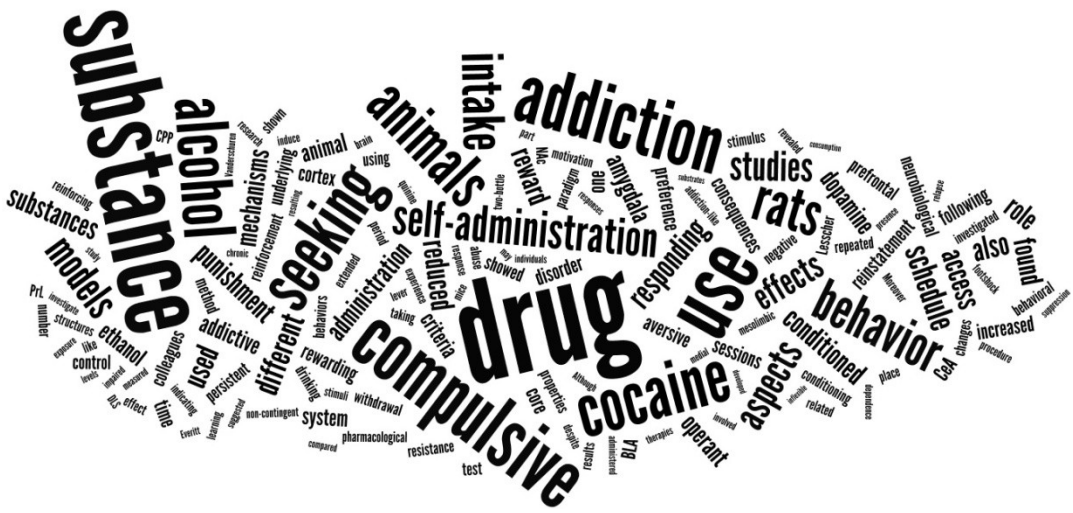
# Compulsive aspects in animal models of addiction and their underlying neurobiological mechanisms

**Abstract**

**Addiction is devastating disease affecting the lives of millions of people worldwide. As there is a need for more pharmacological therapies targeting the core aspects of addiction, research should focus on the severe compulsive aspects of addiction. Here, an overview is given on the existing animal models of addiction and the compulsive aspects in particular which can be modeled by punishment and aversive-resistant behavior during self-administration. Further, this review discusses the neurobiological mechanisms underlying these compulsive behaviors, revealing the prefrontal cortex regions as key structures in the development of compulsive drug use.**

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# Introduction

Addiction is a chronic relapsing disorder that is characterized by compulsive drug seeking, loss of control over intake (dependent substance use) and a negative emotional state during withdrawal periods. It is a devastating brain disease that affects the live of the individual drug addicts, their social environment and the society at large. Only in the Netherlands almost 600.000 individuals meet the criteria for a substance use disorder (table 1). Worldwide more than 50 million people suffer from the addictive effects of illicit drugs (table 2). Since drug seeking and taking occupies most of their time, most drug addicts are not able to participate in professional and social activities, with devastating consequences for their families and society. Moreover, substance use affects people from a medical perspective. Many substances are part of a component cause of life-threatening diseases such as cardiovascular diseases, cancer, and depression, thereby contributing to their prevalence. For society, the dealership of addictive substances is an alarming source of crime-related activity as most substances are prohibited by law. Moreover, addicted individuals and the addiction in general affect the economy due to a loss of productivity, health care and the fight against drug-associated crime. Taken together, there is need for the treatment of addictive people to overcome the substance use related problems in healthcare, justice and economy.

Table 1. Number of Dutch inhabitants aged 18-64 year with a substance use disorder in the last 12 months. Number of inhabitants aged 18-64 year at 1-1-2009: 10.486.000 (de Graaf et al., 2012)

| **Substance use disorder** | **597.000** |
|---|---|
| **Alcohol Abuse** | 395.600 |
| **Alcohol Dependence** | 82.400 |
| **Drugs Abuse** | 92.900 |
| **Cannabis abuse** | 40.200 |
| **Drug Dependence** | 77.000 |
| **Cannabis Dependence** | 29.300 |

Table 2. Estimated number of worldwide cases and age-standardized and sex-standardized prevalence of cannabis, amphetamine, cocaine, and opioid dependence in 2010 (Degenhardt et al., 2013)

| **Substances** | **N** |
|---|---|
| **Cannabis** | 13 073 000 |
| **Amphetamine** | 17 184 000 |
| **Cocaine** | 6 891 000 |
| **Opioids** | 15 479 000 |

Besides behavioral (psycho)therapy, self-help groups and residential treatment programs, medication can potentially help addicted individuals. Most of the existing pharmacological therapies for addiction are counteracting the rewarding effects, the withdrawal symptoms or the comorbidity symptoms of addictive substances (Edens et al., 2010). The few

pharmacological therapies that have been approved cover only a small fraction of substance use disorders and their efficacies are suboptimal (Del Re et al., 2013; Yahn et al., 2013; Yoshimura et al., 2013). New therapeutic agents targeting the core aspects of full-blown addiction are therefore essential. The *Diagnostic and Statistical Manual of Mental Disorders* (DSM) IV manual described two categories of substance related disorders, namely substance abuse and substance dependence. Substance abuse was assumed to be the precursor of substance dependence, which in turn is a term that is used interchangeably with addiction. Recently, a new version of the DSM has been published in which criteria for substance use disorders have been revised. In the DSM V version, substance abuse and dependence are combined into one substance use disorder classification consisting of 11 criteria (Table 3). 2 to 3 criteria define a mild substance use disorder, in case of 4 to 5 criteria we speak of moderate substance use disorder and with 6 or more criteria a severe substance disorder is diagnosed.

