Factors contributing to the intake of alcohol and cocaine by rats:

Role of genetic background, early-life events and stressors

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Factors contributing to the intake of alcohol and cocaine by rats:

Role of genetic background, early-life events, and stressors

een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

Proefschrift

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Contents

Chapter 1

General introduction.

This chapter reviews (some of) the factors involved in the onset of drug addiction and will, thus, summarize research concerning the shift from drug use to abuse and the development of drug addiction. Moreover, an overview of the factors involved in the susceptibility as well as the origin of individual differences in this susceptibility will be given. Before dealing with these factors, it is necessary to give a definition of drug use, drug abuse, and, most importantly, drug addiction. The following section will therefore discuss these definitions as well as the wide-spread prevalence of drug addiction and its burden on our society.

1. A definition of addiction

Addiction proves difficult to define because each health-care organisation has its own set of criteria to define it (see box 1). However, some features are mentioned repeatedly. In general, addiction can be defined as drug seeking- and taking- behavior in a compulsive manner. There is no medical indication for the use of this drug, and, after prolonged use, impairment of health and social functioning occur. Drug use and subsequent abuse progresses through several stages; preoccupation/ anticipation (acquisition-maintenance), binge-intoxication (maintenance), and withdrawal/ negative affect (withdrawal).

As a consequence of its compulsive nature, drug addiction is typically a chronic relapsing disorder: addicts can, with difficulty, achieve abstinence, but most of them relapse, even after prolonged periods. These features make drug addiction a serious problem for our society, mostly due to its widespread prevalence (for an example of drug use; see table 1) and its economical, sociological and health-care burden. For instance, the economical burden of illicit (illegal) drug use in the United States has been estimated to be more than 66 billion dollars per year [1,301], and in Europe more than 57 million euro per year [9].

Table 1: *The (estimated) numbers of cocaine and alcohol (ab)users in the United States of America and the Netherlands (2003). The numbers were obtained from the websites and year reports of the Department of Health & Human Services USA [301] and the National Drug Monitor the Netherlands. Note that the table states drug use, and not drug abuse for alcohol; this is due to the fact that the line between use and abuse for alcohol is very difficult to establish since it is a socially excepted and legal drug. However, heavy use is most likely to fall under the category drug abuse, whilst binge use is most likely to fall under the category drug use. All numbers listed in this table are an estimate, real numbers are suspected to be higher.*

Cocaine abuse in the general population

	Year	Age group	Current user (within the last year)
The Netherlands	2003	> 12 years	1.1%
United States	2003	> 12 years	1.5%

Alcohol use in the general population

	Year	Age group	Current user (within the last year)		
The Netherlands	2003	> 12 years	11.7 %	(2.4 million)	Binge use
			3.7 %	(0.8 millions)	Heavy use
United States	2003	> 12 years	22.6 %	(54 million)	Binge use
			6.4 %	(14.8 million)	Heavy use

Box 1: Definitions used in this thesis:

Use = take or consume (regularly or habitually)

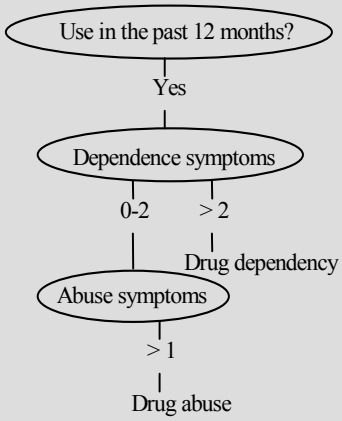
Abuse = misuse: improper or excessive use; abuse of a substance if he or she meets one or more of the four criteria for abuse included in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), but does not meet the definition for dependence for that substance.

Dependency = lack of independence or self-sufficiency; this is a psychiatric and sometimes physical state, a result from the reaction between a certain living organism and a particular substance. It can be categorized by reactions, which always include striving for long-term reception of the substance in order to feel its psychic effects or to avoid the discomfort of its absence.

Addiction = A term referring to an uncontrolable compulsive drug use, psychological dependence, and continuing use regardless of its harmful consequences; being abnormally tolerant to and dependent on something that is psychologically or physically habit-forming; addiction is considered to be a chronic, relapsing brain disorder characterized by neurochemical and molecualr changes as well as behavioral features like craving and an increased compulsive and impulsive sensation. Addiction is often used synonymously with "dependence". However, dependency is a part of an addiction. Therefore, addiction is both dependency and drug abuse, but not either alone.

Diagnostic and Statistical Manual of Mental Disorders -IV (DSM-IV) Definition:

"A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period: (1) Tolerance (= need for increased amounts of the same drug/ less effect of the same amount), (2) (2) Withdrawal/ withdrawal syndrome, (3) Loss of control, (4) Preoccupation with obtaining the drug, use of the drug, and recovery from its effects, (5) Important social, occupational, or recreational activities are given up or reduced because of substance use (continuation despite adverse consequences), and (6) Use if continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance (adverse consequences)." The DSM-IV definition makes a clear-cut separation between drug abuse and drug dependency. Given the fact that most DSM-IV-classified dependency symptoms are of a subjective nature, it is extremely difficult to classify an animal as drug dependent.



World Health Organisation (WHO) Definition:

"Substance dependence is a chronic and relapsing behavioral disorder, caused by repeated and often prolonged and/or heavy use of psychoactive substances. It is characterized by the continued use of these substances despite physical and mental problems, strong desire to take the substance(s), difficulties in controlling substance use, neglect of other activities and interests in favour of using or seeking the drug, increased tolerance and sometimes withdrawal syndrome once drug use is abruptly ceased. Dependence is not the result of the lack of will power or moral weakness. Complete abstinence is not easily achieved, and is often followed by relapse to drug use."

Given the fact that the WHO definition of drug dependence is more or less based on psychiological symptoms, it is easier to classify an animal as drug dependent than with the DSM-IV definition.

It should be noted that none of the two definitions mentioned the term addiction. For this thesis the term addiction will be used when referring to humans, whilst the term drug abuse will be used when referring to animals.

Interestingly, not all drug users become drug addicts. For instance, around 90% of the American population and around 85% of the Dutch population have used a drug (including alcohol) once in their lifetime, but the lifetime prevalence of drug (ab)use is relatively small (between 10 and 40%, actual percentage is highly dependent on the type of drug). Of course, with prolonged use the risk of an addiction becomes greater, but the fact remains that drug use does not inevitably lead to drug addiction. Thus, one of the key questions in the field of drug addiction is why some people are more susceptible to undergo a transition from

use to abuse (and subsequently to addiction), whilst others are relatively unaffected. Identifying factors that are involved in these difference in the susceptibility for developing a drug addiction, can help to identify people that are at risk, making it possible to develop prevention strategies. Moreover, the identification of these factors might contribute to our understanding of these differences and, by such, help to treat addicts. So why are there differences between individuals in drug addiction? And what underlies these differences?

Because of its compulsive nature as well as its etiology, drug addiction is often classified as an psychiatric disease. Most psychiatric diseases are best described by the so-called 'three hit model of psychopathology' [80]. This 'three hit model' states that the onset of a psychiatric disease is the result of an interaction between three factors, namely (a) **genetics**, (b) **early-life events,** and (c) **late environmental factors**. Epidemiological research has, indeed, shown that addiction has a genetic component. For instance, Merikangas et al have reported that relatives from drug abusers have an eight-fold increased risk to become drug addicts themselves [147,212,213,317]. The strongest information for a genetic component comes from research with monozygotic twins that were separated from birth, revealing an estimation of the heritability for drug abuse of around 45% [127,310-312]. Although these studies have revealed that genetic factors are indeed involved in the etiology of addiction, the genes involved remain to be elucidated. Despite the fact that many genetic factors are still to be resolved, certain genes related to the dopaminergic system have been reported. For instance, an association between the *Taq*1 A1 allele of the dopaminergic D2 receptor (DrD2) and alcohol addiction exists [103,226]. However, not all association studies have yielded the same results [226]. Even though these human studies have shown that genetic factors are of great importance in the susceptibility to develop an addiction, these studies have also shown that non-genetic factors play a crucial role. In fact, the heritability score of monozygotic twins for addiction reveals that around 55% is determined by other factors than genetics. Unfortunately, little epidemiological research has been done to study these factors. There are some studies that have reported an increased risk to develop an addiction following childhood abuse, childhood neglect (physical and emotional), parental loss, and other forms of household dysfunction [36,77,89,162,193,285,332]. Thus, these studies all seem to indicate that adverse early life events can increase the risk of an addiction.

It is well accepted that stressful life events at adult age can contribute to the onset of an addiction [116,118], though the effects of stressful life events on individual differences is less well-known. Nevertheless, addiction and trauma often co-occur [104,214,277]. Moreover, research has shown that the occurrence of alcoholism can best be predicted by the occurrence of the *Taq*1 A1 allele of the dopaminergic D2 receptor (DrD2) in combination with a low socio-economical status [189], but not by either factor itself. This could explain why certain researchers could not find the association between this gene and addiction. So, human research has shown that these three factors seem to determine a person's addictive profile. However, the exact nature of these factors, as well as the interaction between these factors, is still largely unknown.

Research concerning the interaction between these factors, is, for obvious reasons, not possible in humans. However, those drugs of abuse (e.g. cocaine, heroin, amphetamine, alcohol) that maintain drug taking in humans are also commonly self-administered by animals, and those avoided by humans are also avoided by animals [1]. The clear division between avoidance and approach of specific drugs is seen in virtually all species and with different routes of administration [1]. Moreover, this resemblance between humans and animals allows researchers to examine the mechanisms that are involved in addiction by using specific animal models. Indeed, like humans, animals show large individual differences in their behavior, including the rate and pattern at which drug self-administration is acquired, maintained, and reinstated [128,142,191,225]. This makes these animals very suitable to study the neurobiological basis of several aspects of individual differences and its influence on abusive behavior. Moreover, animal research has the possibility to study the interaction of the above mentioned factors in determining the susceptibility to drugs of abuse.

However, before discussing these individual differences in the susceptibility to develop a drug addiction, it becomes necessary to understand which processes underlie addiction. Or more specifically: how does controlled substance use advance to a loss of control (section 2), what neuropharmacological processes underlie addiction (section 3), what neuropharmacological processes underlie individual differences (section 4), and, finally, how can one study the interaction between the above-mentioned factors (section 5)?

2. A loss of control over substance use

In an effort to provide an answer to the question why use shifts to abuse, and to ultimately find a treatment for drug addiction, clinicians and researchers have defined addiction as a disease affecting the brain's reward system. This brain reward system includes, amongst others, dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (Nacc) and striatum, as well as glutamatergic inputs from the prefrontal cortex (PFC), and amygdala (box 2). This nucleus accumbens-related circuitry is normally involved in processes of reward, motivation and decision-making, which regulate sexual behavior, safety, hunger, thirst, and nutrient requirements. Thus, this circuitry is essential to the survival of an organism. Not only do drugs of abuse make use of this circuitry, they also alter it at a molecular, cellular and neuronal level [92,222,223,256,321]. These alterations are thought to be critical in the transition from use to abuse. Indeed, the majority of the theoretical explanations for this shift incorporate the idea that drugs change the brain and thereby change some psychological function in the organism.

The most traditional explanation is that a drug is taken because of its pleasant nature, but, with repeated exposure, 'homeostatic' adaptations lead to tolerance and dependence. In such a case, cessation of use would result in unpleasant side effects. Compulsive drug taking is thus maintained to avoid unpleasant effects. Many researchers have postulated this explanation, although all under different names.

Box 2: Circuitry related to the nucleus accumbens [57,68,69,91,92,108,115,168,249,282,321,333]

The nucleus accumbens (Nacc) is a basal forebrain structure that has received a considerable amount of attention over the last years, mostly because this region has been implicated in a great variety of disorders, including drug addiction. The nucleus accumbens, together with the adjacent regions of the olfactory tubercle (OT) and the ventral section of the neostriatum, is called the ventral striatum. The ventral striatum is, together with the dorsal striatum which comprises of the putamen and the rest of the neostriatum, a part of the striatum. Most drug (ab)use research focusses on the ventral striatum, and then particularly on the nucleus accumbens. The accumbens itself consists of two distinct areas, namely the shell and core. Each of these two areas has a specific function: the core seems to be mostly involved in response-learning and locomotion, whilst the shell seems to be mostly involved in motivation. The ventral striatum receive input from many cortical and limbic areas. Dopaminergic neurons, which have their cellbodies in the ventral tegmental area (VTA), terminate in the nucleus accumbens and to a lesser extent in the caudate putamen (the mesolimbic dopaminergic system). Moreover, these dopaminergic neurons terminate in the (medial) prefrontal cortex (PFC), the prepiriform and piriform, entorhinal, and anterior cingulate cortices which are all components of the limbic system (mesocortical dopaminergic system). Both the VTA as well as the PFC project towards the nucleus accumbens by dopaminergic and glutamatergic projections, respectively. This output towards the nucleus accumbens is regulated by the neurotransmitter GABA (gamma-amino butyric acid). The GABAergic neurons in the PFC and VTA are interneurons that form inhibitory synapses with glutamaterigic and dopaminergic cellbodies respectively and that exert an inhibitory influence on these neurons. The VTA has, in addition, GABAergic efferents projecting to the accumbens. This complex pathway results in a strongly regulated system with both excitatory control (via glutamate) as well as inhibitory control (via GABA) that, in the case of abusive substances, regulate motivational (= the drive to obtain or avoid) and rewarding (=pleasurable) processes.

The ventral striatum also receives input from the hippocampus (Hipp), the amygdala (Amyg), and indirectly (via the VTA) of the bed nucleus of the stria terminalis (BNST). The latter two projections are often referred to as the 'extended amygdala' system, and have connections to brain stem circuits involved in arousal, neuroendocrine regulation, and consummatory behavior. The previously mentioned dopaminergic neurons from the VTA also project the amygdala and hippocampus and, thus, form a feedback/ control mechanism of the nucleus accumbens. All these structures together are referred to as the nucleus accumbens-related circuitry and have been implemented in the actions of drugs of abuse.

Although not directly involved, the dorsal striatum is often considered to play a role in switching between behaviors, because it connects (via the substantia nigra) limbic information (emotion) to nigrostriatal information (action). Next to dopamine, glutamate and GABA, two other neurotransmitter systems are considered to be important in the actions of the nucleus accumbens-related circuitry, namely the serotonergic and noradrenergic system.

Serotonergic projections from the dorsal and median raphe nuclei, innervate the SN, the VTA, several thalamic nuclei, many hypothalamic areas, the medial habenula, and the dorsal and ventral striatum. This wide-spread presence of serotonergic projections amongst the nucleus accumbens-related circuitry suggests a role in directing its response. Indeed, research has shown that serotonergic innervation can alter motivational processes either directly or indirectly (through alterations of other neurotransmitter system).

The most important noradrenergic projections originate from the locus coeruleus (LC), the caudal extension of the LC, the (dorsal) medullary group, and the lateral tegmental area, and terminate in, amongst others, the cortex, the hippocampus, the amygdala, several thalamic nuclei, and in the ventral striatum. Like serotonin, noradrenaline can also alter the response of the nucleus accumbens-related circuitry. Noradrenaline can have both a direct or indirect effect on, for instance, accumbal dopamine levels via noradrenergic projections arising in the locus coeruleus or via serotonergic cell bodies arising in the raphe-nigral and the raphe-neostriatal fibers.

One other system needs to be mentioned, and that is the hypothalamic pituitary adrenal (HPA)- axis. This axis regulates the stress response of an animal by means of ACTH and corticosterone release as well as by modulating the dopaminergic, serotonergic and noradrenergic system via stimulation of two corticoid receptors, namely the glucocorticoid receptos (GR) and the mineralocorticoid receptor (MR), and via corticotrophin-releasing factor (CRF) and its receptors. Stress-induced modulation of MR, GR and CRF receptors (and the subsequent release of CRF and corticosterone as well as several other peptides) results in an alteration of the activity of those systems that regulate the (re)activity of the nucleus accumbens. Taken together, multiple systems and neurotransmitters modulate the (re)activity of the nucleus accumbens-related circuitry.

The nucleus accumbens itself projects to several destinations. Some of the relevant destinations for drug abuse research are: (1) towards the ventral pallidum (VP) and then to the VTA (feedback loop), (2) towards VP and then the thalamus, which sends signals to the cortex, (3) towards the ventral tegmental area, (4) towards the substantia nigra pars compacta and then towards the dorsal striatum, and (5) minor projections to the amygdala.

The oldest concept is called homeostatic drive. It simply states that each individual has a fixed set-point for each behavior and through detection and corrections, maintains this set-point. Although this concept explains some aspects of motivation (for instance: when body fluid levels and blood pressure drops, specific hormonal responses are activated, resulting in thirst and drinking behavior), it does not explain anticipatory behavior (for instance: drinking water without being thirsty in anticipation of thirst).

Several studies have shown that under baseline conditions the brain reward circuits are stable (by measuring the circuit's electrical thresholds by means of intracranial self-stimulation (ICSS) procedure and by measuring extracellular levels of neurotransmitters by means of microdialysis) [196,325]. Although these findings support the concept of a homeostatic drive, these studies have also indicated that, during withdrawal of a drug of abuse, ICSS levels increase and neurotransmitters levels decrease and then slowly return to baseline values [90,196,325,336].

This after-reaction is opposite in direction to the drug-induced reaction [333,335], and is an integrative part of two other concepts, namely the opponent-process drive and allostatic concept.
The opponent-process drive concept posits that all hedonic stimuli, if strong and prolonged, activate not only their own direct reaction in the brain, but also an opponent process of opposite value [295]. If the stimulus is pleasant (like a drug of abuse), the opponent is unpleasant. This process is actively generated by the brain to counteract the effects of the stimulus and thus to return to a neutral status (homeostasis). In case of a prolonged intake of a strong hedonic stimulus, the opponent reaction is strengthened whilst the hedonic reaction does not alter. At a certain point, the unpleasant reaction is more intense and longer lasting than the original hedonic reaction: resulting in an increase of intake of the original hedonic stimulus. The allostatic concept states that reward thresholds can alter due to over-activation of brain neurotransmitter systems (opponent effect) that functionally antagonize the brain reward pathway. Taking the drug would, in such a case, lead to more activation of these neurotransmitter systems and result in more negative reinforcement, resulting in a vicious circle. Thus, both concepts state that, after some time, chronic and escalating intake will occur in order to avoid the negative effects [5,167]. However, research has shown that not all (human) addicts continue taking drugs to avoid these negative effects [254].

Another explanation for the shift from use to abuse, is the incentive motivational/ incentive salience concept. This concept is based on the formation of an association between the 'true' reward of the drug and a predictive stimulus of this reward (cues/ environment). Due to this formation, a neutral stimulus can become an incentive motivational stimulus by itself [21]. The reward of the drug itself has been called 'liking', whilst the motivation to obtain the drug has been called 'wanting' or incentive salience (both are considered to be regulated by the nucleus accumbens: liking by the shell-region, wanting by the core-region). In this respect, 'wanting' is assessed as goal directedness to pursue particular events, whilst 'liking' is pleasure

driven behavior. In most cases 'wanting' and 'liking' go together. But with prolonged drug use, the incentive salience (wanting) can become sensitized, resulting in an abnormal desire to take and seek the drug, even though the hedonic value (liking) is not altered [255].

Taken together, these concepts propose that prolonged drug use results in long-term alterations in the rewarding effects of the drugs as well as opposite processes. This drug-induced plasticity explains acquired vulnerability to a drug that eventually will result in a loss of control over the drug-taking habit. However, these concepts do not explain why certain individuals seem to be more addictive-prone than others from the start. For instance, Cloninger has revealed that, in a human population, two personalities can be found that relate to a high predisposition for drug abuse [3,46,129,341].
Individuals that score high on novelty-seeking/ sensation-seeking are characterized by impulsive behavior, risk taking, and an excessive effort towards reward seeking. These individuals seem to need an extraordinary stimulation, like a drug of abuse, to feel arousal and happiness [45,129]. On the other hand, individuals that score high on harm avoidance and are characterized by a worried state of mind, fatigue, and depressive-like symptoms, seem to need a drug to self-medicate some form of emotional distress [129]. Indeed, numerous studies have shown that psychiatric disorders are common among drug addicts [28,197]. It should however be noted that it is not clear if a common underlying neurochemical deficit leads to these psychiatric disorders and drug abuse or whether drug abuse leads to abnormalities mediating these psychiatric disorders (or vice versa). The division into two types of drug users has also been described in the two-affect model: drug use to alleviate an unpleasant mood (negative affect) or to induce a pleasant feeling (positive affect) [13].
In this respect, one other theory needs to be mentioned. This theory is based on the assumption that the hedonic impact of a drug is highly dependent on the physiological status of an individual, even though the absolute hedonic value of the reward is unaltered [30,31,308]. For instance, a hot bath feels more pleasant on a cold day than on a warm day and a salty drink is very unpleasant unless you're thirsty. This phenomenon, called "alliesthesia", has also been shown in animals: certain environmental changes (stressors) can increase the intake of a drugs of abuse [118], which is assumed to be due to an alteration in the hedonic impact of the drug. These theories describe why drug use starts, but they do not clarify why drug use persists and progresses towards a compulsive habit. Thus, all above-mentioned theories together might explain the specific (psychological and behavioral) components of a drug abuse and addiction.

Although the above-mentioned theories give an explanation for the psychological and behavioral aspects of an addiction, they do not explain which neurochemical factors underlie these aspects. The next section will therefore (briefly) discuss the neurochemical factors that are involved in drug addiction in general. Because this thesis focusses on the acquisition phase of addiction, the next section will discuss the neurochemical factors underlying this acquisition phase (without addressing individual differences - section 4). The neurochemical factors that underlie prolonged exposure to a drug are excellently reviewed by Berridge &

Robinson, 1998; Shalev et al, 2002; Koob, 2004 (and some findings are summarized in box 3). Moreover, this thesis will only focus on two types of drugs, namely alcohol and cocaine. The first drug was chosen because of its widespread prevalence, its well-known characteristics, and the existence of genetic animal models. The second drug was chosen because of on its unique, relatively straightforward mechanism of action.

3. The neurochemical factors involved in an alcohol or cocaine addiction

To understand the neuropharmacological and neuroanatomical circuits associated with the action of drugs of abuse, and to understand the specific action of these drugs during the acquisition phase of an addiction, several experimental methods have been developed. These methods can roughly be divided into two classes: the neurobiological (like patch clamp and microdialysis techniques) and the behavioral class (like self-administration). With the aid of these techniques, researchers have been able to study the effects of drugs of abuse. In the next two paragraphs, (some of) these findings will be summarized.

3.1. Alcohol

Alcohol is, by far, the most commonly used drug of abuse in the world. This is mostly because of its socially excepted status and its long history of use. The consumption of alcohol goes as far back as 8000 B.C. (in the form of mead – a fermentation product of honey). Alcohol's properties include its psychoactive, sedative, anxiolytic, and euphoric actions – and this makes it so desirable to consume. These actions come from its unique pattern of activation of the central nervous system (CNS). Alcohol is a CNS depressant, which results in a loss of inhibition over certain systems, and by such resulting in a perception of activation (loss of negative feedback and restraint). This disruption of normal functioning is caused by alcohol's widespread actions on the CNS. Indeed, alcohol does not work on one substrate or substrate-group like cocaine does (see 3.2).
For instance, alcohol enhances the effects of the neurotransmitter GABA on its receptors in the prefrontal cortex, hippocampus, and amygdala [165,205]. This enhancement of activity actually results in the above-mentioned loss of inhibition, and by such, stimulates the release of other neurotransmitters. This loss of negative feedback results in one of the behavioral features of alcohol intoxication, namely a loss of cognition, decision-making and memory. Moreover, alcohol interacts with the opioid receptors and opiate system: alcohol stimulates the release of -endorphins, and alcohol consumption is reduced by the opiate antagonist naloxone [169,316]. These effects are considered to result in feelings of euphoria. Alcohol also stimulate the release of other neurotransmitters, like glutamate, serotonin and noradrenaline [58,157,205,211,235,290,338].
Even though alcohol has many effects in the CNS, the reinforcing (and rewarding) capacity is considered to be due to the activation of the dopaminergic system. There is ample evidence that this system is directly or indirectly involved in the acute reinforcing effects of alcohol in

animals (as well as in humans). Alcohol, either given systematically or intra-accumbal, results in a dose-dependent release of dopamine in the nucleus accumbens [67,164]. Moreover, pharmacological manipulations of the dopaminergic system in the nucleus accumbens alter alcohol consumption: both D1 and D2 dopamine antagonists are known to reduce consumption [58,141], whilst D2 agonists are known to stimulate consumption [141]. Accumbal dopamine does, however, not primarily regulate the consumption of alcohol. Lesions of the accumbens do not alter alcohol consumption [58,66], indicating that more brain areas are involved. Research has, indeed, shown that the alcohol-induced increase in accumbal dopamine is strongly regulated by the VTA and the PFC [121,272,273]. Alcohol also increases the dopamine release from the amygdala [202,338]. Moreover, the dopaminergic system of the VTA plays a role in determining the amount consumed. For instance, injecting a D2 agonist in the VTA decreases alcohol consumption [227]. Thus, ethanol consumption leads to a stimulation of the mesolimbic and mesocortical dopaminergic system. Alcohol, however, also indirectly increases the dopamine release in the brain through stimulation of the opioid receptors in the VTA and the nucleus accumbens (NAcc) [54]. This increase of dopamine release from the mesocorticolimbic system is also considered to result in some of the euphoric effects of alcohol, and by such, leading to an enhancement of consumption.

Next to these effects of neurotransmitter systems, alcohol also seems to interact with the HPA-axis. For instance, numerous studies have indicated that there is a positive association between stressful life events and alcohol consumption [104,189,244]. Animal studies have also shown that adrenalectomy (removal of the adrenal glands- the primary source of corticosterone) decreases alcohol consumption, whilst corticosterone treatment increases alcohol consumption [93-96]. This interaction between stress and alcohol consumpton is not completely clear, but research suggests that stress, via activation of the HPA-axis, alters the (re)activity of the dopaminergic, serotoneric and noradrenergic system [93,244,245], and by such resulting in an increase of alcohol consumption.

3.2. Cocaine

Cocaine is a psychostimulant, indicating that it has an activating effect on the central nervous system, resulting in increased locomotion, feelings of euphoria, and a state of arousal [99]. As the dose of cocaine increases, the user may experience respiratory arrest, convulsions, cardiac arrest and death [323,327]. Cocaine primarily blocks the reuptake of extracellular dopamine from the synaptic cleft back into the neuron; this results in a prolonged effect of extracellular dopamine on its post- and presynaptic dopaminergic receptors. This blockade is accomplished by a direct binding of cocaine to the dopamine transporter (DAT) [262,333,334]. More recently, cocaine has been found to have a direct effect on the dopaminergic storage pools in the neuron (the reserpine-sensitive pool) [102,156]. Thus, suggesting that cocaine has an additional effect on the release of dopamine.
Both clinical and non-clinical studies have shown that the primary site for these actions is the (dopaminergic) mesocorticolimbic system [166,324,333]. Studies have shown that animals self-

administer cocaine directly into the (medial) prefrontal cortex [204,315], the shell section of the nucleus accumbens [204,258], the olfactory tubercle [150], and the ventral tegmental area [56,204]. Moreover, microdialysis and electro-physiological studies have shown that cocaine injections result in an increase of dopamine levels (or activity of neurons) in the nucleus accumbens, the (medial) prefrontal cortex, the ventral tegmental area, and the amygdala [8,148,149,325]. Behavioral studies have also shown that the amount of cocaine self-administered, the occurrence of place preference, and the locomotor-activating effects of cocaine can be altered by injecting an animal with dopaminergic agents (both in- and decreases have been found) [4,33,34,105,132,204,221].

Cocaine is, however, not specific for the dopamine transporter: it also blocks the noradrenaline (NET) and serotonin transporter (5-HTT). Microdialysis experiments have shown that a single injection of cocaine enhances extracellular levels of dopamine (DA), serotonin (5-HT) and noradrenaline (NE) in the ventral tegmental area and the nucleus accumbens [250]. It has also been shown that adrenergic and serotonergic agents can alter cocaine-induced hyperactivity [75,101,138]. In addition, the GABAergic and glutamatergic system are involved in the response to cocaine [25,154]. Their exact role in mediating the effects of cocaine, is, however, not (completely) clear.

There is one 'substrate' that specifically needs to be mentioned in respect to (the effects of) cocaine, and that is the hypothalamic pituitary adrenal axis (HPA-axis). Cocaine is known to stimulate the release of corticotropin releasing factor (CRF) from the hypothalamus, resulting in increased levels of adrenocorticotrophin hormone (ACTH) and corticosterone [275,276]. Moreover, corticosterone administration, or stress (which enhances corticosterone levels), facilitates cocaine self-administration [117,192]. In this respect, cocaine and stress seem to be able to cross-sensitize with respect to their effects on the mesocorticolimbic dopaminergic system [296].

Taken together, the response to alcohol and cocaine involves many neurotransmitters. It is apparent that two of the factors in determining the effects of alcohol and cocaine are the involvement of the dopaminergic mesocorticolimbic system and the HPA-axis.

4. Individual differences

As stated before, the 'addictive profile' of an individual has been attributed to the interaction between three factors, namely genetics, early life events and late environmental factors. However, human research can not elucidate the precise nature of these factors, as well as the interaction between them. For that reason, over the past couple of decades, several animal models have been developed for studying individual differences in the susceptibility to drugs of abuse. The next paragraphs will discuss the current animal models that are available for studying alcohol and cocaine abuse.

Box 3: (Neurochemical) features involved in the (late)maintenance phase, withdrawal and relapse
As stated before, this thesis only focusses on the acquisition phase of an addiction. However, it needs to be noted that other brain area's are involved when examining other phases of an addiction. Moreover, the role of certain neurotransmitters is altered. This box will therefore summarize some of these findings for both alcohol and cocaine consumption.

When substance of abuse are consumed for a prolonged period of time, two distinct processes can occur, namely tolerance and sensitization. Tolerance is a process in which the response to the substance decreases with repeated exposure to the same dose, whilst sensitization is a process in which the response to the substance increases with repeated exposure to the same dose. It should, however, be noted that these processes can occur at a behavioural and neurochemical level. The occurrence of, for instance, behavioral sensitization, can be the result of neurochemical tolerance and/ or sensitization. An example of behavioral sensitization: repeated cocaine exposure with the same dose can result in greater motor activating effects of the drug than when exposed once. An example of neurochemical tolerance: chronic alcohol consumption leads to, amongst others, a reduction of DA release of around 80% in comparison to controls (DA hypofunction), an increased baseline activity of GABAergic neurons in the VTA , an increased affinity of dopaminergic D1 receptors, an increased GABA release in the amygdala, an increased sensitivity to GABA agonists, and a reduction in the number of -adrenergic receptors[71,165,326,334]. An example of neurochemical sensitization: chronic cocaine use leads to, amongst others, an increased number of dopaminergic D3 receptors in the striatum, an increased binding to dopaminergic D1 receptors, an increased opioid receptor binding, an increase in SERT density in the CP and Nacc shell, and an increase in DAT density in the CP [47]. The overall effect of these adaptive processes is that the intake of either cocaine or alcohol needs to be increased in order to obtain the same pleasant effects. The maintenance phase seems to be relatively unaffected by stressors. For instance, Goeders et al have shown that footshock stress and corticosterone injections do not affect ongoing self-administration of cocaine [117].
When subjects stop using a drug, many counterreactions occur. This is mostly due to the fact that the neurotransmitters involved are altered in such a way that cessation of use leads to very adverse effects. Some researchers state that it is these adverse effects that maintain drug taking and drug seeking behaviour in the first place (see section 2).
The last phase of an addiction (although not always present) is relapse/ reinstatement. There are three factors that are suggested to reinstate drug use, namely (a) drug use itself, (b) cues, and (c) stress. Drug-induced and cue-induced reinstatement seem to be mediated mostly by the amygdala and some portions of the PFC[36]. Moreover, both the basolateral amygdala as well as the nucleus accumbens core section seem to be involved in cue-induced reinstatement, whilst the basolateral amygdala is not involved in drug-induced reinstatement [206]. In contrast to the maintenance phase, stress seems to play a crucial role in the reinstatement. Reinstatement can be accomplished by exposing an animal to repeated footshocks or fooddeprivation [284,287]. This stress-induced reinstatement seems to be mediated by the nucleus accumbens shell and the PFC: lesions of both areas prevent stress-induced reinstatement. Moreover, unique for stress-induced reinstatement, is the involvement of noradrenergic neurotransmission from neurons in the lateral tegmental nucleus to the BNST and central amygdala, as well as CRF projections from the central amygdala to the BNST[286,287].
Taken together, the factors involved in the other 3 phases of an addiction (maintenance, withdrawal and relapse) are different or act in a different fashion than those involved in the acquisition phase.

4.1. Animal models for alcohol abuse

Currently, 5 sets of alcohol-preferring and alcohol-non-preferring lines of rats are available through selective breeding on basis of their response to alcohol. These sets include the ALKO alcohol/ non-alcohol (AA vs. ANA) line [292], the alcohol-preferring/ alcohol-non-preferring (P vs. NP) line [219], the University of Chile B and A (UchB vs. UchA) line [203], the high-/ low-alcohol drinking (HAD vs. LAD) replicate line [55], and the Sardinian alcohol-preferring/ alcohol-non-preferring (sP vs. sNP) line [39].
In addition to these models, other genetic rodent lines, which were not selectively bred on alcohol consumption, exhibit innately high alcohol-drinking characteristics. These include

the C57BL/6 mouse strain [259], the Fawn hooded rat strain [251], the Lewis rat strain [107], and the apomorphine unsusceptible (APO-UNSUS) ratline [294]. There is some evidence that the caloric value of alcohol and taste sensation may play a significant role in the high intake pattern of the C57BL/6 mouse and in the Fawn hooded rat [207,208], making these high-alcohol drinking rodents less desirable as a model for alcohol consumption. A study examining the effects of taste on alcohol consumption of the apomorphine unsusceptible ratline or the Lewis rat strain has not been performed.

With the aid of these genetic animal models, several innate neurobiological differences have been found between high alcohol- and low alcohol-consuming rats. The major findings are summarized in table 2.

Table 2: *Summary of some of the research findings with the genetic lines that differ in the amount of alcohol consumed* [38,39,79,203,219,220]

Neurotransmitter system	Differences			
Dopaminergic system				
DA content, innervation nucleus accumbens	NP > P	LAD > HAD		
D1 receptors basal ganglia	NP = P	LAD = HAD		sNP > sP
D2 receptors basal ganglia	NP > P	LAD = HAD	AA = ANA	sNP > sP
DAT levels striatum				sNP > sP
TH staining striatum (enzyme necessary for making dopamine)				sNP > sP
Noradrenergic system				
DBH staining (enzyme necessary for making noradrenaline)				sNP > sP
Serotonergic system				
5-HT content, innervation nucleus accumbens	NP > P	LAD > HAD		
5-HT receptors: 1B				
2	NP > P			
2C	NP > P	LAD = HAD	AA = ANA	
1A	NP < P			
postsynaptic	NP < P	LAD = HAD	AA = ANA	
HPA-axis (basal conditions)				
Corticosterone			AA < ANA	
CRF (corticosterone releasing factor)	NP < P			

Surprisingly, there is a considerable variation in factors that are suspected to be involved in directing the amount consumed. For instance, research considering the influence of the amount of dopaminergic D2 receptors on directing the amount of alcohol consumed has yielded different results: some have reported that low levels of D2 receptors are correlated with an increased alcohol consumption, whilst others have found no correlation. The only correlations that have consistently been found, are: high-consuming rats have (a) less dopaminergic activity/innervation in the accumbens, (b) less serotonergic activity/innervation in the accumbens, and (c) higher activity of the HPA-axis. Some studies have also suggested a correlation between rats consuming more alcohol and lower striatal TH immunostaining, lower striatal DAT levels, lower DBH staining, less postsynaptic 5-HT1a

receptors, and less 5-HT1b receptors. These findings have, however, only been investigated in one genetic animal model.
Research with rat strains that were not selectively bred for high and low alcohol consumption, but do show (stress-induced) differences in alcohol drinking, has also contributed to finding neurobiological features that are correlated with alcohol consumption. For instance, these studies have shown that high alcohol consumption can be correlated with (a) lower levels of striatal dopamine under baseline conditions [138], (b) lower levels of dopamine transporters (throughout the dopaminergic terminal fields) [39] , and (c) lower levels of D2 receptors in the basal ganglia circuitry [237,303]. Moreover, rats, selectively bred for high plasma (nor)adrenaline levels, have a higher preference for alcohol than their counterparts [302]. Research has also revealed that high plasma corticosterone levels are positively correlated with high alcohol intake [93,95,182].

Taken together, these findings suggest that a high consumption of alcohol is correlated with: (a) a decreased innervation and/or function of dopamine in the nucleus accumbens, (b) lower levels of dopaminergic D2 receptor density and/or binding, (c) lower levels of dopamine transporter (DAT) density and/or binding (d) a decreased innervation and/or function of serotonin in the nucleus accumbens, and (e) an increased activity of the HPA-axis (as indicated by the levels of CRF and corticosterone) and possibly (f) an increased activity of the noradrenergic system. The dopaminergic correlations are very similar to those found in humans: alcoholics have lower levels of dopaminergic D2 receptors and DAT in the striatum [313]. Lower levels of dopaminergic D2 receptors can been linked to the *Taq*1 A1 allele of the dopaminergic D2 receptor (DrD2) [226], and by such explain why an association exist between this gene and alcoholism [103,226]. As stated previously, not all human studies have found this association [226]. This could, however, be due to the fact that human research has shown that the actually outcome of the genetic factor is largely dependent on the environmental conditions surrounding the subject [189]. This dependency on the environment can also be seen in animal research. For instance, a number of studies have shown that the adrenal steroid hormone corticosterone (integral part of the stress system) plays a significant role in the modulation of alcohol consumption [93].
It has been shown that surgical [93] or drug-induced [96] adrenalectomy (which is the removal of the adrenal glands- the primary source for the corticosterone) attenuates voluntary ethanol intake. Furthermore, states associated with enhanced corticosterone secretion, such as food restriction or social stress, increase alcohol intake [96,97,189,244]. Indeed, stress during or preceding use is known to (re)direct the vulnerability to all drugs of abuse. Surprisingly, the role of the environment (stress) has hardly been researched with respect to individual differences in alcohol consumption. This is probably due to the fact that research concerning the effects of stress on alcohol consumption has yielded conflicting results as both in- or decreases of intake after stress have been described [93,244]. However, it is not unlikely that this discrepancy is due to the type of stressor used and/ or the genetic background of the strain used. For instance, animal studies have used stressors like cold-water immobilization [257] or prolonged

restraint-stress [184]. These type of stressors are quite extreme and they do not always mimic "normal" environmental stressors.
Given the fact that the HPA-axis (re)activity seems to be involved in directing the consumption of alcohol as well as the fact that human studies have shown that the outcome (alcoholic or not) of the DRD2 genotype is dependent on the magnitude of stress exposure [189], it would be extremely interesting to investigate the role of mild stressors on alcohol consumption in individually different rats.

4.2. Animal models for cocaine abuse

Currently, no genetic animal model, consisting of either mice or rats that were specifically bred for a difference in cocaine consumption, is available. There are however some animal strains that strongly differ in either their reaction to psychostimulants or their intake pattern of a psychostimulant. For instance, the C57BL/6J (C57) mouse strain shows psychostimulant-induced behavioral sensitization (a process in which the animal responds stronger the second time than the first time to the same dose), increased hyperactivity, a higher susceptibility for cocaine-induced seizures, and self-administer more cocaine whilst the DBA2J (DBA) mouse strain does not (or in a reduced fashion) [177,199,231]. When considering rats, Lewis rats (LEW) show a greater behavioral sensitization to cocaine, a greater cocaine-induced increase in dopamine levels, and Lewis rats self-administer cocaine more rapidly and with lower doses than Fischer344 rats [35,174].

Another method to select individuals that differ in the susceptibility to drugs of abuse, is to use a behavioral characteristics as read-out parameter. One of these characteristics is the novelty-seeking component [45,62,341]. Indeed, when placing rats into a forced-exploratory paradigm, two extreme phenotypes will occur: one extreme travels a greater distance and shows less habituation (high responder – HR) whilst the other extreme travels a smaller distance and habituates fast (low responder – LR) [50,62,238]. HR rats, are more predisposed to self-administer amphetamine than LR rats [238]. Since this finding, many studies have used these HR and LR rattypes to study both amphetamine as well as cocaine addiction. Research concerning cocaine addiction has shown that HR rats show a larger cocaine-induced dopamine release in the nucleus accumbens, and show a greater cocaine-induced activity [42,143]. Furthermore, HR rats are known to acquire low-dose cocaine self-administration more rapidly and take far greater amounts of cocaine than LR rats [153]. Research with this animal model has shown that high dopamine levels in the mesocorticolimbic system, and especially in the nucleus accumbens are correlated with a high intake of cocaine [114,143,270]. Moreover, research has shown that HR rats have a higher and prolonged increase in corticosterone levels after a stressor than LR rats [243], a feature that can be correlated to their increased acquisition of cocaine self-administration [63,117,118,192].
Although these studies have clearly shown that mesocorticolimbic dopamine and corticosterone are involved in the reaction of these animals to cocaine and contribute to the line-specific differences in the intake of cocaine, the interaction between the genetic make-up

of an animal and the reaction to the environment has not be investigated. The matter and fashion in which this gene * environment interaction takes place, could well contribute to individual differences in the susceptibility to drugs of abuse. For instance, research has shown that HR and LR rats can reach the same levels of cocaine self-administration if corticosterone levels are increased through drug treatment or severe stress [153,268]. This would indicate that corticosterone levels (stress) is a strong determinant of individual differences. Unfortunately, the genetic features that determine the phenotype HR or LR have never been researched. It is therefore (currently) not possible to investigate this gene-environment interaction. Moreover, when considering the above-mentioned human factors involved in the onset of an addiction (genetics, late environmental factors, and early life events), the effects of early life events can not be investigated in HR and LR rats.

It would therefore be desirable to have an animal model with a known genetic background as well as knowledge of its specific neurochemical and neuroendocrinological features, that can easily be manipulated both late as well as early in life.

