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Compulsive drug use and its neural substrates

Abstract: Drug addiction is a chronic relapsing brain disease, characterized by compulsive drug use. Despite the fact that drug addiction affects millions of people worldwide, treatments for this disorder are limited in number and efficacy. In our opinion, understanding the neural underpinnings of drug addiction would open new avenues for the development of innovative treatments for this disorder. Based on an awareness that drug use and drug reward do not equal drug addiction, there has been increasing interest in developing animal models of addiction that mimick the loss of control over drug use more closely than existing models aimed at studying drug reward. The present review provides an overview of animal studies of compulsive drug use and the neural mechanisms involved. First, the employed models are summarized, with a particular emphasis on models of escalation of drug use and resistance to punishment. Next, we discuss mechanisms within the (ventral and dorsal) striatum and (central) amygdala that have recently been implicated in the compulsive seeking and taking of alcohol and cocaine. The studies discussed here provide a promising line of research that will advance our knowledge of the neural circuits involved in the self-destructive behavior that characterizes drug addiction.

Keywords: alcohol; amygdala; animal model; cocaine; drug addiction; striatum.

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Introduction

Drug addiction is an enormous public health problem with major socio-economic and legal repercussions that affects millions of people worldwide. Drug addiction has been calculated to account for more than 40% of the financial cost to society of all major neuropsychiatric disorders (Uhl and Grow, 2004). However, only a small minority of drug addicts receive any form of treatment, which indicates tremendous room for improvement in reducing the burden of this disorder in terms of individual health, quality of life and costs for society.

Drug addiction is a chronic relapsing disorder that is characterized by loss of control over drug intake (American Psychiatric Association, 2000). During the development of addiction, casual drug use escalates to inappropriate and problematic drug use ('abuse'), ultimately culminating in loss of control over drug use and full-blown addiction, which is characterized by, among others, the occurrence of drug-related activities at the expense of previously important social and professional activities and continued drug use despite awareness of its adverse consequences. The last 5 decades have seen enormous progress in our knowledge of the neural underpinnings of the rewarding and motivational properties of drugs of abuse (e.g., Wise, 1996; Van Ree et al., 1999; Everitt and Robbins, 2005; Pierce and Kumaresan, 2006; Spanagel, 2009; Koob and Volkow, 2010). However, we are only beginning to understand the neurobiological mechanisms that underlie the genuine of loss of control over drug use that characterizes drug addiction (and contrasts it from mere drug use) (Everitt and Robbins, 2005; Kalivas and Volkow, 2005; Kenny, 2007; Koob and Volkow, 2010; Pierce and Vanderschuren, 2010; Ahmed, 2012). Quite clearly, it is not unreasonable to expect that advancing our insight into the neural underpinnings of addictive behavior may contribute to the development of better therapies that target the core of this devastating disorder.

The aim of this review is to describe recent progress in neurobiological research with respect to compulsive drug use. We will discuss recent developments in animal models for addiction, with particular focus on the escalation of drug intake and resistance to punishment, and discuss the evidence for addiction-like phenotypes in laboratory animals. Subsequently, we will discuss the neurobiological substrates that may govern the development of drug addiction, focusing on the (ventral and dorsal) striatum and amygdala, as recent animal studies have implicated these structures in the loss of control of drug seeking and taking.

Animal models for addiction

For many years, studies into the behavioral and neural background of drug addiction have employed models in which animals voluntarily ingest, or self-inject, a variety of drugs of abuse (Weeks, 1962; Thompson and Schuster, 1964; Panlilio and Goldberg, 2007). As stated above, these models have been invaluable in advancing our understanding of the neural mechanisms of drug reinforcement and of the neural changes that result from repeated drug intake (Hyman et al., 2006; Kalivas and O'Brien, 2008). However, there is an emerging awareness that drug use and drug taking, although essential stages in the development of addiction, are not equivalent to full-blown addiction. This has sparked research aimed at the development of animal models in which genuine aspects of addictive behavior can be observed (for reviews see Vanderschuren and Everitt, 2005; Ahmed, 2012; Vanderschuren and Ahmed, 2012). For the most part, the design of these models has been based on the diagnostic criteria outlined for drug addiction in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2000). These criteria include escalation of drug use, neurocognitive deficits, resistance to extinction, increased motivation for drugs, preference for drugs over non-drug rewards and resistance to punishment (Vanderschuren and Ahmed, 2012). Below, we will outline the experimental evidence for the occurrence of escalation of drug use and resistance to punishment. Arguably, these two factors can be regarded as early and late stages, respectively, in the descent from drug use to drug addiction. We focus on cocaine and alcohol, as they represent substances with great addictive potential, the abuse of which inflicts enormous damage on our society (O'Brien, 2001; Nutt et al., 2007) – yet one of these drugs is illicit, whereas the use of the other is legal and well-accepted in large parts of the world.

