Addiction and its brain science

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ABSTRACT

Aims To illustrate how modern neurobiological approaches will help to identify the neurocircuits and genes involved in addictive behavior.

Background The current disorder concept of addiction includes neurobiological foundations and neurobiological research assuming irreversible molecular and structural changes within the brain dopamine reinforcement system, constituting the 'molecular and structural switch' from controlled drug intake to compulsive drug abuse. However, those irreversible changes have not so far been identified and it is suggested that in addition to the mesolimbic dopamine system, other brain systems including the mesocortical and nigrostriatal pathways as well as their non-dopaminergic feedback-loops might be involved in addictive behavior.

Neurobiological approach A three-step neurobiological approach is described that allows in a first step via novel animal models and imaging techniques to identify the neuroanatomical sites mediating voluntary drug intake, reinstatement of drug-seeking behavior, relapse, loss of control and drug intake despite negative consequences. In a subsequent step, forward genetic approaches including quantitative trait loci (QTL)-analysis and gene expression profiling are helpful in identifying so-called candidate genes. In a final step, conditional animal mutants and selective pharmacological tools are used to functionally validate candidate genes. Following this validation process, the transfer to the human situation has to be made and candidate genes have to be verified further in well-phenotyped cohorts of addicted patients.

Conclusion This three-step neurobiological approach, that must involve an interdisciplinary team including experimental psychologists, geneticists, molecular biologists and finally clinical addiction researchers, will allow us to understand where and how the addicted brain goes awry.

KEYWORDS Animal models of addictive behavior, association studies, conditional mutants, gene expression profiling, neuroimaging, QTL-analysis.

INTRODUCTION

Addiction can best be defined as a behavioral syndrome, characterized by compulsive drug seeking with repeated relapses into drug use. Addictive behavior may even recur after many years of abstinence in spite of obviously disastrous consequences for the individual, including his death. Other phenomena, such as physical dependence and withdrawal, have to be strictly separated from addictive behavior as an individual can be physically dependent on a drug without being addicted to it and vice versa. In fact, transient and demonstrably reversible adaptive processes within the central nervous system (CNS) and other physiological systems underlie physical dependence and tolerance to a drug. Also, addicted patients do not typically relapse while in a state of withdrawal. Instead, most relapse events occur when withdrawal symptoms have long dissipated. Clearly, more

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persistent, perhaps irreversible changes within specific neuronal systems must bring about addictive behavior. Among these neuronal systems, the last two decades have seen a tremendous focus on those which mediate positive drug reinforcement as the underpinnings of addiction. One of the major findings to have been observed is that the mesolimbic dopamine system constitutes the core brain reinforcement system, which highlights and predicts important environmental stimuli. Indeed, blockers of the dopamine system induce a 'lock-in situation' in humans, in which relevant environmental stimuli are no longer salient to the organism. Also, drug stimuli and conditioned stimuli that become associated to drug-taking behavior beget their salience to the organism through the mesolimbic dopamine system (Spanagel & Weiss 1999). Further, studies on the interaction of drugs of abuse and the mesolimbic dopamine system have additionally furnished a major hypothesis in the neurobiology of addiction, suggesting that irreversible synaptic alterations on the molecular and/or structural level (i.e. persistent drug-induced synaptic plasticity within the dopaminergic reinforcement system) may underlie addictive behavior.

DO IRREVERSIBLE MOLECULAR AND STRUCTURAL CHANGES WITHIN THE MESOLIMBIC DOPAMINE SYSTEM UNDERLIE ADDICTIVE BEHAVIOR?

Irreversible changes of behavior due to chronic drug use must have neural substrates at the molecular and/or structural level. Before focusing on these, however, we need to consider that a substantial number of addicted individuals spontaneously recover from drug dependence as defined by *Diagnostic and Statistical Manual* version IV (DSM-IV) criteria, often without formal treatment or participation in self-help groups (Dawson *et al.* 2005).

This prompts the question of whether the transition from controlled drug taking to an addiction really is a uniformly irreversible process. One important possibility is that current diagnostic criteria capture a highly heterogeneous population, within which treatment-seeking subjects may be very different from those who fulfil diagnostic criteria in epidemiological studies but who do not seek treatment. In cases where addiction does persist, irreversible changes at the molecular level might indeed be implied.