5. APO-SUS vs. APO-UNSUS rats

Since dopamine plays a crucial role in the different stages of an addiction [68,166,168,324], an animal model based on a difference in this system would be ideal for studying addiction. The department of psychoneuropharmacology therefore started to select Wistar rats on the basis of their response to the dopamine D_1/ D_2 receptor agonist apomorphine, injected subcutaneously in a standard dose of 1.5 mg/kg [50,86]. This resulted in a bimodal shape of variation with approximately 40 - 45 % showing a weak gnawing response and 40 – 45 % showing an intense gnawing response [50,86]. This bimodal variation can also be found in other outbred strains, like the Sprague Dawley rat, but not in inbred strains (unpublished data).
Through a selective and specific breeding program (in order to minimize inbreeding), two distinct lines of rats were created, termed APO-SUS (apomorphine susceptible, i.e. rats that show a strong gnawing response to apomorphine) and APO-UNSUS (apomorphine unsusceptible, i.e. rats that show a weak gnawing response to apomorphine) rats [50]. Ten years after the original selection, the department of Psychoneuropharmacology has replicated the selection procedure and has found the same separation in APO-SUS and APO-UNSUS rats [86]. Although the clear-cut separation of the two lines and the replication line indicate that apomorphine (un)susceptibility is a heritable trait, it was not until recently that the major genetic difference between APO-SUS and APO-UNSUS rats was found.
Using a cDNA/ oligonucleotide micro-array approach, it was found that APO-SUS rats only have one or two copies of the gene *Aph-1b,* whilst APO-UNSUS rats have three copies of this gene [48]. This dosage imbalance is caused by an unequal crossing-over event and, interestingly, the site of recombination was a segmental duplication within the *Aph-1b* locus. The *Aph-1b* gene codes for a small protein that is an integral part of the γ-secretase complex which plays an important role in cleaving type-1 transmembrane proteins [170]. Although the

exact function of the Aph-1 component in the γ-secretase complex is not completely clear, it may play a role in stabilizing the γ-secretase complex and possibly in determining its specificity. The complex is involved in cleaving at least 14 different transmembrane proteins, including amyloid precursor protein, notch, neuregulin and the neuregulin receptor ErbB4 [170]. These substrates, many of which are critically involved in various aspects of development may explain why APO-SUS and APO-UNSUS rats show such fundamentally different properties in the nervous, endocrine and immune system. Indeed, over the last 20 years these rats have been characterized behaviourally, neurochemically, immunologically, and endocrinologically [50,52,53,86]. It would be beyond the scope of this introduction to discuss or even summarize all the characteristics of these rats; only those characteristics that are (suggested to be) involved in the susceptibility to develop an addiction will be discussed.

In comparison to their counterparts, APO-SUS rats are characterized by higher levels of tyrosine hydroxylase (TH) immunoreactivity in the ventral striatum under non-challenged conditions [87], higher levels of TH mRNA in the substantia nigra [267], a higher density of dopaminergic D2 receptors in the striatum [267], and by the same behavioural response to a novelty challenge as the HR rats [50]. Furthermore, APO-SUS rats have a functionally lower noradrenergic activity in the ventral striatum as determined by (α) adrenergic agents induced locomotor activity by accumbal infusions [50,81], and have lower plasma levels of free corticosterone under non-challenged conditions than APO-UNSUS rats [264]. However, under challenged conditions their neurochemical and endocrinological status shifts: after a stressor, APO-SUS rats have a higher stress-induced dopaminergic activation of the ventral striatum [88], a functionally higher noradrenergic activity in the ventral striatum [50,81], and a stronger and longer lasting increase in adrenocorticotropic hormone (ACTH) and corticosterone than APO-UNSUS rats [264]. So, under a challenged condition, APO-SUS and APO-UNSUS rats show a differential response which is inverse to their response under non-challenged conditions.

Not only stressful circumstances during adulthood are known to alter the phenotypic expression of these animals, adverse early life events are also known to alter these animals. Thus, maternal deprivation of APO-UNSUS pups on postnatal day 9 for 24 hours results in sensitized striatal dopamine D2 receptors, an enhanced corticosterone release, and enhanced striatal tyrosine hydroxylase staining [86,263,266]. Moreover, maternally deprived APO-UNSUS rats displayed APO-SUS-like behavior at the adult age when examining apomorphine-induced gnawing behavior [86]. On the other hand, cross fostering of APO-SUS pups to a APO-UNSUS mother on postnatal day 1 results in a decreased the apomorphine-induced gnawing response of these APO-SUS rats [86]. Thus, maternal deprivation alters the phenotypic expression of APO-UNSUS rats, but not APO-SUS rats, and cross fostering alters the phenotypic expression of APO-SUS rats, but not APO-UNSUS rats [86]. The fact that one method only alters one phenotype is not surprising considering the fact that not only the nature of these methods is different, but also the timing. Recently, it has been found that APO-SUS and APO-UNSUS rats have different speeds of development, with APO-

UNSUS rats developing faster than APO-SUS rats [59]. This has led to the proposal of a development model: the genetic background of these animals determine the speed of development of the various brain structures (and therefore their function) and due to the differential development, different structure are 'mature' when the manipulation is performed. This would explain why one manipulation has an effect in one type, but not in the other.

Taking into account the systems that are suggested to be involved in the onset of drug abuse, namely the dopaminergic and to a lesser extent the noradrenergic system as well as the HPA-axis, and the fact that the status of these systems is environmentally dependent in APO-SUS and APO-UNSUS rats, the role of **genes** and **late environmental factors** in directing the intake of addictive substances can be investigated. Moreover, given the fact that adverse early life manipulations are known to alter these systems as well as the status of these animals, the impact of **adverse early life events** can also be investigated in these animals.

6. Aim and outline of this thesis

The aim of this thesis is to investigate the influence of and interaction between (a) **genetics**, (b) **early-life events** (c) and **late environmental factors** in determining the susceptibility for alcohol and cocaine abuse in the ratmodel consisting of apomorphine susceptible and apomorphine unsusceptible rats [53].

Given the status of the above-mentioned systems, a hypothesis was formulated about the intake of alcohol and cocaine: APO-UNSUS rats consume more cocaine and alcohol than APO-SUS rats under non-challenged conditions, whilst the reverse holds true under challenged conditions [53]. It was also hypothesized that the intake of an abusive substance at adult age by APO-SUS and APO-UNSUS rats would alter after an adverse early-life event, dependently on the type of early life event used [53].

Previous experiments in our laboratory have already demonstrated that male APO-UNSUS drink considerably more alcohol than male APO-SUS rats under non-challenged conditions [294]. Moreover, research with Nijmegen HR and LR rats has shown the same profile; under non-challenged conditions Nijmegen LR rats consumed more alcohol and showed a stronger response to amphetamine than Nijmegen HR rats did, whilst under challenged conditions Nijmegen HR rats showed a stronger response to amphetamine than Nijmegen LR rats did [109,110]. Even though Nijmegen HR and LR rats are not the same as APO-SUS and APO-UNSUS rats, they do share some features [50,52]. This suggests that APO-SUS rats will consume more alcohol than APO-UNSUS rats under challenged conditions.
Taken together, previous research has shown that, indeed, APO-UNSUS rats consume more alcohol than APO-SUS rats under non-challenged conditions, whilst the consumption of alcohol under challenged conditions still needs to be elucidated. The experiments in **chapter 2** were, thus, designed to investigate the role of the environment (stress) on alcohol

consumption. By investigating the intake under non-challenged conditions and the impact of mild acute and mild sub-chronic stress on this intake, it was possible to investigate the interaction between the genetically determined features of these rats and the impact of the environment (chapter 2).

One of the questions that remained from the previously performed study as well as the study described in chapter 2, was whether taste sensation contributed to these differences. As stated before, the suggestion has been made that innate taste differences could determine the individual differences in alcohol consumption. In order to rule out this possibility, the experiments in **chapter 3** examined whether APO-UNSUS and APO-SUS rats differed in saccharin and quinine consumption. Moreover, the consumption of the rewarding substance sucrose was investigated.

Given the fact that APO-SUS and APO-UNSUS rats differ in the amount of alcohol consumed and that this difference is due to a complex gene-environment interaction [294], the question arose whether this interaction was unique for alcohol or whether this interaction was consistent for other drugs of abuse, like cocaine. In order to study the role of the genetic basis of the animals and the impact of the environment in directing the amount of cocaine consumed, the experiments in **chapter 4** examined whether APO-UNSUS and APO-SUS rats differed in the acquisition and maintenance phase of cocaine self-administration under two environmental conditions (challenged vs. non-challenged).

As discussed above, early life events have often been implicated in the etiology of an addiction. A previous experiment at our department has revealed that neither maternal deprivation nor cross fostering had an effect on alcohol consumption in APO-UNSUS and APO-SUS rats [294]. The effect of these manipulations on cocaine consumption was, however, unknown. Because early life events are known to alter those systems that are involved in the effects of cocaine, namely the dopaminergic system and HPA-axis [134,148,243,306], one would expect that adverse early life events alter cocaine consumption. In order to investigate the role of adverse early life events in directing the intake of cocaine, the experiments in chapter 5 examined the long-term consequences of an early life stressor on cocaine self-administration in APO-UNSUS rats. Moreover, the experiments in **chapter 5** also incorporated female rats. In the studies performed so far, cocaine self-administration was only investigated in male rats, even though human studies have shown that drug addiction is also very common in females. In fact, cocaine abuse amongst women has increased rapidly over the last few years, and the intake patterns are often different from those of men [301].

Although not mentioned previously, one other factor is also suggested to be involved in a heightened susceptibility to drug addiction, namely prenatal cocaine exposure. Indeed, many animal studies have shown that prenatal cocaine exposure results in long-term alterations in the mesocorticolimbic dopamine system [216,218,278] and that prenatal cocaine-exposure enhances cocaine self-administration, reduces the reinforcing efficacy of cocaine as assessed by breakpoint analysis, and increases striatal extracellular dopamine release after an injection with cocaine [139,158,159]. Until so far, nobody has investigated the effects of prenatal cocaine exposure on the onset of an addiction and its contribution to individual differences in the susceptibility to drugs of abuse. The experiments in **chapter 6**, therefore assessed cocaine self-

administration behavior at adult age of both male and female APO-UNSUS and APO-SUS rats that were exposed *in utero* to repeated cocaine challenges.
Because research suggests a correlation between the amount of DAT, and possibly the amount of NET and SERT, in directing the amount of cocaine consumed, the experiments in **chapter 7** investigated the amount of DAT, NET and SERT in the striatum of non-challenged APO-SUS and APO-UNSUS rats. One other structure was analyzed, namely the hippocampus. This structure was chosen because APO-SUS and APO-UNSUS rats differ in HPA-axis regulation and the origination of schizophrenia-like symptoms, two functions that are both thought to be regulated by the hippocampus.
The previous chapters (chapter 2, chapter 4), together with previous research [294], have shown that under challenged circumstances APO-SUS rats have a higher consumption of alcohol and cocaine than APO-UNSUS rats whilst under non-challenged circumstance APO-SUS rats have a lower consumption of alcohol and cocaine than APO-UNSUS rats. The question that arose from this research is why one rat type consumed more of an addictive substance than the other rat type did. The motivation (willingness) to self-administer more or less of addictive substance is said to be determined by reward [166] (see section 2 and 3 of the introduction). There is, however, some debate about what it means to consume less or more of addictive substance: some state that consuming more of an addictive substance indicates that this substance has a high rewarding value [144,150], whilst others state that cocaine has a low rewarding value and that consumption needs to increase in order to obtain the desired level of reward [330,337]. The experiments in **chapter 8**, therefore, assessed the rewarding properties of cocaine by investigating the occurrence of cocaine-induced conditioned place preference in APO-UNSUS and APO-SUS rats. Finally, **chapter 9** of this thesis summarizes and discusses these results and their significance and relevance.

Chapter 2

The effects of stress on alcohol consumption: mild acute and sub-chronic stressors differentially affect apomorphine susceptible and unsusceptible rats.

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Abstract
The aim of this study was to investigate the effects of mild acute and mild sub-chronic challenges on alcohol intake and preference in the genetically selected ratlines of apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) animals. Animals from both lines were subjected to the 24 hr continuous alcohol vs. water paradigm under baseline conditions, after a single stressor and after multiple stressors. The intake of alcohol in ml was measured and converted to two values, namely intake in g/kg/24 hour of, and preference for, alcohol. This study shows that under baseline conditions the APO-UNSUS animals consume/prefer more alcohol than the APO-SUS animals. After an acute challenge the APO-SUS animals show a large increase in consumption, whereas the APO-UNSUS animals display only a small increase. Furthermore, sub-chronic challenges can further increase the consumption of the APO-UNSUS rat, but not that of the APO-SUS rat. The APO-SUS/ APO-UNSUS rats represent a good model to study the interaction between genetic factors and stress on directing alcohol consumption.

Introduction

Individual susceptibility to the use and misuse of drugs of abuse, like alcohol, is a well-known phenomenon in animals [244]. Studies have shown that different strains of an animal species can be selectively bred using their differential preference for alcohol [123,291]. These strains have subsequently been used to study the role of genetic and environmental factors in directing alcohol consumption [238,294,309].

For instance, studies with the genetically selected alcohol-preferring rats have shown that lower dopamine levels in the striatum, a reduced number of TH-immunoreactive fibers in the ventral striatum, and lower levels of D2 receptors in the basal ganglia circuitry are correlated to high alcohol consumption [38,120,120,203,219,299]. Furthermore, Taylor et al have shown that rats, selectively bred for high plasma (nor)adrenaline levels, have a higher preference for alcohol than their counterparts [302]. Since several studies have suggested an interaction or even a direct link between peripheral and central adrenergic systems [72,188,200], it is not unlikely that a high brain noradrenergic activity is also associated with high alcohol consumption.

Another factor that has been shown to be important in (re)directing the vulnerability to drugs of abuse is the amount of stress during or preceding use [62,93,93,94,96,98,241,242,244]. However, knowledge on the specific effects of stress on alcohol addiction is contradictory as both in- or decreases of intake after stress have been described [93,244]. Research, however, has revealed that plasma corticosterone levels are positively correlated with alcohol intake [93,95,182]. From these data an association is suggested between alcohol consumption and the genetically determined function of the striatal dopaminergic and central noradrenergic system as well as the reactivity of the stress system.

However, the currently available studies on the influence of stress have used extreme stressors like for instance cold-water immobilization prior to alcohol consumption [257], and the effects of daily mild stressors on alcohol consumption remain undisclosed.

This study will therefore investigate the role of mild acute and mild sub-chronic challenges on alcohol intake and preference in the genetically selected ratlines of apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) animals to further elucidate the impact of both genetic as well as environmental factors in directing alcohol consumption. The APO-SUS/APO-UNSUS rat model is based on the characteristic behavior response to a single injection of the selective dopaminergic D1/D2 agonist apomorphine [50]. Subsequent selective breeding has resulted in two distinct rat types that are divergent in the structure and function of, amongst others, the dopaminergic and noradrenergic system and the stress sensitivity. Under non challenged conditions, APO-SUS rats are characterized by higher levels of TH immunoreactivity in the ventral striatum [87], and a higher amount of dopaminergic D2 receptors in the striatum in comparison to APO-UNSUS rats [267]. Furthermore, APO-SUS rats have a functionally lower noradrenergic activity in the ventral striatum than APO-UNSUS rats as determined by (α)adrenergic agents induced locomotor activity by accumbal infusions [50,81], and have lower plasma levels of free corticosterone [264]. After a stressor, APO-SUS rats have a higher stress-induced dopaminergic activation of the ventral striatum [88], a functionally higher noradrenergic activity in the ventral

striatum [50,81], and a stronger and longer lasting increase in ACTH and corticosterone than APO-UNSUS rats [264].
Given the above mentioned negative association between high striatal D2 levels, high levels of TH, low noradrenaline levels and low plasma corticosterone levels on the one hand and alcohol consumption on the other hand, APO-SUS rats are expected to consume considerably less alcohol than APO-UNSUS rats under non challenged conditions. Indeed, a study with APO-SUS/ APO-UNSUS rats has already revealed that non-challenged APO-SUS rats consume considerably less alcohol and have a much smaller preference for alcohol than non-challenged APO-UNSUS rats[294].
To investigate the interaction between genetic factors and stressors on alcohol consumption, we subjected APO-SUS/ APO-UNSUS rats to different unpredictable stressors during alcohol presentation in the two-bottle alcohol vs. water paradigm [109,294]. We hypothesized that, due to their enhanced stress-sensitivity, APO-SUS rats will react stronger and longer to the challenges presented and therefore increase their intake. Since APO-UNSUS rats are relatively stress-insensitive, we predicted that APO-UNSUS rats will not, or only slightly, increase their intake after the challenges.

Methods and Materials

Animals

Adult male Wistar rats from the replicated line (18th generation APO-SUS and APO-UNSUS) were obtained from the Central Animal Laboratory (CDL), University of Nijmegen, The Netherlands. These rats have been selected on the basis of their behavioral response to a single dose of the partial dopaminergic D1/D2 agonist apomorphine [50], which, by selective breeding, resulted in the apomorphine susceptible (APO-SUS) and the apomorphine unsusceptible (APO-UNSUS) ratline. Animals were housed two to three per cage (Macrolon® type 3; 37 x 22 x 18 cm) in temperature controlled rooms (20 ± 2° C) with a standard 12/12-h day/night-cycle (lights on at 7.00 am) and food and water available *ad lib*. All experiments were performed with drug and experimentally naïve rats. All experiments were performed in accordance with institutional, national, and international guidelines for animal care and welfare.

Free choice two-bottle paradigm

After transportation, animals were allowed to acclimatize for 10 days, and from day 5 onwards they were isolated and subsequently weighted daily. APO-SUS and APO-UNSUS rats were matched for weight at the beginning of each experimental protocol. After these 10 days, animals from all experiments were habituated to the two-bottle paradigm by offering them water in two plastic drinking cylinders on top of the cage, one on each side, for a total of 5 days. If a consumption of less than 2 ml/24 hour was observed during this period, animals were excluded from the experiment. Immediately after this 5 day period, the two-bottle, free-choice, 24 hour alcohol vs. water paradigm started [294]. In short, animals were presented with either water in both bottles or, on alternating days, with water and increasing

alcohol percentages (2% to 10%). Alcohol-solutions (2% through 10%) were prepared from the 96% ethanol stock-solution (Genfarma BV, the Netherlands) by diluting it with tap water. Bottles were swapped on alcohol presentation days to prevent bias.
Fluid consumption was measured daily and used to calculate two measurements, namely preference of alcohol above water (as determined by the alcohol intake in ml divided by total intake x 100%) and intake in grams of a 100% alcohol solution per kg bodyweight (intake in ml corrected for the gravity of alcohol and recalculated towards a 100% solution divided by bodyweight in kg).

Experiment 1: no challenges
Animals were given the free choice, two bottle alcohol vs. water paradigm as described above with access to solutions with increasing alcohol percentages or water. Bodyweights were measured during the switch from alcohol to water with a frequency of once a week. This was done to minimize the amount of handling and stress. After verification of a linear progression of bodyweight, daily bodyweights were extrapolated for intake calculation.

Experiment 2: mild acute challenge at 4%
Animals were given the experimental procedure as described for experiment 1, with two exceptions. At 4% alcohol presentation animals were subjected to a single mild challenge by transferring the animals to cages with a stainless steel 2mm grid floor. After 24 hour the grid floor was removed and animals were returned to their cages. Furthermore, animals were not only weighed during the switch from alcohol to water presentation but also at the switch from water to the 4% alcohol solution to better monitor the effect of the challenge. The 4% alcohol concentration was chosen on basis of previously done research that showed that, under non challenged conditions, the animals show a stable intake and preference from 4% on [109,294].

Experiment 3: mild sub-chronic challenges from 4% to 10%
Animals were given the experimental procedure as described for experiment 2. Additional to the challenge at 4%, animals were also presented with the following mild challenges: at 5% wet sawdust bedding, at 6% housing in rat metabolism cages (20 x 12 x 10 cm), at 7% tilting the cage 30-45°, at 8% lights on/off with a 30 minute interval, at 9% housing on a stainless steel grid floor and tilting the cage 30-45°, and at 10% reversed day-night cycle [131]. The challenges given were chosen on basis of their ease to be executed, the possibility to administer them simultaneously with alcohol, and their unpredictability [131]. Bodyweights were measured on each day during the switch from water to alcohol or vice-versa.

Statistical analysis
This experiment yielded 4 measurement values, namely intake of alcohol in grams per kilogram per 24 hours, preference scores for alcohol, intake of water in ml on water days, and bodyweights of the animals. Bodyweights measured at the onset of isolation and at the end of the experiment were used to determine whether initial differences in weight between APO-

SUS and APO-UNSUS in each experimental procedure were present. Analysis was done by means of a one-way ANOVA for each time point. Fluid intake (total water intake per day) was analyzed over the complete duration of the experiment by means of a two-way ANOVA with the factor day as the repeated measure and the factor genotype or treatment as the between subject variable. Due to a constant total fluid intake for APO-SUS and APO-UNSUS during all three experimental procedures, preference values were only analyzed for the non challenged protocol. For the other experiments an increase or decrease in intake automatically indicated an increase or decrease in preference values. Preference data and intake data for the non challenged conditions were analyzed by means of a two-way ANOVA with the factor alcohol percentage as the repeated measure and the factor genotype, followed by an independent samples t-test on percentage where appropriate. Since APO-SUS and APO-UNSUS animals have a different alcohol consumption under basal conditions, the effect of acute and sub-chronic challenges was analyzed per genotype. For each genotype, either APO-SUS or APO-UNSUS intake data were analyzed with a two-way ANOVA with the factor alcohol percentage as the repeated measure and the factor type of stressor (either acute or sub-chronic), followed by an independent samples t-test where appropriate. A probability level of $p < 0.05$ was taken as statistically significant.

Results

Bodyweight

At isolation and at the end of each experimental procedure no difference in bodyweight was seen between APO-SUS and APO-UNSUS (table 1). All animals gained weight during the experiments.

Table 1: *Bodyweight in grams (mean ± sem) for APO-UNSUS and APO-SUS at isolation and at the end of the experiment for all experimental procedures. Bodyweight at isolation was compared between APO-SUS and APO-UNSUS for each experiment by means of an independent samples t-test, which yielded no significant differences.*

	APO-UNSUS		**APO-SUS**	
	isolation weight	*end of experiment*	*isolation weight*	*end of experiment*
Experiment 1: **no challenges**	338 ± 4 (n = 14)	398 ± 4	329 ± 10 (n = 12)	379 ± 8
Experiment 2: **mild acute challenge at 4%**	276 ± 11.8 (n = 9)	347 ± 12.5	296 ± 7.6 (n = 8)	358 ± 6
Experiment 3: **mild sub-chronic challenges from 4 to 10%**	316 ± 4 (n = 9)	346 ± 5	300 ± 6.5 (n = 12)	328 ± 6

Total consumption of water and alcohol during the experiment

Water consumption on intervening water days did not undergo any change (average of 30 ml). On alcohol days the consumption of water was lower than on water days for both ratlines, but stable and equal between the lines throughout the experiment. Total fluid intake was also constant. Therefore, an increase in the intake of alcohol also indicated an increase in preference.

Alcohol consumption under basal conditions

Non-challenged APO-UNSUS rats consumed significantly more alcohol in time than APO-SUS rats (genotype-day interaction: $F_{(8,192)} = 2.9$; $p < 0.01$). Analysis with the independent samples t-test revealed that this difference was present from 4% to 10% ($p < 0.05$: figure 1). In addition to an increased intake of alcohol, APO-UNSUS animals had a higher preference for alcohol in time than APO-SUS animals (genotype-day interaction: $F_{(8,192)} = 1.9$; $p = 0.05$). Analysis with the independent samples t-test revealed that the preference for alcohol above water was significantly larger in APO-UNSUS animals from 4% to 10% ($p < 0.01$: figure 2).

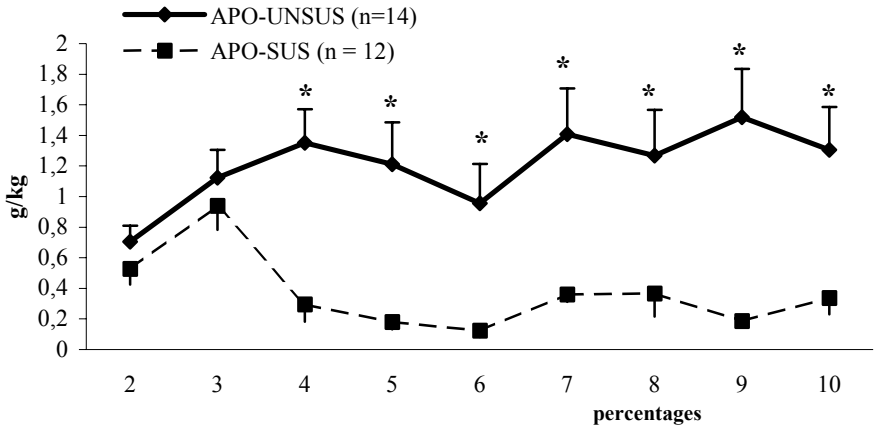

Figure 1: *Intake of alcohol in g/kg alcohol for each presentation day of the percentage 2% to 10% for APO-SUS (dashed line) and APO-UNSUS (straight line) animals. Intake was significantly elevated in APO-UNSUS from 4% to 10% alcohol presentation (independent samples t-test p < 0.05; marked by an asterisks)*

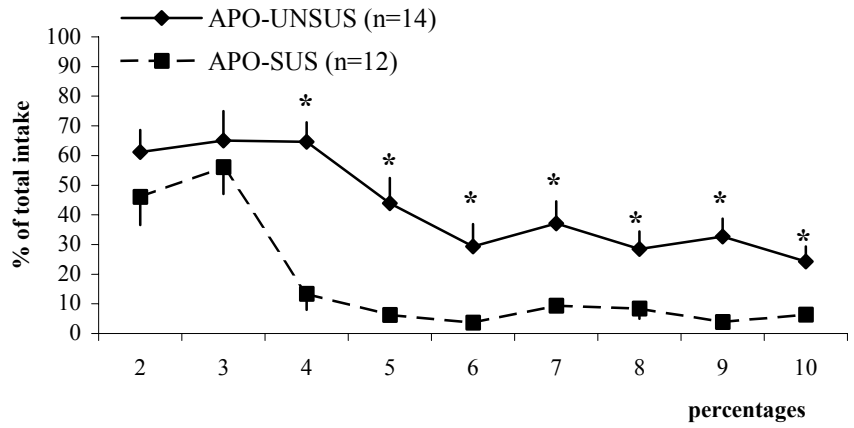

Figure 2: *Preference for alcohol above water in percentages for APO-SUS (dashed line) and APO-UNSUS (straight line) animals. APO-UNSUS animals had a higher preference for alcohol above water than APO-SUS animals from 4% to 10% alcohol presentation (independent samples t-test: p < 0.05; marked by an asterisks).*

APO-UNSUS: the effects of mild acute and mild sub-chronic challenges

The acute challenge at 4% resulted in an overall higher consumption of APO-UNSUS animals when comparing them to the non challenged animals (treatment: $F_{(1,21)} = 4.5$ $p <$

0.05). Analysis with the independent samples t-test revealed that the intake was significantly elevated only at 4% and 5% alcohol presentation ($p < 0.05$: figure 3).

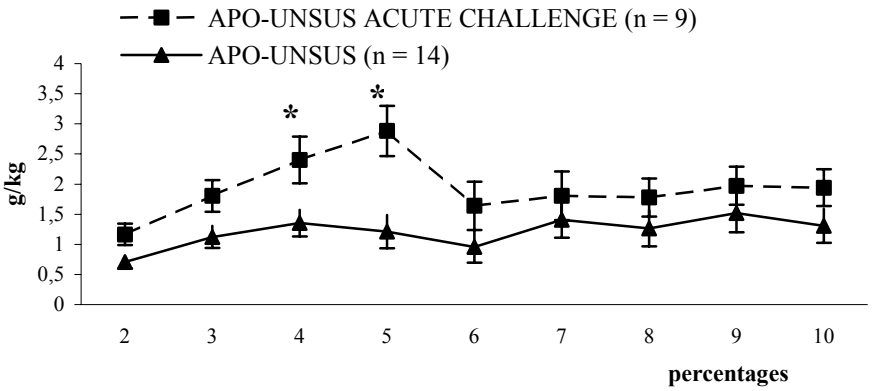

Figure 3: *Intake of alcohol in g/kg alcohol for each presentation day of the percentage 2% to 10% for the APO-UNSUS ratline under normal, non challenged conditions and during the presentation of an acute challenge during presentation of 4% alcohol. APO-UNSUS rats increased their intake during presentation of the challenge at 4% and at 5% alcohol presentation (independent samples t-test $p < 0.05$; marked by an asterisks)*

Sub-chronic stress from 4% to 10% alcohol presentation resulted in an increase in intake over time of the stressed animals in comparison to the non challenged animals (treatment-day interaction: $F_{(8,168)} = 8.6$; $p < 0.05$). This increase in intake was present from 4% to 10% alcohol presentation ($p < 0.05$: figure 4). Remarkably, the effects of the different stressors was constant, resulting in an increase in intake (and preference) of alcohol towards a stable high level.

Comparing the effect of acute and sub-chronic challenges with one another revealed that APO-UNSUS animals responded differently to the two protocols over time (treatment-day interaction: $F_{(8,128)} = 7.8$ $p < 0.01$). The independent samples t-test revealed that sub-chronically challenged APO-UNSUS animals had a larger increase in intake from 6% to 10% ($p < 0.05$). There was no difference in the response to the challenge at 4% between the two groups.

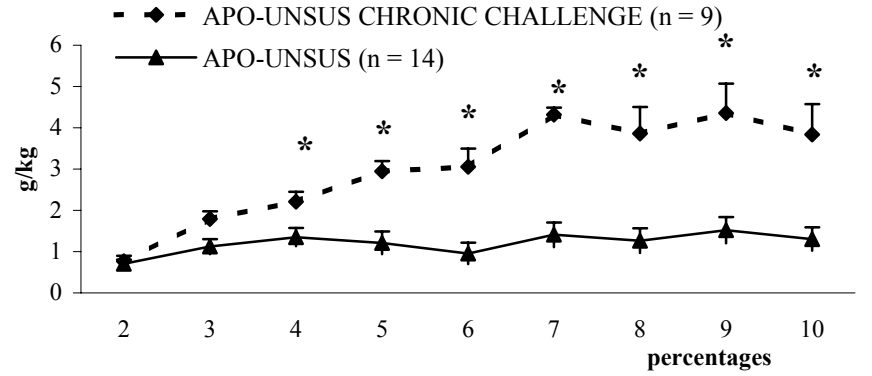

Figure 4: *Intake of alcohol in g/kg alcohol for each presentation day of the percentage 2% to 10% for the APO-UNSUS ratline under normal, non challenged conditions and during the presentation of sub-chronic challenges during presentation at 4% to 10% alcohol. Intake was elevated after administration of the sub-chronic challenges from 4% to 10% (independent sample t-test $p < 0.05$; marked by an asterisks).*

APO-SUS: the effects of mild acute and mild sub-chronic challenges

The acute challenge at 4% resulted in an overall higher consumption of APO-SUS animals when comparing them to the non challenged animals (treatment: $F_{(1,18)} = 12.3$ $p < 0.05$). This increase in intake was present from 4% to 10% ($p < 0.05$: figure 5).

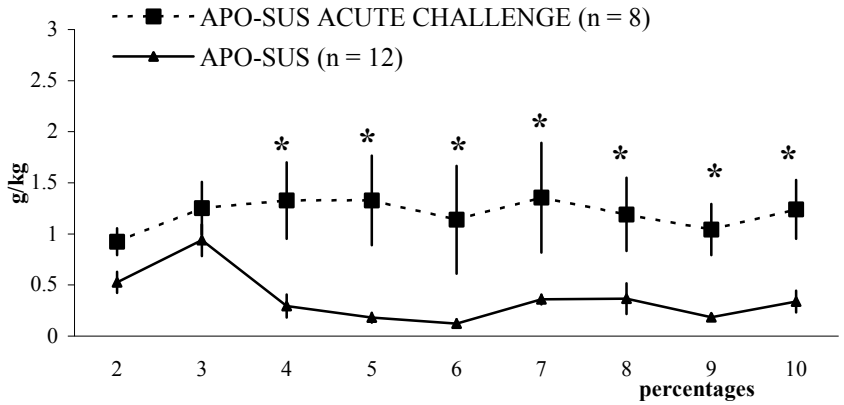

Figure 5: *Intake of alcohol in g/kg alcohol for each presentation day of the percentage 2% to 10% for the APO-SUS ratline under normal, non challenged conditions and during the presentation of an acute challenge during presentation of 4% alcohol. APO-SUS rat increased intake after the acute challenge at 4%, to stay elevated for the remainder of the experiment (independent samples t-test p < 0.05; marked by an asterisks).*

Sub-chronic stress from 4% to 10% alcohol presentation resulted in an increase in alcohol consumption over time when comparing the non challenged with the challenged animals (treatment-day interaction: $F_{(8,176)} = 11$ $p < 0.01$). Analysis revealed that this increase was present from 4% to 10% ($p < 0.05$: figure 6). Surprisingly, although the intake of alcohol during the exposure to the different stressors increased in time, this increase was marked by an oscillating pattern. When comparing the effect of acute and sub-chronic challenges with one another, no difference in intake was seen at any presentation day.

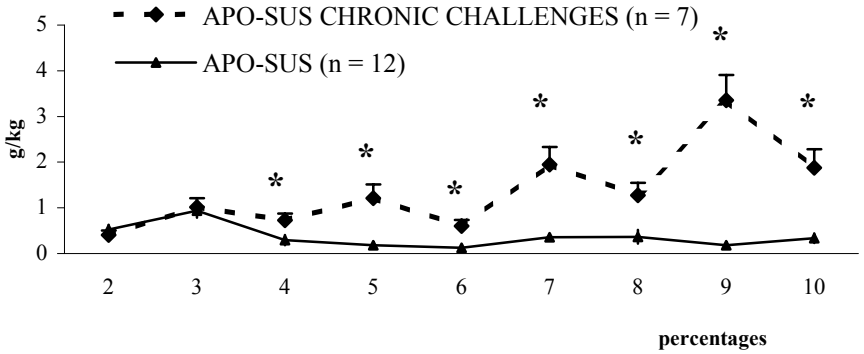

Figure 6: *Intake of alcohol in g/kg alcohol for each presentation day of the percentage 2% to 10% for the APO-SUS ratline under normal, non challenged conditions and during the presentation of sub-chronic challenges during presentation at 4% to 10% alcohol. Intake was elevated after administration of the challenges from 4% to 10% alcohol presentation (independent samples t-test: p < 0.01; marked by an asterisks).*

Discussion

The present study showed that under basal conditions APO-SUS and APO-UNSUS rats differed in the intake of, and preference for, alcohol, in which the former had a lower intake and preference than the latter. This finding is in agreement with the study of Sluyter et al [294]. Furthermore, this study provides more insight into the interaction between genetic and environmental factors in directing alcohol consumption. An acute challenge at 4% alcohol presentation resulted in a prolonged increase in intake of alcohol for the APO-SUS animals and a short-term increase in the APO-UNSUS animals. Sub-chronic stress during 4% to 10% alcohol presentation resulted in an increase in intake and preference for both genotypes.

The differences in alcohol consumption under non challenged conditions between the APO-SUS and APO-UNSUS animals can be associated with their specific features. As mentioned in the introduction, the APO-SUS rats are characterized by higher levels of TH immunoreactivity in the ventral striatum [87], a higher amount of dopaminergic D2 receptors in the striatum [267], a functionally lower noradrenergic activity in the ventral striatum [50,81], and lower free plasma levels of corticosterone [264] than the APO-UNSUS rats. All these features are associated with less alcohol consumption [38,120,120,203,219,220,299,302], and this study therefore gives more weight to this correlation.

Currently, 5 rat models for alcoholism exist, with, amongst others the alcohol avoiding/ non-avoiding line (AA/ANA), the preferring/ non-preferring line (P/NP), the Sardinian preferring/ non-preferring line (sP/ sNP), the University of Chile B and A (UChB/UChA) lines, and the high/ low-alcohol drinking line (HAD/LAD). Although these models have provided evidence for the association between genetics and alcohol consumption and between plasma levels of corticosterone and alcohol consumption [93,95,182,203], the interaction between genetic background and stress was, until now, not investigated.

The present study revealed that stressors can, dependent on the genetically determined characteristics of the rats, change the alcohol consumption. The differential response of the APO-SUS and the APO-UNSUS rats to the acute challenge can be attributed to the interline differences in the reactivity of their HPA axis. APO-SUS rats are marked by a higher and prolonged stress-induced response of the HPA-axis, as measured by the release of ACTH and corticosterone [50,267], which might explain why the relatively stress-sensitive APO-SUS rats have a strong and long-term increase in alcohol consumption after the acute challenge, whilst the stress-insensitive APO-UNSUS rats have a small and short-term increase in intake. Alternatively, the noradrenergic activity of the ventral striatum might also be involved in the stress-induced change in consumption. After a single challenge it is known that the functional noradrenergic activity in the APO-SUS animals increases [50]. Since Taylor et al have found an association between high activity and high intake, one would therefore expect a relatively increased intake in the APO-SUS rats. However, at the same time the functional noradrenergic activity decreases in the APO-UNSUS rats and this would, according to the same principle, have resulted in a decreased intake. Since the APO-UNSUS rats show a moderate increase in alcohol intake, the functional noradrenergic activity of the ventral striatum is suggested to be relatively unimportant in the stress-induced alteration of alcohol consumption. Further research however is necessary to determine the role of both

corticosterone and noradrenergic activity in directing the stress-induced response of both APO-SUS and APO-UNSUS rats.
Sub-chronic challenges increased the intake of alcohol in both genotypes. The APO-UNSUS rats increased the intake of alcohol to very high amounts for the duration of the experiment. If indeed the stress-induced activation of the HPA-axis is involved in modulation of alcohol consumption (see above), the ongoing increase in consumption of alcohol in sub-chronically stressed rats, especially in the APO-UNSUS rats, might indicate that the chosen set of stressors produced a progressively increasing stimulation of the HPA-axis. Further research is required to (in)validate this hypothesis.
Apart from the differential reaction of the APO-SUS and APO-UNSUS to the administration of mild acute and mild sub-chronic challenges, the present data reveal an interesting phenomenon. The sub-chronic stress protocol led to a very stable intake pattern in the APO-UNSUS rats, whilst the intake pattern of the APO-SUS rats followed an ascending pattern of in- and decreases. Apart from the fact that the response to a single stressor is quantitatively greater in APO-SUS rats than in APO-UNSUS rats, the present findings on the different response patterns to exposure to a series of distinct stressors in both types reveal that the response to different stressors is also qualitatively different between APO-SUS and APO-UNSUS rats. To what extent a line-specific difference in the development of sensitization and subsequent desensitization following each stressor underlies the noted difference in the response patterns remains to be investigated.

Conclusions
In conclusion, we replicated the original finding that under baseline conditions the APO-UNSUS rats consume more alcohol than the APO-SUS rats. This finding is now extended by knowledge of the influence of different stressors on alcohol consumption. After an acute challenge the APO-SUS rats show a large and prolonged increase in consumption, whereas the APO-UNSUS rats show a small and short increase. Furthermore, we show that sub-chronic challenges can further increase the intake of APO-UNSUS rats. Thus, these data nicely illustrate that the APO-SUS/ APO-UNSUS ratlines represent an attractive model to study the interaction between genetic factors and stress on directing alcohol consumption.

Chapter 3

Sweet and bitter taste sensation in individually different rats: the consumption of sweetened but not bitter substances parallels to the consumption of drugs of abuse.

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Abstract
Several studies have suggested that alcohol consumption can be predicted by the intake of sweet and/or bitter solutions. Research from our department has shown that genetically selected apomorphine unsusceptible (APO-UNSUS) rats consume considerably more alcohol and cocaine under non-challenged conditions (not stressed) than apomorphine susceptible (APO-SUS) rats do, whilst the reverse holds true under challenged conditions. The main purpose of the present series of experiment was to determine whether the suggested relation between the consumption of sweetened and bitter solutions and drugs of abuse is also present in these genetically selected APO-UNSUS and APO-SUS rats. Animals, both APO-UNSUS and APO-SUS rats, were allowed to consume ascending series of sucrose and quinine by means of the 24-hour access two-bottle paradigm. Each flavored solution was tested in a naive group of rats. Moreover, the impact of neophobia on drinking behavior was investigated by allowing both APO-UNSUS and APO-SUS rats to consume the same concentration of saccharin thrice. The results indicated that the consumption of sucrose parallels to the consumption of drugs of abuse, namely non-challenged APO-UNSUS rats consumed considerably more low-concentration sucrose than non-challenged APO-SUS rats did. There were no line-specific differences found in the consumption of the bitter substance quinine nor did neophobia interfere with drinking behavior.

Introduction

Many studies have suggested that there is a relationship between the preference for, and intake of, sweet solutions and the consumption of abusive drugs like alcohol or psychostimulant consumption [64,155,232]. For instance, sucrose intake has consistently been found to be a predictor for both alcohol as well as cocaine and amphetamine intake [64,124,180,300]. For instance, Stewart et al have reported that rats with a high preference for alcohol also consume more of a sucrose solution [300]. And several studies have shown that rats which consume high amounts of sucrose, also self-administer considerably more cocaine and amphetamine than rats which consume smaller amounts of sucrose [64,124].

Research from our department has shown that the genetically selected apomorphine unsusceptible (APO-UNSUS) rats consume considerably more alcohol (and cocaine) under non-challenged conditions (not stressed) than the apomorphine susceptible (APO-SUS) rats do, whilst the reverse holds true under challenged conditions [294,318,319]. Until so far, taste sensation of APO-UNSUS and APO-SUS rats was not investigated. Considering the above-mentioned correlation between sucrose and alcohol consumption, the present study investigated the intake of different concentrations of sucrose in APO-UNSUS and APO-SUS rats under non-challenged conditions. Because sucrose consumption is suggested to correlate with drug intake, and because APO-UNSUS rats consume more alcohol (and cocaine) under non-challenged conditions [294,318,319], it is hypothesized that non-challenged APO-UNSUS rats will consume more sucrose than non-challenged APO-SUS rats.

In contrast to sucrose intake, the correlation between saccharine consumption and alcohol intake is less unequivocal. For instance, some researchers have shown that saccharin consumption predicts alcohol intake [125,232], and that rats selectively bred for high or low intake of alcohol show parallel drinking patterns of saccharin [291], whilst others have found that the relationship between the intake of saccharin and alcohol is reversed [122]. Moreover, saccharine intake does not predict the intake of other drugs of abuse, like cocaine [106,126]. Closer inspection of the available literature, however, shows that these correlations between either saccharin and alcohol are also highly dependent on the rat strain and protocol used [2,123,291]. Furthermore, some studies have suggested that difference in taste sensation of especially saccharine and quinine results in the differences found in alcohol consumption, and thus making animals with a differential intake of these substances less desirable as a model for alcohol consumption [207,208].

Given this contradiction in the available data on the correlation between saccharine and/or quinine and alcohol intake as well as the suggestion that individual differences in alcohol consumption could be a result of a difference in taste sensation of substance with a bitter component (saccharin consists of both a sweet and bitter component [65]), the present study also investigated the intake of different concentrations of quinine and a single concentration of saccharine in APO-UNSUS and APO-SUS rats under non-challenged conditions.

To circumvent problems of successive series of different flavored solutions and differences in the duration of fluid access [2], the consumption of each different flavored solution was examined in a naive group of non-challenged APO-UNSUS and APO-SUS rats for the duration of 24 hours. Moreover, to evade potential effects of successive series of the same

flavored solutions, the consumption of different ascending and descending concentrations of the same solution was investigated. Finally, to verify that neophobia did not contribute to differences in the consumption of flavored solutions with an extended access protocol, non-challenged APO-UNSUS and APO-SUS rats were also presented with the same saccharin solution thrice [111]. It was expected that with an extended access protocol, the (known) individual differences in neophobia between APO-UNSUS and APO-SUS rats will not be reflected in their drinking behavior [111].

