

Genetic Bases of Addictive Behaviors

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Introduction, Clinical Features and Diagnostic Evaluation

Addiction is not a medical term, and the range of disorders included varies according to the beliefs of the beholder. Nonetheless, its lay usage and dictionary definitions reflect the common belief that at its core is surrender to habitual, self-destructive behavior. Like most psychiatric disorders, attempts to define diagnostic criteria must use terms and constructs from entirely different languages, descriptions of biological factors and descriptions of intrapsychic events whose basis is unknown. The term addiction does not appear in the *Diagnostic and Statistical Manual-IV*, but in that manual, “substance use disorders” would be on nearly everyone’s list of addictions, while such disorders as Bulimia Nervosa, Pathological Gambling, etc. would make the list for many [1].

This review will focus on drug addiction for several reasons. Most data on genetic contributions to addictive behaviors relates to drug dependence. Neurobiological insights have been derived almost entirely from rat and mouse studies employing genetic models of addiction. This is because drugs, given systematically in an experimentally controlled setting to genetically defined subjects, exert their effects in increasingly well-understood pharmacological pathways, often via specific neurotransmitter receptors. Many pharmacological agonist and antagonist drugs and genetic manipulations are available to the experimenter.

Once the global boundaries of the addictions have been agreed on, to ascertain genetic contributions will require, as for any complex trait, that specific diagnostic heterogeneities and comorbidities be selectively measured. The ultimate goal of genetic studies is to identify specific genes, and for these complex traits there are many genes conferring only a small degree of risk for or protection against diagnosis. Finding any such gene presents a large signal-to-noise problem because of the small effect it has, but also because genes interact with each other. For example, a gene possessed by an individual whose risk is being assessed might itself be nearly

irrelevant to diagnosis, yet could potentiate the risk conferred by a specific other gene (i.e., two risk-promoting genes might increase risk 10-fold). Thus, the drug addiction phenotype might consist of individuals from a very heterogeneous population.

Even within the substance abuse and dependence diagnoses, the path is not clear. The National Institute on Alcohol Abuse and Alcoholism defines alcoholism as synonymous with alcohol dependence, and as including four symptoms: craving, loss of control, physical dependence, and tolerance. These symptoms include both physical sequelae of chronic drug use and internal feelings and thoughts about ability to control one's behavior. There is a high comorbidity between alcoholism and smoking, as well as between alcohol abuse and abuse of other drugs that appear to reflect the influence of some genes in common, a condition known as *pleiotropism*. However, each abused substance has individualized genetic risk as well. Some personality traits, such as depression, may predispose to addictive behaviors; although a causal relationship is difficult to ascertain.

More problematic yet for genetic analyses, research shows that about half of addiction risk is attributable to genes with the other half stemming from environmental factors. There is strong evidence [2] that environmental cues associated with drug-taking behavior are strong triggers for relapse and many therapies advocate removing the addicted individual from his or her environment to minimize these drug-associated cues. Inevitably, genotype interacts with environment to produce individual risk, which of course will change developmentally as gene actions and environmental milieu change. The picture is not entirely bleak, however. Genetic studies have helped to refine diagnostic issues. For example, an "addictive personality" is often invoked to "explain" a wide variety of maladaptive habits (if not outright overindulgences). Systematic studies reveal little support for a genetic basis for this postulated disorder [1].

In the following sections, we will point the reader to reviews of the human molecular genetic evidence regarding addiction risk and discuss examples. We will review theories of the neurobiological bases of drug dependence, most of which have studied dysregulation in the brain

circuits underlying both normal and pathological reward. We will focus on genetic animal models as they have been directed toward multiple aspects relevant to understanding drug dependence. The targets of such models take pharmacology as their template, and include the initial response of a naive organism to a drug; neuroadaptations related to chronic drug administration which includes either reduced (tolerance) or increased (sensitization) sensitivity; drug dependence, which is inferred from withdrawal symptoms that occur when the drug is removed; the reinforcing effects of the drug (which include craving in humans and may be either positive or negative); and the area of pharmacokinetics, which describes drug absorption, distribution, metabolism and elimination. Finally, we will discuss individual differences in gene expression and its potential role in the emerging field of pharmacogenomics.

Homeostatic Changes and Neuroadaptations

A core attribute of drug dependence is the emergence of various *withdrawal* symptoms after the abrupt cessation of drug use. Some drugs, such as alcohol and barbiturates, give rise to physical withdrawal symptoms as severe as life-threatening seizures. All addictive drugs, however, can produce psychological withdrawal symptoms such as anxiety, dysphoria and anhedonia. The behavioral withdrawal syndrome appears to result from compensatory neuroadaptations that occur during chronic drug administration. In response to potent neuronal stimulation resulting from the chronic administration of abused drugs, long-term neuroadaptations act to maintain organismal homeostasis and lead to reduced sensitivity to drug effects (i.e., tolerance). With abrupt cessation of drug administration, these neuroadaptations are both unmasked and unopposed by the drug, leading to withdrawal symptoms that are generally opposite to the acute drug effects. One example is the neuronal hyperexcitability evidenced during alcohol withdrawal that may involve the hippocampus, prefrontal cortex and the piriform cortex. Another is the dysphoria accompanying withdrawal, which has been termed “reward dysregulation” [3] thought to involve the nucleus accumbens and the ventral tegmental area-accumbens (mesolimbic) system. The nucleus accumbens is an integral part of the extended

amygdala. A third relates to long-term changes underlying susceptibility to relapse or reduced inhibitory control, and may involve the prefrontal and parietal cortical regions.