Yet this 'molecular switch', which defines the irreversible transition from controlled to compulsive drug use, has so far not been identified. It has been claimed that transcription factors such as ΔFosB may constitute such a molecular switch (Nestler 2004). A transcription factor is a protein required to initiate or regulate the expression of specific target genes through sequence specific binding to a regulatory element of a gene. Usually, a transcription factor such as ΔFosB controls the expression of a variety of target genes and may thereby induce synaptic plasticity. ΔFosB has been shown to accumulate in the mesolimbic dopamine system in response to chronic application of most drugs of abuse, but it does not remain in the brain for more than a few weeks and can therefore hardly account for relapse events months or years into abstinence. A modulator of transcription factors named Per2, however, does remain upregulated in the brain for several weeks following the cessation of chronic drug treatment (Chen *et al.* 2004; Ammon-Treiber & Höllt 2005). Moreover, the importance of Per2 in regulating cocaine reinforcement (Abarca *et al.* 2002) and alcohol reinforcement (Spanagel *et al.* 2005) has been demonstrated recently. Evidently, more work needs to be undertaken, focusing on long-term changes of transcription factors and their modulators in order to more understand clearly whether those changes do, in fact, constitute a molecular switch from controlled to compulsive drug use. Another possibility, of course, is that even transient activation of certain transcription factors may be sufficient in inducing persistent changes in their downstream late response genes, which in turn may encode the phenotypic switch. No such changes, however, have yet been identified within the mesolimbic dopamine system.

Irreversible structural changes within the mesolimbic dopamine system could be another level of regulation behind the long-term behavioral dysregulation in addiction. In fact, long-lasting structural changes after the discontinuation of cocaine, morphine or nicotine exposure have been described. In particular, microstructural changes on dendrites of medium spiny neurons, which constitute an essential cell population within the mesolimbic dopamine system, have been identified (Robinson & Kolb 2004). However, these dendritic microstructural changes were not evident beyond 3 months after discontinuation of drug treatment. This argues against a 'structural switch' within the brain reinforcement system underlying an irreversible transition to addictive behavior.

In summary, neither irreversible molecular nor irreversible structural changes within the mesolimbic dopamine system have been identified so far. The lack of a demonstration of persistent changes within the core of the brain reinforcement system could be due to the possibility that we are not looking at the right neuronal system. The fact that, over the years, a multitude of publications have studied drug-induced changes within the mesolimbic dopamine system in relationship to addictive behavior, should not suggest automatically that there ought to be a causal link between these specific neurobi-

ological changes and addictive behavior. It is not that there is a lack of other candidate systems. Obviously good contenders, which are finally beginning to receive their deserved attention, are systems, pathologically recruited, mediating negative affect and thus setting the scene for negative rather than positive reinforcement by drugs (Ahmed & Koob 2005). Every clinician is well aware that negative affective states dominate in the late, clinical stages of addiction, and the lack of this insight portrays well the insufficient communication between basic and clinical research in addiction biology.

But there is more. Drugs of abuse produce strong habits, rendering the possibility that we might need to look into the nigrostriatal pathway, known to mediate those behaviors (Gerdeman *et al*. 2003). Indeed, some drug abuse researchers suggest that synaptic alterations within the midbrain reinforcement system appear to play a prominent role only in the early stages of controlled drug use, whereas synaptic plasticity in the dorsal striatum may contribute to the formation of persistent drug-related habits, when casual drug use progresses towards compulsive drug use and addiction. Yet another interesting, less explored system to look at could be a neuronal one, which mediates aversion and resistance to acute drug effects. The intake of a drug usually produces an intoxication signal, which conveys a negative feedback on further drug taking behavior. Thus, in most individuals, this is a powerful mechanism which effectively protects a person from uncontrolled drug taking. In fact, attenuated initial response to some alcohol effects is a highly predictive heritable risk factor for developing alcohol dependence (Schuckit *et al*. 2004). However, the neuroanatomical sites mediating intoxication signals are currently not well defined.