Table 3. Criteria for substance use disorder according the DSM V.

| | **A maladaptive pattern of substance use leading to clinically significant impairment or distress, as manifested by 2 (or more) of the following, occurring within a 12-month period:** |
|---|---|
| **1** | Recurrent substance use resulting in a failure to fulfill major role obligations at work, school, or home |
| **2** | Recurrent substance use in situations in which it is physically hazardous |
| **3** | Continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance |
| **4** | Tolerance, as defined by either of the following:<br>a. a need for markedly increased amounts of the substance to achieve intoxication or desired effect<br>b. markedly diminished effect with continued use of the same amount of the substance |
| **5** | Withdrawal, as manifested by either of the following:<br>c. The characteristic withdrawal syndrome for the substance (refer to Criteria A and B of the criteria sets for withdrawal from the specific substances)<br>d. The same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms |
| **6** | The substance is often taken in larger amounts or over a longer period than was intended |
| **7** | There is a persistent desire or unsuccessful efforts to cut down or control substance use |
| **8** | A great deal of time is spent in activities necessary to obtain the substance, use the substance, or recover from its effects |
| **9** | Important social, occupational, or recreational activities are given up or reduced because of substance use |
| **10** | The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance |
| **11** | Craving or a strong desire or urge to use a specific substance. |

Even in individuals suffering from a severe substance disorder not all symptoms are present from the disease onset. Addiction develops as user experience increases, indicating that the disease worsens over time. Supported by the interplay between positive reinforcement (pleasurable effects) and negative reinforcement (alleviating negative emotional symptoms) repeated intake of the substance can lead in vulnerable individuals to loss of control over

drug taking and compulsive drug use. In fact, from the eleven DSM criteria for substance use disorder, criteria number 6 to 10 comprise (Table 3) a loss of control over substance use, due to compulsive drug seeking and taking. Most representative for compulsive action is the continued use of a drug despite its negative consequences. Important in this respect is the fact that none of the existing pharmacological therapies target the compulsive character of addiction. Animals models provide the opportunity to elucidate the underlying neurobiological mechanisms of the compulsive aspects of addiction. Therefore, the aim of this thesis is to evaluate the available animals models for addiction and their capability of approaching this core characteristics of addiction, compulsive drug use. Subsequently the most recent discovered understandings of the neurobiological mechanisms underlying the compulsive behavior in addiction will be discussed.

# Animal models of addiction

Multiple animal models for addiction have been established and used in the past, assessing different aspects of addiction. Animal models enable the study of addiction from the point of early onset to the point of severe dependence, in contrast to clinical observations that start with already addicted individuals. Early models for addiction focused mainly on the acute euphoric and rewarding effects of a drug. Only very recently, several research groups have started to investigate the core characteristics of more advanced stages of addiction, including reinstatement or relapse and loss of control over drug use. In parallel, animal models developed a stronger face validity regarding the seeking aspects of drug addiction.
This chapter will describe both involuntary and voluntary models of drug administration that have been used over the years and which aspects and stages of addiction they assess. This part will also include some of their most important findings. The final part of this chapter will address models that capture aspects of full-blown addiction.

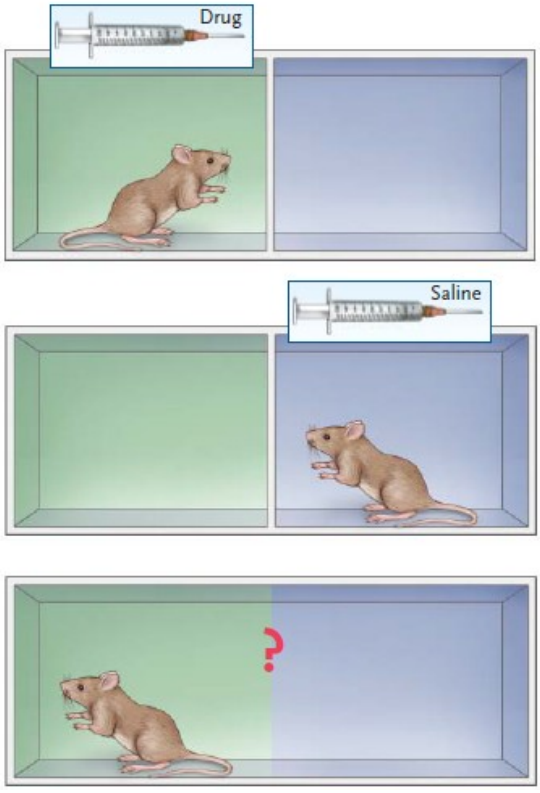


**Fig. 1. Schematic view of a conditioned place preference setup and procedure. The box divided in two distinct compartments differentiating in color and textures. During conditioning sessions the animal is infused with the drug in one compartment and with a control substance in the other compartment. In a following test session the time the animal spend in both compartments is measured and demonstrates the preference or aversion for the drug (Cami and Farre, 2003).**

### Non-contingent administration

The most straightforward method to study both behavioral, cellular and molecular effects of an addictive substance, is the forced administration by the experimenter. In most studies, substances are administered systemically via intravenous, intraperitoneal, or subcutaneous infusions. To study the specific involvement of a brain region in the effect of a substance on behavior and neurobiological is measured by direct infusions into a certain brain region. Early non-contingent drug studies focused on the acute effects of a substance by single infusions. In particular molecular studies have identified the neurochemical targets of different drugs (receptor pharmacology), their signaling pathways and the following changes in gene and protein expression (Nestler and Aghajanian, 1997; Nestler, 2001). Since addiction is a disease that develops with chronic drug exposure recent studies often use a repeated drug administration method. This allows the investigation of neuroadaptations that occur over time (sensitization) and the behavioral consequences of these changes (Robinson and Berridge, 1993; Vanderschuren and Pierce, 2010). One of the behavioral consequences is the increased reinforcement and motivation. Following non-contingent drug-administration a conditioned place preference test can examine these issues.

