Gene-environment interactions in early life and adulthood : implications for cocaine intake

Veen, R. van der

Gene-environment interactions in early life and adulthood

Implications for cocaine intake

Gene-environment interactions in early life and adulthood *Implications for cocaine intake*
Rixt van der Veen

Thesis, Leiden University
October 2008

ISBN: 978-90-6464-285-2

Cover: designed by Rixt van der Veen & Kees van der Veen, photography by Paul van Goor
Layout: Henk van der Veen & Rixt van der Veen
Printing: Ponsen en Looijen, Wageningen, The Netherlands

Gene-environment interactions in early life and adulthood

Implications for cocaine intake

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden
op gezag van de Rector Magnificus Prof. Mr. P.F. van der Heijden
volgens het besluit van het College voor Promoties
te verdedigen op woensdag 1 oktober 2008
klokke 13:45 uur

door

Rixt van der Veen
geboren te Leeuwarden in 1977

Promotiecommissie

Promotores	Prof. Dr. ER de Kloet
	Prof. Dr. PV Piazza (Université de Bordeaux II)
Referent	Dr. LJMJ Vanderschuren (Rudolf Magnus Instituut, Utrecht)
Overige Leden	Prof. Dr. M Danhof
	Dr. MH van IJzendoorn
	Prof. Dr. MS Oitzl
	Dr. AM Pereira Arias
	Prof. Dr. RRJM Vermeiren
	Prof. Dr. FJ Walther
	Prof. Dr. FG Zitman

The studies described in this thesis have been performed at the INSERM unit 259: Psychobiology of adaptive behavior, later named INSERM unit 588: Physiopathology of behavior at the University of Bordeaux II, France. This research was part of a collaboration with the division of Medical Pharmacology of the Leiden/Amsterdam Center for Drug Research (LACDR) and Leiden University Medical Center (LUMC), The Netherlands. This research was financially supported by a collaboration grant (AH002G and 985-10-01) of The Dutch Organisation for Scientific Research (NWO/ZonMw) and the French National Institute of Health and Medical Research (INSERM/Mildt) and the University of Bordeaux II.

Financial support for the printing of this thesis was kindly provided by:
Leiden/Amsterdam Center for Drug Research
JE Jurriaanse Stichting
Noldus Information Technology BV
Top Institute Pharma

What we observe is not nature itself,
but nature exposed to our method of questioning

Werner Heisenberg (1901-1976)

Table of contents

Chapter 1

General Introduction

A central issue in addiction research is who is most at risk and why. Long considered the result of a lack of willpower, addiction is now understood to be a chronic, progressive and relapsing brain disease that today is prevented and treated using relatively untargeted and only partially effective methods. The transition from drug use to drug abuse and addiction implies several steps. From first contact with the drug, to experimentation with use, followed by regular use and eventually leading to abuse (excessive use) and addiction. Drug addiction is defined by two major characteristics: a compulsion to take the drug, despite negative consequences which can be severe, and a loss of control in limiting intake (American Psychiatric Association 1994). Physical dependence on a substance is not necessary or sufficient to define addiction. There are some substances that don't cause addiction but do cause physical dependence (for example, some blood pressure medications) and in contrast, substances that cause addiction but not classic physical dependence. Cocaine withdrawal, for example, doesn't have symptoms like vomiting and chills, but is mainly characterized by depression.

A drug like cocaine has a high probability for the transition from regular use to abuse (Goldstein and Kalant 1990; Tsuang et al. 1999). This is troubling, since cocaine is a widely used drug, with an estimated 14 million users worldwide (United Nations Office on Drugs and Crime 2005). The burden of substance abuse and addiction to society is enormous. Not only are there high costs due to the medical consequences linked to the destructive physical and psychological effects of drugs (including a high risk of HIV contamination), but also loss of productivity, accidents and crime. But why can some people, at least for some period of time, restrict their use of cocaine, while others are vulnerable to becoming compulsive heavy users, stopping only with great difficulty if at all, and relapsing readily? An important challenge for neuroscience is to understand these individual differences in drug use and the development of abuse, this will help to understand the molecular, cellular and system processes that lead to addiction.

1.1 Genes & Environment

Both environmental and genetic factors contribute to individual differences in vulnerability to the initial use of addictive agents as well as the shift from use to addiction. Twin studies have shown that these risk factors seem to be largely non-specific and are shared between different classes of drugs (Kendler et al. 2003). Moreover, a substantial overlap in risk factors is shared between major classes of psychiatric disorders (Krueger et al. 2002; Compton et al. 2005) and comorbidity is often seen between psychiatric and substance use disorders (Kendler et al. 2003). At the genetic level, there is a substantial overlap in the genetic liability for antisocial behavior and substance use problems, as well as with temperamental features and attention deficit/hyperactivity disorder (Cadoret et al. 1995; Krueger et al. 2002; Kendler et al. 2003). Thus, the association between genetic risk factors and psychopathology outcome are weak and often nonspecific (Kendler 2005). Genes that have been found to provide susceptibility to mental disorders are mostly normal allelic variations of common genes. In combination with other susceptibility genes, they may affect particular

physiological pathways that make a psychiatric condition more or less likely. In addition, linkage studies have shown that these genetic risk factors are located on multiple loci and are widely dispersed across the genome (Lewis et al. 2003; Mathews and Reus 2003). Evidently, genes do not cause a mental disorder directly, but they are implicated under the influence and regulation of environmental risk factors.

Environmental risk factors for creating a vulnerable phenotype for substance use and other mental disorders include priming early life adversities that can occur during the prenatal, natal or postnatal period, and adult stressful life events or chronic stressors. Early life adversities that are reported to constitute risk factors include maternal distress and maternal influenza during pregnancy, birth complications, loss of a parent, a depressed or addicted parent, exposure to family conflict and violence, neglect or physical maltreatment (Kessler et al. 1997; De Bellis 2002; Gordon 2002; Heim et al. 2004; King et al. 2005; Talge et al. 2007). Risk factors in adulthood include loss of a beloved one, low socio-economic status and daily life hassles (Kendler et al. 2002). An obvious additional environmental factor that influences substance use and abuse is the availability of the drug. Indeed, increased availability of cocaine and methamphetamine has contributed to the epidemics of addiction to these drugs today (Volkow and Li 2005). So it is obvious that both environmental and genetic risk factors are implicated in the causal process leading to substance abuse disorders.

One of the crucial questions is how these factors interact to explain individual differences in vulnerability to psychopathology. In this context, not only vulnerability but also resistance is important and the concept of resilience is gaining more attention (Luthar et al. 2000; Charney 2004; Cicchetti and Blender 2006; Yehuda et al. 2006). In psychiatry, resilience refers to "a dynamic process encompassing positive adaptation within the context of significant adversity" (Luthar et al. 2000). In other words, resilience implies the exposure to adverse experiences and the ability to positively adapt to them in order to reach and maintain an acceptable level of functioning. Important to note is that resilience is a dynamic concept and not a static state; vulnerabilities and strengths can emerge at each point of the life course and are not automatically valid for different domains of functioning or competence (Luthar et al. 2000). Studying the mechanisms underlying both resilience and vulnerability, that is, both positive and negative adaptation to adversity may reveal the origins of individual differences in behavior.

1.1.1 Gene-environment interaction

Co-action or interplay between genetic risk and environmental risk influences behavior in many ways. In psychiatry, several different concepts of gene-environment interplay are discerned: heritability-environment interaction, gene-environment correlation, epigenetic programming and the pure ***gene-environment interaction***, that ***occurs when the effect of exposure to an environmental factor on health and behavior is conditional upon a person's genotype, or conversely, when the genotype's effect is moderated by the environment*** (Moffitt et al. 2006). Studies on gene-environment interactions specifically address the question of individual variation in susceptibility to environmental events, in contrast to studies on *heritability* that apply to a population variance and not to individuals or to traits as a fixed feature. A high heritability means that genetic factors account for much of

the variation in the liability to show a particular trait. Interestingly, heritability is shown to differ across sub-segments of the population and thus seem to interact with the environment. A clear example is the finding that heritability estimates of IQ levels vary with socio-economic status (Rowe et al. 1999; Turkheimer et al. 2003). When a person's genotype influences his or her probability of exposure to environmental risks, we talk about *gene-environment correlation* (Plomin et al. 1977). This means that people to some extent shape and select their environment through their behavior (Rutter et al. 1997). And finally, *epigenetic programming* provides a way in which environmental influences can have important, heritable effects on gene expression without changing DNA sequence. Epigenetics refers to the regulation of various genomic functions that are controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure. DNA methylation and histone acetylation (two linked chemical processes) shield or expose genes, thereby allowing or preventing parts of the genome to be translated. Central in this thesis is the concept of gene-environment interactions, as we focus on the question of individual variation in susceptibility to environmental events.

An elegant demonstration of gene-environment interaction in rodents goes back to 1958, to a study of Cooper and Zubek that used rat lines selectively bred for a distinct difference in performance in a maze, these lines were originally developed by Tolman and Tryon (for review Innis 1992). In selective breeding, individuals with desirable traits are selected for use in breeding over several generations. The 'maze bright' and 'maze dull' rats were raised in either a normal (cage with bedding), a restricted (in an empty cage with gray walls) or an enriched environment (in a cage with designs on its walls that contained objects such as ramps, mirrors, swings, balls, slides, and tunnels). When raised in a normal environment the rats were clearly different in maze performance. However, when raised in the restricted environment, the dull rats made many errors as usual, but the bright rats also made many errors and when raised in the enriched environment, both groups made few errors *(figure 1.1)*. The authors argued that heredity (defined as the genetic transmission of characteristics from parent to offspring) and environment always interact to produce final behavior (Cooper and Zubek 1958).

A decade later, Henderson showed that genetic influences on the behavior of mice can be obscured by laboratory rearing (1970) and short exposures to an enriched environ-

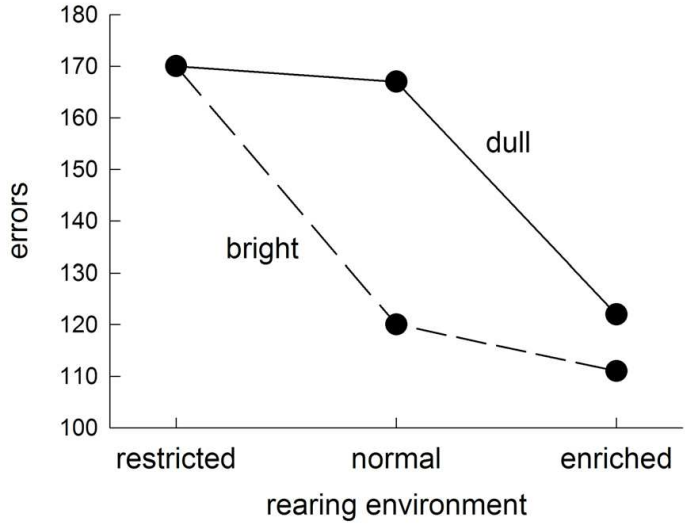


Figure 1.1: Gene-environment interaction in maze learning. Maze-bright and maze dull rats were raised in a normal, a restricted or an enriched environment. *From Cooper and Zubek (1958).*

ment can increase genetic variability in mice (1976). More recently, Crabbe and colleagues (Crabbe et al. 1999; Wahlsten et al. 2003) and Cabib and colleagues (Cabib et al. 2000) showed that the expression of behavioral differences linked to the genetic make-up of mice depends upon the environment.

So, over time it is repeatedly demonstrated that gene-environment interaction is a crucial determinant for behavioral responses. Meanwhile, psychiatry went through several periods of reasoning. The 1950s to early 1960s were characterized by environmentalism, when emphasis was placed on the role of adverse experiences in the development of mental disorders and 'constitutional' factors were given little attention, The following years were a period of acceptance of the importance of genetic influences on variations in the individual liability to mental disorders (1960s to the 1980s) and finally led to periods of denial of the importance of environmental influences (1980s to the early 1990s) (Rutter et al. 2006). Today is characterized by the acceptance of the multifactorial origin of most mental disorders and the importance of gene-environmental interplay.

1.1.2 Gene-environment interaction in psychopathology

Gene-environment interaction has long been a useful theoretical concept in behavioral sciences, but recently it has gained interest in the field of psychiatry (Caspi and Moffitt 2006) and studies try to specifically address this type of interplay in relation to psychopathology. A striking example is a functional polymorphism in the promoter region of the serotonin transporter (5-HTT) gene that moderates the influence of stressful life events on depression. Individuals with one or two copies of the 5-HTT short allele exhibited more depressive symptoms, diagnosable depression, and suicidality following stressful life events than individuals homozygous for the long allele (Caspi et al. 2003; Eley et al. 2004; Grabe et al. 2005; Kendler et al. 2005; Cervilla et al. 2007). However, good social support protected children from the adverse effects of maltreatment-genotype interaction (Kaufman et al. 2004). Other polymorphisms have been shown to play a role in the environmental influence on antisocial disorders (Caspi et al. 2002; Foley et al. 2004) and schizophrenia (Caspi et al. 2005).

Also twin and adoption studies start to more specifically address gene-environment interactions by using statistical models like 'Structural equation modeling', which is an extension of the general linear model and simultaneously estimates relationships between multiple independent, dependent and latent variables. This is how Silberg and colleagues (Silberg et al. 2001) found a significant effect of independent life events on adolescents' depression, but only in the presence of parental emotional disorder (a measure of genetic vulnerability). Likewise, gene-environment interactions were found for antisocial disorders (Cadoret et al. 1995; Jaffee et al. 2005) and schizophrenia (Tienari et al. 2004).

Direct evidence of gene-environment interactions in substance abuse disorders is more scarce, although adoption studies tend to show an interaction (Cutrona et al. 1994; Sigvardsson et al. 1996) and recently a polymorphism in the GABA-A2 receptor subunit gene has been shown to interact with the influence of marital status on alcohol dependence (Dick et al. 2006). There is a substantial association between antisocial behavior and substance use/misuse, although it is unlikely that the gene-environment interactions operate in exactly the same way (Rutter et al. 2006).

Thus, epidemiological studies have identified gene-environment interactions in psychopathology, but are limited to study underlying mechanisms. Indeed, these studies often rely on questionnaire measures, so it is difficult to get a precise and reliable measure of risk conditions (e.g. stress intensity or amount of substance administered). Moreover, gene-environment correlations have to be taken into account and complex statistical models employed (Whisman and McClelland 2005). Controlled human studies in this field are rare, since ethics prohibit exposing humans to risk. A cry for collaboration with neuroscience was recently heard, to try to understand how genes and environment interact and how vulnerability and resilience to psychopathology emerge (Caspi and Moffitt 2006).

1.2 Drugs of abuse

The study of gene-environment interaction in drug abuse and addiction is particularly difficult in humans. An evident and possibly confounding environmental risk factor for substance abuse is the availability and use of the drug. Genetic susceptibility for substance abuse might involve the liability to engage in risk-taking behavior (such as taking drugs), but also the psychophysiological response to a particular substance. Moreover, there is marked heterogeneity within the group of addicted individuals (e.g. demographic factors, level of education, comorbid medical, neurological and/or psychiatric conditions), as well as differing patterns of drug use (e.g. type, duration, frequency, and dosage). Animal models exist for many aspects of substance use and abuse, and these might be very useful in bringing knowledge about the biological aspects of individual differences in drug use and addiction.

1.2.1 Animal models of substance use

Clearly, the drug self-administration (SA) model represents the most obvious and face-relevant model of drug use and abuse. Animals readily self-administer most drugs of abuse and conversely, drug SA in animals is highly predictive of drug abuse liability in humans (Spealman and Goldberg 1978; Johanson and Fischman 1989; Rose and Corrigall 1997; Van Ree et al. 1999). Moreover, individual differences in the propensity to develop drug intake have been demonstrated in the laboratory rat (Piazza et al. 1989; Deminiere et al. 1989). This implies that similar molecular substrates are shared between species and opens the possibility to study voluntary drug use in an animal model.

While alcohol preference and intake is mostly studied by adding an alcohol/sucrose solution to the drinking water, cocaine intake is mostly studied by using the intravenous route of administration. That is, a catheter is placed in the jugular vein and animals either press a lever or poke their snout in a hole to obtain an infusion of cocaine. The basic assumption of this method, which is derived from the operant conditioning paradigm (Skinner 1953), is that psychoactive drugs, like natural reinforcers (e.g., food, water, sex), can control behavior by functioning as positive reinforcers (Brady 1991). Indeed, opiate and psychostimulant drugs support intravenous drug SA in mice, rats and monkeys, indicating that they serve as positive reinforcers in an operant procedure (Brady 1991). Moreover, un-

derlying (physical) drug dependence is not necessary for intravenously administered drugs to serve as reinforcers and addictive drugs are voluntarily self-administered intracerebrally only into brain loci known to be associated with the brain's reward substrates (Wise 1996; Gardner 2000).

Drug SA under fixed-ratio (FR) reinforcement is the most commonly used schedule in animal studies of addiction. Under a FR reinforcement schedule, the animal receives either drug (as in intravenous SA experiments) or access to drug (as in oral SA experiments) according to a fixed number of responses (e.g. lever presses or nose pokes) emitted by the animal. Thus, under a fixed-ratio-1 (FR1) reinforcement schedule the animal receives an administration or presentation of the drug after a single instrumental response, while under an FR2 schedule, two instrumental responses are required to obtain the same drug infusion. FR reinforcement for drug administration or presentation has the merits of simplicity and of an unambiguous relationship between the animal's behavior and obtaining the drug. While low FR levels are used to measure the reinforcement of a drug, higher FR levels are used to measure reinforcement efficacy (O'Brien and Gardner 2005).

The reinforcing effects of drugs under simple schedules (like FR) most often are characterized by an inverted U-shaped dose-response function. This type of function relating dose and effect reflects the balance of reinforcing effects and behaviorally disruptive effects of drugs. Indeed, high doses that rapidly satiate brain reward substrates, can induce response inhibition (sedating effects) and have aversive effects like vasoconstriction and an increase in heart rate (O'Brien and Gardner 2005). Reinforcing efficacy and motivation to self-administer drugs can be measured under progressive-ratio (PR) reinforcement. Here, the animal has to furnish a progressively increasing amount of work (number of responses) to receive an administration of the drug. Relapse can be measured in SA experiments using different 'reinstatement' protocols. After behavioral extinction of drug-taking behavior (by withholding the drug reinforcer), the animal is tested to see whether a triggering stimulus (e.g. drug, stress, or drug-paired environmental cues) will reinstate the nose-poking or lever-pressing behavior (which is now not reinforced by the drug anymore and thus qualified as 'drug-seeking' behavior rather than 'drug-taking' behavior). Finally, persistent drug seeking despite negative consequences can be studied by coupling the drug infusion to an adverse stimulus, like foot-shock.

Other behavioral responses to cocaine relevant to drug use and addiction are sensitization and conditioned place preference (CPP). Sensitization is primarily seen with psychostimulants such as cocaine and amphetamine and refers to the progressive augmentation of the behavioral (locomotor) and neurochemical (alterations in extracellular dopamine) effects of the drug with successive administrations. The occurrence of sensitization depends on the pattern of administration; intermittent/low doses mostly produce sensitization, while chronic continuous or high-dose regimens produce tolerance (O'Brien and Gardner 2005). Importantly, sensitization is seen following SA of addictive drugs (Hooks et al. 1994).

The CPP procedure is used to measure the reinforcing effects of unconditioned stimuli in a classical conditioning paradigm. In CPP studies, subjects are trained to associate one distinctive environment with a drug injection and a different environment with a vehicle injection. Following training, rats spend more time in the drug-paired environment, when given a choice between the two environments, on a drug-free test day (for review

Bardo and Bevins 2000). Many studies have shown that opiate and psychostimulant drugs can be rewarding as measured in the CPP procedure. An advantage of the CPP procedure is that testing for preference to an environment previously paired with a drug is conducted in a drug-free state. Thus, the results thereby obtained are not confounded by the unconditioned effects (e.g. changes in activity levels) of the drug. However, in the CPP procedure, exposure to the drug is not under the control of the subject and total drug exposure is very low. The advantage of drug SA is the possibility to measure the reinforcing properties of the drug by voluntary intake that has been shown to touch distinct biological circuits compared to forced injections (Jacobs et al. 2003; Greenwald and Roehrs 2005; Dumont et al. 2005).

1.2.2 Neurobiological basis for drug reward

Virtually every drug of abuse influences dopamine-mediated neurotransmission by affecting directly or indirectly the activity of the dopamine cells (Bonci et al. 2003). The midbrain dopamine system, which is the main source of dopamine in the central nervous system, is composed of two major projections: the *nigrostriatal* system, which projects from the substantia nigra to the corpus striatum (caudate putamen) and the core region of the nucleus accumbens (NAcc), and the *mesocorticolimbic* system, which projects from the ventral tegmental area (VTA) to the shell region of the NAcc, olfactory tubercle, ventral pallidum, bed nucleus of the stria terminalis (BNST), frontal cortex, and amygdala. While the nigrostriatal system is classically implicated in motor control and linked to the activating effects of drugs of abuse, it is the mesocorticolimbic system that has been primarily implicated in the reinforcing actions of drugs of abuse *(figure 1.2)* (Le Moal and Simon 1991).

The mesolimbic circuit includes projections from cell bodies of the VTA to limbic structures, such as the NAcc shell, amygdala, and hippocampus. This circuit has been implicated in acute reinforcing effects, memory, and conditioned responses linked to craving and the emotional and motivational changes of the withdrawal syndrome. The mesocortical dopamine circuit includes projections from the VTA to the prefrontal cortex (PFC), orbitofrontal cortex, and anterior cingulate. It is involved in the conscious experience of the effects of drugs, drug craving, and the compulsion to take drugs. The mesolimbic and the mesocortical dopamine circuits operate in parallel and interact with each other and with other areas by means of projections from the GABA neurons of the NAcc to the VTA and glutamatergic projections from the PFC, amygdala and hippocampus to the NAcc and VTA. Opioid interneurons modulate the GABA-inhibitory action on the VTA. Serotonergic (5-HT) projections from the raphe nucleus extend to the VTA and the NAcc (for review Koob 1992; Jentsch and Taylor 1999; Hyman and Malenka 2001; Cami and Farre 2003).

The impact of the acute effects of drugs of abuse on neuronal circuits of reward can contribute to the subsequent neuroadaptations in the reward system that define an individual sensitivity to drug reward and liability to drug abuse (Koob and Le Moal 2001). This impact depends on the initial predisposition to self-administer drugs of abuse, but also on individual differences in brain functioning on a molecular, cellular and system level, interacting with reward processes. A system closely interacting with reward is the stress system. Both drug administration and withdrawal activate central and peripheral stress systems (Sarnyai et al. 2001; Shalev et al. 2002). Short-term administration elevates central

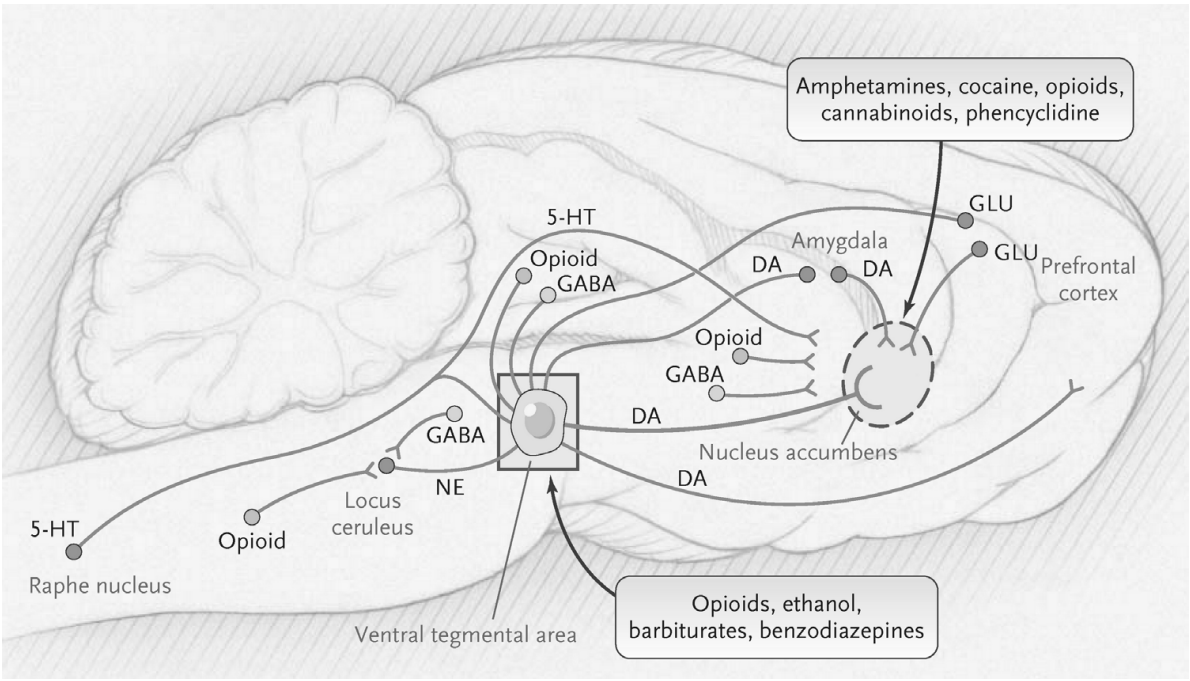


Figure 1.2: Mid sagittal diagram of the rat brain showing neural reward circuits important in the reinforcing effects of drugs of abuse. Mesocorticolimbic dopamine (DA) systems originating in the VTA include projections from cell bodies of the VTA to the NAcc, amygdala, and PFC; glutamatergic (GLU) projections from the PFC to the NAcc and the VTA; and projections from the g-aminobutyric acid (GABA) neurons of the NAcc to the PFC. Opioid interneurons modulate the GABA-inhibitory action on the VTA and influence the firing of norepinephrine (NE) neurons in the locus ceruleus. Serotonergic (5-HT) projections from the raphe nucleus extend to the VTA and the NAcc. The figure shows the proposed sites of action of the various drugs of abuse in these circuits. *Figure taken from Cami and Farré 2003.*

corticotropin-releasing factor levels and peripheral glucocorticoid levels. These hormonal elevations have been related to the rewarding properties of drug use (for review Sarnyai et al. 2001; Marinelli and Piazza 2002). During withdrawal, an increase in corticotropin-releasing factor in the amygdala has been related to stress and negative effects of abstinence (Shalev et al. 2002). Inversely, stress has been shown to facilitate drug intake and human studies suggest that exposure to (early) life stressors is an environmental risk factor for the development of addiction.

An interaction between the reward and stress systems in drug addiction was conceptualized by Koob and le Moal (2001). They have postulated the concept of an allostatic state of the reward system. Allostasis has been defined as the ability to re-establish homeostasis through change. It is based on neuroplasticity allowing the brain to constantly adapt in structure and function to environmental demands. In the concept of Koob and le Moal this allostatic process could lead to an allostatic state of the reward system. This state represents a chronic deviation from the normal operating level that could more easily evolve into a state of dependence and relapse. This dysregulation is thought to be fuelled by maladaptive changes in the stress system. The extended amygdala (EA), a macro anatomical structure within brain reward circuitry (Heimer 2003; Alheid 2003), is hypothesised by these authors form the central point where the stress and reward systems interact (Koob and Le Moal 2001; Koob 2003). The extended amygdala encompasses the central and me-

dial nuclei of the amygdala and the bed nucleus of the stria terminalis (BNST), and forms a continuum with the shell of the NAcc. Each of these regions shares certain cytoarchitectural and circuitry similarities (Heimer and Alheid 1991). Although the shell of the NAcc, like the core, is part of the ventral striatum, it also has features that are reminiscent of the extended amygdala (e.g. opiate and dopamine receptor binding properties). These features lead to an anatomical and functional distinction of both parts of the NAcc (Heimer 2003). It is the dopamine activity in the shell of the NAcc that has shown to be involved in the reinforcing properties of drugs of abuse (Marinelli and Piazza 2002).

The extended amygdala components are highly interconnected by long associative pathways, and have prominent projections to autonomic and somatomotor centers in the lateral hypothalamus and brainstem (central division) and to the endocrine-related medial hypothalamus (medial division). The extended amygdala, like the ventral striatum, receives input primarily from nonisocortical regions of the greater limbic lobe, including the

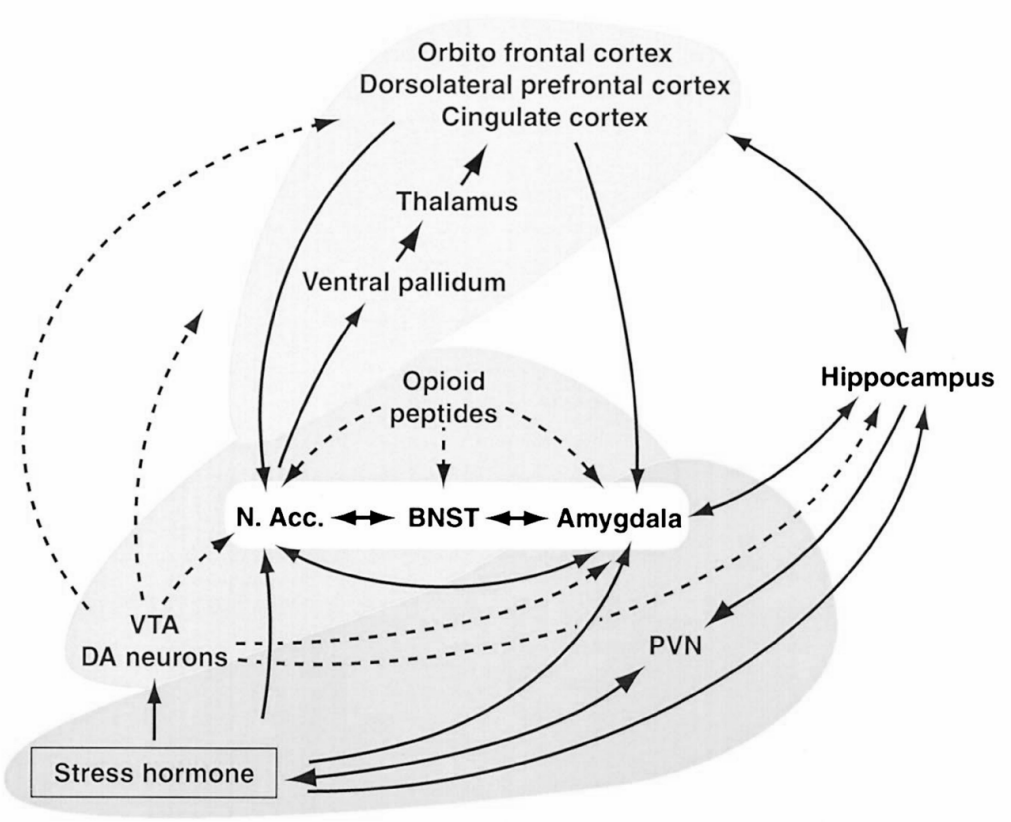


Figure 1.3: The extended amygdala as a central point for the interaction between the stress and the reward circuit. Elements of the NAcc, BNST and central nucleus of the amygdala have cytoarchitectural, circuit and functional similarities that have been hypothesized to form a key component of the brain reward circuit. Critical neurochemical components of the brain reward circuit include the mesolimbic DA system and opioid peptides (dotted lines). The HPA stress axis and brain stress systems would fuel dysregulation within the brain reward system, whereas the ventral cortico-striatal-pallidal-thalamic loop circuit expresses and consolidates the dysregulation of reward. This latter circuit originates in the prefrontal cortex area, runs through the ventral striatal-pallidal system and relays in the medial dorsal nucleus of the thalamus (Heimer 2003). BNST: bed nucleus of the stria terminalis, DA: dopamine, N. Acc.: nucleus accumbens, PVN: paraventricular nucleus, VTA: ventral tegmental area. *Figure taken from Koob & Le Moal 2001*.

lateral basal cortical amygdala. Although heavy interconnections between the central and medial amygdalar nuclei, caudal substantia innominata and BNST are widely recognized, the concept of the EA however, has met with some resistance (Dong et al. 2001; Swanson 2003). Nevertheless, its components play key roles in drug reward and interact with the stress system *(figure 1.3)* (Koob and Le Moal 2001; Koob 2003).

1.2.3 Environmental stress and drug self-administration

a) Stress response and the HPA axis

Environmental stressors (either physical or psychological) trigger a cascade of reactions in the body in order to deal with the threatening situation. The severity of a stressor is judged by limbic brain structures (including the hippocampus, amygdala and PFC) and subsequently, there is a surge in arousal, vigilance, focused attention and cognitive processes. This 'alert' state is made possible by the rapid activation of the sympathetic nervous system, that leads to the release of noradrenaline from widely distributed synapses and adrenaline from the adrenal medulla and activation of the HPA axis which leads to the release of corticosteroids from the adrenal cortex. This allows a rapid increase in blood flow to the central nervous system and muscles, mobilization of lipid and glucose reserves and suppression of immune and reproductive functions. Past the stressful situation, the body regains its normal functioning. These reactions are crucial to the survival of the organism, but might become maladaptive, if they are triggered too often or for a too long time, like when stress is chronic and uncontrollable. Whether the environment is perceived as stressful and uncontrollable and the ability of stressors to cause lasting effects on health and behavior is strongly dependent on individual differences in the reactivity of the systems involved in the stress response. The reactivity of these systems is fine-tuned during development and consequently, stressful situations in developmentally vulnerable periods can have a big impact on later life stress reactivity.

A part of the stress system that has been shown to be particularly sensitive to environmental stimuli is the hypothalamic-pituitary-adrenal (HPA) axis. During a stressful situation, this system is activated by the stimulation of parvocellular cells in the paraventricular nucleus (PVN) of the hypothalamus. Upon stimulation, these cells produce corticotropin releasing hormone (CRH) and vasopressin (AVP) that are released in the hypophyseal portal system, and in turn activate the production and release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary. ACTH, via the bloodstream, arrives in the adrenals, where it stimulates the synthesis and release of glucocorticoids (cortisol in humans and primates, corticosterone in rodents) from the adrenal cortex. Glucocorticoids enable the body to deal with the metabolic demands of a stressful situation by enhancing catabolism, mobilizing lipid and glucose reserves, suppressing the immune system and increasing cardiovascular tone. Secondly, they mediate recovery from stress by reducing HPA axis activity on the level of the PVN (negative feedback). Finally, they affect emotional responses and promote cognitive processes, serving behavioral adaptation to stressful situations. However, the inability to cope with life events, which leads to the excessive secretion of corticosteroids, leads to damage and increases the risk for mental disorders like depression, as well as increased abdominal obesity, osteoporosis and cardiovascular problems (for review de Kloet et al. 2005).

b) Stress and drug intake

Much research has been devoted to the interactions between stress and drug intake. The effect of several stressful experiences on drug reinforcement has been studied, mostly in rats. Stressors such as food restriction, social defeat, electric foot shock, prenatal stress, social isolation and tail pinch, can increase opioid, psychostimulant and alcohol SA in rats (for review Campbell and Carroll 2000; Lu et al. 2003). A quite reliable and potent effect on drug SA is that induced by acute or chronic food deprivation or restriction. Acute food restriction (typically 24h of either deprivation or restriction) or chronic food restriction (several days or weeks of limited access to food) significantly increases acquisition and maintenance of intravenous opiate and psychostimulant SA (Carroll et al. 1979; Carroll 1999). Food deprivation does not produce a typical shift to the left in the dose-response curve, but rather induces an upward shift in the curve across a wide dose range. Changes in drug efficacy due to environmental manipulations are likely to be reflected as an upward shift in the dose-response curve, rather than a shift to the left (Piazza et al. 2000). It is not clear whether the stress-like physiological effects of food restriction (e.g. activation of HPA axis) are involved in the observed changes on drug SA. Carroll and Meisch (1984) conclude that the basic mechanism is rather a 'reinforcer interaction', whereby decreased availability or non-availability of one reinforcer increases responding maintained by another. So the effects of food restriction on drug reward seem to form a separate category of stress-induced increase of drug reinforcement. Next to strong environmental stressors, environmental context in which a drug is administered can also influence cocaine SA in the rat (Caprioli et al. 2007). In this latter study, novelty (*vs.* habituation) of the SA cage, which can be considered as an arousing event rather than a stressful situation, was shown to facilitate acquisition of cocaine SA at low doses.

Despite many years of research, the neuronal mechanisms involved in the modulation of the effect of different stressors on drug SA are largely unknown, although corticosterone is shown to be involved (for review Piazza and Le Moal 1996; Goeders 1997; 1997; Marinelli and Piazza 2002). Inhibition of circulating corticosterone by adrenalectomy or synthesis inhibitors of the hormone decreases intravenous cocaine SA in rats (for review Piazza and Le Moal 1996; Goeders 1997; 1997), an effect that was dose-dependently reversed by corticosterone replacement (Deroche et al. 1997b). In addition, as observed for stress, glucocorticoid hormones have a facilitatory role on behavioral responses to psychostimulant drugs such as locomotor activity, SA and relapse (for review Marinelli and Piazza 2002). These behavioral effects of glucocorticoids were shown to involve an action on the meso-accumbens dopamine system, and to be state dependent (Piazza et al. 1996; Cho and Little 1999), that is, corticosterone can only increase NAcc dopamine if the hormone is administered in conditions in which the dopamine system is activated. Moreover, cocaine stimulates the HPA axis through hypothalamic CRF secretion (Sarnyai et al. 1993). Glucocorticoids are most likely a component of both the endogenous reward system and the stress system. Its primary role in the reward system would be to 'energize' goal-directed behaviors and their action on the reward system during the stress response would reduce the aversive effects of stress and thus increase coping capacities (Marinelli and Piazza 2002). Indeed, glucocorticoids, in the range of concentrations induced by stress, have posi-

tive reinforcing effects (Piazza and Le Moal 1997). The role of these hormones in drug abuse is probably related to the long-lasting sensitization they induce in the reward system when they are repeatedly activated during stress.

c) Gene-environment interactions in drug intake

Not all individuals are equally sensitive to the effects of stress on drug intake. There are some rodent studies (especially on alcohol intake) pointing to gene-environment interactions in the effect of environmental stressors on drug SA. The effect of adult food restriction and isolation housing on alcohol intake has been shown to be strain dependent (Schroff et al. 2004; Ehlers et al. 2007) and the impact of maternal separation on alcohol intake can depend on genetic background (Roman et al. 2005). In these studies, environmental stressors seem to influence alcohol intake preferentially in alcohol preferring strains: Isolation housing and maternal separation increased limited access drinking only in high drinking alcohol-preferring rats (Roman et al. 2005; Ehlers et al. 2007) and food deprivation increased alcohol intake in the C57BL/6 strain (high alcohol preference), but not in the DBA/2 strain (low alcohol preference) (Schroff et al. 2004). Studies on gene-environment interactions in psychostimulant drug SA are rare. To my knowledge there is only one study that tried to address this issue by showing a differential influence of 'lights off during acquisition' on cocaine intake in two rat lines selected for their behavioral response to apomorphine (van der Kam et al. 2005). In this study, 'lights off' was defined as a stressful situation that took place during the light phase. Clearly, evidence is limited and additional animal models of gene-environment interactions are needed to study the biological processes underlying individual differences in drug taking behavior.

Several inbred strains of mice have been shown to differently self-administer drugs abused by humans, indicative of a genetic component in drug use in rodents (Meliska et al. 1995; Grahame and Cunningham 1995; Grahame and Cunningham 1997; Crawley et al. 1997; Deroche et al. 1997a; Risinger et al. 1998; Rocha et al. 1998; Stolerman et al. 1999; David et al. 2001). Two of the most widely used inbred strains, the C57BL/6 (C57) and DBA/2 (DBA) strain, are reported to react particularly differently to most drugs of abuse, both after forced injections (Womer et al. 1994; Zocchi et al. 1998; Orsini et al. 2005) and in several SA paradigms (Grahame and Cunningham 1995; Rocha et al. 1998; Kuzmin and Johansson 2000). Both strains acquire non-restrained intravenous SA of cocaine, but a higher demand for cocaine was found in C57 mice (Grahame and Cunningham 1995; Rocha et al. 1998). Also faced to solutions of morphine, alcohol and cocaine in a two bottle choice, C57 showed a higher preference for all drugs compared to DBA (Horowitz et al. 1977; Morse et al. 1993; Belknap et al. 1993a; Belknap et al. 1993b; Meliska et al. 1995; Risinger et al. 1998). Using these inbred strains, Cabib et al. (2000) nicely showed a gene-environment interaction in rewarding behavior of amphetamine as measured in a CPP test. In this study, the initial aversive response of DBA mice to amphetamine in a CPP test was reversed to a preference after having experienced a short period of food restriction. In contrast, C57 mice kept their initial preference for the amphetamine coupled environment. This study formed an important motivation to use these two mouse strains in our experiments.

1.3 Early life environment

1.3.1 Brain development

The early life period is a critical and sensitive period of brain development. The brain develops in multiple stages (cell birth, differentiation and migration of neurons to their final location), axons and dendrites branch and form important synaptic connections that set the stage to encoding information potentially for the rest of life. Once the initial phase of innervation has occurred in the prenatal period, approximately 50% of all neurons are eliminated during the period immediately before birth, in a process known as programmed cell death or apoptosis. During this period, dramatic morphological rearrangements occur with the hypothesized goal to increase efficiency of synaptic transmission (Purves and Lichtman 1980).

The postnatal period is an important period of adaptation of systems to environmental demand. Different brain regions have a unique course of ontogeny, and late developing structures, including cortex, hippocampus and the cerebellum, set the stage for differential periods of vulnerability in a regionally specific matter (Andersen 2003). The dopamine system, that plays an important role in reward, changes importantly during the postnatal period. At birth dopaminergic markers, including tyrosine hydroxylase activity, dopamine uptake sites, and dopamine content, are at approximately 10% of levels found in the adult rat (Broaddus and Bennett 1990). These markers increase importantly during postnatal life to attain adult levels between 28 and 35 days in the rat. Firing rates of nigrostriatal neurons increase gradually (Pitts et al. 1990; Tepper et al. 1990) and dopamine D1 and D2 receptor density increases in a linear fashion during the first 4 weeks of life before reaching their adult-like density (Murrin and Zeng 1986; Rao et al. 1991).

Stress responsiveness also dramatically changes during development. Stress-mediated changes within the dopamine system have a wider magnitude of effect during development, showing an important neuronal activation in NAcc, compared to adulthood, where PFC activation is predominant (Lyss et al. 1999). HPA axis responses to stress importantly change during development. While fetal rats have high basal blood levels of corticosterone and respond to multiple stressors, there exists a time window in the first two weeks of postnatal life where corticosterone levels are low and the system is hypo-responsive to mild stressors; only severe physiological or psychological stressors can provoke prolonged activation of the HPA axis. In rodents, this period of low stress system activity is known as stress hypo-responsive period (SHRP), and lasts depending on the species (rat or mouse) for about the first two weeks following birth (Schapiro et al. 1962; Levine 1970; Sapolsky and Meaney 1986; Schmidt et al. 2003). This relative inactivity of the adrenocortical axis can be viewed as adaptive, since it limits catabolic processes initiated by circulating glucocorticoids and favors the predominating anabolic events (de Kloet et al. 1988). In the third week of life, corticosterone basal levels rise and animals show an ACTH and corticosterone response to a mild novelty stressor. The SHRP is believed to be of vital importance for normal development of the brain and HPA axis, and possibly the dopamine system. Indeed, stressful experiences in this period, like long separations from the mother,

can counteract this hypo-responsivity of the HPA axis (Levine 2001) and induce long-term changes in adult stress responsiveness. Moreover, manipulations lowering the effect of the long term maternal separation on HPA-axis activity during the SHRP, can reverse the long-term changes in stress reactivity (Suchecki et al. 1993; van Oers et al. 1998; van Oers et al. 1999).

1.3.2 Early life manipulations and maternal behavior

Early life manipulations in the developmentally critical perinatal period, can affect the adult phenotype and the lifetime vulnerability to disease. In humans, high levels of early life stress (e.g. loss of a parent, parental neglect or abuse, or a depressed parent), can impact on the long-term neurobiological and psychosocial development of the offspring and markedly increase vulnerability to the development of psychiatric disorders like anxiety and depressive disorders and substance abuse (Sameroff 2000; De Bellis 2002; Gordon 2002; Heim et al. 2004)

The importance of the early days of life has also been extensively shown in animal studies. Models based on the impact of disrupted maternal care show marked chronic effects on neurobiology, physiology and behavior in adulthood and findings bear striking similarities to findings for stress-related illnesses in humans, including major depression (Newport et al. 2002; Matthews and Robbins 2003; Pryce et al. 2005). Research on the chronic effects of postnatal manipulation of the infant–mother relationship started half a century ago with the pioneering rat studies by Seymour Levine (Levine et al. 1956; Levine 1957; 1960; 1962) and Victor Denenberg (Denenberg and Smith 1963; Denenberg et al. 1967; DeNelsky and Denenberg 1967). The first postnatal manipulation model that was investigated in detail in the laboratory rat was the comparison between early handling (EH) and early non-handling (NH) (Levine et al. 1956; Levine 1960). In these studies, EH was defined as the experimenter picking up the pup, removing it from the breeding cage and isolating it in a small compartment for several minutes, repeated across days between birth and weaning. NH was defined as the complete absence of handling (both experimental and husbandry related) in the breeding cage between birth and weaning. As adults, early handled rats were more active, explored more, and defecated and urinated less in the open field. They exhibited a lower plasma corticosterone response to stressors like injection or placement in water and demonstrated a more rapid maximal corticosterone response to electro-foot shock followed by a more rapid return to baseline levels. These initial studies with the EH-NH model on anxiety like behavior and hormonal stress response suggested a reduced activation of the sympathetic branch of the autonomic nervous system and altered activity of the limbic HPA system.

The long-term changes associated with EH have since been replicated and completed by many laboratories, and most extensively by a series of studies by Michael Meaney, Paul Plotsky and colleagues (for review Meaney 2001; Pryce and Feldon 2003). It was demonstrated that EH relative to NH show a reduced plasma corticosterone and ACTH stress response, reduced CRF expression in the hypothalamus and increased binding capacity of glucocorticoid receptors (GRs) in hippocampus (negative feedback) (Meaney et al. 1989; Plotsky and Meaney 1993). Handling was shown to have the strongest effect when administered during the first week of life, with minimal effect when pups are handled after

the first 2 postnatal weeks (Meaney and Aitken 1985). Their work has led to the apparition of the maternal separation (MS) model. This model, that consists of separating the pups from the mother for a longer period of time (with protocols varying widely between laboratories from 1h up to 24h of separation over 1 to 14 or even 21 postnatal days), demonstrated often, but not always, opposite effects to EH, that is, increases in anxiety related behavior and hyperactivity of the HPA axis in response to stressors (for review Ladd et al. 2000; Pryce and Feldon 2003; Holmes et al. 2005). Moreover, after prolonged MS (24h) during the SHRP, the HPA axis of the pups was shown to be activated and responded to mild stressors (Stanton et al. 1988; Pihoker et al. 1993; Schmidt et al. 2004).

Although handling was classically defined as a short period of isolation (from both mother and littermates), the term is thereafter also used to define a short period of separation (3-15min) of the litter from the mother, often used as a control group of MS. When litters are maternally separated for example for 15 min, this group is often named MS15 and referred to as the 'handled' group. Mid-long periods of MS (1 to 6 hours) are usually called 'maternal separation', while the long periods of MS (24h) are often described as 'maternal deprivation'. The terms 'early isolation' and 'early deprivation' are employed to define periods of complete isolation from both mother and littermates, these periods can also range from 1h to 24h. Altogether, there are many forms of manipulation that are currently in use and a large number of potentially very important variables (duration and time window of manipulation, control group, rearing conditions) are being manipulated differently in the many laboratories active in this area. The nature of the control group is also an important point of discussion (Levine 1960; Pryce and Feldon 2003). It was argued that NH rats (complete absence of any human interference) represents a 'non-manipulation' compared to the manipulation in EH, rather than a control group for the MS. Moreover, NH animals would be below the minimal amount of stress and/or stimulation needed in order to develop into adult rats that exhibit a behavioral profile typical for laboratory rats (Weiner et al. 1987). Thus, the animal facility rearing (AFR) group was introduced, that experiences at least the human interventions that come with cage cleaning. Thus, this research area has become quite complex, both conceptually and methodologically (Pryce and Feldon 2003).

Apart from using very different protocols, these early life manipulations have in common that they interfere with maternal care. The mother plays an important role in the early environment of the neonatal rat pup, providing protection, food, warmth and grooming necessary for immediate survival and transmitting environmental demands by changes in behavior and milk constitution (energetic value and hormones) thereby regulating the development of systems underlying adult behavior and physiology (Hofer 1994). In the course of normal mother-pup interactions, the dam is regularly away from the nest for periods of 20-30 min (Jans and Woodside 1990). Thus the handling manipulation does not result in an abnormal period of separation or loss of maternal care. However, handling was found to stimulate an increase in pup licking, not only upon reunion of mother and pups, but during the whole following day-night cycle. Based on this observation it was hypothesized that under normal conditions maternal care actively contributes to the development of neural systems that mediate stress responses (for review Meaney 2001).

Indeed, individual differences in spontaneous frequency of licking/grooming (LG) pups and arched-back nursing (ABN) predicted behavioral outcome of offspring in adult-

hood. ABN is a position where the mother is crouched over the pups in an active position with the pups attached to her nipples. The offspring of the 25% highest LG-ABN mothers showed an EH like phenotype (Liu et al. 1997). This was shown for neurochemical traits (e.g. increased central benzodiazepine receptor density in the amygdala and locus coeruleus; decreased CRF receptor density in the locus coeruleus), neuroendocrine traits (e.g. increased hippocampal GR mRNA; decreased hypothalamic CRF mRNA; reduced ACTH and corticosterone stress reactivity), and behavioral traits (e.g. increased exploration; reduced novelty-induced suppression of feeding) (for review Meaney 2001). Meaney and colleagues further demonstrated that individual differences in stress reactivity induced by maternal programming are transmitted across generations (Francis et al. 1999), and that the down regulating effects of high levels of LG-ABN might be mediated by epigenetic changes in gene expression (Weaver et al. 2004).

Disruption of normal maternal behavior appears to contribute to the effects of MS in rats. A reversal of the effects of MS on later stress hyper-reactivity was established by artificially stroking (anogenital stroking to enable urination and defecation) and feeding rat pups during the separation period (Suchecki et al. 1993; van Oers et al. 1998; van Oers et al. 1999). Blocking plasma glucocorticoid release did not have the same effect indicating that corticosterone is not solely responsible for the effects of MS. In addition, a recent study found that if dams were provided with a foster litter during the period of separation from their own pups, the offspring did not subsequently exhibit hyper emotionality, suggesting that the long term consequences of MS on rat pups stem in part from the stressful effects of the manipulation on the mother (Huot et al. 2004). But, although very important, it is argued that maternal care is not the only factor determining stress reactivity (Macri and Wurbel 2006; Parker et al. 2006). Maternal care is sensitive to different forms of environmental challenge and both EH and MS stimulate maternal care (Liu et al. 1997; Pryce et al. 2001; Macri et al. 2004). Moreover, in primates, brief intermittent infant stress induces neuroendocrine stress resistance, without changing maternal care (Parker et al. 2006). This raises the possibility that environmental stress imposed by adverse environmental conditions (e.g., long mother offspring separations) affects the development of the HPA system in the pups independently of the amount of maternal care received, and that the resulting phenotype in the offspring will depend on the relative weight of these two factors (Macri and Wurbel 2006).

Overall, the mother can be seen as the most important environment that a rodent encounters during its (early) life period and changes in maternal care can have a major impact on adult phenotype.

1.3.3 Early life experience and drug use in rodents

Although not very extensive, there is growing evidence in rodents that early life stress disrupts the development of neural systems mediating reward related behaviors. The first observations in this field showed that prenatal stress (restraint of the mother during the last week of pregnancy) induced higher amphetamine SA in adulthood (Deminiere et al. 1992), facilitated amphetamine-induced sensitization and induced long-lasting changes in dopamine receptors in the NAcc (Henry et al. 1995). A recent study using this model found no influence of prenatal stress on the acquisition of cocaine SA, but prenatally stressed

animals showed a higher cocaine-induced reinstatement and differences in basal and drug-induced dopamine and glumate levels in the NAcc (Kippin et al. 2008). Certain prenatal stress effects were shown to be reversed by adoption (Maccari et al. 1995) and modified maternal behavior was supposed to be of influence in this effect. This observation led to a focus on the behavioral impact of postnatal manipulations on adult behavior. It was shown that handling attenuated CPP for amphetamine (Campbell and Spear 1999) and decreased sensitivity to the locomotor stimulating effects of cocaine (Meaney et al. 2002). Maternally separated animals showed stress induced sensitization to the effects of amphetamine on locomotor activity (while EH and NH did not) and showed lower DA transporter levels in NAcc and caudate putamen (Meaney et al. 2002). Dopamine receptor binding and sensitivity was either affected (Ploj et al. 2003a; Kosten et al. 2005) or not affected (Matthews et al. 1999; Meaney et al. 2002) by MS and alterations in the opioid peptide systems were seen (Ploj et al. 2003b). After a single 24h maternal deprivation on postnatal day 3 (PD3), rats showed an enhanced apomorphine induced stereotypic gnawing in adulthood, which can be seen as a measure for functional activity of the dopamine system (Rots et al. 1996).

MS generally increases voluntary ethanol consumption, but only at moderate to relatively high doses (8-10 % solutions) (Huot et al. 2001; Ploj et al. 2003a; Roman et al. 2005) and depending on strain and gender (Roman et al. 2004; Roman et al. 2005). A 24h maternal deprivation on PD14 did not influence alcohol intake (Vazquez et al. 2002), but the same study showed that repeated injections over PD7-13 (chronic intermittent injection stress) enhanced intake. Neither cross-fostering nor maternal deprivation influenced the voluntary alcohol intake in two ratlines selectively bred for high susceptibility to apomorphine (Sluyter et al. 2000). MS induced CPP for morphine at lower doses and increased oral morphine intake (Vazquez et al. 2005).