Methods and Materials

Animals

Adult male Wistar rats from the 18th and 19th generation (replicated line) of APO-UNSUS and APO-SUS rats were obtained from the Central Animal Laboratory (CDL), Radboud University Nijmegen, The Netherlands [86]. These rats have been selected on the basis of their behavioral response to a single dose of the partial dopaminergic D1/D2 agonist apomorphine [50], which, by selective breeding, has resulted in the apomorphine unsusceptible (APO-UNSUS) and the apomorphine susceptible (APO-SUS) ratline. Animals were housed two to three per cage (Macrolon® type 3; 37 x 22 x 18 cm) in temperature-controlled rooms (20 ± 2° C) with a standard 12/12-h day/night-cycle (lights on at 7.00 am) and food and water available *ad lib*. All ascending and/or descending series of the solutions were performed with a new group of naïve rats. All experiments were performed in accordance with institutional, national, and international guidelines for animal care and welfare.

Free choice two-bottle paradigm

Animals were isolated and left undisturbed for 5 days. After these 5 days, all animals were habituated to the two-bottle paradigm by offering them water in two plastic drinking cylinders on top of the cage, one on each side, for another 5 days. Immediately after this 5 day period, the two-bottle, free-choice, 24 hour solution vs. water paradigm started [294]. In short, habituated (non-challenged) animals were presented with either water in both bottles or, on alternating days, with water in one bottle and the flavored solution in the other bottle. Bottles were swapped on flavored solution presentation days to prevent bias for one side. Each experiment yielded 2 measures, namely intake of the flavored solution in grams per kilogram per 24 hours and the preference scores for the flavored solution (intake in ml divided by total fluid intake x 100%).

Experiment 1: neophobia

This experiment determined whether non-challenged APO-UNSUS (n = 7) and APO-SUS (n = 9) rats differed in the consumption of a 0.25% saccharin solution (figure 1). More importantly, this experiment investigated whether the known differential neophobic response of APO-UNSUS and APO-SUS rats could be prevented by extending the access period in which rats were allowed to consume the solution. Therefore, all animals were allowed to consume the 0.25% saccharin solution for 24 hours and the same animals were presented

with the same saccharin concentration twice more (after 72 hours and 144 hours). This was done to verify that the consumption was unaltered during these three presentations days with the extended access protocol [111].

Experiment 2: sucrose consumption

To investigate whether non-challenged APO-UNSUS and APO-SUS rats differed in the consumption of the sweet solution sucrose, distinct naive groups of APO-SUS and APO-UNSUS rats were allowed to consume different concentrations of sucrose (figure 1). In experiment 2A, APO-UNSUS (n = 9) and APO-SUS (n = 8) rats were presented with a 0.5%, 1.0%, 3.0%, 7.0% and 15.0% sucrose solution (wt/vol) on every other day. In experiment 2B, APO-UNSUS (n = 7) and APO-SUS (n = 9) rats were presented with 1.0%, 3.0%, 7.0%, 15.0% and a 25.0% sucrose solution (wt/vol) on every other day. And in the last experiment (2C), APO-UNSUS (n = 8) and APO-SUS (n = 7) rats were presented with the 15.0% sucrose solution (wt/vol) once. The purpose of these three different experiments was to determine whether the 'drinking history' of an animal affects subsequent drinking as has been suggested by Ackroff et al [2].

Experiment 3: quinine consumption

Since saccharin has both a sweet and bitter gustatory effect [65] and since research has suggested that a difference in the consumption of quinine can result in a differential intake of alcohol [207,208], the consumption of the bitter solution quinine was also investigated (figure 1). Naive groups of APO-UNSUS and APO-SUS rats were presented with different series of quinine solutions. In experiment 3A, APO-UNSUS (n = 9) and APO-SUS (n = 8) rats were presented with a 0.0005%, 0.001%, 0.005%, 0.01% quinine solution (wt/vol) on consecutive solution presentation days. In experiment 3B, APO-UNSUS (n = 9) and APO-SUS (n = 8) rats were presented with a 0.001% quinine solution followed by a 0.0005% quinine solution (wt/vol) on consecutive solution presentation days.

Statistical analysis

Bodyweights, the preference score and the intake values (g/kg/24 hr) of each flavored solution were compared within and between each experimental procedure by means of a two-way ANOVA with the factor bodyweight or concentration as the repeated measure and the factor genotype (APO-SUS vs APO-UNSUS) or experiment (different protocols), followed by a post-hoc independent samples t-test where appropriate (analysis was done with SPSS 11.0). Since intake and preference values of the sucrose experiment reached a ceiling at 3.0% sucrose, the data were also analyzed for the lowest three concentrations (0.5%, 1.0%, and 3.0%). The same was done for the lowest concentrations of quinine (0.0005% and 0.001%). Again these data were analysed with a one-way ANOVA for repeated measures. The 'drinking history' of either APO-UNSUS or APO-SUS rats was analyzed by means of a two-way ANOVA with the factor concentration as the repeated measure and the factor experiment, followed by a post-hoc independent samples t-test where appropriate. A probability level of $p < 0.05$ was taken as statistically significant

			day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9
saccharin	exp 1	side A	0.25%	H_2O	H_2O	H_2O	H_2O	H_2O	0.25%		
		side B	H_2O	H_2O	H_2O	0.25%	H_2O	H_2O	H_2O		
sucrose	exp 2A	side A	H_2O	H_2O	1.0%	H_2O	H_2O	H_2O	7.0%	H_2O	H_2O
		side B	0.5%	H_2O	H_2O	H_2O	3.0%	H_2O	H_2O	H_2O	15%
	exp2B	side A	1.0%	H_2O	H_2O	H_2O	7.0%	H_2O	H_2O	H_2O	25%
		side B	H_2O	H_2O	3.0%	H_2O	H_2O	H_2O	15%	H_2O	H_2O
	exp 2C	side A	H_2O								
		side B	15%								
quinine	exp 3A	side A	0.0005%	H_2O	H_2O	H_2O	0.005%	H_2O	H_2O		
		side B	H_2O	H_2O	0.001%	H_2O	H_2O	H_2O	0.01%		
	exp 3B	side A	0.001%	H_2O	H_2O						
		side B	H_2O	H_2O	0.0005%						

Figure 1: *Schedule of the three experiments. Before the presentation of the first flavored solution, animals were allowed to drink water (H_2O) for 5 days. Each animal was presented with the flavored solution on one side (side A) and water on the other side (side B). After 24 hours, both bottles were replaced by two new waterbottles. Subsequent presentation of the flavored solution was always on the opposite side (in comparison to the first presentation). The protocol of the saccharin experiment was slightly different from the other two protocols: instead of an 24-hour interval between flavored solutions, the saccharine protocol had a 48-hour interval. This difference was based on previous experiments researching neophobia [111].*

Results

In general

Prior to the start of experiment (presentation of the first flavored solution) all animals consumed around 30 ml of water per day. APO-UNSUS and APO-SUS rats of the saccharin experiment and the different sucrose experiments weighted the same throughout the experiment. There were no differences in bodyweight between the two rattypes during the quinine experiments. However, when comparing APO-UNSUS rats from experiment A with B, APO-UNSUS rats from experiment B were lighter than APO-UNSUS rats from experiment A during the presentation of 0.0005% and 0.001% ($p < 0.05$; table 1).

Experiment 1: neophobia

Both APO-UNSUS and APO-SUS rats consumed equally large amounts of the 0.25% saccharin solution during the first presentation (APO-UNSUS: 51.7 ± 4.5 ml, APO-SUS: 61.2 ± 7.7 ml). The preference score for the 0.25% saccharin solution was 90.8 ± 2.3 % for APO-UNSUS rats and 96.9 ± 0.6% for APO-SUS rats (ind. samples t-test; $t = 2.5$, $p = 0.04$). Since the intake of the 0.25% saccharine solution was equal between APO-UNSUS and APO-SUS rats, the difference in the preference score was due to a differential consumption of water (preference score = flavored solution divided by total fluid consumption). During the second and third presentation no differences in intake and preference were visible (figure 2). Since both APO-UNSUS and APO-SUS rats consumed equal amounts of the same solution during each presentation, the influence of neophobia as a contributing factor to individual differences in drinking behavior was neglectable

Table 1: *Bodyweight of APO-SUS and APO-UNSUS rats throughout the experiments. There were no differences between APO-SUS and APO-UNSUS rats during the saccharine, sucrose and quinine experiments. There was, however, a difference between the APO-UNSUS rats of experiment 3A and 3B during the presentation of 0.0005% and 0.001%: APO-UNSUS rats of experiment 3b weighted less than APO-UNSUS rats of experiment 3a.*

				0.25%	0.25%	0.25%			
saccharin	exp 1	APO-SUS	(n = 9)	344.2 ± 6.6	348.2 ± 6.7	351.8 ± 6.7			
		APO-UNSUS	(n = 7)	358.5 ± 7.9	367.1 ± 8.3	373.3 ± 7.9			
				0.5%	1.0%	3.0%	7.0%	15.0%	25.0%
sucrose	exp 2A	APO-SUS	(n = 8)	332.4 ± 3.1	339.8 ± 3.1	340.2 ± 3.5	344.6 ± 3.1	353.1 ± 3.8	
		APO-UNSUS	(n = 9)	343 ± 4.8	350.5 ± 4.9	349.8 ± 5.1	356.5 ± 4.8	365 ± 4.6	
	exp 2B	APO-SUS	(n = 9)		317.7 ± 11	323.9 ± 11.3	329 ± 11.6	330.9 ± 10.6	337.1 ± 11
		APO-UNSUS	(n = 7)		320.2 ± 8.3	329 ± 8.3	332.2 ± 8.4	336.6 ± 8.4	343.1 ± 8.5
	exp 2C	APO-SUS	(n = 7)					357.6 ± 6	
		APO-UNSUS	(n = 8)					374.4 ± 12	
				0.0005%	0.001%	0.005%	0.01%		
quinine	exp 3A	APO-SUS	(n = 8)	366.3 ± 12.7	373 ± 12.7	375.2 ± 13	377.8 ± 12.9		
		APO-UNSUS	(n = 9)	358.6 ± 8.5	366.2 ± 8.2	371.4 ± 8.3	374.7 ± 7.9		
	exp 3B	APO-SUS	(n = 8)	373.1 ± 17.8	367.8 ± 17.4				
		APO-UNSUS	(n = 9)	337 ± 3.5	330.5 ± 4.2				

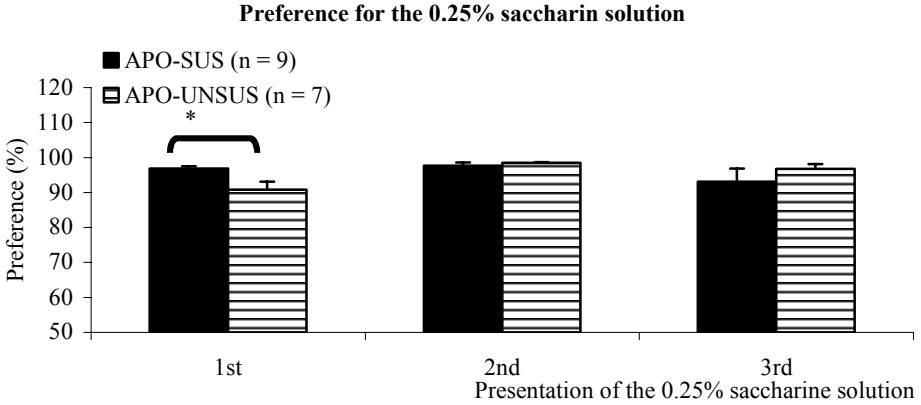

Figure 2: *Preference for the presented 0.25% saccharin solutions (mean ± sem). The preference score during the first presentation of the 0.25% saccharin solution was significantly different between APO-UNSUS and APO-SUS rats (ind. samples t-test p < 0.05; marked by *). During the second and third presentation no differences in the preference scores were visible.*

Experiment 2: sucrose consumption

Experiment 2A: *presentation of 0.5% sucrose followed by 1%, 3%, 7% and 15%*

There were no overall differences in the intake (g/kg) of and preference for the different sucrose solutions between the two rat types. The rats consumed almost only sucrose, yielding

preference scores from around 80% at the start of the experiment to 98% at the end of the experiment (figure 3).

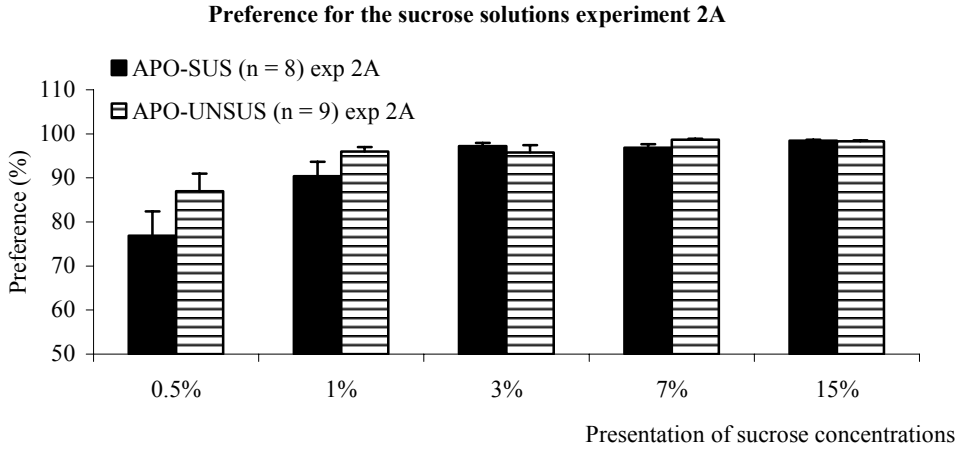

Figure 3: *Preference for the presented sucrose solutions (0.5%-1.0%-3.0%-7.0%-15.%). There were no differences in the preferences score between APO-UNSUS and APO-SUS rats throughout the experiment.*

Because intake and preferences values had reached a maximum at 3.0%, the data were also analyzed for the lowest three concentrations (0.5%, 1.0%, and 3.0%). This revealed that there were no differences in the preference score, but the intake score of the lowest two concentrations was different (0.5% and 1.0%). Non-challenged APO-UNSUS rats consumed considerably more of the 0.5% and 1.0% sucrose-solutions than non-challenged APO-SUS rats did ($F_{(1,15)} = 8.1$ $p < 0.05$; post-hoc t-test $p < 0.05$ 0.5% and 1%; figure 4).

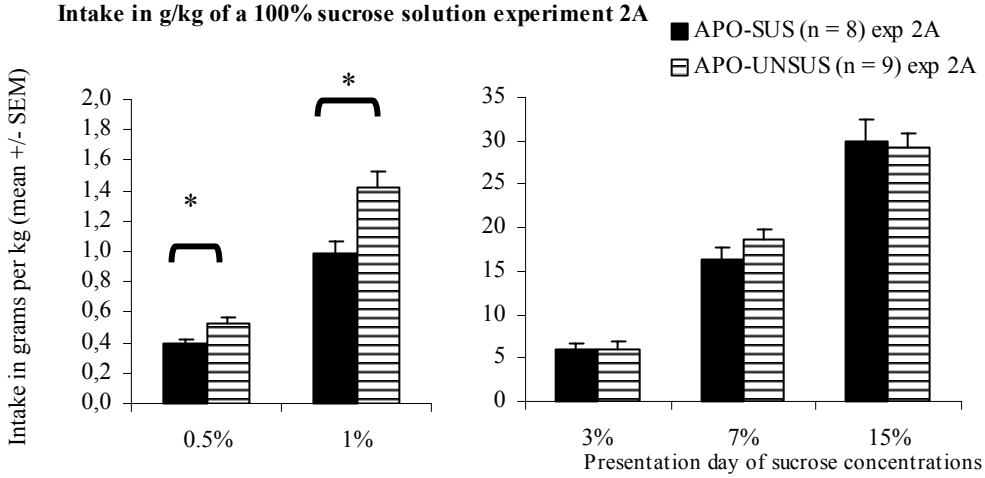

Figure 4: *Intake (gram/kg/24 hour) of the presented sucrose solutions (0.5%-1.0%-3.0%-7.0%-15.%). APO-UNSUS rats consumed considerably more sucrose than APO-SUS rats did when presented with 0.5% and 1.0% sucrose (p < 0.05, marked by *). The left side of the graph shows the intake of the lowest concentrations, namely 0.5% and 1.0%. The right side shows the other concentrations.*

Experiment 2B: *presentation of 1% sucrose followed by 3%, 7%, 15% and 25%*
There were no differences in the intake of and preference for the sucrose solutions between the two rat types. As with experiment A, APO-UNSUS and APO-SUS rats almost only consumed sucrose, yielding preferences scores from around 90% at the beginning of the experiment to 98% at the end of the experiment (figure 5). Again, because the intake and preferences values had reached a maximum at 3.0%, the data were also analyzed for the lowest two concentrations (1.0%, and 3.0%). As before, non-challenged APO-SUS and APO-UNSUS rats had the same preference score, but non-challenged APO-UNSUS rats consumed considerably more of the 1% sucrose solution than non-challenged APO-SUS rats did ($F_{(1,14)} = 4.5$ $p < 0.05$; post-hoc t-test $p < 0.05$ 1%; figure 6). There was no difference in intake when the rats were presented with 3% sucrose.

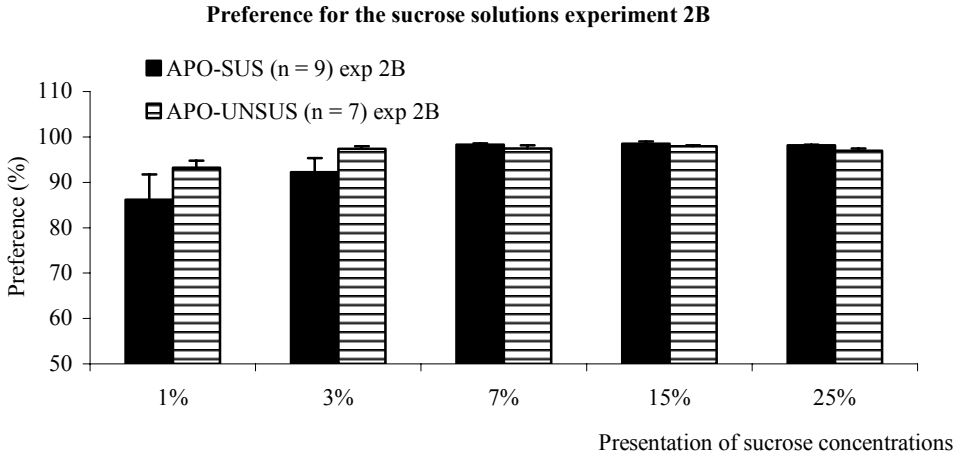

Figure 5: *Preference for the presented sucrose solutions (1.0%-3.0%-7.0%-15.0%-25.0%). There were no differences in the preferences score between APO-UNSUS and APO-SUS rats.*

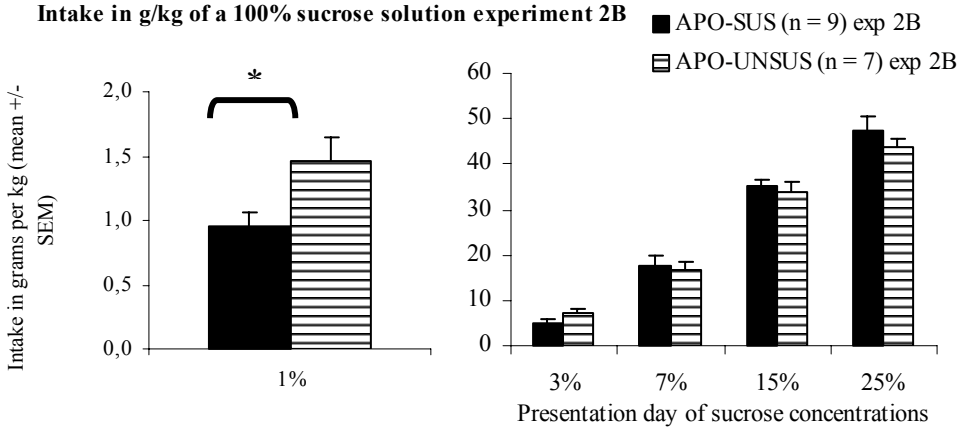

Figure 6: *Intake (gram/kg/24 hour) of the presented sucrose solutions (1.0%-3.0%-7.0%-15.0-25.0%%). APO-UNSUS rats consumed considerably more sucrose than APO-SUS rats did when presented with 1.0% sucrose ($p < 0.05$).*

Experiment 2C: presentation of 15%
There was no difference in the intake of and preference for the 15% sucrose solution between APO-UNSUS and APO-SUS rats. APO-UNSUS rats had a preference score of 94% ± 2.7% whilst APO-SUS rats had a preference score of 98% ± 0.4%.

Comparing experiment 2A, 2B and 2C
To investigate whether the 'drinking history' of either rattype had an effect on subsequent drinking, the intake and preference values of the different experiments were compared. Even though APO-UNSUS and APO-SUS rats started with a different concentration, no differences in intake or preference were found between experiment 2A, 2B, and 2C. For instance, the consumption of 1.0% sucrose was equal between experiment 2A and 2B for both APO-SUS and APO-UNSUS rats. The same holds true when examining the intake of 15.0% (figure 7).

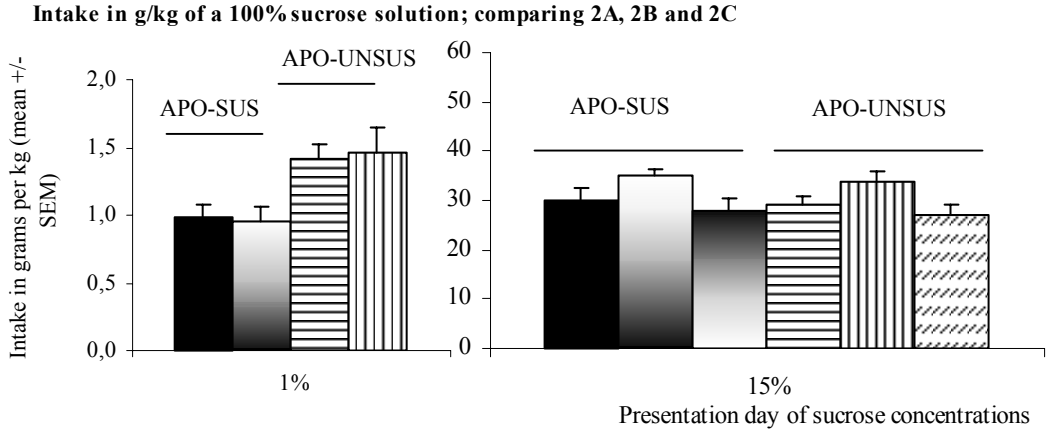

Figure 7: *Comparison between sucrose experiment 2A, and 2B for the intake of 1.0% and between sucrose experiment 2A, 2B and 2C for 15.0% (other concentrations were also compared, but are not shown in a graph). Even though the rats had a different 'drinking history', no differences were seen in the intake of 1.0% and 15.0% sucrose, indicating that the 'drinking' history did not play a role in deteremining the intake of sucrose.*

Experiment 3: quinine consumption
Experiment 3A; presentation of 0.0005% followed by 0.001%, 0.005% and 0.01%
The consumption of the 0.005% and the 0.01% quinine solution was below detection level, indicating a severe aversion for these concentrations of quinine (consumption was less than 2 ml/24hr). Analysis of the data was therefore done only on 0.0005% and 0.001% quinine. It should, however, be noted that the intake values (in ml) of 0.001% were just above detection level. Both APO-UNSUS and APO-SUS rats consumed equally small amounts and had the same preference for the 0.0005% and the 0.001% quinine solutions (table 2).

Experiment 3B; presentation of 0.001% followed by 0.0005%
Again, APO-UNSUS and APO-SUS rats consumed equally small amounts and had the same preference for the 0.001% and the 0.0005% quinine solution (table 2). Both rat types

increased their intake and preference when the lower concentration was presented (concentration effect: $F_{(1,15)} = 12.6$ $p < 0.05$).

Comparing experiment 3A and 3B

Even though the order of presentation of the 0.0005% and the 0.001% quinine solution was different between experiment A and B, the amount consumed was equal for both APO-UNSUS and APO-SUS rats, indicating that 'drinking' history did not play a role.

Table 2: *Preference for and intake (ml and g/kg) of the two quinine experiments (mean ± sem). In experiment 3A, APO-SUS and APO-UNSUS rats were allowed to consume a 0.0005% quinine solution, followed by 0.001%, 0.005% and 0.01% quinine. Since the consumption of the 0.005% and 0.01% quinine solutions was below detection level, the data were not analyzed further. In experiment 3B, APO-SUS and APO-UNSUS rats were allowed to consume a 0.001% quinine solution followed by a 0.0005% quinine solution. There were no differences in the intake values (ml and g/kg) and preference score between APO-SUS and APO-UNSUS or between the experiments.*

			0.0005%	0.001%	0.005%	0.01%
ml	exp 3A	APO-SUS (n = 8)	12.4 ± 3	2.5 ± 0.5	1.7 ± 0.1	1.6 ± 0.1
		APO-UNSUS (n = 9)	7.1 ± 1.4	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.2
	exp 3B	APO-SUS (n = 8)	8.4 ± 2.7	4.5 ± 0.9		
		APO-UNSUS (n = 9)	8 ± 3.5	3.8 ± 1.1		
gram/kg	exp 3A	APO-SUS (n = 8)	1.73 ± 0.4	0.7 ± 0.15		
		APO-UNSUS (n = 9)	0.97 ± 0.2	0.5 ± 0.05		
	exp 3B	APO-SUS (n = 8)	1.14 ± 0.4	1.23 ± 0.25		
		APO-UNSUS (n = 9)	1.19 ± 0.5	1.19 ± 0.4		
preference	exp 3A	APO-SUS (n = 8)	38.7 ± 9.8	8.7 ± 1.8		
		APO-UNSUS (n = 9)	20.5 ± 4.1	5.2 ± 0.6		
	exp 3B	APO-SUS (n = 8)	27.9 ± 9.8	15.6 ± 3.8		
		APO-UNSUS (n = 9)	24.2 ± 9.9	12.6 ± 3.8		

Discussion

The main purpose of this study was to determine whether the enhanced consumption of both cocaine and alcohol by the non-challenged APO-UNSUS rats [294,318,319], parallels to the consumption of sucrose. The present study clearly shows that non-challenged APO-UNSUS rats consumed considerably more of low-concentration sucrose solutions than non-challenged APO-SUS rats. When animals were presented with higher concentrations of sucrose, the line-specific differences disappeared. In addition, the study also investigated whether the intake of different concentrations of quinine and a single concentration of saccharine in non-challenged APO-UNSUS and APO-SUS rats was different. This was done to investigate whether differences in taste sensation, as suggested by some researchers [207,208], was the causative factor for the previously found differences in alcohol consumption. The present study shows that there were no line-specific differences in the consumption of saccharine and quinine.

The present study showed that there were no line-specific differences in the intake of the 0.25% saccharin solution and that repeated presentation did not alter the drinking pattern of these rats. The fact that there were no differences in saccharin intake indicated that neither

APO-UNSUS nor APO-SUS rats experienced neophobia in the current extended access protocol. This is in contrast to the previously performed study at our department with rats that have many features in common with APO-SUS and APO-UNSUS rats, namely the high responder (HR) and low responder (LR) to novelty rats respectively [111]. That study showed that LR rats display a strong neophobic response when presented with a new solution [111]. However, that study employed a 10-min access period instead of a 24-hr access period, indicating that with an extended access protocol, neophobia is not a causative factor for individual differences in drinking behavior. Moreover, the finding that the order of presentation (either ascending or descending) did not alter intake and preference, allows for a comparison between the different concentrations and between the different experiments. This is best illustrated with the intake patterns of 1.0% and 15.0% sucrose (see results); even though both rattypes had a different 'drinking history', no differences were seen in the intake of 1.0% and 15.0% sucrose. This indicates that with a 24-hour access protocol the effects of a 'drinking' history are neglectable. Moreover, it indicates that the intake and preference values of substances given for the first time are 'true' values (and not distorted by learning processes, novelty-induced drinking and/or avoidance, and neophobia).

The finding that non-challenged APO-UNSUS and APO-SUS rats did not differ in the consumption of 0.25% saccharin, indicates that the found differences in alcohol consumption are not caused by difference in taste sensation for the sweet-bitter substance saccharine. The same holds true for the intake of the bitter substance quinine since both APO-UNSUS and APO-SUS rats did not differ in the consumption of 0.0005% and 0.001% quinine solutions. The concentrations used in this study were based on research performed in our laboratory as well as by others [111]. One could argue that the low intake values (in ml) at 0.001% and 0.0005% did not allow for the detection of differences. However, the concentrations, and then especially 0.0005%, did yield 'high intake values' as well as representative preference scores (around 30-40%). It is expected that with lower concentrations, like 0.0001% (0.1 mg quinine/liter), the preference score will reach 50%, indicating that the quinine solution is not distinctly different from water.

The fact that non-challenged APO-UNSUS rats consumed more of low-concentration sucrose solutions than non-challenged APO-SUS rats did, is in line with our hypothesis that non-challenged APO-UNSUS rats would consume more sucrose since this rattype also consumes more cocaine and alcohol [318,319]. It should be noted that, even though the intake of low-concentration sucrose was different, the preference scores were not. This indicates that non-challenged APO-UNSUS and APO-SUS rats equally 'like' the sucrose solutions, but that the rewarding properties of these sucrose concentrations are divergent between the two rat types. It is known that sucrose activates orosensory substrates that are coupled to the central dopaminergic and opioidergic systems [180], and both systems play a role in motivation and reward [160]. For instance, sucrose feeding leads to a dose-dependent increase in dopamine levels in the ventral striatum [133]. It is therefore likely that the differences found in the consumption of low-concentration sucrose are due to individual differences in the structure and/or function of those brain systems that are associated with reward sensation. This difference in the consumption of low-concentration sucrose parallels the previously found

results of both alcohol- and cocaine self-administration [294,318,319], thus indicating that non-challenged APO-UNSUS rats consume more of a rewarding substance than their counterparts, non-challenged APO-SUS rats. The question that remains is whether non-challenged APO-UNSUS rats, which consumed more of the low sucrose concentrations, experienced less or more reward than APO-SUS rats did.

The APO-UNSUS/APO-SUS rat model is based on the characteristic behavioral response to a single injection of the selective dopaminergic D1/D2 agonist apomorphine [50]. Subsequent selective breeding has resulted in the distinct rat types that are divergent in the structure and/or function of, amongst others, the dopaminergic and noradrenergic system and the HPA-axis. Non-challenged APO-UNSUS rats are characterized by lower levels of TH immunoreactivity in the ventral striatum, a lower amount of dopaminergic D2 receptors in the striatum, a functionally higher noradrenergic activity in the ventral striatum, and higher free plasma levels of corticosterone [81,87,264,267]. Since both a heightened dopaminergic activity and heightened HPA-axis activity are considered to contribute to a heightened consumption of alcohol and cocaine [117,143,203,299], it is therefore not unlikely that these two factors also contributed to the high consumption of low-concentration sucrose of these rats.

This study also showed that the line-specific differences in sucrose consumption disappeared with increasing sucrose concentrations. Some researchers state that rats, when confronted with higher concentrations of sucrose, experience more aversion than reward [330]. However, this study has found no evidence that either APO-UNSUS or APO-SUS rats experienced aversion when presented with either 15 or 25% sucrose. It is however possible that the extended access protocol provides animals with the opportunity to 'strategically' plan their intake pattern to avoid negative side effects. In contrast, the previously executed limited access protocol did yield an aversive response to the 15% sucrose solution [112].

In conclusion, the present study demonstrated that there were line-specific differences in the consumption of low-concentration sucrose whereby non-challenged APO-UNSUS rats consumed more sucrose than non-challenged APO-SUS rats. There were no differences in the consumption of either quinine or saccharin. The difference in the consumption of low-concentration sucrose parallels the previously found results of both alcohol- and cocaine self-administration, namely that non-challenged APO-UNSUS rats consumed more of a rewarding substance than their counterparts, suggesting that similar mechanisms might be involved.

Chapter 4

Gene – environment interactions determine the individual variability in cocaine self-administration.

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Abstract
Research into factors that determine the propensity to self-administer cocaine have shown that stressors can determine the amount of cocaine self-administered as well as the rate of acquisition. However, the interaction between the genetic make-up of the animal and stress is unknown. This study investigated this interaction by using the genetic animal model consisting of apomorphine susceptible (APO-SUS) and unsusceptible (APO-UNSUS) rats. Animals were allowed to self-administer 0.25 mg/kg cocaine under stressful and habituated conditions. This study revealed that the amount of cocaine consumed was highly dependent on the genetic make-up of the animal as well as the amount of stress during self-administration. Under habituated circumstances APO-UNSUS rats took far more cocaine than APO-SUS rats. Under stressful circumstances, however, the APO-SUS rats took far more cocaine than APO-UNSUS rats. This difference in the amount consumed by APO-SUS and APO-UNSUS rats is likely to be due to the specific neurobiological features of their dopaminergic and, possibly, noradrenergic system as well as the reactivity of their HPA-axis. It is suggested that the amount of a drug consumed and, accordingly, its addictive potential and 'drug-vulnerability' are determined by the interaction between the genetic make-up of the animals and stress, and not by either component alone.

Introduction

It is known that there are marked individual differences in both humans and animals in the sensitivity to the reinforcing properties of cocaine [129,271] . As in humans, amongst those animals that learn to self administer cocaine, there are considerable differences in the amount of daily intake as well as the rate of acquiring self-administration.

Several studies have identified behavioral and neuro-chemical determinants that are predictive of the liability to self-administer cocaine and the amount of cocaine taken. One approach that has been utilized to detect phenotypes that predict an increased sensitivity to the effects of cocaine is the selection of animals based on their locomotion in a novel environment [62,143,153,238]: high responders to novelty (HR) are known to acquire low-dose cocaine self-administration more rapidly and take far greater amounts of cocaine than low responders to novelty (LR). Secondly, several studies have shown that the dopaminergic systems that mediate stress responsiveness, locomotion and natural reinforcement are involved in determining these individual differences in drug intake. These studies have revealed that high dopamine levels are correlated with a high intake of cocaine [114,143,270]. Thirdly, the plasma corticosterone level at the time of cocaine self-administration has also been identified as a determinant of the acquisition of self-administration [63,117,118,192]. Indeed, pretreatment with corticosterone can greatly enhance self-administration, even to the point that differences in self-administration between the phenotypically selected HR and LR rats are abolished [268]. Other studies using physical stressors such as intermittent tail pinch prior to self-administration, unpredictable foot shocks during self-administration, social defeat prior to self-administration, and long-term neonatal isolation have shown that these stressors can enhance the acquisition of cocaine self-administration and the amount of cocaine taken [118,153,171]. Although these studies have clearly indicated that stress can enhance self-administration of cocaine, the interaction between the genetic make-up of an animal and stress in directing cocaine self-administration is not known.

Therefore, the present study used the genetic rat model consisting of apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) rats. This model is based on the differences in gnawing behavior after a single injection of the selective D1/D2 agonist apomorphine. Subsequent selective breeding has resulted in two distinct rat types that are marked by differences in the determinants that are suggested to be predictive of the liability to self-administer cocaine and the amount of cocaine taken.

APO-SUS animals are, in comparison to their counterpart APO-UNSUS, characterized by higher levels of tyrosine hydroxylase (TH) immunoreactivity in the ventral striatum under normal conditions [87], higher levels of TH mRNA in the substantia nigra [267], a higher density of dopaminergic D2 receptors in the striatum [267], a higher stress-induced dopaminergic activation of the ventral striatum [88], and by the same behavioural response to a novelty challenge as the HR rats [50]. In addition, APO-SUS and APO-UNSUS rats are marked by differences in the amount of free plasma corticosterone under normal and challenged conditions. Under normal conditions APO-UNSUS rats have more free plasma corticosterone, but after a single mild challenge (novelty challenge) APO-SUS rats have a stronger and longer lasting increase in corticosterone [264].

These differences in the (re)activity of the dopaminergic system and the HPA-axis make APO-SUS/ APO-UNSUS rats a good model to study the interaction between genes and stress on the acquisition of cocaine self-administration and the amount of cocaine taken. Previous studies with APO-SUS and APO-UNSUS rats have already shown that these animals, dependent on the environment, differ in the amount of alcohol consumed [294]. This study therefore investigated the acquisition and maintenance phase of cocaine self-administration under several behavioral conditions, ranging from stressful to habituated circumstances in the genetically selected APO-SUS and APO-UNSUS rats. We hypothesized that under stressful circumstances APO-SUS rats would consume more cocaine than APO-UNSUS rats due to their heightened activation of the dopaminergic system and the HPA-axis. We hypothesized that APO-UNSUS rats would consume larger amounts of cocaine than APO-SUS rats due to higher levels of free plasma corticosterone under habituated circumstances [53].

Methods

General Methods

Adult male Wistar rats (n = 160) of two pharmacogenetically selected rat lines (33rd generation; original line) were obtained from the Central Animal Housing, Radboud University of Nijmegen, The Netherlands. By selective breeding, two ratlines were obtained consisting of apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) rats [50,52]. Animals were housed two to three per cage (Macrolon® type 3; 42 x 26 x 20 cm) in temperature-controlled rooms (21° ± 2° C) with a standard 12/12-h day/night-cycle (lights on at 7.00 am) and food and water available *ad libitum*. Each experiment was conducted with a new group of drug-naïve animals. All experiments were performed in accordance with institutional guidelines and national as well as international laws for animal care and welfare.

Drugs

Cocaine hydrochloride (Pharmacy, UMC St Radboud, Nijmegen, the Netherlands) was dissolved in 0.9% sodium chloride and solutions were made for each individual animal on a weekly basis. Furthermore, bodyweights were measured each week and if necessary solutions were adjusted to fit individual bodyweights.

Surgery

Animals were surgically implanted with an intracardiac silicon catheter (0.3 mm i.d., 0.7 mm o.d.) under isoflurane anesthesia. The catheters were inserted into the right external jugular vein, subcutaneously passed and fixed to the skull. Following surgery, rats were individually housed. Catheter patency was maintained by daily infusions of 0.1 ml of a saline-heparin (50IU/ml) solution. After completion of experimental procedures catheter placement and patency was confirmed by infusion of 0.1 ml pentobarbital (6 mg). Rats that failed to lose

muscle control within 5-10 seconds were discarded from further analysis [153]. Loss of animals due to misplacement and/or obstruction of the catheter was less than 4% (n = 7).

Self-administration cages
The operant self-administration cages (30.5 (l) x 24.1 (w) x 29.2 (h) cm) were equipped with two small nose-poke holes located opposite to one another, a house-light located above the "drug-active" hole, and a stainless steel grid floor (Med Associates Inc).

General protocol self-administration
A nose poke into the drug-active hole resulted in an infusion of 35,4 µl of a cocaine-solution (250 µg/kg) over 2 seconds followed by a 20 second time-out (TO) period. During the infusion and time-out period the house-light was illuminated. A nose poke during the time-out period or in the "drug-inactive" hole was recorded but without consequence [153]. After a 7 day recovery period, animals were allowed to self-administer cocaine during 15 daily (7 days per week) 1-hour sessions on a fixed ratio 1 (FR1) schedule of reinforcement, with a maximum of 100 infusions per session. All tests were done between 9:00 am and 18:00 pm (light phase). Acquisition of cocaine self-administration was established when animals reached a stable infusion pattern (less than 10% deviation) over three consecutive days on the drug-active hole in combination with a minimum number of 6 infusions [247].

Self-administration procedure, experiment 1
This experiment was done to determine whether APO-SUS (n = 17) and APO-UNSUS (n = 13) rats differed in the rate of acquisition and the amount of cocaine taken after a 7-day period of habituation to the cages and the experimental procedure of attaching the animals to the infusion line, including the ability to freely self-administer cocaine. From day 8 onwards animals were shaped for the drug-active hole [247]. In short, animals were placed with their nose into the active nose-poke hole and were given one forced infusion at the beginning of each session, marked by illumination of the light cue (table 1). Shaping proved to be an essential procedure for the animals to acquire self-administration (see results). Henceforward, all following experiments used the procedure of shaping.

Self-administration procedure, experiment 2
This experiment was done to test the effect of stress, caused by a lack of habituation to the cages, and attaching the animals to the infusion line (table 1). Therefore, APO-SUS (n = 16) and APO-UNSUS (n = 18) rats were shaped from day 1 onwards and were allowed to self-administer cocaine freely.

Self-administration procedure, experiment 3
Experiment 2 revealed that the stress caused by a lack of habituation resulted in an equal amount of cocaine taken and the same rate of acquisition for both rat types (see results). Apparently, the lack of habituation was not enough stress to result in differences between the two types. Therefore, an additional stressor was introduced into the protocol. Both APO-SUS (n = 13) and APO-UNSUS (n = 13) rats, were shaped from day 1 onwards and all the

lights in the self-administration room were turned off (reversal day-night) during the session (table 1). Turning off the lights during the light phase is considered to be stressful as stated by Willner and Haile et al [131,331].

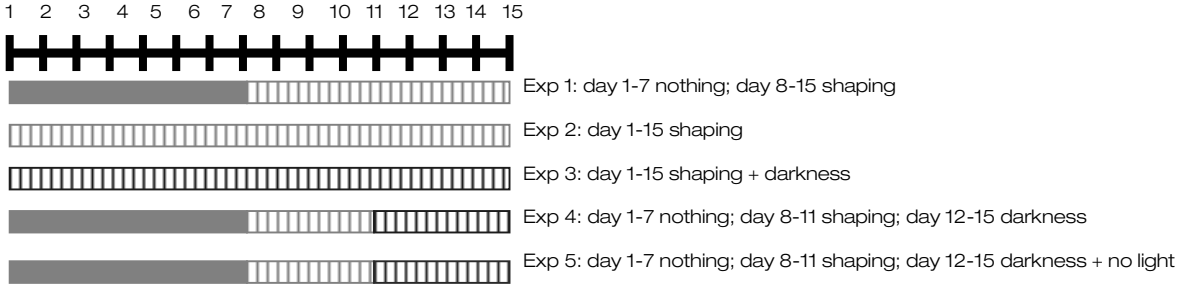
Self-administration procedure, experiment 4

Since experiment 3 revealed that the combination of both stressors, namely the stress caused by the lack of habituation and the stress caused by the light-dark switch, turned out to be effective, the question arose whether the light-dark switch by itself produced sufficient stress to uncover line-specific differences. Thus, APO-SUS (n = 8) and APO-UNSUS (n = 7) rats were first habituated to the cages and attaching the animals to the infusion line for a period of 7 days. From day 8 onwards, animals were shaped for the active nose poke hole and from day 12 onwards lights were turned off during the sessions (table 1).