*Conditioned Place Preference*

Already in 1940 Spragg showed that morphine–dependent chimpanzees preferred a white box with a morphine syringe above a black box with a banana. As one of the core aspects of addiction, drug reward can be assessed using a conditioned place preference (CPP) paradigm (Bardo and Bevins, 2000; Tzschentke, 1998). The CPP paradigm measures the preference or aversion for a place where a substance has been presented. By way of classical (Pavlovian) conditioning the place becomes associated with the rewarding or aversive effects of the substance. Following conditioning, the time spent in the drug-paired compartment is a measure for the rewarding and motivational properties of a substance.

A place preference setup normally consists of a box with two compartments, that differ in color (black/white) and texture of the floor (grid/bars). During conditioning sessions, animals are infused with a substance following confinement in one of the compartments. In a successive session, animals receive a control substance and are placed in the other compartment. After repeated conditioning sessions animals are tested for the time spent in both compartments (Fig 1). Using this procedure the rewarding effects of cocaine, amphetamine and morphine have been demonstrated showing an increased preference after repeated conditioning sessions (Lett, 1989; Shippenberg and Heidbreder, 1995). Since then, CPP has been successfully used to investigate the pharmacological and neuroanatomical substrates of reward (Tzschentke, 1998; Tzschentke, 2007).

Two of the main advantages of CPP are that animals are tested in a drug-free state, and only one single drug-pairing can be enough to induce CCP. A disadvantage of the CPP pro-

cedure is its incapability of measuring the reinforcing properties of a drug, unlike different models of self-administration, and is likewise unsuitable for investigating drug-seeking behavior. More disadvantageous is that in most CPP experiments the substance is administered in a non-contingent way. There is an increasing amount of evidence supporting the presence of discrepancies between voluntary and non-voluntary intake of substances regarding both behavioral and neurobiological aspects of rewarding. For instance, it was found that contingent administration of cocaine triggers an increased incentive motivation as measured with cue-evoked food seeking (Leblanc et al., 2013), which was not observed in animals with a yoked administration of cocaine. Furthermore, non-contingent has been shown to decrease responding under a second-order schedule of cocaine reinforcement (Markou et al., 1999). At the molecular level, contingent administration of cocaine in rats showed to induce significantly higher acetylcholine levels in the nucleus accumbens compared to non-contingent administration of cocaine (Mark et al., 1999). Additionally, decreases in dopamine transporters were found after withdrawal in rats after contingent administered and not after non-continent administered methamphetamine (Stefanski et al., 1999). As voluntary intake has more potential regarding the face validity of the procedure, CPP paradigm by way of non-contingent drug-administration is not preferable regarding  Still, this CPP paradigm can provide useful information when implemented in a model of contingent drug administration.

**Contingent administration**

Voluntary drug administration is currently the most widely used model in current addiction research. The procedure of operant self-administration has proved to be effective in assessing aspects of both drug seeking and drug taking behavior, beyond the rewarding aspects that have been studied with the CPP paradigm.

*Operant self-administration*

Originally designed to examine the reinforcing properties of a substance, operant self-administration has been used since the early sixties (Clark et al., 1961; Weeks, 1962). By an operant response such as a lever press or a nose poke into a hole, animals are able to self-administer a substance. Most substances are infused systemically by implanting a catheter into the jugular vein. For that purpose, a catheter is implanted and connected to a back mount that can in turn be connected to tubing and a syringe pump. A liquid swivel the between pump and the catheter avoids immobility of the animal. For fluids like alcohol, which may be administered orally, liquid dippers have been developed. Following an operant response, the dipper cups deliver a volume of the substance into a receptacle that is positioned in the near vicinity of the lever or nose poke hole.

*Reinforcement*

Different schedules may be applied in operant self-administration to assess various effects of drugs of abuse. A fixed ratio (FR) schedule will deliver a reward after a fixed number of responses. Most commonly used is the FR 1 schedule which requires one response to obtain a reward. With a fixed ratio schedule the reinforcing properties of a substance may be demonstrated. Although closely related to reward, reinforcement refers not to the same concept. Reinforcement is the strengthening effect of a behavior (i.e. response) as a consequence of a preceding stimulus (i.e. reward). In other words, the stimulus increases the future probability of the behavior. The reinforcing properties of a substance are thought to be indicative for the positive-subjective effects and therefore this method has been frequently used to test the abuse liability of substances (O'Connor et al., 2011). Additionally, the FR1 schedule in operant self-administration has revealed many of the neurobiological mechanisms related to reinforcement.

Regarding addiction, the fixed ratio schedule has been used to study the transition from controllable to uncontrollable drug use by varying intake patterns. Ahmed and Koob investigated the effect of drug availability on cocaine seeking behavior in rats using an operant self-administration model. Animals were trained for 1 or 6 hours per day to press the levers for an infusion of cocaine. The intake of cocaine in the 1 hour access group remained stable but intake for the 6 hour access group was increasing per session (Ahmed and Koob, 1998). Similar results were found for heroin (Ahmed et al., 2000) and methamphetamine (Kitamura et al., 2006), showing that escalation of substance use emerges in an intake-dependent manner.

