ED, Tilders FJ, Janszen AW, Binnekade R, De Vries TJ, Schoffelmeer AN. Intermittent cocaine exposure causes delayed and long-lasting sensitization of cocaine-induced ACTH secretion in rats. *Eur J Pharmacol* 1995; 285(3): 317-321.

[611] Seale TW, Carney JM. Genetic determinants of susceptibility to the rewarding and other behavioral actions of cocaine. *J Addict Dis* 1991; 10(1-2): 141-162.

[612] See RE, Kruzich PJ, Grimm JW. Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. *Psychopharmacology (Berl)* 2001; 154(3): 301-310.

[613] Self DW, Barnhart WJ, Lehman DA, Nestler EJ. Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. *Science* 1996; 271(5255): 1586-1589.

[614] Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ. Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. *J Neurosci* 1998; 18(5): 1848-1859.

[615] Selye H. A syndrome produced by diverse nocuous agents. *Nature* 1936; 138: 32.

[616] Setlow B, Roozendaal B, McGaugh JL. Involvement of a basolateral amygdala complex-nucleus accumbens pathway in glucocorticoid-induced modulation of memory consolidation. *Eur J Neurosci* 2000; 12(1): 367-375.

[617] Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor1 receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. *Psychopharmacology (Berl)* 1998; 137(2): 184-190.

[618] Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Res Brain Res Rev* 2000; 33(1): 13-33.

[619] Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J. Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. *J Neurosci* 1997; 17(7): 2605-2614.

[620] Shaham Y, Highfield D, Delfs J, Leung S, Stewart J. Clonidine blocks stress-induced reinstatement of heroin seeking in rats: an effect independent of locus coeruleus noradrenergic neurons. *Eur J Neurosci* 2000; 12(1): 292-302.

[621] Shalev U, Marinelli M, Baumann MH, Piazza PV, Shaham Y. The role of corticosterone in food deprivation-induced reinstatement of cocaine seeking in the rat. *Psychopharmacology (Berl)* 2003; 168(1-2): 170-176.

[622] Shanks N, Griffiths J, Zalcman S, Zacharko RM, Anisman H. Mouse strain differences in plasma corticosterone following uncontrollable footshock. *Pharmacol Biochem Behav* 1990; 36(3): 515-519.

[623] Shilling PD, Kelsoe JR, Segal DS. Dopamine transporter mRNA is up-regulated in the substantia

nigra and the ventral tegmental area of amphetamine-sensitized rats. *Neurosci Lett* 1997; 236(3): 131-134.

[624] Shippenberg TS, Heidbreder C. Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. *J Pharmacol Exp Ther* 1995; 273(2): 808-815.

[625] Shoaib M, Swanner LS, Beyer CE, Goldberg SR, Schindler CW. The GABAB agonist baclofen modifies cocaine self-administration in rats. *Behav Pharmacol* 1998; 9(3): 195-206.

[626] Simar MR, Saphier D, Goeders NE. Differential neuroendocrine and behavioral responses to cocaine in Lewis and Fischer rats. *Neuroendocrinology* 1996; 63(1): 93-100.

[627] Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ. Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. *Arch Gen Psychiatry* 2006; 63(3): 324-331.

[628] Sinha R, Lacadie C, Skudlarski P, Fulbright RK, Rounsaville BJ, Kosten TR, Wexler BE. Neural activity associated with stress-induced cocaine craving: a functional magnetic resonance imaging study. *Psychopharmacology (Berl)* 2005; 183(2): 171-180.

[629] Smith JE, Co C, Yin X, Sizemore GM, Liguori A, Johnson III, Martin TJ. Involvement of cholinergic neuronal systems in intravenous cocaine self-administration. *Neurosci Biobehav Rev* 2004; 27(8): 841-850.

[630] Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* 1990; 347(6289): 146-151.

[631] Sonuga-Barke EJS. Causal models of attention-deficit/hyperactivity disorder: from common simple deficits to multiple developmental pathways. *Biol Psychiatry* 2005; 57(11): 1231-1238.

[632] Sora I, Wichems C, Takahashi N, Li XF, Zeng Z, Revay R, Lesch KP, Murphy DL, Uhl GR. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. *Proc Natl Acad Sci U S A* 1998; 95(13): 7699-7704.

[633] Sorg BA, Chen SY, Kalivas PW. Time course of tyrosine hydroxylase expression after behavioral sensitization to cocaine. *J Pharmacol Exp Ther* 1993; 266(1): 424-430.

[634] Sorg BA, Davidson DL, Kalivas PW, Prasad BM. Repeated daily cocaine alters subsequent cocaine-induced increase of extracellular dopamine in the medial prefrontal cortex. *J Pharmacol Exp Ther* 1997; 281(1): 54-61.

[635] Sorg BA, Kalivas PW. Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum. *Brain Res* 1991; 559(1): 29-36.

[636] Spencer RL, Young EA, Choo PH, McEwen BS. Adrenal steroid type I and type II receptor binding: estimates of in vivo receptor number, occupancy, and activation with varying level of steroid. *Brain Res* 1990; 514(1): 37-48.