In conclusion, although long-lasting changes may occur within the mesolimbic system, which probably cause the enhanced salience of the drug and conditioned drug stimuli, other neuronal systems may finally be involved in drug craving and relapse. Thus at the neuronal circuit level, alterations in the mesocortical system including its glutamatergic feedback loop may cause compulsive drug-seeking and loss of control and may therefore trigger relapse behavior (Volkow & Li 2004). In addition, alterations in the nigrostriatal system may contribute to the habit-forming properties of drugs of abuse and may therefore be involved in stereotypic, rigid behavioral patterns contributing to relapse. Therefore, in order to understand addictive behavior, molecular and structural changes within different neuronal circuits should be considered. In addition, we should realize that a majority of pre-clinical addiction research is carried out in acute paradigms using non-dependent subjects. It is clinically clear that the time-course for developing addiction encompasses a period of years to decades, and that it may therefore require a long time for ultimately irreversible synaptic alterations to occur. Thus, for understanding the neurobiological foundation of drug addiction, it is an essentially primary precondition that we use appropriate animal models which mimic addictive behavior.

ANIMAL MODELS FOR ADDICTIVE BEHAVIOR ARE A PREREQUISITE FOR THE SEARCH OF A NEUROBIOLOGICAL FOUNDATION OF DRUG ADDICTION

Most of the research conducted in the pre-clinical addiction research field does not address addictive behavior *per se*, but rather examines chronic drug consumption or administration and associated consequences such as tolerance and physical dependence. Although drug self-administration procedures have high face validity to what is observed in humans in respect to drug intake behavior, it may be questioned as to whether they provide us further insights into addictive behavior. However, as described in the following section, some of the characteristics of an addictive behavioral syndrome such as reinstatement of drug-seeking behavior, relapse, loss of control and drug intake despite negative consequences, can be modeled satisfactorily in laboratory animals.

Drug seeking in animals—the reinstatement model

The most common procedure to study drug-seeking behavior has been the so-called reinstatement model (Shaham *et al*. 2003). In this procedure, an animal is trained to self-administer a drug and the behavior is then subjected to extinction—that is, the animal is tested under conditions of non-reinforcement until operant responding appears to be extinguished. When the animal reaches some criterion of unresponsiveness, various stimuli are presented. A stimulus is said to reinstate the drug-seeking behavior if it causes renewed responding, i.e. lever pressing, without any further response-contingent drug reward. Reinstatement of drug-seeking can be used to study the neurobiological and molecular basis of craving and relapse, because there appears to be a good correspondence between the events that induce drug-seeking in laboratory animals and those that provoke craving and relapse in humans.

At least three events can reinstate responding: (i) drug priming—that is, the injection of a small dose of the drug, (ii) stress and (iii) conditioned stimuli. The data derived from studies using the reinstatement model suggest that the neuronal substrates that mediate drug-, stress- and cue-induced reinstatement are not identical (Shaham

et al. 2003) and therefore imply different neurobiological pathways in provoking relapse. Importantly, the reinstatement model already has some degree of pharmacological validation. Thus, acamprosate and naltrexone are known to reduce craving and relapse in alcoholic patients and can also reduce or even block cue-induced reinstatement of alcohol-seeking behavior (Katner *et al*. 1999; Bachteler *et al*. 2005).

The reinstatement paradigm is nowadays a well-established model in rats, and it is only recently that it has become possible to transfer this model to mice. A few preliminary studies with mice have been reported so far. However, in the future this paradigm will be used frequently to study genetically modified mice in order to identify precisely the genes and brain sites involved in drug craving and relapse. However, the usefulness of the reinstatement model in modeling human craving and relapse displays some limitations. First, the reinstatement test is performed under drug-free conditions. Thus, reinstatement occurs if the animal presses a lever, even though the lever-pressing does not result in drug delivery. In contrast, a typical relapse in drug addicts is defined as compulsive drug consumption following a period of abstinence; a relapse therefore cannot happen under drug-free conditions. Thus, having this definition in mind, it remains unclear why the reinstatement model was put forward as a model of relapse. This does not diminish the value of this model in measuring drug-seeking behavior, which is without a doubt one behavioral dimension of craving. In conclusion, the reinstatement model should be considered as a model of craving rather than relapse. Secondly, it appears that extinction of drug-seeking behavior usually plays only a minor role in addicted patients trying to achieve and maintain abstinence. Consequently, the animal reinstatement model may not reflect accurately the situation of abstinent addicts experiencing craving and/or relapse.