Neonatal isolation (1h/day from PD2-PD9) has been shown to induce acquisition of cocaine SA at lower doses of the drug in males, and to increase cocaine intake in a dose response study in females (Kosten et al. 2000; Kosten et al. 2006). Under extended access conditions of a high cocaine dose (24h access, 4-10min trials/h, 1.5 mg/kg), isolation (1h/day from PD2-PD12) did not alter cocaine intake, but isolated rats responded at higher levels during both extinction and cue-induced reinstatement testing (Lynch et al. 2005). Either handling or a 10 min. aversive stimulation (alternating bright light, low temperature and noise) from PD1-PD10 increased initial preference for oral cocaine and promoted changes in intake pattern (Marquardt et al. 2004). MS of 3h/day (MS180) induced cocaine SA at lower doses of the drug (Moffett et al. 2007), while animals undergoing long periods of maternal separation (6h) tend to self administer less cocaine (Matthews et al. 1999).

Taken together, studies on this topic are very diverse and no clear picture emerges yet concerning the effect of postnatal manipulation on drug SA and reinforcement.

1.4 Scope and outline of the thesis

1.4.1 Rationale and objective

Individual differences in drug taking behavior and liability to develop drug dependence are clearly observed, but the underlying mechanism is still poorly understood. A role for gene-environment interactions has been proposed but remains mainly hypothetical and difficult to study in humans. Pertinent animal models that could help to understand how genes and environment interact and how vulnerability or resilience to psychopathology emerge are much needed.

The objective of the research described in this thesis is to demonstrate the role of gene-environment interactions in the emergence of individual differences in cocaine use. For this purpose I focus on the impact of early life experience as well as a later life psychosocial stressor with the goal to create a mouse model that enables the study of the biological basis underlying such individual vulnerabilities.

To achieve this objective studies are designed:
i. To test the hypothesis that the impact of early life environmental factors on adult cocaine taking behavior depends on the genetic background of the individual.
ii. To test the hypothesis that a short-lasting stressful experience in later life can differentially affect cocaine taking behavior, depending on the genetic background of the individual.
iii. To examine basal changes in gene expression in drug-relevant brain structures of adult individuals that display profound individual differences in changing their cocaine intake after environmental experiences in early or adult life.

1.4.2 Experimental approach

The impact of early life environmental factors and a short lasting stressful experience during adulthood on cocaine taking behavior was studied in two inbred mouse strains: C57BL/6 and DBA/2. These strains differ in the behavioral responsiveness to both addictive drugs and stressors and also differ in the anatomy and functioning of the mesocorticolimbic dopamine system, an important brain target of cocaine, involved in its reinforcing properties.

To study the impact of early life factors, without profoundly disturbing mother and offspring by human intervention, the maternal environment of the mice was manipulated by cross-fostering to non-related mother strains showing either high or low pup-oriented behavior *(figure 1.4 left panel)*. Since very little is known about the spontaneous maternal behavior of inbred mouse strains, we performed a pilot study (not further detailed in this thesis) with dams of four different strains, that were chosen based on their pup retrieval behavior as measured by Carlier et al. (1982). In this pilot study, we analyzed the spontaneous maternal behavior of the dams when raising either C57 or DBA pups. Dams of the AKR and C3H/HeN (C3H) strain represented the most extreme mothers in terms of maternal care and were chosen to serve as maternal environments in our experiments. To study the

impact of an adult environmental experience, adult C57 and DBA mice were transiently group housed with same sex, same strain animals *(figure 1.4 right panel)*.

First, mice from both experimental set-ups (early life and late life), were tested on intravenous SA of cocaine. Then, changes in gene expression in the extended amygdala were analyzed in (drug-naïve) animals from both experimental set-ups by micro-array hybridization and quantitative PCR.

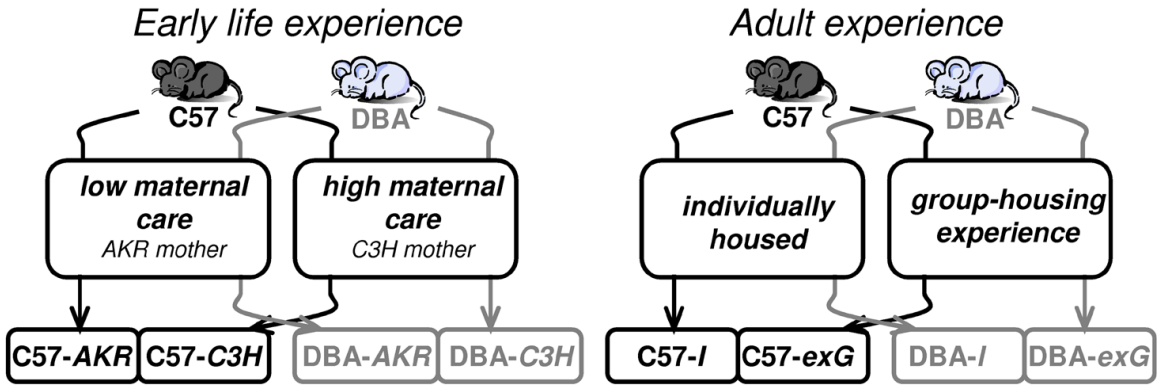


Figure 1.4: Experimental approach. Mice of the C57BL/6 and DBA/2 mice strain were either exposed to different early life environments (left panel) or a group housing experience in adulthood (right panel).

1.4.3 Outline of the thesis

Chapter 2 gives a detailed characterization of mouse maternal behavior under normal and different cross-fostering conditions. First, we analyzed the spontaneous maternal behavior of C57 and DBA dams toward their biological offspring. Second, C57 and DBA mice were cross-fostered to a mother of the same strain (*intra*-strain cross-fostering), and the maternal behavior of C57 and DBA foster dams was analyzed. Finally, C57 and DBA mice were cross-fostered to non-related mothers, coming from the AKR and C3H strain (*inter*-strain cross-fostering). Spontaneous maternal behavior of these non-related mothers with both foster pups was analyzed.

Chapter 3 describes the impact of early life experiences on cocaine taking behavior in adult C57 and DBA mice. Mice were tested on intravenous cocaine SA after *inter*-strain cross-fostering to a mother showing either high (C3H mother) or low (AKR mother) pup-oriented behavior. Acquisition of cocaine SA and a dose-response study are shown. Next to this, a forced swim test and an elevated plus maze were performed to test for motivational and anxiety aspects in the behavior of the mice.

Chapter 4 describes the impact of a past short-lasting adult experience on cocaine taking behavior in C57 and DBA mice. Adult mice were group housed for 19 days, and intravenous cocaine SA was conducted one week after the end of group housing. Acquisition of cocaine SA and a dose-response study are shown for both ex-group housed and control animals. To control for possible alterations in cocaine metabolism, a second experiment

was performed. Here, one week after the end of group housing, total brain cocaine levels were measured after a single programmed intravenous cocaine infusion.

Chapter 5 reports on basal changes in gene expression in a drug-relevant brain structure of adult C57 and DBA mice, after environmental experiences in early or adult life. Gene expression profiles in the extended amygdala were studied in pooled samples using Affimetrix micro-arrays and confirmed on individual samples with quantitative PCR.

The results obtained are summarized and discussed in **chapter 6**.

References

Alheid GF (2003) Extended amygdala and basal forebrain. Ann N Y Acad Sci 985: 185-205

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th ed. Washington, D.C.: American Psychiatric Association edn.

Andersen SL (2003) Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci Biobehav Rev 27: 3-18

Bardo MT, Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology (Berl) 153: 31-43

Belknap JK, Crabbe JC, Riggan J, O'Toole LA (1993a) Voluntary consumption of morphine in 15 inbred mouse strains. Psychopharmacology (Berl) 112: 352-358

Belknap JK, Crabbe JC, Young ER (1993b) Voluntary consumption of ethanol in 15 inbred mouse strains. Psychopharmacology (Berl) 112: 503-510

Bonci A, Bernardi G, Grillner P, Mercuri NB (2003) The dopamine-containing neuron: maestro or simple musician in the orchestra of addiction? Trends Pharmacol Sci 24: 172-177

Brady JV (1991) Animal models for assessing drugs of abuse. Neurosci Biobehav Rev 15: 35-43

Broaddus WC, Bennett JP (1990) Postnatal development of striatal dopamine function. I. An examination of D1 and D2 receptors, adenylate cyclase regulation and presynaptic dopamine markers. Brain Res Dev Brain Res 52: 265-271

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

Cadoret RJ, Yates WR, Troughton E, Woodworth G, Stewart MA (1995) Genetic-environmental interaction in the genesis of aggressivity and conduct disorders. Arch Gen Psychiatry 52: 916-924

Cami J, Farre M (2003) Drug addiction. N Engl J Med 349: 975-986

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

Campbell UC, Carroll ME (2000) Acquisition of drug self-administration: environmental and pharmacological interventions. Exp Clin Psychopharmacol 8: 312-325

Caprioli D, Paolone G, Celentano M, Testa A, Nencini P, Badiani A (2007) Environmental modulation of cocaine self-administration in the rat. Psychopharmacology (Berl) 192: 397-406

Carroll ME (1999) Interaction between food and addiction. In: Niesnik RJM, Hoefakker RE, Westera W, Jaspers RMA, Kornet LMW, Boobis S (eds) Neurobiobehavioral toxicology and addiction: food, drugs and environment. Boca Raton: CRC Press, pp 286-311

Carroll ME, France CP, Meisch RA (1979) Food deprivation increases oral and intravenous drug intake in rats. Science 205: 319-321

Carroll ME, Meisch RA (1984) Increased drug-reinforced behavior due to food deprivation. In: Thompson T, Dews PB, Barrett JE (eds) Advances in behavioral pharmacology. New York: Academic Press, pp 47-88

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A et al. (2002) Role of genotype in the cycle of violence in maltreated children. Science 297: 851-854

Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 7: 583-590

Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A et al. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry 57: 1117-1127

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J et al. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301: 386-389

Cervilla JA, Molina E, Rivera M, Torres-Gonzalez F, Bellon JA, Moreno B, Luna JD et al. (2007) The risk for depression conferred by stressful life events is modified by variation at the serotonin transporter 5HTTLPR genotype: evidence from the Spanish PREDICT-Gene cohort. Mol Psychiatry 12: 748-755

Charney DS (2004) Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. Am J Psychiatry 161: 195-216

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

Cicchetti D, Blender JA (2006) A multiple-levels-of-analysis perspective on resilience: implications for the developing brain, neural plasticity, and preventive interventions. Ann N Y Acad Sci 1094: 248-258

Compton WM, Thomas YF, Conway KP, Colliver JD (2005) Developments in the epidemiology of drug use and drug use disorders. Am J Psychiatry 162: 1494-1502

Cooper RM, Zubek JP (1958) Effects of enriched and restricted early environments on the learning ability of bright and dull rats. Can J Psychol 12: 159-164

Crabbe JC, Wahlsten D, Dudek BC (1999) Genetics of mouse behavior: interactions with laboratory environment. Science 284: 1670-1672

Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, Hitzemann RJ et al. (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology (Berl) 132: 107-124

Cutrona CE, Cadoret RJ, Suhr JA, Richards CC, Troughton E, Schutte K, Woodworth G (1994) Interpersonal variables in the prediction of alcoholism among adoptees: evidence for gene-environment interactions. Compr Psychiatry 35: 171-179

David V, Polis I, McDonald J, Gold LH (2001) Intravenous self-administration of heroin/cocaine combinations (speedball) using nose-poke or lever-press operant responding in mice. Behav Pharmacol 12: 25-34

De Bellis MD (2002) Developmental traumatology: a contributory mechanism for alcohol and substance use disorders. Psychoneuroendocrinology 27: 155-170

de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6: 463-475

de Kloet ER, Rosenfeld P, Van Eekelen JA, Sutanto W, Levine S (1988) Stress, glucocorticoids and development. Prog Brain Res 73: 101-120

Deminiere JM, Piazza PV, Guegan G, Abrous N, Maccari S, Le Moal M, Simon H (1992) Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers. Brain Res 586: 135-139

Deminiere JM, Piazza PV, Le Moal M, Simon H (1989) Experimental approach to individual vulnerability to psychostimulant addiction. Neurosci Biobehav Rev 13: 141-147

DeNelsky GY, Denenberg VH (1967) Infantile stimulation and adult exploratory behaviour in the rat: effects of handling upon visual variation-seeking. Anim Behav 15: 568-573

Denenberg VH, Brumaghim JT, Haltmeyer GC, Zarrow MX (1967) Increased adrenocortical activity in the neonatal rat following handling. Endocrinology 81: 1047-1052

Denenberg VH, Smith SA (1963) Effects of infantile stimulation and age upon behavior. J Comp Physiol Psychol 56: 307-312

Deroche V, Caine SB, Heyser CJ, Polis I, Koob GF, Gold LH (1997a) Differences in the liability to self-administer intravenous cocaine between C57BL/6 x SJL and BALB/cByJ mice. Pharmacol Biochem Behav 57: 429-440

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

Dick DM, Agrawal A, Schuckit MA, Bierut L, Hinrichs A, Fox L, Mullaney J et al. (2006) Marital status, alcohol dependence, and GABRA2: evidence for gene-environment correlation and interaction. J Stud Alcohol 67: 185-194

Dong HW, Petrovich GD, Swanson LW (2001) Topography of projections from amygdala to bed nuclei of the stria terminalis. Brain Res Brain Res Rev 38: 192-246

Dumont EC, Mark GP, Mader S, Williams JT (2005) Self-administration enhances excitatory synaptic transmission in the bed nucleus of the stria terminalis. Nat Neurosci 8: 413-414

Ehlers CL, Walker BM, Pian JP, Roth JL, Slawecki CJ (2007) Increased alcohol drinking in isolate-housed alcohol-preferring rats. Behav Neurosci 121: 111-119

Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, Plomin R et al. (2004) Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol Psychiatry 9: 908-915

Foley DL, Eaves LJ, Wormley B, Silberg JL, Maes HH, Kuhn J, Riley B (2004) Childhood adversity, monoamine oxidase a genotype, and risk for conduct disorder. Arch Gen Psychiatry 61: 738-744

Francis D, Diorio J, Liu D, Meaney MJ (1999) Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 286: 1155-1158

Gardner EL (2000) What we have learned about addiction from animal models of drug self-administration. Am J Addict 9: 285-313

Goeders NE (1997) A neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinology 22: 237-259

Goldstein A, Kalant H (1990) Drug policy: striking the right balance. Science 249: 1513-1521

Gordon HW (2002) Early environmental stress and biological vulnerability to drug abuse. Psychoneuroendocrinology 27: 115-126

Grabe HJ, Lange M, Wolff B, Volzke H, Lucht M, Freyberger HJ, John U et al. (2005) Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden. Mol Psychiatry 10: 220-224

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

Grahame NJ, Cunningham CL (1997) Intravenous ethanol self-administration in C57BL/6J and DBA/2J mice. Alcohol Clin Exp Res 21: 56-62

Greenwald MK, Roehrs TA (2005) Mu-opioid self-administration vs passive administration in heroin abusers produces differential EEG activation. Neuropsychopharmacology 30: 212-221

Heim C, Plotsky PM, Nemeroff CB (2004) Importance of studying the contributions of early adverse experience to neurobiological findings in depression. Neuropsychopharmacology 29: 641-648

Heimer L (2003) A new anatomical framework for neuropsychiatric disorders and drug abuse. Am J Psychiatry 160: 1726-1739

Heimer L, Alheid GF (1991) Piecing together the puzzle of basal forebrain anatomy. Adv Exp Med Biol 295: 1-42

Henderson ND (1970) Genetic influences on the behavior of mice can be obscured by laboratory rearing. J Comp Physiol Psychol 72: 505-511

Henderson ND (1976) Short exposures to enriched environments can increase genetic variability of behavior in mice. Dev Psychobiol 9: 549-553

Henry C, Guegant G, Cador M, Arnauld E, Arsaut J, Le Moal M, Demotes-Mainard J (1995) Prenatal stress in rats facilitates amphetamine-induced sensitization and induces long-lasting changes in dopamine receptors in the nucleus accumbens. Brain Res 685: 179-186

Hofer MA (1994) Early relationships as regulators of infant physiology and behavior. Acta Paediatr Suppl 397: 9-18

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

Hooks MS, Duffy P, Striplin C, Kalivas PW (1994) Behavioral and neurochemical sensitization following cocaine self-administration. Psychopharmacology (Berl) 115: 265-272

Horowitz GP, Whitney G, Smith JC, Stephan FK (1977) Morphine ingestion: genetic control in mice. Psychopharmacology (Berl) 52: 119-122

Huot RL, Gonzalez ME, Ladd CO, Thrivikraman KV, Plotsky PM (2004) Foster litters prevent hypothalamic-pituitary-adrenal axis sensitization mediated by neonatal maternal separation. Psychoneuroendocrinology 29: 279-289

Huot RL, Thrivikraman KV, Meaney MJ, Plotsky PM (2001) Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology (Berl) 158: 366-373

Hyman SE, Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci 2: 695-703

Innis NK (1992) Tolman and Tryon. Early research on the inheritance of the ability to learn. Am Psychol 47: 190-197

Jacobs EH, Smit AB, de Vries TJ, Schoffelmeer AN (2003) Neuroadaptive effects of active versus passive drug administration in addiction research. Trends Pharmacol Sci 24: 566-573

Jaffee SR, Caspi A, Moffitt TE, Dodge KA, Rutter M, Taylor A, Tully LA (2005) Nature X nurture: genetic vulnerabilities interact with physical maltreatment to promote conduct problems. Dev Psychopathol 17: 67-84

Jans JE, Woodside BC (1990) Nest temperature: effects on maternal behavior, pup development, and interactions with handling. Dev Psychobiol 23: 519-534

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

Johanson CE, Fischman MW (1989) The pharmacology of cocaine related to its abuse. Pharmacol Rev 41: 3-52

Kaufman J, Yang BZ, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal JH, Gelernter J (2004) Social supports and serotonin transporter gene moderate depression in maltreated children. Proc Natl Acad Sci U S A 101: 17316-17321

Kendler KS (2005) "A gene for...": the nature of gene action in psychiatric disorders. Am J Psychiatry 162: 1243-1252

Kendler KS, Gardner CO, Prescott CA (2002) Toward a comprehensive developmental model for major depression in women. Am J Psychiatry 159: 1133-1145

Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B (2005) The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. Arch Gen Psychiatry 62: 529-535

Kendler KS, Prescott CA, Myers J, Neale MC (2003) The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. Arch Gen Psychiatry 60: 929-937

Kessler RC, Davis CG, Kendler KS (1997) Childhood adversity and adult psychiatric disorder in the US National Comorbidity Survey. Psychol Med 27: 1101-1119

King S, Laplante D, Joober R (2005) Understanding putative risk factors for schizophrenia: retrospective and prospective studies. J Psychiatry Neurosci 30: 342-348

Kippin TE, Szumlinski KK, Kapasova Z, Rezner B, See RE (2008) Prenatal stress enhances responsiveness to cocaine. Neuropsychopharmacology 33: 769-782

Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 13: 177-184

Koob GF (2003) Neuroadaptive mechanisms of addiction: studies on the extended amygdala. Eur Neuropsychopharmacol 13: 442-452

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24: 97-129

Kosten TA, Miserendino MJ, Kehoe P (2000) Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. Brain Res 875: 44-50

Kosten TA, Zhang XY, Kehoe P (2005) Neurochemical and behavioral responses to cocaine in adult male rats with neonatal isolation experience. J Pharmacol Exp Ther 314: 661-667

Kosten TA, Zhang XY, Kehoe P (2006) Heightened cocaine and food self-administration in female rats with neonatal isolation experience. Neuropsychopharmacology 31: 70-76

Krueger RF, Hicks BM, Patrick CJ, Carlson SR, Iacono WG, McGue M (2002) Etiologic connections among substance dependence, antisocial behavior, and personality: modeling the externalizing spectrum. J Abnorm Psychol 111: 411-424

Kuzmin A, Johansson B (2000) Reinforcing and neurochemical effects of cocaine: differences among C57, DBA, and 129 mice. Pharmacol Biochem Behav 65: 399-406

Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM (2000) Long-term behavioral and neuroendocrine adaptations to adverse early experience. Prog Brain Res 122: 81-103

Le Moal M, Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev 71: 155-234

Levine S (1957) Infantile experience and resistance to physiological stress. Science 126: 405

Levine S (1960) Stimulation in infancy. Sci Am 202: 81-86

Levine S (1962) Plasma-free corticosteroid response to electric shock in rats stimulated in infancy. Science 135: 795-796

Levine S (1970) The pituitary-adrenal system and the developing brain. Prog Brain Res 32: 79-85

Levine S (2001) Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. Physiol Behav 73: 255-260

Levine S, Chevalier JA, Korchin SJ (1956) The effects of early shock and handling on later avoidance learning. J Pers 24: 475-493

Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, Williams NM et al. (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. Am J Hum Genet 73: 34-48

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S et al. (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277: 1659-1662

Lu L, Shepard JD, Hall FS, Shaham Y (2003) Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci Biobehav Rev 27: 457-491

Luthar SS, Cicchetti D, Becker B (2000) The construct of resilience: a critical evaluation and guidelines for future work. Child Dev 71: 543-562

Lynch WJ, Mangini LD, Taylor JR (2005) Neonatal isolation stress potentiates cocaine seeking behavior in adult male and female rats. Neuropsychopharmacology 30: 322-329

Lyss PJ, Andersen SL, LeBlanc CJ, Teicher MH (1999) Degree of neuronal activation following FG-7142 changes across regions during development. Brain Res Dev Brain Res 116: 201-203

Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H, Le Moal M (1995) Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J Neurosci 15: 110-116

Macri S, Mason GJ, Wurbel H (2004) Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. Eur J Neurosci 20: 1017-1024

Macri S, Wurbel H (2006) Developmental plasticity of HPA and fear responses in rats: a critical review of the maternal mediation hypothesis. Horm Behav 50: 667-680

Marinelli M, Piazza PV (2002) Interaction between glucocorticoid hormones, stress and psychostimulant drugs. Eur J Neurosci 16: 387-394

Marquardt AR, Ortiz-Lemos L, Lucion AB, Barros HM (2004) Influence of handling or aversive stimulation during rats' neonatal or adolescence periods on oral cocaine self-administration and cocaine withdrawal. Behav Pharmacol 15: 403-412

Mathews CA, Reus VI (2003) Genetic linkage in bipolar disorder. CNS Spectr 8: 891-904

Matthews K, Robbins TW (2003) Early experience as a determinant of adult behavioural responses to reward: the effects of repeated maternal separation in the rat. Neurosci Biobehav Rev 27: 45-55

Matthews K, Robbins TW, Everitt BJ, Caine SB (1999) Repeated neonatal maternal separation alters intravenous cocaine self-administration in adult rats. Psychopharmacology (Berl) 141: 123-134

Meaney MJ (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 24: 1161-1192

Meaney MJ, Aitken DH (1985) The effects of early postnatal handling on hippocampal glucocorticoid receptor concentrations: temporal parameters. Brain Res 354: 301-304

Meaney MJ, Aitken DH, Viau V, Sharma S, Sarrieau A (1989) Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. Neuroendocrinology 50: 597-604

Meaney MJ, Brake W, Gratton A (2002) Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? Psychoneuroendocrinology 27: 127-138

Meliska CJ, Bartke A, McGlacken G, Jensen RA (1995) Ethanol, nicotine, amphetamine, and aspartame consumption and preferences in C57BL/6 and DBA/2 mice. Pharmacol Biochem Behav 50: 619-626

Moffett MC, Vicentic A, Kozel M, Plotsky P, Francis DD, Kuhar MJ (2007) Maternal separation alters drug intake patterns in adulthood in rats. Biochem Pharmacol 73: 321-330

Moffitt TE, Caspi A, Rutter M (2006) Measured gene-environment interactions in psychopathology. Perspect Psychol Sci 1: 5-27

Morse AC, Erwin VG, Jones BC (1993) Strain and housing affect cocaine self-selection and open-field locomotor activity in mice. Pharmacol Biochem Behav 45: 905-912

Murrin LC, Zeng W (1986) Postnatal ontogeny of dopamine D2 receptors in rat striatum. Biochem Pharmacol 35: 1159-1162

Newport DJ, Stowe ZN, Nemeroff CB (2002) Parental depression: animal models of an adverse life event. Am J Psychiatry 159: 1265-1283

O'Brien CP, Gardner EL (2005) Critical assessment of how to study addiction and its treatment: human and non-human animal models. Pharmacol Ther 108: 18-58

Orsini C, Bonito-Oliva A, Conversi D, Cabib S (2005) Susceptibility to conditioned place preference induced by addictive drugs in mice of the C57BL/6 and DBA/2 inbred strains. Psychopharmacology (Berl) 181: 327-336

Parker KJ, Buckmaster CL, Sundlass K, Schatzberg AF, Lyons DM (2006) Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. Proc Natl Acad Sci U S A 103: 3000-3005

Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 245: 1511-1513

Piazza PV, Deroche-Gamonent V, Rouge-Pont F, Le Moal M (2000) Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. J Neurosci 20: 4226-4232

Piazza PV, Le Moal M (1996) Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annu Rev Pharmacol Toxicol 36: 359-378

Piazza PV, Le Moal M (1997) Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Res Brain Res Rev 25: 359-372

Piazza PV, Rouge-Pont F, Deroche V, Maccari S, Simon H, Le Moal M (1996) Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. Proc Natl Acad Sci U S A 93: 8716-8720

Pihoker C, Owens MJ, Kuhn CM, Schanberg SM, Nemeroff CB (1993) Maternal separation in neonatal rats elicits activation of the hypothalamic-pituitary-adrenocortical axis: a putative role for corticotropin-releasing factor. Psychoneuroendocrinology 18: 485-493

Pitts DK, Freeman AS, Chiodo LA (1990) Dopamine neuron ontogeny: electrophysiological studies. Synapse 6: 309-320

Ploj K, Roman E, Nylander I (2003a) Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. Neuroscience 121: 787-799

Ploj K, Roman E, Nylander I (2003b) Long-term effects of short and long periods of maternal separation on brain opioid peptide levels in male Wistar rats. Neuropeptides 37: 149-156

Plomin R, DeFries JC, Loehlin JC (1977) Genotype-environment interaction and correlation in the analysis of human behavior. Psychol Bull 84: 309-322

Plotsky PM, Meaney MJ (1993) Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Brain Res Mol Brain Res 18: 195-200

Pryce CR, Bettschen D, Feldon J (2001) Comparison of the effects of early handling and early deprivation on maternal care in the rat. Dev Psychobiol 38: 239-251

Pryce CR, Feldon J (2003) Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms. Neurosci Biobehav Rev 27: 57-71

Pryce CR, Ruedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, Feldon J (2005) Long-term effects of early-life environmental manipulations in rodents and primates: Potential animal models in depression research. Neurosci Biobehav Rev 29: 649-674

Purves D, Lichtman JW (1980) Elimination of synapses in the developing nervous system. Science 210: 153-157

Rao PA, Molinoff PB, Joyce JN (1991) Ontogeny of dopamine D1 and D2 receptor subtypes in rat basal ganglia: a quantitative autoradiographic study. Brain Res Dev Brain Res 60: 161-177

Risinger FO, Brown MM, Doan AM, Oakes RA (1998) Mouse strain differences in oral operant ethanol reinforcement under continuous access conditions. Alcohol Clin Exp Res 22: 677-684

Rocha BA, Odom LA, Barron BA, Ator R, Wild SA, Forster MJ (1998) Differential responsiveness to cocaine in C57BL/6J and DBA/2J mice. Psychopharmacology (Berl) 138: 82-88

Roman E, Gustafsson L, Hyytia P, Nylander I (2005) Short and prolonged periods of maternal separation and voluntary ethanol intake in male and female ethanol-preferring AA and ethanol-avoiding ANA rats. Alcohol Clin Exp Res 29: 591-601

Roman E, Ploj K, Nylander I (2004) Maternal separation has no effect on voluntary ethanol intake in female Wistar rats. Alcohol 33: 31-39

Rose JE, Corrigall WA (1997) Nicotine self-administration in animals and humans: similarities and differences. Psychopharmacology (Berl) 130: 28-40

Rots NY, de Jong J, Workel JO, Levine S, Cools AR, de Kloet ER (1996) Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. J Neuroendocrinol 8: 501-506

Rowe DC, Jacobson KC, Van den Oord EJ (1999) Genetic and environmental influences on vocabulary IQ: parental education level as moderator. Child Dev 70: 1151-1162

Rutter M, Dunn J, Plomin R, Simonoff E, Pickles A, Maughan B, Ormel J et al. (1997) Integrating nature and nurture: implications of person-environment correlations and interactions for developmental psychopathology. Dev Psychopathol 9: 335-364

Rutter M, Moffitt TE, Caspi A (2006) Gene-environment interplay and psychopathology: multiple varieties but real effects. J Child Psychol Psychiatry 47: 226-261

Sameroff AJ (2000) Developmental systems and psychopathology. Dev Psychopathol 12: 297-312

Sapolsky RM, Meaney MJ (1986) Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res 396: 64-76

Sarnyai Z, Biro E, Telegdy G (1993) Cocaine-induced elevation of plasma corticosterone is mediated by different neurotransmitter systems in rats. Pharmacol Biochem Behav 45: 209-214

Sarnyai Z, Shaham Y, Heinrichs SC (2001) The role of corticotropin-releasing factor in drug addiction. Pharmacol Rev 53: 209-243

Schapiro S, Geller E, Eiduson S (1962) Neonatal adrenal cortical response to stress and vasopressin. Proc Soc Exp Biol Med 109: 937-941

Schmidt M, Enthoven L, van der MM, Levine S, de Kloet ER, Oitzl MS (2003) The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse. Int J Dev Neurosci 21: 125-132

Schmidt M, Enthoven L, van Woezik JH, Levine S, de Kloet ER, Oitzl MS (2004) The dynamics of the hypothalamic-pituitary-adrenal axis during maternal deprivation. J Neuroendocrinol 16: 52-57

Schroff KC, Cowen MS, Koch S, Spanagel R (2004) Strain-specific responses of inbred mice to ethanol following food shortage. Addict Biol 9: 265-271

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 54: 1-42

Sigvardsson S, Bohman M, Cloninger CR (1996) Replication of the Stockholm Adoption Study of alcoholism. Confirmatory cross-fostering analysis. Arch Gen Psychiatry 53: 681-687

Silberg J, Rutter M, Neale M, Eaves L (2001) Genetic moderation of environmental risk for depression and anxiety in adolescent girls. Br J Psychiatry 179: 116-121

Skinner BF (1953) Science and Human Behavior, Free Press, New York edn.

Sluyter F, Hof M, Ellenbroek BA, Degen SB, Cools AR (2000) Genetic, sex, and early environmental effects on the voluntary alcohol intake in Wistar rats. Pharmacol Biochem Behav 67: 801-808

Spealman RD, Goldberg SR (1978) Drug self-administration by laboratory animals: control by schedules of reinforcement. Annu Rev Pharmacol Toxicol 18: 313-339

Stanton ME, Gutierrez YR, Levine S (1988) Maternal deprivation potentiates pituitary-adrenal stress responses in infant rats. Behav Neurosci 102: 692-700

Stolerman IP, Naylor C, Elmer GI, Goldberg SR (1999) Discrimination and self-administration of nicotine by inbred strains of mice. Psychopharmacology (Berl) 141: 297-306

Suchecki D, Rosenfeld P, Levine S (1993) Maternal regulation of the hypothalamic-pituitary-adrenal axis in the infant rat: the roles of feeding and stroking. Brain Res Dev Brain Res 75: 185-192

Swanson LW (2003) The amygdala and its place in the cerebral hemisphere. Ann N Y Acad Sci 985: 174-184

Talge NM, Neal C, Glover V (2007) Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? J Child Psychol Psychiatry 48: 245-261

Tepper JM, Trent F, Nakamura S (1990) Postnatal development of the electrical activity of rat nigrostriatal dopaminergic neurons. Brain Res Dev Brain Res 54: 21-33

Tienari P, Wynne LC, Sorri A, Lahti I, Laksy K, Moring J, Naarala M et al. (2004) Genotype-environment interaction in schizophrenia-spectrum disorder. Long-term follow-up study of Finnish adoptees. Br J Psychiatry 184: 216-222

Tsuang MT, Lyons MJ, Harley RM, Xian H, Eisen S, Goldberg J, True WR et al. (1999) Genetic and environmental influences on transitions in drug use. Behav Genet 29: 473-479

Turkheimer E, Haley A, Waldron M, D'Onofrio B, Gottesman II (2003) Socioeconomic status modifies heritability of IQ in young children. Psychol Sci 14: 623-628

United Nations Office on Drugs and Crime (2005) UN World Drug Report.

van der Kam EL, Ellenbroek BA, Cools AR (2005) Gene - environment interactions determine the individual variability in cocaine self-administration. Neuropharmacology 48: 685-695

van Oers HJ, de Kloet ER, Levine S (1999) Persistent effects of maternal deprivation on HPA regulation can be reversed by feeding and stroking, but not by dexamethasone. J Neuroendocrinol 11: 581-588

van Oers HJ, de Kloet ER, Whelan T, Levine S (1998) Maternal deprivation effect on the infant's neural stress markers is reversed by tactile stimulation and feeding but not by suppressing corticosterone. J Neurosci 18: 10171-10179

Van Ree JM, Gerrits MA, Vanderschuren LJ (1999) Opioids, reward and addiction: An encounter of biology, psychology, and medicine. Pharmacol Rev 51: 341-396

Vazquez DM, Eskandari R, Zimmer CA, Levine S, Lopez JF (2002) Brain 5-HT receptor system in the stressed infant rat: implications for vulnerability to substance abuse. Psychoneuroendocrinology 27: 245-272

Vazquez V, Penit-Soria J, Durand C, Besson MJ, Giros B, Dauge V (2005) Maternal deprivation increases vulnerability to morphine dependence and disturbs the enkephalinergic system in adulthood. J Neurosci 25: 4453-4462

Volkow N, Li TK (2005) The neuroscience of addiction. Nat Neurosci 8: 1429-1430

Wahlsten D, Metten P, Phillips TJ, Boehm SL, Burkhart-Kasch S, Dorow J, Doerksen S et al. (2003) Different data from different labs: lessons from studies of gene-environment interaction. J Neurobiol 54: 283-311

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S et al. (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7: 847-854

Weiner I, Feldon J, Ziv-Harris D (1987) Early handling and latent inhibition in the conditioned suppression paradigm. Dev Psychobiol 20: 233-240

Whisman MA, McClelland GH (2005) Designing, testing, and interpreting interactions and moderator effects in family research. J Fam Psychol 19: 111-120

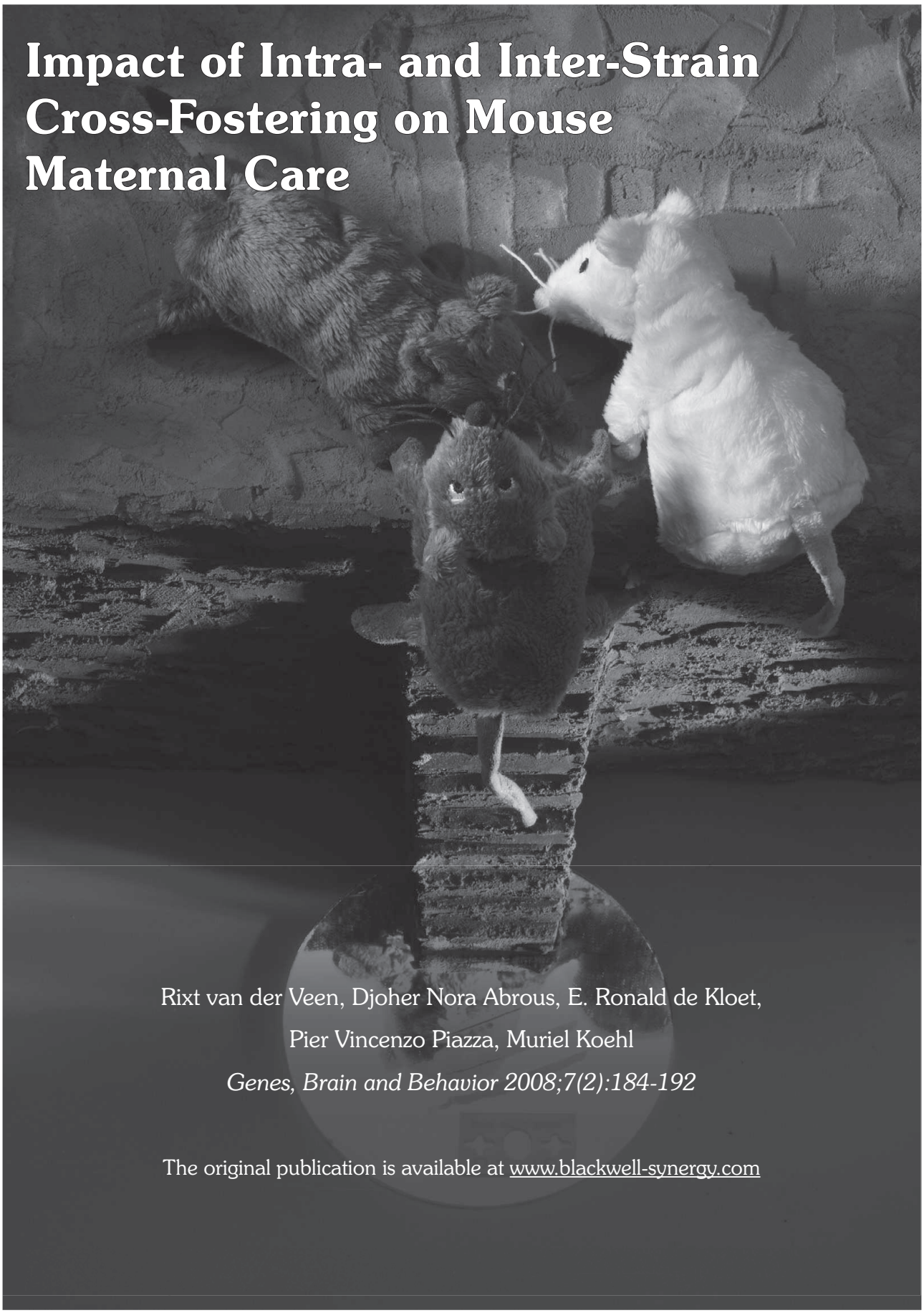
Wise RA (1996) Addictive drugs and brain stimulation reward. Annu Rev Neurosci 19: 319-340

Womer DE, Jones BC, Erwin VG (1994) Characterization of dopamine transporter and locomotor effects of cocaine, GBR 12909, epidepride, and SCH 23390 in C57BL and DBA mice. Pharmacol Biochem Behav 48: 327-335

Yehuda R, Flory JD, Southwick S, Charney DS (2006) Developing an agenda for translational studies of resilience and vulnerability following trauma exposure. Ann N Y Acad Sci 1071: 379-396

Zocchi A, Orsini C, Cabib S, Puglisi-Allegra S (1998) Parallel strain-dependent effect of amphetamine on locomotor activity and dopamine release in the nucleus accumbens: an in vivo study in mice. Neuroscience 82: 521-528

Chapter 2



Impact of Intra- and Inter-Strain Cross-Fostering on Mouse Maternal Care

Rixt van der Veen, Djoher Nora Abrous, E. Ronald de Kloet,
Pier Vincenzo Piazza, Muriel Koehl

Genes, Brain and Behavior 2008;7(2):184-192

The original publication is available at www.blackwell-synergy.com

Abstract

The importance of maternal care in shaping an individual's phenotype in health and disease is becoming more and more apparent in both human and animal studies. However, in mouse studies using inbred strains or knockout mice to analyze the genetic influences on the development of normal and aberrant behavioral phenotypes, maternal behavior is very poorly characterized and often ignored. This study provides an extensive analysis of spontaneous maternal behavior of inbred mice in three conditions: (1) comparing two commonly used strains, (2) analyzing the impact of adopting pups from the same strain (*intra*-strain cross-fostering) and (3) analyzing the impact of adopting pups from a different strain (*inter*-strain cross-fostering). For each condition, maternal behavior was analyzed continuously over 23h periods on postnatal days 2, 4, 6, and 9. We report that a) the maternal behavior of C57BL/6J and DBA/2J dams toward their biological offspring is highly similar, b) *intra*-strain cross-fostering has minimal impact on maternal behavior of C57BL/6J and DBA/2J dams, and c) *inter*-strain cross-fostering does not modify the strain differences in maternal care observed between AKR and C3H/He mothers, but d) the pup strain does influence the amount of maternal behavior shown by both mothers in *inter*-strain cross-fostering. These latter findings demonstrate that both mother strain and pup strain are key determinants of maternal behavior.

Introduction

There is increased awareness that gene-environment interactions are key determinants of an individual's phenotype. An elegant illustration of this interaction is shown in an extensive study by Crabbe and colleagues, which demonstrated that the expression of strain-related behavioral differences depends upon the laboratory environment (Crabbe et al. 1999; Wahlsten et al. 2003). Among environmental factors influencing phenotype, maternal behavior is of major importance and variations in maternal behavior were shown to be essential in the development of the pup and its phenotype in adulthood (Liu et al. 1997; 2000). Moreover, postnatal interventions such as handling (Meaney et al. 1991; Levine 2005), maternal separation (Ladd et al. 2000), environmental enrichment (Chapillon et al. 1999; Francis et al. 2002) and cross-fostering (Carlier et al. 1991; Anisman et al. 1998; Caldji et al. 2004; Priebe et al. 2005), which all change the quality and quantity of mother-pup interactions, were found to influence adult behavior and brain morphology.

Although these reports clearly demonstrate the impact of maternal care on shaping the individual's phenotype, its importance is often overlooked in studies using knockout or inbred strains of mice, the most common models to study genetic influences on the development of behavioral phenotypes. For example, in studies using knockout mice it is not unusual that cross fostering to a wildtype mother is performed due to low maternal care of the knockout strain. But this procedure can induce unexpected behavioral changes in the adult knock-out mice (Weller et al. 2003), and interfere with conclusions on the role of the targeted gene. Thus, differences found between knockout and wildtype mice that are usually assigned to the gene of interest, might instead reflect differential maternal care received.

Cross-fostering is also widely used with inbred strains of mice to determine the relative contributions of genetic and early environmental factors in shaping phenotypes, but the procedures in use pose important problems. First, experimental designs lack consistency, since either entire litters or only some pups per litter are fostered. Second, litters of mothers from two different strains are usually exchanged and postnatal effects are attributed to differences in maternal behavior. In this case, the control group consists of litters fostered to a mother of the same strain, which may be inappropriate. Indeed, pups may benefit more from the environment provided by a mother of the same strain compared to a mother of another strain (Yamazaki et al. 2000; Hager and Johnstone 2003). And finally, maternal behavior is very poorly characterized in mice, and the extent to which maternal care depends upon pups remains largely unknown in this species.

To address these issues, we performed an extensive analysis of maternal behavior in three conditions. We first analyzed the maternal behavior of two of the most commonly used mouse strains, C57BL/6J (C57) and DBA/2J (DBA), toward their biological offspring. Second we analyzed the impact of fostering pups from the same strain (*intra*-strain cross-fostering) on C57 and DBA dams. Finally we analyzed the impact of fostering pups from a different strain (*inter*-strain cross-fostering). We chose AKR and C3H/HeN dams for this *inter*-strain cross-fostering, since they exhibit important differences in their maternal care (Carlier et al. 1982). Pups from the C57 and DBA strains were fostered to these mothers

in order to determine whether the genetic background of the mother or that of the pups is determinant in defining maternal behavior.

Materials and Methods

Subjects

All mice used in the experiments were bred in our animal facilities. Three of the original strains (C57BL/6JOlaHsd, AKR/OlaHsd, C3H/HeNHsd) were obtained from Harlan France (Gannat, France), and the DBA/2J@Ico mice were obtained from Charles River Laboratories (Arbresle, France). Animals were kept in our temperature (23°C) and humidity (60%) controlled animal facilities. All experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Breeding & cross-fostering

From mating until weaning, dams were fed on a protein (23.5%) and fat (5%) enriched diet (M03 breeding diet, UAR, Bordeaux, France). A 14h light-10h dark cycle was installed, as is common in reproductive facilities. Each female was paired with a male of her own strain that was removed from the cage when pregnancy was ascertained by visual observation and body weight recording. All males were removed from the cage at least 1 week before birth. Cross-fostering was conducted between 4-7 hours after both biological and adoptive dams had given birth. The procedure consisted in removing the biological mother, weighing and sexing all the pups, culling the litter to 5-8 pups, placing the litter in a clean cage containing bedding of the adoptive mother, and finally placing the adoptive mother in the cage. Culled litters contained a balanced amount of males and females thus reaching 5-8 pups depending on the sex counting the smallest number of pups. Average litter size did not differ between experimental groups [group effect $F(1,7)=1.58$ p=ns].

The whole procedure of fostering lasted on average 2 min, and never took more than 4 min. Biological litters were treated as cross-fostered litters, but were returned to their original nest. Four experimental groups per pup strain were thus constituted: pups of the C57 and DBA strains raised by their biological mother, a mother of the same strain as their biological mother, a mother of the AKR strain or a mother of the C3H strain. The breeding cage (29x11x13 cm) contained a transparent Plexiglas separation at 9 cm from the wall with a small hole to go in and out, to create a nest compartment (9x11x13 cm). This nest compartment occupied approximately one-third of the cage.

Maternal behavior

The breeding cages were placed in sound safe video-equipped chambers to record maternal behavior (Imetronic, Pessac, France). An infra-red camera was placed facing the back wall of the breeding cage where the nest compartment was located. During both the day and the night phase a clear view of the dam-pups dyad was available, and the different maternal behaviors could be clearly distinguished. Given that maternal behavior is rhythmic and might

be differently organized in different mouse strains, analysis were performed over the entire light-dark cycle except for the last hour of the dark period since other cages were placed in the recording boxes during this period to allow recording on the next day. Continuous recordings were thus made over 23h periods on postnatal days 2, 4, 6 and 9. The day of birth was considered postnatal day 1 (PD1). Only dams with litters between 10h and 24h of age at the start of PD2 were included in behavioral scoring. Scoring of maternal behavior was performed off line by an experimenter unaware of the experimental groups using The Observer® (Noldus Information Technology, Wageningen, The Netherlands). The following behaviors were scored: *Pup licking:* Licking any part of the pups' body, including anogenital parts. *Nursing posture:* Immobile arched-back posture over the pups, with the abdomen actively elevated from the floor and pups attached to the nipples. *Nest reorganizing:* Nest disturbance and pup scattering by a push forward movement of the mothers' head in the sawdust. *Self-grooming on the nest:* Self-grooming while being in contact with the pups. '*Passive' nest presence:* Being in contact with the pups without showing any of the other behaviors. *Nest absence:* Not being in physical contact with the pups.

To obtain a reasonable estimation of behavior while maintaining the time needed for scoring within a certain limit, we chose to make an observation (instantaneous sample) every 2.5 min, which means 24 observations per animal per hour, or 552 observations per animal per postnatal day. Results were expressed as the percentage of time spent in individual behaviors over 23h. Furthermore, since the time spent out of the nest also represents the period the mother is most active, we analyzed this parameter in bouts of 75 minutes over the light-dark cycle in order to evaluate the activity rhythm of the mothers. Finally the circadian rhythms of the most explicit maternal behaviors (licking, nursing, and nest reorganizing) were analyzed as well. However, since their occurrence was not high enough for analysis in bouts of 75 minutes, the light-dark cycle was split into 4 periods; two in the light phase and two in the dark phase.

Weaning & housing

Pups were weaned at 21 days of age, and placed together with same sex group mates (4-6 per cage). At 10 weeks of age the animals were individually housed and returned to a standard 12h light-dark cycle. Bodyweight was recorded before the cross-fostering procedure (birth body weight), and then on weeks 3, 5, 7, 10 and 16.

Data analysis

Differences in maternal behavior over the 4 postnatal days were examined with an analysis of variance (ANOVA) using Statistica 6.0©. Strain (either mother strain or pup strain) served as a between factor, and time (either postnatal day (PD), 75 min timeblocks, or light-dark period (LD) depending upon the analysis performed) served as a within factor. Possible differences in bodyweight at birth and in litter size at fostering were analyzed using a one way ANOVA. For analyzing the influence of mother on pup bodyweight from weaning to 16 weeks of life, mother served as a between subject factor and age served as a within subject factor. Whenever adequate, Duncan's test was used as a post hoc analysis. For clarity purpose, only statistics yielding significant results have been reported.

Results

Maternal behavior was assessed by analyzing a) pup-oriented behaviors (licking, nursing, and nest reorganizing) and b) non pup-oriented behaviors (self-grooming, passive nest presence, and nest absence).

Maternal behavior of C57 and DBA mothers toward their biological offspring

The analysis of pup-oriented behaviors revealed that the daily amounts of pup licking and nursing posture, the two most relevant pup-oriented behaviors, were similar for the two mother strains *(figure 2.1 bar graphs)*. Thus, both strains showed higher amounts of pup licking on PD2 compared to the other postnatal days [PD effect $F(3,63)=9.67$ $p<0.001$; PD2 $p<0.01$], whereas nursing posture remained at a similar level over the first nine days of the postpartum period. Mothers differed however in nest reorganizing, with C57 dams displaying higher levels than DBA although this difference decreased over days [mother effect $F(1,21)=5.86$ $p<0.05$; PD effect $F(3,63)=15.5$ $p<0.001$; PD*mother interaction $F(3,63)=4.14$ $p<0.01$ with C57 ≠ DBA on PD2].

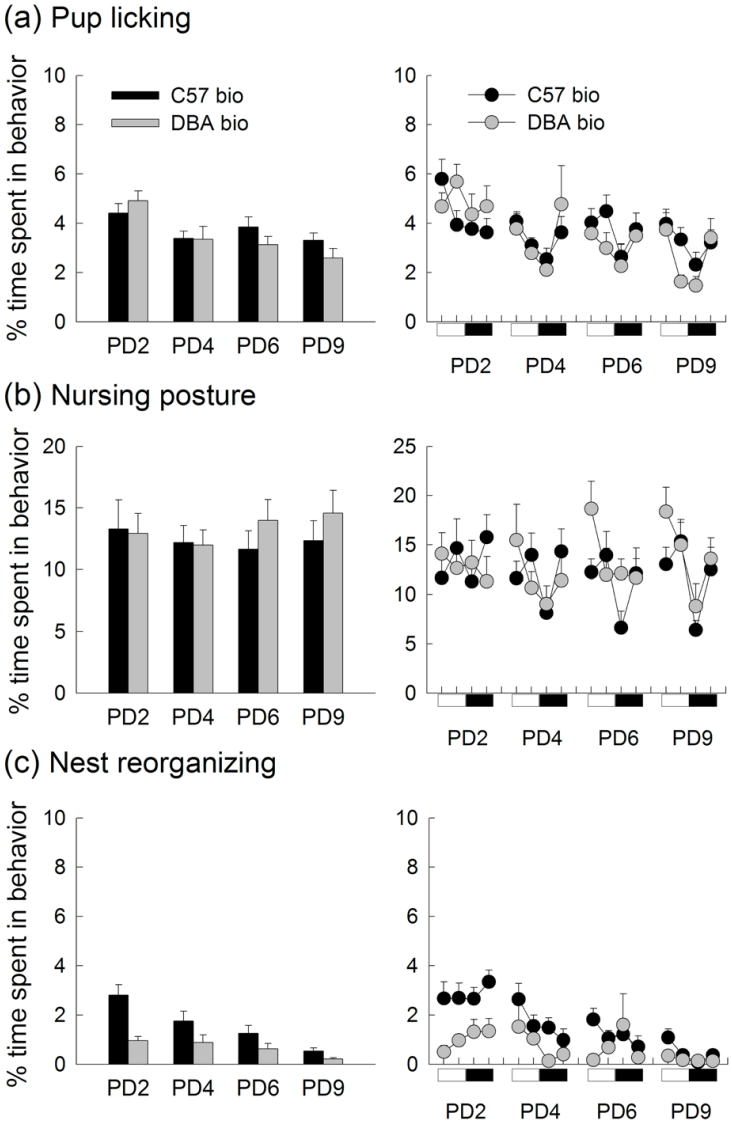


Figure 2.1: Pup-oriented maternal behavior of C57 and DBA mothers with their biological (bio) offspring. Graphs represent the percentage of time spent in ***(a)*** pup licking, ***(b)*** nursing posture and ***(c)*** nest reorganizing over the four postnatal days. Bar graphs show the mean percentage of time spent in different behaviors over 23h and line graphs show the circadian rhythms (two light and two dark measurements per day) of the pup-oriented behaviors. The white bar represents the light period, the black bar represents the dark period. Note that nest reorganizing is represented on the same scale as pup licking, which is half the scale of nursing posture, in order to indicate the proportional amounts of pup-directed behaviors. Values represent mean ± SEM. C57 bio, n=15; DBA bio, n=8.

The analysis of the circadian rhythms of licking, nursing, and nest reorganizing revealed that pup care followed a day-night rhythm *(figure 2.1 line graphs)*. Most marked were the lower amounts of licking and nursing in the first part of the dark phase [light-dark (LD) effect $F(3,63)=9.72$ and 13.68, both $p<0.001$], which coincide with the peak of nest absence (*see figure 2.2*). Both mother strains had a similar circadian rhythm of licking, but a different rhythm of nursing [LD*mother interaction $F(3,63)=7.42$ $p<0.001$], with DBA showing more nursing in the first part compared to the second part of the light phase, and C57 showing the opposite. Nest reorganizing only showed a rhythm on PD4 [PD*LD interaction $F(9,189)=2.75$ $p<0.01$; PD4 $p<0.05$], which did not differ between mothers.