Escalation of drug use

Loss of control over drug use is, in most if not all cases of addiction, preceded by marked increases in drug intake. This makes the escalation of drug intake one of the earliest clearly visible signs of addictive behavior. Increasing drug intake is thought to evoke changes in brain function that facilitate the development of compulsive drug use (for reviews, see Hyman et al., 2006; Kalivas and O'Brien, 2008; e.g., Ahmed et al., 2005). However, it is also possible that escalation of drug intake is caused by the same changes in brain function that underlie the development of compulsive drug use. Human studies have shown escalation of drug use over time in certain individuals to be a precedent for developing drug addiction. For example, a longitudinal study by Palmer et al. (2009) reported a gradual increase in drug use over time, reflected by increased prevalence rates of occasional and repeated drug use as well as drug addiction. This study suggests that escalation of drug use indeed precedes the development of drug addiction. In fact, in another study, escalating smoking intensities were shown to predict the development of tobacco addiction. In particular, early rapid escalation of smoking posed the greatest risk for subsequently developing addiction (Karp et al., 2005). Thus, escalation of drug use is a hallmark feature in the development of drug addiction.

To the best of our knowledge, the earliest evidence of escalation of drug intake after repeated or prolonged drug use in rodents comes from a study by Wise (1973). In that study, rats that received intermittent access to alcohol (i.e., every other day) showed a gradual increase in alcohol intake over time. This escalation of alcohol intake over time in intermittent/every-other-day models has been confirmed by recent studies that have reintroduced this approach in the field of alcoholism research (Simms et al., 2008; Loi et al., 2010; Hwa et al., 2011; Melendez, 2011; Gilpin et al., 2012). Likewise, classic studies by Wolffgramm (1991), Wolffgramm and Heyne (1991, 1995) demonstrated that rats increase their levels of alcohol intake after lengthy periods (i.e., months) of relatively stable ethanol intake. Subsequent studies (Hölter et al., 1998; Spanagel and Hölter, 1999) employed periods of deprivation from ethanol to evoke escalation of ethanol intake, which is consistent with findings obtained in rats lever-pressing for alcohol (Rodd et al., 2003). Similar increases in operant ethanol self-administration emerge after repeated deprivations from forced ethanol exposure (Roberts et al., 2000; Valdez et al., 2002; Becker and Lopez, 2004; and, for review, see Koob, 2003; Crabbe et al., 2011). In 2005, the drinking-in-the-dark paradigm was introduced, in which mice consume high amounts of alcohol when water was replaced by alcohol in daily 2–4 h sessions early in the dark phase (Rhodes et al., 2005). Extending these studies to a limited-access choice paradigm, Lesscher et al. (2009a,b) demonstrated that mice that had access to alcohol for 2 h/day (with water always available) gradually increased alcohol intake over 4 weeks of testing.

Escalation of cocaine self-administration has received a large amount of research attention during the last decade. Early studies in rats and monkeys (Deneau et al., 1969; Johanson et al., 1976; Bozarth and Wise, 1985) had already shown that animals with unlimited access to

psychostimulant drugs or opiates would show erratic patterns of responding, with increases in drug intake over time sometimes clearly visible. Studies into the escalation of cocaine intake were sparked by a landmark study by Ahmed and Koob (1998), who demonstrated that rats with extended access to cocaine self-administration (i.e., 6 h/day) gradually increased their cocaine intake across days, whereas in rats with limited drug access (i.e., 1 h/day), cocaine self-administration was stable for months (Ahmed and Koob, 1999). This finding has since been replicated by many other laboratories (Ben-Shahar et al., 2008; Mantsch et al., 2008; Oleson and Roberts, 2009; Quadros and Miczek, 2009; Hao et al., 2010; Hollander et al., 2010; Pacchioni et al., 2011; for review see Ahmed, 2012).

The escalation of drug intake with prolonged or extended access is not exclusive for cocaine and ethanol. In both operant self-administration and oral ingestion setups, escalation of drug intake has been observed for other drugs of abuse, including the psychostimulants methamphetamine, amphetamine and methylphenidate (e.g., Kitamura et al., 2006), the opiates heroin and etonitazene (Wolffgramm and Heyne, 1995; Heyne, 1996; Ahmed et al., 2000) but, remarkably, much less so for nicotine (Paterson and Markou, 2004; Kenny and Markou, 2006).