Self-administration procedure, experiment 5

To ensure that the behavioral effects of the light-dark switch were indeed caused by stress, and not because of secondary changes, such as an enhanced visibility of the light above the drug active nose poke hole, experiment 4 was repeated apart from one minor change in the protocol. During the light-dark switch the light above the hole remained switched off when either APO-SUS (n = 8) or APO-UNSUS (n = 8) rats gave a nose poke into the drug active hole (table 1).

Table 1: *overview different experimental protocols*



Locomotion

To control for the occurrence of locomotor effects of the light-dark switch that could underlie alterations in self-administration behavior, the effect of the switch on locomotor behavior was measured in a separate experiment. APO-SUS (n = 8) and APO-UNSUS (n = 8) rats were isolated and after 7 days activity was measured in activity cages for 60 minutes per session. The activity cages measure 36 x 24 x 25 cm and are equipped with three photoelectric cells placed 2 cm above the grid floor. Interruptions of the infrared beams were electronically calculated and represented as activity counts per block of 10 minutes. APO-SUS and APO-UNSUS rats were first habituated to the cages (day 1 through 3) and were then tested twice, once in the light and once in the dark (day 4 and day 5; separated by 24 hours).

Statistical analysis

Differences between APO-SUS and APO-UNSUS rats in bodyweight at the start of each experiment were compared by means of a one-way ANOVA. Self-administration sessions resulted in three variables, namely (1) the number of infusions, (2) the number of active nose pokes, and (3) the number of inactive nose pokes. Furthermore, each experimental protocol resulted in a percentage of animals that reached acquisition criteria. The number of animals that reached the acquisition criteria was analyzed by means of a χ^2 - test for independency per experiment. The number of inactive nose pokes was used to detect possible differences in this parameter. Analysis was done by means of a two-way ANOVA with genotype as the fixed factor and days as the repeated measure. The number of active nose pokes was also analyzed, but data are not shown because the number of active nose pokes followed the same pattern as the number of infusions for both genotypes. The number of infusions was analyzed per experimental protocol by means of a two-way ANOVA with genotype as the fixed factor and days as the repeated measure. Experiment 1, 4, and 5 were divided in either 2 (experiment 1) or 3 (experiment 4 and experiment 5) data sets, one for the first 7 days, one for day 8 through 15 (experiment 1) or day 8 through 11 and day 12 through 15 (experiment 4 and 5). This was done because in these experiments different protocols were used. Where appropriate, data were further analyzed by means of an post-hoc independent samples t-test on days. Furthermore, APO-SUS and APO-UNSUS rats from experiments 4 and 5 were compared by means of a two-way ANOVA for repeated measures to examine the effects of the omission of the light above the active nose poke hole. Analysis of the data was done only for those animals that reached criteria. Data from the locomotion experiment was analyzed per day by means of an independent samples t-test to compare the activity scores between APO-SUS and APO-UNSUS rats. Secondly, the data from day 4 and 5 were compared with one another per genotype and between genotypes by means of an independent samples t-test. A probability of $p < 0.05$ was taken as significant.

Results

Bodyweight

APO-SUS and APO-UNSUS rats did not differ in bodyweight prior to the start and at the end of the self-administration procedures for all experimental protocols.

Acquisition criteria

As shown in table 2, the number of APO-SUS rats that reached acquisition criteria did not significantly differ from the number of APO-UNSUS rats that reached criteria in all experiments ($\chi^2 < \chi_\alpha{}^2$: χ^2 values between 0.02 and 0.6, critical $\chi_\alpha{}^2$ value 3.8 df 1).

When considering the time point at which animals reached criteria, it should be noted that in experiment 1, 4 and 5 none of the animals reached criteria prior to shaping at day 8. In all experiments shaping proved to be an essential procedure for the rat types to acquire self-administration. Additionally, in experiment 4 and 5, none of the APO-SUS rats reached

criteria before the light-dark switch. This resulted in differences in the time-point at which the APO-SUS and APO-UNSUS rats reached criteria in experiment 4 and 5 (figure 1).

Table 2: *number of animals reaching criteria (< 10% deviation over three consecutive days, minimum of 6 infusions)*

	APO-SUS	APO-UNSUS
Experiment 1	63% (10 of 16)	46% (6 of 13)
Experiment 2	56% (9 of 16)	66% (12 of 18)
Experiment 3	62% (8 of 13)	46% (6 of 13)
Experiment 4	100% (8)	100% (7)
Experiment 5	100% (8)	100% (8)

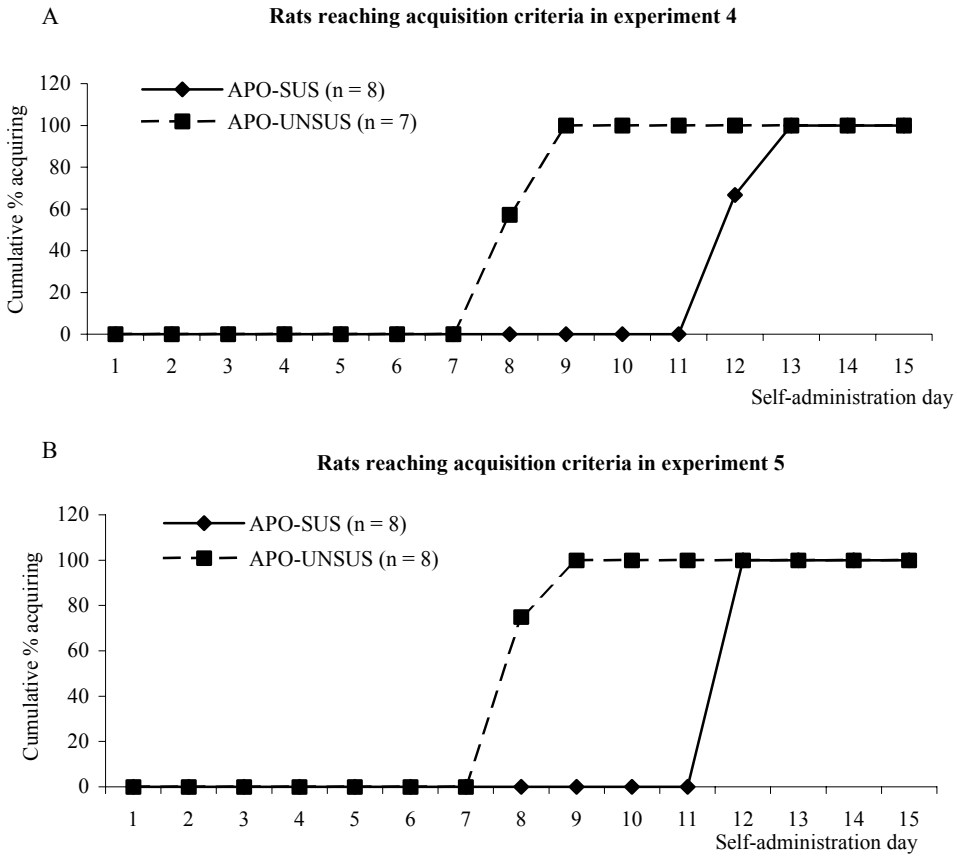


Figure 1: *Time span for animals reaching acquisition criteria (less than 10% deviation over 3 consecutive days, minimum of 6 infusions). The total number of animals reaching criteria is set at 100% and per session the relative number of animals that reached criteria is shown. This results in a graph showing the cumulative percentage of animals acquiring cocaine self-administration in time. It should be noted that the first day of the three day period has been used as set point for these graph. (a) experiment 4: 7 days of habituation, followed by shaping from 8 to day 15, from day 12 to 15 animals are subjected to a stressor. (b) experiment 5 followed the same protocol as experiment 4 except for omission of the cue light during the presentation of the stressor. Both graphs show that none of the animals acquired self-administration prior to shaping, whilst after shaping all APO-UNSUS rats (open line) acquired self-administration within a two-day period. APO-SUS (straight line) rats all acquired cocaine self-administration after introduction of a stressor.*

Inactive nose pokes for all experimental protocols

No differences in the number of inactive nose pokes were found between APO-SUS and APO-UNSUS rats at any time point nor between rats of the same genotype for the different experimental protocols.

Experiment 1: habituation

Data were analyzed for the first 7 days and for day 8 through day 15. The APO-SUS (n =10; weight 307 ± 9 grams) and APO-UNSUS (n = 6; weight 294 ± 6 grams) rats did not differ in intake of cocaine for the first seven days of self-administration. All rats administered some cocaine, but never consistently and they all failed to reach the acquisition criteria (figure 2). Shaping from day 8 onwards resulted in an overall increase in intake of the APO-UNSUS rats, but the APO-SUS rats only slightly increased their intake (genotype: $F_{(1,14)} = 12.6$ $p < 0.01$). Post hoc analysis by means of an independent samples t-test revealed that the APO-UNSUS rats had a higher intake of cocaine from day 10 to 15 ($p < 0.05$). The APO-SUS rats did increase their intake of cocaine in comparison to day 1 through 7 ($p < 0.05$), but their intake remained small.

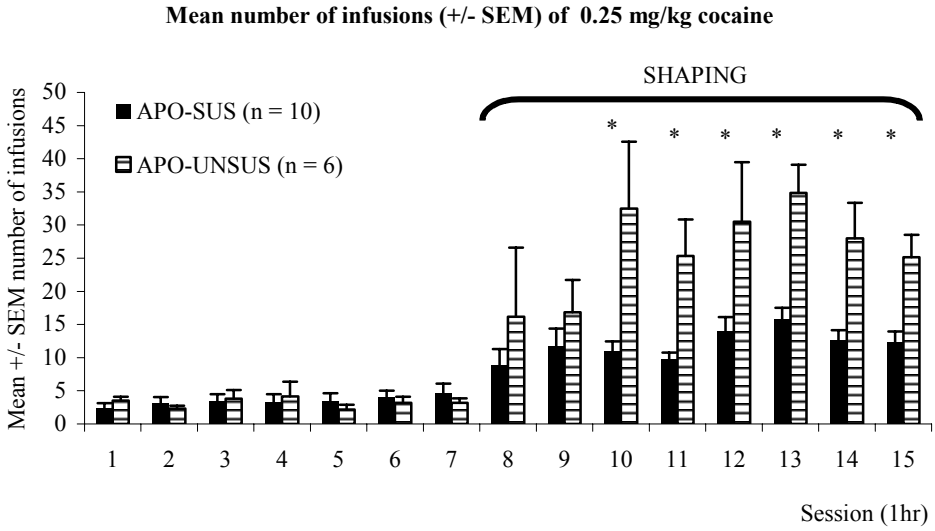


Figure 2: *Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for APO-SUS (n =10) and APO-UNSUS (n = 6) rats for experiment 1. Animals were habituated to the self-administration cages and the experimental protocol for 7 days, followed by shaping from day 8 onwards. APO-UNSUS rats (arced columns) consumed considerably more cocaine after shaping commenced than the APO-SUS (filled columns) rats (independent samples t-test $p < 0.05$; marked by *)*

Experiment 2: lack of habituation

Data from this experiment were analyzed for the complete duration of the experiment since no alterations in the protocol were made during the experiment. Both APO-SUS (n = 9; weight 290 ± 6 grams) and APO-UNSUS (n = 12; weight 275 ± 4 grams) rats self-administered cocaine. There were, however, no differences in the amount of cocaine consumed (figure 3).

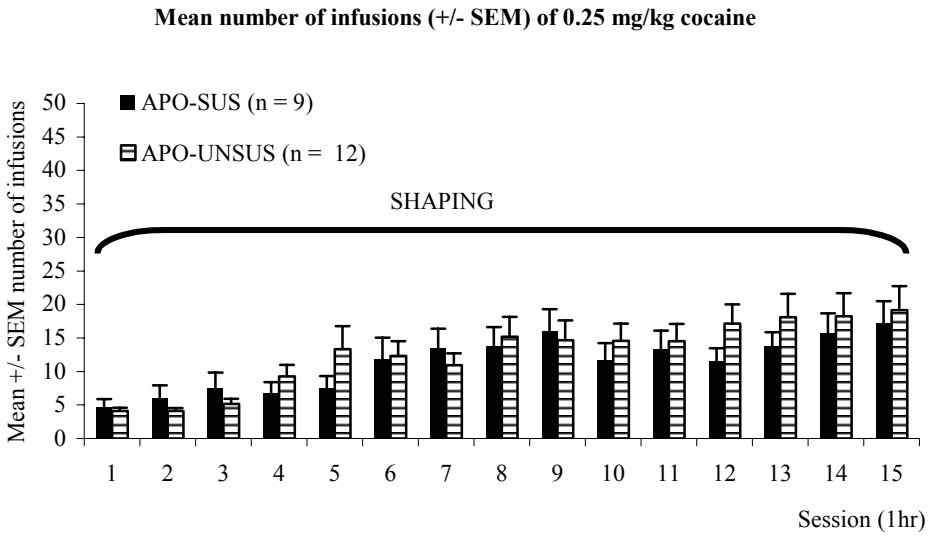


Figure 3: *Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for APO-SUS (n = 9) and APO-UNSUS (n = 12) rats for experiment 2. Animals were shaped from day 1 onwards. APO-UNSUS rats (arced columns) consumed the same amount of cocaine as the APO-SUS (filled columns) rats.*

Experiment 3: stress

Again, data from this experiment, in which the lack of habituation was combined with stress caused by the light-dark switch, were analyzed for the complete duration of the experiment since no alterations in the protocol were made. When comparing APO-SUS (n = 8; weight 275 ± 14 grams) and APO-UNSUS (n = 6; weight 272 ± 5 grams) rats that reached acquisition criteria, an overall higher intake of cocaine of the APO-SUS rats was seen (genotype: $F_{(1,12)} = 5.1$ $p < 0.05$) (figure 4). APO-UNSUS rats did take cocaine, but in smaller amounts than the APO-SUS rats. Post hoc analysis by means of an independent samples t-test revealed that this difference was present at day 7, day 9 and 10, and day 14 and 15 ($p < 0.05$). Intake values were almost significant at day 6, day 8, and day 11 through 13 ($p = 0.06$).

Experiment 4: habituation followed by stress

During the first seven days of the experiment, when the rats had free access to the cocaine, but were not shaped, no difference in intake between APO-SUS (n = 8; weight 304 ± 5 grams) and APO-UNSUS (n = 7; weight 286 ± 7 grams) rats was seen. All rats administered some cocaine, but never constant and they all failed to reach the acquisition criteria. After shaping was introduced, the APO-UNSUS rats, but not the APO-SUS rats, increased their intake of cocaine. This resulted in an overall difference in intake of cocaine (genotype: $F_{(1,13)} = 18.3$ $p < 0.05$). Post hoc analysis by means of an independent samples t-test revealed this differences to be present at day 8 through 11 ($p < 0.01$). The APO-SUS rats remained at the same level of intake as before shaping. After the habituated rats were exposed to stress caused by the light-dark switch, the APO-SUS rats readily increased their intake to match levels of the APO-UNSUS rats. No change in intake of the APO-UNSUS rats was seen. Due to this

increase in intake of the APO-SUS rats, differences between the two lines were abolished (figure 5).

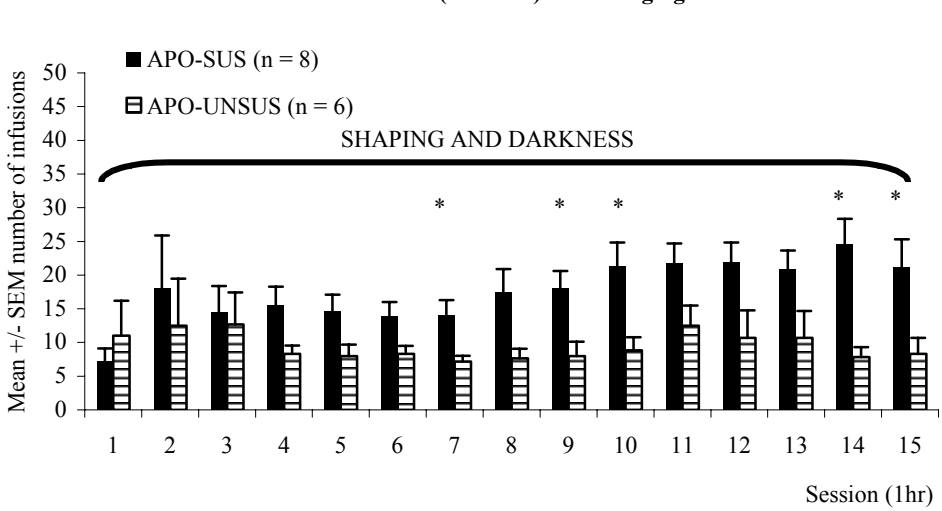


Figure 4: *Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for APO-SUS (n = 8) and APO-UNSUS (n = 6) rats for experiment 3. Animals were shaped from day 1 onwards and animals were tested in the darkness (stressor).APO-SUS rats (filled columns) consumed considerably more cocaine under stressful conditions than the APO-UNSUS (arced columns) rats (independent samples t-test $p < 0.05$; marked by ✶)*

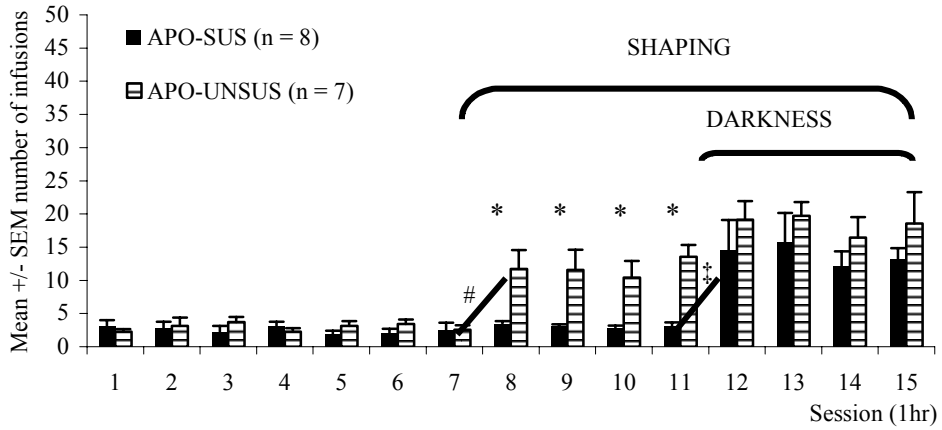


Figure 5: *Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for APO-SUS (n =8) and APO-UNSUS (n = 7) rats for experiment 4. Animals were habituated to the self-administration cages and the experimental protocol for 7 days, followed by shaping from day 8 onwards and darkness (stressor) from day 12 onwards. APO-UNSUS rats (arced columns) consumed considerably more cocaine after shaping commenced than the APO-SUS (filled columns) rats (independent samples t-test $p < 0.05$; marked by ✶), but after darkness was introduced the APO-SUS rats increased their intake to match levels of the APO-UNSUS rats. The increase in consumption was significant for APO-UNSUS rats from day 7 to 8 (independent samples t-test $p < 0.05$; marked by #) and for APO-SUS rats from day 11 to 12 (independent samples t-test $p < 0.05$; marked by ‡)*

Experiment 5: habituation followed by stress

Experiment 5, in which the effect of stress caused by the light-dark switch was tested in habituated rats, yielded the same results as experiment 4. Apparently, the effects of the light-dark switch on self-administration behavior were not confounded by an increased visibility of the light above the drug active nose poke hole. Again during the first 7 days of the protocol, no differences between APO-SUS (n = 8; weight 334 ± 7 grams) and APO-UNSUS (n = 8; 310 ± 12 grams) rats existed. When shaping was introduced, all APO-UNSUS rats showed an overall increase in intake (genotype: $F_{(1,14)} = 11.1$ $p < 0.01$), whilst the APO-SUS rats remained at the same level of intake. This difference in intake was present from day 8 to day 11 (post hoc independent samples t-test; $p < 0.01$). Again, after the light-dark switch, all APO-SUS rats increased their intake to levels matching that of APO-UNSUS rats, whereas no change in the intake of APO-UNSUS rats occurred (figure 6).

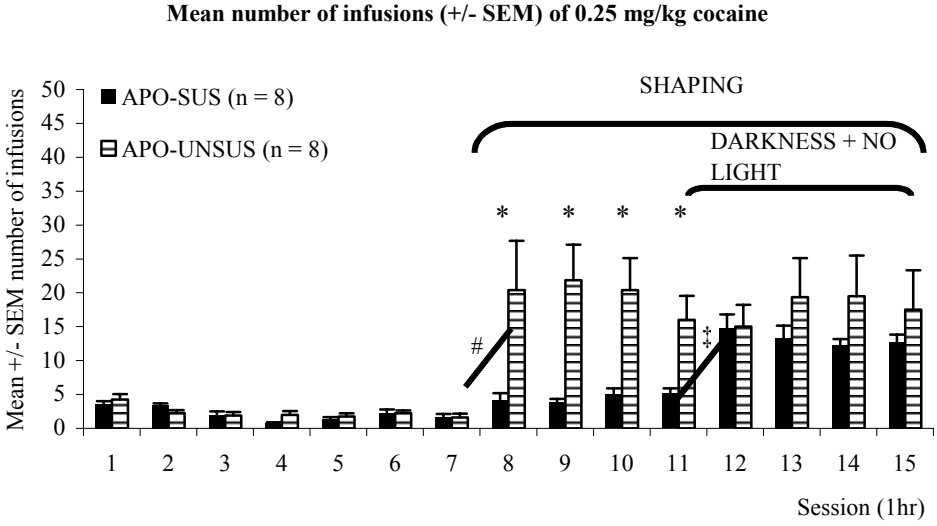


Figure 6: *Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for APO-SUS (n = 8) and APO-UNSUS (n = 8) rats for experiment 5. Animals were habituated to the self-administration cages and the experimental protocol for 7 days, followed by shaping from day 8 onwards and darkness (stressor) from day 12 onwards. The cue light was not presented from day 12 onwards. APO-UNSUS rats (arced columns) consumed considerably more cocaine after shaping commenced than the APO-SUS (filled columns) rats (independent samples t-test $p < 0.05$; marked by *), but after darkness was introduced the APO-SUS rats increased their intake to match levels of the APO-UNSUS rats. The increase in consumption was significant for APO-UNSUS rats from day 7 to 8 (independent samples t-test $p < 0.05$; marked by #) and for APO-SUS rats from day 11 to 12 (independent samples t-test $p < 0.05$; marked by ‡)*

Locomotion

There was no difference in the locomotion from day 1 through day 3 (habituation to the cages). The switch from light to darkness on day 5 increased the activity of both rat types in an equal manner (comparing day 4 to day 5: APO-SUS 39% ± 12%; APO-UNSUS 22% ± 10%, figure 7).

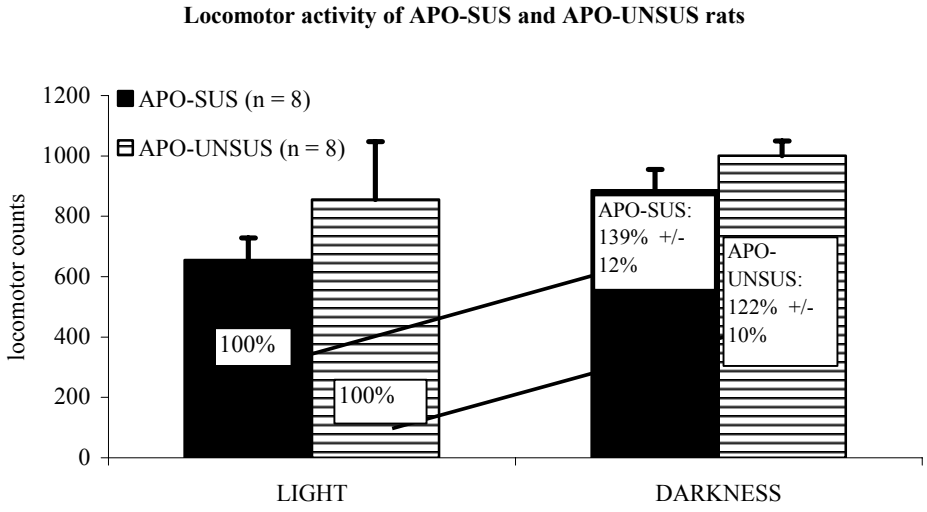


Figure 7: *Activity score for APO-SUS (n = 8) and APO-UNSUS (n = 8) rats in locomotor activity cages. Animals were first habituated to the cages (day 1-3; data not shown) and then tested for the response to the switch from light to dark. APO-SUS rats showed an increase of 139% ± 12%, whilst the APO-UNSUS rats showed an increase of 122% ± 10% (when comparing day 4 to day 5). There were no differences between the increase in activity or between the activity score of day 4 (light) and day 5 (dark).*

Discussion

As stated in the introduction, we hypothesized that APO-UNSUS rats would consume more cocaine than APO-SUS rats under habituated circumstances whilst during stressful circumstances the reverse would hold true. The present study indeed demonstrates that the amount of cocaine taken during acquisition and maintenance was different between both genotypes and that this difference was dependent on the environment. Under habituated circumstances APO-UNSUS rats took far more cocaine than APO-SUS rats (figure 2: total number of infusions of APO-UNSUS rats was 231 ± 43 and of APO-SUS rats 118 ± 8.3). And under stressful circumstances APO-SUS rats took far more cocaine than APO-UNSUS rats (figure 4: total number of infusions of APO-UNSUS rats was 142 ± 26 and of APO-SUS rats 265 ± 29.9). Secondly, the present study shows that the rate of acquisition was dependent on the environment.

The environmentally-induced differences in the rate of acquisition were most obviously shown in experiment 4 and experiment 5 (figure 1). In both experiments, habituated APO-UNSUS rats acquired self-administration faster than APO-SUS rats. After the light-dark switch was introduced, however, APO-SUS rats quickly acquired self-administration. This difference in the time-point of acquisition indicates that cocaine self-administration is indeed dependent on the environmental context. Even though both genotypes learned to self-administer cocaine at a different rate, these two experiments, in combination with the other experiments conducted, clearly showed that APO-SUS and APO-UNSUS rats did not differ in the capability to learn the self-administration behavior.

It should be noted though, that all animals needed to be shaped for the drug active nose poke hole. Shaping was a forced 'priming' infusion at the beginning of each session, which can strengthen both the spatial recollection of the correct nose poke hole and the 'emotional' memory of a cocaine delivery [247]. These two processes are apparently necessary for learning self-administration behavior, especially since animals did not learn to self-administer of cocaine without shaping.

Experiment 1 clearly showed that after habituation to the self-administration cages and the experimental procedures, APO-UNSUS rats took more cocaine than APO-SUS rats. When APO-SUS and APO-UNSUS rats were subjected to a mild stress or caused by a lack of habituation, no differences in the amount of cocaine consumed was found. This suggests that the lack of habituation by itself, is not sufficient to create interline differences. Thus, in the next experiment a second stressor was introduced by switching from a light to a dark surrounding during the self-administration session. This light-dark switch in combination with a lack of habituation resulted in a higher intake of cocaine by APO-SUS rats in comparison to APO-UNSUS rats. Experiment 4 and 5 showed that the light-dark switch alone was a sufficiently strong stressor to enhance self-administration in APO-SUS rats.

As mentioned earlier, placing an animal in a darkened surrounding during the light phase is considered to be stressful [131,331]. An additional experiment was done to control for the occurrence of line-specific, darkness-induced exploratory behavior after the switch from light to dark. It was clearly shown that both APO-SUS and APO-UNSUS rats increased their activity in an equal manner, ruling out that the differences found in the amount of cocaine consumed after the light-dark switch were due to differences in darkness-induced locomotion (figure 7).

We hypothesized that APO-SUS rats would consume more cocaine than APO-UNSUS rats under stressful conditions due to their specific, genetically determined neurobiological features. Under stressful conditions APO-SUS rats have both a high dopaminergic reactivity in the ventral striatum and high corticosterone levels [88,264], two features that have been suggested to be correlated with a high intake of cocaine [153,168,174,195,239,268-270]. It is, therefore, not surprising that APO-SUS rats consumed more cocaine than APO-UNSUS rats under stressful circumstances. The finding that APO-UNSUS rats consumed more cocaine under habituated circumstances becomes understandable in view of the fact that the levels of free corticosterone are higher than that of APO-SUS rats under these conditions [264].

An additional feature that could possibly contribute to the amount of cocaine taken is the level of noradrenergic activity in the ventral striatum. Recently, research has suggested that (stress-induced) activation of the noradrenergic system plays a role in altering reward sensation and in reinstatement of cocaine self-administration under stressful circumstances [179,233]. It is known that under stressful conditions APO-SUS rats have higher levels of functional noradrenergic activity in the ventral striatum, whilst the same holds true for APO-UNSUS rats under habituated circumstances [50,311]. Since APO-SUS rats consume more cocaine under stressful conditions and APO-UNSUS rats under habituated circumstances, these data raise the possibility that there might be a correlation between heightened noradrenergic activity and the consumption of cocaine.

It should be noted that this study only tested one dose of cocaine, namely 0.25 mg/kg. This dose of cocaine resulted in environmentally-modulated differences between APO-SUS and APO-UNSUS rats. It is possible that with other doses different results are obtained, since several researchers have suggested a dose-dependency of the differences between their rat types [191,240].

The present study also revealed another interesting result, namely APO-UNSUS rats seem to be unaffected by any alteration in the environment as can be seen with the light-dark switch in experiment 4 and 5 and the removal of the light in experiment 5. This indicates that the intake of cocaine is very stable and compulsive once an animal has learned to self-administer and has reached the maintenance phase. Preliminary data on the effects of environmental alterations during the maintenance phase on the self-administration behavior of APO-SUS rats has revealed the same phenomenon, namely that the intake of cocaine remains very stable and compulsive once the behavior has been learned (data not shown).

In conclusion, the present study demonstrates that differences in the acquisition of cocaine self-administration between APO-SUS and APO-UNSUS rats are context-dependent. Under habituated circumstances APO-UNSUS rats take more cocaine than APO-SUS rats, whilst the reverse is true under challenged conditions. We therefore postulate that the amount of a drug consumed, and, accordingly, its addictive potential and 'drug-vulnerability' is determined by the interaction between genes and environment, and not by either component alone [53].

Chapter 5

The role of sex and early postnatal manipulations in directing cocaine self-administration in apomorphine unsusceptible rats.

Abstract
This study investigated the effects of both sex and an early postnatal manipulation in apomorphine unsusceptible (APO-UNSUS) rats. We have recently found that this rat type more readily consumes alcohol and cocaine under non-challenged conditions than its counterpart, the apomorphine susceptible (APO-SUS) rat type. In our studies we have, until so far, never incorporated female rats. We therefore investigated whether the self-administration pattern of non-challenged female APO-UNSUS rats differed from that of non-challenged male APO-UNSUS rats (exp 1). Secondly, we investigated the effects of maternal deprivation on postnatal day 9 on cocaine self-administration behavior of male APO-UNSUS rats (exp 2). Because this early postnatal manipulation is known to alter the dopaminergic sensitivity of these male rats, and because the structure and function of the dopaminergic system is implicated in the onset of addiction, we hypothesized that this early postnatal manipulation would alter cocaine self-administration behavior in these male rats. For that reason, male APO-UNSUS rats were maternally deprived at postnatal day 9 for 24 hours. At postnatal day 65, all animals (exp 1 and 2) were operated and after a 7-day recovery period subjected to the self-administration protocol. Animals were first allowed to freely self-administer cocaine for 7 days, followed by shaping from day 8 through day 15. From day 12 onwards, animals were subjected to an environmental challenge that is known to trigger self-administration behavior in these rats. The present study showed that non-challenged female APO-UNSUS rats were faster at acquiring cocaine self-administration than non-challenged male APO-UNSUS rats, but that the amount of cocaine consumed was equal. Maternal deprivation of male APO-UNSUS rats resulted in a shift in cocaine self-administration behavior of these rats, resulting in APO-SUS-like behavior. The present data together with earlier published data allow the conclusion that genetic background, sex, and both early as well as late environmental factors direct the acquisition of cocaine self-administration behavior in rats.

Introduction

A recent study has revealed that pharmacogenetically selected male apomorphine unsusceptible (APO-UNSUS) rats consume considerably more cocaine than male apomorphine susceptible (APO-SUS) rats under non-challenged conditions [319]. This study, however, only investigated cocaine self-administration in non-challenged male rats, but not in non-challenged female rats.

Cocaine abuse amongst women has increased rapidly over the last few years, and their intake patterns are often different from those of men [301]. Moreover, many studies have found that female animals acquire self-administration of drugs of abuse faster than males [37,73,187,260,261,283]. On the other hand, some studies have found the opposite or no differences at all [32,73]. This discrepancy could, however, be caused by the dose of cocaine used (low vs. high doses). Indeed, the findings that females acquire self-administration faster than males seems to be dose-dependent. With low doses, females acquire self-administration faster than males, but, with high doses, opposite effects or no differences are found [261].

We therefore investigated whether the pattern of low-dose cocaine self-administration was different between non-challenged female and male APO-UNSUS rats. Given the above-mentioned literature, we hypothesized that non-challenged female APO-UNSUS rats would acquire self-administration faster than non-challenged male APO-UNSUS rats.

As discussed elsewhere, not only stressful events at adulthood, but also stressful events early at life, have been implicated in most psychiatric diseases, including addiction [85,189,228,234]. It, therefore, became interesting to investigate the role of adverse early life events on the intake of cocaine in APO-UNSUS rats.

Previous research on male rats has shown that early maternal deprivation for 24 hours results in sensitized striatal dopamine D2 receptors, an enhanced corticosterone release, and enhanced striatal tyrosine hydroxylase staining in these rats [86,236,266]. In agreement with this, maternally deprived male APO-UNSUS rats, in comparison to non-deprived male APO-UNSUS rats, have been found to display increased apomorphine-induced gnawing behavior[86]. Moreover, maternal deprivation of male APO-UNSUS rats has been found to result in an increased sensitivity to develop periodontitis [293]; namely an inflammatory disease that is positively correlated with the reactivity of the HPA-axis [26].

Thus, maternal deprivation of male APO-UNSUS rats is known to alter the reactivity of the dopaminergic system as well as the HPA-axis. Because the reactivity of these systems has been implicated in directing the intake of cocaine [63,116,143,192], we investigated the effects of maternal deprivation of male APO-UNSUS rats on the intake of cocaine at adulthood.

Given the fact that an increased apomorphine-induced gnawing score and an increased sensitivity to develop periodontitis are two characteristics of male APO-SUS rats [27,50], we hypothesized that maternally deprived male APO-UNSUS rats would display cocaine self-administration behavior of male APO-SUS rats, i.e. maternally deprived male APO-UNSUS rats will need a challenge to start cocaine self-administration [319]. For that reason, we assessed a challenge that has been found to be effective in triggering cocaine self-administration behavior of male APO-SUS rats [319].

Methods

In general

All animals were housed in Macrolon® type 3 cages (42 x 26 x 20 cm) in temperature-controlled rooms (21°) with a standard 12/12-h day/night-cycle (lights on at 7.00 am) and food and water available *ad libitum*. All experiments were performed in accordance with institutional guidelines, national, and international laws for animal care and welfare.

Breeding

Adult female and male Wistar rats of the pharmacogenetically selected rat line (33rd generation; age approx. 3 months) consisting of apomorphine unsusceptible rats (APO-UNSUS) were obtained from the Central Animal Housing, Radboud University Nijmegen, the Netherlands [50]. Male-female pairs of the APO-UNSUS ratline were allowed to breed. At postnatal day 9 (birth is day 0), litters were randomly assigned to be either controls (CTR) or to undergo maternal deprivation (MD). The maternal deprivation procedure used in the present study has been described in detail elsewhere and is known to be highly effective in producing long-term after-effects in male APO-UNSUS rat type [86]. In short, the pups were weighed and either placed back with their mother (CTR) or placed back in their cage without their mother (MD) with the mother remaining in the vicinity, but out of sight of the pups. After 24 hours the mother was placed back with the pups and the pups were left undisturbed until postnatal day 28. After weaning at postnatal day 28, animals were housed two to three per cage.

Surgery

At postnatal day 65 animals were surgically implanted with an intracardiac silicon catheter (0.3 mm i.d., 0.7 mm o.d; Rubber Bv, the Netherlands) under isoflurane anesthesia. The catheters were inserted into the right external jugular vein, subcutaneously passed and fixed to the skull. Following surgery, rats were individually housed. Catheter patency was maintained by daily infusions of 0.1 ml of a saline-heparin (50IU/ml) solution. After completion of experimental procedures catheter placement and patency was confirmed by infusion of 6 mg sodium pentobarbital (0.1 ml Nembutal). Rats that failed to lose muscle control within 5-10 seconds were discarded from further analysis (4%, n = 1).

Drugs

Cocaine hydrochloride (Pharmacy, UMC St Radboud, Nijmegen, the Netherlands) was dissolved in 0.9% sodium chloride and solutions were made for each individual animal on a weekly basis in order to correct for small bodyweight changes.

Self-administration cages

The operant self-administration cages (30.5 (l) x 24.1 (w) x 29.2 (h) cm) were equipped with two small nose-poke holes located opposite from one another, a house-light located above the "drug-active" hole, and a stainless steel grid floor (Med Associates Inc.). Cages were illuminated by normal ambient lighting. A nose poke into the drug-active hole resulted in an

infusion of 35.4 µl of a cocaine-solution (0.25 mg/kg) over 2 seconds followed by a 20 second time-out (TO) period (Syringe pump PHM-100, Med Associates Inc.). During the infusion and time-out period the house-light was illuminated. A nose poke during the time-out period or in the "drug-inactive" hole was recorded but without consequence. Acquisition of cocaine self-administration was established when animals reached a stable infusion pattern (less than 10% deviation) over three consecutive days on the drug-active hole [247]. In order to avoid 'false positives' (rats that have a stable intake of 2 infusions), a minimum number of 6 infusions was incorporated into the criterium. This amount, being around 25% of the maximum number of infusions, was chosen on the basis of previous research [319].

Self-administration procedure

Each self-administration session was conducted between 9:00 am and 6.00 pm: animals were, thus, allowed to self-administer cocaine during the light phase of the day and in normal ambient lighting of the room unless stated elsewise. After a 7 day recovery period from surgery, animals were allowed to self-administer cocaine during 15 daily (7 days per week) 1-hour sessions on a fixed ratio 1 (FR1) schedule of reinforcement.

The used protocol assessed both the effect of habituation as well as a challenge on the self-administration of both male and female APO-UNSUS rats (exp 1) and male maternally deprived APO-UNSUS rats (exp 2). Thus, animals were first habituated to the cages and the procedure of attaching them to the infusion line for a period of 7 days. During these 7 days, animals were allowed to freely self-administer cocaine. From day 8 onwards, animals were shaped for the active nose poke hole. In short, animals were placed with their nose into the active nose-poke and were given one forced infusion at the beginning of each session, marked by the illumination of the house-light. Shaping is a forced 'priming' infusion at the beginning of each session and it has proven to be essential for APO-SUS and APO-UNSUS rats to acquire cocaine self-administration under the given circumstances [319]. The fact that APO-SUS and APO-UNSUS rats fail to acquire self-administration without shaping is probably due to the fact that these rats were tested during the inactive phase of their day (lightphase). It was specifically chosen not to reverse the day-night rhythm of APO-SUS and APO-UNSUS rats because this is known to interfere with the status of the dopaminergic and noradrenergic system (unpublished observation). In this respect, shaping can strenghten both spatial recollection of the correct nose poke hole and the 'emotional' memory of a cocaine delivery [247].

From day 12 through day 15, the lights in the self-administration room (reversal day-night) were turned off. Previous research has shown that this stressor results in an increase of cocaine and alcohol self-administration in the, more dopaminergic reactive, APO-SUS rat [318,319]. Because the maternally deprived male APO-UNSUS rats are known to have an increased dopaminergic sensitivity at adult age [86], the effect of this stressor was investigated.

Statistical analysis

The number of males and females per group (CTR or MD), the weight before maternal deprivation, and the weight before self-administration were compared with one another by

means of an independent samples t-test. Self-administration sessions resulted in three variables, namely (1) the number of infusions, (2) the number of active nose pokes, and (3) the number of inactive nose pokes. Statistical analysis was done on gender (male versus female CTR) and on the effect of early postnatal manipulations (EPM; MD versus CTR). Analysis was done by means of a two-way ANOVA with either gender or EPM as the between subject factor and days as the repeated measure for the number of inactive nose pokes, the number of active nose pokes, and the number of infusions. The data per experiment were divided into three data sets, one for the first 7 days, one for day 8 through 11, and one for day 12 through 15. This division was made because the protocol altered during the experiment. Where appropriate, data were further analyzed by means of a post-hoc independent samples t-test for each day. The number of active nose pokes was also analyzed, but the data are not shown because the number of active nose pokes followed the same pattern as the number of infusions for all groups at all days. The number of inactive nose pokes was also analyzed to investigate whether the animals had a significant preference for the active nose poke hole and whether male rats differed from female rats or from maternally deprived male rats. All data were analyzed with SPSS 12.0.1, and a probability of $p < 0.05$ was taken as significant.

Results

Experiment 1: sex differences

General

The self-administration behavior of the male APO-UNSUS rats that were not maternally deprived (see below) were compared with the self-administration behavior of female APO-UNSUS rats (n = 9). Male APO-UNSUS rats weighted significantly more than female APO-UNSUS rats (male 227 ± 9 grams, female 156 ± 2 grams; $p < 0.01$). After completion of the self-administration procedure, one male APO-UNSUS rats (4%) had to be discarded since it failed to lose muscle control after an injection with sodium pentobarbital.

Cocaine self-administration.

The number of inactive nose pokes during the self-administration sessions was significantly lower than the number of active nose pokes; moreover, no differences were found between male and female APO-UNSUS rats at any time point (on average 3 nose pokes; data not shown). Female APO-UNSUS rats consumed considerably more cocaine during the first seven days of cocaine self-administration than male APO-UNSUS rats (gender; $F_{(1,16)} = 62.1$, gender-time interaction; $F_{(6,96)} = 5.5$ $p < 0.01$). Further post-hoc analysis by means of a post-hoc independent samples t-test revealed that the female rats consumed significantly more cocaine from day 3 to day 7 (figure 1). The intake of cocaine was almost significantly higher at day 2 as well ($p = 0.09$). The difference in intake between male and female rats was abolished after shaping started, since shaping significantly increased the intake of male APO-UNSUS rats. As expected, no change in the intake pattern of either male or female APO-UNSUS rats was seen after the lights were turned off during self-administration sessions (day 12-15).