[637] Stachowiak MK, Rigual RJ, Lee PH, Viveros OH, Hong JS. Regulation of tyrosine hydroxylase and phenylethanolamine N-methyltransferase mRNA levels in the sympathoadrenal system by the pituitary-adrenocortical axis. *Brain Res* 1988; 427(3): 275-286.

[638] Stein-Behrens BA, Lin WJ, Sapolsky RM. Physiological elevations of glucocorticoids potentiate glutamate accumulation in the hippocampus. *J Neurochem* 1994; 63(2): 596-602.

[639] Steketee JD. Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Res Brain Res Rev* 2003; 41(2-3): 203-228.

[640] Stern E, Silbersweig DA, Chee KY, Holmes A, Robertson MM, Trimble M, Frith CD, Frackowiak RS, Dolan RJ. A functional neuroanatomy of tics in Tourette syndrome. *Arch Gen Psychiatry* 2000; 57(8): 741-748.

[641] Sternberg DB, Isaacs KR, Gold PE, McGaugh JL. Epinephrine facilitation of appetitive learning: attenuation with adrenergic receptor antagonists. *Behav Neural Biol* 1985; 44(3): 447-453.

[642] Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol* 2005; 75(6): 406-433.

[643] Sun W, Akins CK, Mattingly AE, Rebec GV. Ionotropic glutamate receptors in the ventral tegmental area regulate cocaine-seeking behavior in rats. *Neuropsychopharmacology* 2005; 30(11): 2073-2081.

[644] Sun W, Rebec GV. The role of prefrontal cortex D1-like and D2-like receptors in cocaine-seeking behavior in rats. *Psychopharmacology (Berl)* 2005; 177(3): 315-323.

[645] Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HH, Niznik HB. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* 1991; 350(6319): 614-619.

[646] Sunahara RK, Niznik HB, Weiner DM, Stormann TM, Brann MR, Kennedy JL, Gelernter JE, Rozmahel R, Yang YL, Israel Y. Human dopamine D1 receptor encoded by an intronless gene on chromosome 5. *Nature* 1990; 347(6288): 80-83.

[647] Suto N, Austin JD, Tanabe LM, Kramer MK, Wright DA, Vezina P. Previous exposure to VTA amphetamine enhances cocaine self-administration under a progressive ratio schedule in a D1 dopamine receptor dependent manner. *Neuropsychopharmacology* 2002; 27(6): 970-979.

[648] Svenningsson P, Nairn AC, Greengard P. DARPP-32 mediates the actions of multiple drugs of abuse. *AAPS J* 2005; 7(2): E353-E360.

[649] Svingos AL, Periasamy S, Pickel VM. Presynaptic dopamine D(4) receptor localization in the rat nucleus accumbens shell. *Synapse* 2000; 36(3): 222-232.

[650] Swanson CJ, Kalivas PW. Regulation of locomotor activity by metabotropic glutamate receptors in the nucleus accumbens and ventral tegmental area. *J Pharmacol Exp Ther* 2000; 292(1): 406-414.

[651] Szantai E, Szmola R, Sasvari-Szekely M, Guttman A, Ronai Z. The polymorphic nature of the human dopamine D4 receptor gene: a comparative analysis of known variants and a novel 27 bp deletion in the promoter region. *BMC Genet* 2005; 6(1): 39.

[652] Szilagyi A, Boor K, Szekely A, Gaszner P, Kalasz H, Sasvari-Szekely M, Barta C. Combined effect of promoter polymorphisms in the dopamine D4 receptor and the serotonin transporter genes in heroin dependence. *Neuropsychopharmacol Hung* 2005; 7(1): 28-33.

[653] Szumlinski KK, Dehoff MH, Kang SH, Frys KA, Lominac KD, Klugmann M, Rohrer J, Griffin III, Toda S. Homer proteins regulate sensitivity to cocaine. *Neuron* 2004; 43(3): 401-413.

[654] Taber MT, Das S, Fibiger HC. Cortical regulation of subcortical dopamine release: mediation via the ventral tegmental area. *J Neurochem* 1995; 65(3): 1407-1410.

[655] Takahashi T, Kimoto T, Tanabe N, Hattori TA, Yasumatsu N, Kawato S. Corticosterone acutely prolonged N-methyl-d-aspartate receptor-mediated Ca2+ elevation in cultured rat hippocampal neurons. *J Neurochem* 2002; 83(6): 1441-1451.

[656] Tanaka SC, Doya K, Okada G, Ueda K, Okamoto Y, Yamawaki S. Prediction of immediate and future rewards differentially recruits cortico-basal ganglia loops. *Nat Neurosci* 2004; 7(8): 887-893.

[657] Tang WX, Fasulo WH, Mash DC, Hemby SE. Molecular profiling of midbrain dopamine regions in cocaine overdose victims. *J Neurochem* 2003; 85(4): 911-924.

[658] Tessari M, Pilla M, Andreoli M, Hutcheson DM, Heidbreder CA. Antagonism at metabotropic glutamate 5 receptors inhibits nicotine- and cocaine-taking behaviours and prevents nicotine-triggered relapse to nicotine-seeking. *Eur J Pharmacol* 2004; 499(1-2): 121-133.