Relapse behavior in animals—the deprivation model

An animal model to study relapse behavior is the deprivation model. Drug-experienced animals show a transient increase in drug intake after a period of forced abstinence, which is termed the 'deprivation effect'. Deprivation effects can be observed in numerous species including rats, mice, monkeys and man, and show a high degree of reliability. An apparent limitation of this paradigm to model relapse is that clinical relapse is characterized by a loss of control and a period of drug intake, lasting many days or weeks, with the deprivation effect being transient. However, repeated cycles of forced drug intoxication and deprivation lead to an upregulated drug preference that persists for very long periods of time, or for the life-time of the individual (Rimondini *et al*. 2002, 2003). The deprivation model has so far been applied only to alcohol to study relapse drinking behavior (Spanagel & Zieglgänsberger 1997) and it needs more systematic work to examine whether a deprivation effect might also occur with other drugs of abuse. Although we do not fully understand the nature of a deprivation effect, the fact that the clinically effective anti-relapse drugs acamprosate and naltrexone also reduce or even abolish the alcohol deprivation effect (Spanagel & Zieglgänsberger 1997) lends predictive value to this animal model for the development of new and better drugs for the treatment of relapse.

Loss of control in animals—the temporal discount procedure

Addictive behavior is usually characterized as compulsive, meaning that there is a progressive loss in the ability to refrain from drug-related behaviors. Loss of control is considered as a core feature of addictive behavior. It has been proposed that loss of control could be characterized by wrong decision-making processes or by alterations in impulsive behavior (Bickel & Marsch 2001). In particular, the so-called 'impulsive choice' can be related to loss of control. Impulsive choice is defined as an abnormally high preference for small, immediate rewards over larger, delayed rewards and can be measured by the principles of temporal discounting. Here, two behavioral alternatives are presented to a subject, one offering access to a small reinforcer after a short delay and another that results in a bigger reinforcer after a larger but variable lapse.

Choosing a bigger but delayed reinforcer is then understood as a reflection of self-controlled behavior, whereas choosing a small but instant reinforcer is considered as an impulsive choice. Operant boxes offer a perfect means to establish temporal discount-based tasks in rodent models. Thus, two different levers are used, each one associated with a different reinforcement alternative. In such a procedure, it is fundamental that the levers representing each alternative are presented simultaneously and that the operant requirements are identical. Therefore, the two available options differ only in the delay and magnitude of the obtained reinforcer. The time between the response and the reinforcer delivery for the delayed reinforcer is then changed across discrete trials and finally discount rates are obtained.

Considering drug consumption as a choice between the immediate rewarding properties of the drug and the deferred benefits of abstinence, temporal discount procedures seem to represent a suitable method in exploring impulsive choice and loss of control in rodents. Face and construct validity of these procedures comes from the higher rate of discount displayed by drug addicts in similar tasks (Kirby & Petry 2004). Thus, heroin and cocaine

abusers have higher discount rates than non-drug-using controls. Interestingly, temporal discounting seems to be dependent on the nature of the delayed reinforcer, as in smokers the discount rates are larger for cigarettes than for money, and cocaine as well as heroin addicts display higher discount rates compared to alcoholics (Kirby & Petry 2004). Furthermore, temporal discounting in rodents is affected by the manipulation of brain substrates previously related to addiction (Cardinal *et al*. 2004). However, among the weaknesses of the temporal discount model, it should be noted that impulsive choice is not an exclusive feature of drug addicts but can also be observed during adolescence or in attention-deficit/hyperactivity disorder, as well as in other neuropsychiatric disorders.

Drug intake in animals despite negative consequences—modified conflict paradigms

Another criterion in defining addiction is the use of the drug despite its negative consequences. Thus, consuming a drug while being aware of its associated harmful and aversive consequences implies an enhanced motivation and a reduced elasticity in the behavioral repertoire of the individual. Any attempt to model this feature of addictive behavior should provide a scenario in which drug-seeking or drug-taking should persist regardless of any adverse consequences. In this respect, in a few experimental attempts traditional 'conflict paradigms' have been adapted to a situation in which the delivery of the self-administered drug results in the simultaneous delivery of an aversive stimulus. For example, Deroche-Gamonet *et al*. (2004) assessed the resistance to punishment during cocaine self-administration by delivering simultaneously the drug and an electrical shock. In this study, the suppression of cocaine self-administration by an electrical, painful shock was reduced in rats with a longer history of drug consumption and high sensitivity to cocaine-induced reinstatement.