*Motivation*

To test the motivational properties of substance in an operant self-administration setup, a progressive ratio schedule can be applied. Motivation is conceived as the amount of work an animal is willing to work for. In this schedule the number of responses required to obtain a reward is progressively increasing. The last number of responses that is executed and wherefore a reward is received is the maximum effort an animal will expend. This phenomenon is known as breaking point. The higher the breaking point, the more animals are motivated to obtain the drug. The schedule has been used to investigate the role of drug availability on motivation. Rats with a history of extended cocaine access showed an increased motivation compared to rats with a short access to cocaine (Paterson and Markou, 2003). Other factors influencing motivation have been found such as social isolation (Baarendse et al., 2013), speed of drug delivery (Minogianis et al., 2013) and locomotor response (Mandt et al., 2008).

*Cue-controlled seeking*

Trough their involvement in craving and relapse environmental and contextual (conditioned) cues are a major subject of investigation. Complex cue-controlled drug seeking behaviors can be approached using a second order schedule of reinforcement. This schedule consists of two simpler schedules. In the first schedule a fixed number of responses results in the presentation a conditioned stimulus (CS), a light or tone previously paired with the drug reinforcer. After completion of a series of responses for the CS presentation, the unit schedule results in the delivery of the original reward (substance). An example of a second order schedule is FR*x*(FR*y*), with x the number of unit schedule requirements, and y the number of lever presses resulting in the presentation of the CS. This allows the investigation of responding for drug-associated cues and the underlying neurobiological substrates (Belin and Everitt, 2008; Vanderschuren et al., 2005). Most advantageous of this model is that drug seeking can be investigated in absence of the pharmacological effects of the drug.

*Extinction & Reinstatement*

Addiction is characterized by a high rate of relapse (McLellan et al., 2000); the recurrent use of the substance after periods of withdrawal. Pharmacological and neuroanatomical studies have revealed many of the mechanisms underlying drug reinstatement (Shaham et al., 2003). Stressful events, drug priming injections and drug-associated cues have the potential to induce a state of craving that may lead to drug seeking behavior and possibly relapse. Resistance to extinction and reinstatement have been implemented in self-administration models of addiction using the different causes of craving.

After a period of drug access, seeking can be extinguished when responding is not followed by a reinforcing event or a conditioned stimulus. These extinction sessions are repeated until responding has disappeared or has been reduced to minimal levels. Reinstatement is achieved by different methods. Priming injections of the substance of abuse after extinction have been shown to reinstate drug seeking, and drugs other than the one of used in self-administration were also able to induce reinstatement (De Vries et al., 1998). The administration of uncontrollable electric footshocks and the administration of the neuropeptide corticotropin releasing factor CRF, both serving as stressors, have been proved to reinstate drug seeking showing an intake-dependent effect on the susceptibility for reinstatement (Mantsch et al., 2008). A third way to induce reinstatement is by the representation of a light and/or as a tone, previously associated with the drug. Re-exposure to conditioned stimulus after a abstinence period of 19 weeks in rats with a history of cocaine self-administration elicited robust responding (Weiss et al., 2001). This has been proven to be more pronounced in rats with extended access compared to rats with short access to cocaine (Kippin et al., 2006). Moreover, it was found that responding during reinstatement

sessions increased as the period of withdrawal was extended (Grimm et al., 2001). The discovery of different stimuli influencing reinstatement in animal models of addiction has contributed to a better understanding of the neurobehavioral mechanism underlying the propensity to relapse in human addicts.

*Two-Bottle Choice Test*

Since alcohol is administered orally a different method to examine its rewarding properties has been developed. In the two-bottle choice paradigm animals have access to a diluted ethanol solution in a drinking bottle of their home cage next to a bottle with normal tap water (Lesscher et al., 2010; Melendez, 2011; Rhodes et al., 2005; Simms et al., 2008; Wise, 1973). The drug-containing solution is sometimes offered in different concentrations. By daily weighing of the bottles the preference for a certain drug solution or concentration is measured. Early studies on alcohol dependence suffered from difficulties to induce excessive ethanol intake in animals. To increase intake amounts early studies used a sucrose fading procedure (Samson, 1986) in which sucrose is gradually replaced by ethanol. Another method to increase drinking volumes proved to be a non-continuous access to alcohol. These intermittent access paradigms resemble the binge-like intake pattern of alcohol in humans in which large amounts are consumed in short periods of time. Different intermittent access drinking paradigms have been developed and improved over time. Rhodes and colleagues (2005) developed a method named ‘drinking in the dark’ in which the alcohol was the only available solution for a limited amount of time during the dark cycle. By using this method blood ethanol concentration of more than 1.0 mg ethanol per ml blood were achieved (Rhodes et al., 2005), by the authors considered as pharmacologically significant drinking. A clear disadvantage of this method is the absence of normal tap water during the availability of ethanol, because ethanol drinking in this method might reflects thirst in these animals. Simms and colleagues adapted (2008) the method of Wise (1973) and provided rats both tap water and ethanol at the same time as part of a two-bottle choice experiment, with the important changes as compared to more conventional two-bottle choice tests that the alcohol was provided to the animals on every other day. With this approach, rats consumed high levels of ethanol, also shown by their blood ethanol concentrations (Simms et al., 2008). The levels that the animals consumed in this intermittent-every-other-day paradigm were higher compared to animals that had continuous access to alcohol. Similar studies showed that this was also true for mice (Lesscher et al., 2009; Lesscher et al., 2010; Lesscher et al., 2012; Melendez, 2011).