[659] Tiberi M, Jarvie KR, Silvia C, Falardeau P, Gingrich JA, Godinot N, Bertrand L, Yang-Feng TL, Fremeau RT, Jr., Caron MG. Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. *Proc Natl Acad Sci U S A* 1991; 88(17): 7491-7495.

[660] Tidey JW, Miczek KA. Acquisition of cocaine self-administration after social stress: role of accumbens dopamine. *Psychopharmacology (Berl)* 1997; 130(3): 203-212.

[661] Tiffany ST. A cognitive model of drug urges and drug-use behavior: role of automatic and nonautomatic processes. *Psychol Rev* 1990; 97(2): 147-168.

[662] Toda S, McGinty JF, Kalivas PW. Repeated cocaine administration alters the expression of genes in corticolimbic circuitry after a 3-week withdrawal: a DNA macroarray study. *J Neurochem* 2002; 82(5): 1290-1299.

[663] Todtenkopf MS, Mihalakopoulos A, Stellar JR. Withdrawal duration differentially affects c-fos expression in the medial prefrontal cortex and discrete subregions of the nucleus accumbens in cocaine-sensitized rats. *Neuroscience* 2002; 114(4): 1061-1069.

[664] Tolliver BK, Belknap JK, Woods WE, Carney JM. Genetic analysis of sensitization and tolerance to cocaine. *J Pharmacol Exp Ther* 1994; 270(3): 1230-1238.

[665] Tolliver BK, Carney JM. Locomotor stimulant effects of cocaine and novel cocaine analogs in DBA/2J and C57BL/6J inbred mice. *Pharmacol Biochem Behav* 1995; 50(2): 163-169.

[666] Tolliver BK, Carney JM. Sensitization to stereotypy in DBA/2J but not C57BL/6J mice with repeated cocaine. *Pharmacol Biochem Behav* 1994; 48(1): 169-173.

[667] Tracy JI, de Leon J, Qureshi G, McCann EM, McGrory A, Josiassen RC. Repetitive behaviors in schizophrenia: a single disturbance or discrete symptoms? *Schizophr Res* 1996; 20(1-2): 221-229.

[668] Trapp T, Holsboer F. Heterodimerization between mineralocorticoid and glucocorticoid receptors increases the functional diversity of corticosteroid action. *Trends Pharmacol Sci* 1996; 17(4): 145-149.

[669] Tricot JP. Cocaine: half a century of therapeutic use (1880-1930). *Verh K Acad Geneeskd Belg* 1991; 53(5): 487-496.

[670] Tsuang MT, Lyons MJ, Eisen SA, Goldberg J, True W, Lin N, Meyer JM, Toomey R, Faraone SV,

Eaves L. Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. *Am J Med Genet* 1996; 67(5): 473-477.

[671] Tsujimoto S, Sawaguchi T. Neuronal activity representing temporal prediction of reward in the primate prefrontal cortex. *J Neurophysiol* 2005; 93(6): 3687-3692.

[672] U'Prichard DC, Bylund DB, Snyder SH. (+/-)-[3H]Epinephrine and (-)[3H]dihydroalprenolol binding to beta1- and beta2-noradrenergic receptors in brain, heart, and lung membranes. *J Biol Chem* 1978; 253(14): 5090-5102.

[673] U'Prichard DC, Synder SH. Differential labelling of alpha and beta-noradrenergic receptors in calf cerebellum membranes with 3H-adrenaline. *Nature* 1977; 270(5634): 261-263.

[674] U'Prichard DC, Greenberg DA, Sheehan P, Snyder SH. Regional distribution of alpha-noradrenergic receptor binding in calf brain. *Brain Res* 1977; 138(1): 151-158.

[675] Ujike H, Akiyama K, Otsuki S. D-2 but not D-1 dopamine agonists produce augmented behavioral response in rats after subchronic treatment with methamphetamine or cocaine. *Psychopharmacology (Berl)* 1990; 102(4): 459-464.

[676] Ungless MA, Whistler JL, Malenka RC, Bonci A. Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* 2001; 411(6837): 583-587.

[677] Upchurch M, Wehner JM. Differences between inbred strains of mice in Morris water maze performance. *Behav Genet* 1988; 18(1): 55-68.

[678] Valadez A, Schenk S. Persistence of the ability of amphetamine preexposure to facilitate acquisition of cocaine self-administration. *Pharmacol Biochem Behav* 1994; 47(1): 203-205.

[679] Valjent E, Corvol JC, Pages C, Besson MJ, Maldonado R, Caboche J. Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J Neurosci* 2000; 20(23): 8701-8709.

[680] Van Bockstaele EJ, Pickel VM. GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res* 1995; 682(1-2): 215-221.

[681] van den Buuse M, Morris M, Chavez C, Martin S, Wang J. Effect of adrenalectomy and corticosterone replacement on prepulse inhibition and locomotor activity in mice. *Br J Pharmacol* 2004; 142(3): 543-550.