Analysis of non pup-oriented maternal behavior *(figure 2.2)* showed that self-grooming on the nest was strain dependent, with DBA mice exhibiting higher amounts starting on day 4 [PD effect $F(3,63)=65.1$ $p<0.001$; PD*mother interaction $F(3,63)=3.31$ $p<0.05$ with C57 ≠ DBA on PD4, PD6, PD9 but not PD2]. For both strains passive nest presence decreased over days, whereas nest absence increased [PD effect $F(3,63)=36.5$ and 123.7, both $p<0.001$].

A detailed analysis of the circadian rhythm of nest absence *(figure 2.2d)* revealed that 1) a rhythm gradually installed over the postnatal days [PD*timeblocks interaction $F(54,1134)=2.07$ $p<0.001$], with a clear peak of nest absence at light onset, followed by a U-shaped absence vale and a second peak at dark onset, and 2) there was a strain effect on the installation of this circadian rhythm [PD*timeblocks*mother interaction $F(54,1134)=1.95$

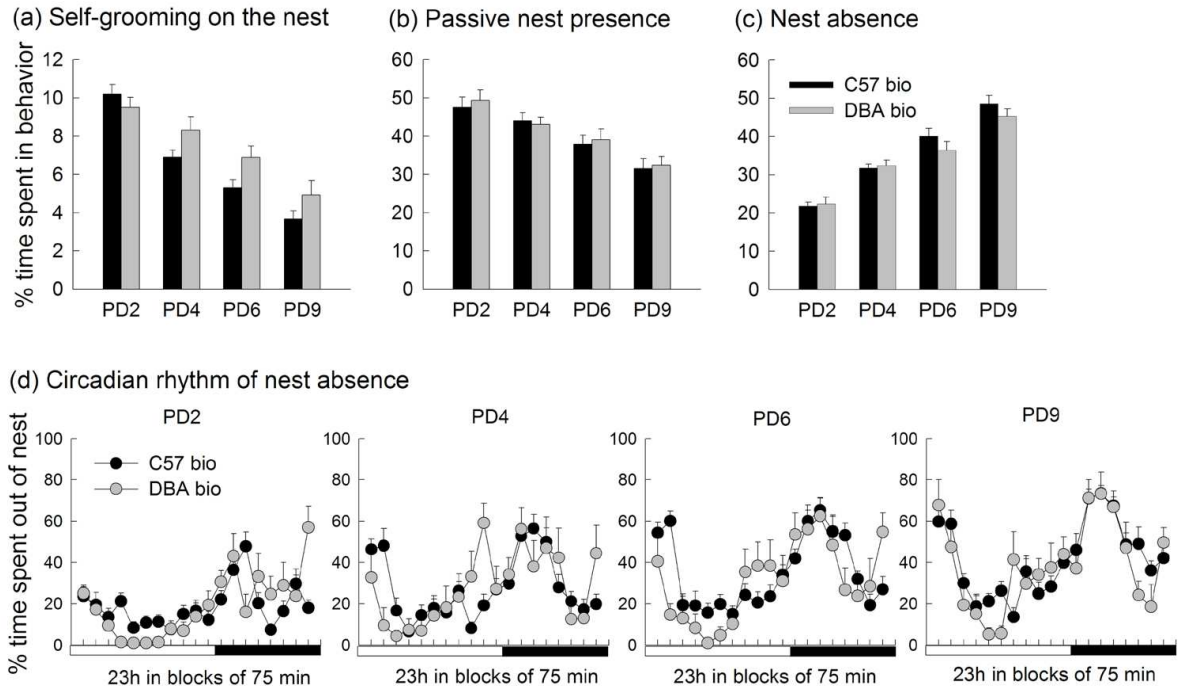


Figure 2.2: Non pup-oriented maternal behavior of C57 and DBA mothers with their biological (bio) offspring. Graphs represent the percentage of time spent in ***(a)*** self-grooming on the nest, ***(b)*** passive nest presence and ***(c)*** nest absence over the four postnatal days (23h measurements). ***(d)*** Graphs show the circadian rhythm of nest absence over the four postnatal days in bouts of 75 min. The white bar represents the light period, the black bar represents the dark period. Values represent mean ± SEM. C57 bio, n=15; DBA bio, n=8.

p<0.001] with DBA mothers being less absent from the nest in the first part of the light phase and more in the second part when compared to C57 mothers.

Impact of intra-strain cross-fostering on maternal behavior

Maternal behavior of C57 and DBA mothers was analyzed after adoption of same-strain pups.

Intra-strain cross-fostering increased pup licking in C57 dams *(figure 2.3a)* regardless of the postnatal day [mother effect $F(1,29)=5.98$ $p<0.05$], but remained without effect on any other behavior in C57 or DBA dams *(figure 2.3b-f)*. Analyzing the circadian rhythms of licking, nursing or nest reorganizing *(figure 2.4)* and of nest absence *(figure 2.5)* did not reveal any differences between foster and biological mothers for both C57 and DBA mice.

Impact of inter-strain cross-fostering on maternal behavior

Maternal behavior of AKR and C3H mothers was analyzed after adoption of C57 or DBA pups. We first verified that AKR and C3H mothers differed in their spontaneous maternal

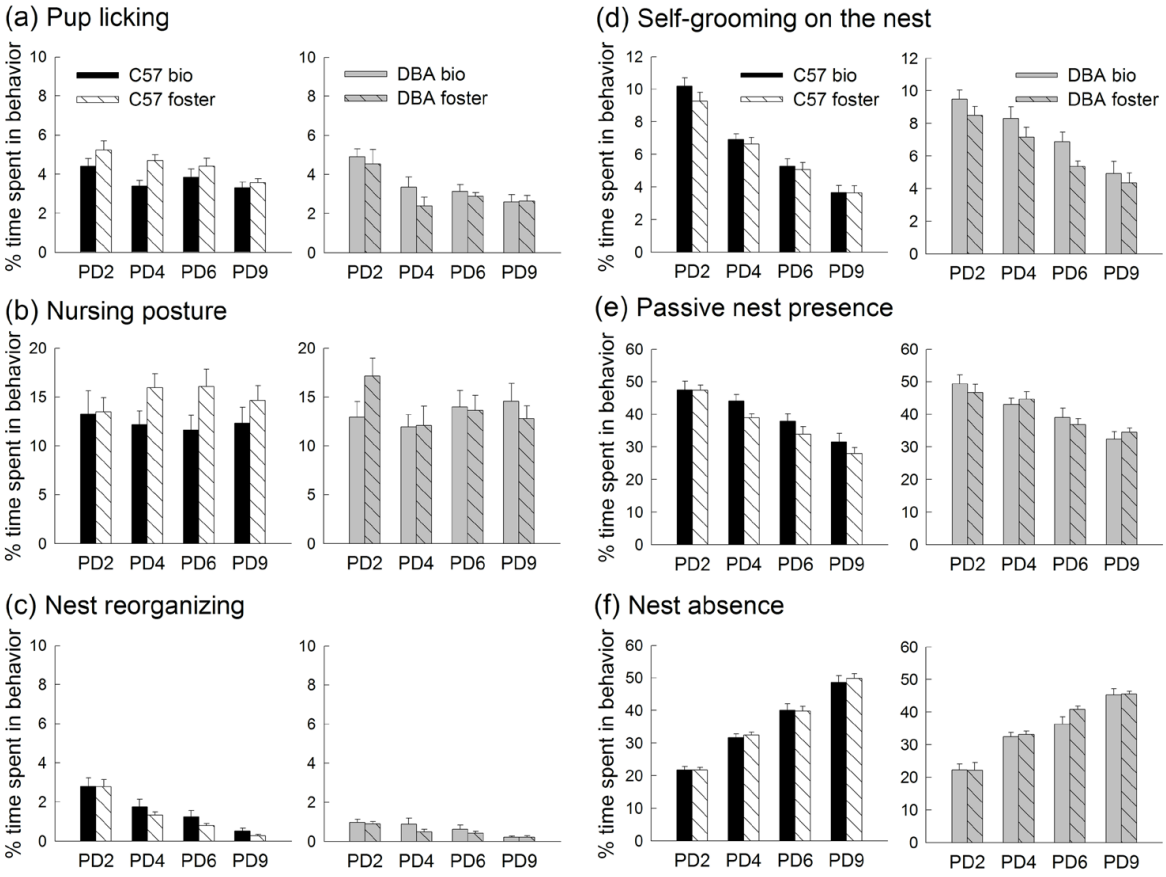


Figure 2.3: C57 and DBA mothers: Impact of intra-strain cross-fostering on maternal behavior. Graphs represent the percentage of time spent in ***(a-c)*** pup-oriented behaviors and ***(d-f)*** non pup-oriented behaviors over 23h on each postnatal day. Each graph shows behavior of dams with biological (bio) and same-strain fostered (foster) offspring. Values represent mean ± SEM. C57 bio, n=15; C57 foster, n=16; DBA bio, n=8; DBA foster, n=10.

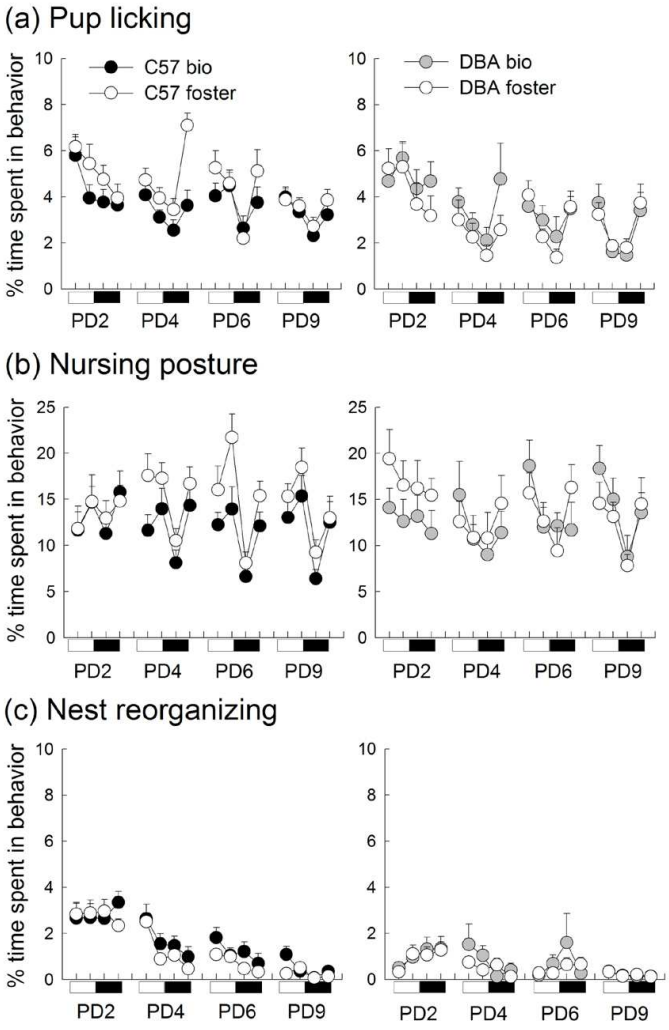


Figure 2.4: C57 and DBA mothers: Impact of intra-strain cross-fostering on the circadian rhythm of pup-oriented behaviors. Graphs show the circadian rhythms of ***(a)*** pup licking, ***(b)*** nursing posture and ***(c)*** nest reorganizing over the 4 postnatal days (two light and two dark measurements per day). Each graph shows behavior of dams with biological (bio) and same-strain fostered (foster) offspring. The white bar represents the light period, the black bar represents the dark period. Values represent mean ± SEM. C57 bio, n=15; C57 foster, n=16; DBA bio, n=8; DBA foster, n=10.

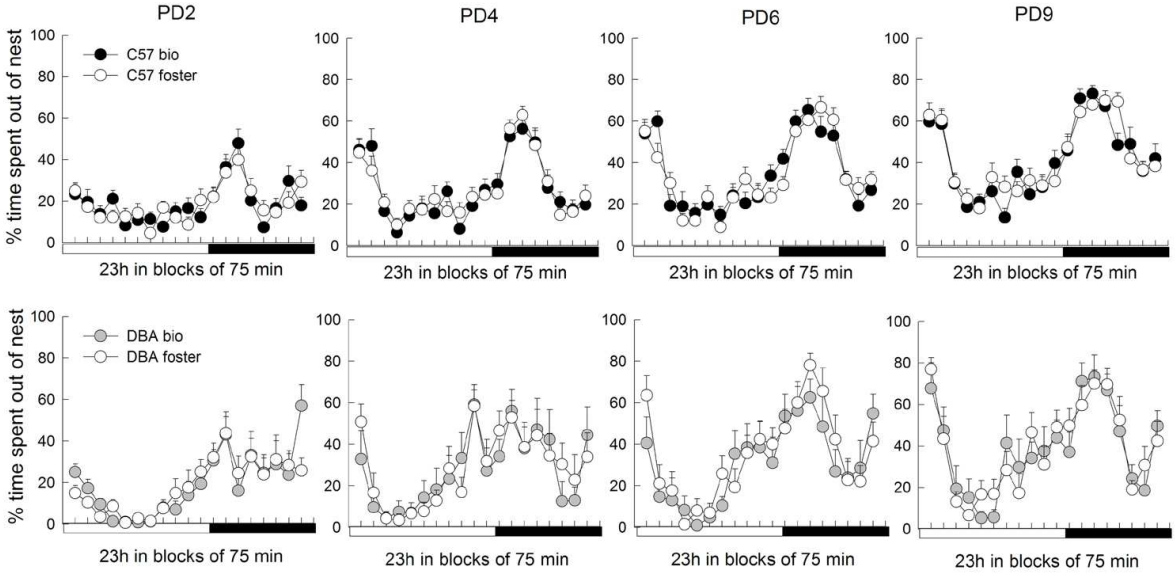


Figure 2.5: C57 and DBA mothers: Impact of intra-strain cross-fostering on the circadian rhythm of nest absence. Graphs represent the circadian rhythm of nest absence over the four postnatal days in bouts of 75 min for C57 (upper panels) and DBA (lower panels) dams. Each graph shows the behavior of dams with biological (bio) and same-strain fostered (foster) offspring. The white bar represents the light period, the black bar represents the dark period. Values represent mean ± SEM. C57 bio, n=15; C57 foster, n=16; DBA bio, n=8; DBA foster, n=10.

behavior as was previously reported for provoked behavior (Carlier et al. 1982). Next, the impact of fostering pups from different strains was analyzed for each mother strain.

When comparing the two adoptive mothers *(figure 2.6, comparing white and grey panels)*, a profound difference in maternal behavior was visible, as expected. Indeed, whatever the strain of pups adopted, AKR mothers spent less time licking and nursing pups, and more time in nest reorganizing and self grooming than C3H mothers [for C57 pups: mother strain effect $F(1,14)=51.24$, 14.89, 6.05, and 11.23 for licking, nursing, nest reorganizing, and self grooming, all $p<0.01$; for DBA pups: mother strain effect $F(1,14)=47.84$, 6.52, 22.35, and 15.56 for licking, nursing, nest reorganizing, and self grooming, all $p<0.01$]. Strain differences were also seen for the evolution over days of passive nest presence and nest absence, AKR mothers remaining at stable levels from PD2 to PD9 while C3H mothers exhibit a decrease in passive nest presence and an increase in nest absence [for C57 pups: PD*mother strain interaction $F(3,42)=13.95$ and 11.05 respectively; for DBA pups: PD*mother strain interaction $F(3,42)=23.19$ and 23.06 respectively, all $p<0.001$]. When comparing AKR and C3H mothers in the circadian rhythm of pup-oriented behaviors *(figure 2.7)* the only clear difference was the lack of a rhythm in nest reorganizing for C3H dams, while a rhythm was present on PD4 for AKR dams [PD*LD interaction

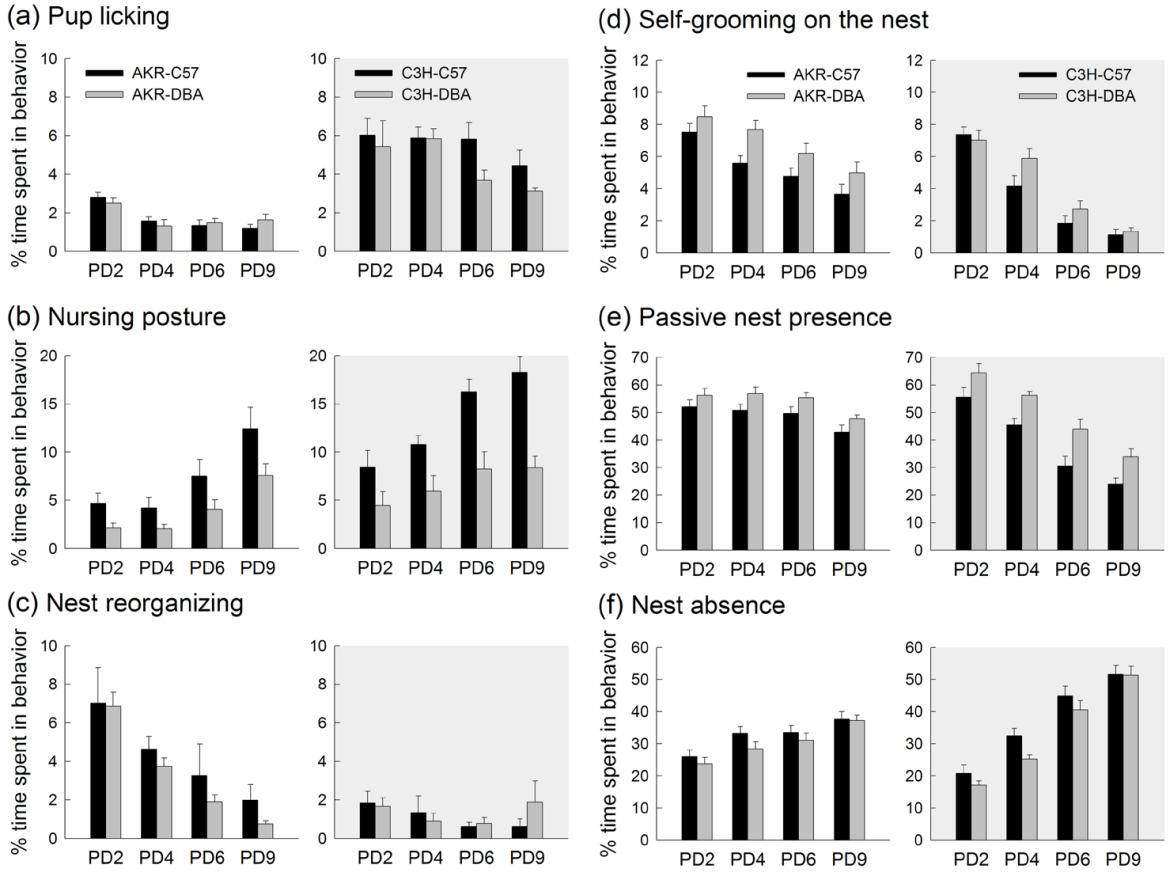


Figure 2.6: AKR and C3H mothers: Impact of inter-strain cross-fostering on maternal behavior. Graphs represent the percentage of time spent in ***(a-c)*** pup-oriented behaviors and ***(d-f)*** non pup-oriented behaviors over 23h on each postnatal day. Graphs show the behavior of AKR (white backplane) and C3H (grey backplane) dams with either C57 or DBA foster pups. Values represent mean ± SEM. AKR-C57, n=9; AKR-DBA, n=9; C3H-C57, n=7; C3H-DBA, n=7

F(9,144)=4.69 p<0.001; PD4 p<0.001]. The circadian rhythm of nest absence *(figure 2.8)* revealed a clear difference during the dark phase, with AKR mothers showing two peaks of nest absence, whereas C3H mothers showed only one wider peak [timeblock*mother strain interaction F(18,252)=6.48 and 4.22 for C57 pups and DBA pups respectively, both p<0.001].

Analyzing the impact of cross-fostering *(figure 2.6, comparing black and grey bars for each mother)* revealed that both AKR and C3H mothers showed an increased maternal behavior toward C57 compared to DBA pups. Indeed, when exposed to C57 pups, both mothers spent more time in nursing posture, and less time in non pup-oriented behaviors while on the nest (self-grooming and passive nest presence) [for AKR mothers: pup strain effect F(1,16)=5.93, 5.20 and 5.28 for nursing behavior, self grooming and passive nest presence, all p<0.05; for C3H mothers: pup strain effect F(1,12)=23.03 and 13.61 for nursing behavior and passive nest presence, PD*pup strain interaction F(3,36)=3.19 for self grooming, all p<0.05]. For both mothers, pup licking, nest reorganizing and total nest absence were unaffected by the pup strain. When the circadian rhythms of the pup-oriented behaviors were analyzed *(figure 2.7),* effects of pup strain were found for both mothers, but these effects remained subtle since they were mostly dependent on postnatal days,

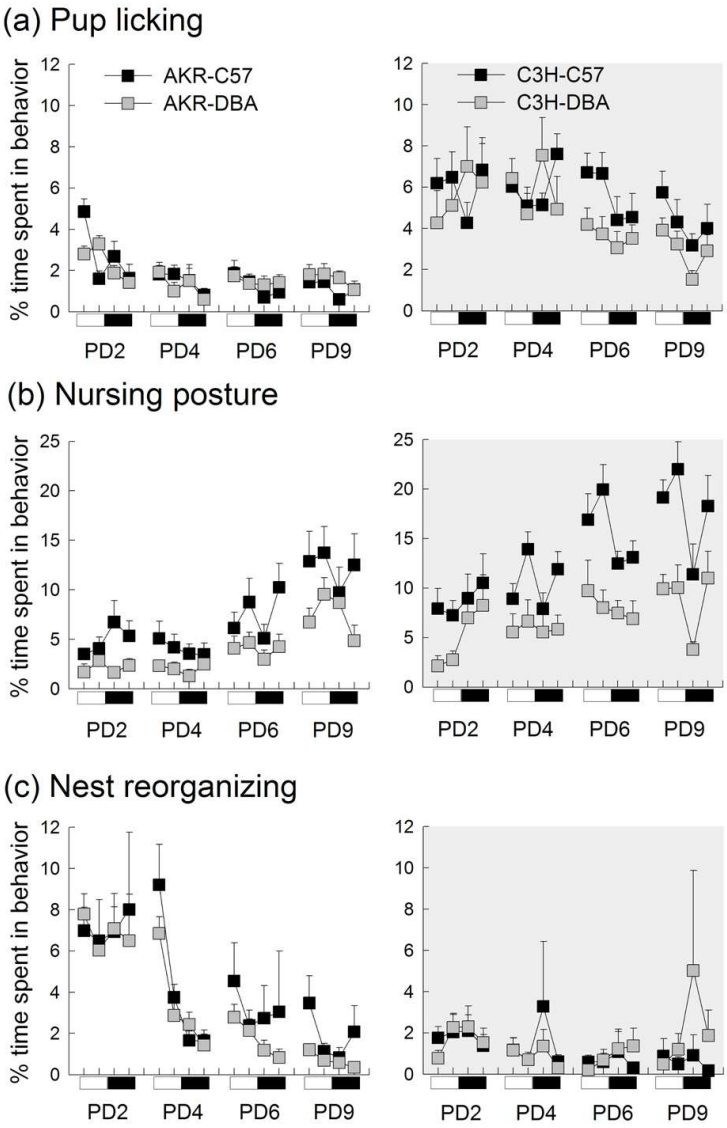


Figure 2.7: AKR and C3H mothers: Impact of inter-strain cross-fostering on the circadian rhythm of pup-oriented behaviors. Graphs show the circadian rhythms of ***(a)*** pup licking, ***(b)*** nursing posture and ***(c)*** nest reorganizing over the 4 postnatal days (two light and two dark measurements per day). Graphs show the behavior of AKR (white backplane) and C3H (grey backplane) dams with either C57 or DBA pups. The white bar represents the light period, the black bar represents the dark period. Values represent mean ± SEM. AKR-C57, n=9; AKR-DBA, n=9; C3H-C57, n=7; C3H-DBA, n=7.

and thus not consistent over the 4 days analyzed. That is, for AKR mothers the strain of fostered pups affected the rhythm of licking on postnatal day 2 [PD*LD*pup strain interaction $F(9,144)=1.49$ $p<0.05$; PD2 $p<0.05$] and the rhythm of nursing on postnatal day 9 [PD*LD*pup strain interaction $F(9,144)=1.49$ $p<0.05$]. For C3H mothers, the circadian rhythm of licking was affected by pup strain regardless of postnatal day [LD*pup strain interaction $F(3,36)=3.84$ $p<0.05$]. The circadian rhythm of nest absence *(figure 2.8)* was not influenced by pup strain.

Bodyweight of pups raised by the different mothers

Bodyweight at birth and a follow-up from weaning to 16 weeks of life is shown in Table 2.1. There were no differences in birth weight among the C57 and the DBA pups that were attributed to the 4 experimental groups. However, the two strains differed in birth weight, with C57 pups weighing on average 0.05 grams more [pup strain effect $F(1,216)=9.36$ $p<0.01$]. This difference between C57 and DBA mice continued from weaning to 16 weeks of life whatever the maternal environment [pup strain effect $F(1,213)=97.6$ $p<0.001$]. *Intra*-strain cross-fostering affected only C57 mice with pups raised by a foster mother weighing more than those raised by their biological mother [mother effect $F(1,74)=9.88$ $p<0.01$]. The analysis of mice issued from *inter*-strain cross-fostering showed that both C57 and DBA pups raised by C3H mothers were heavier than pups raised by AKR mothers [mother strain effect $F(1,46)=62.8$ and $F(1,43)=16.7$ for C57 and DBA mice respectively, both $p<0.001$]. These differences persisted until the last measurement at 16 weeks of life.

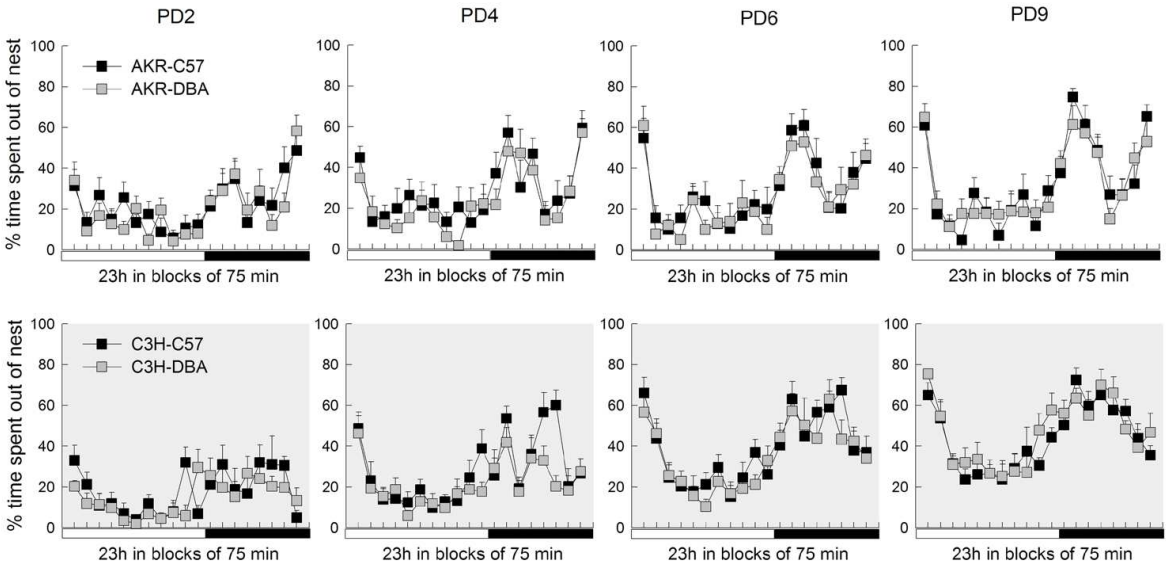


Figure 2.8: AKR and C3H mothers: Impact of inter-strain cross-fostering on the circadian rhythm of nest absence. Graphs represent the circadian rhythm of nest absence over the four postnatal days in bouts of 75 min. Graphs show the behavior of AKR (white backplane) and C3H (grey backplane) dams with either C57 or DBA pups. The white bar represents the light period, the black bar represents the dark period. Values represent mean ± SEM. AKR-C57, n=9; AKR-DBA, n=9; C3H-C57, n=7; C3H-DBA, n=7.

Table 2.1: Follow up of bodyweight (g) from birth to 16 weeks of life. The table shows bodyweight data of C57 and DBA offspring raised by their biological mother (C57 bio and DBA bio), a foster mother of the same strain (C57 foster and DBA foster), or a foster mother of a different strain (AKR or C3H).

			Bodyweight (g)					
Pup strain	Mother strain	*n*	Birth	3 weeks	5 weeks	7 weeks	10 weeks	16 weeks
C57	C57 bio	39	1.34 ± 0.015	9.15 ± 0.32	20.23 ± 0.42	24.85 ± 0.27	27.62 ± 0.29	29.44 ± 0.31
	C57 foster	37	1.35 ± 0.018	8.03 ± 0.13	18.84 ± 0.33	23.57 ± 0.27	26.95 ± 0.30	28.32 ± 0.29
	AKR	26	1.39 ± 0.019	7.38 ± 0.17	17.81 ± 0.34	22.15 ± 0.20	25.54 ± 0.27	26.88 ± 0.25
	C3H	22	1.35 ± 0.018	10.14 ± 0.28	21.64 ± 0.42	24.68 ± 0.41	28.14 ± 0.38	29.68 ± 0.35
DBA	DBA bio	21	1.36 ± 0.036	7.81 ± 0.25	17.05 ± 0.49	22.00 ± 0.41	23.48 ± 0.36	26.38 ± 0.32
	DBA foster	31	1.31 ± 0.021	7.90 ± 0.16	17.29 ± 0.29	22.00 ± 0.30	24.19 ± 0.26	26.58 ± 0.29
	AKR	27	1.30 ± 0.027	7.59 ± 0.19	16.74 ± 0.36	21.89 ± 0.30	23.56 ± 0.26	26.37 ± 0.26
	C3H	18	1.25 ± 0.040	9.22 ± 0.21	18.56 ± 0.41	23.00 ± 0.42	24.83 ± 0.32	27.83 ± 0.41

Values represent mean ± SEM

Discussion

We show after a thorough study of spontaneous maternal behavior with biological and fostered pups, that a) the maternal behavior of C57 and DBA dams toward their biological offspring is highly similar; b) cross-fostering pups of the same strain to C57 or DBA dams has minimal effects on maternal behavior; c) the AKR and C3H strains exhibit very distinct maternal behaviors, with AKR dams showing less pup-directed behavior than C3H dams, and d) the strain of foster pups influences maternal behavior, but does not influence the strain differences in maternal behavior.

Strain comparisons in mice have confirmed that genetic factors influence maternal behavior and differences in maternal care have been reported between C57BL/6J and DBA/2J inbred mice, two widely utilized strains to study the genetic basis of behavior. Thus, C57 mice have been described as better retrievers than DBA mice when reexposed to their pups after a short period of separation (Carlier et al. 1982; Brown et al. 1999), although clear differences in retrieving behavior were not always detected (Wainwright 1981). Such discrepancies were also evident in observations of spontaneous maternal behavior, where DBA mice were found to display either less nursing and pup grooming than C57 (Ward 1980) or to exhibit more nursing without any differences in pup licking (Shoji and Kato 2006). These data contrast with our results, since we did not find strain differences in either pup licking or nursing over 23h periods. A possible explanation for these discrepancies might be the difference in sampling frequency of behavior. Indeed, maternal behavior is rhythmic, with nursing and pup licking occurring more frequently during the light phase while non-maternal components of behavior increase during the dark phase (see Shoji and Kato 2006). Despite knowledge of this rhythm, most conclusions on maternal behavior are drawn from observations of 10-30 min/day during either the light or the dark

phase, with the exception of the study by Shoji & Kato (2006), where maternal behavior was evaluated for 2h during both the light and the dark phase. In our study, maternal behavior was assessed every 2.5 min during 23h per postnatal day, and although we did not find differences in the overall daily occurrence of nursing between C57 and DBA dams, we did find a strain difference in the circadian rhythm of nursing, which might explain findings of strain differences when short sampling periods are used. These observations are crucial and stress the importance of behavioral measures during the totality of the light-dark cycle.

Differences between the two strains were also reported for nest building behavior, but again results are far from consensual. Indeed, C57 mice were reported to have lower (Broida and Svare 1982; Shoji and Kato 2006), similar (Wainwright 1981), or higher (Brown et al. 1999; Bond et al. 2002) nest ratings and nest building behavior than DBA mice. We did not assess nest building behavior *per se* since we did not provide nest material. However, we found that C57 dams engaged in more nest reorganizing than DBA mice on postnatal day 2. This behavior is characterized by a push forward movement of the head in the sawdust during which pups are often discarded and regrouped afterwards. Since nest material is not available, this might represent a displaced nest building behavior. Alternatively, it might also represent a behavior aimed at regulating temperature, since the duration of each nest bout is limited by the rise of maternal temperature when the dam huddles with her litter (Woodside and Jans 1988).

Altogether, we thus found that DBA and C57 mothers are highly similar in their maternal behavior when raising their biological offspring. This contrasts with the literature, but these discrepancies can be partly imputed to differences in methodology. Indeed, many studies have analyzed maternal behavior following pup removal and replacement (Wainwright 1981; Carlier et al. 1982; Brown et al. 1999), a procedure which may differently affect C57 and DBA dams. For instance, C57 and DBA mice differ in the morphology of the medial preoptic nucleus, a central region in the regulation of maternal behavior (for review Numan 2007), and a differential expression of Fos immunoreactivity was observed in this region following the return of pups after displacement in DBA and C57 dams (Mathieson et al. 2002). Furthermore, pup displacement may represent a stressor for females, and DBA and C57 mice have been shown to differ in their corticosterone response to stress (Cabib et al. 1990; Shanks et al. 1990; Jones et al. 1998), which deserves attention since glucocorticoids were found to increase pup licking and grooming in rats (Rees et al. 2004).

We report that *intra*-strain cross-fostering has very limited impact on maternal behavior in our conditions, both for C57 and DBA dams. Of all behaviors tested, only a slight increase in licking was found for C57 adoptive mothers, a result that is consistent with rat studies (Darnaudery et al. 2004). In line with this, cross-fostering did not affect the maternal behavior of outbred Swiss CD-1 or Swiss Webster mice (Meek et al. 2001; Bartolomucci et al. 2004).

Classically, cross-foster studies with mice interchange pups between strains, meaning that the control group is constituted of pups fostered to a same-strain mother. However, this experimental design ignores the influence of the pup-mother interaction on maternal behavior and pup development. Indeed, mothers do not represent the same care environment for pups of their own strain compared to pups of another strain (Yamazaki et al. 2000; Hager and Johnstone 2003), and an optimal behavioral coordination between mother and

young is clearly needed for a healthy development (Fleming et al. 1999). Thus, to determine whether spontaneous maternal behavior in inbred mice is dependent on pup strain, we performed *inter*-strain cross-fostering with two strains of mothers and two strains of pups.

When comparing the two adoptive mothers, AKR and C3H, we found important differences in maternal behavior. Consistent with a study from Carlier et al. (1982), showing that C3H were better retrievers than AKR, we found here that C3H mothers showed more pup-directed behavior (licking and nursing), whereas AKR mothers exhibited more non pup-directed behavior (self grooming) when on the nest. Nest reorganizing behavior was very pronounced and some more violent in AKR dams that often showed intense bouts of this behavior after 'circling behavior', a stereotypical running around in circles. The interpretation of excessive nest reorganizing in AKR mothers is thus more likely negative than positive in terms of maternal care. AKR mothers displayed inferior maternal behavior than C3H with both foster pups, indicating that the strain differences in maternal care were unaffected by cross-fostering. This result is in accordance with a recent study in which cross-fostering BALB/cBy pups to C57BL/6By mothers and vice versa did not abolish the profound differences in the maternal style of these two strains (Prakash et al. 2006), and emphasizes that maternal behavior is highly determined by the mother herself. However, we found a major pup strain effect on nursing behavior; both AKR and C3H mothers showed less nursing posture with DBA than with C57 pups. Differences in quality and quantity of ultrasonic vocalizations (USV) of the pups might be important, although both strains emit USV in a high frequency range, which is in the sensitive range for at least the C3H/He mothers (Cohen-Salmon et al. 1985).

In order to determine whether the changes in maternal behavior induced by cross-fostering would affect the development of the pup, we followed their body-weight. After *intra*-strain cross-fostering of C57 mice, we found a reduction in bodyweight from weaning to adulthood. Could this be the result of more licking shown by the foster mother? This seems unlikely since a study with Swiss CD-1 mice showed the opposite effect, with a higher bodyweight in fostered pups compared to biological pups without changes in maternal behavior (Bartolomucci et al. 2004). Influence of *intra*-strain cross-fostering on bodyweight thus seems strain dependent and unrelated to visible maternal behaviors. Stronger differences were seen following *inter*-strain cross-fostering since both C57 and DBA pups raised by C3H mothers showed a higher bodyweight compared to same-strain pups raised by AKR mothers. This could be a consequence of the inferior maternal behavior of AKR mothers, but a difference in milk constitution -including different hormone levels- cannot be ruled out.

In conclusion, we report that C57 and DBA dams exhibit a comparable maternal behavior towards their biological offspring, and that their behavior is not considerably changed after *intra*-strain cross-fostering. We also show that *inter*-strain cross-fostering does not modify the maternal style of the foster mothers -i.e. highly-maternal mothers display higher amounts of maternal care than poorly-maternal mothers independently of the foster strain-, but that the pup strain does influence the amount of maternal behavior a mother shows. Therefore, both mother strain and pup strain are key determinants of maternal behavior. Furthermore, we show that the phenotype of the mother influences the

development of the pup. This raises an important issue: whether later adult behavior will be influenced by these early environmental influences.

Acknowledgements

We would like to thank Cedric Dupuy for excellent animal care and Dr Melly S. Oitzl for early scientific discussion. This research was supported by the NWO/INSERM/ZON grants AH002G and 985-10-014 and the university of Bordeaux 2.

References

Anisman H, Zaharia MD, Meaney MJ, Merali Z (1998) Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci 16: 149-164

Bartolomucci A, Gioiosa L, Chirieleison A, Ceresini G, Parmigiani S, Palanza P (2004) Cross fostering in mice: behavioral and physiological carry-over effects in adulthood. Genes Brain Behav 3: 115-122

Bond TL, Neumann PE, Mathieson WB, Brown RE (2002) Nest building in nulligravid, primigravid and primiparous C57BL/6J and DBA/2J mice (Mus musculus). Physiol Behav 75: 551-555

Broida J, Svare B (1982) Strain-typical patterns of pregnancy-induced nestbuilding in mice: maternal and experiential influences. Physiol Behav 29: 153-157

Brown RE, Mathieson WB, Stapleton J, Neumann PE (1999) Maternal behavior in female C57BL/6J and DBA/2J inbred mice. Physiol Behav 67: 599-605

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

Caldji C, Diorio J, Anisman H, Meaney MJ (2004) Maternal behavior regulates benzodiazepine/GABAA receptor subunit expression in brain regions associated with fear in BALB/c and C57BL/6 mice. Neuropsychopharmacology 29: 1344-1352

Carlier M, Roubertoux P, Cohen-Salmon C (1982) Differences in patterns of pup care in Mus musculus domesticus l- Comparisons between eleven inbred strains. Behav Neural Biol 35: 205-210

Carlier M, Roubertoux PL, Pastoret C (1991) The Y chromosome effect on intermale aggression in mice depends on the maternal environment. Genetics 129: 231-236

Chapillon P, Manneche C, Belzung C, Caston J (1999) Rearing environmental enrichment in two inbred strains of mice: 1. Effects on emotional reactivity. Behav Genet 29: 41-46

Cohen-Salmon C, Carlier M, Roubertoux P, Jouhaneau J, Semal C, Paillette M (1985) Differences in patterns of pup care in mice. V--Pup ultrasonic emissions and pup care behavior. Physiol Behav 35: 167-174

Crabbe JC, Wahlsten D, Dudek BC (1999) Genetics of mouse behavior: interactions with laboratory environment. Science 284: 1670-1672

Darnaudery M, Koehl M, Barbazanges A, Cabib S, Le Moal M, Maccari S (2004) Early and later adoptions differently modify mother-pup interactions. Behav Neurosci 118: 590-596

Fleming AS, O'Day DH, Kraemer GW (1999) Neurobiology of mother-infant interactions: experience and central nervous system plasticity across development and generations. Neurosci Biobehav Rev 23: 673-685

Francis DD, Diorio J, Plotsky PM, Meaney MJ (2002) Environmental enrichment reverses the effects of maternal separation on stress reactivity. J Neurosci 22: 7840-7843

Hager R, Johnstone RA (2003) The genetic basis of family conflict resolution in mice. Nature 421: 533-535

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

Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM (2000) Long-term behavioral and neuroendocrine adaptations to adverse early experience. Prog Brain Res 122: 81-103

Levine S (2005) Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology 30: 939-946

Liu D, Diorio J, Day JC, Francis DD, Meaney MJ (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci 3: 799-806

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S et al. (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic- pituitary-adrenal responses to stress. Science 277: 1659-1662

Mathieson WB, Wilkinson M, Brown RE, Bond TL, Taylor SW, Neumann PE (2002) FOS and FOSB expression in the medial preoptic nucleus pars compacta of maternally active C57BL/6J and DBA/2J mice. Brain Res 952: 170-175

Meaney MJ, Mitchell JB, Aitken DH, Bhatnagar S, Bodnoff SR, Iny LJ, Sarrieau A (1991) The effects of neonatal handling on the development of the adrenocortical response to stress: implications for neuropathology and cognitive deficits in later life. Psychoneuroendocrinology 16: 85-103

Meek LR, Dittel PL, Sheehan MC, Chan JY, Kjolhaug SR (2001) Effects of stress during pregnancy on maternal behavior in mice. Physiol Behav 72: 473-479

Numan M (2007) Motivational systems and the neural circuitry of maternal behavior in the rat. Dev Psychobiol 49: 12-21

Prakash P, Merali Z, Kolajova M, Tannenbaum BM, Anisman H (2006) Maternal factors and monoamine changes in stress-resilient and susceptible mice: cross-fostering effects. Brain Res 1111: 122-133

Priebe K, Brake WG, Romeo RD, Sisti HM, Mueller A, McEwen BS, Francis DD (2005) Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: A cross-fostering study. Dev Psychobiol 47: 398-407

Rees SL, Panesar S, Steiner M, Fleming AS (2004) The effects of adrenalectomy and corticosterone replacement on maternal behavior in the postpartum rat. Horm Behav 46: 411-419

Shanks N, Griffiths J, Zalcman S, Zacharko RM, Anisman H (1990) Mouse strain differences in plasma corticosterone following uncontrollable footshock. Pharmacol Biochem Behav 36: 515-519

Shoji H, Kato K (2006) Maternal behavior of primiparous females in inbred strains of mice: A detailed descriptive analysis. Physiol Behav 89: 320-328

Wahlsten D, Metten P, Phillips TJ, Boehm SL, Burkhart-Kasch S, Dorow J, Doerksen S et al. (2003) Different data from different labs: lessons from studies of gene-environment interaction. J Neurobiol 54: 283-311

Wainwright PE (1981) Maternal performance of inbred and hybrid laboratory mice (Mus Musculus). J Comp Physiol Psychol 95: 694-707

Ward R (1980) Some effects of strain differences in the maternal behavior of inbred mice. Dev Psychobiol 13: 181-190

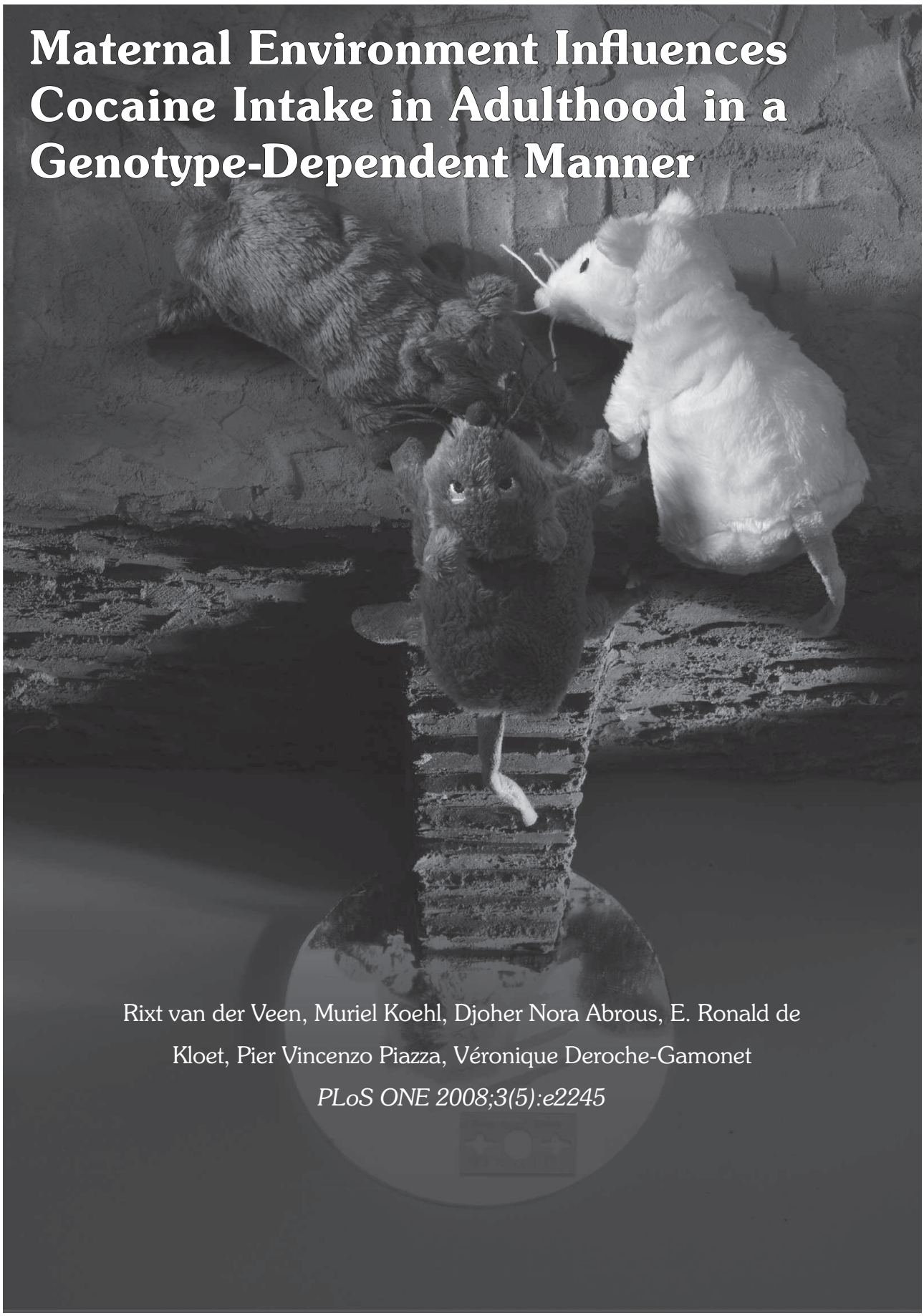
Weller A, Leguisamo AC, Towns L, Ramboz S, Bagiella E, Hofer M, Hen R et al. (2003) Maternal effects in infant and adult phenotypes of 5HT1A and 5HT1B receptor knockout mice. Dev Psychobiol 42: 194-205

Woodside B, Jans JE (1988) Neuroendocrine basis of thermally regulated maternal responses to young in the rat. Psychoneuroendocrinology 13: 79-98

Yamazaki K, Beauchamp GK, Curran M, Bard J, Boyse EA (2000) Parent-progeny recognition as a function of MHC odortype identity. Proc Natl Acad Sci U S A 97: 10500-10502

Chapter 3

Maternal Environment Influences Cocaine Intake in Adulthood in a Genotype-Dependent Manner



Rixt van der Veen, Muriel Koehl, Djoher Nora Abrous, E. Ronald de Kloet, Pier Vincenzo Piazza, Véronique Deroche-Gamonet

PLoS ONE 2008;3(5):e2245

Abstract

Accumulating epidemiological evidence points to the role of genetic background as a modulator of the capacity of adverse early experiences to give rise to mental illness. However, direct evidence of such gene-environment interaction in the context of substance abuse is scarce. In the present study we investigated whether the impact of early life experiences on cocaine intake in adulthood depends on genetic background. In addition, we studied other behavioral dimensions associated with drug abuse, i.e. anxiety- and depression-related behaviors. For this purpose, we manipulated the maternal environment of two inbred mouse strains, the C57BL/6J and DBA/2J by fostering them with non-related mothers, i.e. the C3H/HeN and AKR strains. These mother strains show respectively high and low pup-oriented behavior. As adults, C57BL/6J and DBA/2J were tested either for cocaine intravenous self-administration or in the elevated plus-maze and forced swim test. We found that the impact of maternal environment on cocaine use and a depression-related behavior depends upon genotype, as cocaine self-administration and behavior in the FST were influenced by maternal environment in DBA/2J, but not in C57BL/6J mice. Anxiety was not influenced by maternal environment in either strain. Our experimental approach could contribute to the identification of the psychobiological factors determining the susceptibility or the resilience of certain individuals to develop psychopathologies.

Introduction

Clinical and epidemiological studies point to an important role of adverse early experiences in the vulnerability to a variety of psychiatric disorders in adulthood. Family environment in particular would affect the long-term neurobiological and psychosocial development of the offspring and modulate the vulnerability to mood-, anxiety-, psychosis- or drug use-related disorders (Oakley-Browne et al. 1995; Sameroff 2000; De Bellis 2002; Newport et al. 2002; Gordon 2002; Heim et al. 2004). But, although their influence may be strong and pervasive, early experiences rarely determine the nature and outcome of the psychopathology (Kendler 2005; Rutter et al. 2006). Indeed, large individual differences exist in susceptibility to the impact of early life events on health and behavior.

Numerous twin and adoption studies have demonstrated a gene-environment interaction in the development of psychiatric disorders (Cadoret et al. 1995; Silberg et al. 2001; Tienari et al. 2004; Jaffee et al. 2005). Moreover, accumulating epidemiological evidence suggest that genetic background can modulate the capacity of an environmental risk factor to give rise to mental illness (Moffitt et al. 2006; Caspi and Moffitt 2006). In particular, functional polymorphisms were found to modulate the influence of adverse early experiences on antisocial disorders (Caspi et al. 2002; Foley et al. 2004), schizophrenia (Caspi et al. 2005) and depression (Caspi et al. 2003). Direct evidence of gene-early environment interactions in substance abuse disorders is more scarce and mostly concerns alcohol abuse (Cutrona et al. 1994; Sigvardsson et al. 1996).

The accessibility to (illegal) drugs, drug effects and the great individual variability in intake makes epidemiological studies on substance abuse particularly difficult. As an alternative to human studies, interesting animal models exist for many aspects of substance use and abuse. In particular intravenous self-administration (SA) is considered as one of the best animal models of human psychopathology that has high predictive validity for detecting compounds with an abuse potential in humans (Ator and Griffiths 2003). Rodent studies using this model suggest that also in the etiology of cocaine abuse, gene-environment interactions could play a role. That is, different adult environmental experiences were shown to influence cocaine SA in a gene-dependent manner (van der Kam et al. 2005; van der Veen et al. 2007). However, it remains to be determined whether long-lasting changes induced by early life experiences can also influence cocaine SA in a genotype-dependent manner.

In the present study we investigated whether the impact of early life environment on cocaine SA in adulthood depends on the genetic background. For this purpose, we manipulated the maternal environment of two inbred mouse strains, the widely used C57BL/6J (C57) and DBA/2J (DBA) by fostering them with non-related mothers. A critical component of the maternal environment is constituted by the behavior of the mother. We therefore chose two strains showing respectively high and low pup-oriented behavior, i.e. the C3H/HeN (C3H) and AKR strains (described in chapter 2; van der Veen et al. 2008). For reference, we included mice of both strains raised by their biological mother. In adulthood, mice were tested on intravenous cocaine SA. In parallel with these SA experiments, in a separate set of animals, we investigated whether genotype influenced the impact of maternal

environment on anxiety- (elevated plus-maze) and depression- (forced swim test) related behaviors; two dimensions potentially associated with the vulnerability to cocaine abuse (Khantzian 1985; Brady et al. 2007).

We show that the impact of early life experiences on cocaine use in adulthood is dependent on the genotype, as we found the DBA strain sensitive and the C57 strain resistant to the influence of maternal environment on cocaine intake in adulthood. Additional behavioral characterization revealed that the alterations in cocaine-taking behavior observed in the DBA strain were accompanied with alterations in depression-related behavior while anxiety was not influenced. These results demonstrate a strong gene-environment interaction during the early life period that affects psychopathology-related behaviors in adulthood.

Materials & Methods

Subjects

All mice used in the experiments were bred in our animal facilities. Three of the original strains (C57BL/6JOlaHsd, AKR/OlaHsd, C3H/HeNHsd) were obtained from Harlan (Gannat, France), the DBA/2J@Ico mice were obtained from Charles River Laboratories (Arbresle, France). Animals were kept in our temperature (23°C) and humidity (60%) controlled animal facilities. Food and water were available *ad libitum.* All experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Breeding & cross-fostering

A 14h light-10h dark cycle was installed (lights on from 08:00 to 22:00) as is common in reproductive facilities. In an individual mouse cage (29x11x13 cm), each female was paired with a male of her own strain that was removed from the cage when pregnancy was ascertained by visual observation and body weight recording. Cross-fostering was conducted between 4-7 hours after both biological and adoptive dams had given birth. The procedure consisted of removing the biological mother, weighing and sexing all the pups, culling the litter to 5-8 pups, placing the litter in an individual mouse cage containing bedding of the adoptive mother, and finally placing the adoptive mother in the cage. This cage contained a transparent Plexiglas separation with a small hole creating a nest compartment that occupied one-third of the cage. Culled litters contained a balanced amount of males and females. The whole procedure lasted on average 2 min, and never took more than 4 min. Biological litters were treated as cross-fostered litters, but were returned to their original mother.