Consistent with the notion that escalation of drug intake is an important step in the development of compulsive drug use, it has been shown that, after escalated cocaine or alcohol self-administration, other behavioral characteristics of addictive behavior can also be observed. These include increased motivation for the drug (Paterson and Markou, 2003; Liu et al., 2005; Lenoir and Ahmed, 2008; Wee et al., 2008; Orio et al., 2009), increased sensitivity for reinstatement of drug seeking after extinction (Mantsch et al., 2004; Ahmed and Cador, 2006; Kippin et al., 2006; Knackstedt and Kalivas, 2007) and reduced sensitivity to punishment (Wolffgramm, 1991; Wolffgramm and Heyne, 1991, 1995; Hölter et al., 1998; Spanagel and Hölter, 1999; Vanderschuren and Everitt, 2004; Lesscher et al., 2010; Ahmed, 2012).

Resistance to punishment

Continued drug use despite knowledge of adverse consequences is a hallmark feature of addictive behavior; it is one of the seven symptoms of addictive behavior listed in the DSM-IV (American Psychiatric Association, 2000). This feature of addiction has been the topic of animal studies in which continued drug use despite adverse consequences was operationalized as resistance to drug seeking and taking of punishment. Various forms of aversive and punishing stimuli have been used. These include adulteration of the drug solution (when it is orally ingested in the experiment) with the bitter tastant quinine or punishment of drug seeking or taking with lithium-induced malaise, mild electric footshocks or footshock-associated stimuli. However, although they are all aversive, footshocks, lithium and quinine, are perceptually and emotionally different stimuli that induce different forms and intensities of aversion or punishment, which makes them difficult to compare. Nonetheless, as will be described below, the use of such aversive/punishing stimuli has revealed that laboratory animals develop addiction-like behavior.

In their pioneering studies on the development of alcohol addiction in rats, Wolffgramm (1991) and Wolffgramm and Heyne (1991), not only demonstrated escalation of intake, they also observed resistance to punishment in the form of insensitivity to quinine. That is, the rats that showed elevated levels of alcohol intake also displayed a smaller reduction of alcohol intake when the solution was made bitter with quinine. This work has recently been followed up in two separate studies (Hopf et al., 2010b; Lesscher et al., 2010). One of these (Hopf et al., 2010b) used rats, to demonstrate that prolonged periods of intermittent access to alcohol [in a setup comparable to Wise (1973)] caused these animals to become insensitive to quinine when they self-administered ethanol under a progressive ratio schedule of reinforcement. Interestingly, this was not found in animals with a shorter alcohol history, or with continuous access to alcohol. Lesscher et al. (2010) used a limited access choice paradigm in mice. Resonating well with the findings of Wolfgramm (1991) and Wolffgramm and Heyne (1991), these mice readily escalated their alcohol intake and also became insensitive to quinine. Remarkably, this was apparent when the devalued solution was the only source of alcohol for the mice (i.e., the choice was between alcohol+quinine and water) after only 2 weeks of alcohol experience. After several more weeks of alcohol drinking, the phenotype became more intense, as the animals also became indifferent to quinine. That is, when they were offered alcohol+quinine vs. alcohol, they drank the bitter ethanol, even though a non-adulterated ethanol solution was simultaneously available.

A different approach that was applied paired oral ingestion of alcohol or cocaine solutions with lithium-induced malaise to evaluate responding for alcohol or cocaine after devaluation (Dickinson et al., 2002; Miles et al., 2003). These studies showed that, unlike responding to food or sucrose, responding to alcohol or cocaine during extinction sessions was insensitive to lithium

devaluation. Interestingly, during aversive conditioning, consumption of the devalued drug solution steadily declined, which is in apparent contrast with the studies above, where animals continued to consume a devalued alcohol solution. This may be the result of the relatively short period of instrumental training in the studies of Dickinson et al. and Miles et al., which rendered drug seeking, but not drug taking, insensitive to devaluation. Alternatively, the different means of devaluation (lithium-induced malaise vs. quinine adulteration) could also induce a different pattern of behavioral effects. Recently, these findings have been extended using a different way of devaluing the alcohol, i.e., by inducing satiety, allowing the animals to freely drink alcohol before a lever pressing test in extinction (Corbit et al., 2012). In keeping with the data outlined above, it was found that after limited alcohol self-administration experience, responding for alcohol was sensitive to satiety-induced devaluation, but that the sensitivity to devaluation was lost in animals with a prolonged alcohol history.