[682] van der Elst MC, Roubos EW, Ellenbroek BA, Veening JG, Cools AR. Apomorphine-susceptible rats and apomorphine-unsusceptible rats differ in the tyrosine hydroxylase-immunoreactive network in the nucleus accumbens core and shell. *Exp Brain Res* 2005; 160(4): 418-423.

[683] van der Elst MCJ, Verheij MMM, Roubos EW, Ellenbroek BA, Veening JG, Cools AR. A single exposure to novelty differentially affects the accumbal dopaminergic system of apomorphine-susceptible and apomorphine-unsusceptible rats. *Life Sci* 2005; 76(12): 1391-1406.

[684] van der Kam EL, Ellenbroek BA, Cools AR. Gene - environment interactions determine the individual variability in cocaine self-administration. *Neuropharmacology* 2005; 48(5): 685-695.

[685] Van Eekelen JA, De Kloet ER. Co-localization of brain corticosteroid receptors in the rat hippocampus. *Prog Histochem Cytochem* 1992; 26(1-4): 250-258.

[686] Van Eekelen JA, Jiang W, De Kloet ER, Bohn MC. Distribution of the mineralocorticoid and the glucocorticoid receptor mRNAs in the rat hippocampus. *J Neurosci Res* 1988; 21(1): 88-94.

[687] Van Ree JM, Burbach-Bloemarts EM, Wallace M. Vasopressin neuropeptides and acquisition of heroin and cocaine self-administration in rats. *Life Sci* 1988; 42(10): 1091-1099.

[688] Van Ree JM, Niesink RJM, Van Wolfswinkel L, Ramsey NF, Kornet M, Van Furth WR, Vanderschuren LJMJ, Gerrits MAFM, Van den Berg CL. Endogenous opioids and reward. *Eur J Pharmacol* 2000; 405(1-3): 89-101.

[689] Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* 1991; 350(6319): 610-614.

[690] Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V. Multiple dopamine D4 receptor variants in the human population. *Nature* 1992; 358(6382): 149-152.

[691] Vanderschuren LJ, Beemster P, Schoffelmeer AN. On the role of noradrenaline in psychostimulant-induced psychomotor activity and sensitization. *Psychopharmacology (Berl)* 2003; 169(2): 176-185.

[692] Vanderschuren LJ, Everitt BJ. Drug seeking becomes compulsive after prolonged cocaine self-administration. *Science* 2004; 305(5686): 1017-1019.

[693] Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berl)* 2000; 151(2-3): 99-120.

[694] Vanderschuren LJ, Schmidt ED, De Vries TJ, Van Moorsel CA, Tilders FJ, Schoffelmeer AN. A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. *J Neurosci* 1999; 19(21): 9579-9586.

[695] Vanderschuren LJ, Schoffelmeer AN, Mulder AH, De Vries TJ. Dopaminergic mechanisms mediating the long-term expression of locomotor sensitization following pre-exposure to morphine or amphetamine. *Psychopharmacology (Berl)* 1999; 143(3): 244-253.

[696] Vanderschuren LJ, Schoffelmeer AN, Van Leeuwen SD, Hof L, Jonker AJ, Voorn P. Compartment-specific changes in striatal neuronal activity during expression of amphetamine sensitization are the result of drug hypersensitivity. *Eur J Neurosci* 2002; 16(12): 2462-2468.

[697] Vanderschuren LJMJ, Di Ciano P, Everitt BJ. Involvement of the dorsal striatum in cue-controlled cocaine seeking. *J Neurosci* 2005; 25(38): 8665-8670.

[698] Vanderschuren LJMJ, Everitt BJ. Behavioral and neural mechanisms of compulsive drug seeking. *Eur J Pharmacol* 2005; 526(1-3): 77-88.

[699] Venero C, Borrell J. Rapid glucocorticoid effects on excitatory amino acid levels in the hippocampus: a microdialysis study in freely moving rats. *Eur J Neurosci* 1999; 11(7): 2465-2473.

[700] Ventura R, Alcaro A, Cabib S, Conversi D, Mandolesi L, Puglisi-Allegra S. Dopamine in the medial prefrontal cortex controls genotype-dependent effects of amphetamine on mesoaccumbens dopamine release and locomotion. *Neuropsychopharmacology* 2004; 29(1): 72-80.

[701] Ventura R, Alcaro A, Mandolesi L, Puglisi-Allegra S. In vivo evidence that genetic background controls impulse-dependent dopamine release induced by amphetamine in the nucleus accumbens. *J Neurochem* 2004; 89(2): 494-502.

[702] Ventura R, Cabib S, Alcaro A, Orsini C, Puglisi-Allegra S. Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and mesoaccumbens dopamine release. *J Neurosci* 2003; 23(5): 1879-1885.

[703] Ventura R, Cabib S, Puglisi-Allegra S. Genetic susceptibility of mesocortical dopamine to stress determines liability to inhibition of mesoaccumbens dopamine and to behavioral 'despair' in a mouse model of depression. *Neuroscience* 2002; 115(4): 999-1007.