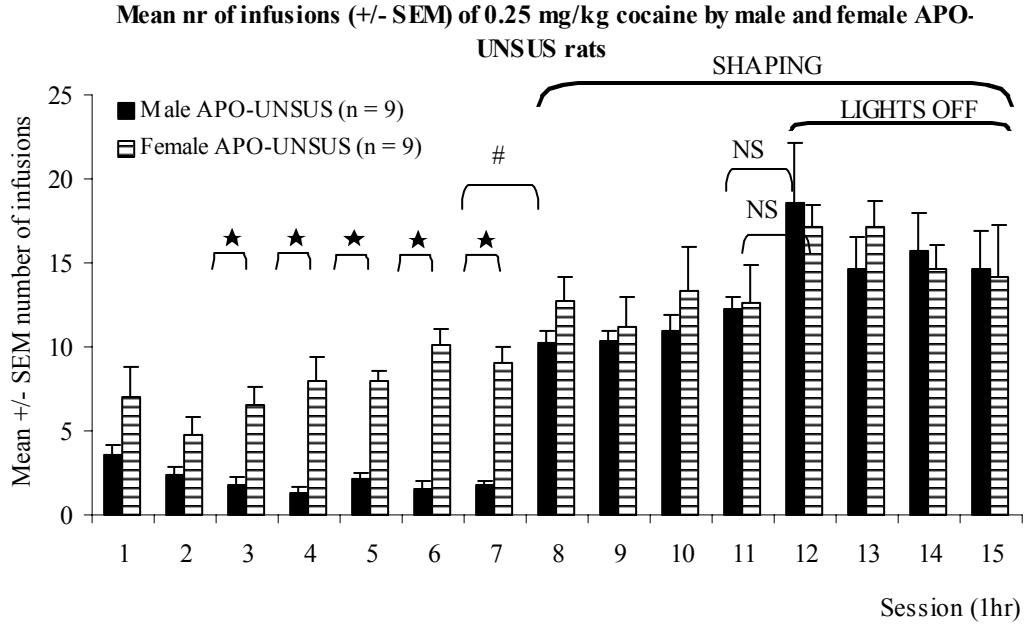


Figure 1: *Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for male (n = 9; filled columns) and female (n = 9; arced columns) APO-UNSUS rats. Female APO-UNSUS rats consumed considerably more cocaine than male APO-UNSUS rats (p < 0.05; marked by ★), but when shaping was introduced the male APO-UNSUS rats quickly increased their intake to match levels of the females rats. The increase in consumption was significant for the male APO-UNSUS rats from day 7 to 8 (p < 0.05; marked by #).*

Experiment 2: the role of adverse early life events

General

The litters that originated from the breeding program had an average of 10.4 ± 0.5 pups. There was no difference in the number of males or females between the groups. There were no differences in bodyweight prior to maternal deprivation (male CTR 14.9 ± 0.4 grams, male MD 14.4 ± 0.2 grams). Thus, there were no differences between the litters at the beginning of the experiment. Only a small fraction of the male animals was used for the self-administration experiment, namely 10 male control APO-UNSUS rats and 13 maternally deprived APO-UNSUS rats, originating from multiple litters. All other rats were used in a different experiment. There was no difference in the bodyweight between the adult male CTR and male MD APO-UNSUS rats (CTR 227 ± 9 grams, MD 223 ± 5 grams).

The effects of an early life manipulation on cocaine self-administration by male APO-UNSUS rats

The number of inactive nose pokes during the self-administration sessions was significantly lower than the number of active nose pokes; moreover, no differences were found between male MD and male CTR APO-UNSUS rats (on average 2 nose pokes; data not shown).

During the first seven days of the experiment, when the rats had free access to cocaine, but were not shaped, no difference in the intake of cocaine was seen between male CTR (n = 9) and male MD (n = 13) APO-UNSUS rats. All rats administered some cocaine (on average 2

infusions per day), but never constant and they all failed to reach the acquisition criteria. After shaping started, male CTR APO-UNSUS rats, but not male MD APO-UNSUS rats, increased their intake of cocaine (independent samples t-test day 7-day 8 for CTR rats, $p < 0.01$). This resulted in a differential intake of cocaine in time (EPM; $F_{(1,20)} = 86.5$, EPM-time interaction; $F_{(3,60)} = 4.9$ $p < 0.01$). Post hoc analysis by means of an independent samples t-test revealed this difference to be present across day 8 – day 11 ($p < 0.01$). The male MD APO-UNSUS rats remained at almost the same level of intake as before shaping (slight non-significant increase). After the lights were turned off during self-administration sessions, the male MD APO-UNSUS rats readily increased their intake to match levels of male CTR APO-UNSUS rats (independent samples t-test day 11 - day 12 for MD rats, $p < 0.01$). No change in intake of male CTR APO-UNSUS rats was seen. Due to this increase in intake of male MD APO-UNSUS rats, differences between the two groups were abolished.

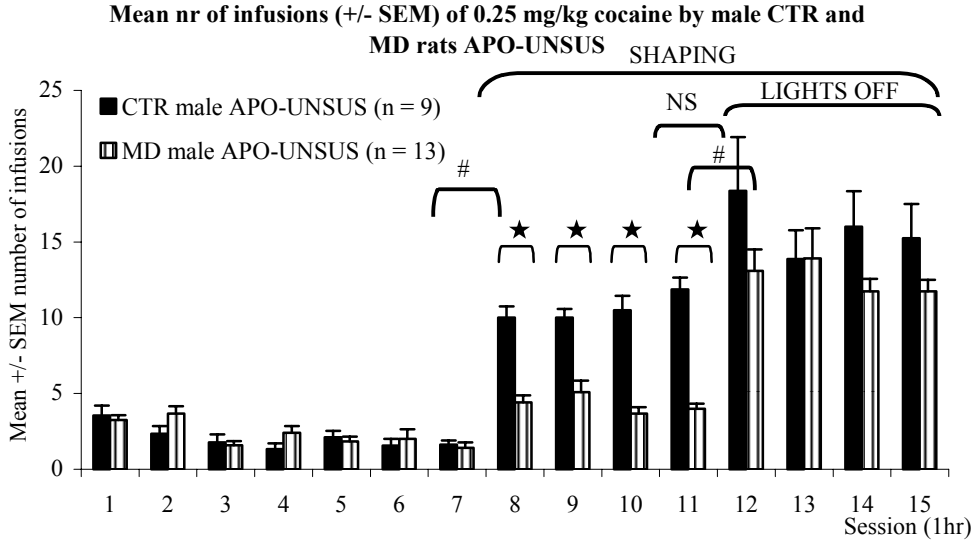


Figure 2: *Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for CTR (n = 9) and MD (n = 13) APO-UNSUS rats. CTR rats (filled columns) consumed considerably more cocaine after shaping started than the MD rats (arced columns) rats (p < 0.01; marked by ✶), but after darkness was introduced the MD rats increased their intake to match levels of the CTR rats. The increase in consumption was significant for the CTR rats from day 7 to 8 (p < 0.05; marked by #) and for the MD rats from day 11 to 12 (p < 0.05; marked by #)*

Discussion

The present study provides two new sets of data. First, this study shows that there were differences between non-challenged male and female APO-UNSUS rats in the rate of acquisition of cocaine self-administration. Female APO-UNSUS rats acquired self-administration without shaping, resulting in a heightened cocaine consumption during the first 7 days of self-administration. This difference in cocaine intake between male and female rats was abolished when shaping started. Second, this study shows that maternal deprivation of male APO-UNSUS rats altered their self-administration behavior, resulting in a different

pattern of self-administration. The control group of male APO-UNSUS rats consumed more cocaine under habituated circumstances, whilst the maternally deprived male rats needed an environmental challenge to start consuming cocaine. These data are discussed below.

The present data clearly showed that non-challenged female APO-UNSUS rats were faster at acquiring self-administration of cocaine than non-challenged male APO-UNSUS rats, but that there were no differences in the amount consumed once self-administration was acquired. There were no differences in the amount of animals that reached acquisition criteria (100%), revealing that both males and females were equally capable to learn self-administration. The finding that female rats acquired self-administration faster than male rats is in concordance with several other studies [32,137,146,186,187,261]. These studies have suggested that this enhanced rate of self-administration is due to higher levels of estrogen. Estrogen is known to influence many drug-related behaviors and the development of behavioral sensitization [19,145]. It is also known to facilitate the amount of dopamine released in the ventral and dorsal striatum [17,18] and to enhance the reuptake of dopamine in the ventral striatum [304]. Because these dopaminergic systems are known to be involved in the response to drugs of abuse [143], it is likely that the facilitating effect of estrogen on dopaminergic activity contributed to the enhanced acquisition of cocaine self-administration in female rats. There is, however, an alternative explanation possible. Non-challenged female APO-UNSUS rats did not require shaping, whilst non-challenged male APO-UNSUS rats did. As stated in the Methods, shaping is a forced 'priming' infusion, which can strengthen both the spatial recollection of the correct nose poke hole as well as the 'emotional' memory of a cocaine delivery [247]. Thus, the fact that female APO-UNSUS rats did not need shaping, could indicate that the emotional memory of the drug (motivation to obtain the drug) is much greater in females than in males. This enhanced motivation could equally be related to the higher levels of estrogen in the female rats.

Because male and female APO-UNSUS rats consumed equal amounts of cocaine once self-administration was acquired, it can be suggested that the presence of estrogen does not determine this parameter (cf: [172,185,186]). However, more research is necessary to establish the exact role of estrogen (and the fluctuations in the estrous cycle) in the rate of acquisition of cocaine self-administration behavior by female APO-UNSUS rats.

The present study also demonstrates that maternally deprived male APO-UNSUS rats needed a challenge to start cocaine self-administration. In contrast, male APO-UNSUS rats that were not maternally deprived did not need a challenge to acquire cocaine self-administration. The finding that this environmental challenge (light-dark switch) can trigger cocaine self-administration has also been found in male APO-SUS rats; these rats do not acquire self-administration under non-challenged conditions, unless they are exposed to the above-mentioned light-dark switch [319]. In other words, the present study extends the previously reported finding that maternally deprived male APO-UNSUS rats display APO-SUS like characteristics such as a stronger apomorphine-induced gnawing behavior and an increased sensitivity to develop periodontitis at adult age. [27,86].

Because the increase in apomorphine-induced gnawing behavior and in the sensitivity to develop periodontitis in maternally deprived APO-UNSUS rats reflect changes in the dopaminergic system and HPA-axis respectively and because all these factors play a crucial role in the response to cocaine [91,114,116,166,253], it becomes important to investigate to what extent the changes in cocaine self-administration induced by maternal deprivation are due to alterations in these factors.

Research has shown similar alterations in the dopaminergic system and HPA-axis with other adverse early life events [11,134,136,209]. However, some studies have found a decrease in low-dose cocaine self-administration, whilst other have found an increase in low-dose cocaine self-administration [175,201]. A factor that most likely has contributed to this discrepancy, is the protocol used. The effect of early postnatal manipulations is critically regulated by the duration of the manipulation, the timeframe in which the manipulation is executed, and the animal strain used [60,83,340]. The present study, thus, used a protocol that has proven its validity and it is known to alter those systems that are correlated with the response to a drug of abuse in APO-UNSUS rats [82,86,266]. The present study did not investigate the effects of maternal deprivation on cocaine self-administration behavior of APO-SUS rats at adult age since previous research has shown that maternal deprivation does not alter the phenotypic expression of APO-SUS rats [86]. However, since early crossfostering was shown to alter apomorphine-induced gnawing behavior and the sensitivity to develop periodontitis of APO-SUS rats [86,293] into the direction of APO-UNSUS rats. it would be interesting to investigate whether such an early life event would also alter cocaine self-administration behaviour in APO-SUS rats..

In conclusion, the present study shows that female APO-UNSUS rats are faster at acquiring cocaine self-administration than male APO-UNSUS rats. It also shows that maternal deprivation of male APO-UNSUS rats results in a shift in cocaine self-administration behavior of these APO-UNSUS rats, resulting in APO-SUS-like behavior. Taken together, these data together with earlier published data allow for the conclusion that genetic background, sex, and both early as well as late environmental factors direct the acquisition of self-administration behavior in rats.

As a final remark in this context, it has recently been found that the APO-UNSUS rats have three copies of the gene Aph-1b in contrast to the APO-SUS rats that have just one or two copies [48]. Accordingly, it is highly interesting to investigate to what extent these genetic differences underlie the noted line-specific differences in cocaine self-administration.

Chapter 6

Prenatal cocaine exposure in individually different rats facilitates cocaine self-administration behavior of adult male and female rats.

Abstract

Cocaine exposure during pregnancy is correlated with premature birth, severe respiratory distress, attention deficits, and an increased risk for addictive disorders as an adult. Animal research has also confirmed that prenatal cocaine exposure enhances cocaine self-administration at adult age. However, until so far, nobody has investigated the effects of prenatal cocaine exposure on cocaine self-administration in individually different rats. This study, therefore, assessed self-administration behavior at adult age in the genetically selected ratlines consisting of apomorphine unsusceptible (APO-UNSUS) and apomorphine susceptible (APO-SUS) rats, which were exposed *in utero* to repeated cocaine challenges. Pregnant APO-UNSUS and APO-SUS rats were injected with either 20 mg/kg cocaine or saline (s.c.) at postgestational day 8, 11, and 14. At postnatal day 65, animals were operated and after a 7-day recovery period were subjected to the self-administration protocol. Animals were first allowed to freely self-administer for 7 days, followed by shaping from day 8 through day 15. Previous research has shown that shaping is an essential procedure for male APO-UNSUS rats to start consuming cocaine. From day 12 onwards, animals were also subjected to an environmental challenge. Previous research has shown that this challenge is necessary for the male APO-SUS rats to start consuming cocaine. None of the cocaine-exposed pregnant APO-SUS rats gave birth (0%), and only a small percentage of the saline-exposed pregnant APO-SUS rats gave birth (33%). On the other hand, all cocaine-exposed as well as all saline-exposed APO-UNSUS rats gave birth to a healthy litter (100%). Subsequently, self-administration was only conducted with APO-UNSUS rats. Prenatal cocaine-exposure resulted in an increase of the rate of acquisition of male APO-UNSUS rats, but in an increase of the amount of cocaine consumed in female APO-UNSUS rats. These data indicate that prenatal stress in the stress-sensitive APO-SUS rats results in a high number of spontaneous abortions, while prenatal stress in the relativelty stress-insensitive APO-UNSUS rats does not affect the birth score. Furthermore, these data indicate that prenatal cocaine exposure has a profound effect on both male and female offspring: both males and females suffer from a long-term alteration in the reinforcing efficacy of cocaine, resulting in a facilitation of cocaine self-administration behavior at adult age.

Introduction

Cocaine abuse is a huge problem due to its high prevalence and high rates of relapse. In 2002, the number of cocaine users in the United States alone was estimated at 5.9 million people, representing 2.5% of the population aged 12 and older. The same survey also revealed that a large number of pregnant women took illicit drugs like cocaine during pregnancy [301].

Considering the fact that cocaine is known to readily cross the blood-placenta barrier and is metabolized slowly in fetuses, a single cocaine exposure can have a large effect on the fetus as well as on the infant [61]. It has been shown that prenatal cocaine-exposure can lead to premature birth, lower birth weight, respiratory distress, and an increased risk for seizures [61,159]. Behaviorally, these neonates are more irritable, over-reactive to environmental stimuli, and have concentration problems. With age, these symptoms decrease, but behavioral disturbances, like attention problems, remain present [43,61,181].

A number of techniques has been used to examine the neurochemical and behavioral consequences of prenatal cocaine exposure on the pup as well as on the adult rat. For instance, microdialysis data from pups prenatally exposed to cocaine has shown that these rats have increased extracellular basal levels of striatal dopamine and its metabolites, DOPAC and HVA. These studies have also shown that these pups have an enhanced striatal dopamine response to acute stressors such as a tail pinch [158,159]. Even though these differences seem to disappear during adulthood, an enhanced dopamine response to a cocaine injection develops in the nucleus accumbens [140]. Moreover, offspring of dams exposed to cocaine have an increase of striatal D2 binding [278], an increase of the behavioral sensitivity to the D2/D3 agonist quinpirole [218], and a decrease of the sponteneous activity of midbrain DA neurons [216]. These findings show that there are long-term alterations in the mesocorticolimbic dopamine system after prenatal cocaine exposure. Since this system is associated with mechanisms of reward and reinforcement [70,91,204], it is not surprising that prenatal cocaine-exposure enhances cocaine self-administration, reduces the reinforcing efficacy of cocaine as assessed by breakpoint analysis, and increases striatal extracellular dopamine release after an injection with cocaine [139,158,159].

In both humans and rats, it is known that there are marked individual differences in the sensitivity to the reinforcing properties of cocaine at adult age [129,271]. For instance, several studies have shown that rats with a high response to a novel environment (HR) are better at acquiring cocaine self-administration and take far greater amounts of cocaine than rats with a low response to a novel environment (LR) [153,268]. These differences have been attributed to a differential structure and/or function of the dopaminergic system and the HPA-axis [118,143,192,270]. Although these studies have clearly shown that several neurochemical and behavioral factors can have an impact on cocaine-self-administration, the interaction between these genetically and environmentally determined factors was, until recently, not investigated. Until now, nobody has investigated the effects of prenatal cocaine exposure on cocaine self-administration at adult age in individually different rats.

The department of psychoneuropharmacology has developed a ratmodel consisting of apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) rats

[50,52]. These rats were selected from a normal Wistar population on the basis of their response to the dopamine D_1/ D_2 receptor agonist apomorphine[50,86]. Through a selective and specific breeding program (in order to minimize inbreeding), two distinct lines of rats were created [50]. APO-UNSUS and APO-SUS rats have been characterized behaviourally, neurochemically, immunologically, and endocrinologically [50,52,53,86]. For instance, APO-UNSUS rats have, under non-challenged conditions, a lower density of dopaminergic striatal D2 receptors [267], a functionally higher noradrenergic activity in the ventral striatum as determined by (-) adrenergic agents induced locomotor activity by accumbal infusions [50,81], higher plasma levels of free corticosterone than APO-SUS rats [264], and show the same behavioural response to a novelty challenged as the LR rats [50]. However, under challenged conditions their neurochemical and endocrinological status is known to alter: after a stressor, APO-UNSUS rats have a lower stress-induced dopaminergic activation of the ventral striatum [88], a functionally lower noradrenergic activity in the ventral striatum [50,81], and a smaller and shorter lasting increase in adrenocorticotropic hormone (ACTH) and corticosterone than APO-SUS rats [264]. The fact that the status of these systems was known to be environmentally dependent as well as the fact that these systems are known to be involved in directing the amount of cocaine consumed [118,143,192,270], allowed for an investigation of the influence of the environment on cocaine self-administration behavior in APO-UNSUS and APO-SUS rats. A recent study has revealed that pharmacogenetically selected apomorphine unsusceptible (APO-UNSUS) rats consume considerably more cocaine than apomorphine susceptible (APO-SUS) rats under non-challenged conditions, whilst the reverse holds true under challenged conditions [319], making them a good model to study the long-term effects of prenatal cocaine exposure on the gene * environment interaction(s) in cocaine self-administration at adult age.

This study, therefore, assessed self-administration behavior at adult age of male and female APO-UNSUS and APO-SUS rats that were exposed *in utero* to repeated cocaine challenges. Given the fact that prenatal cocaine exposure has been shown to sensitize the dopaminergic system that is involved in the onset of an addiction, we hypothesized that both male and female APO-SUS and APO-UNSUS rats that are prenatally exposed to cocaine will increase cocaine self-administration. Moreover, since cocaine-exposed females show greater behavioral and neurochemical alterations in the dopaminergic system than males do [74], it is expected that female APO-SUS and APO-UNSUS rats will have a higher increase in cocaine self-administration than male APO-SUS and APO-UNSUS rats do. This differential effect of prenatal cocaine-exposure on the dopaminergic system in females could be due to the presence of estrogen: estrogen is known to influence drug-related behaviors [19,145], mostly because it facilitates the release and reuptake of dopamine from the striatum [17,18,304]. Moreover, some researchers have stated that prenatal cocaine exposure alters sexual differentiaton of the brain [215], and this could result in the differential effect on the dopaminergic system between females and males after cocaine-exposure.

In addition, since male, and presumably female, APO-SUS rats are already characterized by heightened striatal dopaminergic activity (in comparison to APO-UNSUS rats) [88], we

hypothesized that prenatal cocaine-exposure will differentially affect cocaine self-administration behavior in APO-SUS and APO-UNSUS rats at adult age.

Methods

In general

Adult female and male Wistar rats of the pharmacogenetically selected outbred rat line (original line 33rd generation; age approx. 3 months) consisting of apomorphine unsusceptible rats (APO-UNSUS) and apomorphine susceptible rats (APO-SUS) were obtained from the Central Animal House, Radboud University Nijmegen, the Netherlands [50]. Male-female pairs of APO-UNSUS rats and APO-SUS rats were housed together in cage with a stainless steel grid floors. The presence of a vaginal plug in the cage was defined as 'day 0' of gestation. After gestation, the female rats were individually housed on normal sawdust bedding. Pregnant rats, both APO-SUS and APO-UNSUS rats, were given a subcutaneous injection of either saline (1ml /kg) or cocaine (20 mg/kg, 1 ml/kg) at gestational day 8, 11 and 14 [158]. The injection sites were given on alternating spots in the neck and on the back to minimize skin lesions. Within one day after injection with either saline or cocaine, animals had small necrotic areas at the injection site. Bodyweight of the pregnant rats was measured from the first injection until the last injection to verify that each animal gained weight during gestation (measurement of nutritional intake during the prenatal period).

Given the fact that early postnatal manipulations, like handling, maternal deprivation, and cross fostering is known to alter the neurochemical and endocrinological status of APO-SUS and APO-UNSUS rats [60,86,293], all pups were, after birth, left undisturbed until weaning at postnatal day 28.

Unfortunatelly, none of the pregnant cocaine-exposed and only a small percentage of the saline exposed APO-SUS rats gave birth. All pregnant APO-UNSUS rats gave birth, and the self-administration experiments could, therefore, be performed only with APO-UNSUS rats (see results). All animals were housed two to three per cage (except during breeding) in standard Macrolon® type 3 cages (42 x 26 x 20 cm) in temperature-controlled rooms (21°) with a standard 12/12-h day/night-cycle (lights on at 7.00 am) and food and water available *ad libitum*. All experiments were performed in accordance with institutional guidelines, national, and international laws for animal care and welfare.

Surgery

At postnatal day 65, a random selection of rats from 3 - 4 litters was chosen to be operated. The remainder of the rats were used for different experiments (not shown). Animals, both cocaine-exposed and saline-exposed males and females were surgically implanted with an intracardiac silicon catheter (0.3 mm i.d., 0.7 mm o.d.) in the right external jugular vein under isoflurane anesthesia. Following surgery, rats were individually housed. Catheter patency was maintained by daily infusions of 0.1 ml of a saline-heparin (50IU/ml) solution. After completion of experimental procedures catheter placement and patency was confirmed

by infusion of 6 mg pentobarbital [(0.1 ml Nembutal©); Narcovet®, Apharma, Arnhem, the Netherlands]. Rats that failed to lose muscle control within 5-10 seconds were discarded from further analysis (4 %, n = 1).

Drugs
Cocaine hydrochloride (Pharmacy, UMC St Radboud, Nijmegen, the Netherlands) was dissolved in 0.9% sodium chloride and solutions were made for each individual animal on a weekly basis.

Self-administration cages and infusion scheme
The operant self-administration cages (30.5 (l) x 24.1 (w) x 29.2 (h) cm) were equipped with two small nose-poke holes located opposite from one another, a house-light located above the "drug-active" hole, and a stainless steel grid floor (Med Associates Inc). A nose poke into the drug-active hole resulted in an infusion of 35.4 μl of a cocaine-solution (250 μg/kg) over 2 seconds followed by a 20 second time-out (TO) period. During the infusion and time-out period the house-light was illuminated. A nose poke during the time-out period or in the "drug-inactive" hole was recorded but without consequence. After a 7 day recovery period from the surgery, animals were allowed to self-administer cocaine during 15 daily (7 days per week) 1-hour sessions on a fixed ratio 1 (FR1) schedule of reinforcement, with a maximum of 100 infusions per session. All tests were done between 10:00 and 15:00 (light phase). Acquisition of cocaine self-administration was established when animals reached a stable infusion pattern (less than 10% deviation) over three consecutive days on the drug-active hole in combination with a minimum number of 6 infusions [247,319].

Self-administration male and female rats
This experiment was done to determine whether prenatal cocaine exposure altered the self-administration behavior of adult male and female APO-UNSUS rats. Therefore, adult male and female cocaine-exposed APO-UNSUS rats [Prenatal Cocaine (PC): male: n = 11, female: n = 8] and saline-exposed APO-UNSUS rats [Prenatal Saline (PS): male: n = 8, female: n = 8] were allowed to self-administer cocaine under three behavioral conditions as described previously [319]. In short, animals were first habituated to the cages and the experimental procedure of attaching the animals to the infusion line, including the ability to freely self-administer cocaine for a period of 7 days. From day 8 onwards, animals were shaped for the drug-active hole [247]. Shaping has previously been shown to be an essential procedure for male APO-UNSUS rats to acquire self-administration [319]. Previous experiments have also shown that a challenge (by turning off the lights during self-administration) can enhance both cocaine as well as alcohol self-administration in the stress-sensitive male APO-SUS rats, but not in the stress-insensitive male APO-UNSUS rats [318,319]. To examine whether, and to what extent, the stress-insensitive male APO-UNSUS rats underwent an alteration in their stress (in)sensitivity due to prenatal cocaine, the animals were also subjected to the challenge of switching of the lights from day 12 onwards.

Statistical analysis

Maternal body weight was measured during the injection procedure. In order to compare the different groups of APO-UNSUS and APO-SUS rats, body weight at the first injection was set to 100%. The groups were compared by means of a three-way ANOVA with the factors injection days, genotype and treatment, followed by a post-hoc bonferroni where appropriate. The number of pups (males and females) born of both rat types and of both treatments was evaluated and, were possible, statistically analyzed by means of an independent sample t-test.

Differences in bodyweight at the start of the self-administration experiment between prenatally cocaine-exposed and saline-exposed of both male and female rats were compared by means of a two-way ANOVA, followed by a post-hoc independent samples t-test. Self-administration sessions resulted in three variables, namely (1) the number of infusions, (2) the number of active nose pokes, and (3) the number of inactive nose pokes. Since a previous experiment has shown that male and female rats have a different self-administration pattern (Van der Kam et al, submitted), the statistical analyses of the variables was done per gender. Analysis was done by means of a two-way ANOVA with prenatal manipulation (PS = prenatal saline, PC = prenatal cocaine) as the between subject factor and days as the repeated measure for the number of inactive nosepokes, the number of active nosepokes, and the number of infusions. In addition, the data were also analysed in three data sets; one for the first 7 days, one for day 8 through 11, and one for day 12 through 15.

To examine the effects of shaping and the challenge, the number of infusions between day 7 and 8 and between day 11 and 12 were compared per group (PC male, PS male, PC female, and PS saline) by means of a paired samples t-test. Where appropriate, data were further analyzed by means of an post hoc independent samples t-test for each day. The number of inactive nose pokes was used to detect differences in this parameter. The number of active nose pokes was analyzed, but data are not shown because the number of active nose pokes followed the same pattern as the number of infusions. All data were analyzed using SPSS10.0. A probability of $p < 0.05$ was taken as significant.

Results

In general

The pregnant APO-UNSUS rats exposed to cocaine and saline gradually gained weight during the injection period (PC weight gain 15% ± 1.5%: PS weight gain 16% ± 1.4%, n.s. different). The pregnant APO-SUS rats exposed to cocaine and saline, on the other hand, did not gain much weight during the injection period (PC weight gain 4% ± 2.1%: PS weight gain 5% ± 1.4%, n.s. different; see figure 1). All pregnant APO-UNSUS rats gained significantly more weight in time than the pregnant APO-SUS rats (injection day-genotype interaction: $F_{(6,60)} = 16.5$ $p < 0.05$).

All pregnant APO-UNSUS rats gave birth to healthy litters of, on average, 10 pups (100% birthscore; cocaine-exposed: 10 ± 1.9 pups, saline-exposed: 9.5 ± 4.5 pups; n.s. different). There were no differences in the amount of male and female pups between saline-exposed

and cocaine-exposed APO-UNSUS rats (50% male, 50% female). None of the cocaine-exposed pregnant APO-SUS rats gave birth (0% birth score). Only a small portion of the saline-exposed pregnant APO-SUS rats gave birth (33% birth score). These rats gave birth to healthy litters of, on average, 8 pups (50% male, 50% female). The number of male and female pups and litters was, however, too small to give an reliable representation of the saline-exposed APO-SUS rat population. These rats were therefore not utilized in the self-administration experiment. At postnatal day 65, a random selection of at least 5 litters was operated (cocaine-exposed: male n = 11, female n = 8; saline-exposed: male n = 8, female n = 8).

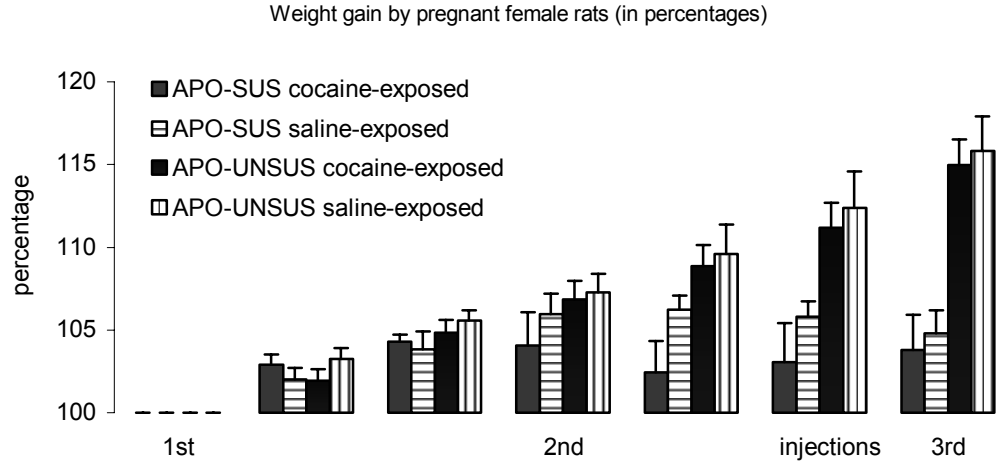


Figure 1*: Weight gain by pregnant female APO-UNSUS and APO-SUS rats in percentages. Bodyweight at the 1st injection was set at 100%. All female APO-UNSUS rats, either exposed to cocaine or saline, gained weight. Female APO-SUS rats, either exposed to cocaine and saline, did not gain much weigth during the injection period.*

Bodyweight

The female APO-UNSUS rats had a significantly lower bodyweight than the male APO-UNSUS rats (two-way ANOVA: $F_{(3,32)} = 72.8$ $p < 0.05$). The PC and PS rats of either the male or female group, however, did not differ in bodyweight at the start of the self-administration procedure [PC male: 231 ± 4.7 grams, PS male 234 ± 8.2 grams: PC female 152 ± 3.7 grams, PS female 155 ± 3.6 grams (± SEM)].

Acquisition criteria

The number of male and female APO-UNSUS rats, either prenatally treated wth cocaine or saline, that acquired self-administration, was not different from one another (100% for all groups). None of the rats failed to reach criteria. There was however a difference in the time-point at which male APO-UNSUS rats reached the acquisition criteria. The PC male APO-UNSUS rats acquired self-administration faster than the PS male APO-UNSUS rats (figure 2). In agreement with previous experiments, none of PS male APO-UNSUS rats acquired self-administration prior to shaping [319]. The PC male APO-UNSUS rats all acquired cocaine self-administration prior to shaping. There was no difference in the time-point at which female APO-UNSUS rats acquired self-administration.

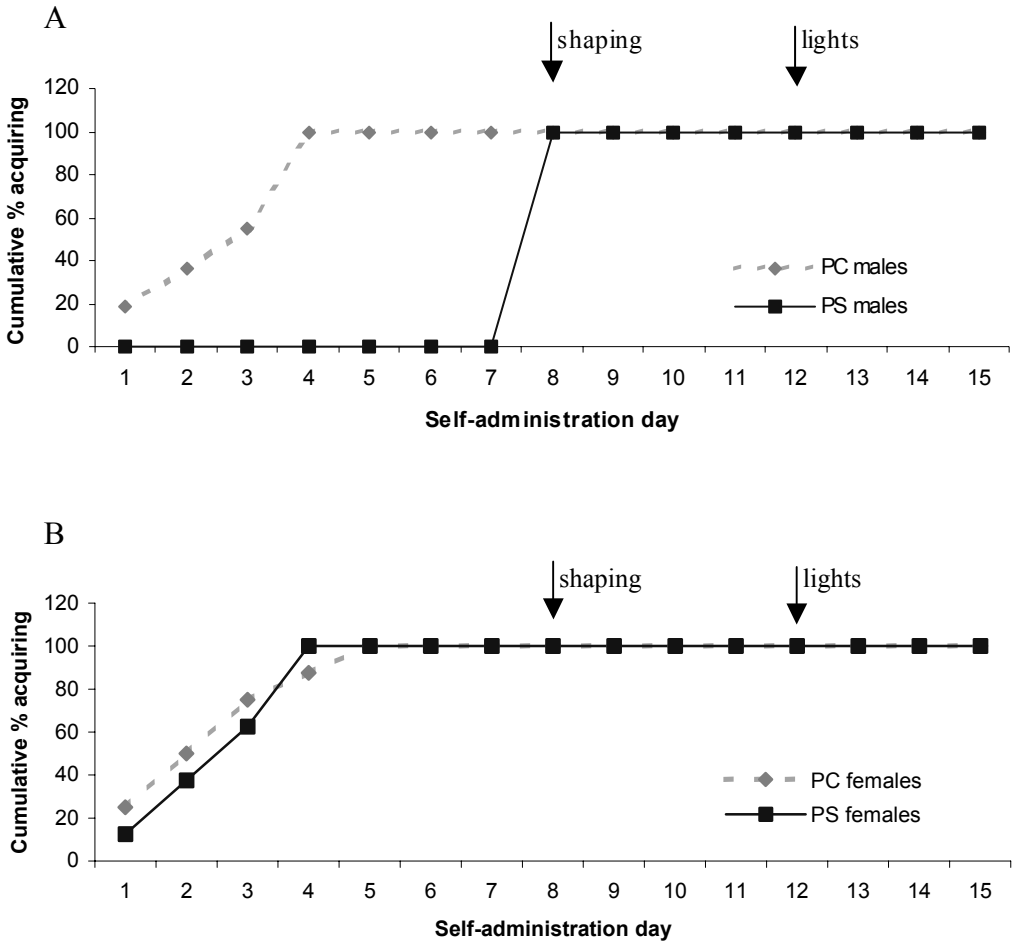


Figure 2*: Time span for male (a) and female (b) APO-UNSUS rats reaching acquisition criteria (less than 10% deviation over 3 consecutive days, minimum of 6 infusions). The total number of animals reaching criteria is set at 100% and per session the relative number of animals that reached criteria is shown. This results in a graph with the cumulative percentage of animals acquiring cocaine self-administration in time. It should be noted that the first day of the three day period has been used as set point for these graph. (a) The graph shows that none of the PS male APO-UNSUS rats acquired self-administration prior to shaping, whilst the PC male APO-UNSUS rats acquired self-administration within 4 days. (b) The graph shows that both PC and PS female APO-UNSUS rats acquired self-administration within 4 days. There were no differences between PC and PS female APO-UNSUS rats. Because all animals had acquired self-administration prior to the stressor, no effect was seen*

Inactive nosepokes for all groups

No differences in the number of inactive nose pokes were found between prenatally cocaine-exposed or saline-exposed male and female APO-UNSUS rats at any time-point during self-administration.

Cocaine self-administration of male rats

The data were analyzed for the complete duration of the experiment. When comparing PC males with PS males, a higher intake of cocaine over time by PC males was seen (session-treatment interaction: $F_{(15,210)} = 2.8$ $p < 0.05$). Post hoc analyis by means of an independent samples t-test revealed that the PC male rats consumed considerably more cocaine during day 1 through 7 ($p < 0.01$, figure 3). The analysis per data set (day 1-7, day 8-11, and day 12-15) provided the same information, namely that from day 1 to 7 the PC males consumed more cocaine than the PS males. When examing the effect of shaping on the PS males, a significant increase of the number of infusions was seen [day 7: 2.2 ± 0.6 (mean ± sem) infusions, day 8: 8.4 ± 0.9 infusions; paired samples t-test: $p < 0.05$]. The challenge at day 12 resulted in a non-significant increase of the amount of infusions of the PS males (day 11: 12.8 ± 2.6 infusions, day 12: 23.4 ± 6.5 infusions; paired samples t-test: $p = 0.05$). The PC males did not increase (or decrease) the number of infusions after shaping or when the challenge was presented (sample t-test $p > 0.05$).

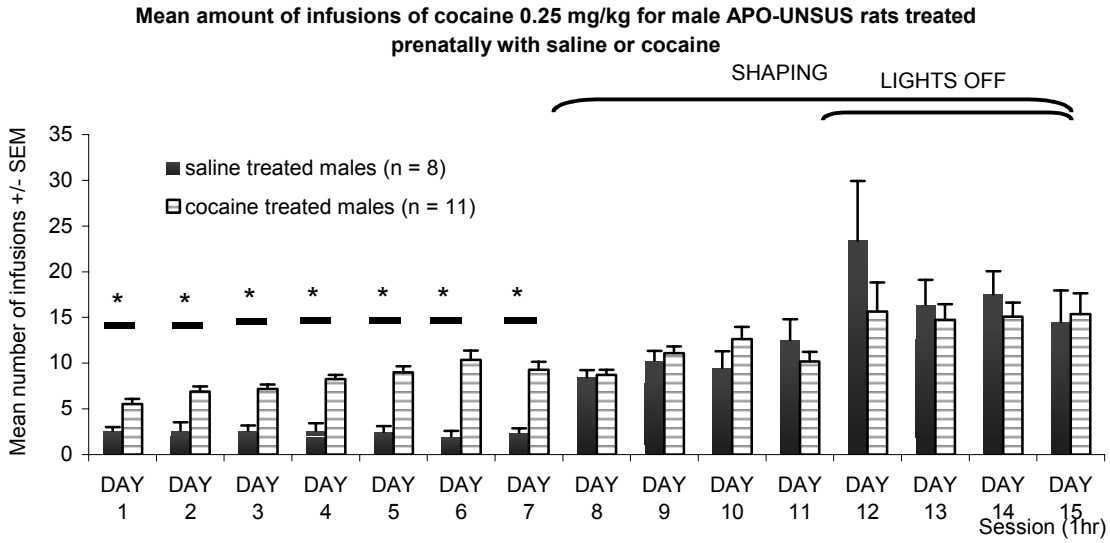


Figure 3*: Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for male PS APO-UNSUS (n = 8) and PC APO-UNSUS (n = 11) rats. Animals were habituated to the self-administration cages and the experimental protocol for 7 days, followed by shaping from day 8 onwards and darkness (stressor) from day 12 onwards. Cocaine-exposed male rats (arced columns) consumed considerably more cocaine during the first 7 days of self-administration than the saline-exposed male (filled columns) rats (independent samples t-test p < 0.05; marked by ✶). The saline-exposed male rats, however, increased their intake to match levels of the cocaine-exposed rats when shaping commenced.*

Cocaine self-administration of female rats

The female APO-UNSUS rats that were prenatally treated with cocaine consumed more cocaine than the female rats that were prenatally treated with saline (treatment: $F_{(1,14)} = 6.4$ $p < 0.05$). Post hoc analyis by means of an independent samples t-test revealed that the PC females consumed considerably more cocaine during day 5 through 11 ($p < 0.05$, figure 4). There was a tendency to consume more cocaine at day 4 ($p = 0.06$). The analysis per data set (day 1-7, day 8-11, and day 12-15) provided the same information, namely that the PC

females consumed more cocaine from day 5 through 11. Neither the PS nor PC female APO-UNSUS rats reacted to shaping (with either a decrease or increase in the number of infusions). Both groups, however, did increase their intake after the challenge at day 12 (PS females day 11: 12.5 ± 2.2 infusions, day 12: 21.1 ± 4.9 infusions; paired samples t-test: p = 0.05; PC females day 11: 18.6 ± 1.4 infusions, day 12: 32.8 ± 8.2 infusions; paired samples t-test: $p < 0.05$).

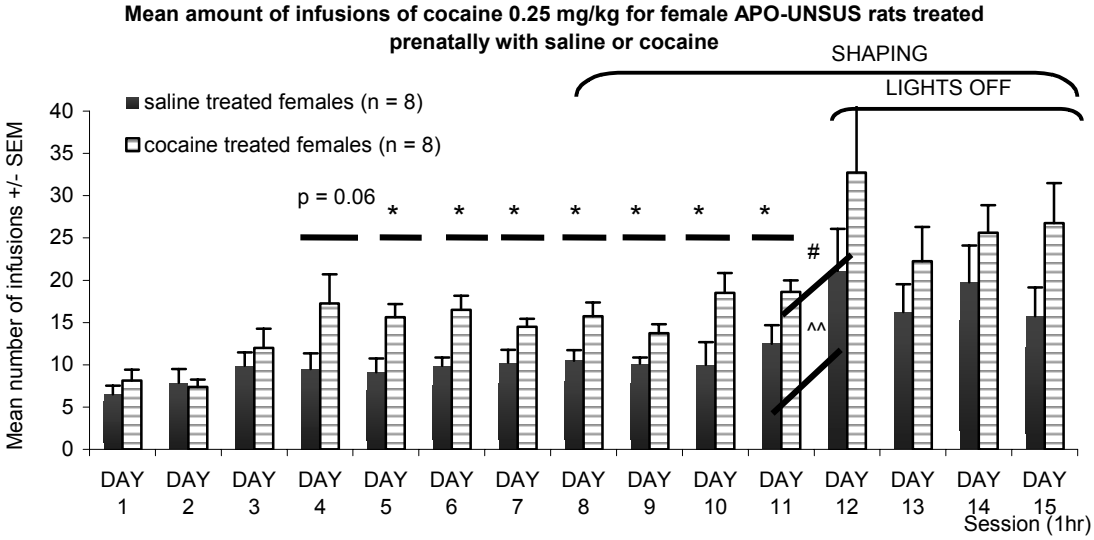


Figure 4*: Mean number of infusions of a 0.25 mg/kg cocaine solution (± SEM) for female PS APO-UNSUS (n = 8) and PC APO-UNSUS (n = 8) rats. Animals were habituated to the self-administration cages and the experimental protocol for 7 days, followed by shaping from day 8 onwards and darkness (stressor) from day 12 onwards. Cocaine-exposed female rats (arced columns) consumed considerably more cocaine from day 5 to day 11 than the saline-exposed female (filled columns) rats (independent samples t-test p < 0.05; marked by ✶). None of the female rats responded to shaping. Both groups, however, did increase their intake after the challenge at day 12 was presented. The increase in intake of both groups (saline-exposed group p = 0.05; marked by ^^: cocaine-exposed group p < 0.05, marked by #) resulted in an equal intake for the remaining sessions.*

Discussion

The present study was done to investigate the effects of prenatal cocaine-exposure on cocaine self-administration at an adult age in the individually different APO-UNSUS and APO-SUS rats. However, due to the fact that none of pregnant cocaine-exposed APO-SUS rats and only a small percentage of saline-exposed APO-SUS rats gave birth, the effects of prenatal cocaine exposure on cocaine self-administration could only be studied in male and female APO-UNSUS rats. The present study showed that prenatal cocaine-exposure resulted in an increase in the rate of acquisition of male APO-UNSUS rats, whilst it increased the amount of cocaine consumed of female APO-UNSUS rats. These data are discussed below.

