Minireview

Amphetamine recapitulates developmental programs in the zebrafish

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Abstract

Addictive drugs hijack the human brain's 'reward' systems. A zebrafish model of addiction has recently been used to query changes in gene expression during this process.

The mammalian brain is characterized by neuroanatomical, biochemical and molecular complexities that drive cognitive and emotional responses. One of the brain's most notable functions is the evaluation of rewards that impact on daily activities and that help the individual to plan for future rewarding experiences. Unfortunately, the brain-rewarding system can be hijacked by psychostimulants that cause drug dependence and addiction in humans. Drug dependence and addiction are complex and vexing neuropsychiatric syndromes characterized by periods of escalated drug use, abstinence, repeated relapses and an array of adverse medical and biopsychosocial consequences [1]. Although efforts to treat addicted patients have met with some degree of success, the molecular neurobiology of these syndromes has remained mysterious.

Several animal models have been devised in attempts to dissect the biochemical and molecular pathways that form the pathobiological substrates of drug addiction. Among these is the conditioned place preference (CPP), which has been used extensively to assess the rewarding effects of both licit and illicit drugs [2,3]. CPP has been used to investigate the motivational properties of an array of pharmacological agents, including amphetamine, cocaine, ethanol, marijuana, methamphetamine, nicotine and opiates [3]. In the CPP paradigm, the primary rewarding properties of a drug represent an unconditioned stimulus (UCS) that is paired to a neutral stimulus that acquires secondary rewarding properties that act as conditioned stimuli (CS) [4,5]. Descriptively, one compartment of a two-chamber apparatus is paired to injections of saline whereas the other compartment is paired to a psychoactive agent given repetitively over several days. Following the period of repeated exposure, the animals are then allowed free choice between the two compartments. This procedure leads to the development of preference for the drug-paired compartment [3,5].

Such studies in rodents, including the use of transgenesis, pharmacological manipulations, and gene-expression studies, have provided only a few hints to the molecular neuropathobiology of drug-induced neuroadaptations [6,7] because of the mysterious nature of the addiction process. Thus, the paper by Webb *et al.* [8] in this issue of *Genome Biology*, which looks at changes in gene expression in a zebrafish model of the addiction process, is a very welcome addition to the armamentarium of behavioral neuroscientists who are trying to illuminate the biological bases for such a complex neuropsychiatric syndrome.

Conditioned place preference and the zebrafish

The zebrafish (*Danio rerio*) is a small cyprinoid teleost that comes from South Asian waters. The fish can be found in aquaria and pet stores throughout the world. It is a model organism for developmental and genetic studies [9-12] because of its short generation time, very large numbers of eggs generated after mating and transparent embryos, among other advantages. More recently, neuroscientists have begun

to make use of the zebrafish in behavioral genetics. Indeed, because genetic mutations can affect brain circuitry by causing dysfunctional patterns of connectivity, it has been possible to use mutagenesis screens to identify some of the molecular substrates of brain development and function using the zebrafish [10,11]. Similar attempts are presently under way to clarify the molecular bases of some behaviors [12]. The unbiased screens used in such experiments should make it possible to identify hitherto unsuspected biochemical and molecular processes that might be involved in the addiction process.

Enter the study by Webb *et al.* [8], which reveals the identification of some novel transcripts that are involved in the rewarding effects of amphetamine in zebrafish. They identified a network of co-regulated genes that might serve as molecular switches during the development of addictive behaviors. Webb *et al.* [8] used the CPP procedure described by Ninkovic and Bally-Cuif [13]. Briefly, this comprises several behavioral steps that include periods of habituation and the determination of the compartment initially preferred by individual animals. This is followed by injection of amphetamine in the non-preferred compartment and of saline in the preferred one. This sequence of events results in amphetamine-induced place preference for the compartment in which the drug was injected. Using the potent mutagen *N*-ethyl-*N*-nitrosourea, the authors generated mutants that failed to exhibit amphetamine place preference in this system [8]. They named the mutant 'no addiction' - *nad3256* or *nad*.

nad zebrafish show differential amphetamine-induced gene expression

The authors then performed systematic microarray experiments that allowed them to identify genes that were differentially expressed between wild-type and mutant zebrafish [8]. They identified 139 transcripts that belonged to a 'reward pool' of genes whose transcription was influenced in a differential fashion between the two groups of fish. A majority of the genes showed dichotomous changes in response to amphetamine, with 24% being upregulated and 35% downregulated in the mutants compared with levels in the wild-type fish. The differentially affected genes were enriched for transcription factors. These results are comparable to those of other studies using various psychostimulants, which have reported that the CPP procedure or self-administration of drugs are accompanied by differential expression of transcription factor genes [6,7,14]. Also of interest are observations by Webb *et al.* [8] that genes involved in cell differentiation, cytoskeletal organization, development and signal transduction were also differentially expressed. The changes in cytoskeletal-associated transcripts are consistent with several studies that have reported alterations in cell structure after ampheta mine administration [15], indicating that structural neuroadaptations are an essential part of addictive processes. Thus, the possibility exists that the amphetamine CPP might differentially affect the structure of the brains of mutant and wild-type zebrafish.