Synaptic Plasticity and Associative Learning

Mechanisms involved in associative learning include stimulation of dopaminergic D₁ receptors, activation of the cAMP/PKA/CREB signal transduction pathway, altered gene expression, and synaptic rearrangements and altered neuronal connectivity. There is much evidence for a role in the activation of adenylyl cyclase with downstream activation of protein kinase A (PKA) and cAMP binding protein (CREB) in the chronic effects of abused drugs [4;5] that results in increased neurotransmitter concentrations in the synapse. After drug administration, gene expression differences would follow the activation of CREB in the network of genes with 5' CRE regulatory elements. One example discussed below is the up-regulation of prodynorphin expression in the nucleus accumbens. This cascade of changes is dependent on new RNA and protein synthesis, and may be related to structural brain changes resulting from regulation of cell adhesion molecules such as nerve cell adhesion molecule (NCAM).

There is an abundance of opioid receptors and processed peptides in both nucleus accumbens and ventral tegmental area. The presence of dopamine- and serotonin-containing fibers also suggests interaction of these neurotransmitter systems. In addition, GABA, glutamate and acetylcholine have all been suggested to be involved in behavioral responses to drugs of abuse. Reviews of the multiplicity of structural and functional changes accompanying chronic drug administration and withdrawal indicate that the specific changes seen depend to some degree on the drug studied, but also reflect broad dysfunction of similar reward pathways [3,6].

Human Molecular and Genetic Data

Classical Genetic Methods-- Twins, Families, Adoptees, and Genetic Epidemiology

Given the complex interplay of genetic and environmental factors predisposing individuals to addiction, much work has concentrated on dissociating these contributions. Comorbidity for various addictive behaviors has been shown in twins and other biological

relatives, and environmental factors inferred from comparisons with adoptees. Substantial genetic influence has been demonstrated for risk of alcoholism, substance abuse (especially smoking), major depression, eating disorders and other putative representatives of addictive behaviors [1]. Each disorder also reflects unique genetic influences [7]. Twin and family studies can identify traits with common genetic and/or environmental influence, and can assess gene-environment interaction as well [8]. However, the generally small number of subjects in such studies makes it difficult for them by themselves to locate relevant genes (but see later).

Molecular Genetic Studies--Association and Linkage

As the Human Genome Project increased information about the specific DNA sequences in the human genome, the power and precision of genetic mapping studies was greatly enhanced [9]. Genetic mapping studies attempt to correlate genetic markers (i.e., sequences of DNA localized to specific locations in the genome, e.g., the mid-region of Chromosome 6) with the phenotypic trait being mapped (e.g., diagnosis) across specific groups of individuals [10;11;12]. Co-occurrence of specific markers with the trait suggests a gene of which the marker sequence is a part, or a separate nearby gene on the same chromosome (i.e., linked), is directing synthesis of a gene product that enhances risk. Because hundreds of comparisons are made in these kinds of analyses, there is a high false-positive rate for detecting such associations or linkages. Also, populations that appear homogeneous may vary in gene frequencies for genes in the associated region. This problem of *stratification* often plagues these types of studies, as the control and addicted groups may actually be drawn from two genetically distinct populations [13]. Further, because addictions are multigenic or polygenic traits, each gene mapped exerts only a small risk or protection, so there is low statistical power to detect linkage. Extremely strong association and linkage data for markers are required for even a single-gene disorder, such as Huntington's disease [14]. It has been argued that implication of a relevant gene or genes requires converging evidence from a variety of genetic and nongenetic techniques [15].

Despite the intrinsic difficulties, hundreds of linkage and association studies have been

performed for alcoholism, drug dependence, and the various other addictive disorders. Linkage and association genome scans for addiction risk have provided converging data implicating several chromosomal regions likely to harbor allelic variants contributing to drug addiction, such as alcoholism and smoking [16]. Most of these studies search the surrounding genomic area for a “candidate gene” as the hypothesized cause of the genetic effect. Candidate genes are nominated based on previously-existing biological data, such as a study that identified a marker near the serotonin 5-HT_{1B} receptor gene in two populations of impulsive-aggressive alcoholics [17]. Much data supports a role of gamma-aminobutyric acid (GABA) in alcohol dependence and related phenotypes [18] and studies have revealed a haplotypic association with the gene encoding the alpha2 subunit of the GABAA receptor with alcohol dependence [19;20;21]. However, it is not currently known whether the candidate genes nominated by linkage and association studies in fact contribute to the disorders being mapped. This inferential problem is shared by studies using animal models, and because the solutions are conceptually similar, we will discuss the next steps toward proving a functional role for a candidate gene in a later section.

Enhancing the Power of Human Genetic Mapping Studies

There are several approaches for solving the problems of low signal-to-noise in gene mapping studies. Obviously, population sizes can be increased, but this is very expensive and may not increase power very much. The use of “endophenotypes” has become popular, as they are postulated to reflect the effects of closely linked genes on a more tractable (and potentially more heritable) trait. Endophenotypes can be measurable components of complex neuropsychiatric diseases that may represent neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive or neuropsychological aspects of the disorder that can be separately measured [22] and also used in the development of preclinical models [23]. In recognition of the comorbidity of many traits (and, more simply, the pleiotropic effects of genes on multiple traits assumed to contribute to comorbidity), it has been suggested that some endophenotypes may be “closer to the gene.” An example is the abnormal P300 sensory evoked potential characterizing

alcoholics and their offspring. On the assumption that the collection of genes contributing to abnormal P300 responses will be simpler than that contributing to the complex trait, alcoholism, mapping studies have identified quantitative trait loci (QTLs) where a gene or genes influencing P300 must reside [24]. Similarly, a long-term epidemiological study demonstrated more than 20 years ago that college-age men with a positive family history for alcoholism were less sensitive than those without to the effect of an acute dose of alcohol to induce body sway and subjective intoxication. Many years later, family history predicted alcoholism diagnosis when the young men entered the age of maximum risk for alcoholism, but the sensitivity to acute ethanol effects was an even better predictor of alcoholism, accounting for most of the individual differences in susceptibility. The low sensitivity to alcohol trait was subsequently mapped to specific genetic markers [25].