The present study showed that pregnant APO-SUS rats reacted stronger to the injections (with or without cocaine) than pregnant APO-UNSUS rats, resulting in loss of their set of offspring. The fact that, independent of whether cocaine or saline was given, female APO-SUS rats lost their offspring, indicates that female APO-SUS rats were more susceptible to

the injection procedure than female APO-UNSUS rats. This is in line with previous findings in adult males that APO-SUS rats are more stress-sensitive than APO-UNSUS rats are [52,267]. Short- and long-term alterations in the offspring elicited by prenatal stress are usually mediated through physiological disturbances in the dam that are likely to result in fetal distress. Indeed, it is known that stress hormones readily cross the blood-brain barrier and, therefore, can have an effect on fetal development [41,329]. Moreover, research has shown that prenatal stress can result in a high mortality of neonates [41,130]. However, there are, to our knowledge, no data available on the effects of stress on birth scores in rats. It is apparent that prenatal stress in the stress-sensitive APO-SUS rats resulted in a reduced birth score in comparison to 'normal' APO-SUS rats [birth score pregnant APO-SUS rats 90% (number of females giving birth, average of last 20 generations), unpublished data]. Given the fact that the treatment commenced after day 8 of gestation, the low birth score must have been due to fetal loss (abortion) or resorption. Unfortunately, female rats usually dispose of stillborns and unhealthy pups by consuming them; leaving no trace of the abortion. In only one case, a small number of pups was found heavily mutilated. Three weeks after the suspected birthdate, an autopsy revealed no remaining pups in the uteri of APO-SUS rats. Interestingly, the pregnant APO-SUS rats did gain some weight at first, but this weight gain stopped around the 2nd injection with either saline or cocaine. This suggests that the pregnant APO-SUS rats lost their offspring at this time-point.

On the other hand, prenatal stress in the relatively stress-insensitive APO-UNSUS rats did not affect the birth score, since it was comparable to the birth score of untreated APO-UNSUS rats [birth score pregnant APO-UNSUS rats 94% (average of last 20 generations), unpublished data]. The data on maternal weight gain revealed the same: all pregnant APO-UNSUS rats, either injected with saline or cocaine, showed a gradual increase in bodyweight, again indicating that the relatively stress-insensitive APO-UNSUS rats were not afffected by the injections.

Recently, it has been found that APO-UNSUS rats have three copies of the gene APH1b in contrast to APO-SUS rats that have just one or two copies [48]. The protein, APH 1b, is an integral part of the γ-secretase complex which cleaves many peptides that are crucial in the various aspects of development. Considering the role of this gene in development, it would be interesting to investigate to what extent this genetic difference gives rise to the line-specific differences found in the birth score and stress sensitivity during gestation.

Interestingly, all rats developed small necrotic areas after cocaine and saline injections. Subcutaneous cocaine injections are known to result in necrosis [139,158,298], but, to our knowledge, nobody has shown that subcutaneous saline injections result in necrosis. It is possible that APO-SUS and APO-UNSUS rats are more sensitive in this respect. The fact that all rats, whether injected with cocaine or saline, developed small necrotic areas could indicate that these injections are stressors by themselves. However, because saline-exposed APO-UNSUS rats had the same pattern, rate and amount of cocaine self-administered as in a previous study[319], it is unlikely that injection stress played an important role in determining cocaine self-administration behavior in APO-UNSUS rats.

It should be noted that we, in contrast to others [158,159], did not cull, weigh, or cross foster the

pups. We left the pups and mothers undisturbed until postnatal day 28 when the pups were weaned. This was done because previous research has shown that early postnatal manipulations, like culling, handling, maternal deprivation, and cross fostering alter those neurochemical and endocrinological systems of APO-SUS and APO-UNSUS rats that are known to be involved in the response to cocaine [60,83,86,293](Van der Kam et al, submitted).

The present data clearly showed that male APO-UNSUS rats that were prenatally exposed to cocaine, were faster at acquiring cocaine self-administration than male APO-UNSUS rats that were prenatally exposed to saline. The finding that saline-exposed APO-UNSUS rats acquired self-administration only after shaping was introduced, is in line with previous research [319]. Even though both groups learned to self-administer cocaine at a different rate, the number of animals that eventually reached acquisition criteria was equal (100%), clearly showing that both groups did not differ in the capability to learn the self-administration behavior. The finding that prenatally cocaine-exposed male rats acquired self-administration faster is in concordance with a study by Keller et al [158]. However, our study also reveals that cocaine-exposed male APO-UNSUS rats did not differ from saline-exposed male APO-UNSUS rats in the amount of cocaine consumed: overall saline-exposed and cocaine-exposed males consumed the same amount of cocaine (figure 3: total number of infusions of PC males was 150 ± 12.7 and of PS males 129.8 ± 18.6). Several experiments have shown that prenatal cocaine exposure seems to augment the dopaminergic response to cocaine [113,139,159,159]. Moreover, research has shown that a heightened striatal dopaminergic response seems to be correlated with a higher acquisition rate of cocaine [114,143,270]. Therefore, the present finding that cocaine-exposed male APO-UNSUS rats acquire cocaine-selfadministration faster is in line with previous studies.

The present study also showed that female APO-UNSUS rats that were prenatally exposed to cocaine, consumed greater amounts of cocaine than saline-exposed female APO-UNSUS rats. This difference in the amount consumed was not due to a difference in either the rate of acquisition or to a difference in the learning capability, since both saline-exposed and cocaine-exposed females acquired cocaine self-administration at the same time-point and capacity (100%). It should, however, be noted that a shift in the acquisition rate was nearly impossible since PS females were extremely fast at acquiring self-administration (within 4 days). The finding that PS female APO-UNSUS rats self-administered cocaine from the beginning of the experiment and did not need shaping is in line with previous experiments (Van der Kam et al, submitted).

PC female APO-UNSUS rats took far greater amounts of cocaine during the first part of the experiment than the PS female APO-UNSUS rats (day 4-day 11; see figure 4). This finding is in line with our hypothesis. As stated above, the increase of the rate of acquisition in male rats is suggested to be due to an augmentation of the dopaminergic response to cocaine [113,139,159,159]. The present study, however, reveals that female rats increased the amount of cocaine consumed. The question remains why male rats show a leftward shift (increased rate of acquisition) whilst female rats show an upward shift (increased amount). Previous research has shown that cocaine-exposed females show greater behavioral and neurochemical abnormalities in the dopaminergic systems than males [74,161,198]. Moreover, some researchers

have suggested that prenatal cocaine exposure may alter sexual differentiaton of the brain [215], which could result in a differential effect (or levels) of circulating hormones. Because the female hormone estrogen is known to facilitate the release and reuptake of dopamine from those systems directly involved in addictive behavior [17-19,145,304], it might explain why females have an increased intake of cocaine, whilst males (only) have an increased rate of acquisition.
An interesting finding is that female APO-UNSUS rats, independent from the treatment received, reacted to the challenge presented at day 12 (reversal day-night). Both saline-exposed as well as cocaine-exposed females increased their intake when presented with the challenge. This is different from male APO-UNSUS rats [319] and a previous study with female APO-UNSUS rats (Van der Kam, et al, submitted). Some studies have suggested that prenatal cocaine exposure not only augments the dopaminergic response to cocaine, but also augments the dopaminergic response to stressors [159,297]. The present study, however, seems to indicate that prenatal stress exposure (injection stress) can also enhance self-administration in female rats. Recent research has indeed revealed that prenatal stress exposure has a significant and differential impact on the dynamics of the stress response of male and females rats: males show an impairment of feedback control of the stress system, while females show a significant increase of adrenal corticosterone secretion [230]. Plasma corticosterone levels at the time of cocaine self-administration have been identified as a determinant of the amount of cocaine consumed [63,117,118,192], and thus, the suggestion can be made that prenatally stressed female rats react stronger to stressors later in life and consume greater amounts of substances of abuse as a consequence.
In conclusion, the present study shows that pregnant APO-SUS rats were extremely susceptible to injection stress, resulting in loss of their set of offspring. Pregnant APO-UNSUS rats, on the other hand, seemed to be unaffected by either saline or cocaine injections. However, male and female offspring of these rats suffered from a long-term alteration in the reinforcing efficacy of cocaine, resulting in a shift in cocaine self-administration behavior at an adult age. Male cocaine-exposed offspring showed an enhanced rate of acquisition (left shift) whilst female cocaine-exposed offspring showed an enhanced intake of cocaine (upward shift). Altogether, these results indicate that prenatal cocaine exposure has life-altering effects in the susceptibility to drugs of abuse, which differs between males and females.

Chapter 7

Monoamine transporters in apomorphine susceptible and unsusceptible rats.

Submitted to Neuroscience
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Abstract

Our department has recently shown that apomorphine unsusceptible (APO-UNSUS) rats have a higher consumption of cocaine than apomorphine susceptible (APO-SUS) rats under non-challenged conditions, whilst the reverse holds true under challenged conditions. Because research has suggested a correlation between the amount of dopamine transporters and, possibly, the amount of noradrenaline and serotonin transporters in directing the amount of cocaine consumed, it became interesting to investigate the levels of these transporters in APO-UNSUS and APO-SUS rats. The striatum was selected because the susceptibility of this structure to dopaminergic agents greatly differs between both rat types, whereas the hippocampus was selected because its function and structure differs between both rat types.

The present study showed that APO-UNSUS rats have a significantly smaller level of dopamine transporters in the striatum than APO-SUS rats do. On the other hand, APO-UNSUS rats had slightly higher, signifcantly different levels of dopamine and serotonin transporters in the hippocampus than APO-UNSUS rats did. There were no differences in striatal serotonin and noradrenaline transporters or hippocampal noradrenaline transporters levels. The results are discussed with respect to the known behavioral and neurochemical differences of these animals.

Introduction

Apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) rats were selected from a normal Wistar population on the basis of their response to the dopamine D_1/ D_2 receptor agonist apomorphine, injected subcutaneously in a standard dose of 1.5 mg/kg [50,86]. Through a selective and specific breeding program (in order to minimize inbreeding), two distinct rat lines were created, termed APO-SUS rats (apomorphine susceptible rats, i.e. rats that show a strong gnawing response to apomorphine) and APO-UNSUS rats (apomorphine unsusceptible, i.e. rats that show a weak gnawing response to apomorphine) [50]. Research has shown that these animals have some fundamentally different properties in, amongst others, the striatal dopaminergic system [50,52], i.e. APO-SUS rats have, in comparison to APO-UNSUS rats, higher levels of tyrosine hydroxylase (TH) immunoreactivity in the ventral striatum [87] and a higher density of dopaminergic D2 receptors in the striatum [267] under non-challenged conditions. Until now, however, the amount of dopamine transporters (DAT) under these conditions was unknown. This is of special interest since recent research has shown that APO-UNSUS rats consume more cocaine than APO-SUS rats under non-challenged conditions [319]. Given the fact that cocaine primarily exerts its action by blocking dopamine transporters [99,333], it became interesting to investigate whether APO-UNSUS and APO-SUS rats differed in the DAT levels. Therefore, we measured DAT levels in the striatum (both dorsal and ventral) of non-challenged APO-UNSUS and APO-SUS rats by means of western blot analysis. The striatum was selected because the susceptibility of this structure to dopaminergic agents (both cocaine as well as apomorphine [52]) greatly differs between both rat types [52] and because of its well-established physiological role in addictive behavior [10,91,149,151,246,256,258]. Because cocaine also exerts an action on the noradrenaline (NET) and serotonin transporter (SERT), the levels of these transporters in the striatum were also analyzed.

Next to the striatum, the hippocampus was analyzed. This structure was chosen because its structure differs between both rat types, i.e. APO-SUS rats have, in comparison to APO-UNSUS rats, more mineralocorticoid receptors [265], a greater dynorphin B expression in the hippocampus [51], and a greater hippocampal weight [50]. Moreover, APO-SUS rats display schizophrenia-like symptoms as can be seen by a reduced prepulse inhibition and latent inhibition [84], namely features that that can be attributed to a disturbance in hippocampal functioning [40,217,252,281].

Methods

Tissue handling

Adult male Wistar rats of the pharmacogenetically selected outbred rat line (original line 33rd generation; age approx. 3 months) consisting of apomorphine unsusceptible rats (APO-UNSUS) and apomorphine susceptible rats (APO-SUS) were obtained from the Central Animal House, Radboud University Nijmegen, the Netherlands [50]. Animals (APO-SUS n = 6, APO-UNSUS n = 6) were sacrificed by deep aneasthesia and decapitation. The brains

were removed and the striata and hippocampi were removed by microdissection, quickly frozen in liquid nitrogen and stored at –80˚ C until needed.

Protein preparation
The frozen tissues were homogenized with a dounce homigenizer in lysis buffer [50mM Tris-HCl (pH 7.5), 150mM NaCl, 1 mM EDTA, supplemented with protease inhibitors (10 µl per ml trypsin and PMSF)] and kept on ice for 30 minutes. Tissues were subsequently centrifuged at 800 x g for 15 minutes at 4˚ C to remove nuclei and cellular debri. The supernatants were further centrifuged at 18,000 x g for 60 minutes at 4˚ C. The obtained pellets were resuspended and sonificated for approximately 3 seconds in lysis buffer containing 1% NP-40 and 0.1% SDS. Protein concentrations were determined by the Bradford method, using the protocol from the manufacturer. Subsequently, samples were stored at –20˚ C until use.

Western immunoblotting analysis
Fourthy micrograms of membrane protein from the hippocampus or the striatum were size-fractioned on an 8% SDS-PAGE gel and transferred to a nitrocellulose membrane. Membranes were washed in 1x PBS, blocked in 1x PBS with 5% nonfat milk and subsequently exposed to the first antibody overnight at 4˚ C in blocking solution. Antibodies were used against (a) the 18 amino-acid peptide sequence near the NH2-terminus of the rat brain dopamine transporter coupled to KLH (rabbit anti-DAT, 1:2500, Chemicon International, AB1591P), (b) a 22 amino-acid peptide sequence mapping the 1st extracellular domain of the rat norepinephrine transporter (rabbit anti-NET, 1:2500, Chemicon International, AB5066P), (c) a 15 amino-acid sequence between transmembrane domain 7 and 8 of the rat serotonin transporter (rabbit anti-SERT, 1:2500, Chemicon International, AB1594P), and (d) against β-tubulin (mouse anti-tubulin, 1: 3000) [44]. The β-tubulin immunoreactivity was used as an internal loading control. Blots were then washed and incubated with goat α-rabbit peroxidase (GαRPO) or goat α-mouse peroxidase (GαMPO) at a 1:5000 dilution for 45 minutes at room temperature. Antibody binding was detected by enhanced chemiluminescence (ECL). For quantification, hybridization signals were analyzed using the Labworks 4.0 program (UVP BioImaging systems, Cambridge, United Kingdom). Band intensities were calculated, and background was subtracted using local averages. Values were normalized for variation in protein loading based on levels of β-tubulin immunoreactivity.

Statistical analysis
The intensity values of the APO-UNSUS rats were recalculated to a percentage of the values of APO-SUS rats (e.g. *(1/apo-sus)* apo-unsus*) for statistical analysis. The values were recalculated since multiple (replication) measurements were done. Both absolute as well as recalculated values for APO-SUS and APO-UNSUS rats were statistically analyzed by means of an independent samples t-test or one-sample t-test respectively per brain structure and per antibody (DAT/ NET/ SERT). For illustrative purposes, the recalculated values are shown

(with APO-SUS set at 1; without SEM). All data were analyzed with SPSS 12.0.1 and a probability level of $p < 0.05$ was taken as statistically significant.

Results

Striatal DAT, NET and SERT levels

Immunoblots showed that the antibodies raised against the DAT, NET and SERT were bound to a band of approximatelly 90 kDA in the striatum. These immuno-reactive bands ran slightly higher than indicated by the manufacturer (around 85 kDA). A representative blot from the immunoblotting experiment for the expression of striatal DAT in APO-UNSUS and APO-SUS rats is shown in figure 1. Data analysis revealed that APO-UNSUS rats have significantly lower levels of DAT than APO-SUS rats do (t-test $p < 0.05$; figure 3). There was no difference in striatal NET and SERT levels between the two rat types.

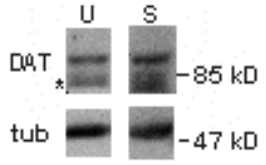


Figure 1*: A representative gel for DAT from striatal tissue of APO-UNSUS (U) and APO-SUS (S) rats. Staining for DAT revealed one background staining (marked by *)Top bands are DAT at around 85 kDA. β-tubulin was used for normalization and are located at around 50 kD. APO-UNSUS rats had signficantly less DAT expression than APO-SUS rats did.*

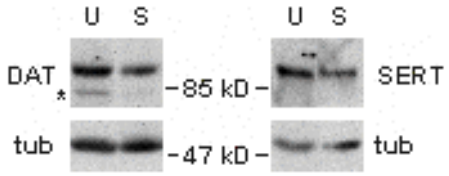


Figure 2*: A representative gel for DAT(left side) and SERT (right side) from hippocampal tissue of APO-UNSUS (U) and APO-SUS (S) rats. Staining for DAT revealed one background staining (marked by *)Top bands are DATor SERT at around 85 kDA. β-tubulin was used for normalization and are located at around 50kD. APO-UNSUS rats had signficantly greater DAT and SERT expression than APO-SUS rats did.*

Hippocampal DAT, NET and SERT expression

As with the striatum, the immunoblots of the hippocampus showed that the antibodies raised against the DAT, NET and SERT were bound to a band of around 90 kDA. A representative blot from the immunoblotting experiment for the expression of DAT and SERT in the hippocampus is shown in figure 2. Data analysis revealed that APO-SUS rats rats have significantly lower levels of DAT and SERT levels than APO-UNSUS rats do (t-test $p < 0.05$; figure 4). There was no difference in NET levels between the two rat types.

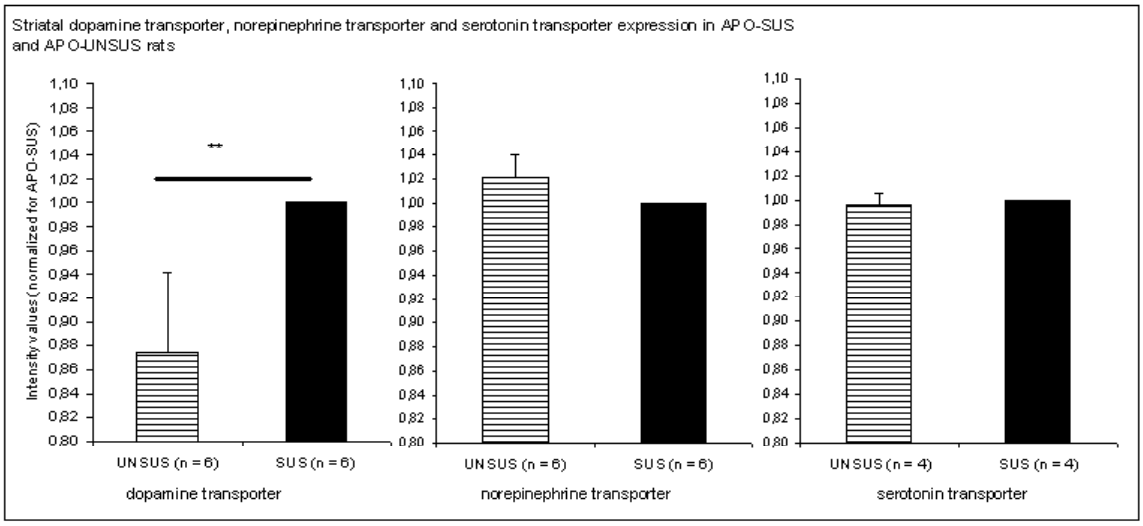


Figure 3: *Western blot analysis of the striatal dopamine transporter, norepinephrine transporter and serotonin transporter expression in APO-SUS (filled columns) and APO-UNSUS (arced columns) rats. Data were normalized toward β-tubulin and APO-SUS rats are expressed as arbitrary units (band intensity). APO-UNSUS rats had significantly less DAT immunoreactivity in the striatum than APO-SUS rats did (t-test p < 0.05 marked by *). There were no differences in NET and SERT immunoreactivity between the two rat types.*

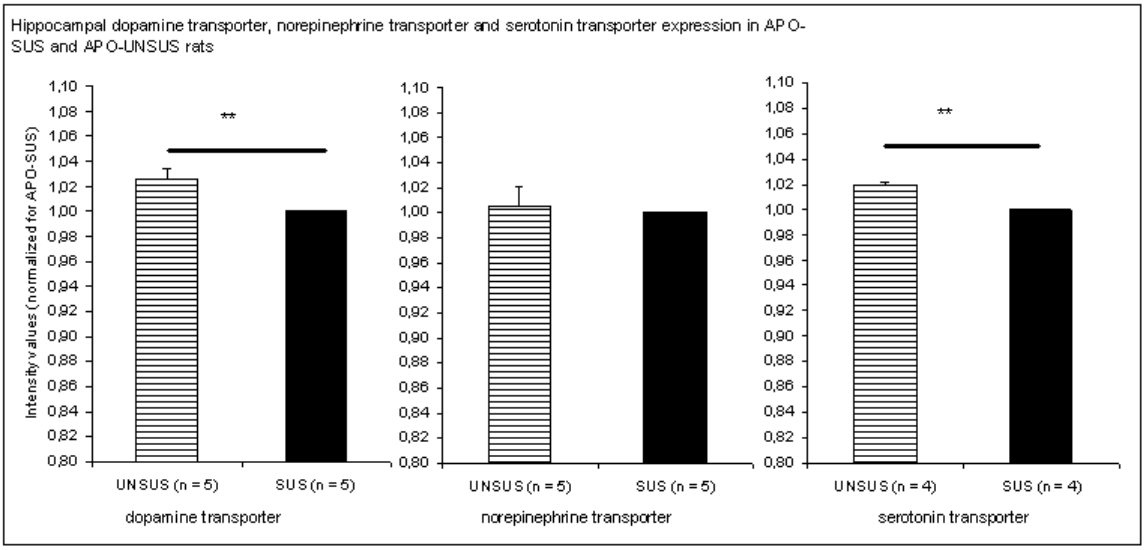


Figure 4: *Hippocampal dopamine transporter, norepinephrine transporter and serotonin transporter expression in APO-SUS (filled columns) and APO-UNSUS (arced columns) rats. Data were normalized toward β-tubulin and APO-SUS rats are expressed as arbitrary units (band intensity). APO-UNSUS rats had slightly (significantly different) more DAT and SERT immunoreactivity in the hippocampus than APO-SUS rats did (t-test p < 0.05 marked by *). There were no differences in NET immunoreactivity between the two rat types.*

Discussion

The present study was done to determine the level of dopamine, noradrenaline, and serotonin transporters in the genetically selected non-challenged APO-UNSUS and APO-SUS rats in two distinct structures, namely the striatum and hippocampus. We showed that

non-challenged APO-UNSUS rats have significantly lower levels of DAT in the striatum than non-challenged APO-SUS rats do. On the other hand, non-challenged APO-UNSUS rats had slightly higher, significantly different, levels of DAT and SERT in the hippocampus than non-challenged APO-SUS rats did. There were no differences in striatal SERT, striatal NET, and hippocampal NET levels between the two rat types.

As stated in the introduction, cocaine exerts its actions primairly by blocking the dopamine transporters [99,333], resulting in an increased action of dopamine and subsequent reward sensation. If there are less dopamine transporters to block, less cocaine is necessary to exert an action. Thus, extracellular dopamine levels are already increased with lower doses of cocaine, resulting in an increase of cocaine intake by animals with less DAT. In other words, the noted differences in striatal DAT levels between non-challenged APO-UNSUS and APO-SUS rats helps to understand the earlier reported finding that non-challenged APO-UNSUS rats consume more cocaine, especially low doses, than non-challenged APO-SUS rats [319].

With increasing doses of cocaine, all dopamine transporters will be blocked, independent of striatal DAT levels. Thus, extracellular dopamine levels are increased in all animals, resulting in an increase of consumption by these animals. It is, therefore, expected that with increasing doses of cocaine, differences in cocaine consumption between APO-UNSUS and APO-SUS rats will disappear. Although this has not been investigated (yet), research with high responder and low responder to novelty rats (which share many features with APO-SUS and APO-UNSUS rats respectively [50]), has shown that differences in the consumption of cocaine between these rats disappeared with increasing doses of cocaine [191].

Although other studies have suggested a role for NET and SERT in directing cocaine consumption [135,288], we did not find any differences in NET and SERT levels between the high-consuming APO-UNSUS rats and the low-consuming APO-SUS rats. It is, however, important to realize that most studies pointing to a role for NET and SERT were done with knockout mice and may, therefore, reflect compensatory mechanisms rather than normal physiological processes.

Interestingly, hippocampal DAT levels are opposite to striatal DAT levels: APO-UNSUS rats have a slightly higher hippocampal DAT levels than APO-SUS rats. Moreover, APO-UNSUS rats also have slightly higher levels of hippocampal SERT than APO-UNSUS rats. The behavioural and/or neurochemical impact of higher DAT and SERT levels in the hippocampus of APO-UNSUS rats is, as yet, unknown.

The hippocampus is involved in, amongst others, the retrieval of (spatial) information, encoding of emotional memory, and the negative feedback of the HPA-axis via glucocorticosteroid receptors [12,49,210,217,252]. Moreover, a disturbance in hippocampal functioning is suggested to produce many schizophrenia-like symptoms (e.g. disrupted prepulse inhibition and latent inhibition) [49,252,281]. This disturbed hippocampal functioning is thought to result from a developmental neuropathology [40].

APO-SUS rats, but not APO-UNSUS rats, display many characteristic features of schizophrenia, including a diminished prepulse inhibition of the acoustic startle response and a diminished latent inhibition [84]. Moreover, APO-SUS rats also have a developmental retardation in comparison to APO-UNSUS rats [59].

Taken together, the small reduction in hippocampal DAT levels in APO-SUS rats could well be a reflection of this developmental retardation and by such play a role in the expression of schizophrenia-like symptoms.

As stated above, the hippocampus is also involved in the negative feedback of the HPA-axis via glucocorticosteroid receptors. This function is of special interest when examining the differences in hippocampal SERT levels between APO-SUS and APO-UNSUS rats. Research with SERT knockout (KO) mice has shown that these animals have an exaggerated response of the HPA-axis [307]. Like SERT-KO mice, APO-SUS rats have a disrupted HPA-axis regulation: i.e. APO-SUS rats have an increased corticosteroid feedback resistance [264], resulting in a prolonged stress-induced release of corticosterone and ACTH [265]. Research has shown that (hippocampal) serotonergic neurotransmission is involved in the regulation of the HPA-axis and vice versa [100,190,322]. These findings, thus, suggest that decreased hippocampal SERT levels in APO-SUS rats could be either a causative factor or an effect of the differences in HPA-axis regulation found between APO-SUS and APO-UNSUS rats.

Research with SERT-KO mice has also revealed that a lack of SERT throughout the brain can result in depression-like symptoms [183]. This finding is corroborated with several imaging studies revealing that patients with major depression have less SERT in the hippocampus, but not in striatal areas [224]. Other imaging studies have also shown that these patients have more DAT in the basal ganglia (of which the striatum is a part) [29]. Moreover, depressive patients often have a hyperreactive HPA-axis [7,305]. Given these data, it becomes interesting to investigate whether APO-SUS rats display more depressive-like symptoms than APO-UNSUS rats do.

In conclusion, the present study revealed that APO-UNSUS rats, in comparison to APO-SUS rats, have a lower DAT levels in the striatum, whilst the reverse holds true in the hippocampus for both DAT and SERT levels. This insight can help to understand at least some behavioural and pharmacological differences between both rat types.

Chapter 8

The amount of cocaine consumed by individually different rats is determined by reward sensation: a study using a conditioned place preferences set-up.

Abstract
Recently, our department has revealed that under challenged conditions the genetically selected apomorphine susceptible (APO-SUS) rats self-administer more cocaine than their counterparts the apomorphine unsusceptible (APO-UNSUS) rats. To investigate whether, and to what extent, APO-SUS and APO-UNSUS rats differentially experience reward from cocaine, both rattypes were tested for the occurrence of cocaine-induced place preference. Given the fact that the rewarding properties of the drug (reward sensation) direct the development of conditioned place preference, it is hypothesized that APO-SUS rats, in comparison to APO-UNSUS rats, have a greater capacity to display conditioned place preference if the latter takes place under challenged conditions. If, however, conditioned place preference takes place under non-challenged conditions, it is hypothesized that APO-SUS rats, in comparison to APO-UNSUS rats, have a smaller capacity to display conditioned place preference. The data clearly show that challenged APO-SUS rats, but not challenged APO-UNSUS rats, had a preference for the cocaine-paired compartment. APO-UNSUS rats never showed place preference. On the basis of these data, it is concluded that challenged APO-SUS rats experience more reward from cocaine than their counterparts do, both during cocaine-induced place preference and during cocaine self-administration.

Introduction

Recently, our department has shown that the genetically selected apomorphine susceptible (APO-SUS) rats self-administer more cocaine under challenged conditions than apomorphine unsusceptible (APO-UNSUS) rats, whilst the reverse is true under non-challenged conditions [319]. The question that arose from that research is why one rat type consumed more cocaine than the other rat type did.

The motivation (willingness) to self-administer more or less cocaine is said to be determined by reward [166]. Some researchers have stated that consuming more of an addictive substance indicates that this substance has a high rewarding value [144,150]. Because challenged APO-SUS rats consume more cocaine than APO-UNSUS rats do, the above-mentioned notion implies that challenged APO-SUS rats experience a greater reward value when consuming cocaine than challenged APO-UNSUS rats. On the other hand, one can argue that cocaine itself has a low rewarding value and that consumption needs to increase in order to obtain the desired level of reward [330,337]. This notion implies that challenged APO-SUS rats experience a smaller reward value when consuming cocaine than challenged APO-UNSUS rats.

Currently, many hypotheses describe reward sensation as a modulatory mechanism to determine the amount of a drug consumed (for an overview of current hypotheses, see [20]). For instance, the allostasis hypothesis states that when an animal consumes cocaine, the 'status' of the animal is altered due to both positive (snowballing effect) and negative feedback (avoiding negative effects) responses. If enough cocaine is taken, the shift will result in an increase of cocaine consumption [6]. The opponent drive concept states almost the same, namely that a stimulus (like cocaine), if strong and prolonged, activates a hedonic reaction as well as an opponent process of opposite hedonic value; a process which is necessary to bring the animal back to a neutral affective balance [90]. With a prolonged intake of cocaine the pleasant effect is reduced whilst the unpleasant effect is not or strenghtened (tolerance). The animal will therefore increase its intake to maintain the rewarding/ pleasant effects. The incentive motivational/ incentive salience concept is based on the formation of an association between the 'true' hedonic reward and a predictive reward stimulus. Due to this formation, the neutral stimulus will become an incentive motivational stimulus [21,254]. After prolonged use, the incentive motivational stimulus can be sensitized, resulting in abnormal drug-seeking and drug-taking behavior, even when the hedonic value of the drug is not altered (incentive sensitization) [255].

Taken together, these hypotheses all state that an animal consumes more cocaine (or any other drug of abuse) after prolonged exposure due to a 'loss' in either reward sensation or a sensitization of drug-seeking behavior (adaptive strategy) [6,20,167]. Cabanac and Toates have argued that the reward (pleasure) of a stimulus is dependent on the physiological state of the animal, although the sensory quality of the reward is unaltered, a phenomemon which is called alliesthesia [30,31,308]. It is quite likely that, in the case of individually different rat types, this state (starting point) is different.

One technique that is available to study individual differences in 'startingpoints', is the conditioned place preference technique. This technique is based on the formation of an association of the rewarding effects of a drug with a specific environment [14]. Once this

association is established, animals are allowed to freely explore the box. If an animal spends more time on the previously drug-paired side, the drug is said to have rewarding properties [14]. In case a particular drug treatment results in conditioned place preference in one type of rats, but not in the other type of rat, one can conclude that the former rat experiences a greater reward sensation than the latter rat.

Most cocaine-induced place preference research investigating individual differences in reward sensation has been done with high and low responder to novelty rats [119,173,289]. Yet, even though the novelty response seems to be a good predictor of psychostimulant self-administration [153], the association between the novelty response and psychostimulant-induced place preference is less evident. For instance, some studies have found a correlation between a high novelty response and psychostimulant-induced place preference [163], whilst others have not [173]. It is possible that this discrepancy is due to interference of novelty-induced locomotor activity on the induction and/or expression of conditioned place preference. For instance, if animals have a different exploration of new environments (like the high responder and low responder to novelty do), it is possible that some compartments in the conditioned place preference box may be more novel than others, which could lead to 'false' psychostimulant-induced place preference [14,22]. However, this 'novelty-seeking' problem as well as some other problems, like drug-induced disturbances in compartment familiarization (a process in which cocaine disturbes contextual recognition of a compartment – state dependent learning [14,178]), can be overcome by adjusting the experimental procedure as described by Bardo, Klebaur and Shimosato [14,163,289].

Our department has recently shown that challenged APO-SUS rats consume more cocaine than challenged APO-UNSUS rats, whereas non-challenged APO-SUS rats consume less cocaine than non-challenged APO-UNSUS rats [319]. Because it was our intention to understand these type-specific differences in term of type-specific differences in reward sensation, it became necessary to establish whether the acquisition and display of conditioned place preference takes place under challenged or non-challenged conditions. For that reason, we analyzed to what extent habituation occurs throughout the test procedure.

Given the fact that the rewarding properties of the drug (reward sensation) direct the development of conditioned place preference, it is hypothesized that APO-SUS rats, in comparison to APO-UNSUS rats, have a greater capacity to display conditioned place preference if the latter takes place under challenged conditions. If, however, conditioned place preference takes place under non-challenged conditions, it is hypothesized that APO-SUS rats, in comparison to APO-UNSUS rats, have a smaller capacity to display conditioned place preference.

Materials and Methods

Animals

Adult male Wistar rats from the 20th generation (replicated line) of APO-SUS and APO-UNSUS rats were obtained from the Central Animal Laboratory (CDL), Radboud University Nijmegen, The Netherlands [86]. These rats have been selected on the basis of their

behavioral response to a single dose of the partial dopaminergic D1/D2 agonist apomorphine, which, by selective breeding, has resulted in the apomorphine susceptible (APO-SUS) and the apomorphine unsusceptible (APO-UNSUS) ratline [50]. Animals were housed two to three per cage (Macrolon® type 3; 42 x 26 x 20 cm) in temperature-controlled rooms (20 ± 2° C) with a standard 12/12-h day/night-cycle (lights on 7.00 am) and food and water available *ad libitum*. Each experiment was performed with drug and experimentally naïve rats. All experiments were performed in accordance with institutional, national, and international guidelines for animal care and welfare.

Drugs

Cocaine hydrochloride (Pharmacy, Radboud University Nijmegen, the Netherlands) was dissolved in 0.9% sodium chloride. Animals were injected intraperitoneally with 0 (control), 5 or 10 mg/kg cocaine in a volume of 1 ml/kg. Each animal was injected approximately 1 minute before placement in the conditioned place preference box.

Apparatus

Place conditioning was performed in a box with three compartments [14]. The two outer compartments (L 30 x W 28 x H 30 cm) were visually distinct from one another by means of either horizontally or vertically black and white stripes (2 cm) on the wall and by means of either smooth or rough floor-texture. The middle compartment (L 10 x W 28 x H 30 cm) had a smooth floor-texture and black walls. All compartments could be separated by means of sliding doors. The third (inner) compartment was added to overcome the problems with novelty seeking [14]. Since animals are not allowed to explore this compartment as much as the two other compartments (see below), this compartment will be the most novel space during testing and subsequently the animals will spend more time and/or travel a greater distance in this compartment if novelty plays a role [14].

As stated in the introduction, it became necessary to establish whether the acquisition and display of conditioned place preference took place under challenged or non-challenged conditions. For that reason, we analyzed locomotor acivity during all phases of the conditioned place preference paradigm by means of the program OpenField© . This program works on the principal of contrast (white rat vs. dark surrounding (or vice versa)) and determines the gravity point of the rat, which can subsequently be traced. This allows for an accurate measurement of displacement (distance traveled and time spent) and entries (crossing into one of the three compartments) into each of the three compartments [50,178].

Conditioned Place Preference

All animals were individually housed, handled and injected daily with 1 ml/kg saline IP to reduce injection stress. After 3 days of handling and injecting, animals were subjected to the conditioned place preference test (figure 1). All testing took place between 10:00 and 14:00. In short, animals were first allowed to freely explore the box for 20 minutes. During this preconditioning session (PC), possible compartment-preferences were determined. If the difference between the time spent in one of the two outer compartments was more than 60

seconds (5% of the time), an animal was said to have a preference; these animals were given cocaine in the non-preferred compartment. The remainder of the rats rats were randomly assigned to receive cocaine in one of the two outer compartments.
One day after PC, animals were subjected to 4 conditioning sessions in the outer compartments for 30 minutes each. Conditioning sessions (C1-C4) were done on 4 consecutive days. Animals received an injection of saline just before the first and third session and were placed in one of the two outer compartments. Just before the second and fourth session, animals received an injection of cocaine in the other outer compartment. After the cocaine sessions, the box was turned round 180° to prevent spatial recollection of the cocaine-paired compartment [14].
Twenty-four hours after conditioning, animals were again allowed to freely explore the complete box for 20 minutes to determine if conditioning had resulted in place preference of either the saline-paired or the cocaine-paired compartment (T1). One day after this test, animals were again allowed to explore the box for 20 minutes (T2). Just before this second test, animals were injected with the appropriate dose of cocaine. This test phase was conducted to control for the possibility that, during the conditioning session, cocaine might have altered familiarization. If familiarization plays a role, distance traveled and/or time spent in the cocaine-paired compartment during the 2nd testphase is less than that of the 1st testphase [14].

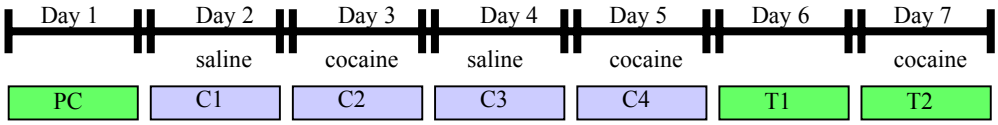


Figure 1: *The conditioned place preference protocol. At day 1 rats were preconditioned (free exploration for 20 minutes), followed by four conditioning sessions (saline, cocaine, saline, cocaine; each 30 minutes) from day 2 to day 5. Testing was done at day 6 and day 7 (20 minutes). Just before placing the rat in the box at day 7 an additional cocaine injection was given.*

Data analysis in general
Only bodyweights assessed at the beginning of the experiment were analyzed by means of a two-way ANOVA with the factor dose and genotype, followed by a post-hoc Bonferroni on dose and genotype. A probability level of $p < 0.05$ was taken as statistically significant.
The conditioned place preference test resulted in two parameters per test phase, namely the distance traveled and time spent in the three compartments.

Challenged vs. non-challenged analysis
The distance traveled during PC was compared with the distance traveled during the 1st test phase (T1) for both APO-SUS and APO-UNSUS rats and per dose of cocaine by means of a paired samples t-test, in order to establish whether or not animals habituated during the experiment.

Place preference analysis

The distance traveled and time spent in each compartment during PC, T1 and T2 was expressed as a percentage of the total distance or time. This was done to compare the different phases of the experiment with one another. Data were subsequently analyzed per genotypes (dose-effect) by means of a two-way ANOVA with the factor session (PC, T1 and T2) as the repeated measure and the factor dose, followed by a post-hoc Bonferroni on session. Subsequently, the preconditioning session was compared with the 1st testphase by means of a paired sample t-test to investigate whether or not place preference had occurred. The 1st testphase was also compared with the 2nd testphase by means of a paired samples t-test

Total distance traveled analysis

The distance traveled during PC between APO-SUS and APO-UNSUS rats was analyzed by means of a two-way ANOVA with the factor dose and genotype, followed by a one-way ANOVA on dose or genotype where appropriate.

To examine the effectiveness of the dose of cocaine used during conditioning, the data from the four sessions were analyzed using a three-way ANOVA with the factor sesion (C1-C4) as the repeated measure and the factors dose and genotype. The data were subsequently analyzed by means of a two-way ANOVA with the factor session as the repeated measure and the factor genotype (APO-SUS vs. APO-UNSUS). The data were also split up per genotype and analyzed by means of a two-way ANOVA with the factor session as the repeated measure and the factor dose, followed by a post-hoc Bonferroni on dose. Data from the effective dose were analyzed per genotype by means of a paired samples t-test (C1-C2, C2-C3, C3-C4) to determine if animals differed in the distance traveled after each injection. The distance traveled during the two test phases was first analyzed by means of a three-way ANOVA with the factor session (T1-T2) as the repeated measure and the factor dose and genotype. Data were then analyzed only for the first test phase (see results) by means of a one-way ANOVA per dose of cocaine (APO-SUS vs. APO-UNSUS rats) and per genotype (dose-effect), followed by an independent samples t-test were appropriate.

Results

In general

During preconditioning, less than 10% of the animals had an initial compartment preference (4 out of 40 animals). There was no difference in the amount of APO-SUS or APO-UNSUS rats that had this initial compartment-preference. There were no differences in bodyweight between the APO-SUS rats and the APO-UNSUS rats at the beginning of the conditioned place preference experiment (two-way ANOVA: $F_{(5,41)} = 1.6$ $p = 0.1$; table 1).

Table 1: *Bodyweight of animals at the beginning of the experiment for APO-SUS and APO-UNSUS rats. No differences in bodyweight existed between APO-SUS and APO-UNSUS rats, and between the different doses of cocaine.*

	APO-SUS	APO-UNSUS
0 mg/kg	296 ± 9.5 (n = 7)	310 ± 7 (n = 8)
5 mg/kg	313 ± 6.2 (n = 6)	297 ± 4.8 (n = 6)
10 mg/kg	292 ± 4.7 (n = 8)	308 ± 5.8 (n = 7)

Challenged vs non-challenged analysis

To investigate whether APO-SUS or APO-UNSUS rats were in a challenged or non-challenged state during the conditioned place preference protocol, the distance traveled during preconditioning was compared with the distance traveled during the first testphase by means of a paired samples t-test. This revealed that the APO-SUS and APO-UNSUS rats that had received 0 mg/kg cocaine did reduce the distance traveled (paired-samples t-test $p < 0.05$). The APO-SUS and APO-UNSUS rats that had received 5 or 10 mg/kg cocaine did not reduce the distance traveled (paired samples t-test $p > 0.05$). Henceforward, the 5 and 10 mg/kg groups did not habituate to the conditioned place preference protocol (figure 2).