Interestingly, this insensitivity to punishment also showed a good correspondence with other proposed measures of addictive behavior, such as drug seeking in the absence of drug delivery and enhanced motivation for the drug. However, the interpretation of data obtained in this model can be difficult, at least when using drugs of abuse which modify pain and/or anxiety thresholds such as morphine or ethanol. Some alternatives have been explored to surmount the problems created by the interaction between the pharmacological actions of drugs and the aversive, harmful events used in such a conflict-based model. Thus, Vanderschuren & Everitt (2004) have proposed the substitution of the harmful event (e.g. footshock) by conditioned stimuli associated to it (e.g. tone). This procedure also produces a diminishment of responding for cocaine that is not apparent in long-term cocaine self-administering rats; however, the suppressant effect of the conditioned stimuli is small. In addition, in this model, the aversive consequences are neither proportional nor contingent to the self-administration behavior, a fact that clearly separates it from the human situation. In the context of alcohol research, alcohol consumption in rats, despite taste adulteration with a bitter flavor such as quinine, has been understood as a rodent model equivalent to human drug consumption, despite harmful and aversive consequences. This procedure has the advantage that the effects of the drugs do not seem to interfere with the aversive properties of quinine. In addition, in this case the magnitude of the aversive event is contingent and proportional to the self-administration behavior. Importantly, the suppressant effect of quinine addition is not observed in long-term experienced rats (Wolffgramm & Heyne 1995). However, this procedure may have some difficulties when using other drugs of abuse because not all drugs are easily orally self-administered, although it should be noted that its suitability for amphetamine and the opiate etonitazene has been proven (Wolffgramm & Heyne 1995).

In summary, in the last decade, advances have been made in developing new animal models that mimic core features of an addictive behavior. The validity of animal models is assessed typically using three evaluation criteria including face, construct and predictive validity. Furthermore, reliability is also a critical issue in complex animal models as described here. Currently, cue-induced reinstatement and alcohol deprivation paradigms are the models that these issues have addressed most systematically. Considerable work remains to be conducted to establish whether measures obtained in these and the other models are valid and reliable. The refinement of these animal models and the characterization of specific reliable phenotypes within these models is a challenging process that should involve interdisciplinary research teams including experimental and clinical psychologists, clinicians and patients themselves. Nevertheless, these models can already be used for studying the neurobiological foundation of reinstatement of drug-seeking behavior, relapse, loss of control and drug intake despite negative consequences. But how can modern brain science use these models to identify the molecular changes underlying the transition from controlled to compulsive drug-seeking and -taking behavior? This search should involve a three-step research program: first, we have to identify where in the brain (i.e. neuroanatomical substrates) this transition takes place and secondly, we have to search for the molecular and neurochemical substrate. In the last step we have to validate candidate systems functionally to demonstrate their causal involvement in addictive processes.

BRAIN IMAGING AND OTHER APPROACHES TO IDENTIFY THE NEUROANATOMICAL SUBSTRATES OF ADDICTIVE BEHAVIOR

In the search for the neuroanatomical substrates of addictive behavior a variety of imaging techniques can be applied. Unfortunately, brain imaging in small laboratory animals such as mice and rats are hampered, as the brain sites of interest are tiny compared to the human brain and measurements can be performed only in anaesthetized animals. In contrast, with the use of a comfortable head restraint device, imaging in well-trained conscious monkeys can be performed and conditioned drug responses can be assessed (Howell & Wilcox 2002). However, the application of various brain imaging techniques to drug addicts have so far provided the most insights into the brain sites involved in craving and loss of control. For instance, while postulated persistent microstructural changes in the brain reinforcement system remain elusive, the 'simple' method of structural magnetic resonance imaging (MRI) has already demonstrated macrostructural changes that are highly likely to be clinically relevant. Pfefferbaum's group has, over the years, clearly documented the loss of frontocortical gray matter in chronic alcoholism (Rosenbloom *et al.* 2003). Given the established role of the frontal lobes in decision making and impulse control, it is clear that impairments here are likely to contribute to the vicious cycle of uncontrolled drug use. Indeed, measures of executive function subserved by the frontal lobes are significant predictors of relapse. Notably, it remains unclear whether non-alcoholic drinking affects the brain in a similar manner; two large epidemiological studies have arrived at conflicting results (Pfefferbaum 2004). Clarifying in press would appear to be of high public health relevance.