Other studies of resistance to punishment of drug seeking and taking have used mild electric footshock as the aversive stimulus. These approaches date back to classic studies using the so-called 'obstruction box', where rats have to cross an electrified grid to reach a reward (Jenkins et al., 1926). In the last decade, several studies have shown that cocaine seeking or taking, although initially very sensitive to punishment, becomes insensitive to the presentation of mild electric footshocks or footshock-associated conditioned stimuli after prolonged cocaine intake (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Pelloux et al., 2007; Belin et al., 2009; Ahmed, 2012; Jonkman et al., 2012a,b). Electric footshocks have also been used to determine the resistance to punishment of the reinstatement of extinguished drug seeking, a model for relapse. After conditioning in operant chambers where the animals had to cross an electrified grid floor to press for cocaine, exposure to a light cue that was previously paired to cocaine infusions induced reinstatement, albeit only in a subset of animals (Cooper et al., 2007). Of note, and in line with this finding, it has been reported that even after prolonged cocaine self-administration, there are large individual differences in the sensitivity to punishment (Deroche-Gamonet et al., 2004; Pelloux et al., 2007; Belin et al., 2009; Jonkman et al., 2012a,b), although resistance to punishment can also be found when the data are analyzed on a group level (Vanderschuren and Everitt, 2004; Ahmed, 2012; Jonkman et al., 2012b). Consistent with the notion put forward above, that escalation of drug intake facilitates the development of other signs of addictive behavior, it has recently been shown that the resistance to footshock punishment after prolonged cocaine self-administration is a result of extended cocaine intake, rather than merely aberrant drug-cue conditioning (Jonkman et al., 2012b).

Neurobiological mechanisms of compulsive drug use

Ventral and dorsal striatum

The striatum, in particular its ventral regions including the nucleus accumbens, is probably the most extensively studied brain region in relation to drug use and drug addiction. Studies into the role of the nucleus accumbens in addictive behavior date back to the 1970s (Roberts et al., 1977; Glick and Cox, 1978; Lyness et al., 1979). On the basis of the pioneering studies by Di Chiara and colleagues in the 1980s that showed that different drugs abused by humans all increase dopamine release within the nucleus accumbens (Di Chiara and Imperato, 1988), numerous pharmacological and molecular studies have substantiated the involvement of the ventral striatum in self-administration of different classes of abused drugs (Ikemoto and Wise, 2004; Pierce and Kumaresan, 2006). More recently, habit-based theories of addiction (Tiffany, 1990; Everitt et al., 1999; Everitt and Robbins, 2005; Pierce and Vanderschuren, 2010) have inspired work on the role of the dorsal striatum in drug use and addiction.

Cocaine

There is an extensive body of evidence implicating nucleus accumbens dopamine in the reinforcing properties of cocaine (for review, see Pierce and Kumaresan, 2006). The initial reinforcing properties of the drug that propel further drug taking likely depend on increases in extracellular dopamine levels in the ventromedial parts of the striatum, i.e., the nucleus accumbens shell and the olfactory tubercle. Thus, drug-naive animals self-administer cocaine into these regions (Rodd-Henricks et al., 2002; Ikemoto, 2003), and dopamine receptor blockade in the ventromedial nucleus accumbens shell inhibits the acquisition of cocaine self-administration (Veeneman et al., 2012). After establishment of cocaine taking, dopamine in both the nucleus accumbens shell and core has been reported to mediate the reinforcing properties of the drug (Caine et al., 1995; Bachtell et al., 2005; Bari and Pierce, 2005; Suto et al., 2009; Veeneman et al., 2012). The nucleus

accumbens core has been implicated in the impact of cocaine-associated conditioned stimuli on behavior (Di Ciano and Everitt, 2001; Fuchs et al., 2004; Ito et al., 2004), although this depends on glutamatergic rather than dopaminergic neurotransmission (Di Ciano and Everitt, 2001, 2004; Backstrom and Hyytia, 2007). With regard to loss of control over cocaine seeking, it was recently demonstrated that rats that display cocaine addiction-like behavior [i.e., exaggerated motivation for the drug, persistent drug seeking during periods of explicit non-availability and resistance to footshock during cocaine taking (Deroche-Gamonet et al., 2004)] show altered neuroplasticity in the nucleus accumbens core (Kasanetz et al., 2010). Thus, whereas a period of cocaine self-administration disrupted the induction of NMDA-receptor-dependent long-term depression, this blunted plasticity remained in animals that subsequently came to show addiction-like behavior, whereas the deficit recovered in animals that kept control over cocaine self-administration. These findings provide important information about the neural underpinnings of addiction, as opposed to mere drug taking. How the deficit in NMDA-receptor-dependent long-term depression in the nucleus accumbens core contributes to the behavioral signs of addiction remains to be demonstrated.