The discovery of single nucleotide polymorphisms (SNPs) led to a new generation of markers. Analyzing the current abundant number of SNP markers has been facilitated by the availability of high throughput database technologies [26]. Use of SNPs, though, is not without disadvantages (e.g., the map of SNPs in the genome is less well articulated than that for standard multi-base DNA markers, the sheer number of SNPs is overwhelming, etc.). Further, there are discrepancies among many case-controlled studies occurring by chance, population stratifications, different definitions of ‘cases’ and improper control groups. SNP patterns associated with adverse drug reactions can also be screened in patients to identify potential risk in situations where a maintenance drug should be avoided [27].

More powerful designs (e.g., the transmission disequilibrium test (TDT) and its variants) can increase detection power. The TDT compares marker frequencies among family members with shared traits of interest versus family members who do not share phenotypes, which reduces the probability of false positive associations. Statistical genetic methods are also being developed to deal with issues in quantitative genomics, such as gene-gene interactions [28]. By conditioning a gene’s effects on the genotype at another locus, genetic signal can be enhanced.

Although such methods are computationally difficult and lead to great increases in the numbers of comparisons made, they may reveal genetic effects when those effects are masked by the presence of other genes. An interesting and thoughtful paper has proposed a synthetic approach to combine the strengths of classical twin designs with those of family-based linkage and association studies, and proposed the value of such an approach for gene mapping studies [29].

A Case of Protective Genes - ADH and ALDH Polymorphisms

Alcoholism is one of the longest-studied complex traits in human genetics, and the findings with alcohol metabolizing enzymes illustrate the potential of genetic studies [7]. Ethyl alcohol is metabolized rapidly to acetaldehyde, which causes symptoms including nausea, facial flushing, dizziness, and headaches. The enzymatic conversion is effected by the enzyme alcohol dehydrogenase (ADH). Most humans then rapidly convert the acetaldehyde to acetate and carbon dioxide, innocuous products that are excreted, because they possess variant alleles for the aldehyde dehydrogenase (ALDH) gene that lead to synthesis of an efficient ALDH protein. There is a variant allele for the ALDH gene that leads to a slow-metabolizing form of the protein, ALDH2*2 that is present at high frequencies in southeast Asian gene pools. When an individual heterozygous (or, more strikingly, homozygous) for ALDH 2*2 drinks alcohol, he or she rapidly accumulates acetaldehyde and frequently experiences the unpleasant resulting symptomatology. After years of population-based screening studies, a single Asian alcoholic was finally found to be an ALDH2*2 homozygote. The variant ALDH allele therefore offers a substantial degree of protection against alcoholism [7]. There is a similar polymorphism in ADH that reduces alcoholism risk, but to a lesser degree, perhaps 20%.

It is an interesting feature of these findings that disulfiram has long been used as a treatment for alcoholism under the trade name Antabuse®. Disulfiram is an ALDH inhibitor, so an individual taking this drug chronically experiences the flushing, nausea, etc. after drinking alcohol, just as if he or she were genetically protected by a variant enzyme. The treatment has seen limited use because of a lack of patient compliance with their medication schedule, but it is

not entirely without efficacy.

Thus, genetic analyses can lead to candidates for pharmacotherapy. The use of the long-acting opioid agonists (methadone and certain of its derivatives) to blunt heroin's reinforcing and physical withdrawal effects is another case where targeting a drug's obvious pharmacologically relevant sites has seen success. Many years have been spent in pursuit of a drug that would pharmacologically "antagonize" cocaine and/or methamphetamine's effects, with little success (e.g., tricyclic antidepressants, selective serotonin reuptake inhibitors, disulfiram, dopaminergic agents and GABA_A-GABA_B ligands). It is not unreasonable to hope that genetic analyses will aid the discovery of novel pharmacotherapies for addiction.

Genetic Animal Models

Why Study Non-Human Animals?

Humans are a very messy species for genetic studies. They have few offspring, small families, the generation time is inconveniently long, they breed with whomever they please, and ascertaining the genetic specifics of their ancestry is difficult. To overcome these problems requires very large population sizes with consequent enormous expense. There is a long history of application of genetic animal models to the study of addiction genetics. The advantages are obvious - on every feature named above, rodents are far more desirable subjects than humans. Unfortunately, rodent behavior can never model the full spectrum of any complex behavioral human trait. There are species-specific differences in physiology, behavior, social structure, and their interactions that ensure that an animal model can never be isomorphic with the human psychiatric trait (or diagnosis) it targets. However, human and mouse genetics appear to share over 80% homology for groups of linked genes because of syntenic conservation due to shared ancestry. That is, knowing where a particular gene is in the genome of one species will predict about 80% of the time where the homologous gene is in the other. Genetic animal models have, therefore, taken the initial, reductionistic approach of attempting to model the various contributing features of complex traits separately, and have had increasing success.

Classical Genetic Animal Model Methods

Selective Breeding

Artificial selection or selective breeding is the one of the oldest and most powerful methods in behavioral genetics. In the late 1940s, high and low preferring lines of rats were bred to drink alcohol solutions in preference to water at the University of Chile. There are now half a dozen sets of rat lines, and mouse lines as well, that differ genetically in their willingness to consume alcohol [1]. Genetically high-drinking rats will self administer intoxicating doses of alcohol under some circumstances. One of the features of selective breeding is that breeding for one trait leads to the development of differences in many other traits. This is because any given gene does not exert a single effect, but rather has many other effects on a variety of traits, a phenomenon termed *pleiotropy*. Thus, genetically preferring rats and mice have been found to have an array of behavioral differences as compared to lines selected to avoid alcohol solutions [30]. For example, alcohol preferring lines appear to be less sensitive to the aversive effects of alcohol, more sensitive to the motor stimulant and electroencephalographic effects of ethanol, and to develop more persistent tolerance. High alcohol consumption appears to be associated with low brain serotonin, which is interesting in that specific serotonin reuptake inhibitors are currently showing some signs of clinical efficacy in the treatment of alcoholism [30]. For several recent reviews of selected rat lines differing in alcohol preference see issue 11 (3-4) of *Addiction Biology* 2006.