**Models of compulsive drug use**

Although the above described models include many addiction-like behaviors such as reward, reinforcement, motivation and reinstatement, none of these models capture the compulsive aspects of addiction. One of the criteria for substance use disorders derived from the DSM-5 manual is that the substance is used despite the knowledge of its negative consequences indicating that both physical and psychological consequences are ignored by the user resulting in a continuation of the intake. This is considered to be hallmark for compulsive drug seeking and can be examined by measuring inflexible drinking in both two-bottle preference test or the punishment resistance in operant self-administration.

Already in the early nineties researchers tried to catch the compulsive aspect of addictive behavior in animals models. In 1991 Wolffgramm and Heyne looked at the consumption of alcohol upon adulteration with an aversive substance, to determine whether rodents would develop inflexible alcohol intake in that it may become insensitive to negative consequences. In their research, Wolffgramm & Heyne used a choice paradigm with four different concentrations of ethanol. After a long period of alcohol intake, the alcohol solutions were adulterated with the bitter substance quinine. Although animals reduced their alcohol intake, they nevertheless consumed still more alcohol than naïve rats in non-adulterated solutions, indicating that rats became insensitive for the bitter taste (Wolffgramm and Heyne, 1991). These experiments were repeated with amphetamine (Heyne and Wolffgramm, 1998) and opiates (Heyne, 1996) with similar results. More recently, Lesscher et al. (2010) investigated the sensitivity for quinine in mice after alcohol consumption. After only mice 2 weeks of alcohol experience in a two-bottle choice paradigm the addition of low concentrations quinine (250 uM) failed to reduce alcohol intake. Yet, high concentration (500 uM) of quinine did reduce alcohol consumption. After 8 weeks of consumption mice showed no preference for quinine-free alcohol over quinine-adulterated alcohol (Lesscher et al., 2010). Further, Hopf and colleagues 2010 showed that this insensitivity also occurs in rats after 3-4 month of intermittent alcohol access. Adulteration with quinine (0.01, 0.03 g/L) did not reduce ethanol intake in rats, whereas in rats with continuous access even small concentration reduced alcohol intake (Hopf et al., 2010). Moreover, breaking points, as assessed with an operant PR schedule responding, were not reduced by quinine adulterations (0.1 g/L). Both studies show that punishment resistance develops depending on both intake pattern and the rate of punishment.

In parallel to the alcohol studies demonstrating persistent alcohol intake despite negative consequences, there are also a number of groups that have investigated punishment resistance in operant self-administration of other drugs of abuse. One of the methods used for punishment resistance is footshock administration in a seeking-taking chain schedule. In this schedule (Olmstead et al., 2000) a response on the seeking lever results in the appearance of the taking lever and a following a response on the taking lever results in a

reward. This enables the separate investigation of both drug seeking and taking behaviors. In their research on compulsive behavior, Vanderschuren and Everitt (2004) used the seeking–taking chain schedule to investigate the aversive properties of a conditioned stimulus. After reaching a training criterion in the self-administration sessions, animals received footshocks paired with a tone resulting in the association between tone (CS) and the footshock. Subsequently, drug seeking was tested in the presence of the CS to see if any suppression would arise. Short experience with cocaine resulted still in a conditioned suppression of cocaine seeking behavior. In contrast, persistent cs-insensitive drug seeking was observed in all animals with prolonged experience with cocaine (Vanderschuren and Everitt, 2004). Instead of using the footshock association, direct footshock administration has also been used to test investigate punishment resistance. Pelloux and colleagues (2007) trained rats for cocaine self-administration following punishment sessions in which half of the responses on the seeking lever was followed by a footshock. A subpopulation of rats with extended cocaine experience showed a significant persistent seeking during footshock sessions (Pelloux et al., 2007). Importantly, these direct footshocks given in a response-contingent manner specifically affected cocaine seeking and not sucrose responding which was tested in parallel. For both studies it should be noted that was tested if fearful behavior still existed in these animals. Using a fear conditioning procedure it was shown that cs-shock associations were still present.

**Concluding remarks**

Although experimenter-administered models of addiction have revealed much of today's knowledge of acute en chronic effects of addictive substances, contingent models are more preferable because of their broad application and strong face validity. Recent studies have evidenced that prolonged use is an important factor that determines the sensitivity for different addiction-related behaviors like reinforcement, motivation and reinstatement.

Both two-bottle preferences test and operant self-administration provide the possibility to look at compulsive drug-seeking related behaviors. The adulteration of alcohol with quinine and the administration of both footshocks and aversive conditioned stimuli have proved to be successful in demonstrating compulsive behavior in animals following extensive drug self-administration. Interestingly, conditioned suppression in animals with extended cocaine experience was impaired in all animals while directs suppression with footshocks affected only a sub-population, suggesting different underlying mechanisms and a certain vulnerability for addiction-like behaviors which is also present in humans. As addiction affects not all human drug users the presence of an animal sub-population in which punishment-resistance occurs seems to represent a true model.