Studies focusing on the role of cocaine-associated stimuli in drug seeking and taking have indicated that the nucleus accumbens core might provide a gateway by which dorsal striatal mechanisms gain control over behavior, perhaps associated with habitual forms of drug seeking and taking (Everitt and Robbins, 2005; Pierce and Vanderschuren, 2010). These findings are buttressed by converging lines of evidence. Repeated exposure to psychostimulant drugs has been shown to facilitate the development of stimulus-response habits (Nelson and Killcross, 2006; Nordquist et al., 2007) that depend on the integrity of the dorsolateral striatum (Balleine et al., 2009). Furthermore, neuroadaptive changes in dopamine function have been shown to spread from ventral to dorsal striatal regions with increasing cocaine self-administration experience in primates (Letchworth et al., 2001; Porrino et al., 2004). These dorsal striatal mechanisms have been implicated in well-established cue-controlled cocaine seeking. Thus, in rats responding for cocaine-associated cues, extracellular dopamine concentrations rise in the dorsolateral, but not the ventral striatum (Ito et al., 2000, 2002), and infusion of a dopamine receptor antagonist into the dorsolateral striatum reduces cue-controlled cocaine seeking (Vanderschuren et al., 2005; Belin and Everitt, 2008). The involvement of dorsal striatal dopamine in cue-controlled cocaine seeking depends on the functional integrity of the nucleus accumbens core (Belin and Everitt, 2008), most likely through its glutamatergic input from the basolateral amygdala (BLA) (Di Ciano and Everitt, 2001, 2004). These findings are in keeping with the observation of increased dopamine activity in the dorsal (but not ventral) striatum during cue-induced cocaine craving in human addicts (Volkow et al., 2006; Wong et al., 2006). Recent studies have also implicated the dorsal striatum in the escalation of cocaine seeking (Hollander et al., 2010; Im et al., 2010). These studies identified a signal transduction pathway, involving the transcription factor MeCP2, the microRNA-212, cAMP response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) within the dorsal striatum (including both the dorsolateral and central dorsal striatum) in increased cocaine intake in animals with extended access to the drug. Although dopamine in the dorsolateral striatum is also involved in the reinforcing properties of cocaine itself (Veeneman et al., 2012), there is also direct evidence that dorsolateral striatal mechanisms contribute to habitual aspects and resistance to punishment of cocaine seeking (Zapata et al., 2010; Jonkman et al., 2012a). After extended cocaine self-administration experience under a seeking-taking chain schedule, drug seeking became insensitive to the devaluation (i.e., extinction) of the taking link, which demonstrates the habitual nature of cocaine seeking under these circumstances.

Pharmacological inactivation of the dorsolateral striatum restored the goal-directedness of cocaine seeking, i.e., rendered it sensitive to the extinction of the taking response (Zapata et al., 2010). In addition, pharmacological inactivation of the dorsolateral striatum reduced cocaine seeking when this was punished by probabilistic footshock, but not under unpunished conditions (Jonkman et al., 2012a).

Together, the data summarized above indicate that mechanisms within the striatum mediate the descent from casual to compulsive cocaine use by contributing to the escalation of cocaine intake, as well as to the resistance to punishment of cocaine seeking and taking.

Alcohol

The nucleus accumbens has also been implicated in alcohol consumption (Chen et al., 2011). Rat strains selected for high or low preferences for alcohol show marked differences in protein expression in the nucleus accumbens (Witzmann et al., 2003). Likewise, rats selected for alcohol preference showed a greater ethanol-induced dopamine release from the nucleus accumbens as compared to selected alcohol-avoiding rats (Bustamante et al.,

2008). These studies suggest that innate differences in the nucleus accumbens function contribute to individual variation in the sensitivity to alcohol reward. Indeed, the nucleus accumbens appears to be more sensitive to alcohol in selected alcohol-preferring rats as compared to Wistar control rats. For example, selected alcohol-preferring rats showed a greater sensitivity to acquisition of alcohol self-administration directly into the nucleus accumbens shell (but not core) in that they responded at lower doses of alcohol compared to Wistar rats (Engleman et al., 2009). Recently, alcohol-preferring rats were also shown to be more sensitive to acquisition of cocaine self-administration into the nucleus accumbens shell (Katner et al., 2011). This suggests that the innate differences between selected alcohol-preferring and non-preferring rats extend to other drugs of abuse and implicate the nucleus accumbens shell as an important neurobiological substrate for drug reward (Ikemoto and Wise, 2004; Pierce and Kumaresan, 2006). With prolonged consumption of alcohol, both chronic and in binges, protein and gene expression changes have been reported in the nucleus accumbens and other regions of the extended amygdala (Bell et al., 2006, 2009; McBride et al., 2010), which suggests that the nucleus accumbens contributes to both the initiation and the maintenance of alcohol drinking. A wide variety of studies indeed support this notion. For example, local knockdown of dopamine D1 receptors in the nucleus accumbens by RNA interference reduced ethanol consumption in previously ethanol-naive mice (Bahi and Dreyer, 2012). Using a similar viral transfer system, overexpression of 5-HT1B receptors in the nucleus accumbens was shown to enhance ethanol consumption in rats trained to consume ethanol (Hoplight et al., 2006). Maintenance of ethanol self-administration is reduced among others after the pharmacological blockade of mGluR5 receptors in the nucleus accumbens, but not the dorsomedial striatum or prefrontal cortex (Besheer et al., 2010) and deep brain stimulation of the nucleus accumbens shell and core (Knapp et al., 2009). Further, the nucleus accumbens also contributes to increased alcohol seeking that is observed during abstinence, i.e., incubation of alcohol seeking. For example, Bowers et al. (2008) showed a region-specific increase in the expression of a novel G-protein modulator AGS3 in the nucleus accumbens core during abstinence from operant ethanol self-administration. Local knockdown of AGS3 reduced the increase in alcohol seeking, normally seen in abstinence, to pre-abstinence levels (Bowers et al., 2008). More recently, the same research group showed reduced small-conductance calcium-activated potassium (SK) channel currents in the nucleus accumbens core of rats after abstinence from prolonged ethanol self-administration. Activation of SK channels reduced alcohol seeking in abstinence (Hopf et al., 2010a), which suggests that SK channels in the nucleus accumbens core facilitate alcohol seeking in abstinence, an important characteristic of alcoholism.