Many lines of rats and mice have now been genetically selected for high and low sensitivity to other important effects of abused drugs including sensitivity, tolerance, and dependence for alcohol and several other drugs of abuse [1]. Selected lines have been used in many studies to elucidate the neurobiological changes that accompany chronic drug administration [31].

Recently, some attempts have also been made to genetically select lines differing in traits thought to be highly comorbid with the addictions. Anxiety, impulsivity, antisocial behavior, and

depression can be modeled in rat and mouse behavioral assays, although some of these behavioral assays have a bit more than the usual level of difficulty in convincing nonbelievers they possess face validity. Given the intrinsic power of this method to assess genetic pleiotropy, it might be worthwhile to develop additional lines of mice or rats that differ in some of the other traits correlated with drug abuse susceptibility in humans.

Inbred Strains

One of the most straightforward genetic animal models is to examine existing genetic variation. There exist well over 100 inbred strains of mice and as many of rats. All same-sex members of such a strain (e.g., C57BL/6 mice) are somewhat like monozygotic twins, essentially genetic clones reproduced through many generations of brother-sister matings, but possess two copies of the same allele at all genes. If many strains are compared for a trait in a controlled environment, mean differences among the strains can be attributed to genetic differences. C57BL/6 strain mice were shown early on to prefer to drink alcohol solutions, while other strains, such as DBA/2, were nearly complete teetotalers. C57BL/6 mice will apparently readily self-administer nearly all drugs of abuse with great avidity, as has been made clear in many subsequent strain comparisons for alcohol and drug sensitivity, tolerance, dependence/withdrawal severity, and self-administration [32].

Because the genotypes of each strain remain nearly invariant over time, studies conducted 40 years ago are still informative today. A recent historical comparison found that in inbred strains, characteristic alcohol preference has been highly stable across 40 years of data, like brain weight data [33]. Some phenotypes, however, show less comparability over time (e.g., anxiety-like behavior in the elevated plus maze) [33]. The specific genes that lead to the strain differences, however, have remained elusive, because the large numbers of alleles for any gene represented in a multi-strain survey make genotyping difficult and expensive.

One boost to strain surveys is a new initiative spearheaded by The Jackson Laboratory, the Mouse Phenome Project [34]. By supplying up to 40 commonly used and genetically diverse

inbred strains to willing phenotypers, and assembling a relational database containing the resulting behavioral and physiological data, centralized access to strain data will vastly improve in the next few years. The database will allow for appropriate strains to be chosen for specific behavioral tasks modeling human diseases, and improved inferences about the influence of environment on genotype. One very powerful use of this database will be to enable correlational analyses of the strain's average phenotypes. Such analyses have already taught us much about codetermination of genetic influence. For example, inbred mouse strains that are efficient at learning to inhibit a signaled nosepoke response were those that in other studies had low ethanol preference. Similarly, high alcohol-preferring strains tend to show minimal withdrawal severity after chronic ethanol intake. Together, these studies might be interpreted to mean that being able to avoid alcohol self-administration is a consequence of a genetic predisposition to experience severe withdrawal coupled with the ability to inhibit responding. Given the difficulties of mapping mouse behavioral assays directly to human traits, this would be quite an interpretational stretch from the data, but it could suggest informative experiments to test the hypothesis.

Quantitative Trait Locus (QTL) Mapping

A quantitative trait is a measurable phenotype emerging from genetic and environmental factors where the trait is distributed in magnitude in a population rather than all or none. A quantitative trait locus (QTL) is a specific chromosomal region or genetic locus within which particular alleles are statistically associated with variation in the trait. These quantitative traits are often influenced by several polymorphic genes and environmental conditions and one or many quantitative trait loci (QTLs) can influence a phenotypic trait [35].

Inbred strains, selected lines, and various other genetically specified populations have been used in studies analogous to the association and linkage studies described above with human populations. The proximal goals of these studies are first to locate a QTL harboring a gene or genes affecting the trait to be mapped, and then refine that genomic map until a single gene or genes can be implicated in the effect on the trait. Of course, each QTL generally

accounts for only a small proportion of the variability in the complex behavioral traits contributing to addiction, so this is a difficult task and cautious interpretation is warranted. The probability of success in QTL mapping depends on: the heritability of the trait; whether the underlying Quantitative Trait Gene (QTG) is dominant, recessive or additive; the number of genes that affect the trait; whether or not their effects are interactive; and most importantly, the number of subjects that can be tested (i.e., the statistical power of the mapping effort).

More than 2,000 QTLs have been identified [35] and over 100 behavioral QTLs have been detected using many diverse mouse breeding strategies [36]. These efforts were undertaken in the early 1990s, and have succeeded for several complex traits of importance to medicine [37]. Although possibly thousands of QTLs have been described for many diverse complex traits in rats (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>) and mice (<http://www.informatics.jax.org/>), very few of these QTLs have been reduced to QTGs or quantitative trait nucleotides (QTNs) and integrative strategies are underway to approach this endpoint [38]. Even though a QTL may be mapped to a chromosomal region with a very high statistical probability, the region still contains many candidate genes, and much further work is required to eliminate all but one or a few of these to arrive at the QTG(s). The more long-term goals of QTL mapping projects are then to move to human populations for studies of the homologous, or orthologous gene, and use information about the biological effects of the gene's product to help design therapeutic agents or other therapies.