This said, it should be noted that these models are limited to only one aspect of addiction, indicating that they do not represent a full blown addiction. Yet, Deroche-Gamonet and colleagues (2004) combined punishment resistance with two other criteria for addiction-like behaviors into one models of addiction. They examined animals for the following three criteria for addiction-like behavior: difficulty stopping, high motivation and the continued use despite aversive consequences. A correlation analysis relate these addiction-like behaviors to the propensity for reinstatement, thereby linking addiction-like aspects with a certain predisposition for relapse (Deroche-Gamonet et al., 2004). Interestingly, the percentage of animals that met all three criteria (17%) is similar to the human cocaine users diagnosed with addiction.

# Neurobiology of compulsive behavior in addiction

The compulsive character of substance use in addiction is a consequence of pathological changes in the brain. But where do these changes take place in the brain? And more importantly, how can we target neurobiological substrates to compensate for these pathological changes? Animal models for addiction can be used address these questions. The multiple animal models that capture different aspects of addiction have revealed genetic, neurochemical, cellular and circuitry mechanisms that contribute to these behavioral characteristics of addiction. Most studied is this respect is reward sensitivity in general, which is mediated by the mesolimbic dopamine system.

**The mesolimbic dopamine system and reward sensitivity**

The mesolimbic dopamine system has been the major focus area for reward and motivational behavior (Wise and Rompre, 1989; Wise, 2004). This part of the dopamine system links the midbrain, in particular the ventral tegmental area (VTA), with different parts of the limbic and cortical structures via dopaminergic transmission. The limbic system contains different nuclei like the hippocampus, amygdala, and nucleus accumbens (NAc). Cortical structures part of the dopamine system are the prefrontal cortex, orbitofrontal cortex and the anterior cingulate. The mesolimbic dopamine system supports goal-directed behavior, indicating that it helps selecting and executing actions according to their predicted consequences. Goal-directed behavior is known to develop in parallel with the dopamine system (Naneix et al., 2012). Regarding reward, dopamine is thought to attach strong motivational value to an external -rewarding or neutral- stimulus(“incentive salience”), thereby preparing the organism to adapt its behavior should a motivational event reoccur (Kalivas and Volkow, 2005). The adaptation is supported by conditioned stimuli that are thought to predict reward by the release of dopamine (Schultz, 1998). Early studies on reward have mainly focused on the mesoaccumbal pathway, which comprises dopaminergic projections from the VTA to the NAc shell and core (Olds and Milner, 1954). Just like natural rewards such as food (McCullough and Salamone, 1992; Yoshida et al., 1992), water (Yoshida et al., 1992; Young et al., 1992) and sex (Pfaus et al., 1995), addictive substances enhance dopamine transmission in the NAc, as measured by increased extracellular dopamine concentrations (Di Chiara and Imperato, 1988). Unlike natural rewards, the effects of repeated exposure to addictive substances is not habituated, but continuously activates dopamine transmission (Di Chiara, 1999), resulting in a persistent effect that promotes further use.

The role of the mesolimbic dopamine system in the reinforcing effects of addictive substances has been extensively investigated by studies of self-administration, self-stimulation or conditioned place preference. By manipulating the dopaminergic system using DA synthesis blockers or antagonists and excitotoxic or 6-OHDA lesions numerous studies have revealed a differential effects of addictive substances. Summarizing these studies, the mesolimbic dopamine system is crucial for the rewarding effects of psychostimulants like cocaine and amphetamine, and is important but not necessary for the rewarding effects of other addictive substances like opiates, nicotine, cannabis and ethanol (Koob and Nestler, 1997; Pierce and Kumaresan, 2006; Wise and Rompre, 1989). This indicates that other neurotransmitter and neuromodulator systems are involved in the acute reinforcing effects including opioids, cannabinoids, glutamate, γ-aminobutyric acid (GABA) and serotonin. In particular, activation of opioid receptors in VTA and NAc directly by opiates like heroine and morphine or trough other mechanisms by alcohol and psychostimulants can mediate the reinforcing properties (Olive et al., 2001; van Ree et al., 1999). Summarizing, both dopamine-dependent and dopamine-independent mechanisms contribute to the direct rewarding and pleasurable effects of addictive substances.

**Changes following chronic drug exposure**

Repeated exposure to addictive substances is known to induce neuroadaptive changes that facilitate learning of association between and the drug-taking process and actions or environmental stimuli related to this process. At the molecular level these neuroadaptations comprise the activation of transcription factors like CREB and ΔFosB and their up and downstream targets (for reviews see Nestler and Aghajanian, 1997; Nestler, 2001). Cellular changes in dendritic and spine morphology in mesolimbic structures have been reported after chronic exposure to amphetamine, cocaine, nicotine and morphine (Robinson and Kolb, 2004). Furthermore it was found that long term depression (LTD) is impaired in the nucleus accumbens of rats meeting the three criteria for addiction-like behavior derived from Deroche-Gamonet (2004), indicating affected synaptic plasticity (Kasanetz et al., 2010). These gradual brain adaptations are thought to contribute to the pathological consequences of repeated drug use.

**Neurobiology of compulsive drug use**

As described in the chapter on Animals models of addiction, compulsive behavior in addiction has been demonstrated in animal models of punishment resistance in operant self-administration and aversive-resistant drinking in the two-bottle tests. Different perspectives about the possible underlying mechanisms of compulsive behavior have been put forward. Theories of impaired cognitive control, pathological habit formation, incentive sensitization

and hedonic allostasis have been implicated in compulsive drug use (Vanderschuren and Everitt, 2005). These suspected mechanisms have different neurobiological substrates, but can all be reduced to the mesocorticolimbic system.