There is also emerging literature supporting a role for the dorsal striatum in alcohol use and addiction. Prolonged alcohol exposure leads to an array of neuroadaptive changes in the dorsal (as well as ventral) striatum (for review, see Chen et al., 2011). Evidence for a role of the dorsal striatum in alcohol drinking comes from studies that have implicated NMDA receptors in the dorsomedial striatum in the operant response to alcohol in rats, as well as in the reinstatement of extinguished alcohol seeking (Wang et al., 2010). Furthermore, BDNF signaling in the dorsolateral striatum controls operant responding for alcohol (Jeanblanc et al., 2009). Recently, the dorsolateral striatum has also been shown to be involved in habitual alcohol seeking (Corbit et al., 2012). In this study, alcohol was devalued by pre-exposing rats to alcohol before testing the response to alcohol in extinction. With a prolonged alcohol self-administration experience, the sensitivity to devaluation was lost, which indicates a habitual structure to this behavior. Pharmacological inactivation of the dorsolateral striatum restored the sensitivity to devaluation of alcohol seeking (Corbit et al., 2012). This latter finding is in keeping with the studies implicating the dorsolateral striatum in habitual or punished cocaine seeking (Zapata et al., 2010; Jonkman et al., 2012a).

Amygdala

It is becoming increasingly clear that the amygdala contributes to the development of compulsive drug use. Human alcoholics and cocaine addicts show reduced amygdala volume (Makris et al., 2004, 2008), which is associated with stronger craving and a greater propensity for relapse (Wrase et al., 2008). Functional MRI studies have repeatedly shown that drug-associated cues elicit stronger activation in the amygdala of drug addicts as compared to control subjects (e.g., Childress et al., 1999; Schneider et al., 2001). The amygdala is most widely studied for its involvement in emotional processing, fear and anxiety (Maren and Quirk, 2004; Phelps and LeDoux, 2005) but preclinical evidence suggests that the amygdala also contributes to the development of drug addiction. In particular, the central nucleus of the amygdala (CeA) has been shown to contribute to the escalation of alcohol intake and to the development of compulsive drug seeking. The CeA will therefore be the focus of this section.

The BLA also plays an important role in drug addiction, albeit in behavioral processes other than those discussed here. Most prominently, the BLA has been widely implicated in the influence of drug-associated cues on drug craving, seeking and relapse (for reviews, see Kalivas and McFarland, 2003; Bossert et al., 2005; See, 2005; Robbins et al., 2008).