Maternal behavior

Cages were placed in sound safe video-equipped chambers equipped with infra-red cameras to record maternal behavior (Imetronic, Pessac, France). Continuous recordings of

maternal behavior were made over 23h periods on postnatal days (PD) 2, 4, 6 and 9. Recordings started at 08:00. Only dams with litters between 10h and 24h of age at the start of recording on PD2 were included in behavioral scoring (AKR mothers: n=9 dams for each pup strain, C3H mothers: n=7 dams for each pup strain, bio mothers: n=15 for C57 dams and n=8 for DBA dams). Behavior was scored off line by an experimenter unaware of the experimental groups using The Observer® (Noldus Information Technology, Wageningen, The Netherlands). Six behaviors were scored. *Pup licking:* Licking any part of the pups' body, including anogenital parts. *Nursing posture:* Immobile arched-back posture over the pups, with the abdomen actively elevated from the floor and pups attached to the nipples. *Nest reorganizing:* Nest disturbance and pup scattering by a push forward movement of the mothers' head in the sawdust. *Self grooming on the nest:* Self grooming while being in contact with the pups. *'Passive' nest presence:* Being in contact with the pups without showing any of the above mentioned behaviors. *Nest absence:* Not being in physical contact with the pups. An observation was made every 2.5 min. Detailed analysis of maternal behavior is described in chapter 2 (van der Veen et al. 2008).

Drugs, Catheters & Surgery

Cocaine (Coopération Pharmaceutique Française, Bordeaux, France) was dissolved in 0.9% NaCl. Catheters were made from 6 cm soft silastic tubing (i.d.=0.30 mm, o.d.= 0.64 mm; Dow Corning Corp., Midland, Michigan, USA), fitted to a 22G steel cannula (Plastics One Inc., Roanoke, VA, USA) bent at a right angle. The cannula was anchored with dental cement onto the plastic part of a 26G needle and provided with a small piece of nylon mesh for support. Surgery was performed under ketamine (80 mg/kg, Imalgène®)/xylazine (16 mg/kg, Rompun®) anesthesia as previously described (Deroche-Gamonet et al. 2003; van der Veen et al. 2007). During surgery, mice were placed on a heat-pad (30°C). The catheter was inserted into the right jugular vein, until the tip reached the level of the right atrium. It was attached to the vein at the entrance point around a little dab of silicone on the tubing. The distal end was passed subcutaneously over the shoulder to be attached in the mid-scapular region. After surgery, mice were placed in a heat-box (27°C) until they woke up from anesthesia. To prevent infection, animals received daily IP injections of a 0.05 ml gentamicine solution (11.4 mg/ml, Gentalline®) during four days. Throughout the experiment, catheters were flushed daily with a saline solution containing heparin (30 IU/ml).

Intravenous self-administration apparatus

The intravenous SA setup (Imetronic, Pessac, France) consisted of 16 chambers made of Plexiglas and metal. Each chamber (18x11x15 cm) was located within a larger exterior opaque box equipped with exhaust fans that assured air renewal and masked background noise. In the SA chamber, the intravenous catheter of the animal was connected to a pump-driven syringe (infusion speed: 20 µl/sec). Two holes (Ø 8.5 mm) were located at opposite sides of the chamber at 2 cm from the grid floor. A white cue light (Ø 2 mm) was located 3 cm above each hole. Experimental contingencies were controlled and data collected with a computerized system (Imetronic, Pessac, France).

Elevated plus maze (EPM)

The EPM has been validated for monitoring anxiety-related behavior (Pellow et al. 1985). The test is based on the creation of a conflict between the exploratory drive of the rodent and its innate fear of open and exposed areas. The apparatus was made of Plexiglas and consisted of a plus-shaped platform elevated 116 cm above the floor. Two of the opposing arms (30x8 cm) were enclosed by 17 cm high transparent walls (closed arms) whereas the other two arms had no walls (open arms). The floor of the maze was covered with black Plexiglas. At the start of the test, the animal was placed at the crossing of the four arms, facing an open arm. The animal could move freely over the maze for 5 min. A camera connected to a computerized tracking system (©VideoTrack, Viewpoint) allowed to measure entries into open and closed arms and time spent in each compartment.

Forced swim test (FST)

The FST was performed according to the method of Porsolt (Porsolt et al. 1977). This test is classically used to measure the effect of antidepressant drugs on the behavior of laboratory animals, but is also used to assess depression-like phenotypes (Porsolt 2000; Cryan and Mombereau 2004). Mice were placed individually in a glass cylinder (height 25 cm; Ø 18 cm) filled with 22 °C water to a depth of 15 cm. This water depth prevented the animals from touching the bottom of the cylinder with hind limbs or tail. Behavior was recorded for 6 min with a camera placed to give a side view of the cylinder. The duration of total immobility was scored off-line by an experimenter unaware of the experimental groups. A mouse was judged to be immobile when it remained floating, in an upright position, making only small movements to keep its head above the water.

Procedures

Pups were weaned and weighed at 21 days of age and placed together with same sex group mates, 4-6 animals per cage (37x21x14 cm). At 10 weeks of age the animals were individually housed under a standard 12h light-dark cycle (lights on from 08:00 to 20:00). Four experimental groups were available for measuring adult behavior *(figure 3.1)*: C57 and DBA mice raised by mothers of the AKR strain (C57-*AKR* and DBA-*AKR)* and C57 and

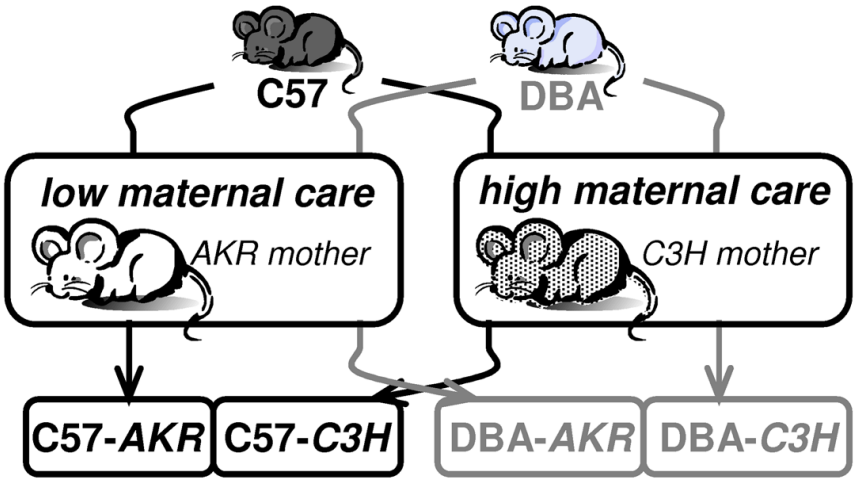


Figure 3.1: Experimental set-up. Manipulation of the early environment by fostering pups of the C57 and DBA strain with mothers of the AKR or C3H strain.

DBA mice raised by mothers of the C3H strain (C57-*C3H* and DBA-*C3H)*. Two reference groups were formed by pups raised by their biological mothers (C57-*bio* and DBA-*bio*). Since animals from these last two groups have an isogenic mother and are not fostered, they can not be considered as fully valid control groups and thus can not be included in the analysis of the impact of maternal behavior. They can, however, be used as reference groups providing indications of the change that might occur in behavior from mice of each strain raised by one or the other foster mother. As such, they will appear in the background of the graphs. At three months of age, animals were attributed to an experiment (1 or 2) and behavioral testing was performed between 3 and 5 months of age.

Experiment 1: Cocaine self-administration

For a first set of animals (n=12 per experimental group), the light-dark cycle was inverted (lights off from 08:00 to 20:00) and mice were given a three weeks acclimatization period. Then catheterization took place followed by 4-6 days of recovery before the start of cocaine SA. Sixteen SA cages were available, which resulted in three SA sessions per day, the first session starting 1.5 hours after lights off. Animals from the different experimental groups were randomly attributed to a session, each individual animal being tested at the same time each day. When the animal introduced its snout into one of the holes (the active device), this turned on the cue light and subsequently switched on the infusion pump (1 sec cue light alone followed by 1 sec cue light plus infusion). 'Nose'-pokes in the other hole (the inactive device) had no scheduled consequences. A fixed ratio 1 (FR1) schedule was applied throughout the experiment, meaning that one nose-poke was necessary to obtain an infusion of cocaine. Each infusion was followed by a 9 sec time-out period during which cocaine was not available. Mice were allowed to acquire cocaine SA during daily 90 min sessions. In an earlier study (see chapter 4; van der Veen et al. 2007) we identified a difference in dose sensitivity for cocaine between the C57 and DBA strains; the latter strain having a dose-response curve shifted to the left. In order to obtain a comparable number of infusions at acquisition, C57 mice acquired at a dose of 1mg/kg/infusion and DBA mice at a dose of 0.5 mg/kg/infusion *(see figure 3.2 for data supporting this choice).*

Criteria for acquisition of cocaine SA were defined by a stable number of self-infusions over at least three consecutive sessions (± 20%) and at least 75% responding for the active hole. After 10 days of SA all animals reached these criteria. Following acquisition, a dose-response test was performed. Cocaine dose was gradually diminished over sessions

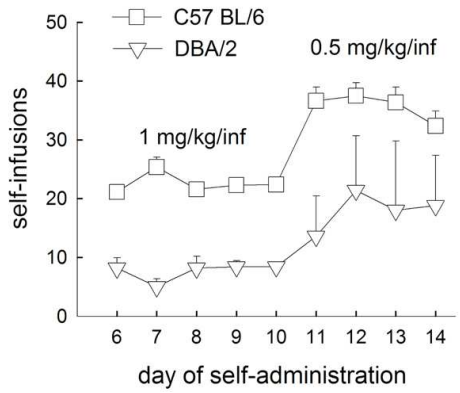


Figure 3.2: Cocaine self-administration in C57 and DBA mice in a study where both strains acquired at 1 mg/kg/infusion. The graph shows the number of self-infusion on the last 5 days of acquisition (day 6-10) and the transition to a 0.5 mg/kg/infusion dose (day 11-14). At the 0.5 mg/kg dose, DBA mice show a number of self-infusions that is comparable to that of the C57 mice at the 1 mg/kg/infusion dose. Symbols represent mean ± SEM. C57 (squares) n=8, DBA (triangles) n=5.

(0.5, 0.25, 0.125, 0.0625 mg/kg/infusion for C57 and 0.25, 0.125, 0.0625, 0.0313 mg/kg/infusion for DBA), maintaining each dose until the animal reached at least two days of stable intake (± 20 %). Considering the results, each strain was tested for an extra seventh dose (2 mg/kg/infusion for C57 and 0.0156 mg/kg/infusion for DBA) in order to obtain a bell-shaped dose-response curve for both strains. Finally, both strains were tested for saline during 5 days. Because of blocked or leaking catheters, some animals were taken out of the experiment. Only animals that completed SA were included in the analyses.

Experiment 2: Elevated plus maze and forced swim test

A second set of animals (n=8-14 per experimental group), bred together with the animals used in experiment 1, was tested on the EPM and the FST successively. Both tests were performed during the light phase (starting 1.5 hours after lights on) under dim light conditions (90 lux). Four weeks separated the two tests.

Data analysis

Analyses were performed using Statistica 6.0© (StatSoft Inc). *Maternal behavior:* Maternal behavior differences between AKR and C3H mothers were globally analyzed with bifactorial ANOVAs using pup strain and mother strain as independent factors. To situate the behavior of these foster mothers relative to that of biological mothers, within each pup strain, one-way ANOVAs were performed with mother strain (C3H, AKR and bio mother) as independent factor. *Bodyweight:* Bodyweight differences at birth of pups attributed to the different experimental groups were analyzed within each strain using one-way ANOVAs. The influence of mother strain on bodyweight at weaning and adulthood was analyzed with a repeated measures ANOVA using mother strain and pup strain as independent variables and age as dependent variable. To situate the reference group *(-bio)* within each pup strain, repeated measures ANOVAs were performed. *Self-administration:* To verify recognition of the active hole in the acquisition phase of SA, and recognition of the different drug doses in the dose-response test, a repeated measures ANOVA was performed within each experimental group, using either hole and day (acquisition) or dose (dose-response) as dependent variables. Within each pup strain, group differences in responding at the acquisition endpoint (mean of the last three acquisition days) were analyzed with unpaired two-tailed t-tests. The reference groups *(-bio)* were situated using one-way ANOVAs. Group differences in responding during the 10-day acquisition period and during the dose-response phase were analyzed with repeated measures ANOVAs, where mother strain served as an independent factor and day or dose as dependent variable. In the dose-response test, we considered the mean of the last two days (stable intake) for each dose. Within each pup strain, the reference group *(-bio)* was situated using repeated measures ANOVAs, where mother strain served as an independent factor and day or dose as dependent variables. *Elevated plus maze and forced swim test:* Mother strain effect on behavior in the EPM and FST was globally analyzed with bifactorial ANOVAs using mother strain and pup strain as independent variables. Within each pup strain, the reference group *(-bio)* was situated using a one-way ANOVA. A significance level of $p<0.05$ was used for all statistical analyses. Whenever appropriate, a Newman-Keuls post-hoc test was performed.

Results

Maternal behavior of foster mothers

Maternal behavior differences between AKR and C3H mothers were globally analyzed including both pup strains. Then, for each pup strain, the behavior of the adoptive mothers was compared to that of biological mothers.

Maternal behavior of AKR and C3H dams was shown to be very different, irrespective of the pup strain raised *(see figure 3.3 for pup-oriented behaviors)*. C3H dams showed more pup licking [mother strain effect $F(1,28)=97.7$ $p<0.001$, mother strain*pup strain interaction $F(1,28)=2.07$ p=ns], more nursing posture [mother strain effect $F(1,28)=21.4$ $p<0.001$, mother strain*pup strain interaction $F(1,28)=3.14$ p=ns] and less nest reorganizing [mother strain effect $F(1,28)=14.7$ $p<0.001$, mother strain*pup strain interaction $F(1,28)=0.69$ p=ns] compared to AKR dams. Although both foster mothers spend an equal amount of time in contact with the pups, AKR mothers spent more time in self-grooming on the nest and 'passive' nest presence, two behaviors that are less directly pup-oriented (graphs not shown). Both mother strains showed less nursing with DBA pups than with C57 pups [pup strain effect $F(1,28)=26.0$ $p<0.001$, mother strain*pup strain interaction $F(1,28)=3.14$ p=ns].

The behavior of biological C57 mothers *(figure 3.3 hatched bands in left panels)* was situated in-between the foster mothers for pup licking [mother strain effect $F(2,28)=31.6$ $p<0.001$ with bio>AKR, bio<C3H and AKR<C3H, all $p<0.001$]. Considering nursing posture and nest reorganizing, behavior of biological C57 mothers was comparable to that

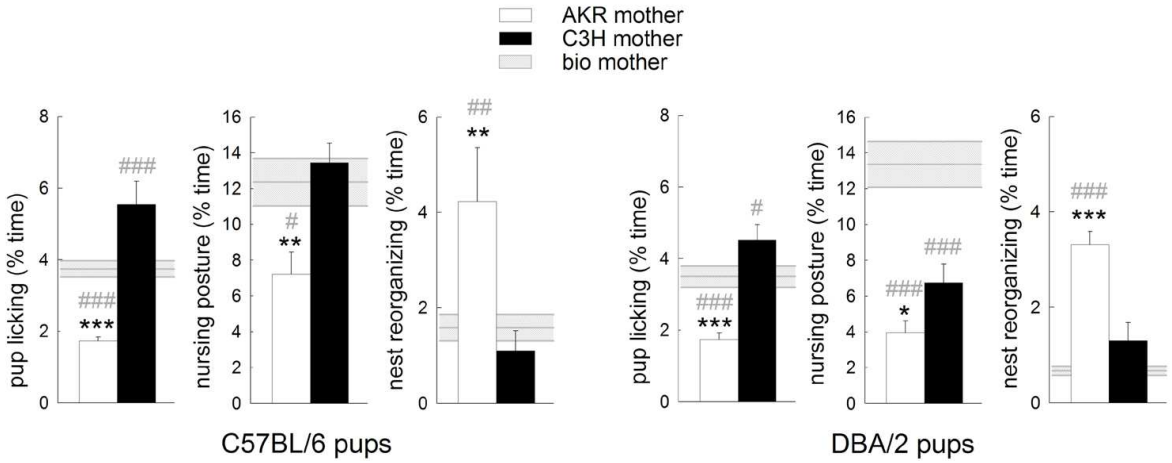


Figure 3.3: Maternal behavior displayed by AKR and C3H mothers toward C57 and DBA pups. Graphs show the percentage of time spent in pup licking, nursing posture and nest reorganizing activity with C57 pups (left panels) and DBA pups (right panels) averaged over 4 postnatal days (PD 2, 4, 6 and 9). Behavior of the reference mothers (bio mothers) is shown as horizontal hatched bands. Bars represent mean ± SEM. AKR mothers (white bars) n=9 dams for each pup strain. C3H mothers (black bars) n=7 dams for each pup strain. Bio mothers (hatched bands) n=15 for C57 dams and n=8 for DBA dams. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ compared to C3H mothers. # $p<0.05$, ## $p<0.01$ and ### $p<0.001$ compared to bio mothers.

of C3H foster mothers [*nursing posture:* mother strain effect F(2,28)=5.7 p<0.01 with bio>AKR p<0.05, bio≈C3H and AKR<C3H p<0.01, *nest reorganizing:* mother strain effect F(2,28)=6.87 p<0.01 with bio<AKR p<0.01, bio≈C3H and AKR>C3H p<0.01].

The behavior of biological DBA mothers *(figure 3.3 hatched bands in right panels)* was equally situated in-between the foster mothers for pup licking [mother strain effect F (2,21)=25.6 p<0.001 with bio>AKR p<0.001, bio <C3H p<0.05 and AKR<C3H p<0.001]. Considering nursing posture, biological DBA mothers showed higher levels compared to both foster mothers [mother strain effect F(2,21)=27.9 p<0.001 with bio>AKR p<0.001, bio>C3H p<0.001 and AKR<C3H P<0.05]. The nest reorganizing behavior of biological DBA mothers was comparable to that of C3H foster mothers [mother strain effect F (2,21)=32.7 p<0.001 with bio<AKR p<0.001, bio≈C3H and AKR>C3H p<0.001].

Bodyweight of offspring

Bodyweight at birth did not differ between pups attributed to the two different maternal environments or pups that stayed with their biological mother [mother effect C57 F(2,73)=1.85 and DBA F(2,62)=0.71, both p=ns] *(table 3.1).*

When comparing the influence of being raised by an AKR or a C3H mother on bodyweight, it appeared that pup strains were differentially affected [mother strain*pup strain interaction F(1,88)=10.46 p<0.01]. This effect was comparable at weaning and adulthood [age*mother strain*pup strain interaction F(1,88)=0.02 p=ns]. Although it was found that being raised by an AKR mother gives a lower bodyweight compared to being raised by a C3H mother for both C57 (p<0.001) and DBA (p<0.01), the influence of mother strain on bodyweight was more important in C57 mice.

The bodyweight of C57 mice raised by their biological mother, was differentially situated at weaning and in adulthood [age*mother strain interaction F(2,73)=11.35 p<0.001]. At weaning C57-*bio* were situated in-between C57-*AKR* and C57-*C3H* (C57-*bio*>C57-*AKR* p<0.01 and C57-*bio*<C57-*C3H* p<0.05). In adulthood, C57-*bio* mice continued to be heavier than C57-*AKR* mice (p<0.001), but now showed a bodyweight comparable to that of C57-*C3H* mice.

The bodyweight of DBA mice raised by their biological mother was lower than that of DBA-*C3H*, but comparable to that of DBA-*AKR* [mother strain effect F(2,62)=10.27

Table 3.1: The impact of maternal environment on bodyweight. The table shows bodyweight data at birth, weaning and 16 weeks of age of male C57 and DBA offspring raised by an AKR, a C3H or their biological mother.

			Bodyweight (g)		
Pup strain	Maternal environment	*n*	Birth	Weaning	16 weeks
C57	AKR mother	24	1.39 ± 0.02	7.54 ± 0.23	26.92 ± 0.27
	C3H mother	26	1.35 ± 0.02	10.31 ± 0.25	29.46 ± 0.36
	C57 bio mother	26	1.34 ± 0.02	9.08 ± 0.20	29.65 ± 0.27
DBA	AKR mother	20	1.31 ± 0.03	7.45 ± 0.21	26.30 ± 0.29
	C3H mother	22	1.28 ± 0.03	8.64 ± 0.27	27.18 ± 0.31
	DBA bio mother	23	1.32 ± 0.03	7.30 ± 0.23	26.04 ± 0.35

Values represent mean ± SEM

p<0.001 with DBA-*bio*≈DBA-*AKR* and DBA-*bio*<DBA-*C3H* p<0.001]. This was seen both at weaning and in adulthood [age* mother strain interaction F(2,62)=0.16 p=ns].

Cocaine intravenous self administration

After ten days of training, both C57 (at 1 mg/kg/infusion) and DBA (at 0.5 mg/kg/infusion) acquired cocaine SA *(figure 3.4 left panels)*. Animals showed a stable responding (± 20%) over at least the last 3 days and reached the criteria of 75% responding for the active hole, indicating that they clearly discern the active from the inactive hole [hole effect C57-*AKR*: F(1,4)=33.21 p<0.01, C57-*C3H*: F(1,7)=9.27 p<0.05, C57-*bio*: F(1,7)=22.4 p<0.01, DBA-*AKR*: F(1,7)=12.37 p<0.01, DBA-*C3H*: F(1,10)=63.16 p<0.001, DBA-*bio*: F(1,9)=31.4 p<0.001, hole*day interaction p=ns for all groups].

The two foster environments did not differentially affect acquisition of cocaine SA in C57 mice. Responding varied over the ten days of acquisition, but this variation was comparable for C57-*AKR* and C57-*C3H* animals [day effect F(9,99)=3.87 p<0.001, day*mother interaction F(9,99)=1.61 p=ns]. At the acquisition end point, C57-*AKR* and C57-*C3H* mice showed a similar number of self-infusions ($t_{1,11}$= -1.10 p=ns). On the contrary, in the DBA strain, acquisition was influenced by maternal environment. Responding varied over the ten days of acquisition, and this variation was dependent on mother strain [day effect F(9,153)=2.82 p<0.01, day*mother interaction F(9,153)=2.12 p<0.05]. At the acquisition endpoint, DBA-*AKR* mice showed a lower cocaine intake compared to DBA-*C3H* mice ($t_{1,17}$= -2.12 p<0.05).

Animals from each experimental group distinguished the different doses of cocaine in the dose-response test (*figure 3.4 right panels)*[dose effect C57-*AKR* F(7,28)=23.65; C57-*C3H* F(7,49)=12.17; C57-*bio* F(7,49)= 16.07; DBA-*AKR* F(6,42)=8.79; DBA-*C3H* F(6,60)=14.79; DBA-*bio* F(6,54)=15.79, all p<0.001]. However, DBA mice still showed important responses to the lower and less reinforcing doses of cocaine as well as to saline infusions. This 'deficit' of extinction resulted in a large variation for these doses. For this reason, only group differences in the descending limb of the dose-response curve (4 highest doses) were analyzed.

Similarly to acquisition, maternal environment did not affect responding in the dose-response test in C57 mice, but it did in DBA mice. C57 animals raised by an AKR or a C3H mother showed a similar dose-response curve for cocaine self-infusions [mother effect F(1,11)=1.00 p=ns, dose*mother interaction F(3,33)=1.50 p=ns]. On the contrary, DBA mice raised by an AKR mother showed a downward shift in the dose-response curve as compared to mice raised by a C3H mother [mother effect F(1,17)=4.6 p<0.05, dose*mother interaction F(3,51)=0.45 p=ns].

SA behavior of C57-*bio* mice *(figure 3.4a, grey lines)* was similar to that of C57 mice raised by either foster mother. This was seen in the variation in intake during acquisition [day effect F(9,162)=6.12 p<0.001, day*mother interaction F(18,162)=1.40 p=ns] as well as the intake at the acquisition endpoint [mother effect F(2,18)=0.73 p=ns], and in the dose-response test [mother effect F(2,18)=0.55 p=ns, dose*mother interaction F(6,54)=0.72 p=ns].

DBA-*bio* mice *(figure 3.4b, grey lines)* showed intermediate levels of responding in SA, more closely resembling the responding of DBA-*C3H* mice in the dose-response

test. Although visually apparent, these differences were not statistically significant [day effect in acquisition F(9,234)=4.46 p<0.001, day*mother interaction F(18,234)=1.34 p=ns, mother effect at acquisition endpoint F(2,26)=2.42 p=ns, mother effect in dose response test F(2,26)=2.45 p=0.11 (ns), dose*mother interaction F(6,78)=0.57].

Elevated plus maze and forced swim test

We measured both locomotor activity and anxiety behavior on the EPM. We used the number of entries into the closed arms as a measure of locomotor activity (File 2001) and the percentage of time spent on the open arms as a measure of anxiety (Pellow et al. 1985). Comparing behavior in the EPM, it appeared that neither locomotor activity *(figure 3.5 left panels)*, nor anxiety behavior *(figure 3.5 middle panels)* was influenced by the foster environments [mother effect F(1,40)=0.35 and 1.89, both p=ns; mother strain*pup strain

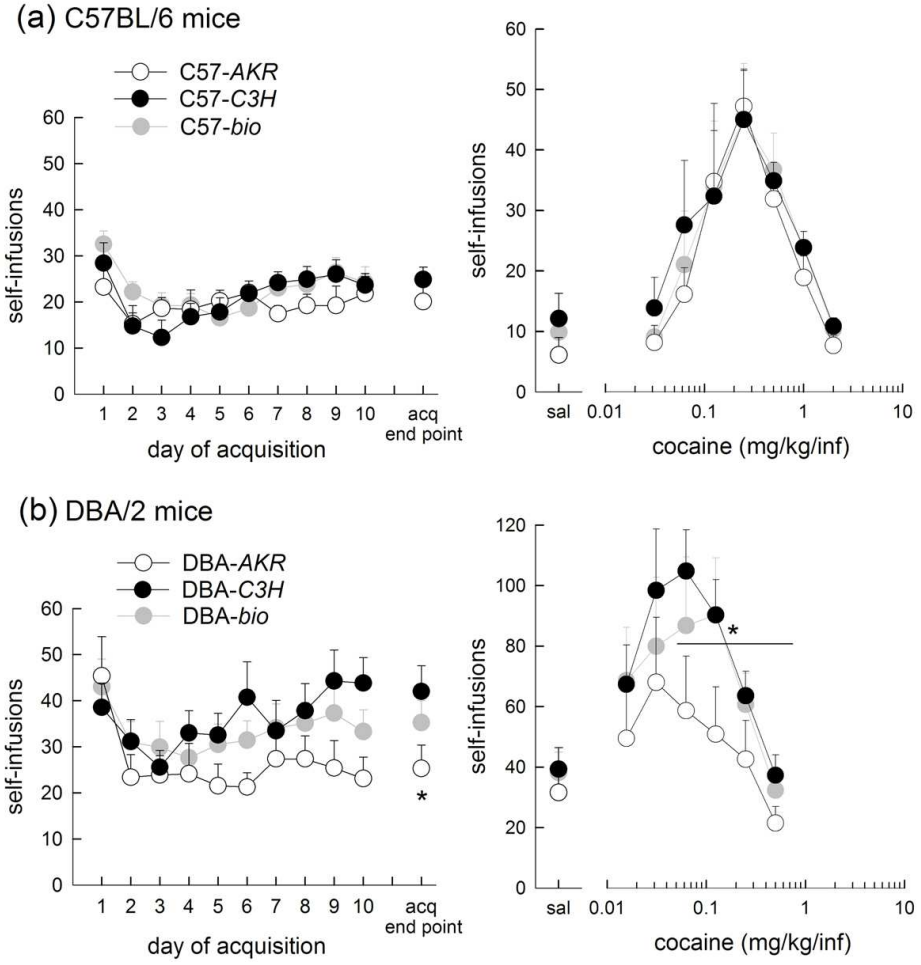


Figure 3.4: Impact of maternal environment on cocaine intravenous SA in C57 and DBA mice. Graphs show the number of self-infusions obtained in ***(a)*** C57 mice in acquisition at 1 mg/kg/infusion (left panel) and dose-response test (right panel) ***(b)*** DBA mice in acquisition at 0.5 mg/kg/infusion (left panel) and dose-response test (right panel). The symbols in the dose-response graph represent the last two days of stable intake on a dose. Behavior of the reference groups *(-bio)* is shown with grey lines and symbols. Horizontal lines show the data submitted to statistical analysis. Symbols represent mean ± SEM. C57-*AKR* (white circles) n=5, C57-*C3H* (black circles) n=8, C57-*bio* (grey circles) n=8, DBA-*AKR* (white circles) n=8, DBA-*C3H* (black circles) n=11, DBA-*bio* (grey circles) n=10. * p<0.05 compared to DBA-*C3H*.

interaction F(1,40)=1.96 and 0.01, both p=ns for respectively entries closed arms and time open arms].

In contrast, C57 and DBA mice were differentially influenced by the two foster environments in immobility behavior in the FST *(figure 3.5 right panels)* [mother strain*pup strain interaction F(1,40)=5.56 p<0.05]. Indeed, DBA-*AKR* mice showed an increased duration of immobility compared to DBA-*C3H* mice (p<0.01), while both C57 groups showed a comparable behavior in this test.

Behavior of C57-*bio* animals *(figure 3.5a hatched bands)* in these two tests was comparable to that of animals raised by either foster mother [mother effect in EPM entries closed arms F(2,37)=1.46, EPM time open arms F(2,37)=0.40, FST immobility F(2,37)=0.61, all p=ns].

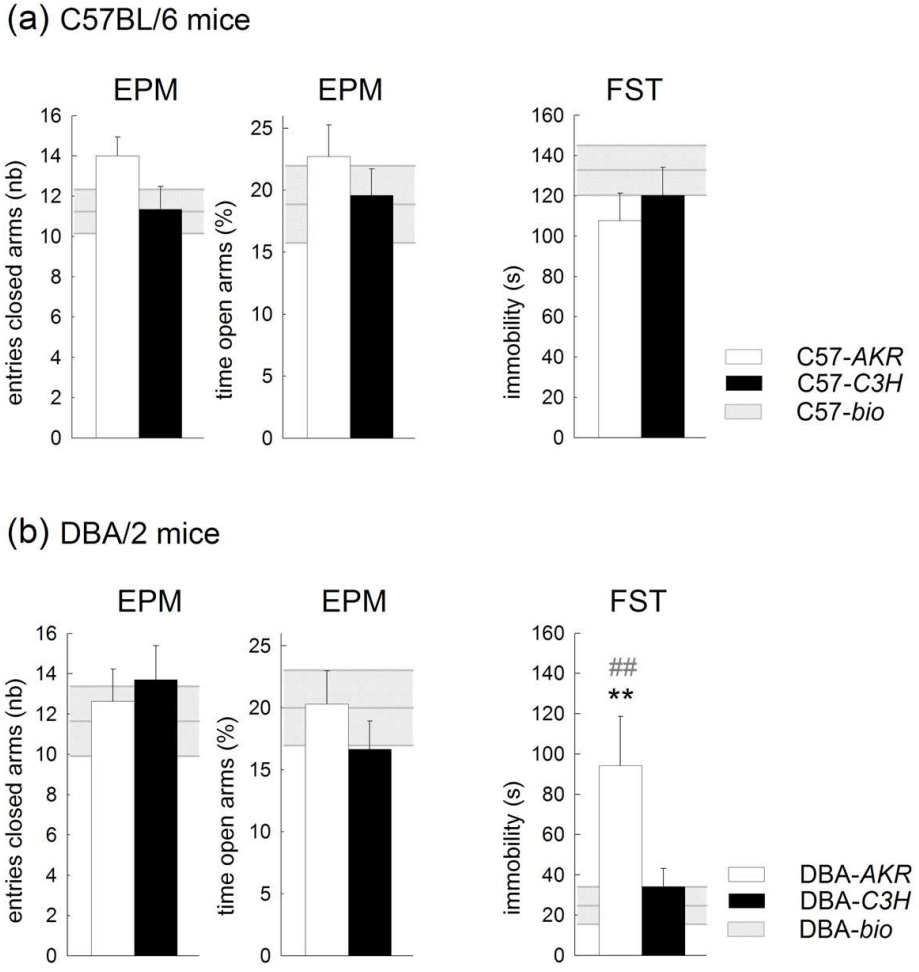


Figure 3.5: Impact of maternal environment on anxiety- and depression-related behaviors in (a) C57 and (b) DBA mice. EPM: Graphs show the total number of entries into the closed arms, an index of exploration (left panels) and the percentage of time spent on the open arms, a measure of anxiety (right panels). FST: Graphs show total immobility time in a 6 min test, a measure of depression-related behavior. Behavior of the reference groups *(-bio)* is shown as horizontal hatched bands. Bars represent mean ± SEM. C57-*AKR* (white bars) n=12, C57-*C3H* (black bars) n=14, C57-*bio* (hatched bands) n=14, DBA-*AKR* (white bars) n=8, DBA-*C3H* (black bars) n=10, DBA-*bio* (hatched bands) n=11. * p<0.05 compared to DBA-*C3H*. ## p<0.01 compared to DBA-*bio*.

In the same way, behavior of DBA-*bio* animals *(figure 3.5b hatched bands)* in the EPM was comparable to that of animals raised by either foster mother [mother effect in EPM entries closed arms $F(2,26)=0.40$, EPM time open arms $F(2,26)=0.55$, both p=ns]. Behavior of DBA-*bio* animals in the FST however, was comparable to behavior of DBA-*C3H* mice, but at a lower level compared to DBA-*AKR* mice [mother effect $F(2,26)=6.48$ $p<0.01$, with DBA-*bio*<DBA-*AKR* $p<0.01$ and DBA-*bio*≈DBA-*C3H*].

Discussion

Epidemiological and clinical studies point a role for negative experiences in the early life as risk factors for the development of psychopathologies in adulthood. However, individuals would be differentially susceptible to the same environmental challenges depending on their genetic background. Our results suggest that such gene-environment interactions can also be observed in rodents. Adult mice, exposed as pups to distinct maternal environments, were examined in cocaine SA and measures for depression- and anxiety- related behaviors, two behavioral dimensions that are frequently associated with human drug abuse (Brady et al. 2007). We report that the outcome of maternal environment for cocaine SA and depression-related behavior in the offspring depends on their genotype. Indeed, C57 mice exhibited similar behaviors when raised in two distinct maternal environments, i.e. a C3H or an AKR mother, indicating a relative resistance to early environmental factors. In contrast, DBA mice raised by an AKR mother significantly differed from DBA mice raised by a C3H mother in cocaine intake and in immobility in the FST, indicating sensitivity to early environmental factors. Anxiety behavior as measured in the EPM was not affected by maternal environment in either strain.

When considering the reference groups (mice raised by their biological mother), it appears that a foster environment *per se*, created by either an AKR or a C3H mother, did not influence behavior in C57 mice, as C57-*AKR*, C57-*C3H*, and C57-*bio* mice were comparable for all behavior tested. Regarding DBA mice, the foster environment created by a C3H mother did not induce behavioral changes (DBA-*C3H* and DBA-*bio* mice are largely comparable), but the foster environment created by an AKR mother did. This latter environment appears to induce a decrease in cocaine intake and an increase in a depression-related behavior.

The design of our study was based on an alteration of the maternal environment of two inbred strains of mice, C57 and DBA, which were fostered with non-related mother strains. Compared to a classical cross-foster experiment, where C57 and DBA mice would have exchanged mothers, our approach reveals two interesting new aspects. Firstly, it allowed testing the outcome of two extremely different maternal environments and secondly, it permitted to minimize potential confounding factors generated by cross-fostering experiments. In classical cross-fostering experiments (exchanging pups between two strains), not only the quality of the maternal environment is manipulated, but also the genetic background of the mother. That is, while the control group is raised by an isogenic mother (e.g. DBA pup raised by a DBA mother), the experimental group is raised by a non-isogenic

mother (e.g. DBA pup raised by a C57 mother). Using our model, we were able to demonstrate that the maternal environment, rather than the fostering to a non-isogenic mother is determinant in producing gene-dependent differences in adult behavior.

A factor that could be responsible for the influence of maternal environment on psychopathology-related behaviors is the maternal behavior (Meaney 2001). The two foster mothers used in this study exhibit very distinct maternal behaviors. AKR mothers showed less pup-oriented behaviors (pup licking and nursing posture), and more nest disturbing behavior (nest reorganizing) and thus can be considered to provide an impoverished maternal environment compared to C3H mothers. Interestingly, the amount of pup licking, which is a very important component of maternal behavior (Meaney 2001), of both C57 and DBA biological mothers was situated in-between the two foster mothers. This underlines the clearly distinct maternal behavior of the AKR and C3H mothers. Our results suggest that this difference in maternal behavior could have played a role in the appearance of behavioral differences in adult DBA mice. However, it should be noted that the maternal environment is a complex ensemble including general behavioral patterns of the mother, but also factors like milk constitution and mother-pup communication through odor cues and vocalizations.

Probably central to gene-environment interactions is that a given environment is not perceived and handled with in the same way by different individuals. The context of maternal environment adds another level of complexity, i.e. the fact that the given environment is not inert but potentially responsive. Indeed, resembling the human situation, the environment furnished by the mother is influenced by the mother-pup dyad. Thus, two pups with different genetic background exposed to the same mother (genetically identical), might generate different behaviors or stimuli which in turn might influence the maternal environment in terms of maternal behavior, milk constitution or vocalizations. For example, the DBA-*AKR* and C57-*AKR* dyads generated the same level of pup licking and nest reorganizing by AKR dams, but not the same level of nursing posture. It remains questioned whether the gene-environment interaction observed in the present study resulted from DBA pups being more sensitive than C57 pups to similar aspects of the foster environment or from differences in the foster environments created by the mother-pup dyad.

Although foster mothers showed less nursing posture with DBA pups compared to C57 pups, this does not necessarily mean that DBA pups are less fed. Indeed, regarding bodyweight differences, undernourishment does not seem to be responsible for the observed gene-environment interaction. In the first place, bodyweight of pups raised by biological DBA mothers (that showed more nursing posture compared to both foster mothers), is similar to DBA-*AKR* pups. Comparing the biological litters of C57 and DBA mice, we can see that the overall lower bodyweight of DBA mice is due to a strain difference, rather than a difference in nursing posture received. In the second place, for bodyweight at weaning, both pup strains are affected in the same way by the foster environments. That is, pups raised by an AKR mother showed a lower bodyweight compared to pups raised by a C3H mother. At adult age, these bodyweight differences were still visible, although less pronounced. Interestingly, the influence of maternal environment on bodyweight was even stronger in C57 than in DBA mice.

Only a few studies investigated the genotype-dependent impact of the early life period on psychopathology-related behaviors in adulthood. To our knowledge, studies regarding drug intake only concern alcohol and support our findings. That is, when tested at the age of weaning, cross-fostering influenced alcohol intake in DBA, but not in C57 mice (Randall and Lester 1975). A recent study using selected rat lines showed a genotype-dependent impact of maternal separation on alcohol intake in rats (Roman et al. 2005). Also anxiety- (Caldji et al. 2004) and depression-related behaviors (Friedman et al. 2006) were shown to be affected by cross-fostering in a genotype-dependent manner.

Interestingly, rodent models in this field resemble the human condition in two aspects. Firstly, a given genotype is not vulnerable or resistant to a given environment in all aspects of its behavior. We for example, showed that DBA are sensitive to the effect of cross-fostering on cocaine use and a depression-related behavior, but not on an anxiety-related behavior. Similarly to our observations, C57 mice are often reported to be resistant to the influence of early life manipulations on several aspects of adult behavior, including cognitive abilities, depression-related and schizophrenia-related behaviors (Anisman et al. 1998; Francis et al. 2003; Caldji et al. 2004; Millstein et al. 2006). However, early life manipulations do influence aggressive behavior in this strain (Veenema et al. 2007). Secondly, a given genotype would not be vulnerable or resistant to all types of early life manipulations. In our study, DBA mice were susceptible to the effect of cross-fostering on a depression-related behavior, while others have shown that maternal separation or handling were ineffective in this strain (Millstein and Holmes 2007). In the same way, cross-fostering of the C57 to a BALB/c mother did not change anxiety-related behavior in the C57 mice (Caldji et al. 2004; Priebe et al. 2005), while maternal separation did (Romeo et al. 2003; Veenema et al. 2007), although the effects of maternal separation on anxiety might depend on the protocol used (Parfitt et al. 2007).

Our data suggest an opposite relation between a depression-related behavior and cocaine intake. The comorbidity between depression and cocaine abuse that is frequently described, appears contradictory with this finding. To explain comorbidity, the self-medication theory proposes that depressed patients seek for the specific effects of cocaine to relieve distress associated with depression (Khantzian 1985). However, the causal links between depression and cocaine use are controversial. It needs to be mentioned that cocaine abuse would rather be associated with bipolar depression rather than with unipolar depression (Weiss 2004). Moreover, in comorbid patients, depression could rather be a consequence than a cause of cocaine abuse (Khantzian 1997). Indeed, depressive symptoms are frequently mentioned as a consequence of drug use or withdrawal (Gawin 1991; Markou and Koob 1992). Furthermore, our observation of an opposite relation between a depression-related behavior and cocaine intake is in accordance with other rodent studies. A decreased sensitivity to reward was found in animals in which a depressive-like behavior was induced either by exposure to chronic mild stress (CMS) (Willner 2005) or olfactory-bulbectomy (OBX) (Willner and Mitchell 2002). Thus, in rats, CMS induces an increased immobility in the FST associated with a decrease in reward sensitivity as measured by increased intracranial self-stimulation threshold, decreased sucrose consumption, decreased preference for alcohol, decreased sexual behavior and decreased amphetamine and morphine rewarding effects (Willner 2005). Similarly, OBX, that exhibits a high de-

gree of neurochemical similarity to depression, induces a decreased sensitivity to reward as shown by a decreased sexual behavior (Lumia et al. 1992), an increased intracranial self-stimulation threshold (Slattery et al. 2007) and a reduced cocaine place preference (Calcagnetti et al. 1996).

In conclusion, we showed for the first time that gene-environment interactions during the early life period can affect cocaine use in adulthood. We further demonstrated an association with a depression-related behavior. Our experimental approach could contribute to the identification of the psychobiological factors determining the susceptibility or the resilience of certain individuals to develop psychopathologies.

Acknowledgements

We would like to thank Cédric Dupuy for excellent animal care and Hélène Woehrle for technical assistance. This research was supported by the NWO/INSERM/ZON grants AH002G and 985-10-014 and the university of Bordeaux 2.

References

Anisman H, Zaharia MD, Meaney MJ, Merali Z (1998) Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci 16: 149-164

Ator NA, Griffiths RR (2003) Principles of drug abuse liability assessment in laboratory animals. Drug Alcohol Depend 70: S55-S72

Brady KT, Verduin ML, Tolliver BK (2007) Treatment of patients comorbid for addiction and other psychiatric disorders. Curr Psychiatry Rep 9: 374-380

Cadoret RJ, Yates WR, Troughton E, Woodworth G, Stewart MA (1995) Genetic-environmental interaction in the genesis of aggressivity and conduct disorders. Arch Gen Psychiatry 52: 916-924

Calcagnetti DJ, Quatrella LA, Schechter MD (1996) Olfactory bulbectomy disrupts the expression of cocaine-induced conditioned place preference. Physiol Behav 59: 597-604

Caldji C, Diorio J, Anisman H, Meaney MJ (2004) Maternal behavior regulates benzodiazepine/GABAA receptor subunit expression in brain regions associated with fear in BALB/c and C57BL/6 mice. Neuropsychopharmacology 29: 1344-1352

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A et al. (2002) Role of genotype in the cycle of violence in maltreated children. Science 297: 851-854

Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 7: 583-590

Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A et al. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry 57: 1117-1127

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J et al. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301: 386-389

Cryan JF, Mombereau C (2004) In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. Mol Psychiatry 9: 326-357

Cutrona CE, Cadoret RJ, Suhr JA, Richards CC, Troughton E, Schutte K, Woodworth G (1994) Interpersonal variables in the prediction of alcoholism among adoptees: evidence for gene-environment interactions. Compr Psychiatry 35: 171-179

De Bellis MD (2002) Developmental traumatology: a contributory mechanism for alcohol and substance use disorders. Psychoneuroendocrinology 27: 155-170

Deroche-Gamonet V, Sillaber I, Aouizerate B, Izawa R, Jaber M, Ghozland S, Kellendonk C et al. (2003) The glucocorticoid receptor as a potential target to reduce cocaine abuse. J Neurosci 23: 4785-4790

File SE (2001) Factors controlling measures of anxiety and responses to novelty in the mouse. Behav Brain Res 125: 151-157

Foley DL, Eaves LJ, Wormley B, Silberg JL, Maes HH, Kuhn J, Riley B (2004) Childhood adversity, monoamine oxidase a genotype, and risk for conduct disorder. Arch Gen Psychiatry 61: 738-744

Francis DD, Szegda K, Campbell G, Martin WD, Insel TR (2003) Epigenetic sources of behavioral differences in mice. Nat Neurosci 6: 445-446

Friedman E, Berman M, Overstreet D (2006) Swim test immobility in a genetic rat model of depression is modified by maternal environment: a cross-foster study. Dev Psychobiol 48: 169-177

Gawin FH (1991) Cocaine addiction: psychology and neurophysiology. Science 251: 1580-1586

Gordon HW (2002) Early environmental stress and biological vulnerability to drug abuse. Psychoneuroendocrinology 27: 115-126

Heim C, Plotsky PM, Nemeroff CB (2004) Importance of studying the contributions of early adverse experience to neurobiological findings in depression. Neuropsychopharmacology 29: 641-648

Jaffee SR, Caspi A, Moffitt TE, Dodge KA, Rutter M, Taylor A, Tully LA (2005) Nature X nurture: genetic vulnerabilities interact with physical maltreatment to promote conduct problems. Dev Psychopathol 17: 67-84

Kendler KS (2005) "A gene for...": the nature of gene action in psychiatric disorders. Am J Psychiatry 162: 1243-1252

Khantzian EJ (1985) The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. Am J Psychiatry 142: 1259-1264

Khantzian EJ (1997) The self-medication hypothesis of substance use disorders: a reconsideration and recent applications. Harv Rev Psychiatry 4: 231-244

Lumia AR, Teicher MH, Salchli F, Ayers E, Possidente B (1992) Olfactory bulbectomy as a model for agitated hyposerotonergic depression. Brain Res 587: 181-185

Markou A, Koob GF (1992) Bromocriptine reverses the elevation in intracranial self-stimulation thresholds observed in a rat model of cocaine withdrawal. Neuropsychopharmacology 7: 213-224

Meaney MJ (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 24: 1161-1192

Millstein RA, Holmes A (2007) Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. Neurosci Biobehav Rev 31: 3-17

Millstein RA, Ralph RJ, Yang RJ, Holmes A (2006) Effects of repeated maternal separation on prepulse inhibition of startle across inbred mouse strains. Genes Brain Behav 5: 346-354

Moffitt TE, Caspi A, Rutter M (2006) Measured gene-environment interactions in psychopathology. Perspect Psychol Sci 1: 5-27

Newport DJ, Stowe ZN, Nemeroff CB (2002) Parental depression: animal models of an adverse life event. Am J Psychiatry 159: 1265-1283

Oakley-Browne MA, Joyce PR, Wells JE, Bushnell JA, Hornblow AR (1995) Adverse parenting and other childhood experience as risk factors for depression in women aged 18-44 years. J Affect Disord 34: 13-23

Parfitt DB, Walton JR, Corriveau EA, Helmreich DL (2007) Early life stress effects on adult stress-induced corticosterone secretion and anxiety-like behavior in the C57BL/6 mouse are not as robust as initially thought. Horm Behav 52: 417-426

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14: 149-167

Porsolt RD (2000) Animal models of depression: utility for transgenic research. Rev Neurosci 11: 53-58

Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229: 327-336

Priebe K, Brake WG, Romeo RD, Sisti HM, Mueller A, McEwen BS, Francis DD (2005) Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: A cross-fostering study. Dev Psychobiol 47: 398-407

Randall CL, Lester D (1975) Cross-fostering of DBA and C57Bl mice. Increase in voluntary consumption of alcohol by DBA weanlings. J Stud Alcohol 36: 973-980

Roman E, Gustafsson L, Hyytia P, Nylander I (2005) Short and prolonged periods of maternal separation and voluntary ethanol intake in male and female ethanol-preferring AA and ethanol-avoiding ANA rats. Alcohol Clin Exp Res 29: 591-601

Romeo RD, Mueller A, Sisti HM, Ogawa S, McEwen BS, Brake WG (2003) Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. Horm Behav 43: 561-567

Rutter M, Moffitt TE, Caspi A (2006) Gene-environment interplay and psychopathology: multiple varieties but real effects. J Child Psychol Psychiatry 47: 226-261

Sameroff AJ (2000) Developmental systems and psychopathology. Dev Psychopathol 12: 297-312

Sigvardsson S, Bohman M, Cloninger CR (1996) Replication of the Stockholm Adoption Study of alcoholism. Confirmatory cross-fostering analysis. Arch Gen Psychiatry 53: 681-687

Silberg J, Rutter M, Neale M, Eaves L (2001) Genetic moderation of environmental risk for depression and anxiety in adolescent girls. Br J Psychiatry 179: 116-121

Slattery DA, Markou A, Cryan JF (2007) Evaluation of reward processes in an animal model of depression. Psychopharmacology (Berl) 190: 555-568

Tienari P, Wynne LC, Sorri A, Lahti I, Laksy K, Moring J, Naarala M et al. (2004) Genotype-environment interaction in schizophrenia-spectrum disorder. Long-term follow-up study of Finnish adoptees. Br J Psychiatry 184: 216-222

van der Kam EL, Ellenbroek BA, Cools AR (2005) Gene - environment interactions determine the individual variability in cocaine self-administration. Neuropharmacology 48: 685-695

van der Veen R, Abrous DN, de Kloet ER, Piazza PV, Koehl M (2008) Impact of intra- and interstrain cross-fostering on mouse maternal care. Genes Brain Behav 7: 184-192

van der Veen R, Piazza PV, Deroche-Gamonet V (2007) Gene-environment interactions in vulnerability to cocaine intravenous self-administration: a brief social experience affects intake in DBA/2J but not in C57BL/6J mice. Psychopharmacology (Berl) 193: 179-186

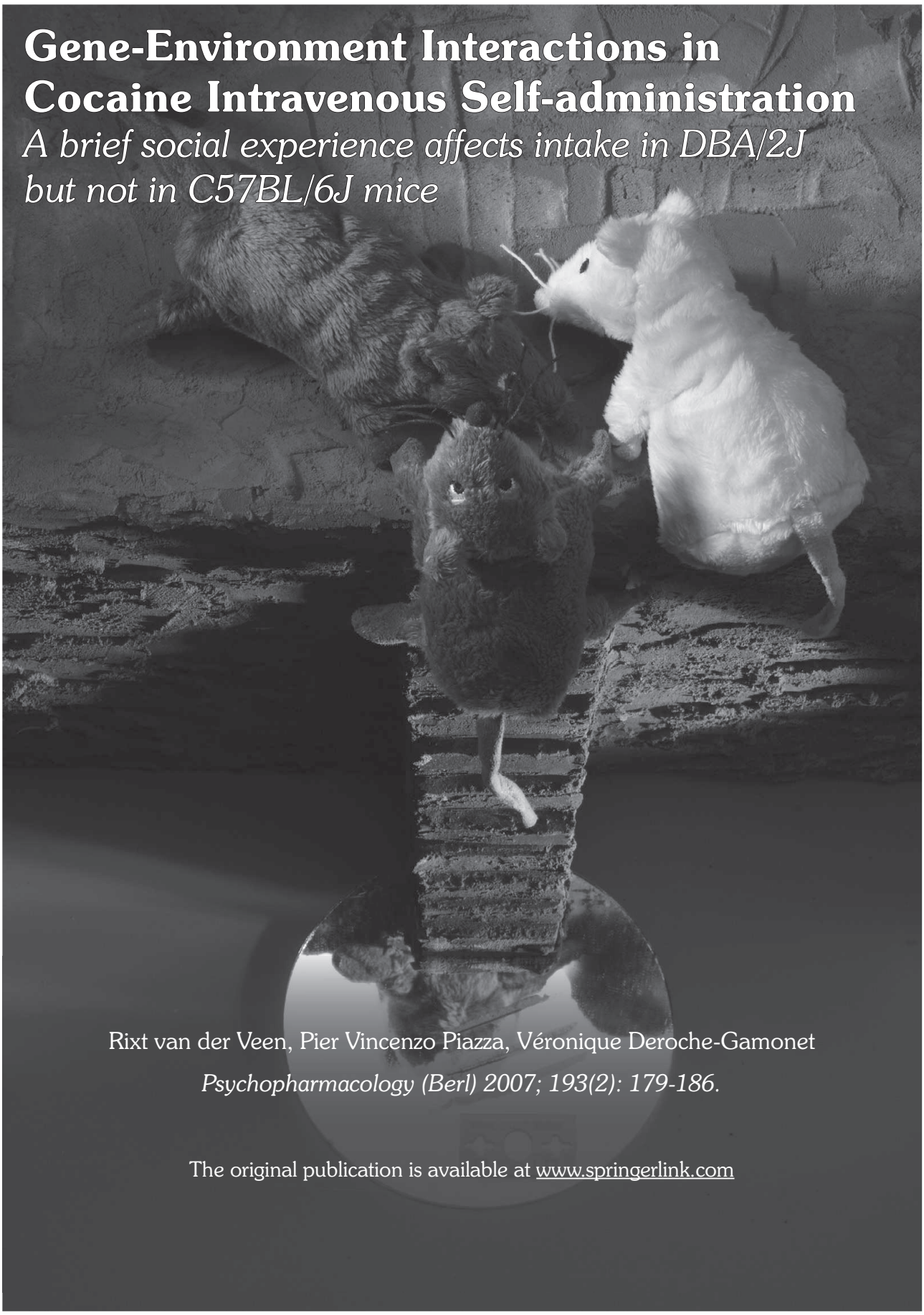
Veenema AH, Bredewold R, Neumann ID (2007) Opposite effects of maternal separation on intermale and maternal aggression in C57BL/6 mice: link to hypothalamic vasopressin and oxytocin immunoreactivity. Psychoneuroendocrinology 32: 437-450

Weiss RD (2004) Treating patients with bipolar disorder and substance dependence: lessons learned. J Subst Abuse Treat 27: 307-312

Willner P (2005) Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52: 90-110

Willner P, Mitchell PJ (2002) The validity of animal models of predisposition to depression. Behav Pharmacol 13: 169-188

Chapter 4



Gene-Environment Interactions in Cocaine Intravenous Self-administration

A brief social experience affects intake in DBA/2J but not in C57BL/6J mice

Rixt van der Veen, Pier Vincenzo Piazza, Véronique Deroche-Gamonet

Psychopharmacology (Berl) 2007; 193(2): 179-186.