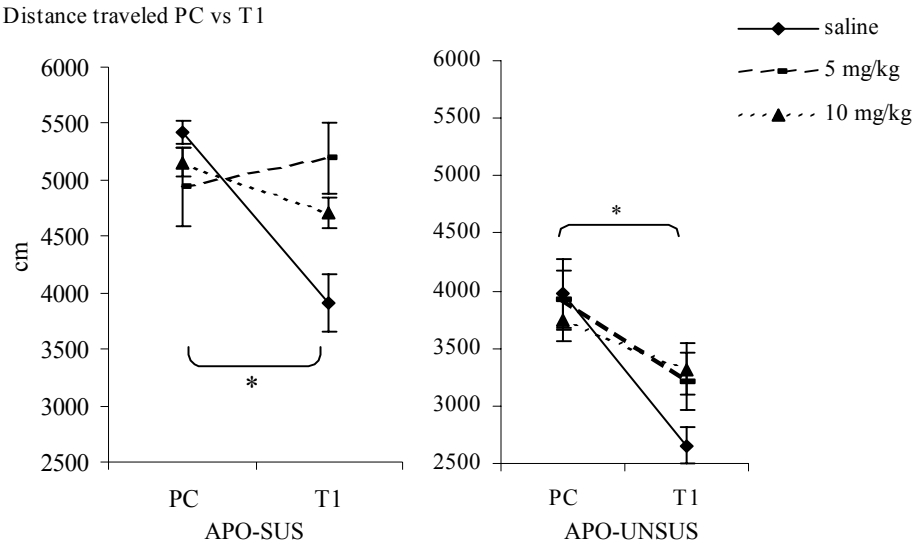
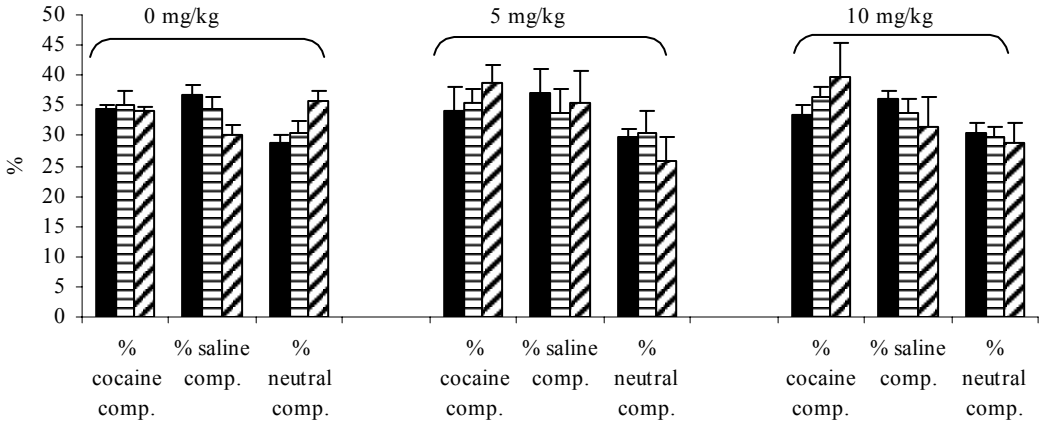


Figure 2*: Distance traveled during preconditioning (PC) and the first testphase (T1) for APO-SUS (left side) and APO-UNSUS (right side) rats. APO-SUS rats always (during PC and T1) traveled a greater distance than APO-UNSUS rats (ind samples t-test $p < 0.05$). APO-SUS and APO-UNSUS rats that had received saline during conditioning habituated to the protocol as can be seen by a reduction in the distance traveled (paired sample t-test $p < 0.05$, marked by *). APO-SUS and APO-UNSUS rats that had received either 5 or 10 mg/kg cocaine during conditioning did not significantly reduced the distance traveled from PC to T1 (paired samples t-test $p > 0.05$).*

Place preference analysis

The APO-SUS rats that had received 10 mg/kg cocaine did show place preference for the cocaine-paired compartment during the test phase. These rats spent significantly more time and traveled a significantly greater distance in the cocaine-paired compartment, combined with a significant reduction in the time spent and traveled distance in the saline-paired compartment (time saline comp. $F_{(2,23)} = 18.8$ $p < 0.01$; distance saline comp. $F_{(2,23)} = 10.9$ $p < 0.01$; time cocaine comp. $F_{(2,23)} = 7.1$ $p < 0.01$; distance cocaine comp. $F_{(2,23)} = 7.4$ $p < 0.01$: figure 3b and figure 4b). There was no change in the time spent and distance traveled in the neutral compartment. When comparing the distance traveled/time spent during the preconditioning phase with the test phase, the data revealed that cocaine-induced conditioned place preference had occurred in the APO-SUS rats that has received 10 mg/kg (paired-samples t-test $p < 0.05$).

A **Distance traveled by APO-UNSUS rats treated with 0, 5, and 10 mg/kg cocaine; comparing PC (filled), T1 (horizontally striped), and T2 (diagonally striped)**



B **Distance traveled by APO-SUS rats treated with 0, 5, and 10 mg/kg cocaine; comparing PC (filled), T1 (horizontally striped), and T2 (diagonally striped)**

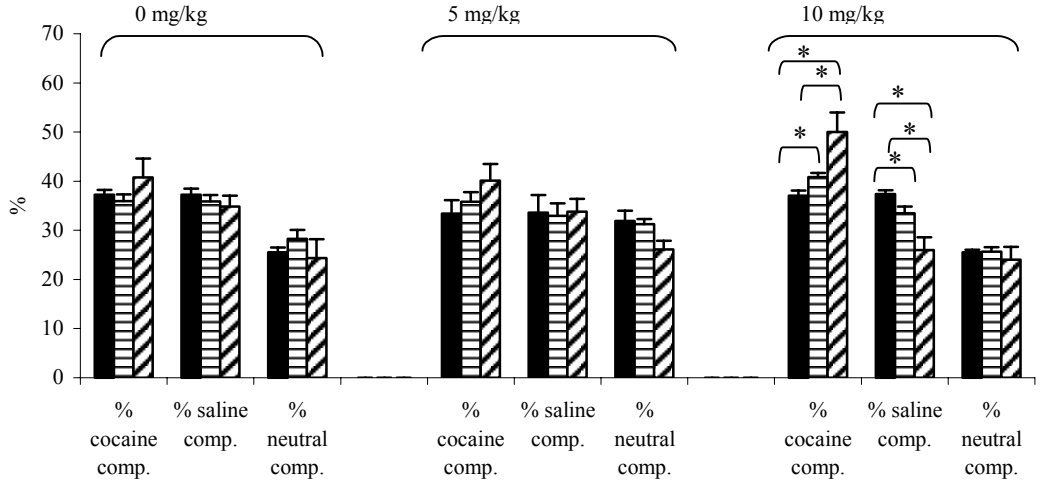
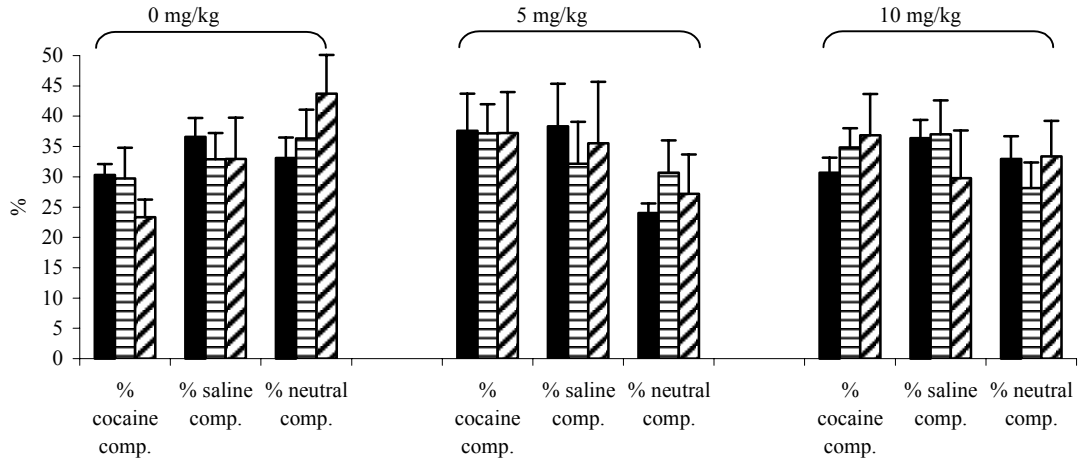


Figure 3a and 3b: *Traveled distance PC, T1 and T2 for either APO-UNSUS rats (A) and APO-SUS rats (B) that were treated with 0, 5 or 10 mg/kg cocaine IP during conditioning. APO-UNSUS rats never showed conditioned place preference. However, analysis by means of a one-way ANOVA on session (PC, T1, T2) revealed that the APO-SUS rats treated with 10 mg/kg cocaine during conditioning traveled a greater distance in the cocaine-paired compartment and traveled a smaller distance in the saline-paired compartment (distance saline comp.* $F_{(2,23)} = 10.9$ $p < 0.01$; *distance cocaine comp.* $F_{(2,23)} = 7.4$ $p < 0.01$*). Further analysis by means of an independent samples t-test revealed that from PC to T1 and from T1 to T2 APO-SUS rats increased the distance traveled in the cocaine-paired compartment progressively more ($p < 0.05$; marked by a star). The distance traveled was reduced in the saline-paired compartment in the same, inverted, matter as the cocaine-paired compartment ($p < 0.05$; marked by a star). The distance traveled in the neutral compartment remained unaltered.*

Time spent by APO-UNSUS rats treated with 0, 5, and 10 mg/kg cocaine; comparing PC (filled), T1 (horizontally striped), and T2 (diagonally striped)



Time spent by APO-SUS rats treated with 0, 5, 10mg/kg cocaine; comparing PC (filled), T1 (horizontally striped), and T2 (diagonally striped)

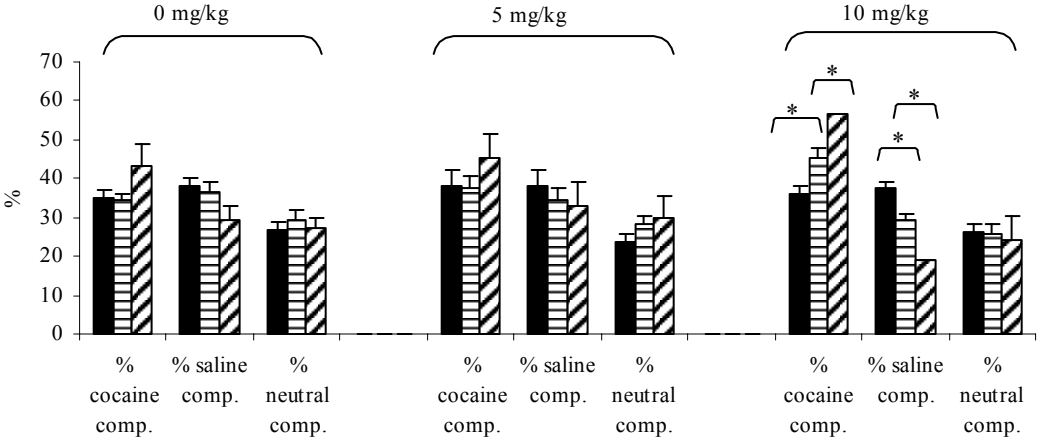


Figure 4a and 4b: *Time spent during PC, T1 and T2 for either APO-UNSUS rats (A) and APO-SUS rats (B) that were treated with 0, 5, and 10 mg/kg cocaine IP during conditioning. APO-UNSUS rats never showed conditioned place preference. However, analysis by means of a one-way ANOVA on session (PC, T1, T2) revealed that APO-SUS rats treated with 10 mg/kg cocaine during conditioning spent more time in the cocaine-paired compartment and less time in the saline-paired compartment (time saline comp.* $F_{(2,23)} = 18.8$ $p < 0.01$; *time cocaine comp.* $F_{(2,23)} = 7.1$ $p < 0.01$*). Further analysis by means of an independent samples t-test revealed that from PC to T1 and from T1 to T2 APO-SUS rats increased the time spent in the cocaine-paired compartment progressively more ($p < 0.05$; marked by a star). The time spent was reduced in the saline-paired compartment in the same, inverted, matter as the cocaine-paired compartment ($p < 0.05$; marked by a star). The time spent in the neutral compartment remained unaltered.*

When comparing T2 to T1, the APO-SUS rats traveled a greater distance and spent the same time in the cocaine-paired compartment, whilst the distance traveled and the time spent in

the saline-paired compartment were further reduced (paired samples t-tests $p < 0.05$). Thus, familiarization/ state-dependent learning did not play a role. The APO-SUS rats that had received 0 or 5 mg/kg cocaine did not show place preference for any of the three compartments (figure 3b and 4b). None of the APO-UNSUS rats showed conditioned place preference (figure 3a and 4a).

Total distance traveled

a. Preconditioning

The APO-SUS rats always traveled a greater distance than the APO-UNSUS rats (two-way ANOVA: genotype $F_{(1,36)} = 61.85$ $p < 0.05$; figure 2). There was no difference in the total distance traveled between the APO-SUS rats or between the APO-UNSUS rats that received different doses of cocaine (two-way ANOVA: dose $F_{(2,36)} = 2.06$ $p = 0.1$).

b. Conditioning

A three-way ANOVA revealed that the different doses of cocaine were effective in both lines (genotype x dose x session interaction $F_{(6,108)} = 2.6$ $p < 0.05$; figure 5). Subsequent analysis by means of a two-way ANOVA revealed that the APO-SUS rats overall (independent of the dose of cocaine) traveled a greater distance than the APO-UNSUS rats (genotype $F_{(1,40)} = 19.6$ $p < 0.05$). Both the APO-SUS and APO-UNSUS rats responded equally to the dose of 0 mg/kg and the dose of 5 mg/kg cocaine, but all animals reacted stronger to the dose of 10 mg/kg cocaine (dose APO-SUS: dose $F_{(1,18)} = 93$ $p < 0.05$; APO-UNSUS $F_{(1,18)} = 58.3$ $p < 0.05$; posthoc Bonferroni 0 = 5 mg/kg, 0 ≠ 10 mg/kg and 5 ≠ 10 mg/kg). All rats, both APO-SUS and APO-UNSUS, increased the distance traveled when injected with cocaine and decreased the distance traveled when injected with saline (paired-samples t-test per genotype $p < 0.05$). The first injection of 10 mg/kg cocaine equally increased the distance traveled by APO-SUS and APO-UNSUS rats (APO-SUS: 142 ± 19%, APO-UNSUS: 160 ± 24%, n.s. different).

c. Test phases

There was a significant difference in the distance traveled between either APO-SUS or APO-UNSUS rats or between the three doses (two-way ANOVA: genotype $F_{(1,36)} = 47.8$; dose $F_{(2,36)} = 10.9$ $p < 0.05$). Further analysis revealed that the APO-SUS rats, as with the preconditioning phase, always traveled a greater distance than the APO-UNSUS rats (one-way ANOVA: 0 mg/kg: $F_{(1,14)} = 17$ $p < 0.01$; 5 mg/kg $F_{(1,11)} = 26.3$ $p < 0.01$; 10 mg/kg $F_{(1,14)} = 34.4$: figure 2 and table 2). There was no difference in the distance traveled between the two test phases (two-way ANOVA: session $F_{(1,36)} = 2.1$ $p = 0.15$).

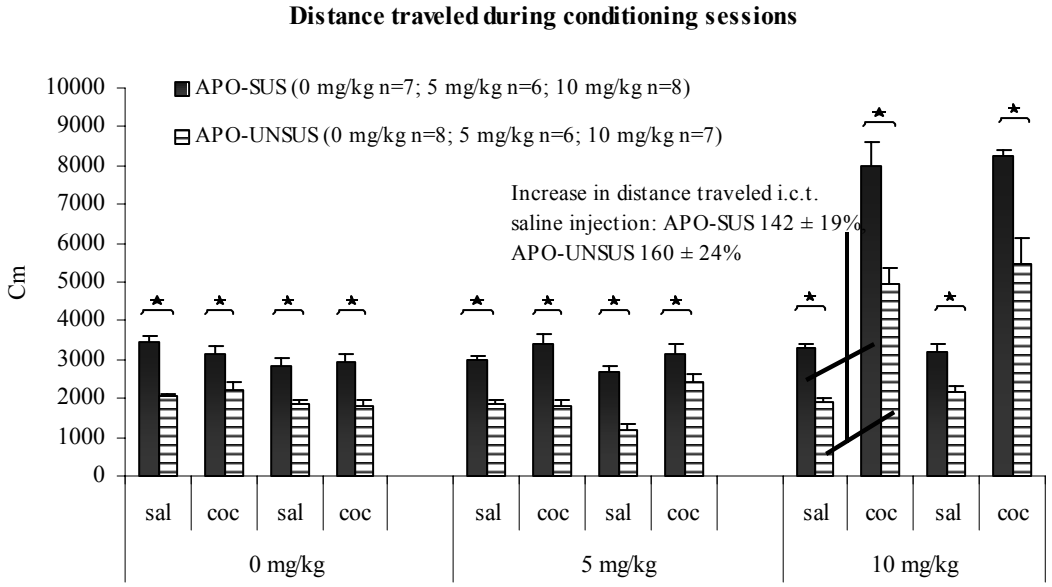


Figure 5: *Traveled distance during conditioning sessions. APO-SUS animals always traveled a greater distance than APO-UNSUS rats (0 mg/kg: genotype $F_{(1,13)} = 69.2$ $p < 0.01$; 5 mg/kg: genotype $F_{(1,10)} = 44.1$ $p < 0.01$; 10 mg/kg: session-genotype interaction: $F_{(3, 39)} = 2.9$ $p < 0.05$). Further analysis by means of an independent samples t-test revealed that APO-SUS rats traveled a greater distance during each conditioning session (one-way ANOVA $p < 0.05$; marked by star). When comparing the effects of each dose of cocaine per genotype (either APO-SUS or APO-UNSUS), it was seen that APO-SUS and APO-UNSUS rats responded differently during the conditioning sessions for the different doses of cocaine. APO-SUS rats that received saline or 5 mg/kg cocaine did not differ from one another, whilst the 10 mg/kg cocaine group did. This same holds true for APO-UNSUS rats. When examining the response to the first 10 mg/kg cocaine injection, APO-SUS and APO-UNSUS rats increased their locomotor response in the same matter (142 ± 19% vs. 160 ± 24%).*

Table 2: *Distance traveled during test phase 2 for APO-SUS and APO-UNSUS rats (mean ± sem). APO-SUS rats traveled a larger distance then APO-UNSUS rats in the 0 and 5 mg/kg groups (independent samples t-test $p < 0.05$; marked by a star). Furthermore, APO-SUS rats that had received 5 and 10 mg/kg during conditioning traveled a greater distance than the group that had received 0 mg/kg (one-way ANOVA $p < 0.05$, marked by ##), and APO-UNSUS rats that had received 10 mg/kg during conditioning traveled a greater distance than the other two groups (one-way ANOVA $p < 0.05$, marked by ^^).*

	APO-SUS		APO-UNSUS	
0 mg/kg cocaine	3512 ± 347	*	2149 ± 247	##
5 mg/kg cocaine	4211 ± 170	*	2770 ± 313	^^
10 mg/kg cocaine	4854 ± 333		3932 ± 632	

Discussion

This research was conducted to answer the question whether, and to what extent, APO-SUS and APO-UNSUS rats that are known to differ in cocaine self-administration [319], experience more or less reward from cocaine. This study clearly shows that APO-SUS rats displayed

cocaine-induced conditioned place preference, whilst APO-UNSUS rats did not, suggesting that APO-SUS rats, but not APO-UNSUS rats, experienced reward from the injections of cocaine [14,314].

This study also shows that, the 'novelty-seeking' problem as well as some other problems, like drug-induced disturbances in compartment familiarization/ state dependent learning [14,178], can indeed be overcome by adjusting the experimental procedure as described by Bardo, Klebaur and Shimosato [14,163,289]. Thus, even though APO-SUS rats, like high responders to novelty rats [50], traveled a greater distance than APO-UNSUS rats throughout the experiment, there was no interference of this component with the occurrence of place preference [14,163,289]. This study is the first to examine the locomotor response during the conditioning sessions. Moreover, the data show that both APO-SUS and APO-UNSUS rats responded equally to each dose of cocaine, thereby ruling out the possibility that cocaine-induced differences in locomotion caused a difference in the occurrence of place preference. Some researchers have suggested that cocaine can decrease familiarization of the cocaine-paired compartment [14,178], resulting in an apparent place preference. Finally, this study clearly shows that familiarization (or state-dependent learning) did not contribute to the occurrence of place preference, since the distance traveled and/or time spent in the cocaine-paired compartment during the 2nd testphase were equal or greater than that of the 1st testphase [14]. Altogether, the influence of either novelty seeking, cocaine-induced differences in locomotion, or differences in familiarization, can be excluded as possible contributing factors to the differences found between APO-SUS and APO-UNSUS rats.

This study was conducted to investigate whether the type-specific differences found in cocaine self-administration were a reflection of type-specific differences in reward sensation. We have recently found that APO-SUS rat self-administer more cocaine under challenged conditions than APO-UNSUS rats, whilst the reverse is true under non-challenged conditions [319]. As stated in the introduction, some researchers argue that consuming more of an addictive substance indicates that this substance has a high rewarding value [144,150], whilst others argue that consuming more of an addictive substance indicates that this substance has a low rewarding value [330,337]. The present study clearly shows that APO-SUS rats experienced reward, whilst the APO-UNSUS rats did not. Because both rattypes did not reduce the distance traveled throughout the experiment (see results), we can conclude that these rats were in a challenged state throughout this experiment. This indicates that challenged APO-SUS rats experience reward from cocaine, whilst challenged APO-UNSUS rats do not experience reward from cocaine, both during cocaine-induced place preference and during cocaine self-administration. It can therefore be concluded that consuming more of an addictive substance is indeed a reflection of its high rewarding value [144,150] .

The question remains why APO-SUS rats showed place preference while APO-UNSUS rats did not? In order to answer this question, it is important to understand that the neurochemical status of several brain systems of these animals is highly dependent on the environmental setting (challenged vs. non-challenged) [52,264]. Given the finding that these rattypes did not habituate (see results), these rats were in a challenged state throughout this experiment. Research with these animals has shown that challenged APO-SUS rats are

characterized by a higher stress-induced dopaminergic activation of the ventral striatum [88], more striatal D2 receptors [267], more mRNA for striatal D1 receptors than APO-UNSUS rats [267], a functionally higher noradrenergic activity in the ventral striatum [81], and higher levels of stress-induced corticosterone and ACTH [264]. Conditioned place preference research has suggested a strong correlation between high amounts of striatal dopamine and a high amount of the dopaminergic D1 and D2 receptors and cocaine-induced place preference [16,204,248,274,328]. Recently, Drouin et al have also revealed that mice without alpha-1 adrenergic receptors do not show cocaine-induced place preference [76], suggesting some role for noradrenaline in the occurrence of place preference. Evidence for the involvement of corticosterone in cocaine-induced conditioned place preference is however scarce [62,152]. In view of these data, it is not surprising that challenged APO-SUS rats indeed showed cocaine-induced place preference.

It should be noted that this study only tested two doses of cocaine, namely 5 and 10 mg/kg cocaine. In this study the highest dose resulted in the occurrence of place preference in challenged APO-SUS rats, but not in challenged APO-UNSUS rats. It is not unlikely that with higher doses of cocaine, APO-UNSUS rats will also show conditioned place preference, since research has suggested a clear-cut dose-dependency [24,78,229].

The present study reveals that challenged APO-SUS rats also have a greater capacity to display conditioned place preference than challenged APO-UNSUS rats, indicating that challenged APO-SUS rats experience a higher reward sensation of cocaine than challenged APO-UNSUS rats. It, of course, remains to be seen whether APO-SUS rats will experience less reward in a non-challenged state than the non-challenged APO-UNSUS rats. Unfortunately, our current set-up of the conditioned place preference experiments does not allow testing under non-challenged conditions. However, since it is known that APO-SUS rats self-administer less cocaine under non-challenged conditions[319], it is predicted that APO-SUS rats under such conditions indeed experience less reward than their counterparts do. Further research is required to (in)validate this prediction. Such a shift in the value of reward has previously been described by Cabanac et al and is called “alliesthesia” (= change in sensation) [31,308]. In the case of APO-SUS and APO-UNSUS rats, this implies that the value of cocaine is high in APO-SUS rats when challenged, whilst it becomes low when APO-SUS rats are not challenged.

In conclusion, the present study shows that APO-SUS rats displayed cocaine-induced conditioned place preference, whilst APO-UNSUS rats did not. On the basis of these data, it is concluded that challenged APO-SUS rats experience more reward from cocaine than their counterparts do, both during cocaine-induced place preference and during the acquisition of cocaine self-administration.

Chapter 9

General discussion and conclusions.

In this thesis, we investigated the influence of (a) **genes,** (b) **early-life events**, and (c) **late environmental factors** in determining the susceptibility for alcohol and cocaine abuse in the apomorphine susceptible (APO-SUS) and unsusceptible (APO-UNSUS) rat model. Given the fact that apomorphine (un)susceptibility is a genetically heritable trait as well as the differences found in the number of *Aph-1b* genes, combined with the specific knowledge of the structure and function of the system that are considered to play a role in directing the onset of an addiction (see introduction), it was possible to study the influence of these three factors on directing the intake of alcohol and cocaine in APO-SUS and APO-UNSUS rats.

The experiments described in this thesis, together with previous research [294], have shown that APO-UNSUS rats consumed far greater amounts of alcohol than APO-SUS rats did under non-challenged conditions. In addition, under the same conditions, APO-UNSUS rats also more readily self-administered cocaine than APO-SUS rats did.
One specific point needs to be discussed in light of the intake of cocaine, namely the use of shaping during cocaine self-administration. In all experiments that were performed, none of the animals learned to self-administer cocaine before shaping had commenced. Several additional experiments (not mentioned in chapter 4) were performed to investigate whether APO-SUS and APO-UNSUS rats would learn to self-administer cocaine if given more time. However, none of the rats learned to self-administer cocaine according to the self-administration criterium. Shaping was, therefore, introduced into the protocol. The fact that rats failed to learn without shaping is due to the fact that no artificial 'boosts' were used like, for instance, food deprivation, priming injections, or (severe) stress. This is in contrast to a large portion of self-administration studies. There are studies that have not used these 'boosting' mechanisms, but these studies have used animals that lived in a reversed day-night rhythm. Thus, these animals were tested during the active-phase of their day (nighttime). We choose specifically NOT to reverse the day-night rhythm of APO-SUS and APO-UNSUS rats because this is known to interfere with the status of the dopaminergic and noradrenergic systems as well as the HPA-axis (unpublished). Our animals were, therefore, tested during the inactive-phase of their day (daytime). Shaping was, however, not always necessary. For instance, male APO-UNSUS rats that were prenatally exposed to cocaine did not need shaping in order to acquire self-administration. Prenatal cocaine exposure is known to enhance the responsiveness of the dopaminergic and serotonergic system [15,139,140,216,297], and by such resulting in an enhanced motivation to obtain cocaine. Thus, shaping by itself seems to enhance the motivation for the drug, which has been described as a boost of the 'emotional' memory of a cocaine delivery [247].
As stated above, the fact that APO-UNSUS rats consume more alcohol and cocaine under non-challenged conditions can be correlated to the specific features of the dopaminergic and noradrenergic system as well as the HPA-axis. For instance, APO-UNSUS rats are characterized by lower levels of TH immunoreactivity in the ventral striatum [87], a lower amount of dopaminergic D2 receptors in the striatum [267], a functionally higher noradrenergic activity in the ventral striatum [50,81], and higher levels of free plasma

corticosterone [264] than APO-SUS rats. Moreover, the experiment in chapter 7 revealed that APO-UNSUS rats also have less dopamine transporters in the striatum than APO-SUS rats.
The status of these features is genetically determined. Recently, it was found that one important genetic difference between these two rat types is the number of copies of the *Aph-1b* gene: APO-UNSUS rats have three copies, whilst APO-SUS rats have one or two copies [48]. This gene codes for a small protein that is a part of the γ-secretase complex, which cleaves substrates that are all critically involved in various aspects of development [170]. Therefore, the dosage imbalance of this gene between APO-SUS and APO-UNSUS rats may explain why these rats are so fundamentally different in those features that are correlated with a higher intake of alcohol and cocaine. It should, however, be noted that, so far, only one third of all genes have been screened. It is, thus, possible that other genes might also contribute to the differences found between APO-SUS and APO-UNSUS rats.
However, if the dosage imbalance in the *Aph-1b* gene is the causative factor for the difference in addictive behavior between APO-UNSUS and APO-SUS rats, it can be hypothesized that the amount of copies of this gene would be directly linked to the amount of alcohol or cocaine consumed. To that extent, the department of Psychoneuropharmacology has started to selectively breed rats with either 1, 2, or 3 copies of the *Aph-1b* gene. Moreover, by back-crossing with the original strains, animals have been obtained that are genetically identical, except for the *Aph-1b* locus. Preliminary research with these animals suggests that the dopaminergic D2 receptor sensitivity in the striatum can be directly linked to the amount of copies of this gene: rats with 1 or 2 copies of the *Aph-1b* gene have (like APO-SUS rats) a higher apomorphine-induced gnawing score than rats with 3 copies (unpublished data). Research is currently underway to determine whether rats with 2 copies have a different sensitivity than rats with 1 copy of the *Aph-1b* gene. In the near future, research will be conducted in order to establish whether the amount of *Aph-1b* copies is related to the amount of drugs consumed.
Even without knowledge of the exact function of this dosage imbalance in directing the intake of alcohol and cocaine, it is apparent that APO-SUS and APO-UNSUS rats differ in some features. This suggests that these features are (directly) responsible for the differences in alcohol and cocaine consumption. However, in order to (in)validate this suggestion, it becomes necessary to manipulate these neurochemical and endocrinological features either pharmacological or behavioral. It is known from the APO-SUS/ APO-UNSUS rat model that, after an environmental manipulation (stressful event), some of these features undergo a shift (figure 1).
Thus, the intake of alcohol and cocaine by APO-SUS and APO-UNSUS rats was investigated under challenged conditions. It was shown that, after a single stressful experience, APO-SUS rats showed a strong and prolonged increase in the intake of alcohol, whilst the APO-UNSUS rats only showed a small and short-lasting increase. Even more interesting, APO-SUS rats consumed considerable more cocaine than APO-UNSUS rats under challenged conditions. These findings, in combination with the known status of several features of these rats under non-challenged and challenged conditions, allow for the

conclusion that these features indeed direct the amount of alcohol and cocaine consumed (see figure 1).

Given the differences between APO-SUS and APO-UNSUS rats in the intake of alcohol and cocaine under challenged and non-challenged conditions, it became interesting to investigate whether the reward sensation was also different between these animals under these circumstances. It was shown that challenged APO-SUS rats, in line with their intake patterns of cocaine, experienced more reward from cocaine than APO-UNSUS rats (chapter 8). Because the administered cocaine interfered with habituation, we were not able to test under non-challenged conditions. It, therefore, remains to be seen whether APO-UNSUS rats will experience more reward than APO-SUS rats under non-challenged conditions. Given the intake pattern of alcohol and cocaine by APO-UNSUS rats under non-challenged conditions, it can be hypothesized that APO-UNSUS rats experience more reward than APO-SUS rats under these circumstances. Further research is required to (in)validate this hypothesis. Such a shift in the value of reward has previously been described by Cabanac et al and is called "alliesthesia" (= change in sensation) [31,308]. In the case of APO-SUS and APO-UNSUS rats, this implies that the value of cocaine is high in APO-SUS rats when challenged, whilst it becomes low when APO-SUS rats are not challenged.

	Normal/ non-challenged	Stress
Dopaminergic system:		
-D2 receptors DS	APO-UNSUS < APO-SUS	No measurement
-Dopamine release VS	APO-UNSUS = APO-SUS	APO-UNSUS > APO-SUS
-DAT levels Str	APO-UNSUS < APO-SUS	No measurement
Noradrenergic system:		
-functional activity VS	APO-UNSUS > APO-SUS	APO-UNSUS < APO-SUS
HPA-axis:		
-ACTH levels	APO-UNSUS = APO-SUS	APO-UNSUS < APO-SUS
-Corticosterone levels	APO-UNSUS > APO-SUS	APO-UNSUS < APO-SUS
Intake of cocaine	APO-UNSUS > APO-SUS	APO-UNSUS < APO-SUS
Intake of alcohol	APO-UNSUS > APO-SUS	APO-UNSUS < APO-SUS (increase)

Table 1: *overview of the specific neurobiological and endocrinological features of APO-SUS and APO-UNSUS rats and the impact of stress on these features, as well as the intake of alcohol and cocaine (VS = ventral striatum, DS = striatum, Str = VS + DS)*

As stated before, it was hypothesized that, after a stressor, APO-SUS rats would consume more alcohol and cocaine than APO-UNSUS rats. The experiments in chapter 2, however, showed that APO-SUS rats had a greater increase in intake of alcohol than APO-UNSUS rats in comparison to the intake under non-challenged conditions. Overall, on the other hand, APO-SUS and APO-UNSUS rats had the same intake of alcohol after stress. This, however, becomes understable in light of the protocol used: APO-SUS and APO-UNSUS rats were first allowed to habituate to the experimental set-up and were already allowed to consume some alcohol prior to the stressor. Thus, APO-UNSUS rats already consumed high amounts of alcohol, and APO-SUS rats did not.

After the stressor, APO-SUS rats increased their intake and matched levels of APO-UNSUS rats. A similar intake pattern was also found with cocaine self-administration when animals were first habituated and then stressed. Thus, both rat lines are equally vulnerable to consume high amounts, but the expression of this vulnerability is dependent on the environment.

Another factor that is suggested to alter the susceptibiliy for drug abuse, is **adverse early postnatal life events** [23,36,77,89,162,175,176,193,279,280,285,332] and **adverse prenatal life events**. Both events are known to alter the systems that are suggested to be involved in directing the intake of alcohol and cocaine, namely the dopaminergic system and the HPA-axis [11,134,136,140,158,159,209,216,218,278]. Thus, we investigated the effects of maternal deprivation at postnatal day 9 on self-administration behavior of APO-UNSUS rats, and the effects of repeated cocaine and saline injections *in utero* on self-administration behavior of APO-SUS and APO-UNSUS rats.

It was shown that maternally deprivation of APO-UNSUS pups altered the phenotypic expression of these rats, giving them the phenotypic appearance of APO-SUS rats when examining cocaine self-administration behavior. This phenotypic alteration is in line with the previous finding with apomorphine-induced gnawing response where maternally deprived APO-UNSUS rats also displayed APO-SUS-like gnawing behavior [86]. Although not examined in this thesis, it is known that cross-fostering of APO-SUS pups to an APO-UNSUS mother on postnatal day 1 alters the phenotypic expression of these rats, giving them the phenotypic appearance of APO-UNSUS rats. It can, therefore, be hypothesized that cross-fostering of APO-SUS pups will result in cocaine self-administration behavior resembling that of APO-UNSUS rats. This, off course, remains to be investigated.

Prenatal cocaine-exposure resulted in an interesting shift in cocaine self-administration behavior of APO-UNSUS rats: shaping, which proved to be essential in all other experiments, was not necessary in these rats. As stated before, this is suggested to be caused by an enhanced motivation for the drug. More interesting though, was the finding that prenatal stress (both cocaine and saline injections) was fatal for almost all the unborn fetuses of APO-SUS mothers: none of cocaine-treated females gave birth and only a small amount of the saline-treated females gave birth. Given the fact that the treatment commenced after day 8 of gestation, the low birth score must have been due to spontaneous abortions. This loss of offspring indicates that female APO-SUS rats were more susceptible to the injection procedure than female APO-UNSUS rats. This is in line with previous findings in male rats, showing that APO-SUS rats are more stress-sensitive than APO-UNSUS rats are [52,267]. In this respect, it is also interesting to note that APO-SUS and APO-UNSUS rats have different speeds of development, with APO-UNSUS rats developing faster than APO-SUS rats [59]. Although this study was done postnatally, it could imply that these animals also have a different developmental speed during gestation. Recent research with rats with 1, 2 copies or 3 copies of the *Aph-1b* gene has shown that the dosage effect is already present during gestation (unpublished data), suggesting that rats with 1 or 2 copies (like APO-SUS rats) indeed develop slower than rats with 3 copies (like APO-UNSUS rats) of the *Aph-1b* gene.

In that respect, an APO-SUS fetus is slightly retarded in its development in comparison to an APO-UNSUS fetus. In humans, severe stress during the first trimester of pregnancy can cause more harm to the developing fetus (or even death) than severse stress during the second or third trimester [320,339]: indicating that less-developed fetuses react stronger to prenatal stress than more-developed fetuses. This phenomenon could explain why the 'retarded' APO-SUS fetuses reacted stronger to prenatal stress than the APO-UNSUS fetuses, resulting in fetal death.

In all of the above-mentioned studies, only male rats were investigated. Actually, most animal research is conducted in male animals, mostly because the hormonal cycle of female animals makes them less desirable and more expensive to utilize. For instance, in rats, four females are necessary (for each day of the hormonal cycle) where one male can be used. Thus, female animals are often not incorporated into experiments. However, human diseases are not limited to men and some diseases are even more common in women. In the case of drug (ab)use, the number of women has increased rapidly over the last decade, and their intake patterns are often different from those of men [301]. Moreover, given the fact that some psychiatric diseases are more common in women and the co-occurrence of these diseases with addiction, it would be interesting to study the impact of sex differences in the onset of an addiction and the origination of individual differences.

The experiments in chapter 5 and 6 were, thus, designed to incorporate female APO-UNSUS rats into the cocaine self-administration protocol. These experiments revealed that female APO-UNSUS rats are faster at acquiring cocaine self-administration than male APO-UNSUS rats; female rats did not require shaping and started self-administration from day 1 onwards. This indicates that the motivational drive for the drug is greater in females than in males. Such an enhancement in motivation is considered to be caused by the presence of the female hormone estrogen [17,18,304]. The data, on the other hand, do suggest that the absolute reward value of the drug is not different between males and females, since both males and females consume equal amounts of cocaine once both had acquired cocaine self-administration. In this respect, it would be interesting to investigate the occurrence of cocaine-induced place preference in female rats.

Unfortunately, female APO-SUS rats were not incorporated in the experiment in chapter 5, simply because no female APO-SUS rats were available. A previous study by Sluyter et al has revealed that female APO-SUS rats consume more alcohol than male APO-SUS rats do [294]. This suggests that the individual differences found in the male population of rats are also present in the female population. Human research also indicates that there are differences amongst women when examing the amount of a drug consumed [194]: the emotional status of a woman (which is determined by, amongst others, female hormones) is important in regulating the rate of alcohol consumption and possibly other drugs of abuse.

Prenatal cocaine-exposure in female APO-UNSUS rats resulted in an increase in the amount of cocaine consumed (in comparison to saline-exposed and naïve female APO-UNSUS rats). Research indeed suggest that prenatal cocaine-exposure has far greater effects in female than in males [74,161,198,215]: this could explain why prenatal cocaine-exposure in female rats resulted in an upward shift in intake (the amount), and in male rats in a leftward shift (the rate).

Even though many aspects of the intake patterns of female rats still needs to be elucidated, it is apparent that female rats are extremely interesting to research with respect to the intake of drugs of abuse. Especially since these rats seem to have a greater motivational drive to obtain the drug as well as an increased response to the drug and stress.

The experiments performed in this thesis revealed that the amount of alcohol and cocaine consumed by APO-SUS and APO-UNSUS rats was, indeed, the result from a **complex interaction** between **genetic factors**, **early (pre- and postnatal) life events** and **late environmental factors**, which is in concordance with the 'three-hit model for psychopathology'. **Sex differences** also seem to play a role in determining the amount of a drug consumed.
The data in this thesis also revealed that an 'addictive personality' is not completely genetically programmed or environmentally deteremined: although the genetic basis of the animals determined the basis for the structure and function of the addiction-related (central nervous) systems, the environmental conditions during the development (prenatal, early postnatal, and adulthood) determined the actual outcome.

General discussion – clinical implications

As stated in the introduction, some individuals score high on harm avoidance and are characterized by a worried state of mind, fatigue, and depressive-like symptoms, and these individuals seem to need a drug to self-medicate some form of emotional distress [129]. Indeed, numerous studies have shown that psychiatric disorders are common among drug addicts [28,197]. For instance, data from the department of Health and Human Services USA has shown that around 4.2 million of the 20 million known drug addicts have a co-occurring serious mental illness (data from national survey 2003). It is however not clear if there is a common underlying neurochemical deficit that led to both diseases or if one of the two led to abnormalities mediating the other. On the other hand, these data also indicate that there is a large group of addicts without a co-occurring mental illness. This group of addicts is often considered to consist of individuals that score high on novelty-seeking/ sensation-seeking and are characterized by impulsive behavior, risk taking, and an excessive effort towards reward seeking. These individuals seem to need an extraordinary stimulation, like a drug of abuse, to feel arousal and happiness [45,129]. The two types of drug users have also been described in the two-affect model: drug use to alleviate an unpleasant mood (negative affect) or to induce a 'high' (positive affect) [13]. Thus, in a human population two distinct types of drug addicts can be defined: addicts with a co-occurring psychiatric disorder (type A), and addicts without a co-occurring psychiatric disorder (type B).

When comparing these two addictive types to APO-SUS and APO-UNSUS rats, one could argue that APO-SUS rats represent type B addicts and APO-UNSUS rats represent type A addicts, since APO-SUS rats are characterized by an increased novelty seeking component whilst APO-UNSUS rats are not [52]. However, APO-SUS rats also display schizophrenia-like

symptoms [84], which would classify them as the first category users. Given the fact that APO-SUS rats display these schizophrenia-like symptoms and consume drugs under challenged conditions, the suggestion can be made that stress is a common trigger for both drug addiction as well as psychiatric conditions. Moreover, novelty-seeking is a stress-induced behavior. Thus, a comparison can be made between APO-SUS rats and drug addicts with a co-occurring psychiatric illness (type A), whilst APO-UNSUS rats can be compared to drug addicts without a co-occurring psychiatric illness (type B). If APO-SUS rats represent type-A addicts, these rats would also have to display more depressive-like symptoms than APO-UNSUS rats.
Interestingly, the amount of dopamine and serotonin transporters in the hippocampus is significantly smaller in APO-SUS rats (in comparison to APO-UNSUS rats), which is suggested to be a reflection of reduced hippocampal functioning [40,281]. This reduced hippocampal functioning is suggested to produce many schizophrenia-like symptoms (e.g. disrupted prepulse inhibition) [40,49,217,252,281], symptoms that are shared by APO-SUS rats. Moreover, research has shown that a downregulation of the serotonergic system results in an exaggerated response of the HPA-axis [190,307] as well as the onset of depression-like symptoms [183]. Research with APO-SUS rats has already shown that these rats, indeed, have an increased corticosteroid feedback resistence [264], resulting in a prolonged stress-induced release of corticosterone and ACTH [265]. Imaging studies have shown that patients with major depression have less serotonin transporters in, amongst other, the hippocampus, but not in striatal areas[224]. Given the fact that depressive patients often have a hyperactive HPA-axis[7,305], like APO-SUS rats, the suggestion can be made that APO-SUS rats indeed will have to display more depressive-like symptoms than APO-UNSUS rats.
Thus, APO-SUS rats can be compared to drug addicts with a co-occuring psychiatric illness, whilst APO-UNSUS rats can be compared to drug addicts without a co-occuring illness. Further research will, however, be necessary to investigate and validate this comparison.