Many addiction-related traits have been targeted for QTL mapping studies. The recent discovery of the addiction relevant QTG, *Mpdz*, which possesses pleiotropic effects on the predisposition to alcohol and barbiturate withdrawal, demonstrates the power of this approach [39]. Traits for which there are significant QTL associations include alcohol preference drinking, acute and chronic alcohol withdrawal, place preference and taste aversion conditioned by alcohol, and acute alcohol-induced hypothermia, locomotor stimulation, and sedation. Other QTLs have been mapped for several responses to morphine, cocaine-induced seizures, and

withdrawal from pentobarbital. These traits and their QTLs, as well as progress toward high-resolution mapping of some of the QTLs, have been discussed elsewhere [1].

Currently, QTL fine mapping usually involves the development of congenic strains. In a congenic strain, a very small sequence of DNA on a chromosome is moved from one genetic background to another, inbred strain background. This is accomplished by examining genetic markers surrounding the QTL and searching for rare cases where a recombination has occurred during cell division. In a recombination event, an exchange of chromosomal material has occurred, termed a crossover, between one of the markers and the QTL. Gradually, with continued breeding, this leads to a narrowing of the confidence interval of DNA sequence surrounding a mapped QTL until only a single gene remains. If a congenic strain containing a single introduced gene differs on the trait versus the background strain, the remaining gene must be responsible for the QTL and the QTL has become a QTG [15].

Newer developments are aiding the investigation of the molecular basis of quantitative variation in the mouse [40]. Analytical tactics such as ancestral probabilistic haplotype reconstruction can be applied to the study of whole genome genetic associations in outbred mouse population studies [36]. Using this method, QTLs are mapped in a single step to regions of less than 1 cM in heterogeneous stock mice (HS). The HS mice are an outbred strain derived from eight inbred strains. Therefore, the chromosomes of the HS are random genetic mosaics of the founding inbred strains and each individual HS animal has a unique genotype [35;41;42].

Chemical N-ethyl-N-nitrosourea (ENU)

QTL mapping relies on existing polymorphisms in the genome. It seeks to associate possession of a particular allele at a particular gene with a high or low score on the trait. An alternative is to create many mutations at random throughout the genome, and then to screen the mutagenized mice (actually, their offspring) to see whether behavioral outliers can be detected. This process of random mutagenesis is now usually induced by treatment of males with the potent chemical carcinogen and mutagen N-ethyl-N-nitrosourea (ENU). There are several large

projects currently underway whose goals are to identify many hundreds of such mutants, and several projects have included behavioral screens for alcohol or drug sensitivity. For example, random mutagenesis resulted in a mouse line with a triple mutant of mouse dopamine transporter (DAT) retaining over 50% uptake activity that was 69-fold less sensitive to cocaine inhibition of DAT activity than the wild-type mouse DAT [43]. Many mutagenesis projects also screen for assays for traits thought to be comorbid, such as anxiety or impulsivity.

Molecular Theories

Features Modeled in Animals

Animal models of addiction have greatly aided the understanding of mechanisms underlying drug addiction and the development of new treatments. In attempts to model human drug-taking behavior, investigators have established reliable patterns of intravenous self-administration with many classes of abused drugs in laboratory animals, demonstrating that they constitute positive reinforcers during acute administration. Progress has been made identifying and characterizing the neuronal circuits and mechanisms mediating the acute reinforcing effects of drugs, particularly as a result of analyses using rodent animal models.

Targeted Mutagenesis

The most important advance in genetic animal model studies during the 1980s was the maturation of the technology for manipulating the genome directly. In a dramatic demonstration as proof of principle, the metallothionein-growth hormone gene was inserted into the pronucleus of mouse eggs and the adult mouse developed from those embryonic cells was shown to express the gene's protein product resulting in very large mice [44]. Mutagenesis has proven to be a powerful technique to dissect the genetic basis of abnormal phenotypes [45]. By introducing engineered genetic material directly into the germ line of mice, investigators have produced hundreds of mouse strains in which a specific gene is overexpressed through the use of specific promoter sequences (i.e., transgenic), underexpressed (i.e., knock down), or where the gene product is only marginally or completely non-functional (i.e., a null mutant or knockout). The

genes targeted for such manipulations were selected based on scientific evidence regarding the biological underpinnings of the trait of interest, and such modification is a test of the direct involvement of the gene product in the trait being analyzed. In the additions, most such targets were neurotransmitters, their enzymes of metabolism, receptors, or transporters. As more understanding of the intracellular messages triggered by receptor interactions has been developed, these signaling proteins have also been targeted.

Production of null mutants is currently a standard and proliferating technology for direct genetic manipulation. Since 1996, approximately 100 genes have been studied for their roles in ethanol-related effects in mice using the array of genetic manipulations [46]. Lists of targeted genes are available online (e.g., <http://165.112.78.65/KOS/KOSearch.taf?function=form> and <http://www.ibngs.org/links.html>). Use of these model strains will continue to be invaluable, especially as the tissue-specific and conditionally-expressed generation of genetically altered mice become more widely used. These strains can have expression of the targeted gene restricted to particular brain regions (spatial), and have gene expression turned on or off at particular developmental stages (temporal), thereby reducing many of the interpretational difficulties with the first generation of null mutants, which largely stem from observed compensatory responses that no doubt occur throughout development. An additional caveat is the observation that the specific mouse strain employed may influence the phenotype that results from such genetic manipulations.