The original publication is available at www.springerlink.com

Abstract

Rationale: Individual differences in cocaine-taking behavior and liability to develop abuse are clearly observed, but underlying mechanisms are still poorly understood. A role for gene-environment interactions has been proposed but remains hypothetical. *Objectives:* We investigated whether gene-environment interactions influence intravenous cocaine self-administration in mice. We tested the effect of a past short group housing experience on cocaine self-administration in two inbred strains of mice, the C57BL/6J and DBA/2J. *Methods:* Adult C57BL/6J and DBA/2J mice were individually housed upon arrival in the laboratory. After 3 weeks, half of the animals of each strain were group housed for 19 days. One week after the end of group housing, cocaine self-administration or measurement of brain cocaine levels took place. *Results:* Individually and ex-group housed C57BL/6J mice did not differ for cocaine self-administration. On the contrary, ex-group housed DBA/2J mice showed an upward shift in the dose-response curve as compared to individually housed DBA/2J. Differences in brain cocaine levels could not account for the observed behavioral differences. *Conclusions*: These results demonstrate that vulnerability to cocaine reinforcing effects can be affected by gene-environment interactions. We propose a mouse model for the characterization of gene-environment interactions in the vulnerability to cocaine-taking behavior.

Introduction

In psychiatry, the concept of gene-environment interactions is receiving more and more attention in understanding individual differences in psychopathology (Caspi and Moffitt 2006). It refers to the situation where the impact of environmental factors on mental health and behavior is conditional upon a person's genotype (Moffitt et al. 2006). Such interactions have been recently demonstrated for depression (Silberg et al. 2001; Caspi et al. 2003), antisocial disorders (Cadoret et al. 1995; Caspi et al. 2002; Jaffee et al. 2005) and schizophrenia (Tienari et al. 2004; Caspi et al. 2005), but remain hypothetical for substance use disorders (Moffitt et al. 2006). Epidemiological studies on substance use disorders are confronted with a difficulty: the genetic susceptibility to develop drug abuse might involve either liability to engage in risk-taking behavior or a particular psychophysiological response to drugs of abuse. Animal studies would thus be very useful to investigate gene-environment interactions in vulnerability to drug use.

Cocaine abuse is a chronic disease for which no effective medication is currently available. Observations suggest that gene-environment interactions could play a role in the etiology of cocaine abuse. In animals, psychostimulants-related behaviors have been shown to be influenced by gene-environment interactions. For example, the same experience differentially alters psychostimulant-induced behaviors in two inbred strains of mice (Badiani et al. 1992; Cabib and Bonaventura 1997; Cabib et al. 2000; Conversi et al. 2006) or selected rat lines (van der Kam et al. 2005). However, studies focused so far on responses to noncontingent administration of the drug (Badiani et al. 1992; Cabib and Bonaventura 1997; Cabib et al. 2000; Conversi et al. 2006) or on manipulation of the context of drug self-administration (van der Kam et al. 2005). Nevertheless, behaviorally contingent and non contingent drug administrations clearly involve distinct psychobiological mechanisms (for review Jacobs et al. 2003). Secondly, in humans, environmental risk factors for substance use disorders are situations to which individuals have been exposed in the past and might still be exposed while starting drug use. These environmental risk factors include maternal stress during pregnancy, parental neglect during childhood, premature parental loss and stressful life events involving loss or threat (Caspi and Moffitt 2006).

In the present study, we investigated the influence of a past period of group housing on intravenous self-administration (SA) of cocaine in C57BL/6J (C57) and DBA/2J (DBA) mice. Manipulations of the social environment such as grouping and isolation constitute stressful experiences for adult mice (Cabib et al. 2002b; Avitsur et al. 2003) and present two main advantages. They have been shown to influence psychostimulant effects in rodents (Michel and Tirelli 2002; Lu et al. 2003) and are relatively simple to perform. The C57 and DBA are two intensively studied inbred strains of mice displaying profound neurobiological and behavioral differences. Regarding cocaine, they differ in the anatomy and functioning of its main neurochemical target, i.e. the mesocorticolimbic dopamine system (for review Puglisi-Allegra and Cabib 1997; Cabib et al. 2002a). They differ in behavioral responsiveness to both addictive drugs (for review Crawley et al. 1997) and stressors (Puglisi-Allegra and Cabib 1997; Alcaro et al. 2002; Cabib et al. 2002a; Pothion

et al. 2004; Capone et al. 2005; de Visser et al. 2007). They thus seem a good choice for the study of gene-environment interactions in vulnerability to cocaine use.

Elucidation of the involvement of gene-environment interactions in cocaine abuse appears as a challenge for psychiatrists and neuroscientists in the search for novel therapeutic approaches. We propose a mouse model that represents a useful tool in identifying gene-environment interactions underlying individual differences in cocaine-taking behavior.

Materials and methods

Subjects

DBA/2J@Ico (n=40) and C57BL/6J@Ico (n=40) mice were obtained at 8 weeks of age (Charles River Laboratories, Arbresle, France). Upon arrival, the animals were individually housed in Plexiglas cages (29x11x13 cm) in our temperature (23°C) and humidity (60%) controlled animal facilities. A reversed 12h light-dark cycle (lights on at 22:00, lights off at 10:00) was installed and all experiments were conducted during the dark phase. The animals were given a 3 week acclimatization period before starting the experiment. When group housed, the mice were four per cage in collective cages (37x21x14 cm). Food and water were available *ad libitum* throughout the whole testing period. All experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs, Catheters & Surgery

Cocaine (Coopération Pharmaceutique Française, Bordeaux, France) was dissolved in 0.9% NaCl. Catheters were made from 6-cm soft silastic tubing (i.d.= 0.30 mm, o.d.= 0.64 mm; Dow Corning, Midland, MI, USA), fitted to a 22-G steel cannula (Plastics One, Roanoke, VA, USA) bent at a right angle. The cannula was anchored with dental cement onto the plastic part of a 26-G needle and provided with a small piece of nylon mesh for support. Surgery was performed under ketamine (80 mg/kg, Imalgène®)/xylazine (16 mg/kg, Rompun®) anesthesia as previously described (Deroche et al. 1997; Deroche-Gamonet et al. 2003). During surgery, mice were placed on a heat-pad (30°C). The catheter was inserted in the right jugular vein, until the tip reached approximately the level of the right atrium. It was attached to the vein at the entrance point around a little dab of silicone on the tubing. The distal end was passed subcutaneously over the shoulder, to come out through a small incision in the skin in the mid-scapular region. It was then fixed to the skin with two stitches. After surgery, the mice were placed in a heat-box (27°C) until they woke up from anesthesia. To prevent infection the animals received daily IP injections of a 0.05 ml gentamicine solution (11.4 mg/ml, Gentalline®) for four days after surgery. The animals were given 4 to 6 days to recover from operation before SA started. Catheters were flushed daily with a saline solution containing heparin (30 IU/ml).

Intravenous self-administration apparatus

The intravenous SA setup (Imetronic, Pessac, France) consisted of 16 SA chambers made of Plexiglas and metal. Each chamber (18x11x15 cm) was located within a larger exterior opaque box equipped with exhaust fans that assured air renewal and masked background noise. Briefly, animals were placed daily in a SA chamber where their chronically implanted intravenous catheter was connected to a pump-driven syringe (infusion speed: 20 µl/s). Two holes, located in opposite sides of the SA chamber at 2 cm from the grid floor, were used as devices to record responding. A white cue light (Ø 2 mm) was located 3 cm above each hole. Experimental contingencies were controlled and data collected with PC-windows-compatible software (Imetronic, Pessac, France).

Cocaine assay

To determine cocaine concentrations, we employed a method similar to the one described by Marinelli et al. (1997). Briefly, each brain was separated from its cerebellum and cut into two hemispheres according to the medial axis. The left hemisphere was sonicated in acetonitrile, and supernatant collected after centrifugation. Cocaine content was measured in the supernatant by high performance liquid chromatography coupled with UV detection. The chromatographic system consisted of a Milton Roy Constametric pump, a refrigerated automatic injector (CMA200, Carnegie Medicine, Stockholm, Sweden), a pre-column and a C18 Kromasil column. Detection of cocaine was made by a UV detector (Shimadzu-SPD-A) at λ=235 nm. Results are expressed as micrograms per gram of brain tissue. The detection limit for cocaine was 1 ng (0.25 µg/g brain tissue).

Procedures

After the 3 weeks period of acclimatization, half of the animals of each strain were group housed during 19 days. Then all mice were individually housed again. Catheterization started the day after the end of group housing and took 3 days, during which the animals were randomly operated. Four experimental groups were thus constituted: C57 individually housed (C57-*I),* C57 ex-group housed (C57-*exG),* DBA individually housed (DBA-*I),* and DBA ex-group housed (DBA-*exG).* Eight days after the end of the group housing, the first SA session or brain cocaine measure was conducted.

Experiment 1: Cocaine self-administration

For each experimental group, 12 animals were catheterized. Due to loss during surgery and catheter failure, 9-12 animals per group actually started cocaine SA (C57-*I* n=12; C57-*exG* n=9; DBA-*I* n=11; DBA-*exG* n=12). Animals were trained for cocaine SA during 90 min daily sessions. The testing started 1.5 h after lights off. Introduction of the animal's snout into one hole (active device) turned on the cue light located above it for 1 sec and subsequently switched on the infusion pump (1 sec during which cue light remained illuminated). 'Nose'-pokes in the other hole (inactive device) had no scheduled consequences. A fixed ratio 1 (FR1) schedule was applied throughout the experiment. The self-infusion volume was 20 µL and contained 1 mg/kg of cocaine. Each infusion was followed by a 9-sec time-out period during which cocaine was not available. Criterion for acquisition of

cocaine SA was defined by a stable number of self-infusions over at least three consecutive sessions (± 20%) and at least 75% responding on the active hole. All animals were allowed to acquire for ten consecutive days. After acquisition, a dose-response study was performed. Cocaine dose (0.5, 0.25, 0.125, 0.0625, 0.0313 mg/kg/infusion) was gradually diminished over sessions, maintaining each dose until the animals reached at least 2 days of stable intake (± 20 %). Considering the results, each strain was tested for an extra seventh dose (2 mg/kg/infusion for C57 and 0.0156 mg/kg/infusion for DBA). Finally, both strains were tested for saline. Catheters were flushed daily and animals with blocked or leaking catheters were taken out of the experiment, which resulted in unequal group numbers.

Experiment 2: Brain cocaine concentration

For each experimental group, 8 animals were catheterized. Due to loss during surgery and catheter failure, 5-7 animals per group were available for cocaine measurements. Measures started 3h after lights off. The animals were placed in the SA chambers and received one single programmed intravenous infusion of cocaine at the dose of 1 mg/kg. Since peak brain levels in C57 and DBA mice are reached within 5 min after IP injection (Womer et al. 1994), we decided to sacrifice our mice 2 min after the programmed IV infusion. The brains were collected and immediately frozen on dry ice for cocaine assay.

Data analysis

Statistical analysis was performed with analysis of variance (ANOVA) using Statistica 6.0© (StatSoft). *Self-administration:* Behavior was analyzed with repeated measures ANOVA. For a significant difference between the active and inactive hole within each experimental group, hole served as a within-subject factor. For group comparisons, group *(-exG vs. -I)* and strain (C57 *vs.* DBA) served as between-subjects factors and day (in acquisition) and dose (in dose-response) as within-subjects factors. For the acquisition endpoint, the last 3 days were analyzed. To obtain a full dose-response curve in both strains, each strain was tested for an extra dose (2 mg/kg/infusion for C57 and 0.0156 mg/kg/infusion for DBA). However to be able to compare C57 and DBA mice in the dose-response test only shared doses (six doses and saline) were taken into account. Consequently, also for within strain comparisons, these common doses were used, which yielded similar results as when using all doses (data not shown). *Brain cocaine concentration:* To compare strain and group differences in brain cocaine concentration a bifactorial ANOVA was performed using strain (C57 *vs*. DBA) and group *(-exG vs. -I)* as between subject factors. A significant level of $p<0.05$ was used for all statistical analyses.

Results

Experiment 1: Cocaine self-administration

The mice acquired cocaine SA. After 10 days of training at a dose of 1 mg/kg/infusion, animals from each experimental group showed a stable responding (± 20%) over the last 3

days and reached the criterion of 75% responding for the active hole, indicating that they clearly discern the active from the inactive hole [hole effect C57-*I* $F(1,9)=139.39$ $p<0.001$ (mean preference 76.7%); C57-*exG* $F(1,4)=65.16$ $p<0.01$ (mean preference 78.6%); DBA-*I* $F(1,7)=54.25$ $p<0.001$ (mean preference 85%); DBA-*exG* $F(1,9)=52$ $p<0.001$ (mean preference 83.2%)]. Housing experience did not affect acquisition of cocaine SA in C57 mice, but it did in DBA mice *(figure 4.1a)*. In the C57 strain, responding varied over the ten days of acquisition [day effect $F(9,117)=4.39$ $p<0.001$], but this variation was comparable for C57-*I* and C57-*exG* animals [day*mother interaction $F(9,117)=0.43$ p=ns]. Moreover, the individually and ex-group housed animals showed a similar number of self-infusions at the acquisition endpoint (last 3 days of the curve) [group effect $F(1,13)=0.74$ p=ns]. On the contrary, in the DBA strain, the pattern of intake during the ten days of acquisition was different for individually and ex-group housed animals [day*mother interaction

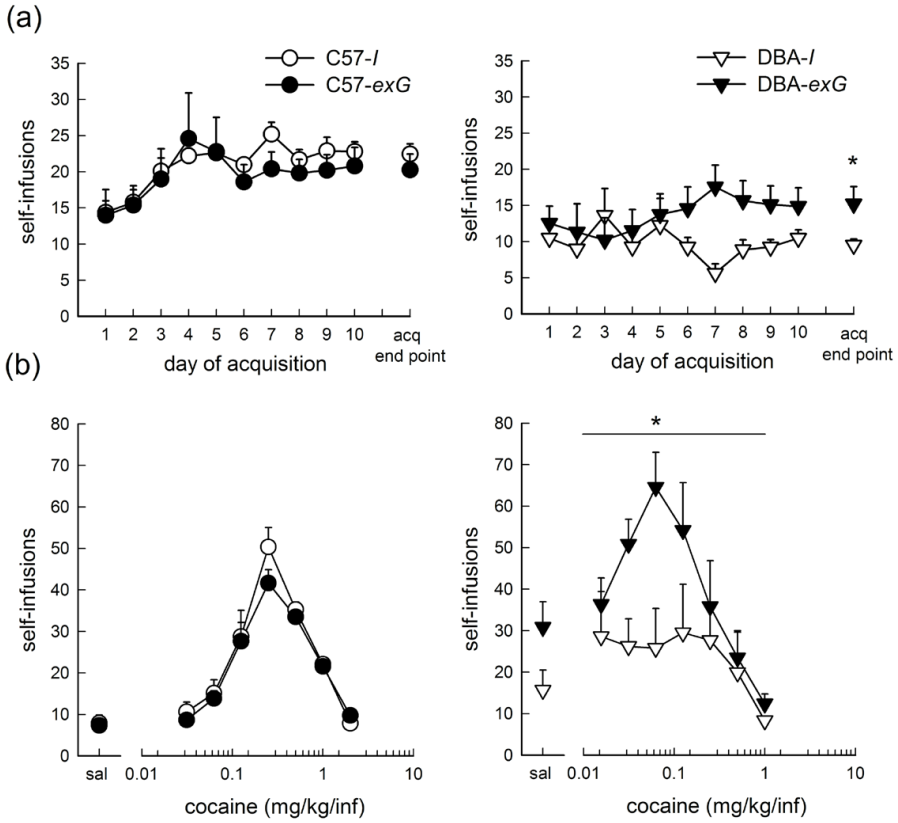


Figure 4.1: Effect of a short group housing experience on cocaine self-administration in C57 and DBA mice. (a) Acquisition of cocaine SA at 1 mg/kg/infusion in individually housed *(-I)* and ex-grouped housed *(-exG)* C57 (left panel) and DBA (right panel). Each point represents the mean sum of self-infusions (± SEM) for each of the ten acquisition sessions. C57-*I*, n=10; C57-*exG*, n=5; DBA-*I*, n=8; DBA-*exG*, n=10. ***(b)*** Dose-response test for cocaine SA in individually housed *(-I)* and ex-group housed *(-exG)* C57 (left panel) and DBA (right panel). Cocaine dose was gradually diminished over sessions maintaining each dose at least 2 days. Considering the results, each strain was tested for an extra seventh dose. Consequently, the dose range was: 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0313 mg/kg/infusion and saline for C57; and 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 mg/kg/infusion and saline for DBA. Each point represents the mean sum of self-infusions (± SEM) of the last 2 days on each dose. C57-*I*, n=8; C57-*exG*, n=5; DBA-*I*, n=5; DBA-*exG*, n=7.

F(9,144)=1.97 p<0.05]. Moreover, DBA-*exG* mice showed a higher cocaine intake at the acquisition endpoint compared to DBA-*I* mice [group effect F(1,16)=4.41 p=0.05].

Similarly, housing experience did not affect responding in the dose-response test in C57 mice, but it did in DBA mice *(figure 4.1b)*. Thus, C57 mice showed a dose-response curve [dose effect F(6,66)=40.35 p<0.001] that was similar in individually and ex-group housed animals [group*dose interaction F(6,66)=0.43 p=ns]. On the contrary, in DBA, ex-group housed mice differed from the individually housed animals [group*dose interaction F(6,60)=2.44 p<0.05]. Although the two groups continued to show a significant discrimination between active and inactive hole [hole effect DBA-*I* F(1,4)=14.17 p<0.05; DBA-*exG* F(1,6)=43.25 p<0.001], DBA-*exG* animals showed a significant dose-response function, clearly distinguishing the different doses [dose effect F(6,36)=10.69 p<0.001], while the DBA-*I* animals did not, and showed a flattened curve [dose effect F(6,24)=1.71 p=ns].

Consequently, the differences in cocaine SA between C57 and DBA mice were strongly dependent on the housing experience. In the individually housed condition *(figure 4.2a)*, the pattern of intake during the ten days of acquisition was different for DBA-*I* and C57-*I* mice [strain*dose interaction F(9,144)=3.47 p<0.001]. Moreover, the C57-*I* self-administered a higher amount of cocaine than DBA-*I* at the acquisition endpoint [strain effect F(1,16)=54.44 p<0.001]. The two strains also differed in responding in the dose-response test in this condition [strain*dose interaction F(6,66)=6.06 p<0.001]. C57-*I* mice showed a classical inverted U-shaped curve [dose effect F(6,42)=23.55 p<0.001], while the DBA-*I* mice showed a flattened dose-effect function [dose effect F(6,24)=1.71 p=ns].

In contrast, in the ex-group housed condition *(figure 4.2b),* C57 and DBA mice did neither differ in pattern of intake over the ten days of acquisition [strain*dose interaction F(9,117)=0.90 p=ns], nor in intake at the acquisition endpoint [strain effect F(1,13)=1.81 p=ns]. They were significantly different in responding in the dose-response test [strain*dose interaction F(6,60)=12.80 p<0.001], but in this case, DBA also showed an inverse U-shape curve [dose effect F(6,36)=10.68 p<0.001], that was shifted to the left compared to C57.

Table 4.1: Brain cocaine concentration. Cocaine concentration in total brain samples, measured 2 min after a 1 mg/kg intravenous infusion of cocaine in individually and ex-group housed C57 and DBA mice.

Strain	Experimental group	*n*	Cocaine (µg/g brain tissue)
C57	*individual*	7	1.32 ± 0.09
	ex-group housed	7	1.48 ± 0.18
DBA	*individual*	5	1.27 ± 0.15
	ex-group housed	5	1.28 ± 0.05

Values represent mean ± SEM

Experiment 2: Brain cocaine concentration

Cocaine concentration in total brain samples was measured 2 min after a 1 mg/kg intravenous infusion of cocaine administered via the catheter *(table 4.1)*. It appeared not to be influenced by the experimental treatment and was comparable between strains [group effect F(1,17)=0.26; strain effect F(1,17)=0.53; strain*group interaction F(1,17)=0.23, all p=ns].

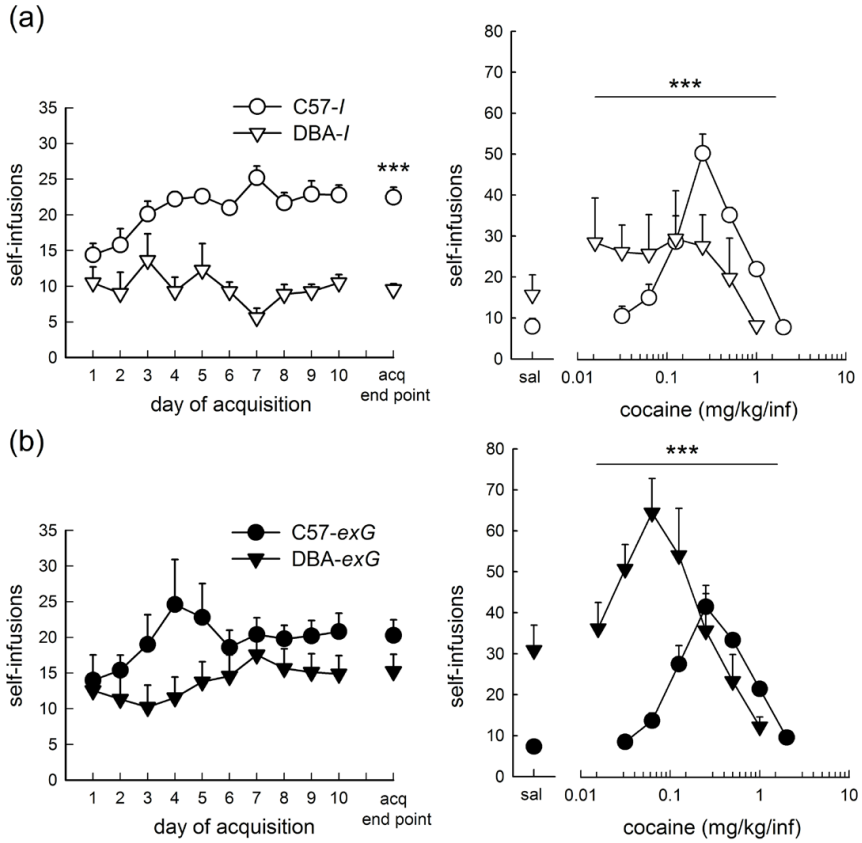


Figure 4.2: Comparing C57 and DBA mice with and without a short group housing experience in cocaine self-administration. Comparison of ***(a)*** individually housed *(-I)* and ***(b)*** ex-group housed *(-exG)* C57 and DBA mice in the acquisition (left panel) and dose-response (right panel) phase of cocaine SA. In the acquisition phase, each point represents the mean sum of self-infusions (± SEM) for each of the ten sessions. C57-*I*, n=10; DBA-*I*, n=8; C57-*exG*, n=5; DBA-*exG*, n=10. In the dose-response test, cocaine dose was gradually diminished over sessions maintaining each dose at least 2 days. Each point represents the mean sum of self-infusions (± SEM) of the last 2 days on each dose. Considering the results, each strain was tested for an extra seventh dose. Consequently, the dose range was: 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0313 mg/kg/infusion and saline for C57; and 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 mg/kg/infusion and saline for DBA. C57-*I*, n=8; DBA-*I*, n=5; C57-*exG*, n=5; DBA-*exG*, n=7.

Discussion

The results reported here indicate that the influence of environmental conditions on cocaine reinforcing effects depends on the genetic background of the subjects. A short group housing experience did not affect cocaine SA behavior in mice with a C57 background, whereas it increased cocaine SA in mice with a DBA background. These results reveal the implication of gene-environment interactions in the vulnerability to cocaine reinforcing effects. These interactions do not involve alterations in cocaine metabolism since both strains show comparable brain cocaine levels whatever the experimental condition.

Our results are in accordance with other studies, showing that DBA are stress-responsive regarding psychostimulant-induced responses, whereas C57 are not (Badiani et al. 1992; Cabib and Bonaventura 1997; Cabib et al. 2000). This could imply that in certain individuals, vulnerability to cocaine abuse results from a genetic vulnerability to environmental stressors. This genetic vulnerability to environmental stressors could affect drug use in different ways. It could either specifically affect sensitivity to drugs of abuse and induce a specific substance-related disorder or it could induce other behavioral disturbances that in turn lead to drug abuse.

Arguments are in favor of disturbances in behavior leading to drug abuse: (1) In DBA and C57, stress affects behaviors other than drug-related behaviors and also in a genotype-dependent manner. For example, DBA, but not C57, show stress-induced anhedonia as measured by intracranial self-stimulation (Zacharko et al. 1990). Oppositely, in C57, but not in DBA, stress might induce a depression-like state as measured in the Porsolt test (Shanks and Anisman 1988; Hwang et al. 1999; Alcaro et al. 2002) and sucrose preference test (Pothion et al. 2004). (2) In DBA and C57, stress affects the response to different classes of drugs in a different way. A past period of group housing affects cocaine SA in DBA, but not in C57 mice (this study). Likewise, a past food shortage increased amphetamine-induced place conditioning and locomotion in DBA without affecting the behavior of C57 mice (Cabib et al. 2000). However, the same food shortage experience increased ethanol preference in C57 but not in DBA (Schroff et al. 2004). Combining these observations, it can be suggested that exposure to environmental stressors could induce different behavioral disturbances in DBA and C57 that would rather be associated with increases in ethanol or psychostimulant preference.

However, the hypothesis of a specific substance-related disorder can not be discarded. The genotype dependent vulnerability to stress regarding psychostimulants and ethanol could result from strain differences in stress sensitivity of distinct neurobiological susbtrates. Thus, although they share common substrates (Phillips et al. 1997; Sarnyai et al. 2001; Marinelli and Piazza 2002; O'Callaghan et al. 2005; de Vries and Schoffelmeer 2005; Pierce and Kumaresan 2006), ethanol and psychostimulants could exert reinforcing effects through distinct mechanisms (Pierce and Kumaresan 2006). Consequently, DBA mice could present a vulnerability to stress of the neurobiological systems involved in psychostimulants reinforcing effects, whereas C57 would present a vulnerability to stress of the neurobiological systems involved in ethanol reinforcing effects. C57 and DBA differ in the activity of the mesocorticolimbic dopaminergic neurons (Puglisi-Allegra and Cabib

1997; Cabib et al. 2002b) that are thought to commonly modulate psychostimulants and ethanol reinforcing effects. However, the two strains also differ in the activity of systems such as opioids (Jamensky and Gianoulakis 1999; Doyle et al. 2006), serotonin (Shanks et al. 1991; Zhang et al. 2004; Fadda et al. 2005; Kelai et al. 2006; Hackler et al. 2006), norepinephrine (Shanks et al. 1991; Hwang et al. 1999) or γ-aminobutyric acid (DuBois et al. 2006), which are more specifically implicated in ethanol reinforcement (Pierce and Kumaresan 2006).

Our results thus demonstrate that differences in cocaine SA between two inbred strains of mice, C57 and DBA, may be highly dependent on a past social experience. This is in accordance with recent studies showing that differences in behavioral responses between inbred strains, including drug related behaviors, are not immutable but influenced by environmental factors (Cabib and Bonaventura 1997; Crabbe et al. 1999; Cabib et al. 2000; Wahlsten et al. 2003; Conversi et al. 2006; de Visser et al. 2007). When animals are individually housed from arrival to our facilities, cocaine appears less reinforcing in DBA than in C57, DBA showing a flattened dose-response curve as classically described in the literature (Grahame and Cunningham 1995; Rocha et al. 1998). This suggests an equivalent reinforcing effect at almost all doses tested, but a deficit of extinction of responding might also play a role. However, when animals have been previously exposed to a short group housing period, DBA appears more sensitive than C57 to cocaine reinforcing effects.

Biological mechanisms underlying gene-environment interactions in vulnerability to cocaine use remain to be investigated. Data suggest that glucocorticoid hormones and the mesocorticolimbic dopaminergic neurons could be important factors. They have been proposed as two key biological mediators of the increased vulnerability to psychostimulants induced by stress (for review Piazza and Le Moal 1996; Marinelli and Piazza 2002; Goeders 2002; de Jong and de Kloet 2004). Now, C57 and DBA differ in the activity of the dopaminergic mesocorticolimbic neurons both in basal conditions and in response to psychostimulants and stressors (Puglisi-Allegra and Cabib 1997; Cabib et al. 2002b; Ventura et al. 2004; McNamara et al. 2006). Interestingly, Ventura et al. (2004) showed that in C57, amphetamine can induce dopamine increases in the NAcc mainly through an impulse-dependant mechanism regulated by prefronto-cortical glutamate transmission, whereas in DBA, this increase is impulse independent. However, food-restriction, which sensitizes DBA mice to the stimulating and rewarding effects of amphetamine, makes DBA mice lose the impulse independent component of this amphetamine DA release and show impulse dependant DA release (Ventura and Puglisi-Allegra 2005). Similar differences might be implicated in cocaine effects, but this remains to be demonstrated. The two strains might also differ in stress-induced corticosterone levels (Cabib et al. 1990; Shanks et al. 1990; Jones et al. 1998), although very few studies have addressed this question and findings are not consistent. Finally, and particularly interesting, de Jong et al. (2007) recently showed that the development of behavioral sensitization to cocaine is depending on corticosterone secretion in DBA but not in C57 mice, suggesting that corticosterone secretion might play a more important role in DBA than in C57 mice in the behavioral responses to cocaine.

In conclusion, our results demonstrate that gene-environment interactions can affect cocaine SA. They comfort the idea that strain differences between C57 and DBA are not immutable but can depend on environmental, even past, conditions. Our results stress the

importance of housing conditions when studying strain differences. Finally and importantly, our model opens the door to the study of the psychobiological mechanisms underlying gene-environment interactions in the vulnerability to cocaine use.

Acknowledgements

This research was supported by the NWO/INSERM/ZON grants AH002G and 985-10-01, Région Aquitaine and the University of Bordeaux 2.

References

Alcaro A, Cabib S, Ventura R, Puglisi-Allegra S (2002) Genotype- and experience-dependent susceptibility to depressive-like responses in the forced-swimming test. Psychopharmacology (Berl) 164: 138-143

Avitsur R, Stark JL, Dhabhar FS, Kramer KA, Sheridan JF (2003) Social experience alters the response to social stress in mice. Brain Behav Immun 17: 426-437

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

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

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

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

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

Cabib S, Ventura R, Puglisi-Allegra S (2002b) Opposite imbalances between mesocortical and mesoaccumbens dopamine responses to stress by the same genotype depending on living conditions. Behav Brain Res 129: 179-185

Cadoret RJ, Yates WR, Troughton E, Woodworth G, Stewart MA (1995) Genetic-environmental interaction in the genesis of aggressivity and conduct disorders. Arch Gen Psychiatry 52: 916-924

Capone F, Venerosi A, Puopolo M, Alleva E, Cirulli F (2005) Behavioral responses of 129/Sv, C57BL/6J and DBA/2J mice to a non-predator aversive olfactory stimulus. Acta Neurobiol Exp (Wars) 65: 29-38

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A et al. (2002) Role of genotype in the cycle of violence in maltreated children. Science 297: 851-854

Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 7: 583-590

Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A et al. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism

in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry 57: 1117-1127

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J et al. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301: 386-389

Conversi D, Bonito-Oliva A, Orsini C, Cabib S (2006) Habituation to the test cage influences amphetamine-induced locomotion and Fos expression and increases FosB/DeltaFosB-like immunoreactivity in mice. Neuroscience 141: 597-605

Crabbe JC, Wahlsten D, Dudek BC (1999) Genetics of mouse behavior: interactions with laboratory environment. Science 284: 1670-1672

Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, Hitzemann RJ et al. (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology (Berl) 132: 107-124

de Jong IE, de Kloet ER (2004) Glucocorticoids and vulnerability to psychostimulant drugs: toward substrate and mechanism. Ann N Y Acad Sci 1018: 192-198

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

de Visser L, van den BR, Stoker AK, Kas MJ, Spruijt BM (2007) Effects of genetic background and environmental novelty on wheel running as a rewarding behaviour in mice. Behav Brain Res 177: 290-297

de Vries TJ, Schoffelmeer AN (2005) Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci 26: 420-426

Deroche V, Caine SB, Heyser CJ, Polis I, Koob GF, Gold LH (1997) Differences in the liability to self-administer intravenous cocaine between C57BL/6 x SJL and BALB/cByJ mice. Pharmacol Biochem Behav 57: 429-440

Deroche-Gamonet V, Sillaber I, Aouizerate B, Izawa R, Jaber M, Ghozland S, Kellendonk C et al. (2003) The glucocorticoid receptor as a potential target to reduce cocaine abuse. J Neurosci 23: 4785-4790

Doyle GA, Sheng XR, Schwebel CL, Ferraro TN, Berrettini WH, Buono RJ (2006) Identification and functional significance of polymorphisms in the mu-opioid receptor gene (Oprm) promoter of C57BL/6 and DBA/2 mice. Neurosci Res 55: 244-254

DuBois DW, Perlegas A, Floyd DW, Weiner JL, McCool BA (2006) Distinct functional characteristics of the lateral/basolateral amygdala GABAergic system in C57BL/6J and DBA/2J mice. J Pharmacol Exp Ther 318: 629-640

Fadda P, Scherma M, Fresu A, Collu M, Fratta W (2005) Dopamine and serotonin release in dorsal striatum and nucleus accumbens is differentially modulated by morphine in DBA/2J and C57BL/6J mice. Synapse 56: 29-38

Goeders NE (2002) The HPA axis and cocaine reinforcement. Psychoneuroendocrinology 27: 13-33

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

Hackler EA, Airey DC, Shannon CC, Sodhi MS, Sanders-Bush E (2006) 5-HT(2C) receptor RNA editing in the amygdala of C57BL/6J, DBA/2J, and BALB/cJ mice. Neurosci Res 55: 96-104

Hwang BH, Kunkler PE, Tarricone BJ, Hingtgen JN, Nurnberger JI, Jr. (1999) Stress-induced changes of norepinephrine uptake sites in the locus coeruleus of C57BL/6J and DBA/2J mice: a quantitative autoradiographic study using [3H]-tomoxetine. Neurosci Lett 265: 151-154

Jacobs EH, Smit AB, de Vries TJ, Schoffelmeer AN (2003) Neuroadaptive effects of active versus passive drug administration in addiction research. Trends Pharmacol Sci 24: 566-573

Jaffee SR, Caspi A, Moffitt TE, Dodge KA, Rutter M, Taylor A, Tully LA (2005) Nature X nurture: genetic vulnerabilities interact with physical maltreatment to promote conduct problems. Dev Psychopathol 17: 67-84

Jamensky NT, Gianoulakis C (1999) Comparison of the proopiomelanocortin and proenkephalin opioid peptide systems in brain regions of the alcohol-preferring C57BL/6 and alcohol-avoiding DBA/2 mice. Alcohol 18: 177-187

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

Kelai S, Hanoun N, Aufrere G, Beauge F, Hamon M, Lanfumey L (2006) Cannabinoid-serotonin interactions in alcohol-preferring vs. alcohol-avoiding mice. J Neurochem 99: 308-320

Lu L, Shepard JD, Hall FS, Shaham Y (2003) Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci Biobehav Rev 27: 457-491

Marinelli M, Piazza PV (2002) Interaction between glucocorticoid hormones, stress and psychostimulant drugs. Eur J Neurosci 16: 387-394

Marinelli M, Rouge-Pont F, Deroche V, Barrot M, Jesus-Oliveira C, Le Moal M, Piazza PV (1997) Glucocorticoids and behavioral effects of psychostimulants. I: locomotor response to cocaine depends on basal levels of glucocorticoids. J Pharmacol Exp Ther 281: 1392-1400

McNamara RK, Levant B, Taylor B, Ahlbrand R, Liu Y, Sullivan JR, Stanford K et al. (2006) C57BL/6J mice exhibit reduced dopamine D3 receptor-mediated locomotor-inhibitory function relative to DBA/2J mice. Neuroscience 143: 141-153

Michel A, Tirelli E (2002) Effects of the social conditions of housing through testing on cocaine-induced contextual sensitisation and conditioned locomotion in C57BL/6J mice. Prog Neuropsychopharmacol Biol Psychiatry 26: 1185-1191

Moffitt TE, Caspi A, Rutter M (2006) Measured gene-environment interactions in psychopathology. Perspect Psychol Sci 1: 5-27

O'Callaghan MJ, Croft AP, Jacquot C, Little HJ (2005) The hypothalamopituitary-adrenal axis and alcohol preference. Brain Res Bull 68: 171-178

Phillips TJ, Roberts AJ, Lessov CN (1997) Behavioral sensitization to ethanol: genetics and the effects of stress. Pharmacol Biochem Behav 57: 487-493

Piazza PV, Le Moal M (1996) Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annu Rev Pharmacol Toxicol 36: 359-378

Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 30: 215-238

Pothion S, Bizot JC, Trovero F, Belzung C (2004) Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behav Brain Res 155: 135-146

Puglisi-Allegra S, Cabib S (1997) Psychopharmacology of dopamine: the contribution of comparative studies in inbred strains of mice. Prog Neurobiol 51: 637-661

Rocha BA, Odom LA, Barron BA, Ator R, Wild SA, Forster MJ (1998) Differential responsiveness to cocaine in C57BL/6J and DBA/2J mice. Psychopharmacology (Berl) 138: 82-88

Sarnyai Z, Shaham Y, Heinrichs SC (2001) The role of corticotropin-releasing factor in drug addiction. Pharmacol Rev 53: 209-243

Schroff KC, Cowen MS, Koch S, Spanagel R (2004) Strain-specific responses of inbred mice to ethanol following food shortage. Addict Biol 9: 265-271

Shanks N, Anisman H (1988) Stressor-provoked behavioral changes in six strains of mice. Behav Neurosci 102: 894-905

Shanks N, Griffiths J, Zalcman S, Zacharko RM, Anisman H (1990) Mouse strain differences in plasma corticosterone following uncontrollable footshock. Pharmacol Biochem Behav 36: 515-519

Shanks N, Zalcman S, Zacharko RM, Anisman H (1991) Alterations of central norepinephrine, dopamine and serotonin in several strains of mice following acute stressor exposure. Pharmacol Biochem Behav 38: 69-75

Silberg J, Rutter M, Neale M, Eaves L (2001) Genetic moderation of environmental risk for depression and anxiety in adolescent girls. Br J Psychiatry 179: 116-121

Tienari P, Wynne LC, Sorri A, Lahti I, Laksy K, Moring J, Naarala M et al. (2004) Genotype-environment interaction in schizophrenia-spectrum disorder. Long-term follow-up study of Finnish adoptees. Br J Psychiatry 184: 216-222

van der Kam EL, Ellenbroek BA, Cools AR (2005) Gene - environment interactions determine the individual variability in cocaine self-administration. Neuropharmacology 48: 685-695

Ventura R, Alcaro A, Cabib S, Conversi D, Mandolesi L, Puglisi-Allegra S (2004) Dopamine in the medial prefrontal cortex controls genotype-dependent effects of amphetamine on mesoaccumbens dopamine release and locomotion. Neuropsychopharmacology 29: 72-80

Ventura R, Puglisi-Allegra S (2005) Environment makes amphetamine-induced dopamine release in the nucleus accumbens totally impulse-dependent. Synapse 58: 211-214

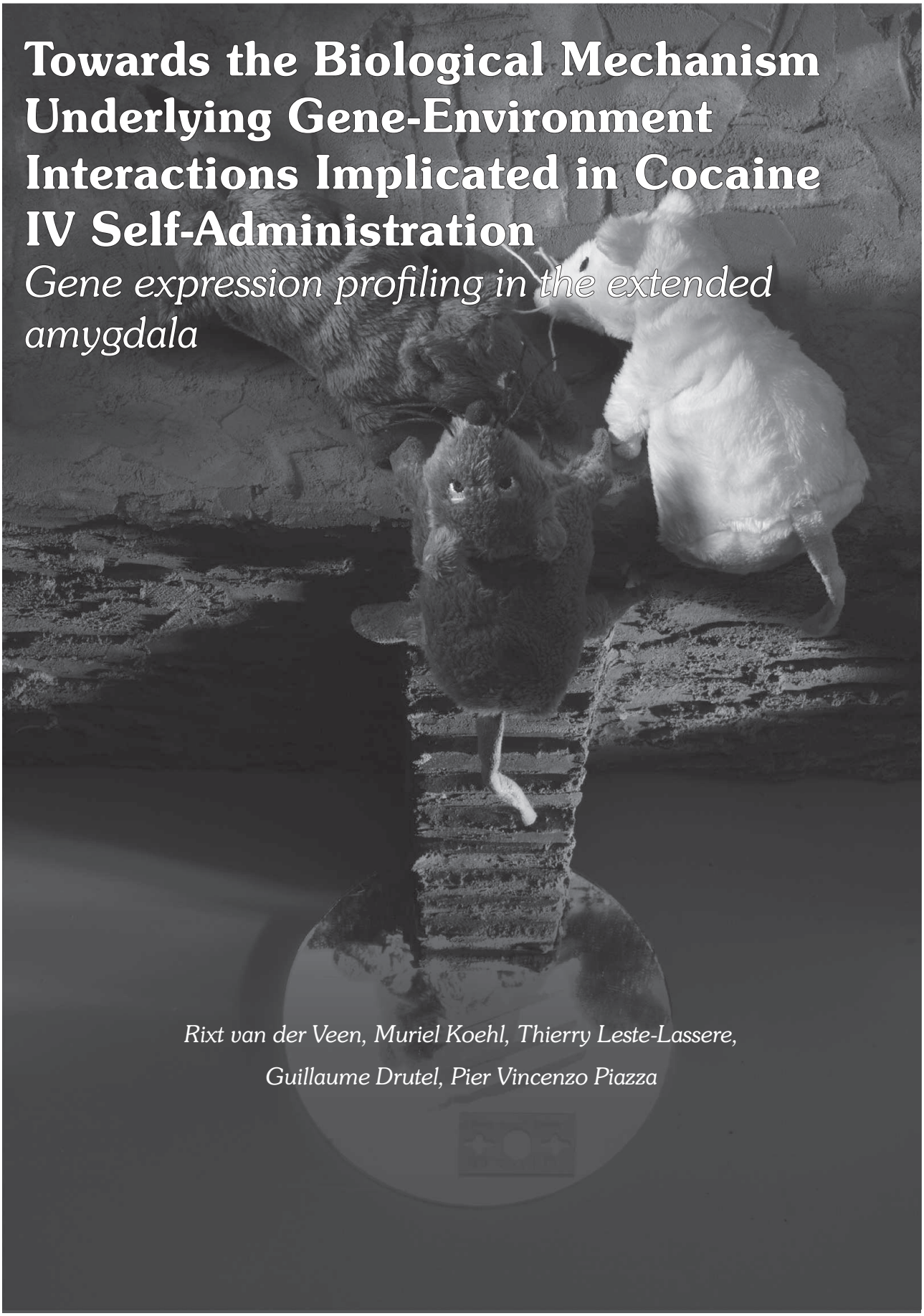
Wahlsten D, Metten P, Phillips TJ, Boehm SL, Burkhart-Kasch S, Dorow J, Doerksen S et al. (2003) Different data from different labs: lessons from studies of gene-environment interaction. J Neurobiol 54: 283-311

Womer DE, Jones BC, Erwin VG (1994) Characterization of dopamine transporter and locomotor effects of cocaine, GBR 12909, epidepride, and SCH 23390 in C57BL and DBA mice. Pharmacol Biochem Behav 48: 327-335

Zacharko RM, Gilmore W, MacNeil G, Kasian M, Anisman H (1990) Stressor induced variations of intracranial self-stimulation from the mesocortex in several strains of mice. Brain Res 533: 353-357

Zhang X, Beaulieu JM, Sotnikova TD, Gainetdinov RR, Caron MG (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science 305: 217

Chapter 5



Towards the Biological Mechanism Underlying Gene-Environment Interactions Implicated in Cocaine IV Self-Administration

Gene expression profiling in the extended amygdala

Rixt van der Veen, Muriel Koehl, Thierry Leste-Lassere, Guillaume Drutel, Pier Vincenzo Piazza

Abstract

Although the importance of gene-environment interactions in the development of psychopathology is widely recognised in psychiatry, the underlying mechanism is still poorly understood. Basic neuroscience studies could help to identify factors that render an individual susceptible or resilient to the impact of environmental stressors on psychopathology-related behaviors. In the previous chapters we have shown clear gene-environment interactions in cocaine taking behavior using two different experimental set-ups with the C57BL/6J and DBA/2J inbred mouse strains. In both the early and late life experiment, mice of the DBA/2J strain proved to be susceptible to the impact of environmental experiences on cocaine self-administration, while mice of the C57BL/6J strain proved to be resilient to the same experiences. We compared RNA expression profiles in the extended amygdala of drug-naïve animals issued from both experiments. Gene expression levels were analyzed with Affymetrix microarrays and group differences were validated with quantitative PCR. In both experimental set-ups, the expression of arginine vasopressin was found to differ significantly between DBA/2J experimental groups, but not between C57BL/6J groups. Thus, vasopressin expression appears to be influenced by early and late life experiences in mice with a DBA/2J background. Differences were inversely related to drug intake, that is, higher amounts of vasopressin transcripts were found in the DBA/2J groups that showed lower cocaine intake.

Introduction

Both environmental risk factors and substantial genetic components have been identified in the etiology of psychopathology, they are strong and pervasive, but, considered separately, rarely determinative in defining disease outcome (Kendler 2005; Rutter et al. 2006). Like mentioned earlier, an involvement of basic neuroscience in this research field has recently been proposed to help understand how genes and environment interact and how susceptibility and resilience to psychopathology emerge (Caspi and Moffitt 2006).

One of the issues in which basic neuroscience studies could participate is the identification of predisposing and protecting factors for psychopathology-related behaviors. In other words, the identification of changes that occur in the individual in response to environmental adversities and that could play a role in the subsequent development or avoidance of psychopathology. For this, changes in the period before the onset of psychopathology-related behavior should be studied. Advances at this pre-state of vulnerability would mean an important step in the understanding of individual differences in the development of psychopathology.

In the previous chapters, we showed a clear gene-environment interaction in cocaine self-administration (SA) using two different experimental set-ups with the C57BL/6J

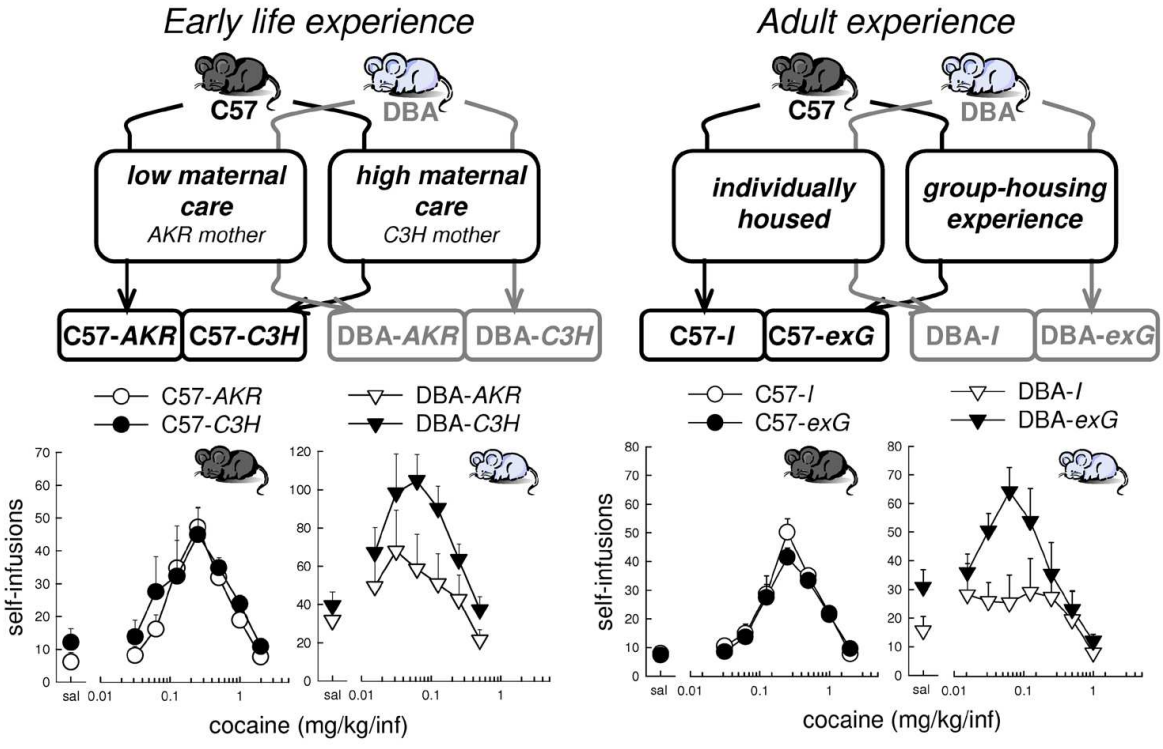


Figure 5.1: Two mouse models revealing a gene-environment interaction in cocaine SA. Different early life environments (left side) were created by cross-fostering mice to mothers differing in maternal care, while a short period of group housing constituted an environmentally 'stressful' situation in adulthood (right side). The DBA strain is represented by the grey mice and the C57 strain by the black mice. In both situations, mice of the DBA strain proved to be susceptible to the impact of environmental experiences on cocaine SA, while mice of the C57 strain proved to be resilient to the same experiences.

(C57) and DBA/2J (DBA) mice *(figure 5.1)*. The first experimental model showed that the impact of early life environment on adult cocaine SA is dependent on the genetic background of the individual. The second model provided the same type of evidence for the impact of an adult stressful environmental experience. In both situations, mice of the DBA strain proved to be susceptible to the impact of environmental experiences on drug taking behavior, while mice of the C57 strain proved to be resilient to the same experiences.

Studying differences in these animals before contact with the drug might enable us to reveal the biological mechanism that underlies this gene-environment interaction that predisposes or protects the animal to a changed drug intake *(figure 5.2)*. In a first approach to identify 'novel' factors in this mechanism, we compared gene expression of drug-naïve animals issued from both the early and late life experiment in a drug relevant brain structure: the extended amygdala. The extended amygdala (EA) is a neuro-anatomical entity that includes the bed nucleus of the stria terminalis (BNST) and the central and medial nuclei of the amygdala, and forms a continuum with the shell of the nucleus accumbens (NAcc) *(figure 5.3)*. Each of these regions shares certain cytoarchitectural and circuitry similarities (Heimer and Alheid 1991). The EA has prominent projections to autonomic and somatomotor centers in the lateral hypothalamus and brainstem (central division of the EA) and to the endocrine-related medial hypothalamus (medial division of the EA). It receives afferents from limbic structures including the basolateral amygdala (BLA) and hippocampus and from medullary structures like the A2 noradrenergic region of the nucleus tractus solitarius (NTS). It thus interfaces classical limbic (emotional) structures and structures transmitting visceral responses linked to emotional processes with the extrapyramidal motor system, and coordinates coherent behavioral responses (Koob 2003; Harris and Aston-Jones 2007).