[704] Ventura R, Cabib S, Puglisi-Allegra S. Opposite genotype-dependent mesocorticolimbic dopamine response to stress. *Neuroscience* 2001; 104(3): 627-631.

[705] Ventura R, Puglisi-Allegra S. Environment makes amphetamine-induced dopamine release in the nucleus accumbens totally impulse-dependent. *Synapse* 2005; 58(3): 211-214.

[706] Vezina P, Stewart J. The effect of dopamine receptor blockade on the development of sensitization to the locomotor activating effects of amphetamine and morphine. *Brain Res* 1989; 499(1): 108-120.

[707] Vezina P, Stewart J. Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. *Brain Res* 1990; 516(1): 99-106.

[708] Vezina P. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neurosci Biobehav Rev* 2004; 27(8): 827-839.

[709] Vezina P, Lorrain DS, Arnold GM, Austin JD, Suto N. Sensitization of midbrain dopamine neuron reactivity promotes the pursuit of amphetamine. *J Neurosci* 2002; 22(11): 4654-4662.

[710] Volkow N, Li TK. The neuroscience of addiction. *Nat Neurosci* 2005; 8(11): 1429-1430.

[711] Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *J Neurosci* 2006; 26(24): 6583-6588.

[712] Vorel SR, Ashby CR, Jr., Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, Gardner EL. Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci* 2002; 22(21): 9595-9603.

[713] Vrana SL, Vrana KE, Koves TR, Smith JE, Dworkin SI. Chronic cocaine administration increases CNS tyrosine hydroxylase enzyme activity and mRNA levels and tryptophan hydroxylase enzyme activity levels. *J Neurochem* 1993; 61(6): 2262-2268.

[714] Wachtel SR, Charnot A, De Wit H. Acute hydrocortisone administration does not affect subjective responses to d-amphetamine in humans. *Psychopharmacology (Berl)* 2001; 153(3): 380-388.

[715] Wagner FA, Anthony JC. From first drug use to drug dependence; developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. *Neuropsychopharmacology* 2002; 26(4): 479-488.

[716] Walker CD, Rivest RW, Meaney MJ, Aubert ML. Differential activation of the pituitary-adrenocortical axis after stress in the rat: use of two genetically selected lines (Roman low- and high-avoidance rats) as a model. *J Endocrinol* 1989; 123(3): 477-485.

[717] Walters CL, Blendy JA. Different requirements for cAMP response element binding protein in

positive and negative reinforcing properties of drugs of abuse. *J Neurosci* 2001; 21(23): 9438-9444.

[718] Wang JB, Liu ZF, Kofuji P, Burt DR. The GABA(A) receptor gamma1-subunit in seizure prone (DBA/2) and resistant (C57BL/6) mice. *Brain Res Bull* 1998; 45(4): 421-425.

[719] Ward AS, Collins ED, Haney M, Foltin RW, Fischman MW. Ketoconazole attenuates the cortisol response but not the subjective effects of smoked cocaine in humans. *Behav Pharmacol* 1998; 9(7): 577-586.

[720] Watanabe Y, Weiland NG, McEwen BS. Effects of adrenal steroid manipulations and repeated restraint stress on dynorphin mRNA levels and excitatory amino acid receptor binding in hippocampus. *Brain Res* 1995; 680(1-2): 217-225.

[721] Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL. Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. *J Pharmacol Exp Ther* 2005; 313(2): 848-854.

[722] Wee S, Wang Z, He R, Zhou J, Kozikowski AP, Woolverton WL. Role of the increased noradrenergic neurotransmission in drug self-administration. *Drug Alcohol Depend* 2006; 82(2): 151-157.

[723] Wei P, Inamdar N, Vedeckis WV. Transrepression of c-jun gene expression by the glucocorticoid receptor requires both AP-1 sites in the c-jun promoter. *Mol Endocrinol* 1998; 12(9): 1322-1333.

[724] Weidenfeld J, Newman ME, Itzik A, Feldman S. Adrenocortical axis responses to adrenergic and glutamate stimulation are regulated by the amygdala. *Neuroreport* 2005; 16(11): 1245-1249.

[725] Weil-Malherbe H, Axelrod J, Tomchick R. Blood-brain barrier for adrenaline. *Science* 1959; 129(3357): 1226-1227.

[726] Weiner DM, Levey AI, Sunahara RK, Niznik HB, O'Dowd BF, Seeman P, Brann MP. D1 and D2 Dopamine Receptor mRNA in Rat Brain. *Proc Natl Acad Sci U S A* 1991; 88(5): 1859-1863.

[727] Weinshank RL, Adham N, Macchi M, Olsen MA, Branchek TA, Hartig PR. Molecular cloning and characterization of a high affinity dopamine receptor (D1 beta) and its pseudogene. *J Biol Chem* 1991; 266(33): 22427-22435.

[728] Weiss IC, Domeney AM, Heidbreder CA, Moreau JL, Feldon J. Early social isolation, but not maternal separation, affects behavioral sensitization to amphetamine in male and female adult rats. *Pharmacol Biochem Behav* 2001; 70(2-3): 397-409.