Using positron emission tomography (PET) imaging, disrupted orbitofrontocortical dopamine function has been shown in conjunction with several categories of addiction, with reductions in dopamine D2 receptor densities being most consistent, and potentially related to the intensity of craving and likelihood of euphorogenic drug effects (Volkow & Li 2004). Although, probably, most important for the initiation phase of addiction and possibly for the initial stages of a relapse episode, these findings demonstrate the ability of PET imaging to gauge behaviorally meaningful parameters of brain function. This approach is particularly attractive due to its potential for translations between animal and human studies, as illustrated by the parallel findings of dopamine D2 receptor downregulation accompanied by increased cocaine intake in subordinate primates (Morgan *et al.* 2002). Equally important are studies that demonstrate the activation of specific brain networks upon presentation of drug-associated cues (Grant *et al.* 1996; Childress *et al.* 1999). Activation of the amygdala seems to be a recurring feature of these paradigms, pointing outside the brain structures considered traditionally to be involved in mechanisms of drug reward and addiction, and into systems well known to the study of stress and negative affective states.

However, we are only at the beginning, and the potential of functional imaging techniques in addiction biology research extends far beyond these initial findings. Currently, functional MRI (fMRI) approaches are being used increasingly. An elegant illustration of their potential was demonstrated in the dissection of brain reward system activation into the incentive versus reward phases postulated on the basis of animal experimentation, and the demonstration of amphetamine modulating incentive-related activity (Knutson *et al.* 2001, 2004). The use of evolving pharmacological MRI offers a considerable potential for those of us who want to develop new treatments. In this context, not only can brain activation patterns, triggered by drug-related cues or the drug itself, be studied but also the way clinically effective drugs modulate them. When these approaches are established, the changes in brain activation patterns induced by existing anti-craving drugs will provide us with a signature which can be used as a short-term surrogate outcome, potentially predictive of clinical efficacy for other candidate drugs. In so doing, it would help us to widen one of the most acute translational bottlenecks between bench and bedside by helping to direct actual intervention trials, of which we can only do so many, to the clinically most promising compounds.

Another promising expansion of MRI that may be on the brink of a breakthrough is its use for spectroscopy of neurochemicals important for the addictive state. While energy metabolites can be quantified with this methodology for quite some time, it is only more recently that measures of central glutamate have begun to appear. It is not perfect at 3 Tesla as we are largely confined to measuring glutamate which comes from the metabolic pool; but at 7 Tesla it is more likely that glutamate can be measured that is directly involved in neuronal communication. One important application of measuring glutamate in the human brain is the search for responders to acamprosate treatment. Recent pre-clinical research demonstrated a hyper-glutamatergic state in the brain of alcohol-dependent animals which is blunted completely by acamprosate treatment (Bolo *et al.* 1998; Dahchour *et al.* 1998; Dahchour & De Witte 2003; Spanagel *et al.* 2005). Spectroscopic measures of glutamate in the human brain might therefore help to identify alcoholic patients that exhibit a hyper-glutamatergic state. According to the preclinical data, those patients should respond to acamprosate treatment. Finally, the marriage of imaging and

human genetics is unavoidable. To ask the question: 'Is there a statistical association between a particular genetic variant and the diagnosis of alcoholism?' requires that hundreds to thousands of subjects be studied. The studies are plagued by numerous sources of error, among which ethnic stratification is probably the most serious. To reverse the question, and to ask if there is a differential release of transmitter *x* or a differential activation of brain structure *y* being dependent on the patient's genotype, seems to have sufficient power so that it can sometimes be answered successfully using a dozen or so patients. This future has been outlined elegantly for us through a landmark study on endogenous opioid release in subjects differing in COMT genotypes (Zubieta *et al*. 2003) and amygdala activation in subjects with different genetic variants of the serotonin transporter (Hariri *et al*. 2002). This approach still awaits the addiction field for it to be put to use.

In summary, over the past decade functional neuroimaging has contributed greatly to our knowledge about the neuroanatomical substrates of addictive behavior. Numerous studies have shown common effects of drugs of abuse such as drug cue-induced activation of the frontal cortex and temporal lobe, and a hypofunction of the dopaminergic system—findings that have also be made by animal experimentation. All this research points to the extended amygdala, including the ventral striatum, the orbitofrontal cortex and possibly the dorsal striatum. It is likely that within these structure or their inputs that long-lasting or even persistent drug-induced synaptic plasticity takes place and it will be the challenge for molecular genetic research to identify those alterations.