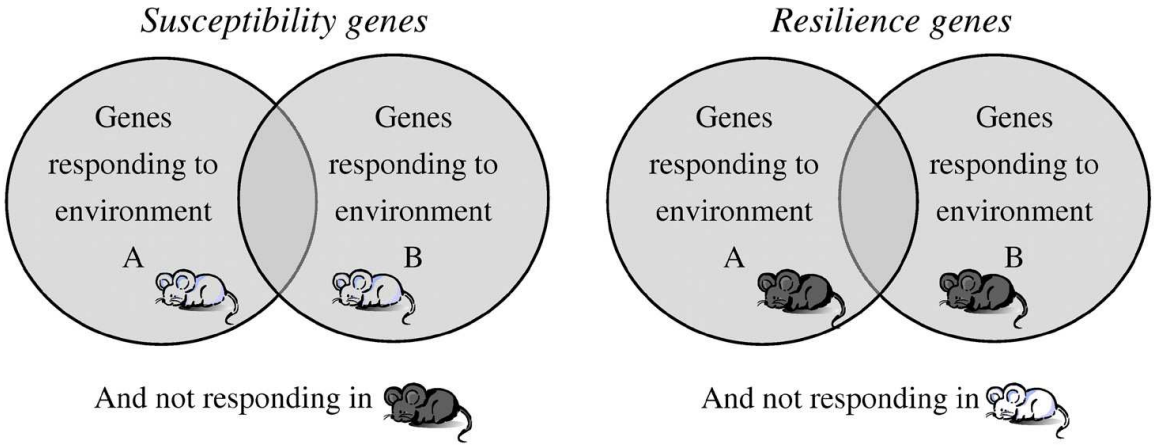


Figure 5.2: Theoretical approach to search for genes possibly implicated in the susceptibility and resilience to the impact of environmental events on cocaine SA. In our experiments, 'A' represents the early environmental experience and refers to the differential expression between animals raised by two different mothers. 'B' represents the adult experience and refers to the differential expression between animals with or without a short group housing experience. The DBA strain is represented by the grey mice and the C57 strain by the black mice. Genes implicated in susceptibility to the environment would then be influenced by both environments (overlapping part) in the DBA mice and not be influenced by either environment in the C57 mice. The opposite would be true for genes implicated in resilience.

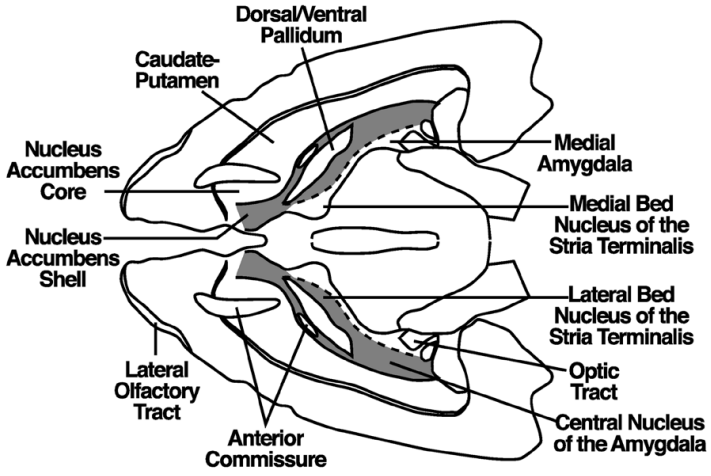


Figure 5.3: Diagram illustrating the extended amygdala with a central division (in grey) and a medial division (in white, interior of the central division). *Figure taken from Koob 2003*.

A large part of the brain reward system is contained within the EA and consequently, it plays a key role in mediating appetitive and adverse behavior. It is hypothesized to mediate goal-directed behavior triggered by reward-related incentives (Koob 2003; Kelley 2004; Waraczynski 2006). The EA might represent a circuitry that computes the value of environmental stimuli, interconnecting stimulus detection (sensory information on stimulus by basolateral and centromedial amygdala and visceral information by NTS) and the translation of motivation into action (by the ventral striato-pallidal system comprising NAcc, olfactory tubercule and ventral pallidum) (Waraczynski 2006; Harris and Aston-Jones 2007). Regarding drugs of abuse, the structures of the extended amygdala were found to be implicated in the acute reinforcing actions of drugs and in the negative effects of compulsive drug administration on reward function (Koob 2003). The decreased preference for cues associated with natural rewards during drug withdrawal is thought to involve heightened activity in stress-related brain areas of the extended amygdala (Koob 2003; Harris and Aston-Jones 2007). The EA is hypothesised by Koob and le Moal (2001; 2003) to form the central point where the stress and reward systems interact (see also introduction).

For the experiments described in this study, mice were sacrificed in adulthood in basal state (at rest). These animals had experienced an environmental challenge, that is, either a different maternal environment or a brief past group housing experience in adulthood, but were not exposed to drugs and had not experienced other behavioral testing. Gene expression profiles of the extended amygdala were analyzed using Affymetrix Microarrays (analysis of the expression level of 34,000 well characterized mouse genes). Since we have shown that both the early and late environmental experiences influence cocaine self-administration in the DBA, but not the C57 strain, genes for which the expression is modified by both experiences in DBA, but not in C57 mice might be potentially implicated in rendering an individual susceptible to environmentally induced changes in drug intake. In a first approach, we focused on these 'susceptibility' genes, that is, the genes that were differentially expressed in DBA, but not in C57 mice. For these genes, quantitative polymerase chain reaction (qPCR) was performed to confirm the Microarray results.

Materials & Methods

Animals

Only male mice were used. Animals of the early life environmental manipulation experiment were bred in our own facilities (see chapter 2 for details) and form 4 of the experimental groups that were tested in this experiment: DBA and C57 mice cross-fostered to an AKR mother (DBA-*AKR* and C57-*AKR),* and DBA and C57 mice cross-fostered to a C3H mother (DBA-*C3H* and C57-*C3H).* At the time of sacrifice, these animals were habituated to a 12h light-dark cycle (lights on at 08:00, lights off at 20:00). Animals of the late life environmental manipulation experiment were obtained at 8 weeks of age (Charles River Laboratories, Arbresle, France) (see chapter 4 for details) and form another 4 experimental groups: DBA and C57 mice individually housed since arrival (DBA-*I* and C57-*I),* and DBA and C57 mice having experienced a short period of group housing (DBA-*exG* and C57-*exG).* At the time of sacrifice these animals were habituated to a reversed 12h light-dark cycle (lights off at 07:30, lights on at 19:30). This second experiment (the late environmental experience) was replicated at a later time point using the same experimental conditions. Food and water were available *ad libitum* throughout the whole experiment.

General procedures

Animals from the 'early life' experiment were sacrificed at 8 months of age. Sacrifice took place during the first part of the light cycle (09:30-12:00), when basal corticosterone levels are low (Dalm et al. 2005). Animals from the 'late life' experiment were sacrificed 8 days after the end of group housing (around 3 months of age). For this group of animals sacrifice took place in the middle of the dark cycle (11:00-13:00), after the nocturnal corticosterone peak, when basal corticosterone levels are relatively low (Dalm et al. 2005). Brains were dissected, RNA was extracted from the extended amygdala, and pooled samples of each experimental group were sent in duplicate for micro-array analysis to *Genome Explorations* (Memphis, USA). Pools were obtained by taking 2 μg of total RNA of each individual sample within a group to obtain a minimum of 10 μg total RNA for microarray hybridization. Upon reception of the microarray hybridization results obtained by *Genome Explorations,* the original data files were re-analyzed with different algorithms and group differences in gene expression were confirmed on individual samples using qPCR in our laboratory.

Dissection and RNA isolation

Animals were sacrificed by decapitation, brains were then rapidly removed from the skull and cut into 1mm thick coronal slices using a precision high grade zinc brain matrice (Braintree Scientific, Inc. Braintree MA, USA) kept cold on ice. Brain structures were dissected from the fresh tissue on a cold plate using a pair of curved tweezers. After dissection, they were immediately frozen on dry ice and stored at -80°C until use. The extended amygdala (including central nucleus of the amygdala, BNST and NAcc) was dissected from three coronal slices (Bregma +1.60 to -1.40) as illustrated in figure 5.4. Due to low

quantity of tissue per mouse, samples of two animals were pooled when possible. This was the case for the group-housing experiment, but in the maternal behavior experiment, animal numbers per experimental group were not sufficient to allow pooling.

RNA extraction

Total RNA was isolated using the TRIzol® reagent (Invitrogen Corp., Cergy Pontoise Cedex, France), according to the manufacturer's instructions. This reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (1987). Trizol® reagent maintains the integrity of the RNA during sample homogenization, while disrupting cells and dissolving cell components.

To remove any contaminating genomic DNA, samples were treated with Turbo DNA-free™ (Ambion/Applied biosystems) according to the manufacturer's instructions. Following extraction the integrity of RNA was verified by capillary electrophoresis using the RNA 600 Nano Lab-on-a-Chip kit (©Agilent technologies) and the Bioanalyzer 2100 (Agilent technologies).

Microarray hybridization

Total RNA was submitted to Genome Explorations Inc. (Memphis, USA) for labeling and hybridization to the *Mouse Genome 430 2.0 Array* (Affymetrix GeneChip®) according to the manufacturer's protocol. The *Mouse Genome 430 2.0 Array* contains 45,000 probe sets to analyze the expression level of over 39,000 transcripts and variants from over 34,000 well characterized mouse genes. The sequences from which these probe sets are derived were selected from GenBank®, dbEST, and RefSeq. Eleven pairs of oligonucleotide probes, including perfect match probes and mismatch probes containing one mismatch each, are used to measure the level of transcription of each sequence represented on the array.

In short, first and second strand cDNA were synthesized from 10 µg of total RNA using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen) and oligo-dT_{24}-T7 (5'-GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG-3') primer (PrOligo). Using the T7 promoter-coupled double stranded cDNA as template, cRNA was

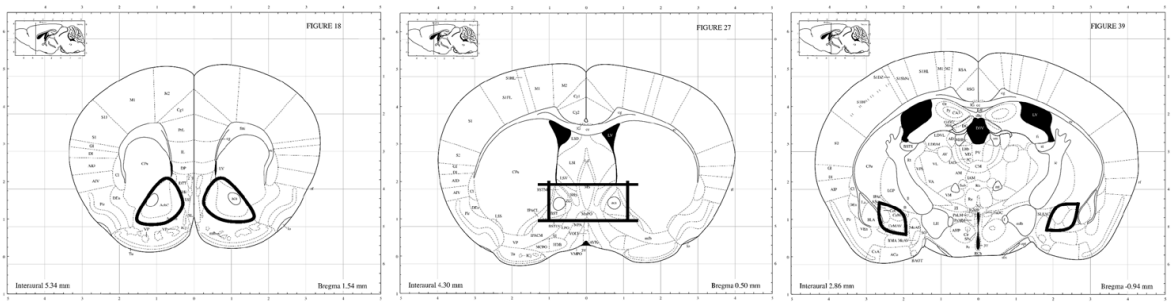


Figure 5.4: Dissection of the extended amygdala. The nucleus accumbens (left), bed nucleus of the stria terminalis (middle) and central nucleus of the amygdala (right) were dissected from 1 mm thick coronal slices of fresh brain tissue (Bregma +1.60 to -1.40 mm). Dissected parts are marked with dark circles and lines. The anterior commissure, corpus callosum and hippocampus were used as reference points. In fresh brain tissue, the central nucleus of the amygdala is recognized as a more translucent part. *Images are taken from The Mouse Brain Atlas (Paxinos and Franklin 2001).*

synthesized and labeled with biotinylated UTP and CTP by in vitro transcription by applying the Bioarray™ HighYield™ RNA Transcript Labeling Kit (ENZO Diagnostics Inc.). cRNA was then fragmented by ion-mediated hydrolysis and hybridized for 16h at 45°C to the *Mouse Genome 430 2.0 Array*. After washing, the arrays were stained with phycoerythrein-conjugated streptavidin (Molecular Probes) and the fluorescence intensities were determined using the Gene Chip Scanner 3000 (Affymetrix), a high-resolution confocal laser scanner. The *GeneChip® Operating Software (GCOS) v1.1.1* (Affymetrix) automatically acquired and analyzed image data.

Real-time quantitative PCR (qPCR)

Quantitative PCR provides an accurate method for determination of levels of specific DNA and RNA sequences. It is based on detection of a fluorescent signal produced proportionally during amplification of a PCR product. Quantitative PCR was preceded by reverse transcription to convert RNA to cDNA. cDNA was synthesized from 2 µg of total RNA using random primers and PowerScript™ Reverse Transcriptase (Clontech, Palo Alto, CA, USA). Real-time PCR was performed with a *DNA Engine Opticon Monitor 2*® fluorescence detection system (MJResearch/Bio-Rad). The PCR amplification was performed in a reaction volume of 10 µl containing: 2 µl cDNA (standard 4 ng, but 10 ng for weakly expressed genes), 3 µl of transcript-specific forward and reverse primers (0.6 µM) and 5 µl of a reaction reagents mix 2x containing modified DyNAmo DNA polymerase, SYBR Green I, optimized PCR buffer, MgCl2 and dNTP mix) (DyNamo™ SYBR® Green qPCR Kit, Finnzymes).

The amplification included an initial hold step of 15 min at 95 °C, followed by 40 cycles of denaturation (for 20 sec at 95°C) and annealing-extension (for 35 sec at 61°C). The annealing temperature varies depending on the used primers. Fluorescence spectra were recorded during the elongation phase of each PCR cycle. A dissociation curve was generated at the end of the 40th cycle to verify that a single product was amplified. Each primer was tested in triplicate.

Transcript-specific primers were designed with Primer Express software (Applied Biosystems) based on GenBank sequence information and verified by NCBI BLAST search. They were designed to generate amplicons of ~70 bp. After custom synthesis (Eurogentec), primer sets were tested by qPCR and gel electrophoresis for the absence of primer dimer artifacts and multiple products. Amplification efficiency of each set was determined by qPCR, using repetitive dilution series of cDNA. Only primers with PCR efficiency uniformly >90% were used. For each primer set, a no-template control was performed. The fluorescence-threshold line was set as described in the DNA Engine Opticon 2 System Operation Manual. Briefly, the threshold line was set manually such that the line intersected the fluorescence traces at the point where signals surpassed background noise and began to increase. The threshold line was used to determine cycle threshold (Ct) values for each well. The same threshold was applied to all wells, thereby ensuring accurate comparison of samples and controls.

Data analysis

Analysis of gene expression in Microarray

A first statistical analysis of microarray data was performed by *Genome exploration In*c using the GCOS software. This software is based on several statistical algorithms (Hubbell et al. 2002; Liu et al. 2002). A statistical significance of p=0.0025 was used to detect the presence of transcripts on the microarray. In our laboratory, we performed a subsequent analysis using ArrayAssist (ArrayAssist Expression software, Stratagene), a software that contains five algorithms (PLIER, RMA, GC-RMA, MAS 5 and LI WONG) that can analyze original data files (fluorescence images) created by GCOS using different criteria, e.g. calculation of background, importance addressed to mismatch probes or number of GC (guanine-cytosine) couples taken into account. This represents a supplementary validation of microarray results. We thus obtained experimental group comparisons using 6 different algorithms. All probe set differences yielding a fold change of $\geq$ 1.5x in the algorithms were retained and of these, only probe sets found to be differentially expressed in more than one algorithm were taken into account. A significant level of $p<0.05$ was used for group comparisons.

Analysis of gene expression in qPCR

Results of the real-time PCR data are represented as "Cycle of threshold" (Ct) values, where Ct is defined as the threshold cycle number of PCRs at which amplified product was first detected. There is an inverse correlation between Ct and amount of target: lower amounts of target correspond to a higher Ct value, and higher amounts of target have lower Ct values. Ct values were normalized to the reference control β-actin (housekeeping gene), obtaining ΔCt. Thus, since each primer was tested in triplicate, ΔCt was determined as 'the mean of the triplicate Ct values for the target gene' minus 'the mean of the triplicate Ct values for β-actin'. Statistical analysis of group differences was performed on the ΔCt values using Statistica 6.0© (StatSoft Inc.). Because of small sample sizes a nonparametric statistic, the Mann-Whitney U test was performed for all ΔCt comparisons. A significant level of $p<0.05$ was used.

The 2^ΔΔCt method (Livak and Schmittgen 2001) allows calculating relative changes (obtaining a fold change difference) in gene expression determined from the real-time quantitative PCR experiments, and allows visualizing group differences. To obtain this relative change between groups, we first calculated the difference in (normalized) expression between the two experimental conditions (mean ΔCt condition A *(either -C3H or -exG)* minus mean ΔCt condition B *(either -AKR or -I)*, represented as ΔΔCt. Since PCR amplification is exponential, the N-fold differential expression of the target gene in experimental condition A compared to condition B, is expressed as 2^ΔΔCt. We thus obtained the relative fold change difference in expression between experimental groups, which gives a single value. Standard error of means (SEM) for graphs were obtained by using the SEM of ΔCt values from the experimental group plotted in the graph (see Livak and Schmittgen 2001).

Results

Analysis of gene expression in microarray

Out of 45,000 probe sets on the micro-array, relatively few were shown to be differentially expressed in the extended amygdala when comparing experimental groups in both DBA and C57 mice *(figure 5.5).*

When comparing DBA mice that were raised in two different maternal environments *(left circle, comparing DBA-AKR to DBA-C3H)*, 50 differentially expressed probe sets were detected. Of these, 44 probe sets coded for known genes and 6 probe sets were expressed sequence tags (ESTs, of unknown function). When comparing DBA mice that had experienced a short period of group housing with DBA mice that had not *(right circle, comparing DBA-exG to DBA-I)*, 45 differentially expressed probe sets were detected. Of these, 35 probe sets coded for known sequences and 10 probe sets were ESTs. When comparing the early life and late life experiments *(overlapping part of circles)*, 4 common probe sets could be found. These were shown to code for 3 known genes: the gene for arginine vasopressin (AVP), oxytocin (OXY) and transthyretin (Ttr).

In the same way we compared the probe sets differentially expressed between C57 experimental groups *(figure 5.5 right side).* When comparing the early life and late life experiments *(overlapping part of circles)* for C57 mice, one common probe set could be found, coding for Ttr. Thus, one gene, the gene coding for Ttr, was found to be differentially expressed after both early and late life environmental experiences in both C57 and DBA mice.

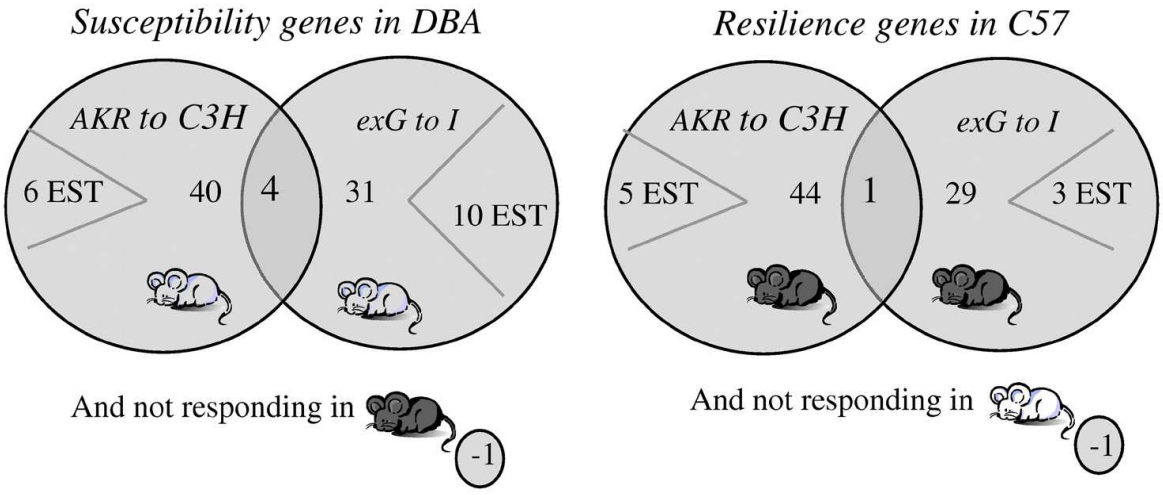


Figure 5.5: Microarray group comparisons of the extended amygdala: The number of probe sets differentially expressed between the different experimental groups. *AKR to C3H*: group comparison in the early life experiment; *exG to I*: group comparison in the late life experiment. EST: Expressed sequence tag. Data were obtained using 6 different algorithms (GCOS, MAS 5, PLIER, GC RMA, RMA, LI WONG); only probe sets found in at least two algorithms were taken into account.

Validation of gene expression by qPCR

The known genes that were differentially expressed between the DBA mice as detected in the microarray experiment were tested in qPCR. Surprisingly, in the early life experiment we could confirm only the differential expression of AVP in qPCR *(see table 5.1 for ΔCt levels and figure 5.6a for fold change differences).* DBA mice raised by an AKR mother showed a higher expression of AVP compared to DBA mice raised by a C3H mother ($U=2$, $p<0.05$). Due to limited efficacy of a first primer couple, a second primer couple was tested, yielding comparable results ($U=4$, $p=0.052$). Since the extended amygdala does not only contain vasopressin neurons, but also receives vasopressinergic fiber input (McEwen 2004), we also verified the expression of the AVP receptor 1a (AVPR1a) in this structure, but found no group differences. The differential expression of OXY and Ttr found in the microarray analysis could not be confirmed by qPCR. Moreover, no other group differences could be confirmed (data not shown).

For the adult life experiment we confirmed the differential expression of AVP and OXY in qPCR *(see table 5.1 for ΔCt levels and figure 5.6b-I for fold change differences)*. Individually housed DBA animals showed a higher expression of both AVP (1st primer: $U=4$, $p<0.01$, 2nd primer: $U=8$, $p<0.05$) and OXY ($U=6$, $p<0.05$) compared to DBA mice that had experienced a period of group housing. Moreover, in a replicate experiment of the group-housing experiment *(figure 5.6b-II)* we confirmed the DBA group differences for AVP (1st primer: $U=6$, $p<0.05$, 2nd primer: $U=4$, $p<0.01$). However, no differential expression of OXY was found in this second experiment. No influence of the group housing experience was found on AVPR1a or OXYR expression and no other group differences

Table 5.1: Expression levels in qPCR. C(t) values were normalized to the β-actin transcript yielding ΔCt. Two primers were tested for AVP. * $p<0.05$ and ** $p<0.01$ compared to DBA animals either raised by an AKR mother or individually housed. See figure 5.6 for fold change differences in group comparisons.

			ΔCt values					
Experiment	Group	*n*	AVP (1)	AVP (2)	AVRR1a	OXY	OXYR	Ttr
Early life experience	C57-*AKR*	6	8.03 ± 0.23	6.66 ± 0.35	8.26 ± 0.16	4.48 ± 0.46	-	8.64 ± 0.16
	C57-*C3H*	7	8.01 ± 0.33	7.05 ± 0.30	8.39 ± 0.11	4.62 ± 0.65	-	9.55 ± 0.45
	DBA-*AKR*	6	8.18 ± 0.35*	6.89 ± 0.52	8.12 ± 0.11	4.64 ± 0.85	-	9.32 ± 0.47
	DBA-*C3H*	5	9.47 ± 0.30	8.11 ± 0.46	8.54 ± 0.29	4.84 ± 0.48	-	8.97 ± 0.53
Adult group-housing experience (I)	C57-*I*	8	8.44 ± 0.45	5.60 ± 0.47	8.80 ± 0.13	3.85 ± 0.28	8.52 ± 0.10	8.92 ± 0.19
	C57-*exG*	7	8.91 ± 0.33	5.96 ± 0.29	9.23 ± 0.24	4.50 ± 0.35	8.74 ± 0.11	8.72 ± 0.65
	DBA-*I*	7	7.02 ± 0.31**	4.20 ± 0.40*	8.57 ± 0.16	2.77 ± 0.25*	8.09 ± 0.13	8.12 ± 0.36
	DBA-*exG*	7	8.48 ± 0.34	5.54 ± 0.40	9.01 ± 0.14	4.11 ± 0.49	8.39 ± 0.08	7.84 ± 0.19
Adult group-housing experience (II)	C57-*I*	8	8.57 ± 0.66	6.50 ± 0.58	8.22 ± 0.21	3.97 ± 0.50	6.65 ± 0.12	-
	C57-*exG*	8	8.68 ± 0.87	6.68 ± 0.65	8.54 ± 0.20	3.90 ± 0.76	6.53 ± 0.08	-
	DBA-*I*	7	7.35 ± 0.28*	5.34 ± 0.26**	8.26 ± 0.13	3.26 ± 0.28	6.16 ± 0.05	-
	DBA-*exG*	7	8.68 ± 0.37	6.61 ±0.20	8.24 ± 0.16	3.48 ± 0.26	6.29 ± 0.06	-

Values represent mean ± SEM

could be confirmed (data not shown). Table 5.2 shows the sequences of each primer couple (forward and reverse primer) used to detect the different genes shown in figure 5.6.

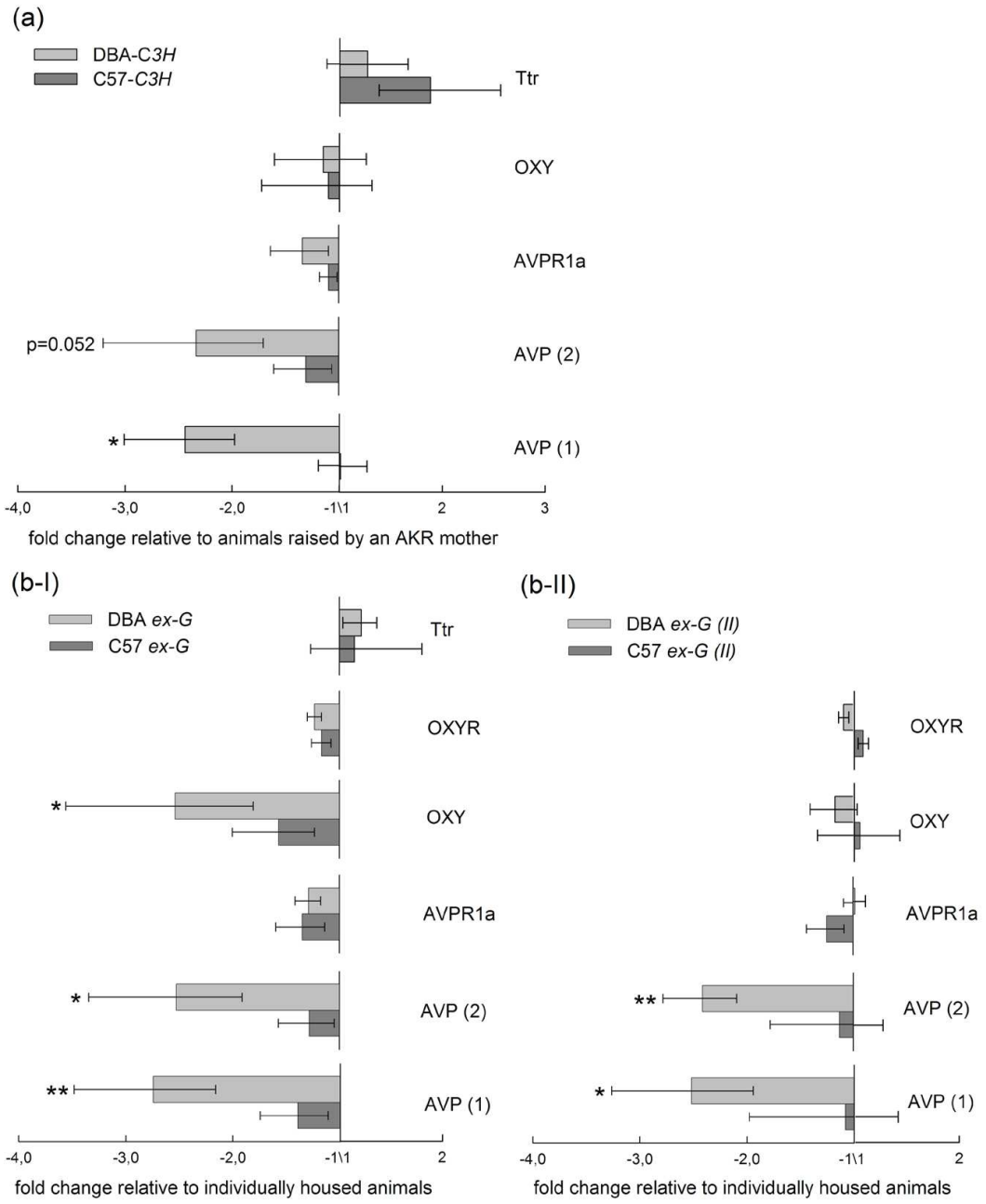


Figure 5.6: Relative expression levels of genes as determined by qPCR. Expression is represented per mouse strain as ***(a)*** mice cross-fostered to a C3H mother relative to mice cross-fostered to an AKR mother and ***(b)*** ex-group housed mice relative to individually housed mice. The group-housing experiment was replicated (I and II). Two primers were tested for AVP expression. Values were normalized to the β-actin transcript. Gene expression levels are presented as fold change ± SEM. AVP: arginine vasopressin; AVPR1a: AVP receptor 1a; OXY: oxytocin; OXYR: oxytocin receptor; Ttr: transthyretin. (a): DBA-*AKR* n=6; DBA-*C3H* n=5; C57-*AKR* n=6; C57-*C3H* n=7; (b-I): DBA-*I* et DBA-*exG* n=7; C57-*I* n=8; C57-*exG* n=7. (b-II): DBA-*I* et DBA-*exG* n=7; C57-*I* et C57-*exG* n=8. *p<0.05 and **p<0.01 compared to DBA animals either cross-fostered to an AKR mother or individually housed.

Discussion

In this study, we analyzed the effects of two different environmental experiences on gene expression in the extended amygdala of two inbred strains of mice that were shown to be differentially affected by these environments in their drug intake. We proceeded by screening the expression level of more than 34,000 genes in the mouse Affymetrix Gene Chip. Despite some initial technical limitations, a promising approach is described.

In the search for genes that could be implicated in the susceptibility of DBA mice to environmental events, an interesting difference appeared in both the early and late life experiment and was confirmed with qPCR: higher amounts of AVP transcripts were seen in the DBA groups that showed lower cocaine intake. The most prominent sites of AVP production in the rat brain are the paraventricular nucleus (PVN), supraoptic nucleus and suprachiasmatic nucleus of the hypothalamus, the bed nucleus of the stria terminalis (BNST), and the medial amygdaloid nucleus (for review de Vries and Miller 1998; McEwen 2004). Two functionally distinct vasopressinergic systems can be defined based on differences in the sites of action and release of AVP. Peripherally circulating AVP is responsible for its classic endocrine functions, like vasoconstriction, glycogen metabolism and antidiuresis. This peripherally acting AVP is synthesized in the magnocellular neurons of the PVN and supraoptic nucleus that project to the posterior pituitary where they secrete their contents as hormones into the peripheral circulation.

The central vasopressinergic system, on the other hand, represents AVP acting as a neuromodulator/neurotransmitter regulating an array of CNS-mediated functions. Its central neuroendocrine reactivity involves AVP produced in the parvocellular neurons of the PVN, projecting to the median eminence and releasing its content in the portal blood, where it can promote the release of certain anterior pituitary hormones like adrenocorticotropin (ACTH), gonadotropic hormones and prolactin. Other central functions of AVP include circadian rhythmicity, thermoregulation and autonomic function, but also higher cognitive functions such as memory and learning (for review Koob et al. 1989; de Wied et al. 1993; Gulpinar and Yegen 2004; McEwen 2004) and complex social behaviors includ-

Table 5.2: Primer sequences. Forward and reverse primers used in the qPCR experiments shown in table 5.1 and figure 5.6. AVP: arginine vasopressin; AVPR1a: AVP receptor 1a; OXY: oxytocin; OXYR: oxytocin receptor; Ttr: transthyretin; β-actin: housekeeping gene.

Coding for	Forward primer	Reverse primer
AVP (1)	GTCCGTGGATTCTGCCAAG	AAAGTTTATTTTCCATGCTGTAGGG
AVP (2)	CGCCAGGATGCTCAACACTA	CCATGTCAGAGATGGCCCTC
AVPR1a	AATTCGCCAAGGATGACTCG	TGTTGGGCTTCGGTTGTTAGA
OXY	TTACTGGCTCTGACCTCGGC	AGACACTTGCGCATATCCAGG
OXYR	TTGTGCTTGTGAATCAGAGACGT	AGCCAGCTAGGATTGATGAGGA
Ttr	AGCAACCCCCAGAATTGAGA	GGGATGCTACTGCTTTGGCA
β-actin	TGACCGAGCGTGGCTACA	CATAGCACAGCTTCTCTTTGATGTC

ing affiliation, aggression, juvenile recognition and parental behavior (for review Young et al. 2001). The neuroendocrine effects of AVP together with the cognitive, emotional and also immunological effects of centrally released AVP are thought to be essential to ensure adequate behavior of the animal during challenging situations and to contribute to the development of efficient coping strategies (de Wied et al. 1993; Landgraf et al. 1998).

AVP has two principal receptors mediating its behavioral effects in the brain: the AVPR1b, found in the anterior pituitary and the AVPR1a, found in numerous brain structures (McEwen 2004). Moreover, central AVP also displays a high affinity to the oxytocin receptors in the central amygdala, ventral hippocampus and olfactory tubercle (Barberis and Audigier 1985; De Kloet et al. 1985; Elands et al. 1988). Polymorphisms in the AVP receptors are possibly linked to autism (Wassink et al. 2004; Yirmiya et al. 2006) and depression (van West et al. 2004).

Although not explaining the differences we found, it is interesting to note that a mutual influence has been shown for AVP and cocaine. Repeated cocaine injections have been shown to decrease AVP content in several brain regions, including the amygdala and hippocampus (Sarnyai et al. 1992b). On the other hand, AVP has been shown to be implicated in cocaine self administration. Interestingly, and in line with our results, subcutaneous (SC) injections of the vasopressin analog desglycinamide-(Arg8)-vasopressin (DGAVP) has been reported to reduce the acquisition of heroin and cocaine self-administration behavior in rats (De Vry et al. 1988; van Ree et al. 1988), and intracerebroventricular (ICV) pre-treatment with vasopressin antiserum increased the acquisition of cocaine self administration (De Vry et al. 1988). Moreover, SC AVP dose dependently attenuated the development of sensitization to cocaine in mice (Sarnyai et al. 1992a), and it antagonized the stimulation of locomotor activity to cocaine and amphetamine after a high cocaine dose (15 and 20 mg/kg) in mice (Chiu et al. 1998). It is not clear whether SC AVP readily enters the brain to exert its actions on drug self administration and where in the brain ICV AVP exerts its drug related actions.

Two prominent sites of AVP production are localized in the extended amygdala: the BNST and the medial amygdala. The AVP-containing cells originating in the medial amygdala project to the ventral hippocampus and the lateral septum (Caffe et al. 1987). The BNST projects to the olfactory tubercle, diagonal band of Broca, lateral septum, anterior amygdala, and lateral habenula and to a number of brainstem sites including the locus coeruleus (Caffe et al. 1989). Vasopressin fiber terminals have also been found in the BNST and several amygdala nuclei, but their cells of origin are not known (for review McEwen 2004).

The vasopressin projections of the BNST and medial amygdala are sexually dimorphic (this system contains more cells and has denser projections in males than in females) and are extremely responsive to gonadal steroids, since both testosterone and estrogen restore AVP expression following castration (de Vries et al. 1984; van Leeuwen et al. 1985; Wang and de Vries 1995). Indeed, vasopressin-producing neurons in the BNST and amygdala were found to possess steroid hormone receptors (Axelson and van Leeuwen 1990). Recently, also progestin receptors were found on these AVP neurons, and progesterone treatment altered AVP immunoreactive labelling within the BNST and central medial amygdala (Auger and Vanzo 2006). AVP expression in BNST and medial amygdala has

been implicated in sexually dimorphic functions such as aggressive behavior as well as in non-sexually dimorphic functions such as social recognition memory (de Vries and Miller 1998). Group housing of male mice has been reported to change sex steroid levels (Nyska et al. 2002). Changes in sex steroid hormones during the group housing experience in our study might thus possibly have influenced AVP expression in these regions. In addition, the effects of cohabitation might be strain specific. Indeed, cohabitation with a female has been shown to influence AVP expression in the BNST in the prairie vole, which changes its social behavior after mating, but not in meadow voles (Wang et al. 1994). It was recently found that also neonatal testosterone and estrogen are important in determining the number of vasopressinergic cells in the BNST in adulthood (Han and de Vries 2003). Moreover, maternal care may influence the expression of oxytocin receptors (in females) and vasopressin receptors (in males) in the central nucleus of the amygdala (Francis et al. 2002). We also tested for potential group differences in the AVP receptor 1a, but found no significant changes.

A potential role for amygdalar AVP was recently proposed in the aversive consequences of early cocaine withdrawal (Zhou et al. 2008) as well as in the effect of negative emotional states on drug-seeking (Zhou et al. 2008). It was found that amygdalar AVP mRNA levels were increased during acute (but not chronic) cocaine withdrawal (Zhou et al. 2005). Moreover, foot shock stress, inducing reinstatement, increased AVP mRNA levels in the amygdala of rats withdrawn from heroin self-administration (Zhou et al. 2008). Ebner et al. (1999; 2002) found that forced swimming triggered vasopressin release within the septum and amygdala to modulate stress-coping strategies in rats. AVP released within the septum seems to be involved in active coping strategies and AVP released in the amygdala seems to be involved in the generation of passive coping strategies in stressful situations. We however, did not examine stress induced AVP peptide changes within the (extended) amygdala and its projection areas (e.g. lateral septum). But the change in basal (non stress induced) expression of AVP mRNA in the extended amygdala that we found in DBA mice might underlie more widespread changes in stress induced expression of AVP that could provoke changes in AVP content in the septum and the amygdala. If this holds true, then stress induced changes in AVP release in the septum or in the amygdala could possibly be involved in the passive coping style of DBA-*AKR* mice seen in the forced swim test. It would be interesting to study stress induced changes in AVP release in these areas in our early life experimental set-up to explore this hypothesis.

Oxytocin, a peptide structurally related to AVP, has also been implicated in drug related behaviors (for review Kovacs et al. 1998). In heroin tolerant rats, intravenous self-administration of heroin was decreased by SC OXY treatment (Kovacs et al. 1985) and ICV injections of OXY in nucleus accumbens or ventral hippocampus disrupted heroin self-administration (Ibragimov et al. 1987). The effect of intra-hippocampal injections lasted longer than the effect of intra-accumbens injections and was found to be prevented by an inhibitor of oxytocin receptors. Interestingly, the ventral hippocampus is an important projection area of the median amygdala vasopressin neurons and AVP sensitive OXY receptors were found in the ventral hippocampus (Barberis and Audigier 1985; Elands et al. 1988). Our results would then be in accordance with these studies, since a higher expression of AVP mRNA in the median amygdala would theoretically mean a higher release of

AVP in the ventral hippocampus, which could influence (decrease) cocaine self administration by its action on OXY receptors. This of course remains highly hypothetical.

In short, stress, cocaine related behaviors and AVP are somehow linked together, and our results suggest that changes in AVP expression in the extended amygdala might be important in the individual vulnerability to stressful events and their impact on drug intake. Specifically blocking the AVP expression in the extended amygdala before and during cocaine self-administration in our experimental set-ups could clarify its possible role in individual differences in drug taking behavior.

Microarray analyses yielded very few group differences in gene expression. It is important to emphasize that Affymetrix technology is not highly sensitive and is unlikely to reveal subtle regulation of low-abundance genes (Evans et al. 2002). This lack of sensitivity will leave a significant number of regulated transcripts undetected, thus representing a significant number of false negatives. This poses a crucial problem for our study, since our environmental conditions most probably induce subtle changes in basal (that is, non stress or drug induced) gene expression. In addition, using qPCR, we could only reliably confirm few of the group differences in gene expression found on the microarrays. The most important reason for this discrepancy between the two techniques is probably the fact that we studied pooled samples for microarrays, which for certain genes masked strong variability (revealed by qPCR) and thus led to false positive results. Research strategy in our laboratory now has changed and when possible, individual samples are sent for microarray analysis. Moreover, we are aware that our dissection technique was 'approximate' (though constant!) and we could not avoid dissecting some 'extra' tissue (outside of the extended amygdala), which might have diluted possible differences. The fact that we found a differential expression of Ttr in each group comparison seems to be an artefact, since this is a prealbumin that is synthesized in the choroid plexus, which is lining the ventricles. Since both the NAcc and the BNST are localised close to the lateral ventricle, we might have dissected some of this tissue.

In conclusion, in both the postnatal maternal behavior and the adult group-housing experiment, DBA appears as a strain that is susceptible to the impact of environmental experiences on drug intake, while the C57 strain is not. We compared the RNA expression profiles in the extended amygdala to identify putative candidate genes that might play a role in the establishment of this susceptibility or resilience to environmental influences on drug taking behavior. Surprisingly few changes in gene expression were detected using microarrays, but when comparing DBA mice, AVP was clearly differentially expressed, while being unchanged in C57 mice. Despite the technological limitations discussed above, the differential expression of AVP in the extended amygdala of DBA mice was found in both the early life and late life experiments and coincides with a differential cocaine self-administration. Does this basal change in extended amygdala AVP play a role in the changed cocaine self-administration? Hence, an interesting and well known candidate molecule has been identified for future experiments.

Acknowledgements

This research was supported by the NWO/INSERM/ZON grants AH002G and 985-10-014 and the university of Bordeaux 2.

References

Auger CJ, Vanzo RJ (2006) Progesterone treatment of adult male rats suppresses arginine vasopressin expression in the bed nucleus of the stria terminalis and the centromedial amygdala. Journal of Neuroendocrinology 18: 187-194

Axelson JF, van Leeuwen FW (1990) Differential Localization of Estrogen-Receptors in Various Vasopressin Synthesizing Nuclei of the Rat-Brain. Journal of Neuroendocrinology 2: 209-216

Barberis C, Audigier S (1985) Vasopressin and oxytocin receptors in the central nervous system of the rat. Ann Endocrinol (Paris) 46: 35-39

Caffe AR, van Leeuwen FW, Luiten PG (1987) Vasopressin cells in the medial amygdala of the rat project to the lateral septum and ventral hippocampus. J Comp Neurol 261: 237-252

Caffe AR, Van Ryen PC, Van der Woude TP, van Leeuwen FW (1989) Vasopressin and oxytocin systems in the brain and upper spinal cord of Macaca fascicularis. J Comp Neurol 287: 302-325

Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 7: 583-590

Chiu J, Kalant H, Lê DA (1998) Vasopressin opposes locomotor stimulation by ethanol, cocaine and amphetamine in mice. Eur J Pharmacol 355: 11-17

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156-159

Dalm S, Enthoven L, Meijer OC, van der Mark MH, Karssen AM, de Kloet ER, Oitzl MS (2005) Age-related changes in hypothalamic-pituitary-adrenal axis activity of male C57BL/6J mice. Neuroendocrinology 81: 372-380

De Kloet ER, Rotteveel F, Voorhuis TA, Terlou M (1985) Topography of binding sites for neurohypophyseal hormones in rat brain. Eur J Pharmacol 110: 113-119

de Vries GJ, Buijs RM, Sluiter AA (1984) Gonadal hormone actions on the morphology of the vasopressinergic innervation of the adult rat brain. Brain Res 298: 141-145

de Vries GJ, Miller MA (1998) Anatomy and function of extrahypothalamic vasopressin systems in the brain. Prog Brain Res 119: 3-20

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

de Wied D, Diamant M, Fodor M (1993) Central nervous system effects of the neurohypophyseal hormones and related peptides. Front Neuroendocrinol 14: 251-302

Ebner K, Wotjak CT, Holsboer F, Landgraf R, Engelmann M (1999) Vasopressin released within the septal brain area during swim stress modulates the behavioural stress response in rats. Eur J Neurosci 11: 997-1002

Ebner K, Wotjak CT, Landgraf R, Engelmann M (2002) Forced swimming triggers vasopressin release within the amygdala to modulate stress-coping strategies in rats. Eur J Neurosci 15: 384-388

Elands J, Barberis C, Jard S (1988) [3H]-[Thr4,Gly7]OT: a highly selective ligand for central and peripheral OT receptors. Am J Physiol 254: E31-E38

Evans SJ, Datson NA, Kabbaj M, Thompson RC, Vreugdenhil E, De Kloet ER, Watson SJ et al. (2002) Evaluation of Affymetrix Gene Chip sensitivity in rat hippocampal tissue using SAGE analysis. Serial Analysis of Gene Expression. Eur J Neurosci 16: 409-413

Francis DD, Young LJ, Meaney MJ, Insel TR (2002) Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. J Neuroendocrinol 14: 349-353

Gulpinar MA, Yegen BC (2004) The physiology of learning and memory: role of peptides and stress. Curr Protein Pept Sci 5: 457-473

Han TM, de Vries GJ (2003) Organizational effects of testosterone, estradiol, and dihydrotestosterone on vasopressin mRNA expression in the bed nucleus of the stria terminalis. J Neurobiol 54: 502-510

Harris GC, Aston-Jones G (2007) Activation in extended amygdala corresponds to altered hedonic processing during protracted morphine withdrawal. Behav Brain Res 176: 251-258

Heimer L, Alheid GF (1991) Piecing together the puzzle of basal forebrain anatomy. Adv Exp Med Biol 295: 1-42

Hubbell E, Liu WM, Mei R (2002) Robust estimators for expression analysis. Bioinformatics 18: 1585-1592

Ibragimov R, Kovacs GL, Szabo G, Telegdy G (1987) Microinjection of oxytocin into limbic-mesolimbic brain structures disrupts heroin self-administration behavior: a receptor-mediated event? Life Sci 41: 1265-1271

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

Kendler KS (2005) "A gene for...": the nature of gene action in psychiatric disorders. Am J Psychiatry 162: 1243-1252

Koob GF (2003) Neuroadaptive mechanisms of addiction: studies on the extended amygdala. Eur Neuropsychopharmacol 13: 442-452

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24: 97-129

Koob GF, Lebrun C, Bluthe RM, Dantzer R, Le Moal M (1989) Role of neuropeptides in learning versus performance: focus on vasopressin. Brain Res Bull 23: 359-364

Kovacs GL, Borthaiser Z, Telegdy G (1985) Oxytocin reduces intravenous heroin self-administration in heroin-tolerant rats. Life Sci 37: 17-26

Kovacs GL, Sarnyai Z, Szabo G (1998) Oxytocin and addiction: a review. Psychoneuroendocrinology 23: 945-962

Landgraf R, Wotjak CT, Neumann ID, Engelmann M (1998) Release of vasopressin within the brain contributes to neuroendocrine and behavioral regulation. Prog Brain Res 119: 201-220

Liu WM, Mei R, Di X, Ryder TB, Hubbell E, Dee S, Webster TA et al. (2002) Analysis of high density expression microarrays with signed-rank call algorithms. Bioinformatics 18: 1593-1599

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408

McEwen BB (2004) General introduction to vasopressin and oxytocin: structure/metabolism, evolutionary aspects, neural pathway/receptor distribution, and functional aspects relevant to memory processing. Adv Pharmacol 50: 1-708

Nyska A, Hester SD, Cooper RL, Goldman JM, Stoker TE, House D, Wolf DC (2002) Single or group housing altered hormonal physiology and affected pituitary and interstitial cell kinetics. J Toxicol Sci 27: 449-457

Rutter M, Moffitt TE, Caspi A (2006) Gene-environment interplay and psychopathology: multiple varieties but real effects. J Child Psychol Psychiatry 47: 226-261

Sarnyai Z, Szabo G, Kovacs GL, Telegdy G (1992a) Opposite actions of oxytocin and vasopressin in the development of cocaine-induced behavioral sensitization in mice. Pharmacol Biochem Behav 43: 491-494

Sarnyai Z, Vecsernyes M, Laczi F, Biro E, Szabo G, Kovacs GL (1992b) Effects of cocaine on the contents of neurohypophyseal hormones in the plasma and in different brain structures in rats. Neuropeptides 23: 27-31

van Leeuwen FW, Caffe AR, de Vries GJ (1985) Vasopressin cells in the bed nucleus of the stria terminalis of the rat: sex differences and the influence of androgens. Brain Res 325: 391-394

van Ree JM, Burbach-Bloemarts EM, Wallace M (1988) Vasopressin neuropeptides and acquisition of heroin and cocaine self-administration in rats. Life Sci 42: 1091-1099

van West D, Del Favero J, Aulchenko Y, Oswald P, Souery D, Forsgren T, Sluijs S et al. (2004) A major SNP haplotype of the arginine vasopressin 1B receptor protects against recurrent major depression. Mol Psychiatry 9: 287-292

Wang Z, de Vries GJ (1995) Androgen and estrogen effects on vasopressin messenger RNA expression in the medial amygdaloid nucleus in male and female rats. J Neuroendocrinol 7: 827-831

Wang Z, Smith W, Major DE, de Vries GJ (1994) Sex and species differences in the effects of cohabitation on vasopressin messenger RNA expression in the bed nucleus of the stria terminalis in prairie voles (Microtus ochrogaster) and meadow voles (Microtus pennsylvanicus). Brain Res 650: 212-218

Waraczynski MA (2006) The central extended amygdala network as a proposed circuit underlying reward valuation. Neurosci Biobehav Rev 30: 472-496

Wassink TH, Piven J, Vieland VJ, Pietila J, Goedken RJ, Folstein SE, Sheffield VC (2004) Examination of AVPR1a as an autism susceptibility gene. Mol Psychiatry 9: 968-972

Yirmiya N, Rosenberg C, Levi S, Salomon S, Shulman C, Nemanov L, Dina C et al. (2006) Association between the arginine vasopressin 1a receptor (AVPR1a) gene and autism in a family-based study: mediation by socialization skills. Mol Psychiatry 11: 488-494

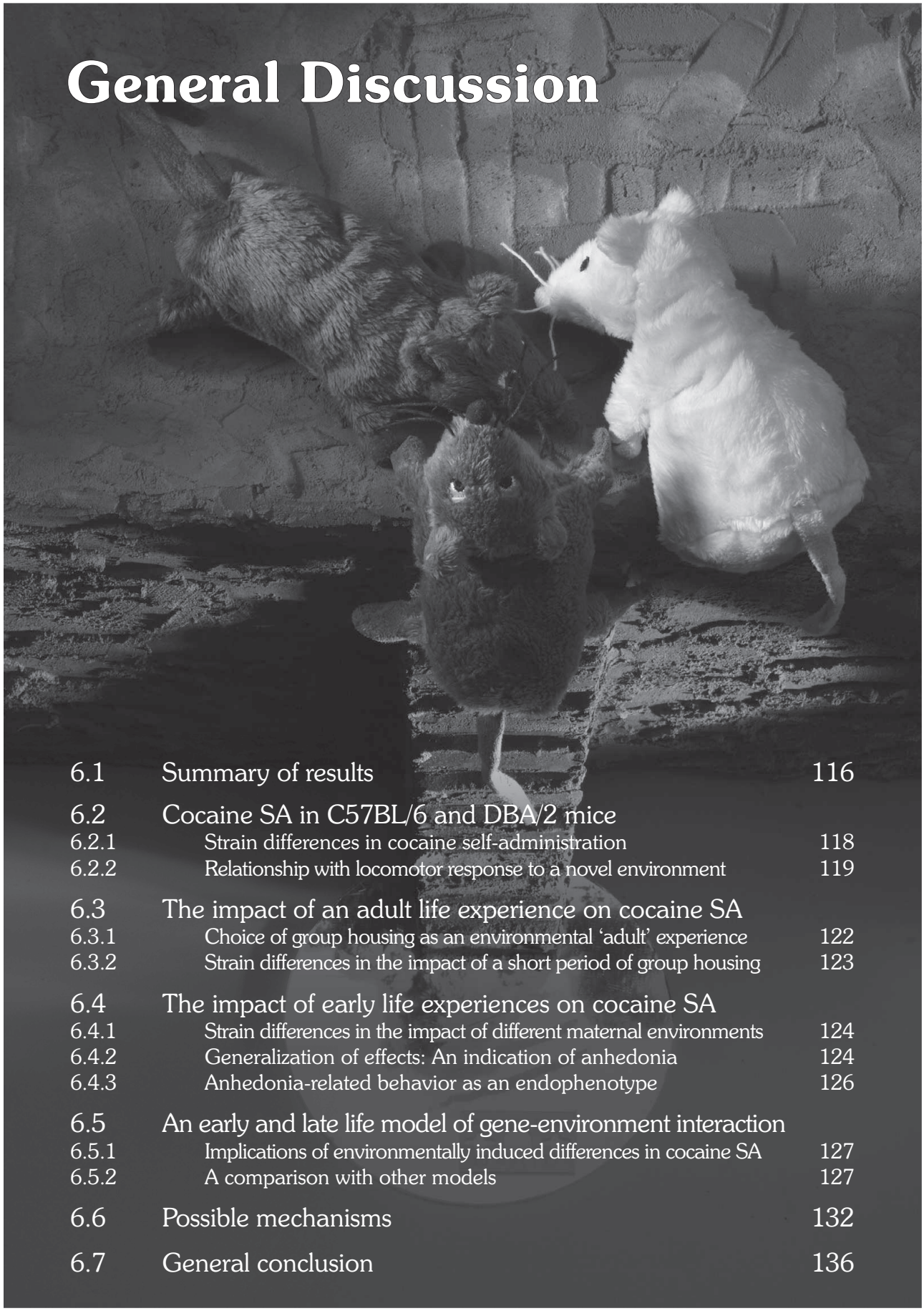
Young LJ, Lim MM, Gingrich B, Insel TR (2001) Cellular mechanisms of social attachment. Horm Behav 40: 133-138

Zhou Y, Bendor JT, Yuferov V, Schlussman SD, Ho A, Kreek MJ (2005) Amygdalar vasopressin mRNA increases in acute cocaine withdrawal: evidence for opioid receptor modulation. Neuroscience 134: 1391-1397

Zhou Y, Leri F, Cummins E, Hoeschele M, Kreek MJ (2008) Involvement of arginine vasopressin and V1b receptor in heroin withdrawal and heroin seeking precipitated by stress and by heroin. Neuropsychopharmacology 33: 226-236

Chapter 6

General Discussion



6.1 Summary of results

The objective of this thesis was to demonstrate the role of gene-environment interactions in the emergence of individual differences in cocaine use. We obtained this by studying the impact of environmental experiences on cocaine intake of two inbred strains of mice. Both an early life experience and a later life psychosocial stressor differentially affected the cocaine intake in the two inbred strains and we thus showed a clear gene-environment interaction. We created two promising mouse models for studying the biological basis underlying individual vulnerabilities to the impact of environmental stressors.