*Amygdala*

For its functioning in negative emotional processing, fear-conditioning and stimulus-reward learning (Baxter and Murray, 2002) increasing studies have hypothesized a role for the amygdala in compulsive behavior. The amygdala has been shown to be reduced in alcoholics (Makris et al., 2008). Both the basolateral amygdala (BLA) en central nucleus of the amygdala (CeA) have been implicated in compulsive drug use. Both regions have distinct roles in fear-conditioning as the CeA is known to facilitate suppression of behavior elicited by an aversive conditioned stimulus and the BLA is suggested to be involved in the formation of the CS-US associations (Killcross et al., 1997). Both structures may therefore have distinct roles in aspects of addiction as well.

The CeA has been implicated in before in different aspects of addiction such as the time-dependent increase in cocaine drug-seeking (Lu et al., 2005; Lu et al., 2005). The role of the CeA in compulsive drug use was only recently investigated by Lesscher and colleagues (2012). They found the adapter protein 14-3-3ζ to be strongly up-regulated in the CeA of mice with escalated ethanol intake. Downregulating this protein in mice elicited increases in alcohol intake and a persistent preference for aversive quinine-adulterated alcohol solutions (Lesscher et al., 2012). Another study investigated the role of the CeA in footshock-resistance drug seeking. Following 2 or 6 hours of cocaine self-administration in animals were punished with a footshock of different intensities. Self-administration was reduced at high intensities in both groups (2 and 6 hours), but remained stable in the 6h group. Inactivation of the CeA during punishment sessions resulted in an increase of responding in mice with both 2h and 6h access to cocaine. However, inhibition of the CeA also increased cocaine self-administration without punishment, suggesting a role for the CeA in reinforcement. Moreover, as punishment sensitivity differs between animals with extended and limited access to cocaine, one would expect also differences in responding after CeA inhibition between both groups. Though, no differences were found during CeA silencing (Xue et al., 2012).

The BLA is well known for its influence on cue-induced reinstatement of drug seeking (Lasseter et al., 2013; Quirk and Gehlert, 2003; Schroeder et al., 2008). The possible functioning of the BLA in compulsive behavior has been suggested by Pelloux and colleagues (2013) provide evidence for BLA involvement in punishment resistance in rats self-administering cocaine. Rats with a BLA lesion and short cocaine experience showed increased levels of self-administration during punishment sessions, at a stage were control

animals reduced their intake (Pelloux et al., 2013). However, fear conditioning was also reduced as a consequence of the BLA lesion, suggesting that impaired fear memory might play a role here. Moreover, it should be questioned if animals were in a state of compulsive drug use since no level of punishment-resistance was achieved. Nevertheless, parts of the amygdala might be involved in other suggested mechanisms underlying compulsive drug use such as behavioral inflexibility as suggested by Stalnaker and colleagues (2007). They demonstrated that lesions of the BLA diminishes the reversal learning deficits in OFC damaged rats (Stalnaker et al., 2007). Subsequently, electrophysiological measurements revealed that neurons in the BLA mediate the decision deficits in cocaine-exposed rats (Stalnaker et al., 2007).

*Prefrontal cortex*

The prefrontal cortex has, through its putative functioning in conflicts situations, decision making and inhibitory control, also been implicated in compulsive drug seeking (Feil et al., 2010; Jentsch and Taylor, 1999; Kalivas and Volkow, 2005; Stalnaker et al., 2009). Just as the amygdala, prefrontal regions have been found to be reduced in size in cocaine addicts (Makris et al., 2008). Direct associations between prefrontal dysfunction and cocaine use have been demonstrated in humans. Cocaine user have been found to show greater activation of OFC and less activation in the dorsolateral prefrontal cortex (DLPFC) and medial prefrontal cortex (MPFC) in an Iowa Gambling task which is a decision-making task (Bolla et al., 2003). In go/no-go tasks and its variant stop-signal reaction time test have evidenced impaired behavioral inhibition in cocaine users and have found reduced activity in the anterior cingulated cortex (ACC)(Fillmore and Rush, 2002; Hester and Garavan, 2004). In animals self-administering cocaine, severe reversal learning impairments were found in a odor-guided go, no-go odor discrimination task after a period of withdrawal (Calu et al., 2007). Using a visual discrimination-reversal learning task and an attentional set shift task these impairments were also found in animals with a history of non-contingent methamphetamine (Izquierdo et al., 2010), but also with self-administration (Parsegian et al., 2011). Different regions of the PFC have been subject of investigation over the past years. Recent findings have evidenced a role for the prelimbic cortex (PrL) in drug seeking in the presence of aversive conditioned stimuli. In animals, it was shown that DA levels increases following aversive taste stimuli (Bassareo et al., 2002). Rats with limited history of cocaine self-administration showed less conditioned suppression after inactivation of the PrL (Limpens et al, unpublished[a]), one of the first studies implicating the PrL in punishment resistance. Using the seeking-taking schedule as described before, Chen and colleagues (2013) investigated the role of the PrL in aversive-resistant responding. They used *ex vivo* electrophysiology to determine the intrinsic excitability of the PrL from shock-sensitive and