[729] Wiener HL, Reith MEA. Correlation between cocaine-induced locomotion and cocaine disposition in the brain among four inbred strains of mice. *Pharmacol Biochem Behav* 1990; 36(3): 699-701.

[730] Williams CL, McGaugh JL. Reversible lesions of the nucleus of the solitary tract attenuate the memory-modulating effects of posttraining epinephrine. *Behav Neurosci* 1993; 107(6): 955-962.

[731] Wills TA, Vaccaro D, McNamara G. Novelty seeking, risk taking, and related constructs as predictors of adolescent substance use: an application of Cloninger's theory. *J Subst Abuse* 1994; 6(1): 1-20.

[732] Windle RJ, Wood SA, Shanks N, Lightman SL, Ingram CD. Ultradian rhythm of basal corticosterone

release in the female rat: dynamic interaction with the response to acute stress. *Endocrinology* 1998; 139(2): 443-450.

[733] Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol Rev* 1987; 94(4): 469-492.

[734] Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB, Jr. Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacologia* 1995; 120(1): 10-20.

[735] Wise RA, Rompre PP. Brain dopamine and reward. *Annu Rev Psychol* 1989; 40: 191-225.

[736] Wolf ME, White FJ, Nassar R, Brooderson RJ, Khansa MR. Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. *J Pharmacol Exp Ther* 1993; 264(1): 249-255.

[737] Womer DE, Jones BC, Erwin VG. Characterization of dopamine transporter and locomotor effects of cocaine, GBR 12909, epidepride, and SCH 23390 in C57BL and DBA mice. *Pharmacol Biochem Behav* 1994; 48(2): 327-335.

[738] Wong DL. Epinephrine biosynthesis: hormonal and neural control during stress. *Cell Mol Neurobiol* 2006; 26(4-6): 889-898.

[739] Woolverton WL. Evaluation of the role of norepinephrine in the reinforcing effects of psychomotor stimulants in rhesus monkeys. *Pharmacol Biochem Behav* 1987; 26(4): 835-839.

[740] Xi ZX, Gilbert J, Campos AC, Kline N, Ashby CR, Jr., Hagan JJ, Heidbreder CA, Gardner EL. Blockade of mesolimbic dopamine D3 receptors inhibits stress-induced reinstatement of cocaine-seeking in rats. *Psychopharmacology (Berl)* 2004; 176(1): 57-65.

[741] Xi ZX, Newman AH, Gilbert JG, Pak AC, Peng XQ, Ashby CR, Jr., Gitajn L, Gardner EL. The novel dopamine D3 receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. *Neuropsychopharmacology* 2006; 31(7): 1393-1405.

[742] Xi ZX, Ramamoorthy S, Baker DA, Shen H, Samuvel DJ, Kalivas PW. Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. *J Pharmacol Exp Ther* 2002; 303(2): 608-615.

[743] Yajie D, Lin K, Baoming L, Lan M. Enhanced cocaine self-administration in adult rats with adolescent isolation experience. *Pharmacol Biochem Behav* 2005; 82(4): 673-677.

[744] Yamada S, Yokoo H, Nishi S. Changes in sensitivity of dopamine autoreceptors in rat striatum after subchronic treatment with methamphetamine. *Eur J Pharmacol* 1991; 205(1): 43-47.

[745] Yamaguchi K, Hama H, Watanabe K, Adachi C. Effect of dopamine injection into the anteroventral third ventricular region and the paraventricular nucleus on vasopressin secretion in conscious rats. *Acta Endocrinol (Copenh)* 1992; 127(5): 420-424.

[746] Yamaguchi M, Suzuki T, Abe S, Hori T, Kurita H, Asada T, Okado N, Arai H. Repeated cocaine administration differentially affects NMDA receptor subunit (NR1, NR2A-C) mRNAs in rat brain. *Synapse* 2002; 46(3): 157-169.

[747] Yentis SM, Vlassakov KV. Vassily von Anrep, forgotten pioneer of regional anesthesia. *Anesthesiology* 1999; 90(3): 890-895.

[748] Yi SJ, Johnson KM. Chronic cocaine treatment impairs the regulation of synaptosomal 3H-DA release by D2 autoreceptors. *Pharmacol Biochem Behav* 1990; 36(3): 457-461.

[749] Yin HH, Knowlton BJ, Balleine BW. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *Eur J Neurosci* 2004; 19(1): 181-189.

[750] Yokel RA, Wise RA. Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Psychopharmacology (Berl)* 1976; 48(3): 311-318.

[751] Young EA, Abelson J, Lightman SL. Cortisol pulsatility and its role in stress regulation and health. *Front Neuroendocrinol* 2004; 25(2): 69-76.

[752] Zaborszky L, Alheid GF, Beinfeld MC, Eiden LE, Heimer L, Palkovits M. Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study. *Neuroscience* 1985; 14(2): 427-453.

[753] Zaborszky L, Vadasz C. The midbrain dopaminergic system: anatomy and genetic variation in dopamine neuron number of inbred mouse strains. *Behav Genet* 2001; 31(1): 47-59.