Chapter 2 describes (1) the spontaneous maternal behavior of C57 and DBA mice when raising biological litters and foster litters of the same strain *(intra*-strain cross-fostering) and (2) the spontaneous maternal behavior of AKR and C3H mice when raising either DBA or C57 pups *(inter*-strain cross-fostering).

Under our experimental conditions, maternal behavior of C57 and DBA mothers toward their biological offspring was surprisingly similar and *intra*-strain cross-fostering had minimal effects on maternal behavior in either strain.

In contrast, the AKR and C3H strains were found to exhibit very distinct maternal behaviors, with C3H mothers showing more pup-directed behavior (licking and nursing), whereas AKR mothers exhibited more non-pup directed behavior (self-grooming) while being present on the nest and an exaggerated 'nest reorganizing' behavior. This maternal style characterizing the dams was seen with both C57 and DBA foster pups. However, although pup strain did not change maternal style, it did have some influence on the amount of maternal behavior. Thus, both adoptive mothers showed less nursing posture with DBA pups than with C57 pups. In addition to the analysis of maternal behavior, we followed bodyweight changes from weaning to 16 weeks of life, and found that both C57 and DBA pups raised by a C3H mother had a higher bodyweight compared to same-strain pups raised by an AKR mother.

Chapter 3 describes the influence of these early (maternal) environments on cocaine taking behavior of the adult C57 and DBA offspring in an intravenous self-administration (SA) experiment. In addition, we studied other behavioral dimensions associated with drug abuse, i.e. anxiety- and depression-related behaviors. We found that the impact of maternal environment on cocaine use and behavioral despair in the FST (a depression-related behavior) depends upon genotype, as cocaine self-administration and floating behavior in the FST were influenced by maternal environment in DBA, but not in C57 mice. Anxiety-related behavior was not influenced by maternal environment in either strain, pointing to a specific influence on motivational aspects of behavior.

When considering the reference groups (mice raised by their biological mother), it appears that a foster environment *per se*, created by either an AKR or a C3H mother, did not influence behavior in C57 mice. Regarding DBA mice, the foster environment created by a C3H mother did not induce behavioral changes, but the foster environment created by

an AKR mother did. This latter environment appears to induce a decrease in cocaine intake and an increase in behavioral despair in the FST.

In conclusion, we showed for the first time that gene-environment interactions during the early life period can affect cocaine use in adulthood. We further demonstrated an association with a depression-related behavior.

In a second experiment we showed that gene-environment interactions in adult life can also play a role in drug taking behavior. **Chapter 4** describes the influence of a short-lasting past experience of group housing in adulthood on cocaine intake in C57 and DBA mice. Cocaine SA in C57 mice did not differ between individually housed *(-I)* and ex-group housed *(-exG)* animals. However, DBA mice were influenced by the group housing expe-

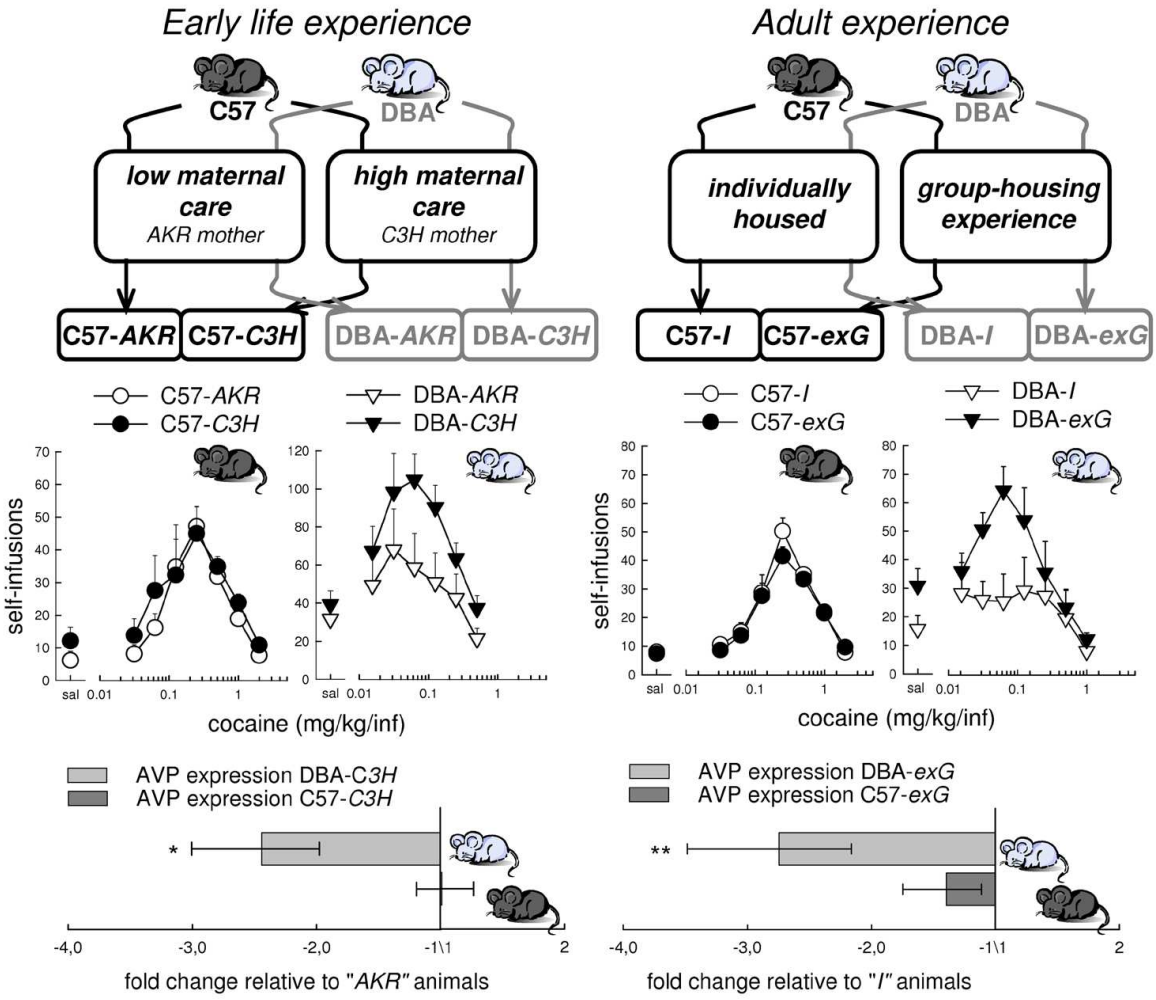


Figure 6.1: Two mouse models demonstrating a gene-environment interaction in cocaine SA. Different early life environments were created by cross-fostering mice to mothers differing in maternal care (left side), a short period of group housing constituted an environmentally 'stressful' situation in adulthood (right side). The DBA strain is represented by the grey mice and the C57 strain by the black mice. In both models, mice of the DBA strain proved to be susceptible to the influence of environmental experiences on cocaine SA, while mice of the C57 strain proved to be resilient to the same experiences (middle graphs). Gene expression in the extended amygdala of drug naïve animals (lower graphs) revealed a higher expression of AVP in the DBA groups showing a lower drug intake.

rience: DBA-*exG* mice showed a higher intake during acquisition and an upward shift in the dose-response curve as compared to DBA-*I*. Differences in brain cocaine levels could not account for the observed behavioral differences. Thus, we showed that the impact of a past adult environmental experience on subsequent drug taking behavior depends on the genetic background of the mice. Here again, DBA mice were shown to be susceptible to the environmental experience, while C57 mice were not.

Finally, **chapter 5** describes a study investigating the mechanism underlying the susceptibility of DBA mice to environmental experiences. Here, changes in gene expression in the extended amygdala of mice from both models were studied. Mice were sacrificed in adulthood after the (early or late) environmental experience, but without having experienced drug SA. Interestingly, we found higher expression levels of arginine vasopressin (AVP) in the extended amygdala of the DBA groups that showed lower cocaine intake *(figure 6.1)*. That is, DBA-*AKR* mice showed higher AVP levels compared to DBA-*C3H* mice and DBA-*I* mice showed higher AVP levels compared to DBA-*exG* mice (note that graphs shows the relative expression of the -*C3H* and -*exG* groups). No differences in the expression of AVP were found between the C57 groups.

Overall, the C57 appears as a strain resistant and the DBA as a strain susceptible to the influence of environmental experiences on cocaine SA. In the following paragraphs, results will be discussed in some more detail.

6.2 Cocaine SA in C57BL/6 and DBA/2 mice

6.2.1 Strain differences in cocaine self-administration

In the group-housing experiment, that was performed before the maternal behavior experiment, both C57 and DBA mice acquired SA of cocaine at a dose of 1 mg/kg/infusion. In accordance with previous findings (Grahame and Cunningham 1995; Rocha et al. 1998), we found that DBA mice (when individually housed upon arrival to our facilities at 8 weeks of age and tested at 15 weeks of age) took less cocaine as compared to C57. It was suggested that differences between C57 and DBA can be interpreted as differences in cocaine-induced intoxicating effects. Rocha and colleagues (1998) reported that "at 2 mg/kg/infusion all DBA mice showed high intoxication symptoms and none of them started lever press". Several observations comfort the intoxication hypothesis by showing that cocaine exerts both higher hepatotoxic (Boyer and Petersen 1992) and neurotoxic (Golden et al. 2001) effects in DBA than in C57. At the acquisition dose of 1 mg/kg/infusion used in our group-housing study, DBA mice acquired, but showed low levels of infusion. The group housing experience increased responding for cocaine in DBA mice. This might suggest that a short experience of group housing induces a decrease in cocaine-induced intoxicating effects in DBA mice.

Since we identified this strain difference in dose sensitivity for cocaine, we decided to lower the acquisition dose for DBA mice in the maternal care experiment. Our objective

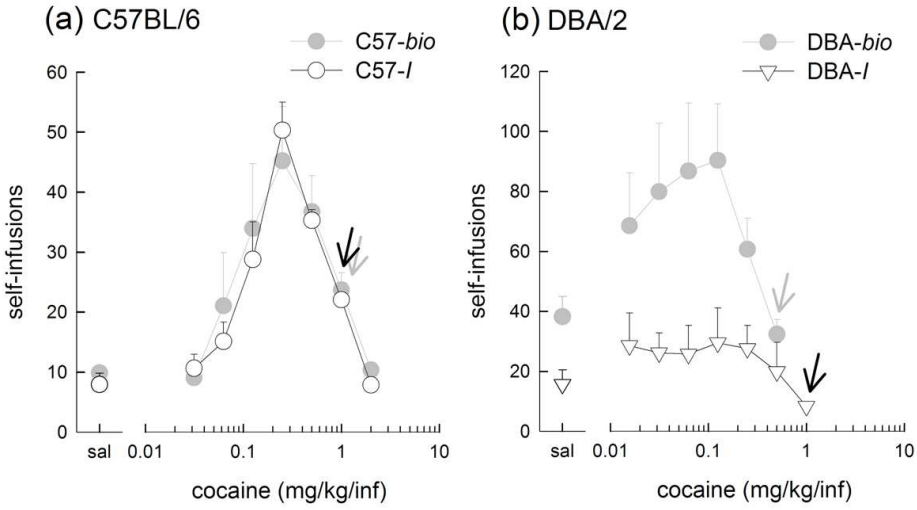


Figure 6.2: Comparing the cocaine dose-response curves in the early and late life experiments in which DBA mice acquired SA at different doses. Shown are the dose-response curves for mice raised by biological mothers *(-bio)* and mice without a grouping experience *(-I)*. ***(a)*** C57 mice acquired at 1 mg/kg/inf dose in both experiments. ***(b)*** DBA mice acquired at 1 mg/kg/inf dose in the late life experiment and at 0.5 mg/kg/inf dose in the early life experiment. Arrows indicate the acquisition dose. Symbols show means ± SEM. Data from chapters 3 and 4 were used to create these graphs.

was to obtain comparative drug responses in acquisition for both strains and avoid a too high 'negative' effect of cocaine in DBA mice. We saw that acquisition at 1 mg/kg/infusion in DBA-*I* mice led to responses comparable with the 0.5 mg/kg/infusion dose in the dose response study for C57-*I* mice *(see figure 3.2 in chapter 3)*. We thus decided to let DBA mice acquire at a dose of 0.5 mg/kg/infusion in the maternal care experiments, while we kept the 1 mg/kg/infusion dose for C57 mice. Indeed, this change in protocol made the DBA mice increase their intake in acquisition, even to a higher level compared to the C57 mice at 1 mg/kg/infusion *(see figure 3.4 in chapter 3 left panels)*. Moreover, in animals that acquired at a lower acquisition dose, the dose-response curve did not show the classical flattened curve often observed for DBA mice. So the dose-response curve of the DBA-*I* starting acquisition at 1 mg/kg/infusion and the DBA-*bio* (reference group) starting acquisition at 0.5 mg/kg/infusion do not have the same appearance *(figure 6.2 right panel)*. Evidently, although theoretically similar, the DBA animals raised by their biological mother in our own animal facilities are not completely comparable to the DBA-*I* animals from the group-housing experiment that arrived in our facilities at 8 weeks of life. But the results in these two experiments together with the data in literature suggest that it is very important to choose the right acquisition dose, especially in this inbred strain, since the acquisition dose influences subsequent cocaine responses. It seems that the acquisition dose sets the stage for responding in the dose-response test.

6.2.2 Relationship with locomotor response to a novel environment

In rats, a relationship between behavior in a novel environment and propensity to self-administer psychostimulant drugs was demonstrated. When outbred Sprague-Dawley rats are exposed to the mild stress of a novel environment, some rats defined as high responders

(HRs) exhibit high rates of exploratory locomotion, while others defined as low responders (LRs) exhibit low rates of locomotor activity (Piazza et al. 1989). HRs were shown to exhibit enhanced psychostimulant SA behavior, sensitization, and basal or drug-induced dopamine release in the nucleus accumbens (NAcc) compared to LRs (Piazza et al. 1989; Piazza et al. 2000; Marinelli and White 2000; Kabbaj et al. 2001). To test whether there would exist a similar relationship between activity and cocaine SA within our mice strains, we examined reactivity to a novel environment in our two models *(figure 6.3)* and separately analyzed the first hour (initial activation, the activity measure in the HR/LR model) and second hour (habituation) of the test.

In the early life experiment *(figure 6.3a),* we found that DBA, but not C57 mice, were affected in their behavior. This was seen in the habituation phase [2nd hour strain*mother interaction $F(1,57)=5.97$ $p<0.05$ with DBA-*AKR*<DBA-*C3H* $p<0.01$ and C57-*AKR*≈C57-*C3H*, timepoint*strain*mother interaction $F(11,627)=0.56$ p=ns], but not during initial activation [1st hour strain*mother interaction $F(1,57)=0.05$ p=ns]. Since DBA-*bio* showed a different habituation of the locomotor response compared to DBA-*AKR*, but not compared to DBA-*C3H* [2nd hour mother effect $F(2,39)=9.81$ $p<0.001$ with DBA-*bio*>DBA-*AKR* $p<0.01$ and DBA-*bio*≈DBA-*C3H*], it seems that being raised by an AKR mother changes locomotor reactivity to a novel environment.

When comparing the influence of a past adult life experience in both strains *(figure 6.3b),* we can see a difference in responding for DBA, but not C57 mice at some timepoints during the initial activity phase, touching significance [1st hour timepoint*strain*group interaction $F(11,473)=1.73$ $p=0.06$]. During the habituation phase the strains were not differentially affected by the group housing experience [2nd hour strain*group interaction $F(1,43)=0.58$, timepoint*strain*group interaction $F(11,473)=1.09$, both p=ns].

Thus, again the C57 and DBA strains were differentially affected in behavior by environmental experiences, with the DBA sensitive and the C57 relatively insensitive to changes. It seems that DBA mice were differently affected by an early- compared to an adult- environmental experience. That is, DBA-*AKR* mice showed a faster habituation compared to DBA-*C3H* mice, while DBA-*I* mice showed a higher initial activation compared to DBA-*exG* mice.

The time of day testing took place might have influenced results and play a role in the differential effects in both experiments. Like HR/LR animals, the animals of the early life experiment were tested in the second part of the light phase, while the animals of the late life experiment were tested in the middle of the dark phase. During these phases, mice differ in both basal activity (Kopp et al. 1998; Hofstetter et al. 2007) and basal corticosterone levels (Dalm et al. 2005), indicating that reactivity/arousal to a novel environment might be different at these different time points.

However, higher locomotor activity to novelty did not correspond to higher cocaine intake in either model. For DBA mice, the initial activation to novelty in the animals with a higher cocaine intake was either similar (DBA-*C3H* compared to DBA-*AKR*) or lower (DBA-*exG* compared to DBA-*I)* compared to the animals with a lower cocaine intake. This finding might not be that surprising, since also in rats higher locomotor activity to novelty does not always predict higher drug intake in SA (see Kabbaj 2006; Kosten et al. 2007). In addition, the behavioral correlation in HR and LR rats is no longer valid after

exposure to a psychosocial stressor. That is, a chronic social defeat stressor was shown to differentially affect both rats and equalize individual differences in cocaine SA (Kabbaj et al. 2001). This again shows that behavior is not static, but depends on environmental conditions and these results underscore the importance of individual differences in reactivity to the environment.

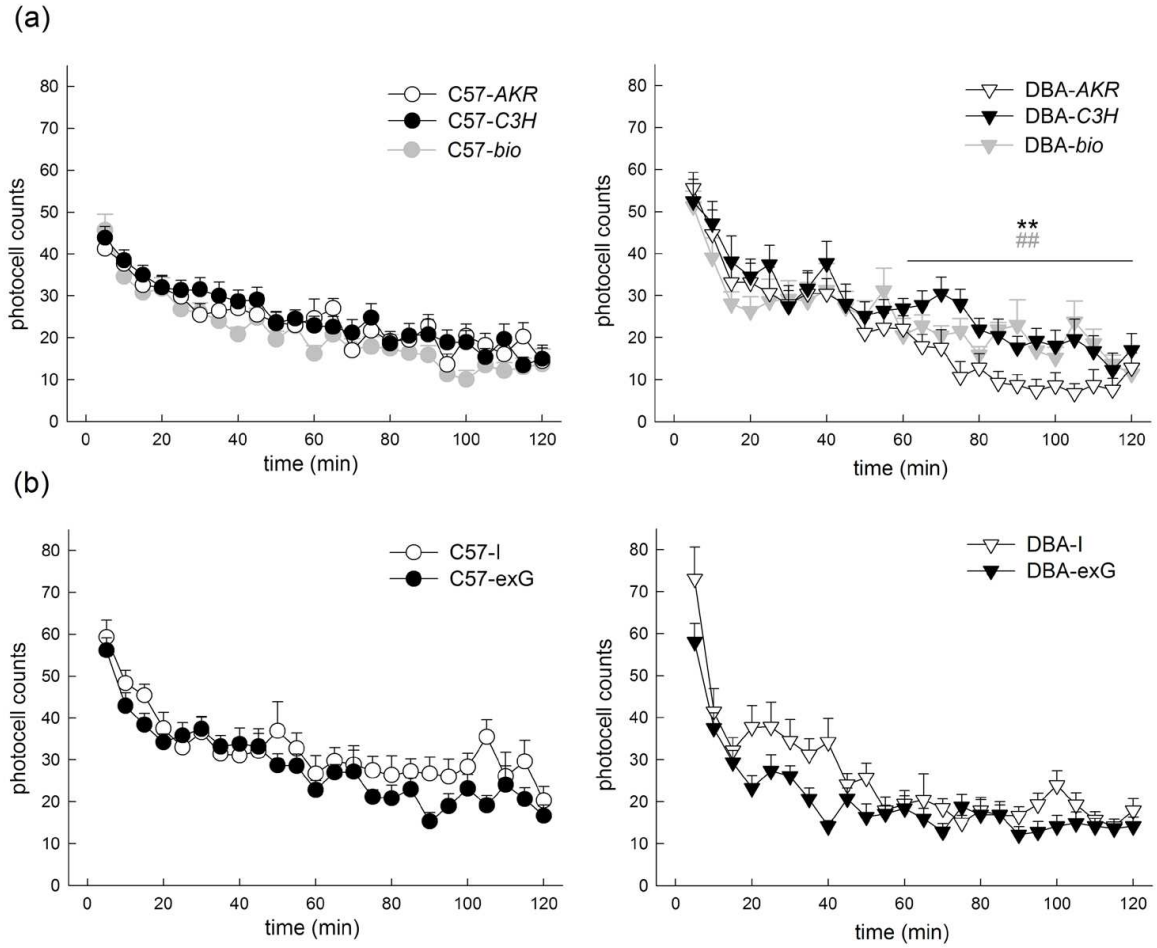


Figure 6.3: Locomotor response to a novel environment in our two experimental models.
(a) C57 (left panel) and DBA (right panel) mice having experienced different maternal environments and ***(b)*** C57 and DBA mice after a short adult group housing experience compared to individually housed animals. Animals were placed for two hours in Plexiglas chambers (21x11x18 cm) with a grid floor. These chambers were located within a larger exterior opaque box and equipped with photoelectric cells to measure horizontal and vertical activity (Imetronic, Pessac, France). Testing took place under dim light conditions. Symbols show means ± SEM over 5 min time-bouts. ANOVAs with repeated measurements were performed over the first hour (initial activation) and second hour (habituation) of the test with strain and mother or group as independent factors. C57-*AKR* n=13, C57-*C3H* n=20, C57-*bio* n=17, DBA-*AKR* n=14, DBA-*C3H* n=14, DBA-*bio* n=14, C57-*I*, n=12; C57-*exG*, n=12; DBA-*I*, n=11; DBA-*exG*, n=12. **p<0.01 compared to DBA-*C3H* and ##p<0.01 compared to DBA-*bio*.

6.3 The impact of an adult life experience on cocaine SA

6.3.1 Choice of group housing as an environmental 'adult' experience

Several environmental 'stressors' can have an impact on psychostimulant SA behavior (for review Lu et al. 2003), but food-restriction in particular has been shown to influence drug intake. Using food restriction as the adult environmental experience in our model would have been a logic choice, since a past period of food restriction was shown to change conditioned place preference for amphetamine in DBA, but not in C57 mice (Cabib et al. 2000). We however, prefered not to use food restriction because of its ambiguous interaction with drug SA. In most IV SA studies, animals are trained to respond for food before starting drug SA (Rocha et al. 1998; e.g. Stolerman et al. 1999; Moffett et al. 2006). This protocol is applied to facilitate SA, but since it implies a food restriction (80-85% of free feeding weight), it introduces an extra 'stress' or 'reward'-confounding factor that can have an (additional) effect on drug SA behavior. There are several studies indicating that acute food deprivation/restriction (typically 24 h of either deprivation or restriction by providing a small food ration of 5-8 g) or chronic food restriction (several days or weeks of limited access to food) significantly increases the initiation and maintenance of IV opiate and psychostimulant SA (Carroll et al. 1979; Papasava and Singer 1985; De Vry et al. 1989). It might be either the stress-like physiological effects of food deprivation (e.g. activation of the HPA axis) that is involved in this effect or the 'reinforcer interaction' effects of food restriction, whereby the decreased availability of one reinforcer increases responding maintained by another (Carroll and Meisch 1984). We thus preferred to use a different environmental experience in our late environment model. And evidently, we did not submit our animals to food restriction at any time during our experiments.

Like food restriction, manipulations of the social environment such as grouping and isolation constitute stressful experiences for adult mice (Cabib et al. 2002b; Avitsur et al. 2003) and present two main advantages. They have been shown to influence psychostimulant effects in rodents (Michel and Tirelli 2002; Lu et al. 2003) and are relatively simple to perform. During the 2.5 week period of group housing in our experiment, no weight loss or any physical injury was observed in either strain and recordings of behavior for 15 min after the weekly cage change did not reveal any fighting behavior. So we did not see evidence of a strong dominance hierarchy, which is an important observation, since social status (dominant and subordinate) is a factor that can impact on drug SA behavior (Morgan et al. 2002; Covington and Miczek 2005).

Since mice are territorial animals, group housing may constitute a stressful situation because of the permanent confrontation to potential rivals. However, it was shown that group housing of mice in small groups of three to five individuals prevents aggressiveness and does not induce changes in fecal corticosterone level (Van Loo et al. 2001; Hunt and Hambly 2006). But rather than the habituated group housing condition *per se*, the change from individual to group housing and back to individual housing again might have constituted a stressful experience. We did not perform extensive 'stress'-measures during group housing and thus cannot conclude about the stressfulness of the experience for the mice.

However, we did perform an elevated plus maze at 2 weeks of group housing, to test for anxiety. It is difficult to say anything conclusive about anxiety behavior of our animals in this test, since an important number of DBA mice fell off the maze when entering an open arm. But interestingly, DBA-*I* mice fell off significantly more frequently than DBA-*exG*. This might suggest that DBA mice do not support individual housing very well, and become more emotionally reactive. This would also fit with the results we found on reactivity to a novel environment *(see figure 6.3)*. Others have found that social isolation in the DBA strain increases behavioral despair in the FST (Cabib et al. 2002b) and increases aggressive responses, which was not seen in the C57 strain (Simler et al. 1982).

Indeed, social isolation can have an important effect on behavior, but has been shown to be highly dependant on the age of onset. For example, isolation during the period just after weaning (termed 'isolation rearing') potentiates locomotor activity induced by psychostimulant drugs, while similar experiences during adulthood ('isolation housing') do not (Hall 1998).

Studies on the effect of isolation housing (without isolation rearing) on drug SA are rare. Isolation housing has been found to have a modest effect on the rate of initiation of heroin, but not cocaine SA in rats (Bozarth et al. 1989). Interestingly, the effect of isolation housing on alcohol intake was influenced by gene-environment interactions. Isolation housing increased limited access drinking only in high drinking alcohol-preferring rats (Ehlers et al. 2007). We show here for the first time that the influence of changes in social housing on cocaine SA is strain dependant.

6.3.2 Strain differences in the impact of a short period of group housing

We showed that a short group housing experience did not affect cocaine SA behavior in mice with a C57 background, whereas it increased cocaine SA in mice with a DBA background. These results reveal the implication of gene-environment interactions in the vulnerability to cocaine reinforcing effects. These interactions do not involve alterations in cocaine metabolism since both strains show comparable brain cocaine levels whatever the experimental condition. Results of this study are thoroughly discussed in chapter 4. In conclusion, our results demonstrate that gene-environment interactions can affect cocaine SA. They support the idea that strain differences between C57 and DBA are not immutable but may depend on environmental conditions and past experiences. Moreover, our results stress the importance of housing conditions when studying strain differences.

The late life group-housing experiment was performed first, although in this thesis, the results are described in chapter 4, following the results of the early life experiment in chapters 2 and 3. The results obtained in this late life experiment strengthened the idea that the DBA and C57 mice are differentially affected by environmental life events in their drug-related behaviors and specifically in cocaine SA behavior. Moreover, in this study we showed that DBA mice can reliably self-administer cocaine, which is not easy to establish according to the literature (see paragraph 6.2.1) and they even show a nice U-shaped dose-response curve after an environmental experience. These observations were important for us to continue with these two strains and to launch the maternal environment model, that was, from a physical and material point of view, a much heavier enterprise.

6.4 The impact of early life experiences on cocaine SA

6.4.1 Strain differences in the impact of different maternal environments

Numerous studies, especially in rats, report on the influence of manipulations (handling, maternal separation or maternal deprivation) during the early postnatal life on adult behavior. Unfortunately, these studies are only rarely comparable, since a lot of different experimental protocols exist with variations in time point and duration of the postnatal experience and differences in control groups used (see also paragraph 1.3.2 of the introduction). In addition, quite some 'human manipulation' is required in these studies which might introduce an extra 'variability' factor that can influence results. Interestingly, changes in maternal behavior were shown to account for a large part of the results in these studies and this is why we decided to expose both the DBA and C57 strain to opposing maternal care environments. These environments were created by cross-fostering pups with either AKR or C3H mothers, two strains clearly showing a different style of maternal behavior. Using this design, we showed that the impact of early life experiences on cocaine taking behavior in adult mice is dependent on genotype. We found that changes in maternal environment that did not affect cocaine SA in mice with a C57 background, persistently altered cocaine SA in mice with a DBA background. Thus, the findings with this early life paradigm showed the implication of gene-early environment interactions in the vulnerability to cocaine reinforcing effects.

6.4.2 Generalization of effects: An indication of anhedonia

Next to differences in drug-intake, we were interested whether there would be other indications for a vulnerable endophenotype in DBA mice. As described in chapter 3, maternal environment did not influence anxiety or locomotor activity as measured in the EPM. Moreover, initial locomotor activity to a novel environment *(see figure 6.3)* was unchanged in either strain. So neither differences in anxiety nor in activity level seems to account for the differences seen in drug intake. However, maternal environment was shown to influence 'depressive'-like behavior as measured in the FST: DBA-*AKR* mice showed less cocaine intake and more 'floating' behavior in the FST compared to DBA-*C3H* mice. This could point in the direction of an anhedonic phenotype.

Anhedonia (loss of interest or pleasure) is a core symptom of the DSM-IV diagnosis of major depression. The FST has some construct validity as an animal model for depression: floating behavior (and thus not trying to escape) in this test is believed to represent a depressive like 'behavioral despair'. However, further characterization of depressive-like behavior is needed to support the idea that DBA-*AKR* mice present a predisposition to depressive-like behavior induced by gene-environment interactions. This characterization could include testing for sucrose preference, immobility in the tail suspension test or changes in the rewarding value of electrical brain stimulation (as measured by intracranial self stimulation: ICSS).

In tests measuring depression-related behavior, a careful interpretation of results is necessary and preferentially several tests should be performed to confirm a depressive-

like phenotype. Indeed, in the FST, decreased immobility behavior might be related to increased anxiety (Ramboz et al. 1998; Parks et al. 1998), which does not seem to be the case in this study since no differences in anxiety were found on the EPM. Decreases in sucrose consumption or preference have been advocated as a behavioral measure that may be associated with anhedonia (Willner 1997), but caution should be taken not to measure metabolic changes (Forbes et al. 1996).

The tail suspension test shares a common theoretical basis and behavioral measure with the FST (Steru et al. 1985). In this test, mice are suspended by the tail, which is attached to a horizontal bar using adhesive tape. Typically, mice immediately engage in several 'agitation'- or 'escape'-like behaviors, followed by increasing bouts of immobility ('behavioral despair'). These immobility bouts are classically decreased by antidepressant treatments. However, although appropriate for DBA mice, it might be less for C57 mice, since they have a tendency to grasp their tails with their front paws and climb up to the horizontal bar (Mayorga and Lucki 2001). This limits the use of this measure in our experimental set-up. Finally, reductions in the rewarding value of electrical brain stimulation of the mesocorticolimbic system may well simulate the anhedonia of human depression (Zacharko and Anisman 1991). Interestingly, it was found that uncontrollable foot shock induced reduced rates of responding for mesocortical brain stimulation in DBA, but not in C57 mice (Zacharko et al. 1990). This would thus be a very interesting anhedonia-related measure to apply in future experiments with our model.

Not much is known about the reinforcing and rewarding effects of psychostimulants in individuals predisposed to depression. It is believed that major depression leads to less sensitivity to reward-related cues, and that dysfunction of the brain reward system and more specifically the mesolimbic dopamine brain circuit, contributes to this anhedonic state (Markou et al. 1998; Naranjo et al. 2001).

The comorbidity often seen between depression and drug abuse is frequently explained in terms of self-medication. This explication is based on the hypothesis that depressed individuals self-administer rewarding drugs to alleviate symptoms, particularly anhedonia (Khantzian 1985). However, the causal links between depression and cocaine use are controversial. It needs to be mentioned that cocaine abuse would rather be associated with bipolar depression rather than with unipolar depression (Weiss 2004). Moreover, in comorbid patients, depression could rather be a consequence than a cause of cocaine abuse (Khantzian 1997). Indeed, depressive symptoms are frequently mentioned as a consequence of drug use or withdrawal (Gawin 1991; Markou and Koob 1992).

Recently, acute administration of a low dose of psychostimulant was used to probe the brain reward system function in major depressive disorder (Tremblay et al. 2002; Tremblay et al. 2005). Severely depressed patients showed a hypersensitivity to the rewarding effects (reported as euphoria) of dextroamphetamine, which can be interpreted as reflecting a hypofunctional state of the brain reward system. However, this hypersensitivity to the rewarding effects was not seen in moderately depressed patients (de Wit et al. 1987; Tremblay et al. 2002), showing that a different severity and type of depression can induce different states. Very low doses of dextroampetamine were used in these studies, which leaves a question on the reaction of depressed patients to moderate or high doses of the drug.

Our observation of an opposite relation between a depression-related behavior and cocaine intake is in accordance with other rodent studies. A decreased sensitivity to reward was found in animals in which a depressive-like behavior was induced either by exposure to chronic mild stress (CMS) (Willner 2005) or olfactory-bulbectomy (OBX) (Willner and Mitchell 2002). The CMS model of depression involves a relatively continuous exposure to a variety of unpredictable mild stressors. In rats, CMS induces an increased immobility in the FST associated with a decrease in reward sensitivity as measured by increased ICSS threshold, decreased sucrose consumption, decreased preference for alcohol, decreased sexual behavior and decreased amphetamine and morphine rewarding effects (Willner 2005). Indeed, after CMS place preference was abolished for morphine at either a 0.7 mg/kg (Papp et al. 1992) or 1.25 mg/kg dose (Valverde et al. 1997) and for amphetamine at 0.5 and 1.0 mg/kg dose (Papp et al. 1991). Similarly, OBX, that exhibits a high degree of neurochemical similarity to depression, induces a decreased sensitivity to reward as shown by a decreased sexual behavior (Lumia et al. 1992), an increased ICSS threshold (Slattery et al. 2007) and a reduced cocaine place preference (Calcagnetti et al. 1996). Although these animals show a greater locomotor response to cocaine (Chambers et al. 2004), the threshold lowering properties of a cocaine administration before ICSS was not different compared to sham operated rats (Slattery et al. 2007), pointing to an unchanged rewarding potency of cocaine. In a first study on drug SA in these animals, OBX rats showed a higher intake in acquisition of a low dose of amphetamine (0.10 mg/kg/inf) compared to sham-operated rats (Holmes et al. 2002). However, at a moderate acquisition dose (0.25 mg/kg/inf), the responding was lower in the OBX rats. This would be in agreement with our study, insofar that we used moderate doses of cocaine in acquisition and lower intake was seen in the group of animals that showed more 'behavioral despair' behavior in the FST.

6.4.3 Anhedonia-related behavior as an endophenotype

Despite major differences in brain anatomy between rodents and humans, there are evolutionary conserved circuits between species, which underlie certain physiological and behavioral responses, like reward, anxiety and fear related behaviors (Cryan and Holmes 2005). Thus, we can study these responses in rodents to elucidate behaviors and the underlying neural circuits and genetic factors, which can contribute to the understanding of human behavior and disease. More recently, it has become clear that a useful strategy might be to model single endophenotypic differences (i.e. one clearly defined behavioral output) relevant to the disease state as opposed to a syndrome (Cryan and Mombereau 2004; Cryan and Holmes 2005; Gould and Gottesman 2006). This 'endophenotype' can be neurophysiological, biochemical, endocrine, neuroanatomical, cognitive or neuropsychological (Gottesman and Gould 2003). As discrete phenotypical features, endophenotypes represent tractable entities to model in rodents and they are assumed to have simpler genetic underpinnings than the disorders themselves. Therefore, it is hypothesized that it will be easier to identify genes associated with endophenotypes than genes associated with their correlated disorders. The outcome of our early environment manipulation in the DBA and C57 mice might be promising as an endophenotype to study the role of gene-environment interactions in anhedonia-related behavior.

6.5 An early and late life model of gene-environment interaction

6.5.1 Implications of environmentally induced differences in cocaine SA

What could this environmentally induced difference in cocaine SA that we saw in DBA mice in both experiments imply? It is clear that cocaine is less reinforcing for DBA-*I* and DBA-*AKR* animals. But do these mice also differ in motivation for the drug (as classically measured using a progressive ratio protocol) and would they react differently in drug or cue (conditioned stimulus) induced relapse? Due to technical limitations inherent to mice studies on intravenous SA, we could perform acquisition (taking 10 days) and a dose-response protocol (taking 25 days for the group-housing and 21 days for the maternal environment experiment) in our mice. After these experiments, there were too few animals with a functional catheter left to test for possible differences in motivation and relapse.

We prioritized the dose-response protocol, since in outbred rats, individual differences in drug self-administration are thought to originate from a vertical shift in the dose-response function over all (low and high) doses (Piazza et al. 2000). Vulnerable subjects, the upward shifted ones, would then have a higher chance to develop drug abuse also when high drug doses are available. Comparing experimental groups, we did not obtain an equally important upward shift in the dose-response function. In the first place, the individually housed DBA mice in the late life experiment that acquired at 1 mg/kg/infusion showed a quite flat dose response function, which is not normally seen in rats. Anxiogenic properties of the drug at higher doses seems more important in (DBA) mice than in rats, which makes interpreting the upward shift more difficult. Moreover, in the early life experiment we rather observed a downward shift for the DBA-*AKR* animals.

It is thus difficult to conclude on the relevance of the observed differences in the light of vulnerability to drugs of abuse and the development of addictive-like behavior. The experiments described in this thesis clearly demonstrate a genotype dependent effect of environmental challenges on cocaine intake, and it would now be very interesting to test for changes in the rewarding value of the drug and in drug- and cue- induced relapse.

6.5.2 A comparison with other models

Several investigators have started to use rodent models of gene-environment interaction in anxiety-, depression- and drug dependence-related behaviors, each with a different approach. Some of these models, touching aspects similar to the models used in this thesis are described in some more detail below in comparison with our approach.

a) The HR/LR rat model

As mentioned before, the outbred high-responder (HR), low-responder (LR) rats were classified based on their levels of exploratory locomotion, which has been shown to be predictive of a range of drug-related behaviors. In addition, HR rats show decreased anxiety-like behavior as compared to LR animals in several tests. The HR/LR phenotype has thus been suggested to reflect a basic difference in novelty seeking and emotional reactivity to the

environment. These behavioral differences are accompanied by basal differences in gene expression of key molecules in the emotional circuitry of the HR *vs.* LR rats (for review Kabbaj et al. 2004). Basal hippocampal expression of glucocorticoid receptors was significantly higher in LRs, an increase that was suggested to be partly responsible for the higher anxiety behavior exhibited by these rats (Kabbaj et al. 2000) with possible consequences for cocaine SA (Deroche-Gamonet et al. 2003). HR and LR rats also differ in terms of expression of the hippocampal serotonergic receptor 5HT1a (receptors that are shown to be dysregulated in depression), and several other genes including CRH in the amygdala (higher in LR, consistent with higher anxiety) and adrenergic receptor subtypes in the BNST. In addition, decreased dopamine D2 receptor binding was shown in the NAcc of HR rats.

However, the distinct difference in behavioral profile of HR and LR tends to disappear after a psychosocial stressor (social defeat). It was suggested that the HR-LR phenotype represents two different pathways to substance use. The HRs might seek drugs because of their high propensity for novelty seeking and the LR might seek drugs as a reaction to stress (Kabbaj et al. 2004). In the search for novel hippocampal genes that might contribute to individual differences in emotional responses in HR and LR rats either basally or following a social stressor, Kabbaj et al. (2004) followed an approach similar to our approach described in chapter 5 and analyzed individual differences using Affimetrix gene chips. They show that HRs and LRs show differential expression of several genes across multiple functional classes. Moreover, social defeat led to differential alterations of gene expression in HRs *vs.* LRs. The relevance of these candidate genes in establishing differences in emotionality and vulnerability to substance abuse is still to be identified. But in a model using outbred animals like the HR/LR, a large number of genes will be found to be differentially expressed, most of which will not be directly implicated in the sensitivity to the environment or the vulnerability to drug intake. In this respect, our approach with inbred mice encountering different environments that impact differently on the mice can be interesting, since (1) the number of differentially expressed genes is reduced and (2) these altered genes more likely are implicated in regulating susceptibility or resilience to the impact of environmental events.

Recently, the HR and LRs were selectively bred and the locomotor response to novelty was shown to be clearly heritable (Stead et al. 2006). In addition, the 8th generation of both lines retained strong differences in spontaneous anxiety related behavior. Drug-related behavior in these lines has not been tested yet. In future studies with these selected lines, the candidate gene approach could be used to identify genetic variants that underlie the observed differences. In addition, these lines will enable to study differences that arise during early development and that may underlie the behavioral differences between HR and LR animals observed in adulthood.

b) The HG/LG rat model

Like the high and low responder rats, high grooming (HG) and low grooming (LG) rats were selected from a population of Wistar rats based on the time spent in self-grooming on the EPM (Homberg et al. 2002). Self-grooming by rodents under stressful conditions has been considered as a 'dearousal' activity, and might therefore reflect a strategy to cope with anxiety or stress. Self grooming would share neurocircuitry with reward related pro-

cesses, since it is mediated by nigrostriatal and tegmento-accumbens dopaminergic systems (Spruijt et al. 1986). During intravenous SA of cocaine, HG rats reached considerably higher breakpoints than LG rats but showed no differences in acquisition rate and dose-response relationships. It seems that stress-induced self-grooming specifically predicts enhanced motivation to self-administer cocaine rather than sensitivity to its reinforcing effects (Homberg et al. 2002). In vitro studies have shown several neurochemical differences between the two ratlines in reward circuitry, both in naïve state and after ten days of either cocaine or saline SA. Interestingly, naive HG rats were shown to exhibit a decreased reactivity of dopaminergic nerve terminals in the medial prefrontal cortex (mPFC), which persisted after the SA procedure (Homberg et al. 2003). Dopamine in the mPFC has been suggested to 'dampen' the effects of aversive as well as rewarding stimuli on subcortical dopamine systems, exerting an inhibitory control (Tzschentke 2001). Loss of this inhibitory control might contribute to an exacerbation of compulsive drug taking behavior or other psychiatric symptoms including impulsivity (Jentsch and Taylor 1999) and depression (Brody et al. 2001). In the NAcc, the activity of the dopamine terminals was diminished in HG rats after exposure to cocaine or saline SA (Homberg et al. 2003). A reduction of tonic dopamine transmission in striatal areas has been implicated in the motivational disturbances (anhedonia) of abstinence in dependent subjects, as well as in the individual vulnerability to drug addiction (Melis et al. 2005).

While several classes of anxiolytic drugs decreased the self-grooming behavior in HG rats, only serotonergic anxiolytics were shown to abolish the pre-existing individual differences in cocaine SA between HG and LG rats (Homberg et al. 2004). So the serotonergic system seems to be involved in the individual difference in motivation to self-administer cocaine in these rats. Moreover, naïve HG and LG rats were shown to differ in the reactivity of serotonergic neurons in the substantia nigra, mPFC and NAcc. These are very interesting findings, but since outbred rats are used, this model would not be very suitable for the candidate gene approach.

Next to higher self-grooming, EPM exposure elicited higher anxiety levels and enhanced plasma corticosterone secretion in HG rats. This reminds of the selected ratlines created by Landgraf and colleagues in the early '90, the high anxiety behavior (HAB) and low anxiety behavior (LAB) rats. These lines were selected on anxiety behavior (time spent on and entries into the open arms) in the EPM (for review Landgraf et al. 2007). Unfortunately, self-grooming on the EPM was (to my knowledge) never measured in these lines. HAB animals, in addition to being extremely anxious, adopt passive and depression-like behaviors including increased floating in the FST and freezing during social defeat. This model is proposed to represent an endophenotype of anxiety and comorbid depression. In both HAB/LAB rats and mice, the behavioral phenotype has been found to be significantly correlated with the expression of AVP at the level of the hypothalamic paraventricular nucleus (PVN), both under basal conditions and upon stressor exposure (Wigger et al. 2004). Prenatal stress has been shown to influence AVP expression in the PVN and alter the phenotype of both HAB and LAB rats in adulthood (Bosch et al. 2006). In addition, antidepressant treatment lowered anxiety behavior and the hyperdrive of AVP in the HAB animals (Keck et al. 2003). Until now, these rats have not been tested on SA of addictive compounds. It would be interesting to see whether cocaine intake in these lines would yield

results similar to those obtained with the HG/LG animals. These lines could then serve as an interesting model to study the genetic variants underlying depressive behavior and the link to drug intake.

c) The APO-SUS/APO-UNSUS rat model

Recently, a selectively bred rat line was proposed as a model for addiction: the apomorphine susceptible (APO-SUS) and unsusceptible (APO-UNSUS) rats (Ellenbroek et al. 2005). These rats (Wistar) were originally classified on the difference in gnawing behavior after a single injection of the dopamine D1/D2 receptor agonist apomorphine (Cools et al. 1990). Subsequent selective breeding has resulted in two distinct rat lines that "differ in the determinants that are suggested to be predictive of the liability to self-administer cocaine". Important differences in the reactivity of the dopamine system and the HPA axis were found. However, experimental proof of strong differences in psychostimulant SA behavior in these two lines is very limited, since only the acquisition of cocaine SA was tested and only at a single dose (van der Kam et al. 2005). Moreover, a 'shaping' procedure was necessary for the animals to acquire cocaine SA. Free choice acquisition at a moderate dose for 1 week yields very low levels of intake not differing between both lines, it is only in the shaping phase of acquisition, where the animals are placed with their nose into the active nose-poke hole and are given one forced infusion at the beginning of each session, that the APO-UNSUS rat starts acquisition. The APO-SUS rat needs an extra 'stressor' (lights off during the acquisition phase) during the shaping phase to acquire SA.

On the basis of these results the authors conclude that gene-environment interactions determine the individual variability in cocaine SA (van der Kam et al. 2005). Clearly, some more characterization of cocaine SA behavior is needed for this model to be credible as a model for addiction or even drug intake. Moreover, testing different acquisition doses might eliminate the necessity to use shaping and eliminate a confounding experimental procedure like forcing the animal's nose in the active hole to obtain a cocaine dose. Moreover, since the 'stressor' (lights off) was applied during the SA session and is a part of the experimental conditions of drug intake, this model does not reveal whether previous exposure to stress can differentially predispose individuals to a changed drug intake. Until now, this model is not exploited yet to study the mechanisms underlying the differential impact of environmental life stressors on vulnerability to drug intake behavior.

d) Comparing C57BL/6 and DBA/2 mice

Several studies have demonstrated a differential impact of environmental stressors on drug-related behaviors in C57 and DBA mice and served as a basis for our experimental models.

A chronic restraint stressor (2 hours daily for 10 consecutive days) was shown to induce sensitization to the behavioral (locomotor) effects of amphetamine in DBA, but not in C57 mice. This difference was seen regardless of the mice being naïve or habituated to the test cage (Badiani et al. 1992). A different stressor, 9 days of food-restriction, was shown to induce the same strain differences in sensitization to amphetamine, measured 24h after free-feeding (Cabib and Bonaventura 1997). A procedural particularity in this latter study was the fact that individually housed food-restricted animals were compared

to group housed free-fed control animals. So the nature of the stressor (food-restriction or isolation housing or a combination of both) inducing the differences cannot be clearly distinguished.

As already mentioned, some years later, Cabib et al. (2000), using these inbred strains, nicely showed a gene-environment interaction in rewarding behavior of amphetamine as measured in a conditioned place preference (CPP) test. In this study, the initial aversive response of DBA mice to amphetamine in a CCP task was reversed to a preference after having experienced a short period (12 days) of food restriction. In contrast, C57 mice kept their initial preference for the amphetamine coupled environment. Interestingly, animals were only tested on CPP once they had regained their initial bodyweight. So, not the reduced bodyweight *per se*, but the impact of a past environmental experience was shown to be important on drug reward in DBA mice.

In a second study, based on these results, Schroff et al. (2004) used the same protocol to study alcohol preference in these two mice strains. They showed that C57, but not DBA heighten their ethanol intake a week after a short period of food shortage. On the other hand, DBA, but not C57 mice were affected in alcohol locomotor stimulating behavior by this experience. Unfortunately only one alcohol dose (10%) was used in this study to test for preference. Considering intake and preference levels, this appears to represent a relatively high dose for DBA mice.

Overall however, these studies show an important strain dependent impact of a past environmental experience on drug-related behaviors and were a great inspiration for our models. Manifestly, drug SA represents the most obvious and face-relevant model of drug use and abuse. So in our studies we assessed the influence of a past stressor on cocaine SA in these inbred strains. As explained in paragraph 6.3.1 we had some hesitations to the use of the food-restriction model and thus chose to use a past period of group housing as an adult environmental experience. In addition, we studied the impact of a change in early postnatal environment in these strains on IV cocaine SA, and thus showed that the impact of several environmental conditions on cocaine SA is strain dependent.

e) Modeling early life influences on behavior in inbred mice

Recently, an attempt to model the influence of early life stress on anxiety- and depression-related behaviors in mice was made by studying the effects of maternal separation (3h from PD0 to PD13) and handling in eight different inbred strains, including C57 and DBA (Millstein and Holmes 2007). As adults, mice were tested on behavior in the open field, EPM, light/dark exploration test and FST. Although this is an interesting approach, showing resemblance with our postnatal maternal behavior model, the results demonstrated no clear and consistent effects. The authors conclude that the maternal separation procedure employed does not provide a robust model of early life stress effects on the anxiety-and depression-related behaviors in the mouse strains tested. We confirm that a change in early life environment does not induce behavioral changes in C57 mice in the EPM and FST and neither in cocaine SA. Our early life experimental procedure did however influence behavior in the DBA mice. The fact that we did find an influence of our relatively 'mild' postnatal manipulation on drug taking behavior of DBA mice and show an indication of a changed motivation in the FST, might be due to the permanent character of the changes we induced

in the maternal environment to which DBA mice appear sensitive. Moreover, the pharmacological effects of cocaine intake might have triggered changes in (a predisposed) reward system, influencing subsequent intake. This would imply that (our) postnatal manipulations induce subtle changes that are revealed in adulthood only after a triggering event, like a severe or chronic stressor or drug intake, in individuals with a vulnerable phenotype. This would also explain the lack of differences we found in EPM, which is in accordance with the Millstein and Holmes study.

In clear contrast with the results in C57 mice, are studies that do show an influence of maternal separation (3h from PD1 to PD14) on anxiety in this strain as measured in the open field, EPM and novel object exploration (Romeo et al. 2003; Veenema et al. 2007). So, even when procedures are similar (like the maternal separation protocol), results on the impact of changes in early life environment on C57 mice on certain aspects of behavior are not consistent over studies. In addition, in C57 mice, maternal separation and/or early handling were shown to influence corticosterone response to an acoustic stressor (Parfitt et al. 2004), change male and maternal aggression (Veenema et al. 2007) and influence sensitivity to physical pathologies like colitis (Varghese et al. 2006), influenza infection (Avitsur et al. 2006) and stroke (Craft et al. 2006). So C57 mice do not seem to be resistant in all aspects of behavior to the impact of different early life environmental changes.

Interestingly, although not an inbred study, maternal deprivation (a single 24h episode on PD12) in the outbred CD-1 mice reduced interest in socio-sexual interaction with peers during adolescence, but did not influence floating time in the FST in adulthood (Macri and Laviola 2004).

Overall, it seems difficult to establish a mouse model of early life influence on adult 'pathology'-like behavior and results in mice are far less consequent than in rats. Unfortunately, a lot of different experimental protocols with varying time and conditions exist that are all described as 'maternal separation' or 'handling', but they might have very different impacts. Interesting to mention in this respect is a study on C57 mice that showed a differential impact of a maternal separation procedure, depending on the time of day of separation (first *vs.* second part of the light phase) (Parfitt et al. 2007). Another great drawback of maternal separation and handling studies is that a lot of 'human manipulation' is required that may lead to additional differences in protocol, even if described procedures are similar. We avoid these problems and propose a mouse model showing clear gene-environment interactions in cocaine intake and a depression-related behavior using a relatively mild early life experimental procedure, without continuous human intervention.

6.6 Possible mechanisms

Which mechanism could underlie the strain dependent susceptibility to environmental influences? Compared to DBA mice, C57 mice show a hyperactive phenotype (Cabib et al. 2002a), passive responses to uncontrollable stressors (Cabib et al. 2002b) and these mice readily self-administer drugs of abuse (Crawley et al. 1997). DBA mice however have shown to be more influenced by environmental stressors especially in psychostimulant-

related behavior (Badiani et al. 1992; Cabib and Bonaventura 1997; Cabib et al. 2000). In this thesis results were shown that confirm such a strain difference in the effect of early and late environmental experiences on adult cocaine SA. Understanding the biological mechanisms underlying this strain difference can represent an important step towards the understanding of individual differences in drug taking behavior and might reveal pathways implicated in the development of psychopathology. Glucocorticoid hormones and the mesocorticolimbic dopaminergic neurons could be important factors in the mechanism underlying these strain differences. They have been proposed as two key biological mediators of the increased vulnerability to psychostimulants induced by stress (for review Piazza and Le Moal 1996; Marinelli and Piazza 2002; Goeders 2002; de Jong and de Kloet 2004).

C57 and DBA differ in the activity of the dopaminergic mesocorticolimbic neurons both in basal conditions and in response to psychostimulants and stressors (Puglisi-Allegra and Cabib 1997; Cabib et al. 2002b; Ventura et al. 2004; McNamara et al. 2006). C57 mice show a facilitated mesoaccumbens DA transmission compared to DBA. An increased density of D2 autoreceptors located on VTA neurons was found in DBA mice compared to C57, together with a decreased density of D2 postsynaptic receptors in the NAcc. This higher ratio of DA autoreceptors/postsynaptic receptors was also found in the nigrostriatal system (Puglisi-Allegra and Cabib 1997). Activation of D2 autoreceptors inhibits impulse flow, synthesis, and release rates of DA neurons, while activation of the postsynaptic D2 receptors leads to the expression of classical DA-dependent responses such as hyperlocomotion and stereotypies. As would be predicted from their higher number of D2 autoreceptors, DBA compared to C57 mice show reduced DA synthesis and release within the mesoaccumbens DA system when challenged with DA direct agonists. This different balance of pre- and postsynaptic DA receptors might play a role in the higher liability to SA drugs of abuse in C57 mice compared to DBA mice (for review Cabib et al. 2002a).