THE USE OF GENETIC APPROACHES IN ANIMAL MODELS OF ADDICTIVE BEHAVIOR HELPS US TO UNDERSTAND THE MOLECULAR SWITCH FROM CONTROLLED TO COMPULSIVE DRUG USE

Reverse genetic approaches using knockout animals has provided the means to test the role of a gene in specific aspects of drug-related behavior. Because the environment can be carefully controlled in experiments with knockout animals, confounds induced by environmental factors such as stress can be avoided in animal genetics. Furthermore, specific 'gene–environment' interactions can be determined in knockout animal models. For example, it has been shown that the corticotropin-releasing hormone system, which mediates endocrine and behavioral responses to stress, contributes to stress-induced alterations in alcohol consumption using mice lacking a functional corticotropin-releasing hormone type 1 receptor (CRH1). Crhr1 mutant animals do not differ from wild-type mice in alcohol intake and preference under stress-free housing conditions. However, after repeated stress, Crhr1 mutant mice display a delayed and enhanced increase in alcohol consumption (Sillaber *et al*. 2002). This study exemplifies that a genetic alteration in a specific gene can be inconspicuous; however, as soon as a specific environmental factor fades in, a full-blown phenotype occurs. In a similar study, the role of the endocannabinoid system on alcohol consumption has been examined. Endocannabinoids are phospholipid metabolites that act as inhibitory retrograde transmitters in the brain. Adult mice with a targeted deletion in the cannabinoid CB1 receptor gene (Cnr1) show a similar preference for alcohol as wild-type animals. When exposed to an environmental stressor, an increase in ethanol consumption occurs only in the wild-type, but not in Cnr1 knockout animals (Racz *et al*. 2003). Thus, with respect to stress-induced alcohol consumption, the Cnr1 and Crhr1 mutations affect the animals' responses in opposite directions.

While studies with knockout animals have provided extremely valuable information about gene functions (Mohn *et al*. 2004), they often do not reflect naturally occurring genetic variations such as hypomorphic alleles with subtle alterations in regulatory sequences or coding regions. Moreover, it is conceivable that compensatory changes in knockout animals contribute equally, or even more, than the mutant allele to the phenotype. However, the most striking limitation of reverse genetics is that it is, by definition, limited to the study of those genes that have already been implicated in addictive behavior through previous studies. Therefore, a forward genetic approach should be favored in which candidate genes are derived from animal models by means of two complementary unbiased hypothesis-free approaches.

First, chromosomal loci that contribute to addictive behavior should be identified through genetic mapping. Evidence from animal experiments and human studies suggests that most naturally occurring allelic variations contribute small quantitative effects to drug-related traits. These allelic variations can be mapped to specific chromosomal regions, which are then called quantitative trait loci (QTL). QTL studies take advantage of the availability of inbred mouse strains that respond differently with respect to the trait of interest. Within an inbred strain, animals of the same gender are genetically just as similar as monozygotic twins. When animals of two different inbred strains are crossed, the F_1 offspring are heterozygous at all alleles in which the parental strains differ. When these F_1 animals are then intercrossed, the F_2 progeny will segregate genetically, thus producing animals with quantitative phenotypic differences. QTLs associated with behavioral phenotypes can be identified by cor-

relating the distribution of microsatellite markers or SNPs in many F_2 individuals with their behavioral responses. If a particular marker is found with a significantly higher frequency in animals of a similar phenotype, a nearby gene is likely to affect the trait.

Secondly, gene expression profiling methods allow the identification of putative candidate genes. A variety of analytical methodologies to investigate gene expression patterns in cells or brain tissues have been developed and applied to drug abuse research (Gebicke-Haerter 2005); however, the so-called microarray technology has, until the present, provided the most insights. This technology fulfils the dreams of many biomedical scientists in gaining detailed simultaneous snapshots of the quantities of all gene transcripts in a given tissue or cell type at certain times or under specific conditions. For example, gene transcripts in brains from rats that are resistant to punishment during cocaine self-administration can be compared to animals that have the exact same drug prehistory but are non-resistant to punishment (Deroche *et al.* 2004). Ideally, tissue from brain regions which seem to be involved in this phenotype, i.e. nucleus accumbens, striatum and prefrontal cortex, will be used in order to identify differentially expressed genes in resistant versus non-resistant rats.