Upon withdrawal from chronic alcohol exposure, rats and mice show increased alcohol intake (Roberts et al., 2000; Valdez et al., 2002; Koob, 2003; Becker and Lopez, 2004; for review, see Lopez and Becker, 2005; Crabbe et al., 2011). These behavioral changes are accompanied by increased levels of corticotropin-releasing factor (CRF), phosphorylated extracellular signal-regulated kinase (ERK) and reduced levels of, among others, neuropeptide Y (NPY), BDNF, glucocorticoid receptor and phosphorylated CREB in the CeA (Merlo Pich et al., 1995; Roy and Pandey, 2002; Roy et al., 2002; Sanna et al., 2002; Pandey et al., 2008). Furthermore, alcohol dependence in rats has been associated with enhanced GABAergic neurotransmission and release in the CeA (Roberto et al., 2004), which was reversible by treatment with CRF antagonists, NPY and nociceptin (Roberto and Siggins, 2006; Roberto et al., 2010; Gilpin et al., 2011). Involvement of the amygdala in alcohol intake in dependent animals is further supported by pharmacological and genetic studies. For example, excessive alcohol consumption and alcohol dependence is associated with gene and protein expression changes in the amygdala (Bell et al., 2006; Rodd et al., 2007; Contet et al., 2011). Further, increasing NPY levels in the CeA or CRF antagonism in the CeA abolished dependence-induced increases in alcohol intake (Funk et al., 2006, 2007; Thorsell et al., 2007; Gilpin et al., 2008a,b), whereas these treatments were without effect in non-dependent rats (Katner et al., 2002; Funk et al., 2007; Gilpin et al., 2008c; Henderson et al., 2010). Although studied most widely for alcohol, these findings also extend to other drugs of abuse. For example, rats withdrawn from chronic cocaine or cannabinoid exposure also display increases in amygdala CRF and vasopressin mRNA (Rodriguez de Fonseca et al., 1997; Richter and Weiss, 1999; Zhou et al., 2003; Zhou et al., 2005). Although the contribution of these changes to the escalation of cocaine or cannabinoid use remains unknown, the CeA has been implicated in the reinforcing properties of cocaine (Caine et al., 1995) and in time-dependent increases in drug seeking that are observed after extended withdrawal, a phenomenon known as incubation of drug craving (Grimm et al., 2001). For example, the increase in cocaine or opiate seeking with prolonged withdrawal is paralleled by increased extracellular dopamine levels (Tran-Nguyen et al., 1998) and AMPA receptor subunits (Lu et al., 2005a) in the CeA. Re-exposure to drug-associated cues caused increases in levels of phosphorylated ERK and similar increases in CREB phosphorylation (Lu et al., 2005b; Li et al., 2008) in the CeA of animals in prolonged withdrawal. Inhibition of ERK phosphorylation in the CeA, in turn, reduced the incubation of cocaine and opiate seeking (Lu et al., 2005b; Li et al., 2008). Together, these studies implicate the involvement of the CeA in the escalation of drug use upon withdrawal.

Recent studies have extended the evidence for the role of the amygdala in the escalation of drug use and the development of compulsive drug use. Using a limited-access choice paradigm, Lesscher et al. (2009a) have demonstrated that when mice had free choice between water and ethanol for 2 h/day, those animals gradually escalated their ethanol intake over 4 weeks of testing. Local knockdown of the protein kinase C (PKC) isozyme PKCepsilon in the CeA completely abolished the escalation of ethanol intake (Lesscher et al., 2009b). The amygdala PKCepsilon control over alcohol intake likely involves the regulation of CRF or GABA release in the CeA (Bajo et al., 2008; Lesscher et al., 2008). More recently, a gene expression study showed that the escalation of ethanol intake was paralleled by gene expression changes in the CeA. This study identified the adapter protein 14–3-3ζ as a novel candidate gene for CeA control over ethanol consumption was identified. Expression of 14–3-3ζ in the CeA was increased during escalation to high ethanol intake (Lesscher et al., 2012). Using RNA interference, the functional contribution of the novel candidate 14–3-3ζ in the escalation of ethanol intake was demonstrated. Local knockdown of 14–3-3ζ in the CeA led to a dramatic increase in ethanol consumption, which suggests that 14–3-3ζ may serve as a protective factor to dampen the escalation of ethanol intake. Moreover, CeA 14–3-3ζ was shown to contribute to the development of alcoholism-like behavior. As described above, in the limited-access choice paradigm, Lesscher et al. (2010) demonstrated that mice became insensitive to quinine adulteration after only 2 weeks of ethanol experience. Thus, the addition of an aversive quinine concentration to ethanol failed to reduce ethanol consumption in mice with 2 weeks of ethanol experience when this was their only source of ethanol, whereas, after 8 weeks, the mice also became indifferent to the aversive taste of quinine-adulterated ethanol. Development of this form of compulsive ethanol consumption involves CeA 14–3-3ζ: upon local knockdown of 14–3-3ζ in the CeA, the mice not only consumed more ethanol, they also showed a persistently high preference for the quinine-adulterated alcohol solution, which is indicative of inflexible alcohol drinking

(Lesscher et al., 2012). There are two likely explanations for amygdala control over the escalation of alcohol use and the development of compulsive drug use. First, the CeA may contribute to sufficient drug exposure for compulsive drug use to develop. As outlined above, multiple studies have demonstrated that the CeA contributes to the escalation to high levels of drug use – ethanol, cocaine and opiates. As a consequence, mechanisms within the CeA may determine the exposure to sufficient levels of drug use for resistance to punishment and, therefore, compulsive drug use to emerge. The second possible explanation for CeA control over the development of compulsive drug use is that the CeA may specifically contribute to punishment insensitivity as is typically seen in drug addicts and drug-dependent animals. The CeA is well-known to contribute to sensitivity to negative emotional stimuli and punishment (Maren and Quirk, 2004; Phelps and LeDoux, 2005; Sehlmeyer et al., 2009; Ciocchi et al., 2010). With regard to the resistance to punishment discussed here, it has been shown that lesions of the CeA reduced conditioned suppression, i.e., the suppression of ongoing operant behavior by a conditioned fear stimulus (Killcross et al., 1997). A recent study extended these findings and showed that the CeA also contributes to the sensitivity to punishment-induced suppression of cocaine self-administration (Xue et al., 2012). Taken together, there is substantial literature demonstrating the involvement of the amygdala in both the escalation of drug use and the development of compulsive drug use. Further studies are required to determine the molecular and behavioral mechanisms through which the amygdala controls the development of drug addiction.