In addition, mice of the C57 and DBA strains undergo opposite alterations of mesocorticolimbic DA functioning in responses to stressors. DBA mice are by far more susceptible than C57s to stress-induced enhanced mesoaccumbens DA release and in stressful situations, they show sustained active behavioral responses while C57 adopt extremely passive responses, as demonstrated by more behavioral despair in the FST (Cabib et al. 2002a). This passive response of C57 mice is thought to be related to a rapid onset of stress-induced inhibition of mesoaccumbens DA release, that is accompanied by a very fast and strong activation of mesocortical DA metabolism in C57 mice (Ventura et al. 2001). Indeed, there exists a strong relationship between susceptibility toward helplessness and mesocortical DA control over subcortical DA transmission in C57 mice (Cabib et al. 2002a). It is argued that the inhibitory mesoaccumbens DA response to stress is due to coping failure following an uncontrollable stressor (Cabib and Puglisi-Allegra 1994). In chapter 3, it was shown that DBA mice raised by an AKR mother show behavioral despair in the FST that is comparable with levels of the C57 mice. This could possibly be related with an impaired coping in these mice accompanied by an inhibition of mesoaccumbens DA release. DA functioning in DBA-*AKR* mice however, is not likely to resemble that of C57 mice in other aspects, since we have shown that cocaine intake is low in these animals.

Food-restriction (9days) promoted stereotyped cage cover climbing behavior and a long-lasting sensitization to the locomotor effects of systemic amphetamine in DBA mice,

while it induced neither of these behaviors in C57 mice (Cabib and Bonaventura 1997). In a following study on the effects of different living conditions in DBA mice, a period of food restriction (13 days of food restriction followed by 48 hours of food *ad libitum*) resulted in a reduced immobility in the FST and a reduced mesocortical and enhanced mesoaccumbens DA response to stress (Cabib et al. 2002b). A comparable imbalance that favors mesoaccumbens DA response to stress challenge was observed in rats exposed to repeated cocaine (Sorg and Kalivas 1991; 1993) in a classical behavioral sensitization procedure. This is in line with the observation that food restriction can promote behavioral sensitization. In the same study, individual housing (15 days), a condition that does not induce behavioral sensitization, was shown to enhance behavioral despair in the FST and to promote a mesocorticolimbic imbalance that favors cortical over subcortical DA response to stress compared to group housed animals (Cabib et al. 2002b). Such an imbalance could have played a role in the lower cocaine SA we observed in the isolated DBA mice compared to the DBA that underwent the grouping experience. Following this reasoning, the group housing experience can then be considered to have counteracted the imbalance caused by isolation housing.

Strain differences in stress- or drug- induced corticosterone levels might also play a role in the different vulnerability of both strains to environmental stressors. We observed a higher corticosterone response in C57 compared to DBA during exposure to a novel environment (data not shown). Such a difference in responding to a change in environment was also found by others, (Cabib et al. 1990; de Jong et al., personal communication), while exposure to a more severe food shock stressor resulted in a prolonged corticosterone response in DBA compared to C57 mice (Shanks et al. 1990). Acute restraint did not result in different corticosterone levels in DBA and C57 mice (Jones et al. 1998). C57 and DBA mice were also found to differ in HPA axis responses to cocaine. Cocaine injections induced an increase in plasma corticosterone levels in DBA, while it rather attenuated corticosterone response in C57 mice (de Jong et al. 2007). Interestingly, the development of behavioral sensitization to cocaine was shown to be dependent on corticosterone secretion in DBA but not in C57 mice (de Jong et al. 2007), suggesting that corticosterone might play a more important role in DBA than in C57 mice in the behavioral responses to cocaine. Thus, it can be hypothesised that lasting changes in HPA axis responses following early life stress or an adult environmental experience influences behavioral responses to cocaine to a greater extent in DBA than in C57 mice.

Considering the data described in chapter 5, AVP expression in the extended amygdala might play a role in the strain differences observed in the vulnerability to environmental stressors. A differential expression of AVP was found in DBA mice in both the early life and late life experiments, while no differences in expression were found in C57 mice. A higher expression of AVP was found in the DBA groups showing a lower cocaine intake. This finding is in line with studies showing that AVP can induce a reduction in drug intake (Van Ree et al. 1988; De Vry et al. 1988, see chapter 5). Where precisely in the brain AVP exerts its drug-related actions is not known. AVP produced in the PVN regulates, in concert with CRH, HPA-axis activity. Evidence has been accumulated in neuroendocrine studies that in depression as well as in chronic stress, there is a gradual shift from CRH- to a more AVP-controlled HPA axis activity (de Goeij et al. 1992; Volpi et al. 2004; Dinan

and Scott 2005). Strain differences were found in AVP expression in the magnocellular and parvocellular neurons of the PVN, the DBA mice having a considerably higher expression compared to C57 mice (de Jong et al., personal communication). This might implicate that also in this structure environmental experiences can differentially affect AVP expression. Thus, the differences we found in expression of AVP in the extended amygdala of DBA mice might implicate more widespread changes in stress induced expression of AVP in the brain.

Neurochemical and electrophysiological studies suggest that norepinephrine, dopamine, serotonin, and glutamate are the neurotransmitters involved in the influence of AVP on brain function (for review de Wied et al. 1993). Although the limbic system (amygdala, hippocampus, septum and some thalamic areas) has shown to play an essential role in the effect of AVP on behavior and memory (Wimersma Greidanus et al. 1983), less is known about the exact mechanism underlying AVP influence on reward behavior. Ventral hippocampus and VTA have shown to be potential sites of action of the reward related effects of AVP (for review de Wied et al. 1993). Some suggestions can be forwarded trying to explain the mechanism underlying an effect of AVP on cocaine intake in the DBA strain, but remain hypothetical until further research.

The behavioral phenotype of high and low grooming (HG and LG) animals, has been found to signifcantly correlate with the expression of AVP at the level of the PVN (Landgraf et al. 2007, see also paragraph 6.5.2). Since serotonergic anxiolytics were shown to abolish the individual differences in drug intake between these HG and LG animals (Homberg et al. 2004), PVN AVP might act on the serotonin system to exert its drug related effects. But this evidence of an AVP link with the serotonin system is ofcourse indirect. Interestingly, strain differences were found in serotonergic functioning between DBA and C57 mice during development (Jazrawi and Horton 1989) and in response to isolation housing (Kempf et al. 1984). However, since we did not find differences in anxiety behavior on the EPM in DBA groups following the early environmental experiences, it remains very speculative to draw a parallel between these studies.

The ventral hippocampus (a projection area of the median amygdala AVP neurons) can be hypothesised to be implicated in the effects of AVP on drug intake. Actions of AVP on oxytocin receptors in this area could account for this implication. Oxytocin (OXY), a peptide structurally related to AVP, was equally shown to be implicated in drug related behaviors (for review Kovacs et al. 1998). Injections of OXY in nucleus accumbens or ventral hippocampus disrupted heroin self-administration (Ibragimov et al. 1987). The effect of intra-hippocampal injections lasted longer than the effect of intra-accumbens injections and was found to be prevented by an inhibitor of OXY receptors. Interestingly, AVP sensitive OXT receptors were found in the ventral hippocampus (Barberis and Audigier 1985; Elands et al. 1988). Our results would then be in accordance with these studies, since a higher expression of AVP mRNA in the median amygdala would theoretically mean a higher release of AVP in the ventral hippocampus, which could influence (decrease) cocaine self administration by its action on OXY receptors.

The ventral hippocampus is an area very sensitive to the behavioral actions of vasopressin (for review de Wied et al. 1993). Relative to its dorsal counterpart, the ventral hippocampus has greater output connections with the prefrontal cortex, bed nucleus of the

stria terminalis, and the amygdala (Henke 1990; Ishikawa and Nakamura 2006). Moreover, it has recently been shown to play a functional role in mediating relapse to cocaine (Rogers and See 2007). AVP acts as a neuromodulator on septal and hippocampal neurons (Urban 1987). It was found to facilitate the response to the excitatory neurotransmitter glutamate (Urban 1987) and was shown to potentiate norepinephrine signalling in both mice and rat hippocampus (Church 1983; Brinton and McEwen 1989).

In conclusion, AVP can have important neuromodulatory influences in the brain and could very well be the most proximal peptide mediating with corticosterone the impact of prolonged HPA activation on behavioral responses to cocaine. AVP can be modified by chronic stress and appears to be implicated in reward related behaviors. Our data support a role for AVP in expressing differential susceptibility to environmental experiences. A major challenge for research in substance abuse is therefore to find clues for the mechanism underlying the putative role of AVP and the HPA axis in the establishment of individual differences in drug intake.

6.7 General conclusion

Overall, the results reported in this thesis indicate that the influence of environmental conditions on cocaine reinforcing effects depends on the genetic background of the subjects. Either changes in postnatal maternal environment or a short group housing experience in adulthood did not affect cocaine SA in mice with a C57 background, but affected cocaine SA in mice with a DBA background. These results reveal the implication of gene-environment interactions in the vulnerability to cocaine's reinforcing effects. Further characterization of reward related behavior in the early life environment model could support the idea that a sphere of 'anhedonia' is touched by our manipulation. The differential expression of AVP in the extended amygdala of experimental DBA groups is a first biological measure defined in our models. Changes in AVP, in concert with changes in HPA-axis function and notably corticosterone can be hypothesized to be implicated in the mechanism underlying gene-environment interactions in drug intake. Finally, our models open the possibility to study the psychobiological mechanism that underlie susceptibility and resilience to the impact of environmental life events on cocaine-intake behavior.

References

Avitsur R, Hunzeker J, Sheridan JF (2006) Role of early stress in the individual differences in host response to viral infection. Brain Behav Immun 20: 339-348

Avitsur R, Stark JL, Dhabhar FS, Kramer KA, Sheridan JF (2003) Social experience alters the response to social stress in mice. Brain Behav Immun 17: 426-437

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

Barberis C, Audigier S (1985) Vasopressin and oxytocin receptors in the central nervous system of the rat. Ann Endocrinol (Paris) 46: 35-39

Bosch OJ, Kromer SA, Neumann ID (2006) Prenatal stress: opposite effects on anxiety and hypothalamic expression of vasopressin and corticotropin-releasing hormone in rats selectively bred for high and low anxiety. Eur J Neurosci 23: 541-551

Boyer CS, Petersen DR (1992) Pharmacokinetic analysis of the metabolism of cocaine to norcocaine and N-hydroxynorcocaine in mice. Drug Metab Dispos 20: 863-868

Bozarth MA, Murray A, Wise RA (1989) Influence of housing conditions on the acquisition of intravenous heroin and cocaine self-administration in rats. Pharmacol Biochem Behav 33: 903-907

Brinton RE, McEwen BS (1989) Vasopressin neuromodulation in the hippocampus. J Neurosci 9: 752-759

Brody AL, Barsom MW, Bota RG, Saxena S (2001) Prefrontal-subcortical and limbic circuit mediation of major depressive disorder. Semin Clin Neuropsychiatry 6: 102-112

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

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

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

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

Cabib S, Puglisi-Allegra S (1994) Opposite responses of mesolimbic dopamine system to controllable and uncontrollable aversive experiences. J Neurosci 14: 3333-3340

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

Cabib S, Ventura R, Puglisi-Allegra S (2002b) Opposite imbalances between mesocortical and mesoaccumbens dopamine responses to stress by the same genotype depending on living conditions. Behav Brain Res 129: 179-185

Calcagnetti DJ, Quatrella LA, Schechter MD (1996) Olfactory bulbectomy disrupts the expression of cocaine-induced conditioned place preference. Physiol Behav 59: 597-604

Carroll ME, France CP, Meisch RA (1979) Food deprivation increases oral and intravenous drug intake in rats. Science 205: 319-321

Carroll ME, Meisch RA (1984) Increased drug-reinforced behavior due to food deprivation. In: Thompson T, Dews PB, Barrett JE (eds) Advances in behavioral pharmacology. New York: Academic Press, pp 47-88

Chambers RA, Sheehan T, Taylor JR (2004) Locomotor sensitization to cocaine in rats with olfactory bulbectomy. Synapse 52: 167-175

Church AC (1983) Vasopressin potentiates the stimulation of cyclic AMP accumulation by norepinephrine. Peptides 4: 261-263

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

Covington HE, Miczek KA (2005) Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: dissociation from corticosterone activation. Psychopharmacology (Berl) 183: 331-340

Craft TK, Zhang N, Glasper ER, Hurn PD, Devries AC (2006) Neonatal factors influence adult stroke outcome. Psychoneuroendocrinology 31: 601-613

Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, Hitzemann RJ et al. (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology (Berl) 132: 107-124

Cryan JF, Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4: 775-790

Cryan JF, Mombereau C (2004) In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. Mol Psychiatry 9: 326-357

Dalm S, Enthoven L, Meijer OC, van der Mark MH, Karssen AM, de Kloet ER, Oitzl MS (2005) Age-related changes in hypothalamic-pituitary-adrenal axis activity of male C57BL/6J mice. Neuroendocrinology 81: 372-380

de Goeij DC, Dijkstra H, Tilders FJ (1992) Chronic psychosocial stress enhances vasopressin, but not corticotropin-releasing factor, in the external zone of the median eminence of male rats: relationship to subordinate status. Endocrinology 131: 847-853

de Jong IE, de Kloet ER (2004) Glucocorticoids and vulnerability to psychostimulant drugs: toward substrate and mechanism. Ann N Y Acad Sci 1018: 192-198

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

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

De Vry J, Donselaar I, Van Ree JM (1989) Food deprivation and acquisition of intravenous cocaine self-administration in rats: effect of naltrexone and haloperidol. J Pharmacol Exp Ther 251: 735-740

de Wied D, Diamant M, Fodor M (1993) Central nervous system effects of the neurohypophyseal hormones and related peptides. Front Neuroendocrinol 14: 251-302

de Wit H, Uhlenhuth EH, Johanson CE (1987) The reinforcing properties of amphetamine in overweight subjects and subjects with depression. Clin Pharmacol Ther 42: 127-136

Deroche-Gamonet V, Sillaber I, Aouizerate B, Izawa R, Jaber M, Ghozland S, Kellendonk C et al. (2003) The glucocorticoid receptor as a potential target to reduce cocaine abuse. J Neurosci 23: 4785-4790

Dinan TG, Scott LV (2005) Anatomy of melancholia: focus on hypothalamic-pituitary-adrenal axis overactivity and the role of vasopressin. J Anat 207: 259-264

Ehlers CL, Walker BM, Pian JP, Roth JL, Slawecki CJ (2007) Increased alcohol drinking in isolate-housed alcohol-preferring rats. Behav Neurosci 121: 111-119

Elands J, Barberis C, Jard S (1988) [3H]-[Thr4,Gly7]OT: a highly selective ligand for central and peripheral OT receptors. Am J Physiol 254: E31-E38

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

Forbes NF, Stewart CA, Matthews K, Reid IC (1996) Chronic mild stress and sucrose consumption: validity as a model of depression. Physiol Behav 60: 1481-1484

Gawin FH (1991) Cocaine addiction: psychology and neurophysiology. Science 251: 1580-1586

Goeders NE (2002) The HPA axis and cocaine reinforcement. Psychoneuroendocrinology 27: 13-33

Golden GT, Ferraro TN, Smith GG, Snyder RL, Jones NL, Berrettini WH (2001) Acute cocaine-induced seizures: differential sensitivity of six inbred mouse strains. Neuropsychopharmacology 24: 291-299

Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160: 636-645

Gould TD, Gottesman II (2006) Psychiatric endophenotypes and the development of valid animal models. Genes Brain Behav 5: 113-119

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

Hall FS (1998) Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. Crit Rev Neurobiol 12: 129-162

Henke PG (1990) Hippocampal pathway to the amygdala and stress ulcer development. Brain Res Bull 25: 691-695

Hofstetter JR, Svihla-Jones DA, Mayeda AR (2007) A QTL on mouse chromosome 12 for the genetic variance in free-running circadian period between inbred strains of mice. J Circadian Rhythms 5: 7 doi:10.1186/1740-3391-5-7

Holmes PV, Masini CV, Primeaux SD, Garrett JL, Zellner A, Stogner KS, Duncan AA et al. (2002) Intravenous self-administration of amphetamine is increased in a rat model of depression. Synapse 46: 4-10

Homberg JR, Arends B, Wardeh G, Raaso HS, Schoffelmeer AN, De Vries TJ (2004) Individual differences in the effects of serotonergic anxiolytic drugs on the motivation to self-administer cocaine. Neuroscience 128: 121-130

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

Homberg JR, Wardeh G, Raaso HS, Schoffelmeer AN, De Vries TJ (2003) Neuroadaptive changes in mesocorticolimbic dopamine and acetylcholine neurons following cocaine or saline self-administration are dependent on pre-existing individual differences. Neuroscience 121: 829-836

Hunt C, Hambly C (2006) Faecal corticosterone concentrations indicate that separately housed male mice are not more stressed than group housed males. Physiol Behav 87: 519-526

Ibragimov R, Kovacs GL, Szabo G, Telegdy G (1987) Microinjection of oxytocin into limbic-mesolimbic brain structures disrupts heroin self-administration behavior: a receptor-mediated event? Life Sci 41: 1265-1271

Ishikawa A, Nakamura S (2006) Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. J Neurophysiol 96: 2134-2138

Jazrawi SP, Horton RW (1989) 5-HT2 receptor binding and 5-HT uptake in mouse brain: developmental changes and the relationship to audiogenic seizure susceptibility in DBA/2J mice. Brain Res Dev Brain Res 45: 257-263

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

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

Kabbaj M (2006) Individual differences in vulnerability to drug abuse: the high responders/low responders model. CNS Neurol Disord Drug Targets 5: 513-520

Kabbaj M, Devine DP, Savage VR, Akil H (2000) Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. J Neurosci 20: 6983-6988

Kabbaj M, Evans S, Watson SJ, Akil H (2004) The search for the neurobiological basis of vulnerability to drug abuse: using microarrays to investigate the role of stress and individual differences. Neuropharmacology 47 Suppl 1: 111-122

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

Keck ME, Welt T, Muller MB, Uhr M, Ohl F, Wigger A, Toschi N et al. (2003) Reduction of hypothalamic vasopressinergic hyperdrive contributes to clinically relevant behavioral and neuroendocrine effects of chronic paroxetine treatment in a psychopathological rat model. Neuropsychopharmacology 28: 235-243

Kempf E, Puglisi-Allegra S, Cabib S, Schleef C, Mandel P (1984) Serotonin levels and turnover in different brain areas of isolated aggressive or non-aggressive strains of mice. Prog Neuropsychopharmacol Biol Psychiatry 8: 365-371

Khantzian EJ (1985) The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. Am J Psychiatry 142: 1259-1264

Khantzian EJ (1997) The self-medication hypothesis of substance use disorders: a reconsideration and recent applications. Harv Rev Psychiatry 4: 231-244

Kopp C, Vogel E, Rettori MC, Delagrange P, Guardiola-Lemaitre B, Misslin R (1998) Effects of a daylight cycle reversal on locomotor activity in several inbred strains of mice. Physiol Behav 63: 577-585

Kosten TA, Zhang XY, Haile CN (2007) Strain differences in maintenance of cocaine self-administration and their relationship to novelty activity responses. Behav Neurosci 121: 380-388

Kovacs GL, Sarnyai Z, Szabo G (1998) Oxytocin and addiction: a review. Psychoneuroendocrinology 23: 945-962

Landgraf R, Kessler MS, Bunck M, Murgatroyd C, Spengler D, Zimbelmann M, Nussbaumer M et al. (2007) Candidate genes of anxiety-related behavior in HAB/LAB rats and mice: focus on vasopressin and glyoxalase-I. Neurosci Biobehav Rev 31: 89-102

Lu L, Shepard JD, Hall FS, Shaham Y (2003) Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci Biobehav Rev 27: 457-491

Lumia AR, Teicher MH, Salchli F, Ayers E, Possidente B (1992) Olfactory bulbectomy as a model for agitated hyposerotonergic depression. Brain Res 587: 181-185

Macri S, Laviola G (2004) Single episode of maternal deprivation and adult depressive profile in mice: interaction with cannabinoid exposure during adolescence. Behav Brain Res 154: 231-238

Marinelli M, Piazza PV (2002) Interaction between glucocorticoid hormones, stress and psychostimulant drugs. Eur J Neurosci 16: 387-394

Marinelli M, White FJ (2000) Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. J Neurosci 20: 8876-8885

Markou A, Koob GF (1992) Bromocriptine reverses the elevation in intracranial self-stimulation thresholds observed in a rat model of cocaine withdrawal. Neuropsychopharmacology 7: 213-224

Markou A, Kosten TR, Koob GF (1998) Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. Neuropsychopharmacology 18: 135-174

Mayorga AJ, Lucki I (2001) Limitations on the use of the C57BL/6 mouse in the tail suspension test. Psychopharmacology (Berl) 155: 110-112

McNamara RK, Levant B, Taylor B, Ahlbrand R, Liu Y, Sullivan JR, Stanford K et al. (2006) C57BL/6J mice exhibit reduced dopamine D3 receptor-mediated locomotor-inhibitory function relative to DBA/2J mice. Neuroscience 143: 141-153

Melis M, Spiga S, Diana M (2005) The dopamine hypothesis of drug addiction: hypodopaminergic state. Int Rev Neurobiol 63: 101-154

Michel A, Tirelli E (2002) Effects of the social conditions of housing through testing on cocaine-induced contextual sensitisation and conditioned locomotion in C57BL/6J mice. Prog Neuropsychopharmacol Biol Psychiatry 26: 1185-1191

Millstein RA, Holmes A (2007) Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. Neurosci Biobehav Rev 31: 3-17

Moffett MC, Harley J, Francis D, Sanghani SP, Davis WI, Kuhar MJ (2006) Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. J Pharmacol Exp Ther 317: 1210-1218

Morgan D, Grant KA, Gage HD, Mach RH, Kaplan JR, Prioleau O, Nader SH et al. (2002) Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nat Neurosci 5: 169-174

Naranjo CA, Tremblay LK, Busto UE (2001) The role of the brain reward system in depression. Prog Neuropsychopharmacol Biol Psychiatry 25: 781-823

Papasava M, Singer G (1985) Self-administration of low-dose cocaine by rats at reduced and recovered body weight. Psychopharmacology (Berl) 85: 419-425

Papp M, Willner P, Muscat R (1991) An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology (Berl) 104: 255-259

Papp M, Lappas S, Muscat R, Willner P (1992) Attenuation of place preference conditioning but not place aversion conditioning by chronic mild stress. J Psychopharmacol 6: 352-356

Parfitt DB, Levin JK, Saltstein KP, Klayman AS, Greer LM, Helmreich DL (2004) Differential early rearing environments can accentuate or attenuate the responses to stress in male C57BL/6 mice. Brain Res 1016: 111-118

Parfitt DB, Walton JR, Corriveau EA, Helmreich DL (2007) Early life stress effects on adult stress-induced corticosterone secretion and anxiety-like behavior in the C57BL/6 mouse are not as robust as initially thought. Horm Behav 52: 417-426

Parks CL, Robinson PS, Sibille E, Shenk T, Toth M (1998) Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci U S A 95: 10734-10739

Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 245: 1511-1513

Piazza PV, Deroche-Gamonent V, Rouge-Pont F, Le Moal M (2000) Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. J Neurosci 20: 4226-4232

Piazza PV, Le Moal M (1996) Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annu Rev Pharmacol Toxicol 36: 359-378

Puglisi-Allegra S, Cabib S (1997) Psychopharmacology of dopamine: the contribution of comparative studies in inbred strains of mice. Prog Neurobiol 51: 637-661

Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ et al. (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci U S A 95: 14476-14481

Rocha BA, Odom LA, Barron BA, Ator R, Wild SA, Forster MJ (1998) Differential responsiveness to cocaine in C57BL/6J and DBA/2J mice. Psychopharmacology (Berl) 138: 82-88

Rogers JL, See RE (2007) Selective inactivation of the ventral hippocampus attenuates cue-induced and cocaine-primed reinstatement of drug-seeking in rats. Neurobiol Learn Mem 87: 688-692

Romeo RD, Mueller A, Sisti HM, Ogawa S, McEwen BS, Brake WG (2003) Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. Horm Behav 43: 561-567

Schroff KC, Cowen MS, Koch S, Spanagel R (2004) Strain-specific responses of inbred mice to ethanol following food shortage. Addict Biol 9: 265-271

Shanks N, Griffiths J, Zalcman S, Zacharko RM, Anisman H (1990) Mouse strain differences in plasma corticosterone following uncontrollable footshock. Pharmacol Biochem Behav 36: 515-519

Simler S, Puglisi-Allegra S, Mandel P (1982) gamma-Aminobutyric acid in brain areas of isolated aggressive or non-aggressive inbred strains of mice. Pharmacol Biochem Behav 16: 57-61

Slattery DA, Markou A, Cryan JF (2007) Evaluation of reward processes in an animal model of depression. Psychopharmacology (Berl) 190: 555-568

Sorg BA, Kalivas PW (1991) Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum. Brain Res 559: 29-36

Sorg BA, Kalivas PW (1993) Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. Neuroscience 53: 695-703

Spruijt BM, Cools AR, Ellenbroek BA, Gispen WH (1986) Dopaminergic modulation of ACTH-induced grooming. Eur J Pharmacol 120: 249-256

Stead JD, Clinton S, Neal C, Schneider J, Jama A, Miller S, Vazquez DM et al. (2006) Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors. Behav Genet 36: 697-712

Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl) 85: 367-370

Stolerman IP, Naylor C, Elmer GI, Goldberg SR (1999) Discrimination and self-administration of nicotine by inbred strains of mice. Psychopharmacology (Berl) 141: 297-306

Tremblay LK, Naranjo CA, Cardenas L, Herrmann N, Busto UE (2002) Probing brain reward system function in major depressive disorder: altered response to dextroamphetamine. Arch Gen Psychiatry 59: 409-416

Tremblay LK, Naranjo CA, Graham SJ, Herrmann N, Mayberg HS, Hevenor S, Busto UE (2005) Functional neuroanatomical substrates of altered reward processing in major depressive disorder revealed by a dopaminergic probe. Arch Gen Psychiatry 62: 1228-1236

Tzschentke TM (2001) Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Prog Neurobiol 63: 241-320

Urban IJ (1987) Brain vasopressin: from electrophysiological effects to neurophysiological function. Prog Brain Res 72: 163-172

Valverde O, Smadja C, Roques BP, Maldonado R (1997) The attenuation of morphine-conditioned place preference following chronic mild stress is reversed by a CCKB receptor antagonist. Psychopharmacology (Berl) 131: 79-85

van der Kam EL, Ellenbroek BA, Cools AR (2005) Gene - environment interactions determine the individual variability in cocaine self-administration. Neuropharmacology 48: 685-695

Van Loo PL, Mol JA, Koolhaas JM, Van Zutphen BF, Baumans V (2001) Modulation of aggression in male mice: influence of group size and cage size. Physiol Behav 72: 675-683

Van Ree JM, Burbach-Bloemarts EM, Wallace M (1988) Vasopressin neuropeptides and acquisition of heroin and cocaine self-administration in rats. Life Sci 42: 1091-1099

Varghese AK, Verdu EF, Bercik P, Khan WI, Blennerhassett PA, Szechtman H, Collins SM (2006) Antidepressants attenuate increased susceptibility to colitis in a murine model of depression. Gastroenterology 130: 1743-1753

Veenema AH, Bredewold R, Neumann ID (2007) Opposite effects of maternal separation on intermale and maternal aggression in C57BL/6 mice: link to hypothalamic vasopressin and oxytocin immunoreactivity. Psychoneuroendocrinology 32: 437-450

Ventura R, Alcaro A, Cabib S, Conversi D, Mandolesi L, Puglisi-Allegra S (2004) Dopamine in the medial prefrontal cortex controls genotype-dependent effects of amphetamine on mesoaccumbens dopamine release and locomotion. Neuropsychopharmacology 29: 72-80

Ventura R, Cabib S, Puglisi-Allegra S (2001) Opposite genotype-dependent mesocorticolimbic dopamine response to stress. Neuroscience 104: 627-631

Volpi S, Rabadan-Diehl C, Aguilera G (2004) Vasopressinergic regulation of the hypothalamic pituitary adrenal axis and stress adaptation. Stress 7: 75-83

Weiss RD (2004) Treating patients with bipolar disorder and substance dependence: lessons learned. J Subst Abuse Treat 27: 307-312

Wigger A, Sanchez MM, Mathys KC, Ebner K, Frank E, Liu D, Kresse A et al. (2004) Alterations in central neuropeptide expression, release, and receptor binding in rats bred for high anxiety: critical role of vasopressin. Neuropsychopharmacology 29: 1-14

Willner P (1997) Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology (Berl) 134: 319-329

Willner P (2005) Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52: 90-110

Willner P, Mitchell PJ (2002) The validity of animal models of predisposition to depression. Behav Pharmacol 13: 169-188

Wimersma Greidanus TB, Bohus B, Kovacs GL, Versteeg DH, Burbach JP, de Wied D (1983) Sites of behavioral and neurochemical action of ACTH-like peptides and neurohypophyseal hormones. Neurosci Biobehav Rev 7: 453-463

Zacharko RM, Anisman H (1991) Stressor-induced anhedonia in the mesocorticolimbic system. Neurosci Biobehav Rev 15: 391-405

Zacharko RM, Gilmore W, MacNeil G, Kasian M, Anisman H (1990) Stressor induced variations of intracranial self-stimulation from the mesocortex in several strains of mice. Brain Res 533: 353-357

Summary

Nature or nurture, a classical debate, but still on the scientific agenda, although it is generally accepted that genes and environment are both important in defining health and behavior. More precise, nature is interacting with nurture and behavior is constantly adapting to deal with environmental demands. In psychiatry, the concept of gene-environment interaction recently gained more interest; specific gene variants were found to heighten the vulnerability to psychopathology, but only in individuals that had encountered (certain) stressful life events. Thus, genetic background was found to influence the impact of stressful life events on mental health. Epidemiological studies are, however, limited to study underlying mechanisms and controlled human studies in this field are rare, since ethics obviously prohibit exposing humans to risk. So there clearly is a need for pertinent animal models that could help to understand how genes and environment interact and how vulnerability or resilience to psychopathology emerges.

Specifically in the field of drug abuse gene-environment interactions are difficult to demonstrate, since the drug itself creates major confounding factors, like accessibility to (illegal) substances and dose-dependant drug effects. Individual differences in drug taking behavior and liability to develop drug dependence are clearly observed, but the underlying psychobiological processes are still poorly understood. In neuroscience, interesting animal models exist for various aspects of substance use and abuse. In particular intravenous self-administration, that is considered as one of the best animal models of human psychopathology. Animals readily self-administer almost every drug abused by humans and inversely, drug self-administration in animals is highly predictive of abuse liability in humans. Moreover, like in humans, individual differences in drug intake are observed in animals. Therefore, animal models could be of great interest to investigate gene-environment interactions in drug use and abuse, but until now such models are rather poorly examined for this purpose.

The objective of the research described in this thesis was to fill this gap and to demonstrate the role of gene-environment interactions in the emergence of individual differences in cocaine use. For this purpose we used two inbred mouse strains, the C57BL/6 (C57) and DBA/2 (DBA), which are known to differ in drug-intake and to be differentially sensitive to several stressors. We studied the impact of early life experiences (long-term influence) as well as a later life psychosocial stressor (short-term influence) on drug intake behavior in these two mouse strains.

An important series of rat studies showed that maternal care influences emotionality and stressor reactivity in adulthood and that a change in maternal care is partly responsible for the impact of early life manipulations on adult behavior. Therefore, to study the impact of the early life environment, we manipulated the maternal environment of the mice by fostering them with non-related mother strains showing either high or low pup-oriented behavior.

Chapter 2 describes the spontaneous maternal behavior of AKR and C3H/HeN (C3H) mice when raising either DBA or C57 pups. These strains were found to exhibit very distinct maternal behaviors, with C3H mothers showing more pup-directed behavior

(licking and nursing), whereas AKR mothers exhibited more non-pup directed behavior (self-grooming) and an exaggerated 'nest reorganizing' behavior. This maternal style characterizing the dams was seen with both C57 and DBA foster pups.

Chapter 3 describes the impact of these early (maternal) environments on cocaine taking behavior in adult C57 and DBA mice in an intravenous self-administration experiment. We demonstrated a clear gene-environment interaction in early life, since C57 mice were not affected in their cocaine intake by the early foster environments, whereas DBA mice were. DBA mice raised by AKR mothers (DBA-*AKR)* showed a lower cocaine intake during the acquisition phase and a downward shift in the dose-response curve compared to DBA mice raised by C3H mothers (DBA-*C3H).* Additional behavioral characterization suggests that DBA-*AKR* mice specifically changed their motivational status, as they also displayed more floating ('depressive'-like behavior) in the forced swim test, but did not differ from DBA-*C3H* mice in anxiety measures on the elevated plus maze. Since DBA-*C3H* mice showed behavior that was close to the reference group (pups raised by their biological mother), DBA-*AKR* mice could be classified as less motivated. Behavior of adult C57-*AKR* and C57-*C3H* mice was comparable in all tests performed and did not differ from behavior shown by the reference group. We thus showed that the impact of early life environment on the motivational status in adulthood depends on the genetic background of the mice.

In a second model, we showed that gene environment interactions in adult life can also play a role in drug taking behavior. **Chapter 4** describes the influence of a short-lasting past experience of group housing in adulthood on cocaine intake in C57 and DBA mice. Adult C57 and DBA mice were transiently group-housed with same sex, same strain animals. For C57, the individually housed *(-I)* mice did not differ in cocaine SA from the ex-group housed *(-exG)* mice. However, DBA mice were again shown to be sensitive to the environmental experience; DBA-*exG* mice showed a higher intake during acquisition and an upward shift in the dose-response curve as compared DBA-*I.* Differences in brain cocaine levels could not account for the observed behavioral differences. Thus, we showed that the impact of a past adult environmental experience on subsequent drug taking behavior depends on the genetic background of the mice.

Finally, **chapter 5** describes a study investigating the mechanisms underlying the susceptibility of DBA mice to environmental experiences. Here, changes in gene-expression in the extended amygdala of mice from both models were studied. Mice were sacrificed in adulthood after the (early or late) environmental experience, but without having experienced drug self-administration. Interestingly, we found higher expression levels of arginine vasopressin (AVP) in the extended amygdala of the DBA groups that showed lower cocaine intake. This is a first biological measure defined in our models and might be implicated in the mechanisms underlying gene-environment interactions in drug intake.

The results described in this thesis reveal the implication of gene-environment interactions in the vulnerability to cocaine reinforcing effects. Further characterization of reward related behavior in the early life environmental influence model could support the idea that a sphere of 'anhedonia' is touched by our manipulation. Finally, our models open the possibility to study the psychobiological mechanisms that underlie susceptibility and resilience to the impact of environmental life events on cocaine-intake.

Samenvatting

Gedrag en gezondheid worden sterk beïnvloed door genetische achtergrond én door omgevingsfactoren. Deze twee factoren staan niet los van elkaar, hun wisselwerking zorgt voor een continue aanpassing van een organisme om zo goed mogelijk om te gaan met nieuwe omgevingssituaties. Ook in de psychiatrie is recentelijk steeds meer aandacht voor de interactie tussen genen en omgevingsfactoren. Het is gebleken dat specifieke genvarianten de kans op psychiatrische aandoeningen verhogen, maar alleen in mensen die ook bepaalde stressvolle gebeurtenissen hebben meegemaakt. Genetische achtergrond beïn-vloedt dus de impact die stressvolle gebeurtenissen kunnen hebben op mentale gezondheid. Epidemiologische studies naar zulke interacties zijn echter maar ten dele geschikt voor het ontdekken van het onderliggende mechanisme. Bovendien heeft om ethische redenen humaan onderzoek uiteraard veel beperkingen. Goed opgezette diermodellen beginnen in dit veld een belangrijke rol te spelen en kunnen bijdragen aan het begrijpen van hoe genen en omgeving communiceren en waarom de één gevoelig is voor psychopathologie en de ander niet.

Met name op het gebied van drugsgebruik en verslaving zijn gen-omgevingsinteracties moeilijk aan te tonen, alleen al omdat de drug zelf een belangrijke variërende factor is, met grote individuele verschillen in inname, kwaliteit en toegankelijkheid. Deze omstandigheden zijn beter te controleren in dierexperimenten en bovendien bestaan er interessante diermodellen voor verschillende aspecten van drugsgebruik. Zo kan in de intraveneuze zelf-toedienings-opzet het dier zichzelf via een katheter een drug (bv cocaine) toedienen door een bepaalde handeling uit te voeren zoals een hendel indrukken of zijn snuit in een kleine opening steken. Zo heeeft het dier zelf de controle over de inname. Deze test wordt veel uitgevoerd met de rat en de muis. Vrijwel alle verslavende middelen die geconsumeerd worden door de mens, worden ook “toegediend” door de rat en de muis en veroorzaken vergelijkbare veranderingen in o.a. het genotcentrum van de hersenen.

Om interacties aan te kunnen tonen tussen genen en omgevingsfactoren, zullen beide factoren gecontroleerd moeten worden en één van de twee getest moeten worden tegen verschillende aspecten van de ander. De genetische achtergrond kan “gecontroleerd” worden door middel van het gebruik van inteelt muisstammen; de genetische opmaak is gelijk voor ieder individu binnen een stam. In dit onderzoek is er gebruik gemaakt van twee stammen, de C57BL/6 (C57) en de DBA/2 (DBA) muis, welke verschillen in drugsinname en stressgevoeligheid. Er is gekeken naar de invloed van verschillende omgevings (levens-) ervaringen op de inname van cocaine in deze dieren in een intraveneuze zelftoedienings-opzet.

Een belangrijke serie experimenten uitgevoerd met ratten, heeft laten zien dat het gedrag van de moeder van invloed is op de stressgevoeligheid en het gedrag van haar jongen op volwassen leeftijd. Wij hebben daarom gekozen voor het manipuleren van de maternale omgeving om de invloed van vroege ervaringen op cocaïne-inname op volwassen leeftijd te bestuderen. Hiertoe hebben we de C57 en DBA pups laten verzorgen (fostering) door moeders afkomstig van twee verschillende muizenstammen. Deze twee muizenstammen, de C3H en de AKR stam, vertonen respectievelijk veel en weinig pup-georiënteerd gedrag, dwz likken en zogen van de pups. We hebben een uitgebreide studie naar maternaal gedrag

uitgevoerd en de twee maternale omgevingen die de AKR en C3H stam creëren voor hun fosterjongen goed gekarakteriseerd. De volwassen C57 en DBA dieren zijn vervolgens getest op cocaïne inname. Duidelijke gen-omgevings interacties werden waargenomen. DBA muizen die zijn opgegroeid in verschillende vroege omgevingen, laten als volwassen dieren een verschillende cocaïne-inname zien. Hiertegenover staat dat de cocaïne-inname van de volwassen C57 muizen niet beinvloed wordt door vroege ervaringen. De impact van vroege omgevingsfactoren op cocaïne-inname is dus afhankelijk van de genetische achtergrond. We hebben de dieren verder gekarakteriseerd in angst- en depressie-gerelateerde testen en de dieren lijken specifiek te zijn veranderd in motivationele componenten van gedrag.

In een tweede experimentele opzet hebben we C57 en DBA muizen onderworpen aan een stressvolle ervaring op volwassen leeftijd. Deze ervaring bestond uit 19 dagen groepshuisvesting en vervolgens terugkeer naar individuele huisvesting. Een week na het eind van de groepshuisvesting werden de dieren getest op cocaïne-inname. Ook in dit experiment werd een duidelijke gen-omgevingsinteractie waargenomen. DBA muizen waren gevoelig voor de omgevingservaring; de dieren die een periode van groepshuisvesting hadden meegemaakt verschilden in cocaïne-inname van de dieren die continu individueel werden gehuisvest. Er werden geen verschillen waargenomen in cocaïne-inname in de C57 muizen.

In een eerste aanpak om te zoeken naar de onderliggende mechanismen van deze gevoeligheid van de DBA muis voor omgevingsfactoren, hebben we gekeken naar genexpressie in de zogenaamde ‘extended amygdala’, een hersenstructuur die betrokken is bij reward (belonings-) processen. Genexpressie werd bekeken in volwassen dieren met een vroege of late omgevingservaring, maar nog drugs-naief, dwz zonder in aanraking te zijn geweest met cocaïne. In beide modellen (vroege en late omgeving) hebben we een hogere expressie van arginine vasopressine (AVP) gevonden in de extended amygdala van de DBA groepen met een lagere cocaïne inname. Verder onderzoek zal uit moeten wijzen of dit verschil in AVP genexpressie ook daadwerkelijk betrokken is bij de gen-omgevings interacties die cocaïne-inname beïnvloeden. Ook is van belang te weten welk epigenetisch mechanisme hier eventueel aan ten grondslag ligt.

In conclusie: de resultaten beschreven in dit proefschrift laten duidelijk zien dat de invloed van omgevingsfactoren op cocaïne-inname afhankelijk is van genetische achtergrond. De modellen die beschreven zijn in het proefschrift openen de mogelijkheid het mechanisme achter deze gen-omgevingsinteracties te bestuderen en individuele verschillen in de invloed van life events op cocaïne-inname beter te begrijpen.

List of abbreviations

Neurotransmitters/neuromodulators

5-HT	serotonin
5-HT T	serotonin transporter
5-HT1a	serotonin receptor 1a
ACTH	adrenocorticotrophic hormone
AVP	arginine vasopressin
AVPR1a/b	AVP receptor 1a/b
CRH/CRF	corticotrophin releasing hormone/factor
D1, D2	dopamine receptor D1, D2
DA	dopamine
GABA	gamma-aminobutyric acid
GLU	glutamate
GR	glucocorticoid receptor
NE	norepinephrine
OXY	oxytocin
OXYR	OXY receptor
Ttr	transthyretin

Animals: strains, experimental groups and selected lines

APO-SUS	apomorphine susceptible
APO-UNSUS	apomorphine unsusceptible
C3H	C3H/HeN mice
C57	C57BL/6J mice
C57-*AKR*	C57 mice raised by an AKR mother
C57-*bio*	C57 mice raised by their biological mother
C57-*C3H*	C57 mice raised by a C3H mother
C57-*exG*	ex group housed C57 mice
C57-*I*	individually housed C57 mice
DBA	DBA/2J mice
DBA-*AKR*	DBA mice raised by an AKR mother
DBA-*bio*	DBA mice raised by their biological mother
DBA-*C3H*	DBA mice raised by a C3H mother
DBA-*exG*	ex group housed DBA mice
DBA-*I*	individually housed DBA mice
HAB	high anxiety behavior
HG	high grooming
HR	high responders
LAB	low anxiety behavior
LG	low grooming
LR	low responders

Early life

ABN	arched-back nursing
AFR	animal facility rearing
EH	early handling
LD period	light/dark period
LG	licking/grooming
MD	maternal deprivation
MS	maternal separation
NH	early non-handling
PD	postnatal day
SHRP	stress hypo-responsive period
USV	ultrasonic vocalizations

Behavioral testing, materials & methods

3h	3 hours
BW	bodyweight
CMS	chronic mild stress
CPP	conditioned place preference
EPM	elevated plus maze
FR	fixed-ratio
FST	forced swim test
ICSS	intracranial self stimulation
ICV	intracerebroventricular
i.d.	inner diameter
IP	intraperitoneal
IV	intravenous
OBX	olfactory-bulbectomy
o.d.	outer diameter
PR	progressive ratio
SA	self-administration
SC	subcutaneous
UV	ultraviolet

Genetics

cDNA	complementary DNA
cRNA	complementary RNA
Ct	cycle threshold
DNA	deoxyribonucleic acid
EST	expressed sequence tag
GCOS	genechip® operating software
mRNA	messenger RNA
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid

Brain structures

BLA	basolateral amygdala
BNST	bed nucleus of the stria terminalis
CNS	central nervous system
EA	extended amygdala
HPA axis	hypothalamic-pituitary-adrenal axis
mPFC	medial prefrontal cortex
NAcc	nucleus accumbens
NTS	nucleus tractus solitarius
PFC	prefrontal cortex
PVN	paraventricular nucleus of the hypothalamus
VTA	ventral tegmental area

Statistics

ANOVA	analysis of variance
n=	number of animals in a group
p=ns	p-value is non-significant
SEM	standard error of the mean

Merci.....

Le temps se permet de prendre celui que nous ne prenons pas...et il passe très vite! Un petit mot pour tous ceux qui m'ont entouré pendant mes presque 7 ans à Bordeaux.

Je vous l'ai déjà dit a mon départ, mais voila encore une belle occasion d'exprimer mes remerciements aux gens de l'unité 259 devenu U588 et aujourd'hui U862….
Muriel et Véronique, avec qui j'ai beaucoup travaillé, et qui m'ont beaucoup appris. Muriel, on a tout fait en grande échelle, beaucoup de souris, beaucoup de nuits au labo pour surveiller les naissances, beaucoup d'heures d'analyse vidéo et beaucoup de tests comportementaux...et finalement un papier avec beaucoup de graphes! Merci pour ton soutien, ça m'a fait plaisir de bosser ensemble. Merci aussi Véro, tu étais là pour la suite, conduire les souris vers la cocaïne...merci pour ton positivisme et ta confiance. Merci à Guillaume et Thierry pour la partie qPCR, c'était bien agréable de passer quelques mois à l'IECB.
Et bien sûr les autres qui était la pour enrichir la vie au labo et en dehors. Ilse, wat werd het gezellig in Bordeaux toen je op het lab stage kwam lopen! Pierre, Jean-Michel, Muriel P. Guillaume, Marie-Christine, Andy et Ryoski pour leur amitié et les weekends de ski super sympas. Monique pour tous les litres de thé qu'on a bu ensemble. Ceux qui ont thésardé avec moi…Pierre, Olivier, Arnaud, Fred, David et Francesco. Patricia et Hélène, qui m'ont fait le plaisir de leur enthousiasme pendant leur stage. Françoise pour le dosage de cocaïne et pour avoir partager ton bureau pendant la phase d'écriture. Cédric pour le soin des animaux, Eric pour l'aide en auto-administration et bien sur Isabelle pour les soins 'administrative'. Je veux aussi exprimer ma gratitude aux ASSEDIC et à l'ANPE, qui m'ont permis de terminer la thèse et de trouver mon chemin vers mon post-doc actuel.

Het was altijd erg leuk om Leiden te bezoeken. Ook vanwege mijn 'mede-strijdster' Inge, vanaf het begin klikte het goed, we zijn in het oorspronkelijke "gezamenlijke project" niet bij elkaar gekomen, maar dat mocht de pret niet drukken. Het was erg goed om samen naar summerschools te gaan, uit eten in Leiden (met stapels grafieken op het terras) en te discussiëren over de telefoon (inderdaad ook voor de nodige peptalks!). Nu weer betrokken bij hetzelfde project, leuk! Super dat je vandaag naast me zit als paranimf.
And in the beautiful city of London, I met this amazing red-haired girl. Maytal, you impressed me! We had a very nice time in the ULLA summerschool, and you and Yair became my "3 étoiles" hotel in Leiden. Lasagna in Friesland, pregnant in Paris, things to never forget...I'm very happy to have you next to me as my paranimf.
Ellen, het contact met Leiden verliep vaak via jou, er werd vlot en efficiënt gehandeld en je was altijd zeer geïnteresseerd. De etentjes in Leiden met Leo, Petra, Inge, Peter, Maytal, Yair en Servane, erg gezellig! Servane, on s'est rencontré à Bordeaux, pour se retrouver dans le même bureau à Leiden, c'est pas mal, non!

To the people outside of the lab: my ‘Dutch’, ‘Swiss’ and ‘French’ friends, ma belle famille, les gens de l’école de sages femmes venant de tous les coins du monde, et bien sûr l’équipe de la Lucarne et ses comédiens pour des belles aventures théâtrales.

En uiteraard hier een speciaal plekje voor mijn familie. Voor mijn ouders voor wie Frankrijk opeens een speciale betekenis kreeg en die me veel hebben opgezocht in “het zuiden”. En uiteraard zo nu en dan terug naar Nederland om weer even op te laden in Friesland met iedereen van de partij. Henk, super dat je me hebt geholpen met de layout!

Nico, tu dis que les chercheurs sont fous, tu as probablement raison. Mais que-est ce que ce monde deviendra sans un peu de folie? On s’est vu à travers la petite Lucarne, puis je continue à avoir envie de te voir et revoir et revoir...

En Madelief, omdat je zo mooi en zo lief en zo alles bent...

Curriculum vitae

Rixt van der Veen werd geboren op 7 maart 1977 te Leeuwarden. Ze haalde in 1995 haar VWO diploma aan het Bogermancollege in Sneek. In september van datzelfde jaar begon ze haar studie biologie aan de Rijksuniversiteit Groningen (RUG). Tijdens de laatste fase van haar studie werden twee onderzoeksprojecten gedaan. Het eerste project werd uitgevoerd bij de vakgroep dierfysiologie aan de RUG onder leiding van Dr. Katalin Horvath en Prof. Dr. Paul Luiten en betrof een studie naar de beschermende effecten van 17β estradiol op leren en geheugen na hersenschade. Een tweede project werd uitgevoerd bij de vakgroep Psychopharmacology aan de HUG (Hôpitaux Universitaires de Genève) onder leiding van Dr. Thierry Steimer. In dit project werd gewerkt met de Roman high avoidance (RHA) and low avoidance (RLA) ratlijnen en gekeken naar aromatase activiteit in specifieke hersengebieden tijdens verschillende ontwikkelingsfasen. Eind 2000 studeerde ze af met als specialisatie medische biologie. Het jaar daarop begon het Frans-Nederlandse avontuur onder leiding van Ron de Kloet en Pier Vincenzo Piazza wat leidde tot dit proefschrift. Ze werd als promovendus aangesteld bij de vakgroep Medische Farmacologie, onderdeel van het Leiden/Amsterdam Center for Drug Research (LACDR) en het Leids Universitair Medisch Centrum (LUMC), maar werkte voornamelijk bij de INSERM U259 (Psychobiologie des Comportements Adaptatifs), later INSERM U588 (Physiopathologie du Comportement) van de Universiteit van Bordeaux II. Dit onderzoek maakte deel uit van een samenwerkingsproject tussen INSERM-Mildt en NWO-ZonMw getiteld “Programming neural stress circuitry by genetic inputs and early experience: implications for individual vulnerability to addiction”. Sinds begin dit jaar is ze werkzaam in Leiden als post-doctoraal onderzoeker bij de vakgroep Medische Farmacologie op een TI-Pharma project getiteld “Novel susceptibility pathways and drug targets for psychosis”.

Rixt van der Veen was born on March 7th, 1977, in Leeuwarden. She attended high school at the Bogermancollege in Sneek and graduated in 1995. She studied biology at the University of Groningen. During the second phase of her study, she specialized in medical biology and performed two research projects. The first project took place in the department of animal physiology at the University of Groningen (RUG) under supervision of Dr. Katalin Horvath and Prof. Dr. Paul Luiten and investigated the protective effects of 17β estradiol on learning and memory after brain damage. The second project was performed at the department of Psychopharmacology at the HUG (Hôpitaux Universitaires de Genève) under supervision of Dr. Thierry Steimer. In this project, brain aromatase activity during different developmental phases was studied in the Roman high avoidance (RHA) and low avoidance (RLA) ratlines. The research work presented in this thesis started in 2001 under supervision of Ron de Kloet and Pier-Vincenzo Piazza and was part of collaboration project between INSERM-Mildt and NWO-ZonMw entitled “Programming neural stress circuitry by genetic inputs and early experience: implications for individual vulnerability to addiction”. She enrolled her thesis at the department of Medical Pharmacology, part of the Leiden/Amsterdam Center for Drug Research (LACDR) and the Leiden University

Medical Centre (LUMC), but performed most of the work at the INSERM unit 259 (Psychobiologie des Comportements Adaptatifs), later named INSERM unit 588 (Physiopathologie du Comportement) at the University of Bordeaux II. Since the beginning of this year she is working as a post-doctoral researcher at the department of Medical Pharmacology in Leiden on a TI-Pharma project entitled “Novel susceptibility pathways and drug targets for psychosis”.

List of publications

van der Veen R, Piazza PV, Deroche-Gamonet V (2007). Gene-environment interactions in vulnerability to cocaine intravenous self-administration: a brief social experience affects intake in DBA/2J but not in C57BL/6J mice. *Psychopharmacology (Berl) 193: 179-186.*

van der Veen R, Abrous DN, de Kloet ER, Piazza PV, Koehl M (2008). Impact of intra- and interstrain cross-fostering on mouse maternal care. *Genes, Brain and Behavior 7: 184-192.*

van der Veen R, Koehl M, Abrous DN, de Kloet ER, Piazza PV, Deroche-Gamonet V (2008). Maternal environment influences cocaine intake in adulthood in a genotype-dependent manner. *PLoS ONE 3(5): e2245.*

Horvath KM, Hartig W, **van der Veen R**, Keijser JN, Mulder J, Ziegert M, Van der Zee EA, Harkany T, Luiten PG (2002). 17beta-estradiol enhances cortical cholinergic innervation and preserves synaptic density following excitotoxic lesions to the rat nucleus basalis magnocellularis. *Neuroscience 110(3):489-504.*

Koehl M, **van der Veen R**, Gonzales D, de Kloet ER, Piazza PV, Abrous DN. Gene-environment interactions in early life impact on adult hippocampal neurogenesis. *In preparation.*