Transgenic overexpression of a wild-type allele driven by a brain-specific promoter can complement results obtained with mice with targeted disruption of the same gene. As noted previously, there is evidence of involvement of cAMP-PKA signaling in the mesolimbic reward pathway, and protein kinases in particular, are critical for alcohol actions [47]. Transgenic mice can be constructed using genes that have been mutated to be either constitutively active or to act as an interfering mutant (dominant negative) to reduce activity of the endogenous product. As an example of this kind of approach, heterozygous mice with targeted disruption of one Gs alpha

allele show increased sensitivity to the sedative effects of alcohol and decreased alcohol consumption [48]. Likewise, different mice with reduced neuronal downstream PKA activity, in which a dominant negative isoform of the regulatory subunit of PKA is expressed in forebrain under control of CAMKII alpha promoter, also demonstrate reduced alcohol consumption and increased sensitivity to the sedative effects of ethanol. In contrast, a third line of mice with increased PKA as a result of transgenic overexpression of a constitutively active form of Gs alpha (with reduced GTPase activity) show reduced sensitivity to the sedative effects of alcohol. Taken together, results obtained with such genetically modified mice to produce opposite phenotypes provide strong and converging evidence of the involvement of a specific pathway in the measured behavioral response.

Role of Gene Expression >

Substantial data show that both acute and chronic drug administration result in changes in the expression of many different genes in the central nervous system--what may be termed a drug-regulated network. With chronic drug abuse, some of these changes and the cellular consequences are quite enduring, even after protracted abstinence and perhaps for a lifetime. The mechanism(s) mediating drug-related changes in gene expression remain elusive, particularly for alcohol. Two areas of investigation into the role of gene expression in drug addiction are currently quite active: individual, pre-existing differences in gene expression that in the presence of drug may pre-dispose to addiction vulnerability; and changes in gene expression after drug administration that may mediate aspects of drug dependence and withdrawal, or drug related reinforcement, and that may also lead to increased risk of relapse. Vulnerability to relapse and withdrawal in the development of addiction may be the result of brain region-specific and long-lived "neuroadaptive" changes in gene expression [4;49]. In this section, we will focus on expression differences in the central nervous system that result from acute and chronic ethanol administration as a paradigm for drug-regulated gene expression. Because of space limitations, we will not discuss naive differences in gene expression that may be predisposing factor(s)

important in alcoholism liability, which have been covered in an excellent review [30]. We will also not focus on translational and post-translational modifications of existing mRNA and proteins, which will require employing a proteomic strategy to identify as outlined below. Such regulation may be a significant factor influencing protein abundance and activity that mediates behavioral and metabolic responses to chronic drug administration, particularly in large post-mitotic cells such as neurons. The reader is instead referred to a review by Lang et al. [50].

The effect of drug administration on gene expression can be evaluated with a variety of methodological approaches. Such techniques can be subdivided on a theoretical basis into those that directly characterize and quantify expression of a manageable number of mRNA transcripts of known sequence chosen based on potential involvement in drug-related responses, and those that globally screen the transcriptome for expression differences - that is, they screen without bias tens of thousands of genes, even fragments of sequence. The complexity of a condition such as human drug addiction endows expression profiling with a substantial number of obstacles but, nevertheless, there have been a number of studies during recent years.

Investigation of Candidate Genes

The first approach has been termed "candidate gene" analysis since these sequences are characterized only after previous results implicate their importance, thus identifying the specific mRNAs as candidates for mediating behavioral responses to drug administration. Many of these genes represent the target proteins for different drugs of abuse. Since changes in expression after chronic administration of drug are often-times opposite in direction to changes observed after acute administration, it has been proposed that long-lived neuroadaptive changes in expression are the result of and in opposition to the acute (rewarding) effects of the drug. Because gene regulation by drug administration may be temporally linked in this fashion, it is important to identify expression differences in both settings.

Changes in mRNA expression after acute or chronic drug exposure have been quantified using Northern analysis, RNase protection analysis or a variety of reverse-transcription

polymerase chain reaction (RT-PCR) approaches for a defined set of known genes. Broad classes of proteins whose transcripts have been characterized as drug-regulated represent traditional candidate genes. These include both ligand-gated and G-protein coupled neurotransmitter receptor subunits and neurotransmitter transporters genes, transcription factors including immediate early genes such as the *c-fos* family and CREB, components of signaling cascades such as PKC and PKA, adenylyl cyclase and G-protein subunits, and certain neuropeptides such as prodynorphin, vasopressin, and brain-derived neurotrophic factor [51]. This step should be followed by a functional confirmation of candidate gene expression changes.

One strong line of evidence for direct involvement of drug-regulated changes in gene expression in behavioral responses to drug administration may be that of corresponding changes in cAMP systems, including CREB-regulated prodynorphin expression [4;49]. After both alcohol and cocaine exposure, prodynorphin expression has been shown to be up-regulated in specific brain areas associated with drug reward, including the nucleus accumbens and caudate putamen [52]. This up-regulation can be mediated by changes in CREB expression and phosphorylation. Thus, elevation of CREB leads to altered gene expression, including increased prodynorphin, and kappa-opioid receptor activation, for which prodynorphin is a ligand. Direct blockade of either CREB expression with viral-mediated gene transfer of a dominant-negative CREB or of kappa-opioid receptor activation with the antagonist norBNI alters signs of dysphoria/depression and aversion observed during cocaine withdrawal [53], directly implicating CREB pathway involvement in drug-induced dysphoria.

Analysis of Global Changes in Gene Expression

Analysis of gene expression differences that allow for global characterization is particularly useful for neurobehavioral syndromes such as drug addiction, since simple single gene effects are unlikely to be the sole underlying mechanism. The number of genes and combination of genes that have been demonstrated to change expression in response to drugs has increased rapidly in recent years [54]. Techniques that have been employed to screen globally

for expression differences without bias and identify novel genes include suppression subtraction hybridization, differential display, RT-PCR, serial analysis of gene expression (SAGE), and gene chip or microarray expression analysis. Early studies characterizing changes in expression after chronic ethanol administration employed material isolated from cultured cells, analyzed by subtractive hybridization studies that enriched the pool of regulated RNA. Heat shock proteins, molecular chaperones and mitochondrial genes were identified as differentially regulated [55]. Differential display analysis, a technique that allows for direct visualization of all gene expression patterns, has been employed to analyze brain expression after chronic ethanol exposure in rodent models. In such studies, mitochondrial and chaperone genes were also identified as targets for ethanol [56;57]. One series of studies has focused on changes in gene expression caused by cocaine and amphetamine, which led to the discovery of a novel class of neuropeptides derived from the CART gene (cocaine and amphetamine-related transcript) [58].