Following this comparison, type A-addicts seem to be highly vulnerable to environmental stress which can trigger drug abuse and possibly relapse, whilst type B-addicts do not seem to be affected by environmental stress. Type B-addicts, on the other hand, seem to have a genetic profile for drug addiction as can be seen in the expression of for instance DrD2 polymorphism in the case of alcohol addiction.

The experiments performed in this thesis have clearly shown that both rat types can have a high intake of alcohol and cocaine. Moreover, the experiments performed in chapter 2, 4, and 5 also clearly showed that both rat types could consume the same amount of alcohol and cocaine. However, the rate at which these types reached this amount was dependent on the environmental conditions during the intake of the drug. This indicates that both types are equally vulnerable to develop a drug addiction. However, the actual outcome of this vulnerability is dependent on the environment. Following the comparison between these two rat types and human drug addicts, this would suggest that all humans are susceptible to develop an addiction.

The difference in environmental dependency also has far reaching consequences for treatment strategies. Currently, there are only a few treatment strategies available and these do not always prove to be effective. This could be caused by this difference in environmental dependency. Indeed, type A-addicts would benefit from a behavioral approach, like for instance prevention of social, economical and environmental stress. On the other hand, type B addicts would not benefit from the behavioral approach as much as type A-addicts. It would, therefore, be desirable to focus on the personal history of a drug addict in order to individualize therapy. More insight into the personal history of a drug addict might even contribute to the development of effective preventive strategies for people that are considered to be at risk. For instance, the occurrence of the *Taq*1 A1 allele of the dopaminergic D2 receptor (DrD2) is often considered to predict whether or not an individual will become addicted. However, research by Madrid et al, has shown that this outcome is also dependent on the presence of a low socio-economical status, and not by the presence of this allele alone [189]. This suggests that people with this genotype benefit greatly from an improvement in their socio-economical status.

Finally, current animal research focuses mainly on males. Research with female animals is hardly being conducted with respect to drug addiction. Given the fact that the group of female addicts has grown over the last decade as well as the fact that their intake patterns are often different than those of male addicts, it would be desirable to conduct more research in this respect. Even more so, the data presented in this thesis revealed that female rats have a greater motivational drive to obtain an abusive substance as well as an increased response to this substance and (prenatal) stress. This enhancement of motivation could have far reaching consequences for treatment strategies of female addicts.

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Summary

The aim of this thesis was to investigate the influence of (a) **genetic background**, (b) **early-life events** (c) and **late environmental factors** in determining the susceptibility for alcohol and cocaine abuse in the rat model consisting of apomorphine unsusceptible (APO-UNSUS) and apomorphine susceptible (APO-SUS) rats.

The fact that apomorphine (un)susceptibility is a genetically heritable trait as well as the differences found in the *Aph-1b* gene, combined with the specific knowledge of the structure and function of those systems that are considered to play a role in directing the onset of drug abuse, namely the dopaminergic and to a lesser extent the noradrenergic system as well as the HPA-axis (see introduction), led us to investigate the role of **genetic background** in directing the intake of alcohol and cocaine. Previous research has shown that the status (high or low activity) of the above-mentioned systems is environmentally dependent in APO-UNSUS and APO-SUS rats.
Under non-challenged conditions, APO-UNSUS rats have the same dopaminergic activation of the ventral striatum, a functionally higher noradrenergic activity in the ventral striatum and higher plasma levels of free corticosterone than APO-SUS rats do. However, under challenged conditions their neurochemical and endocrinological status is altered. After a stressor, APO-UNSUS rats have a lower stress-induced dopaminergic activation of the ventral striatum, a functionally lower noradrenergic activity in the ventral striatum, and a smaller and shorter lasting increase in adrenocorticotropic hormone (ACTH) and corticosterone than APO-SUS rats do. This shift in the status of these systems allowed for an investigation of the role of **late environmental factors** in directing the intake pattern of alcohol and cocaine by APO-UNSUS and APO-SUS rats.
Because research has suggested a positive correlation between the activity of the dopaminergic and noradrenergic system as well as the HPA-axis and the intake of alcohol and cocaine, we hypothesized that, in view of the above-mentioned features of these systems, APO-UNSUS rats consume more alcohol and cocaine than APO-SUS rats under non-challenged conditions, which is in contrast to challenged conditions during which APO-UNSUS rats are hypothesized to consume less alcohol and cocaine than APO-SUS rats [53]. Given the fact that early life events are known to alter the dopaminergic and noradrenergic system as well as the HPA-axis reactivity in these animals, the impact of such **early-life events** on directing the intake of alcohol and cocaine could also be investigated. It was hypothesized that the intake of an abusive substance at adult age by APO-SUS and APO-UNSUS rats would alter after an adverse early life event, dependent on the type of early life event used [53].

Although a previous study has already shown that APO-UNSUS rats consume more alcohol than APO-SUS rats do under non-challenged conditions, the consumption of alcohol under challenged conditions still needed to be elucidated. This was of special interest since research has shown that the (re)activity of the HPA-axis is involved in directing the intake of alcohol. A recent human study has shown that the outcome of the *Taq*1 A1 allele of the dopaminergic D2 receptor (addicted to alcohol or not) was dependent on the socio-economical status of an individual: a low socio-economical status was suggested to produce,

amongst others, stress which was correlated to the outcome of the *Taq*1 A1 allele. This research, thus, suggested that the outcome of a specific genotype was dependent on stress exposure (**no stress vs. stress**). We, therefore, examined the role of stress on alcohol consumption in APO-UNSUS and APO-SUS rats (**chapter 2**).
By investigating the intake under non-challenged conditions and the impact of mild acute and mild sub-chronic stress on this intake, it was possible to investigate the interaction between the genetically determined features of these rats and the impact of the environment. It was shown that after a mild acute stressor, APO-UNSUS rats showed a small increase in consumption, whereas APO-SUS rats displayed a large and long-lasting increase in consumption. These experiments also showed that sub-chronic mild stressors, in comparison to the acute mild challenge, increased the intake of alcohol even further in APO-UNSUS rats, whilst the intake remained relatively stable in APO-SUS rats. These data showed that the amount of alcohol consumed by APO-UNSUS and APO-SUS rats was dependent on both the presence as well as the duration of stress, preceding and during alcohol consumption.

One of the questions that remained was whether differences in taste sensation had contributed to the differences in alcohol consumption. Some researchers have suggested that innate differences in taste sensation, and not neurochemical differences in reward-related circuitries, determine individual differences in alcohol consumption. In order to rule out this possibility, we examined the intake of saccharin and quinine by non-challenged APO-UNSUS and APO-SUS rats (**chapter 3**).
Another question that remained was whether the intake of the abusive and rewarding substance alcohol by APO-UNSUS and APO-SUS rats under non-challenged conditions held true for non-abusive, rewarding substances like sucrose. We, therefore, assessed the intake of sucrose by APO-UNSUS and APO-SUS rats under non-challenged conditions (**chapter 3**). Given the fact that both substances have a rewarding value, we hypothesized that, like with alcohol, APO-UNSUS rats would consume more sucrose than APO-SUS rats under non-challenged conditions.
It was shown that non-challenged APO-UNSUS and APO-SUS rats did not differ in the amount of saccharin and quinine consumed, ruling out the above-mentioned possibility of taste sensation interference in determining alcohol consumption. Moreover, it was shown that non-challenged APO-UNSUS rats consumed considerably more low-concentration sucrose than non-challenged APO-SUS rats did. Since sucrose is known to activate orosensory substrates that are coupled to the central dopaminergic and opioidergic systems, it is likely that the differences in sucrose consumption between APO-UNSUS and APO-SUS rats were due to the differences of those brain systems that are associated with reward sensation. Moreover, it was shown that the intake of non-abusive, rewarding substances was similar to the intake of abusive, rewarding substance in non-challenged APO-UNSUS and APO-SUS rats.

Given the fact that APO-UNSUS and APO-SUS rats differed in the amount of alcohol consumed and that this difference was dependent on the environmental conditions in which these animals were tested (stress vs. no stress), the question arose whether this interaction was unique for alcohol or whether this interaction held true for other drugs of abuse (like cocaine) as well. In order to investigate the role of the genetic background and the impact of the environment in directing the amount of cocaine self-administered, we examined whether APO-UNSUS and APO-SUS rats differed in the acquisition and maintenance phase of cocaine self-administration under non-challenged and challenged conditions (**chapter 4**).
It was shown that, under non-challenged conditions, APO-UNSUS rats acquired cocaine self-administration and subsequently self-administered more cocaine than non-challenged APO-SUS rats. On the other hand, APO-SUS rats needed a challenge to acquire cocaine self-administration, and, under these challenged conditions, APO-UNSUS rats self-administered less cocaine than challenged APO-SUS rats. This was in line with previous data on alcohol consumption. Interestingly, these experiments also revealed that, through manipulation of the environment, APO-UNSUS and APO-SUS rats were able to self-administer equal amounts of cocaine. This suggested that the amount of cocaine self-administered and, accordingly, its addictive potential and 'drug-vulnerability' were determined by the interaction between the genetic make-up of the animals and stress, and not by either component alone.

As stated above, another factor has often been implicated in the etiology of an addiction, namely (**adverse**) **early life events**. Previous research at our department has shown that an adverse early life event, namely maternal deprivation for 24 hours at postnatal day 9, enhances the reactivity of the dopaminergic system as well as the HPA-axis in male rats. In agreement with this, previous research has also shown that maternally deprived male APO-UNSUS rats, in comparison to non-deprived male APO-UNSUS rats, display an increase in apomorphine-induced gnawing behavior as well as an increase in the sensitivity to develop periodontitis (an inflammatory disease that is positively correlated with the reactivity of the HPA-axis).
Because these systems are also involved in the effects of cocaine, it became interesting to investigate the effects of maternal deprivation on cocaine self-administration in adult male APO-UNSUS rats (**chapter 5**) Given the fact that an increased apomorphine-induced gnawing score as well as an increased sensitivity to develop periodontitis are two characteristics of male APO-SUS rats, we hypothesized that maternally deprived male APO-UNSUS rats would display cocaine self-administration behavior of male APO-SUS rats, i.e. maternally deprived male APO-UNSUS rats would need a challenge to start cocaine self-administration.
It was shown that this adverse early postnatal life event, indeed, caused a shift in the intake pattern of cocaine from APO-UNSUS like to APO-SUS like behavior. As a consequence, maternally deprived male APO-UNSUS rats needed a challenge in order to acquire cocaine self-administration. The fact that maternally deprived APO-UNSUS rats needed a challenged to acquire self-administration suggested that maternal deprivation altered the

status of the dopaminergic system and HPA-axis (as also indicated by an increased response to apomorphine and increased sensitivity to develop periodontitis). This alteration also indicated that the intake of cocaine was not only determined by the genetic background and late environmental factors, but also early life events. More importantly, the data clearly showed that it is the interaction between these three factors that ultimately determine whether an individual will start to self-administer cocaine.

Many animal studies have shown that prenatal cocaine exposure results in long-term alterations in the mesocorticolimbic dopamine system and that it enhances cocaine self-administration. Given the already mentioned influence of early postnatal life events on cocaine self-administration, we, therefore, assessed cocaine self-administration behavior at adult age by male APO-UNSUS and APO-SUS rats that were exposed *in utero* to repeated cocaine or saline injections (**chapter 6**). Given the fact that APO-UNSUS rats are relatively stress-insensitive (in comparison to APO-SUS rats), we expected that both pregnant APO-UNSUS rats as well as their offspring would react less strong to repeated cocaine (and saline) injections than APO-SUS rats.
Pregnant APO-UNSUS rats, in comparison to pregnant APO-SUS rats, did not react strong to saline or cocaine injections (100% birthscore, and normal weight gain). On the other hand, prenatal cocaine exposure, but not prenatal saline exposure, resulted in an increase in the rate of acquisition of adult male APO-UNSUS rats. This finding indicated that prenatal cocaine exposure had a profound effect on the offspring: male APO-UNSUS rats suffered from a long-term alteration in the reinforcing efficacy of cocaine, resulting in facilitation of cocaine self-administration behavior at adult age. Pregnant APO-SUS rats reacted much stronger to prenatal stress (saline or cocaine injections) than APO-UNSUS rats: none of the cocaine-exposed pregnant APO-SUS rats gave birth (0%), and only a small percentage of the saline-exposed pregnant APO-SUS rats gave birth (33%). Therefore, cocaine self-administration could only be assessed in APO-UNSUS rats.

In the studies performed so far, cocaine self-administration was only investigated in male rats, even though human studies have shown that drug abuse is also very common in females. In fact, cocaine abuse amongst women has increased rapidly over the last few years, and their intake patterns are often different from those of men. We, therefore, incorporated female APO-UNSUS rats in the experiments described in **chapter 5 and 6**.
It was shown that female APO-UNSUS rats were faster at acquiring cocaine self-administration than male APO-UNSUS rats were (**chapter 5**). Moreover, prenatal cocaine-exposure resulted in an increase in the amount of cocaine consumed of female APO-UNSUS rats (**chapter 6**). These findings suggested that female rats had a greater motivational drive to obtain a drug of abuse, and were more affected by prenatal cocaine exposure than males.

Because research has suggested a positive correlation between the amount of cocaine self-administered and levels of dopamine transporter (DAT), and, possibly, levels of noradrenaline (NET) and serotonin transporters (SERT), we decided to investigate the levels

of these transporters in the striatum and hippocampus of non-challenged APO-UNSUS and APO-SUS rats (**chapter 7**). The striatum was selected because the susceptibility of this structure to dopaminergic agents (apomorphine and cocaine) greatly differs between both rat types, whereas the hippocampus was selected because its function and structure differs between both rat types.
This experiment showed that non-challenged APO-UNSUS rats had significantly lower striatal DAT levels than non-challenged APO-SUS rats did: a feature that gives more insight into why APO-UNSUS rats consumed more cocaine under non-challenged conditions than APO-SUS rats (chapter 4).This experiment also showed that non-challenged APO-SUS rats had slightly lower, but significantly different, hippocampal DAT and SERT levels than APO-UNSUS rats did: two features that may be related to the occurrence of schizophrenia-like symptoms and HPA-axis dysregulation in APO-SUS rats.

Chapters 2 and 4, together with previous research, have shown that under non-challenged conditions APO-UNSUS rats consumed more alcohol and cocaine than APO-SUS rats did, whilst under challenged conditions APO-UNSUS rats consumed less alcohol and cocaine than APO-SUS rats did. The question that arose from this research is why one rat type consumed more of an addictive substance than the other rat type did.
The motivation to self-administer more or less of addictive substance is said to be determined by reward (introduction). There is, however, some debate about what it means to consume less or more of addictive substance: some state that consuming more of an addictive substance indicates that this substance has a higher rewarding value, whilst others state that the substance has a lower rewarding value and that consumption needs to increase in order to obtain the desired level of reward. We, therefore, assessed the rewarding properties of cocaine by investigating the occurrence of cocaine-induced conditioned place preference in APO-UNSUS and APO-SUS rats (**chapter 8**). Given the finding that APO-SUS consumed more cocaine under challenged conditions than APO-UNSUS rats, we assessed the occurrence of cocaine-induced conditioned place preference under these conditions..
The data showed that challenged APO-SUS rats displayed cocaine-induced conditioned place preference, whilst challenged APO-UNSUS rats did not. Because the occurrence of place preference is said to be a measurement of reward sensation, it was concluded that challenged APO-SUS rats experienced more reward from cocaine than their counterparts did. Thus, challenged APO-SUS rats consumed more cocaine (and possibly alcohol) than challenged APO-UNSUS rats because of the high rewarding value of cocaine.

Taken together, the experiments performed in this thesis have revealed that an 'addictive personality' is not genetically programmed or environmentally determined: although the **genetic background** of the animals determined the basis for the structure and function of the addiction-related (central nervous) systems, the **environmental** conditions during development (prenatal, early postnatal) and adulthood determined the actual outcome.

The finding that the amount of a drug consumed is dependent on both the genetic background as well as the environment, has far reaching consequences for treatment strategies. Currently, only a few treatment strategies for drug addicts are utilized, but their effectiveness is debatable. It is possible that a specific treatment did not work, because it did not focus on those factors that determined the addictive profile of the patient. It would, therefore, be desirable to focus on the personal history and circumstances of a drug addict in order to individualize therapy. More importantly, insight into the personal history of a drug addict might even contribute to the development of effective preventive strategies for people that are considered to be at risk (i.e. people with a specific genetic profile). For instance, the occurrence of the *Taq*1 A1 allele of the dopaminergic D2 receptor (DrD2) is often considered to predict whether or not an individual will become addicted to alcohol. As mentioned before, a study by Madrid et al (2001), has shown that individuals with a low socio-economical status resulting in a large amount of stress (i.e. moneyproblems), together with the presence of the *Taq*1 A1 allele, determined the actual outcome of this genotype, and not the presence of this allele alone. This indicates that individuals with such a genotype would benefit greatly from a reduction in the amount of stress through, for instance, an improvement of their socio-economical status.

Moreover, the data presented in this thesis also revealed that **sex differences** play a role in determining the amount of a drug consumed. Most (animal) research is conducted with male animals, mostly because the hormonal cycle of female animals makes them less desirable and more expensive to utilize. For instance, in rats, 4 females are necessary (for each day of the hormonal cycle) where 1 male can be used. Thus, female animals are often not incorporated into experimental designs.
However, human diseases are not limited to men and some diseases are even more common in women. In the case of drug (ab)use, the number of women has increased rapidly over the last decade, and their intake patterns are often different from those of men. Moreover, given the fact that some psychiatric diseases are more common in women and the co-occurrence of these diseases with addiction (i.e. depression), it would be extremely interesting to also study the impact of sex differences in the onset of these diseases. Indeed, the data presented in this thesis revealed that female rats have a greater motivational drive to obtain an abusive substance as well as an increased response to this substance and (prenatal) stress. This enhancement of motivation could have far reaching consequences for treatment strategies of female addicts. It would, therefore, be desirable to conduct more research in this respect.

Samenvatting

Het doel van deze thesis was om de invloed van (a) **genetische aanleg**, (b) **vroege levensgebeurtenissen**, en (c) **late levensgebeurtenissen** vast te stellen in het bepalen van de gevoeligheid voor alcohol en cocaïne in het rat model bestaande uit apomorfine ongevoelige (APO-UNSUS) en apomorfine gevoelige (APO-SUS) ratten.

Het feit dat apomorfine (on)gevoeligheid een erfelijke trek is, alsook de gevonden verschillen in het *Aph 1b* gen, in combinatie met specifieke kennis van de structuur en functie van die systemen die een rol spelen in drugsmisbruik, namelijk het dopaminerge en, in mindere mate, het noradrenerge system evenals de HPA-as (zie introductie), maakte het mogelijk de rol van een genetische aanleg in het inname-patroon van alcohol en cocaïne te onderzoeken. Uit eerder onderzoek is gebleken dat de status (hoge of lage activiteit) van de bovengenoemde systemen bij APO-UNSUS en APO-SUS ratten afhankelijk is van de omgeving.
Het is bekend dat, in rust, APO-UNSUS ratten, in vergelijking met APO-SUS ratten, een vergelijkbare dopaminerge activiteit in het ventraal striatum, een functioneel hogere noradrenerge activiteit in de ventral striatum en een hogere concentratie van (vrij) corticosteron hebben. Echter, onder stressvolle omstandigheden verandert de neurochemische en endocrinologische status van deze dieren. Na stress, hebben APO-UNSUS ratten, in vergelijking met APO-SUS ratten, een lagere dopaminerge activiteit en functioneel lagere noradrenerge activiteit in de ventral striatum alsmede een kleinere en korter durende toename van de stresshormonen ACTH en corticosteron. De verandering in de status van die systemen maakte het mogelijk de rol van de omgeving (stress vs. geen stress) te onderzoeken in het bepalen van de geconsumeerde hoeveelheid alcohol en cocaïne door APO-UNSUS en APO-SUS ratten.
Omdat onderzoek een positieve correlatie tussen de activiteit van de dopaminerge en noradrenerge systeem en de HPA-as en de inname van alcohol en cocaïne heeft gesuggereerd, stelde wij de hypothese op dat, gezien de status van deze systemen in rust en stress bij APO-UNSUS en APO-SUS ratten, in rust APO-UNSUS ratten meer alcohol en cocaïne zouden consumeren dan APO-SUS ratten. Dit is in contrast met de consumptie in stress. Wij veronderstelden daarom dat in stress APO-UNSUS ratten minder alcohol en cocaïne zouden consumeren dan APO-SUS ratten.
Eerder onderzoek heeft aangetoond dat vroege levensmanipulaties de status van de bovengenoemde systemen alsook het gedrag van APO-SUS en APO-UNSUS ratten kan veranderen. Hierdoor kon ook de invloed van dergelijke vroege levensgebeurtenisssen op de inname van alcohol en cocaïne onderzocht worden. Daarom veronderstelden wij dat een aversieve vroege levensmanipulatie het innamepatroon van APO-UNSUS en APO-SUS ratten op volwassen leeftijd zou veranderen, afhankelijk van het soort manipulatie.

Hoewel een vorige studie al liet zien dat in rust APO-UNSUS ratten meer alcohol dan APO-SUS ratten consumeerden, was de consumptie van alcohol onder stressvolle omstandigheden onbekend. De rol van stress op de consumptie van alcohol is van speciaal belang omdat onderzoek heeft laten zien dat de (re)activiteit van de HPA-as een rol speelt bij het bepalen

van de ingenomen hoeveelheid alcohol. Recent humaan onderzoek heeft laten zien dat de uitkomst van het Taq1 A1 allel van de dopaminerge D2 receptor (verslaafd aan alcohol of niet) afhankelijk was van de sociaal-economische status van een individu. Een lage sociaal-economische status zou, onder meer, stress opleveren die gecorreleerd kon worden aan de uitkomst van het Taq1 A1 allel. Deze bevinding suggereerde dat de uitkomst van een specifiek genotype afhankelijk was van de aanwezigheid van stress tijdens, of net voor, de consumptie van alcohol. Wij onderzochten daarom de rol van stress in het bepalen van alcohol consumptie door APO-UNSUS en APO-SUS ratten (**hoofdstuk 2**).
Door het inname van alcohol in rust te onderzoeken en deze te vergelijken met de inname van alcohol na een enkelvoudige, milde stressor en herhaaldelijke, milde stressoren, was het mogelijk om de wisselwerking tussen de genetisch aanleg van deze ratten en de rol van de omgeving te onderzoeken in het bepalen van de geconsumeerde hoeveelheid alcohol. Na een enkelvoudige, milde stressor, lieten APO-UNSUS ratten een kleine toename in de inname van alcohol zien, terwijl APO-SUS ratten een grote en langdurige toename in inname lieten zien. Na subchronische, milde stressoren, in vergelijking met de enkelvoudige milde stressor, nam de inname van alcohol door APO-UNSUS ratten steeds verder toe, terwijl de inname van alcohol door APO-SUS ratten stabiel bleef. Deze resultaten lieten zien dat de geconsumeerde hoeveelheid alcohol afhankelijk was van zowel de aanwezigheid van stress én de duur van deze stress tijdens, en net voor, de consumptie van alcohol.

Eén van de vragen die uit dit onderzoek naar voren kwam, was of de gevonden verschillen in alcohol inname veroorzaakt werden door verschillen in smaaksensatie. Een aantal studies hebben gesuggereerd dat verschillen in smaaksensatie, en niet neurochemische verschillen in die hersengebieden die betrokken zijn bij beloning, de consumptie van alcohol kunnen beïnvloeden en sturen. We onderzochten daarom of de inname van saccharine en kinine in rust niet verschilde tussen APO-UNSUS en APO-SUS ratten (**hoofdstuk 3**).
Een andere vraag was of de consumptie van de verslavende en belonende substantie alcohol door APO-UNSUS en APO-SUS ratten ook opging voor de niet-verslavende, belonende substantie sucrose. Daarom onderzochten we ook de inname van sucrose in rust door APO-UNSUS en APO-SUS ratten (**hoofdstuk 3**). Aangezien beide stoffen een belonende waarde hebben, veronderstelden wij dat, net als bij alcohol, APO-UNSUS ratten meer sucrose zouden consumeren dan APO-SUS ratten.
APO-UNSUS en APO-SUS ratten verschilden niet wat betreft in de geconsumeerde hoeveelheid saccharine en kinine. Hiermee werd bevestigd dat de gevonden verschillen in alcohol consumptie niet veroorzaakt waren door verschillen in smaaksensatie. Ook lieten de experimenten zien dat in rust APO-UNSUS ratten meer lage-concentraties sucrose consumeerden dan APO-SUS ratten. Aangezien sucrose orosensorische systemen activeert die, via centrale dopaminerge en opiode systemen, een rol spelen bij motivatie en beloning, is het zeer waarschijnlijk dat de verschillen in sucrose inname door APO-UNSUS en APO-SUS ratten een weerspiegeling waren van de verschillen in die systemen. Daarnaast lieten deze experimenten zien dat het innamepatroon van de niet-verslavende en belonende substantie sucrose gelijk was aan dat van de verslavende en belonende substantie alcohol.

Gegeven het feit dat APO-UNSUS en APO-SUS ratten verschilden in de consumptie van alcohol en dat de richting van dit verschil afhankelijk was van de omgeving (stress vs. geen stress) waarin de dieren getest werden, rees de vraag of deze wisselwerking uniek was voor alcohol, of dat deze wisselwerking zou gelden voor alle verslavende middelen, inclusief cocaïne. Om de rol van zowel een genetische aanleg als de omgeving als ook de wisselwerking tussen deze twee te onderzoeken, onderzochten wij de inname van cocaïne door APO-UNSUS en APO-SUS ratten in rust en in stress (**hoofdstuk** 4).
In rust hadden APO-UNSUS ratten een grotere acquisitiesnelheid en daardoor dienden ze zichzelf ook meer cocaïne toe in vergelijking met APO-SUS ratten. Daarentegen hadden APO-SUS ratten een stressor nodig om de acquisitiefase te voltooien. Onder deze stressvolle omstandigheden dienden APO-UNSUS ratten zichzelf, in vergelijking met APO-SUS ratten, een kleinere hoeveelheid cocaïne toe. Dit was in lijn met de eerder gevonden data van alcoholconsumptie. Verder lieten deze experimenten zien dat, door manipulatie van de omgeving, APO-UNSUS en APO-SUS ratten zichzelf een gelijke hoeveelheid cocaïne konden toedienen. Dit suggereerde dat de ingenomen hoeveelheid cocaïne en, overeenkomstig, het verslavend potentieel van cocaïne werd bepaald door een wisselwerking tussen de genetische aanleg van deze dieren en de omgeving, en niet door één van de twee componenten alleen.

Zoals bovengenoemd, is er nog een factor die in de etiologie van een verslaving geïmpliceerd wordt, namelijk (aversieve) vroege levensgebeurtenissen. Eerder onderzoek van onze afdeling heeft laten zien dat een aversieve vroege levensgebeurtenis, namelijk maternale deprivatie voor 24 uren op postnatale dag 9, de reactiviteit van het dopaminerge systeem en de HPA-as in mannelijke ratten versterkt. In overeenkomst met dit onderzoek, werd gevonden dat maternaal gedepriveerde mannelijke APO-UNSUS ratten, in vergelijking met niet-gedepriveerde mannelijke APO-UNSUS ratten, een toename lieten zien in apomorfine-geïnduceerd knaaggedrag en in de gevoeligheid om periodontitis te ontwikkelen (een ontsteking die positief gecorreleerd is met de reactiviteit van de HPA-as).
Gegeven het feit dat deze systemen ook betrokken zijn bij het ontstaan van cocaïne misbruik, werd het interessant om de effecten van maternale deprivatie op cocaïne zelftoediening door mannelijke APO-UNSUS ratten te onderzoeken (**hoofdstuk 5**). Aangezien een toename in apomorfine-geïnduceerd knaaggedrag en de gevoeligheid om periodontitis te ontwikkelen twee kenmerken zijn van mannelijke APO-SUS ratten, werd verwacht dat maternaal gedepriveerde APO-UNSUS ratten cocaïne zelftoedieningsgedrag van mannelijke APO-SUS ratten zouden vertonen. D.w.z. maternaal gedepriveerde mannelijke APO-UNSUS ratten zouden een stressor nodig hebben om cocaïne zelftoediening te starten.
De experimenten lieten inderdaad zien dat het cocaïne zelftoedieningsgedrag van maternaal gedepriveerde mannelijke APO-UNSUS ratten veranderd was in het zelftoedieningsgedrag van APO-SUS ratten, namelijk maternaal gedepriveerde APO-UNSUS ratten hadden een stressor nodig om zelftoediening van cocaïne te starten. Het feit dat maternaal gedepriveerde APO-UNSUS ratten een stressor nodig hadden om cocaïne zelftoediening te starten, suggereerde dat maternale deprivatie de status van het dopaminerge systeem en de HPA-as

veranderd had (zoals ook aangegeven door een toename in apomorfine-geïnduceerd knaaggedrag en de gevoeligheid om periodontitis te ontwikkelen). Tevens liet deze verandering in cocaïne zelftoedieninsgedrag zien dat, naast een genetische achtergrond en late omgevingsfactoren, ook vroeg levensgebeurtenissen de inname van cocaïne konden bepalen. Nog belangrijker, deze data toonden duidelijk aan dat de wisselwerking tussen de drie bovengenoemde factoren uiteindelijk vaststelt welk individu de inname van cocaïne zal starten.

Veel dier-experimentele studies hebben laten zien dat prenatale blootstelling aan cocaïne resulteert in, onder andere, veranderingen van het dopaminerge systeem en cocaïne zelftoediening versterkt. Gegeven het feit dat vroeg postnatale effecten al zoveel invloed hebben op cocaïne zelftoedieningsgedrag, onderzochten wij de inname van cocaïne door volwassen, mannelijke APO-UNSUS en APO-SUS ratten die prenataal herhaaldelijk waren blootgesteld aan cocaïne of fysiologisch zout (**hoofdstuk 6**).
Gegeven het feit dat APO-UNSUS ratten relatief stress-ongevoelig zijn (ten opzichte van APO-SUS ratten) werd verwacht dat zowel zwangere APO-UNSUS ratten als ook hun nakomelingen minder sterk zouden reageren op prenatale blootstelling aan cocaïne (en fysiologisch zout) dan zwangere APO-SUS ratten en hun nakomelingen. Prenatale stress (fysiologisch zout of cocaïne injecties) had weinig effect op de zwangere APO-UNSUS ratten (100% geboortescore (=aantal zwangere vrouwtjes dat nakomelingen kreeg) en normale gewichtstoename). Daarentegen resulteerde prenatale blootstelling aan cocaïne, maar niet prenatale blootstelling aan fysiologisch zout, in een toename van de snelheid waarmee de acquisitiefase van cocaïne zelftoediening door mannelijke APO-UNSUS ratten voltooid werd. Deze bevinding liet zien dat prenatale blootstelling aan cocaïne een verregaand effect had op de nakomelingen: mannelijke APO-UNSUS ratten ondergingen een langetermijn-verandering in de versterkende werking van cocaïne waardoor er een versoepeling van cocaïne zelftoedieningsgedrag op volwassene leeftijd ontstond.
In tegenstelling tot zwangere APO-UNSUS ratten, resulteerde prenatale stress in de relatief stress-gevoelige APO-SUS ratten in een hoog aantal spontane abortussen: geen van de zwangere APO-SUS ratten die blootgesteld waren aan cocaïne kreeg nakomelingen (0%), en slechts een klein percentage van de zwangere APO-SUS ratten die blootgesteld waren aan fysiologisch zout kreeg nakomelingen (33%). Hierdoor kon het experiment helaas alleen worden uitgevoerd met APO-UNSUS ratten.

In de tot nu toe verrichtte studies werd cocaïne zelftoediening alleen onderzocht in mannelijke ratten, ook al laten menselijke studies zien dat ook vrouwen vaak drugs misbruiken. Cocaïne misbruik onder vrouwen is de laatste jaren snel toegenomen en de consumptiepatronen van vrouwen verschillen dikwijls van die van mannen. Daarom onderzochten wij ook vrouwelijke APO-UNSUS ratten in de experimenten beschreven in hoofdstuk 5 en 6.
Vrouwelijke APO-UNSUS ratten waren sneller in het aanleren van cocaïne zelftoediening dan mannelijke APO-UNSUS ratten (**hoofdstuk 5**). Bovendien resulteerde prenatale

blootstelling aan cocaïne in een toename in de ingenomen hoeveelheid cocaïne door vrouwelijke APO-UNSUS ratten (**hoofdstuk 6**). Deze bevindingen suggereerden dat vrouwelijke ratten een grotere motivatie (behoefte) hadden om cocaïne te gebruiken en dat vrouwelijke ratten een groter effect hadden van prenatale cocaïne blootstelling dan mannelijke ratten.

Omdat onderzoek een positieve correlatie heeft gesuggereerd tussen de hoeveelheid geconsumeerde cocaïne en de hoeveelheid dopamine (DAT), en mogelijk, noradrenaline (NET) en serotonine (SERT) transporters, besloten wij om de hoeveelheid van deze transporters te meten in het striatum en de hippocampus van APO-UNSUS en APO-SUS ratten in rust (**hoofdstuk** 7). Het striatum werd geselecteerd omdat de gevoeligheid van deze structuur voor dopaminerge farmaca (zoals apomorfine en cocaïne) tussen beide typen rat verschilt. De hippocampus werd geselecteerd omdat deze zowel in functie en structuur verschilt tussen beide rat typen.
In rust hadden APO-UNSUS ratten veel minder striatal DATs dan APO-SUS ratten; een kenmerk dat inzicht verschafte in de reden waarom in rust APO-UNSUS ratten zichzelf meer cocaïne toe dienden dan APO-SUS ratten (hoofdstuk 4). In rust hadden APO-UNSUS ratten meer hippocampale DATs en SERTs dan APO-SUS ratten: twee kenmerken die mogelijk zijn gerelateerd aan het voorkomen van schizofrenie-achtige symptomen en een ontregelde HPA-as bij APO-SUS ratten.

Hoofdstukken 2 en 4, samen met eerder onderzoek, hebben laten zien dat in rust APO-UNSUS ratten meer alcohol en cocaïne consumeerden dan APO-SUS ratten, terwijl in stress APO-UNSUS ratten minder alcohol en cocaïne consumeerden dan APO-SUS ratten. De vraag die uit deze studies voortkwam, is waarom één rat type meer alcohol of cocaïne consumeerde dan het andere rat type.
Uit onderzoek is bekend dat de motivatie om een verslavend middel in te nemen, wordt vastgesteld door de belonende waarde van dit middel (zie introductie). Er is, momenteel, enige discussie gaande over de betekenis van het meer of minder innemen van een verslavend middel ten aanzien van de belonende waarde van dit middel. Sommige onderzoekers hebben gesuggereerd dat een grotere inname van een middel aanduidt dat dit middel een hogere belonende waarde heeft. Anderen hebben gesuggereerd dat het middel juist een lagere belonende waarde heeft waardoor meer moet worden ingenomen om een plezierig effect te bewerkstelligen. Om te bepalen welke van deze twee standpunten waar was, onderzochten wij het voorkomen van cocaïne-geïnduceerde 'conditioned place preference' (**hoofdstuk 8**).
Aangezien APO-SUS ratten zichzelf meer cocaïne toedienden dan APO-UNSUS ratten onder stressvolle omstandigheden, werd het voorkomen van cocaïne-geïnduceerde 'conditioned place preference' onder die omstandigheid onderzocht. In stress lieten APO-SUS ratten cocaïne-geïnduceerde 'conditioned place preference' zien, terwijl APO-UNSUS ratten dat niet lieten zien. Omdat het voorkomen van 'conditioned place preference' een maat is voor de belonende waarde van het middel, liet dit experiment zien dat onder stressvolle omstandigheden APO-SUS ratten een hogere beloning ervoeren van cocaïne dan

APO-UNSUS ratten. Daarom kon geconcludeerd worden dat onder stressvolle omstandigheden APO-SUS ratten meer cocaïne consumeerden (en misschien ook alcohol) dan APO-UNSUS ratten vanwege de hoge(re) belonende waarde van cocaïne.

Alles bij elkaar genomen, hebben de uitgevoerde experimenten laten zien dat het fenomeen 'een verslavende persoonlijkheid' niet alleen bepaald wordt door een genetische aanleg of de omgeving, maar een combinatie van die twee. Alhoewel de **genetische achtergrond** van een individu de basis vormt voor de structuur en functie van de verslaving-verwante (centraal zenuwstelsel) systemen, stelt de omgeving tijdens de **ontwikkeling (prenataal en vroeg postnataal) en tijdens het leven** het uiteindelijke resultaat vast.

De bevinding dat de ingenomen hoeveelheid van een verslavend middel afhankelijk is van zowel een genetische aanleg als ook de omgeving, heeft verstrekkende gevolgen voor mogelijke behandelmethoden. Momenteel bestaan slechts enkele behandelmethoden voor verslaafden. De effectiviteit van die behandelingen is echter twijfelachtig. Het is mogelijk dat een specifieke behandeling bij een bepaald individu niet werkte, omdat de behandeling niet toegespitst was op die factoren die betrokken waren bij het ontstaan van zijn/ haar verslaving. Het is daarom wenselijk meer focus te leggen op de persoonlijke geschiedenis en levensomstandigheden van een drugsverslaafde om daarmee de behandeling te individualiseren.
Meer inzicht in deze omstandigheden kan zelfs bijdragen aan de ontwikkeling van doeltreffende preventieve strategieën voor risicogroepen (bv. door een bepaald genetisch profiel). Bijvoorbeeld, het voorkomen van het Taq1 A1 allel van de dopaminergic D2 receptor (DrD2) wordt geacht te voorspellen wie verslaafd raakt aan alcohol. Onderzoek door Madrid et al (2001) echter laten zien dat de uitkomst van dit gen afhankelijk was van de mate van stress die voortkwam uit een lage sociaal-economische status. Dit duidt erop dat individuen met een dergelijk genotype voordeel hebben van een reductie in de hoeveelheid stress door bijvoorbeeld een verbetering van hun sociaal-economische status.

De uitgevoerde experimenten laten ook zien dat **sexe verschillen** een rol spelen bij het vaststellen van de ingenomen hoeveelheid van een verslavend middel. Momenteel wordt het merendeel van het dierlijk onderzoek uitgevoerd op mannelijke ratten, voornamelijk omdat de hormonale cyclus van vrouwelijke ratten de resultaten kan beïnvloeden waardoor meerdere ratten nodig zijn voor hetzelfde resultaat. Er zijn bijvoorbeeld 4 vrouwelijke ratten nodig (voor elke dag van de cyclus) waar slechts 1 mannelijke rat gebruikt hoeft te worden. Hierdoor worden vrouwelijke dieren vaak niet gebruikt in studies.
Menselijke ziektes komen echter niet alleen bij mannen voor, en sommige ziektes komen zelfs vaker bij vrouwen dan bij mannen voor. Bovendien is het aantal verslaafde vrouwen de laatste decennia sterk gegroeid en laat onderzoek zien dat hun inname patroon vaak anders is dan die van mannen.
Tevens is bekend dat bepaalde psychiatrische aandoeningen vaker voorkomen bij vrouwen en, gezien de wisselwerking tussen deze aandoeningen en verslavingen (bv. depressie), zou het

zeer interessant zijn om meer onderzoek te doen naar sexe verschillen in het ontstaan van deze ziektes. De gegevens uit dit proefschrift laten zien dat vrouwelijke ratten een grotere motivatie hadden om een verslavend middel in te nemen alsook een grotere reactie hadden op dit middel en (prenatale) stress. Deze hogere motivatie kan verstrekkende gevolgen hebben voor de behandelmethoden van vrouwelijke verslaafden. Er zou daarom meer aandacht moeten komen voor sexe-verschillen in (dierlijk) verslavingsonderzoek.

List of publications

Full papers:

Kam, E.L. van der, Coolen, M.C.J., Ellenbroek, B.A., Cools, A.R. (2005). The effects of stress on alcohol consumption: mild acute and sub-chronic stressors differentially affect apomorphine susceptible and unsusceptible rats. *Life Sciences, 76, 1759-1770.*

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Kam, E.L. van der, Ellenbroek, B.A., Cools, A.R. Sweet and bitter taste sensation in individually different rats: the consumption of sweetened but not bitter substances parallels to the consumption of drugs of abuse. *Behavioral Pharmacology (submitted).*

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Book publications:

Kam, E.L. van der (2001) Absence of evidence is not evidence of absence; do we still have to fear BSE? Wolters Noordhoff Groningen, ISDN nr. 9001 457 010

Oral presentations:

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Curriculum Vitae

Elizabeth Louise van der Kam was born on the 25th of February 1978 in Beek en Donk, The Netherlands. She completed her high school (gymnasium) education in 1996 at the 'Doctor Nassau College' in Assen. From 1996 until 2001, she studied Medical Biology at the University of Groningen. Her first research topic concerned the effects of food deprivation and sex on ischemic/ hypoxic infarct size. This neuroanatomical and behavioural research project was conducted at the Department of Biological Psychiatry of the Academic Hospital Groningen under supervision of prof dr. J. Korf. Her second research project concerned the effects of the obstetric history and type of infant food on the quality of motor behaviour in young children. This epidemiological research project was conducted at the Department of Developmental Neurobiology of the Academic Hospital Groningen under supervision of prof. dr. M. Hadders-Algra. In the last year of her study, she wrote a term paper about Bovine Spongiform Encephalopathy at the Department of Neurobiology of the University of Groningen. This paper was honored by the University of Groningen and Wolters-Noordhoff with the title 'Best term-paper 2001' and was published as a book.
Elizabeth Louise van der Kam graduated in 2001 at the University of Groningen. In October 2001 she started working as a PhD student at the Department of Psychoneuropharmacology of the Radboud University Nijmegen under the supervision of prof. dr. A.R. Cools and dr. B.A. Ellenbroek. The experiments performed at this department resulted in this thesis. She will continue her scientific carreer as a post-doc at the Department of Pharmacology of Grunenthal GmbH in Aachen under the supervision of dr. T. Tzschentke and dr. J de Vry.

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"Pooh, pooh", zuchtte Iejoor. "Dat was een zware klus. Een van groot formaat".

De alcoholconsumptie is sterk gestegen
In de laatste jaren, dus ook de gevaren kom je tegen
In het dagelijks leven, in je eigen omgeving
Een ieder heeft zo z'n eigen alcohol beleving
We hebben drinkers en probleemdrinkers
Die maatschappelijk vaak ook extreem wegzinken
Het is verschil is nou nog moeilijk te zien
Maar jij bent niet meer zo clean
En dagelijks meer dan tien
Acht glazen per dag betekent leverschade
En als je zo drie tot vijf jaar door blijft laden
Maar bij twaalf of meer dan stopt de beschaving
Dan leidt je hele lichaam aan alcoholverslaving
Als dan de alcohol jouw bloed verlaat
Dan voel je dat het mis gaat
Dat je weer aan het drinken slaat
Gewenning, dat is hoe verslaving ontstaat
Je gaat steeds meer drinken voor hetzelfde resultaat
Bekende situatie?
Besef dan goed
Dat je het niet meer voor de smaak, maar voor de alcohol doet!

Songtekst 'De Alcohol-list' Osdorp Posse
Courtesy Osdorp Posse & Ramp Records, Amsterdam