[754] Zahm DS, Brog JS. On the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neuroscience* 1992; 50(4): 751-767.

[755] Zhang L, Zhou R, Li X, Ursano RJ, Li H. Stress-induced change of mitochondria membrane potential regulated by genomic and non-genomic GR signaling: a possible mechanism for hippocampus atrophy in PTSD. *Med Hypotheses* 2006; 66(6): 1205-1208.

[756] Zhang XY, Sanchez H, Kehoe P, Kosten TA. Neonatal isolation enhances maintenance but not reinstatement of cocaine self-administration in adult male rats. *Psychopharmacology (Berl)* 2005; 177(4): 391-399.

[757] Zhou QY, Grandy DK, Thambi L, Kushner JA, Tol HHMV, Cone R, Pribnow D, Salon J, Bunzow JR, Civelli O. Cloning and expression of human and rat D1 dopamine receptors. *Nature* 1990; 347(6288): 76-80.

[758] Zhou Y, Spangler R, Ho A, Jeanne Kreek M. Increased CRH mRNA levels in the rat amygdala during short-term withdrawal from chronic 'binge' cocaine. *Mol Brain Res* 2003; 114(1): 73-79.

[759] Zhu BG, Zhu DH, Chen YZ. Rapid enhancement of high affinity glutamate uptake by glucocorticoids in rat cerebral cortex synaptosomes and human neuroblastoma clone SK-N-SH: possible involvement of G-protein. *Biochem Biophys Res Commun* 1998; 247(2): 261-265.

[760] Zocchi A, Orsini C, Cabib S, Puglisi-Allegra S. Parallel strain-dependent effect of amphetamine on locomotor activity and dopamine release in the nucleus accumbens: an in vivo study in mice. *Neuroscience* 1998; 82(2): 521-528.

[761] Zubieta JK, Gorelick DA, Stauffer R, Ravert HT, Dannals RF, Frost JJ. Increased mu opioid receptor binding detected by PET in cocaine-dependent men is associated with cocaine craving. *Nat Med* 1996; 2(11): 1225-1229.

List of abbreviations

ACTH:	Adrenocorticotrophic hormone
ADX:	Adrenalectomy
Amy (BLA/CE):	Amygdala (basolateral, central nuclei)
ANOVA:	Analysis of variance
ANS:	Autonomic sympathetic nervous system
AVP:	Arginine vasopressin
BNST:	Bed nucleus of the stria terminalis
CNS:	Central nervous system
COC:	Cocaine
COMT:	Catechol-O-methyl transferase
CORT:	Corticosterone
(r/c) CP (Lat, DM, VM):	(rostral/caudal) Caudate putamen (lateral, dorso-medial, ventromedial regions)
CRH:	Corticotrophin releasing hormone
DA:	Dopamine
DAT:	Dopamine transporter
DOPA:	Dihydroxyphenylalanine
DOPAC:	Dihydroxyphenylacetic acid
EPI:	Epinephrine
GABA:	Gamma-aminobutyric acid
GR:	Glucocorticoid receptor
GRE:	Glucocorticoid response element
HPA-axis:	Hypothalamic-pituitary-adrenal axis
HVA:	Homovanillic acid
i.p. / i.v.:	Intraperitoneal / intravenous
LC:	Locus coeruleus
MAO:	Monoamine oxidase
MR:	Mineralocorticoid receptor
NAc:	Nucleus accumbens
NET:	Noradrenaline transporter
NTS:	Nucleus of the solitary tract
(v/m/d) PFC (MO, Prl, IL):	(ventral/medial/dorsal) Prefrontal cortex (medial orbital, prelimbic, infralimbic subdivisions)

POMC:	Pro-opiomelanocortin
PTSD:	Post-traumatic stress disorder
PVN:	Paraventricular nucleus of the hypothalamus
QTL:	Quantitative trait locus
RIA:	Radio-immuno-assay
RU:	Mifepristone, RU38486
SAL:	Saline
SERT:	Serotonin transporter
SHAM:	Sham surgery
SNr / SNc:	Substantia nigra pars reticulata / compacta
TH:	Tyrosine hydroxylase
VEH:	Vehicle
VP:	Ventral pallidum
VTA:	Ventral tegmental area