Currently, the most powerful and challenging approach to global screening is to use a DNA chip that contains arrays of tens of thousands of DNA sequences on a solid substrate. Complex RNA probes are simultaneously hybridized to complementary sequence on the chip; quantification of the hybridization pattern thus identifies gene expression differences between two samples. These kinds of analyses are still in their infancy, but some interesting trends are emerging. Gene chip studies comparing human alcoholic samples from frontal cortex to matched controls have confirmed some subtractive hybridization and differential display observations, particularly with respect to chaperone proteins [59]. Somewhat surprisingly, a major system influenced by lifelong drug abuse comprised many myelin-related genes. These data illustrate the power of bioinformatic analysis to suggest when several genes that have impact on a particular pathway or system are identified as regulated by drug administration the more likely the finding is to be biologically meaningful. Animal models are also being employed in expression array studies. One study reported changes in expression of several genes related to intracellular signalling cascades, including multiple protein kinases in nuclear accumbens tissue taken from

primates exposed to cocaine for over a year [60]. Another experiment used a rodent model derived from a genetic selection for alcohol sensitivity. Comparison of untreated, whole brain tissue revealed 41 genes differentially expressed out of more than 18,000 nonredundant mouse cDNA clones in lines selected for high versus low alcohol sensitivity [61].

Interestingly, in studies to date, the most robustly regulated genes are often not neurotransmitter system components or genes that had been previously examined as likely "candidates." Many of the genes now shown to be regulated were not anticipated to be relevant to drug-mediated responses. This is both the great benefit and the daunting challenge of an unbiased approach. Understanding the significance of these unanticipated changes in gene expression in a larger context, and the role played by the translated proteins as a network in a brain region-specific fashion, thus remains a significant challenge for future studies [51;58].

Because of the difficulties in determining molecular factors important for excessive alcohol consumption in humans, including the modest regulation observed after alcohol exposure, several rodent models have been utilized to examine the genetic predisposition to drink alcohol in the alcohol naïve state. Microarray analyses of basal whole brain gene expression in three selected lines and six isogenic strains of mice that differ in ethanol consumption were conducted in a meta-analysis [62]. Three thousand eight hundred genes were found to be significantly and differentially expressed across all models of high vs low alcohol consumption groups. These studies support the importance of identifying functional groups through bioinformatic approaches, such as, mitogen-activated protein kinase signaling and transcription regulation pathways in voluntary ethanol drinking in these mouse models. Further data filtering using a congenic strain microarray set implicated several cis-regulated genes as promising candidates for an alcohol preference trait QTL on chromosome 9. Combined, this report and additional studies that include effects of alcohol [63;64;65] demonstrate the power of such a strategy to analyze a complex behavioral phenotype, such as alcohol preference [62], and similar analyses have been employed for other alcohol related phenotypes [66].

It should be noted that the focus of work thus far has been at the level of the gene, i.e., DNA. It is the gene products, proteins, that accomplish the neurobiological business of the organism, and expression of a gene does not necessarily translate directly into production of a functional protein. The new field of proteomics, in which complex patterns of protein expression are analyzed, is widely touted as the next frontier for expression-based analyses. Proteomic approaches allow the study of global protein expression profiles of disease states or drug effects in the absence of a priori hypotheses. Because of the complexity of proteins and their frequent post-translational processing and/or alternative splicing into multiple active forms, studies of protein expression, which are directly analogous to the gene-based approaches just discussed, are even more complex. A disease state, such as drug addiction, involves multiple interacting proteins in the central nervous system so is well suited for proteomic analysis [67]. The application of subcellular proteomics has led to the identification of proteins associated with different components of the synapse, including synaptosomes, synaptic membranes, postsynaptic densities, synaptic vesicles and the presynaptic terminal. Thus, a more detailed understanding of the structure and function of the synapse proteome is rapidly formulating that will be important for examining the role of synaptic proteins in drug addiction.

Therapy

Existing Therapy

Addictions treatment strategies are varied and often applied in combination. These include the familiar twelve-step self-help programs, psychosocial therapies, brief interventions, behavior therapies, psychodynamic therapies, cognitive-behavioral therapy, and multiple forms of couples/family/group/community-targeted therapies. Nearly all medical disorders are currently treated with drugs, and the addictions are no exception: Although psychotherapy is sometimes offered alone, it is often coupled with a drug. The drugs used include disulfiram (Antabuse®, discussed above, for alcoholism), specific serotonin reuptake inhibitors and other drugs affecting serotonin function (e.g., fluoxetine, sertraline, ondansetron), opioid agonists

(methadone, LAAM) and antagonists (naltrexone), nicotine replacement, and acamprosate, a drug with multiple effects including affecting NMDA receptors. In addition, there are many complementary, or alternative, medical treatments applied to the addictions whether by practitioners or by the patient, including acupuncture, yoga, and herbal therapies. These alternative therapies are often administered in conjunction with routine medical treatment and are increasingly used. As they are generally not studied in a scientifically controlled design, their efficacy is hard to evaluate.