Concluding remarks

In the present review, we have summarized studies aimed at investigating signs of addiction-like behavior in animals. Over the past 15 years, evidence has accumulated that animals not only seek and take drugs, but also show behaviors that resemble certain aspects of addiction in humans (Vanderschuren and Ahmed, 2012). Here, we have focused on two aspects of addiction-like behavior, i.e., escalation of intake and resistance to punishment, and discussed the evidence to support the existence of these two aspects of addiction-like behavior for cocaine and alcohol. More recently, researchers have used these models to study the neural substrates of addiction-like behavior in animals. These studies have indicated the involvement of the dorsal striatum and CeA in the escalation of cocaine and alcohol intake and the resistance to punishment of cocaine and alcohol seeking and taking. This provides us with important new leads to understand the neural basis of drug and alcohol addiction.

Several outstanding questions remain. First, there are marked individual differences between animals in the development of addiction-like behavior that may be related to specific behavioural traits and their neural underpinnings. For example, high impulsivity and high novelty preference (but not high novelty responsivity) have been shown to predict the development of compulsive cocaine seeking (Belin et al., 2008, 2011). It is therefore of interest to investigate whether the neural changes underlying compulsive drug use described here more readily develop in these vulnerable individuals. Second, future studies must address the issue of how the brain regions discussed in the present review interact to mediate the loss of control over drug use that characterizes addiction. There is no direct projection between the striatum (either dorsal or ventral) and the CeA, but there is an indirect projection from the CeA to the dorsal striatum through its projection to the substantia nigra pars compacta (Han et al., 1997; Pitkanen, 2000). Interestingly, a recent study has shown that disconnection of the CeA and the dorsolateral striatum as well as inactivation of the anterior CeA restored sensitivity of sucrose seeking to devaluation with lithium chloride, which demonstrates the importance of a CeA-dorsolateral striatal circuit for habitual behavior (Lingawi and Balleine, 2012). It is therefore quite conceivable that adaptive changes in this pathway are involved in the development of compulsive drug use. Clearly, the neural network that becomes engaged during the descent from drug use to full-blown addiction is far more extensive than this. Thus, neuroplastic changes in the nucleus accumbens core contribute to a loss of control over cocaine self-administration (Kasanetz et al., 2010), and these may be conveyed to dorsal striatal substrates of compulsive drug seeking, perhaps through spiraling striato-nigro-striatal loops (Nauta et al., 1978; Haber et al., 2000) or other pathways that indirectly connect the dorsal and ventral striatum (Ferreira et al., 2008; van Dongen et al., 2009). Furthermore, there are several neural mechanisms of compulsive drug seeking and taking that have not been discussed in depth here, but that may contribute to such a network. For example, marked gene expression changes within the lateral hypothalamus have been found after the escalation of cocaine self-administration in rats (Ahmed et al., 2005), and the lateral hypothalamus is known to receive a dense projection from the nucleus accumbens shell (Heimer et al., 1991) and to send excitatory projections to dopaminergic neurons in the ventral tegmental area (Geisler et al., 2007; Watabe-Uchida et al., 2012). Last, the prefrontal cortex,

which is thought to underlie the cognitive control over drug seeking (Kober et al., 2010) that breaks down in addiction (Everitt and Robbins, 2005; Kalivas and Volkow, 2005; Robbins et al., 2008; Koob and Volkow, 2010) should be incorporated into the amygdalo-striatal circuit of addiction presented here. In particular, dysfunction of the prefrontal cortex is thought to underlie the neurocognitive deficits associated with drug addiction (Rogers and Robbins, 2001; Goldstein and Volkow, 2002; Bechara, 2005; Dom et al., 2005; Garavan and Stout, 2005; Robbins et al., 2008), as well as the risk for relapse to drug seeking (Kalivas and McFarland, 2003; Bossert et al., 2005; Paulus et al., 2005; See, 2005; Beck et al., 2012). Indeed, neuroplastic changes in the prefrontal cortex have recently been found to be associated with compulsive cocaine self-administration in rats (Kasanetz et al., 2012). Concerted research into the prefrontal-striatum-hypothalamus-amygdala circuit that underlies addiction should reveal how altered function in this network causes the loss of control over drug use that characterizes addiction.

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