shock-resistant rats. A reduced excitability was found in both shock-sensitive and in greater amount in shock-resistant rats. Furthermore it required more current to elicit action potential in shock-resistant rats compared to shock-sensitive and naïve rats. To test if activation could reverse compulsive drug seeking prelimbic region was stimulated using optogenetics, a technique that uses light-activated channels to excite or inhibit neurons. Therefore, PrL cortex neurons of shock resistant rats were injected with adeno-associated virus (AAV) encoding Channelrhodopsin 2 fused with eYFP (ChR2-eYFP). Photoactivation with optic fibers during the seek part of shock sessions reduced responding in the compulsive animals. Inhibition of the PrL using AAV encoding Halorhodopsin-eYFP (eNpHR3.0-eYFP) increased responding of shock-sensitive animals (Chen et al., 2013). These results suggest a prelimbic hypofunction mediating compulsive behavior in a subpopulation of rats self-administering cocaine. In addition to the PrL, Seif and colleagues (2013) studied the glutamatergic input from both insula (INS) and mPFC into the NAc core in aversion-resistant alcohol intake. Following a prolonged period of alcohol consumption, rats receiving the NMDAR blocker AP5 into the NAc core showed significantly reduced quinine-adulterated alcohol intake, with no effect in saline-treated animals or quinine-free alcohol solutions. Inhibiting mPFC and INS inputs into the NAc core using optogenetic silencing decreased adulterated alcohol intake in aversive-resistant rats, but not quinine-free alcohol intake. To test if the same mechanism drives footshock-resistant responding, rats were trained for operant self-administration of alcohol. Inhibiting the input from both mPFC and INS to the NAc core reduced responding in footshock-resistant rats. A following electrophysiological experiment discovered the presence of NMDARs active at hyperpolarized potentials in the mPFC and INS to the NAc core of alcohol drinking rats. By blocking the Grin2c subunit of NMDARs quinine-adulterated alcohol intake was reduced. Importantly, intake was not altered when alcohol was presented in the absence of aversive stimuli (Seif et al., 2013). Both studies suggests that compulsive intake is rather a consequence of impaired inhibitory control and decision making resulting from medial prefrontal dysfunction than an impairment of striatal area's involved in the habitual intake. Remarkably, contrary results were obtained in the inhibition by optogenetics of both medial prefrontal circuits, namely an increased responding for cocaine in punishment-resistant animals and a decreased responding for alcohol in aversive-resistant animals, reflecting perhaps the difference in substance or the site of stimulation. Recently, more evidence was found for the involvement of glutamate receptors in compulsive drug seeking behavior. LTD in the metabotropic glutamate receptor 2/3 (mGlu2/3) was diminished in rats showing addiction-like-behavior including aversive-resistant drug seeking. The same animals showed also an increased AMPA/NMDA ratio, indicating the presence of postsynaptic plasticity and perhaps the result of impaired LTD as earlier mentioned (Kasanetz et al., 2013).

*Dorsolateral Striatum*

The putative role for a the DLS in stimulus-response habitual processes suggests also engagement in the compulsive aspects of addiction. Although its role in habitual drug seeking has been suggested for quite some years (Everitt et al., 2001), only recently is was shown that the shift from goal directed to habitual drug seeking after extended cocaine access is mediated by the DLS (Zapata et al., 2010). Only recently, it was discovered that the DLS might has a function in compulsive drug seeking as well.

A study by Jonkman and colleagues revealed that the inactivation of the DLS reduced responding under punishment, but not under normal conditions (Jonkman et al., 2012). In addition, it was shown that inactivation of the DLS reduces responding during aversive conditioned stimulus presentation in rats with extended access to cocaine (Limpens, unpublished[b]). Both studies confirm the role of the DLS in compulsive drug seeking.

**Concluding remarks**

Although the amygdala has been associated with compulsive drug seeking behavior in different studies, its primary role in compulsive, punishment-resistant drug seeking behavior has not been evidenced yet, as in different studies manipulation of amygdala functioning not only affected punishment-resistant behavior but also fear conditioning and punishment-independent drug intake. Therefore, fear-related functioning of the amygdala seem not to be cause of compulsive drug seeking as fear is still present in animals showing punishment resistance in operant self-administration (Pelloux et al., 2007; Vanderschuren and Everitt, 2004). Related to its role in reward and aversion, a more distal role for the amygdala in addiction seem plausible.

Increasing amounts of evidence have implemented the medial PFC in compulsive drug seeking. These studies have shown that the medial PFC mediates aversive-resistant drug seeking and not drug seeking in general. Moreover, a the Grin2 subunit of NMDAR was found mediating aversion-resistant behavior in alcohol self-administration. This molecular substrate is potential target for pharmacological therapies as it seem very specific in this case.

In addition to the critical role for the prefrontal cortex in compulsive drug use, the complete circuitry of the prefrontal cortex and other structures of the mesocorticolimbic system including the amygdale and striatum is likely to be important during the advanced stages of a full-blown addiction. Interestingly, functional connectivity between amygdala and prefrontal areas and striatum has been found to be decreased in chronic cocaine users (Gu et al., 2010; Ma et al., 2010). Future preclinical research investigating circuit has to confirm these impairments.

# Conclusion

The continued use of addictive substances despite the negative consequences represents a core feature of compulsive drug seeking. Here, I describe the animal models that have been used to investigate compulsive behavior and the underlying neurobiological mechanisms, showing evidence of a critical involvement of prefrontal regions in this behavior.

As more and more knowledge is gathered about the mechanisms underlying compulsive drug use, pharmacological therapies targeting substrates related to these mechanisms are in close proximity of clinical use.

The current use of the powerful tool of in vivo optogenetics in behavioral neuroscience offers also opportunities for more practice in addiction research. As there is increasing knowledge about the brain circuitries involved in compulsive drug use, optogenetics provides the possibility for profound research by selectively exciting or silencing these circuitries.

Although beyond the scoop of my research, it seem more and more interesting to explore the link between the neurobiological pathology described in this review and the genetic, perhaps epigenetic and environmental factors defining the vulnerability of addicted individuals.

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