To an increasing extent, different treatment modalities are being offered in combination; for example, multiple drugs with different actions combined with psychotherapy, or using vouchers in a community reinforcement approach in patients on methadone maintenance. Potentially problematically, self-treatment with herbal preparations can influence metabolism of medically prescribed drugs. Because we are neither clinicians nor involved in human subjects research, our opinion is derived from the literature rather than experience. It appears to us that nearly all of the conventional therapies will help a percentage of the individuals who try them, on the order of 20-25% in most large controlled studies. It has proven difficult to predict who will be helped by which therapy, and the majority of addicted patients seeking therapy of any sort will not succeed in remaining abstinent for at least a year. Thus, while there is clear value to the existing therapies, there is also ample room and need for improvement. The potential value in combining these treatments is largely unknown. The objective of the COMBINE study was to evaluate the efficacy of medication, behavioral therapies, and their combinations for therapeutic efficacy in alcohol dependence [68]. These studies reported that patients receiving treatment with naltrexone, behavioral therapy, or both had positive therapeutic effects, but acamprosate was ineffective (with or without behavioral therapy). No combination was more efficacious than either naltrexone or behavioral therapy alone in the presence of medical management. In addition, placebo pills and meeting with a clinician also had a positive effect beyond that of behavioral therapy during treatment.

Prospects for Genomically-Driven Therapies

“Pharmacogenomics” is one term given to the emerging field that seeks to use genetic information to discover new drugs as therapeutic targets and to individualize treatment based on an individual’s genetic fingerprint. It has assumed the knowledge from the field of “pharmacogenetics,” which has long sought to identify polymorphisms in drug enzymes and receptors that result in genetically-based, individual differences in drug metabolism, and builds upon that knowledge. A useful review of the field [69] describes the central feature of pharmacogenomics as its focus on groups of genetic polymorphisms used to predict both efficacy and toxicity of a drug for an individual. We know of no cases to date where this method has been applied to addiction-related traits, but the enterprise is a logical extension of the wealth of accumulating Human Genome Project-driven genomic data. Pharmaceutical companies have used discoveries from QTL mapping and gene expression studies in attempts to identify novel targets for treatment of complex trait disorders. A slightly absurd example might illustrate the general idea. For example, a gene expression array experiment might show that a large group of genes involved in histamine metabolism, catabolism and function (e.g. enzymes, receptors) showed greatly increased or decreased expression in animals chronically treated with alcohol. This would be a very surprising result, for our understanding of the likely neurobiological targets that are dysregulated by chronic alcohol include GABA, NMDA, catecholamines, endocannabinoid, opiate and serotonin systems, but not histaminergic systems. The hope is that this might lead to a clinical trial treating alcoholics with a specific histaminergic receptor antagonist drug, and a cure for alcoholism. The target pathway would never have been identified using the usual neurobiological approaches, which mostly look where the past light was, i.e., in the already-suspect systems.

Major pharmaceutical companies are avidly pursuing characterizing many complex disease traits using this strategy. However, there has been a historical, and continuing, major lack of interest by the companies in pursuing the addictions, which we find very puzzling. Given the

very high prevalence of addiction, and the fact that most of the afflicted are gainfully employed and could thus pay for their drugs, it is troubling that these disorders have not been a subject of greater interest.

Conclusions and Future Directions

Molecular and genetic studies of complex diseases have experienced spectacular progress in the last few years, much of it enabled by the proliferation of genomic approaches. Both knowledge of the genome, and the ability to manipulate it, have contributed. Nonetheless, progress has been greatest for those disorders with simpler genetic structure, i.e., those where a gene or a few genes are of great importance to risk. The addictions are in many ways paradigmatic for complex psychiatric disorders. Their very high prevalence and diversity mirrors, and is probably due in part to, their complex genetics. As we have tried to show in this chapter, a great advantage to studies of the addictions is a historical wealth of genetic animal models, which have offered many insights into the neuroadaptive dysregulation that accompanies the descent into an addicted state and potentially to genetic predisposition that may underlie behavioral differences in these models. Experience has given us a clear idea of the strengths and weaknesses of the various genetic animal models [37].

Important advances would include development of additional animal models not only for ethanol seeking and relapse, but also for ethanol's effects on emotional states. While many aspects of addictive behavior have been modeled in the laboratory, such as reinstatement of drug-seeking behavior, relapse, loss of control and drug self-administration despite adverse consequences, some of the key features of these disorders remain little studied. For example, the best predictors of eventual substance abuse are a family history, and the age at which abusive use was initiated. We know a great deal about the interactions of genetic predisposition and their modulation by environmental factors in humans, but the limited power of human genetic studies has made it very difficult to trace these influences to specific genes. Given this knowledge, it is troubling that there is so little work in experimental neuroscience devoted to exploring the

developmental onset of the addictions, in particular the risk factors -- biological, behavioral, and genetic--that distinguish animals that will go on to display addictive behavior. The potential interaction with other drugs of abuse, such as nicotine, is an important issue because of comorbidity. Finally, an underexplored area is the contribution of gender to multiple aspects of drug abuse, including severity and sites of organ damage, addiction liability, and risk of relapse.

Additionally, confirmation of brain regional differences in expression and functional significance of those changes needs to be established. Changes in receptor subunit assembly at the protein level at distinct, discrete sites have not been reliably established. Further characterization of post-translational changes in brain expression (phosphorylation and targets of kinases or CREB) or in receptor function through changes in subcellular routing and distribution (membrane, cytoskeletal, or internalization) will also be important.

Another area that is currently insufficiently studied is the complexity of the interactions across the levels of genes, their proteins, and environmental influences. It will not be enough merely to produce lists of expressed genes, or proteins, that accompany an addicted state. Rather, the combined, interactive influence of these factors should be explored systematically at the whole organism level, whose most integrated expression is behavior, a perspective that has been called behavioral genomics [1]. The new tools of bioinformatics, which are currently largely confined to data at the level of proteins and genes, will need to be married to the sophisticated multivariate statistical methods of epidemiology and to the behavioral sciences. Only after this advance will we achieve a behavioral genomics perspective allowing discernment of pathways that lead from genetic risk to addictive, or addiction-free, behavior. This is a large challenge, but the basic data are in place, and the tools are being improved.

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