Although this is a promising approach, it is hampered by the fact that brain tissue is usually very heterogeneous. In fact, in a brain area such as the nucleus accumbens numerous different cell populations are present and only a few specific cell populations are embedded in neurocircuits involved in addictive behavior. It is the challenge of the future to identify those cell populations and to conduct gene expression profiling in these cells. Nevertheless, that gene expression profiling, particularly in combination with QTL analysis, is a powerful complementary strategy in drug abuse research, has been demonstrated recently by the laboratory of Lucinda Carr (Spence *et al.* 2005). In their studies, QTL analysis was implemented in alcohol-preferring versus non-preferring rats (iP/niP) to identify chromosomal regions that are associated with alcohol preference. A highly significant QTL on chromosome 4 was identified. In parallel, in their gene expression profiling study in the same animals, differentially expressed genes that map exactly to the chromosome 4 QTL region were identified. Among those genes α-synuclein was the most relevant candidate gene for enhanced alcohol consumption in alcohol-preferring iP rats. In other alcohol-preferring rat lines α-synuclein was also found to be upregulated. α-Synuclein is involved in dopaminergic neurotransmission and α-synuclein knockout mice show reduced alcohol intake compared to wild-type control mice.

Subsequent association studies revealed that, indeed, genetic variations of the α-synuclein gene are associated with alcoholism (Bonsch *et al.* 2005) and elevated α-synuclein levels are associated with craving in alcoholic patients (Bonsch *et al.* 2004). Thus, this study perfectly exemplifies our proposed approach in pinning down genes involved in addictive behavior. In summary, the combination of QTL-studies and gene expression profiling studies in animal models of addictive behavior can yield putative candidate genes. Their exact function can be studied in knockout mice and can finally be verified in cohorts of addicted patients. It should be mentioned that functional validation of candidate genes can be also brought about by highly selective pharmacological tools, e.g. the antagonist of a receptor protein applied to the drug-related phenotype of interest. However, in most cases pharmacological tools are not available and alternative tools such as genetic animal models have to be applied. It has already been mentioned that conventional knockout models are not ideal tools for functional validation, but the new generation of conditional mouse mutation lacks these limitations. Thus, it has become possible to delete a gene of interest in a specific cell population in the brain, and further, it has become possible to switch genes on and off, if desired. Although these developments in mouse engineering appear to make it sound like a miracle, it has become reality and has, in a limited way, already been applied to drug abuse research (Spanagel & Sanchis-Segura 2003).

THE VISION IN RE-STATED OUTLINE: A THREE-STEP NEUROBIOLOGICAL APPROACH TO PIN DOWN WHERE AND HOW THE ADDICTED BRAIN GOES AWRY

Addictive behavior is driven by a variety of factors. Clearly, besides environmental and social factors, genetic factors and neurobiological mechanisms contribute to drug-related behaviors. It is a scientific challenge for the future to pin down the genes and gene products, as well as to identify neurocircuits involved in specific phenotypes such as voluntary drug intake, reinstatement of drug-seeking behavior, relapse, loss of control and drug intake despite negative consequences. Here, we are describing a three-step neurobiological approach that allows in a first step, via novel animal models and imaging techniques, to identify the neuroanatomical sites mediating those drug-related behaviors. The foundations for such an approach are animal models that enable us to study specific behavioral phenotypes. In fact, the reinstatement model allows us to study drug-seeking behavior, the deprivation model mimics relapse-like behavior, the temporal discount procedure can be used to study loss of control and, finally, modified conflict paradigms can be

applied to model drug intake in animals despite negative consequences. Because the environment can be controlled carefully in these animal models, confounds induced by environmental factors can be avoided or, alternatively, the impact of environmental factors can be studied specifically. Despite the fact that these models are valuable tools for experimental psychologists and behavioral pharmacologists, further refinements are needed and only the future will see whether these models provide predictive validity. In order to pin down the neuroanatomical sides involved in those drug-related phenotypes, a variety of imaging techniques can be applied to these animal models; however, as these are non-invasive techniques, imaging in drug addicts provides more direct information. Thus, abnormalities in the orbitofrontal cortex and in the anterior cingulate cortex have been detected repeatedly in addicted patients and it is suggested that those alterations could underlie compulsive drug-seeking and -taking behavior.

In a subsequent second step, forward genetic approaches including QTL-analysis and gene expression profiling are helpful for the identification of genes and gene products involved in those specific behavioral phenotypes. In a third step, candidate genes derived thereby must be validated functionally by pharmacological means or conditional knockout models. Following this validation process, the transfer to the human situation has to be made and candidate genes have to be verified further in well-phenotyped cohorts of addicted patients. If a putative candidate gene has been validated and verified by this strategy, the acid test will be that a specific pharmacological intervention can finally normalize this specific pathological state.

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