Curriculum Vitae

Inge Elisabeth Maria de Jong werd op 31 maart 1978 geboren te Leidschendam. Zij behaalde in 1996 haar VWO diploma aan het Veursch College te Leidschendam. Aansluitend begon zij aan de studie Biomedische Wetenschappen aan de Universiteit van Leiden. Als onderdeel van deze studie werden drie wetenschappelijke onderzoeksstages voltooid waarin zij zich specialiseerde in de neurobiologie. Bij de vakgroep Medische Farmacologie (LACDR/LUMC) van de Universiteit Leiden werd onder begeleiding van dr. Roel H. de Rijk, dr. Paul J. Lucassen, dr. Nicole A. Datson en prof. dr. E. Ronald de Kloet onderzoek verricht naar expressie van de α en β splicevarianten van de glucocorticoid receptor in humane lymfocyten en hippocampus. De tweede doctoraalstage werd uitgevoerd bij TNO preventie en gezondheid te Leiden onder begeleiding van dr. Jeffrey J. Bajramović en dr. Hans M. van Noort en betrof onderzoek naar de expressie van het heat-shock eiwit α-B crystalline in humane lymfocyten en astrocyten. De laatste onderzoeksstage vond plaats aan het Mental Health Research Institute of Victoria te Melbourne, Australië onder begeleiding van dr. Maarten van den Buuse en prof. dr. E. Ronald de Kloet en betrof onderzoek naar de rol van dopamine in de prefrontale cortex in diermodellen voor schizofrenie. In januari 2001 werd het doctoraalexamen Biomedische Wetenschappen *cum laude* afgelegd. Aansluitend werd begonnen met het in dit proefschrift beschreven promotieonderzoek bij de vakgroep Medische Farmacologie (Universiteit Leiden). Dit onderzoek werd uitgevoerd onder begeleiding van prof. dr. E. Ronald de Kloet en prof. dr. Melly S. Oitzl. Sinds 1 februari 2007 is de auteur van dit proefschrift werkzaam als post-doc bij de afdeling Psychopharmacology van H. Lundbeck A/S te Valby in Denemarken waar zij werkt aan de ontwikkeling van diermodellen voor de negatieve symptomen van schizofrenie.

Inge Elisabeth Maria de Jong was born on March 31, 1978 in Leidschendam, The Netherlands. She attended secondary school at the Veursch College in Leidschendam and graduated in 1996. In the same year she commenced the study Biomedical Sciences at the University of Leiden, The Netherlands. During three internships she specialised in neurobiology. At the division of Medical Pharmacology (LACDR/LUMC) of Leiden University she investigated the expression of the α and β splice variants of the glucocorticoid receptor in human lymphocytes and hippocampus under supervision of dr. Roel H. de Rijk, dr. Paul J. Lucassen, dr. Nicole A. Datson

and prof. dr. E. Ronald de Kloet. During the second internship, she investigated the expression of the heat-shock protein α-B crystallin in human lymphocytes and astrocytes at TNO 'preventie en gezondheid' in Leiden under supervision of dr. Jeffrey J. Bajramović and dr. Hans M. van Noort. The final internship was performed at the Mental Health Research Institute of Victoria, in Melbourne, Australia. Under supervision of dr. Maarten van den Buuse and prof. dr. E. Ronald de Kloet she investigated the role of dopamine in the prefrontal cortex in animal models for schizophrenia. In January 2001 she graduated with honours (*cum laude*). In the same year she started the PhD studies described in this thesis at the division of Medical Pharmacology (Leiden University). This work was supervised by prof. dr. E. Ronald de Kloet and prof. dr. Melly S. Oitzl. At present, the author of this thesis is employed as a post-doc at the department of Psychopharmacology of H. Lundbeck A/S in Valby, Denmark where she is involved in developing animal models for the negative symptoms of schizophrenia.

Publications

De Jong IEM, Oitzl MS, De Kloet, ER. Adrenalectomy prevents behavioural sensitisation of mice to cocaine in a genotype-dependent manner.
Behav Brain Res 2007; 177(2): 329-339.

De Jong IEM, Van den Buuse M. SCH 23390 in the prefrontal cortex enhances the effect of apomorphine on prepulse inhibition of rats.
Neuropharmacology 2006; 51(3): 438-446.

De Jong IEM, De Kloet ER. Glucocorticoids and vulnerability to psychostimulant drugs: toward substrate and mechanism.
Ann N Y Acad Sci 2004; 1018: 192-198.

De Rijk RH, Schaaf M, Stam FJ, **De Jong IEM**, Swaab DF, Ravid R, Vreugdenhil E, Cidlowski JA, De Kloet ER, Lucassen PJ. Very low levels of the glucocorticoid receptor beta isoform in the human hippocampus as shown by Taqman RT-PCR and immunocytochemistry.
Brain Res Mol Brain Res 2003; 116(1-2): 17-26.

Bajramovic JJ, **De Jong IEM**, Bsibsi M, Quinlan RA, De Groot CJA, Van Noort JM. Phosphorylation of the small heat shock protein αB-crystallin at serine-59 causes a major shift in intracellular distribution of the protein in human astrocytes.
In: *The expression of αB-crystallin in multiple sclerosis brains: where, when how and why?* Bajramović JJ. Febo Druk, Enschede: 2000; 118-128.

De Jong IEM, Steenbergen PJ, De Kloet ER. Behavioural sensitisation to cocaine: cooperation between glucocorticoids and epinephrine.
Submitted for publication.

De Jong IEM, De Kloet ER. Critical time-window for the actions of adrenal glucocorticoids in behavioural sensitisation to cocaine.
Submitted for publication.

De Jong IEM, Steenbergen PJ, De Kloet ER. Strain differences in the effects of adrenalectomy on the midbrain dopamine system: implication for behavioural sensitisation to cocaine.
Submitted for publication.

Have no fear of perfection,
you will never reach it.

Salvador Dali (1904-1989)