Amphetamine CPP and altered developmental gene expression

Webb *et al.* [8] chose to confirm the amphetamine-induced expression changes for several of the transcription factor genes, including four that were also assigned to the 'developmental' category by quantitative PCR and *in situ* hybridization studies. These four are *her15*, *foxg1*, *emx1* and *dlx1a*, which are counted among the handful of genes known to play significant roles in brain development and axonal guidance [10]. Systematic *in situ* hybridization experiments showed that *foxg1*, which plays an essential role in the development of the telencephalon (the fore-brain), showed significant amphetamine-induced regulation in the ventricular zone of the adult zebrafish (a region from which new neurons arise in the adult).

These are notable findings, and suggest that developmental processes that have not so far been investigated in models of drug abuse and addiction might trigger the switch from a state of exposed brain to that of an addicted brain after recurrent exposure to a rewarding, although addictive, drug. Brain development is dependent on very intricate interactions between cell proliferation, differentiation, and formation of neuronal connections at various stages that can be perturbed by endogenous and/or environmental stimuli [16]. Thus, the report by Webb *et al.* [8] suggests that repeated use of amphetamine might hijack developmental processes in such a way that the switch to drug dependence may occur through a process of dedifferentiation and structural reorganization in an attempt to maintain homeostasis in the brain's reward system. This suggestion is supported by the observation of overrepresentation, in the 'reward-pool' of genes involved in neurogenesis, which might also attempt to compensate for subtle amphetamine-induced neuronal damage. This suggestion is also consistent with the authors' findings that cytoskeletal genes that are known to be involved in brain development are also highly represented in their 'reward pool' [8].

It is also of interest to relate these changes to potential amphetamine stimulant-induced epigenetic changes in gene promoters, as demonstrated with cocaine [14], changes that might have served to influence the pathological re-induction of development-regulatory genes during chronic exposure to amphetamine. This discussion relates, in part, to the observed increases in the expression of brain-derived neurotrophic factor (BDNF) in the brains of rodents exposed to drugs of abuse (see [17] for further discussion), as BDNF has pleiotropic effects on brain development and on the developmental connectivity of reward pathways [18].

As reported by Webb *et al.* [8], amphetamine-induced regulation of several 'developmental' transcription factors sug-

gests the very attractive idea that drugs of abuse might trigger the re-expression of specific developmental genes that might participate in the development of structural plasticity reported in the drug-exposed brain [15]. These observations extend those of other investigators who have investigated patterns of gene expression in the presence of drugs of abuse [6,7] and support the idea that repeated administration of drugs is associated with complex molecular responses that influence the functional connectivity of the mammalian brain. Some of these changes might involve epigenetic regulation of structural changes, as these processes play important roles in the effects of drugs [14] and neuronal differentiation [19]. These suggestions are shown in a schematic format in Figure 1.

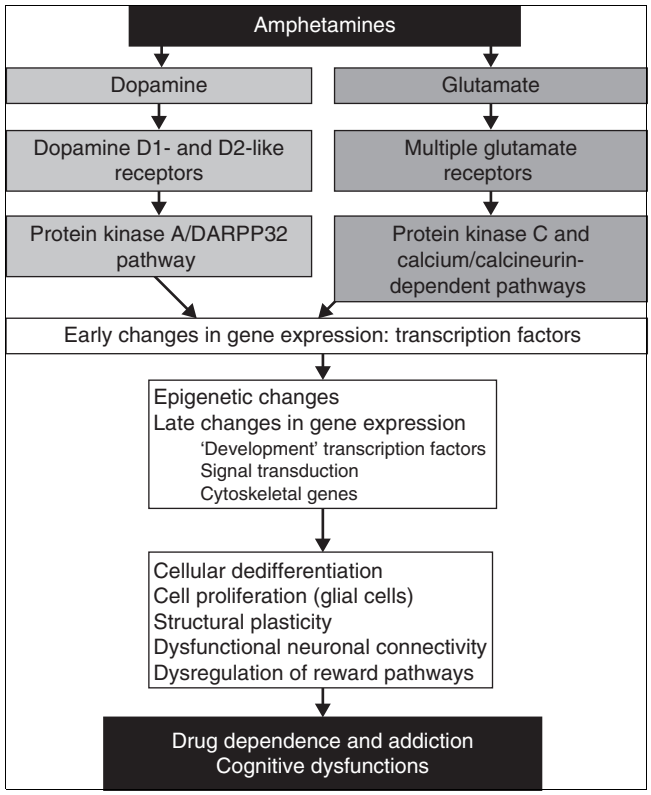

Figure 1
Molecular pathways involved in the development of amphetamine addiction. The scheme represents a working hypothesis identifying potential molecular events that occur in the brain after repeated exposure to amphetamine. The amphetamines are known to cause substantial and early increases in the expression of several transcription factors, in part via the activation of dopaminergic and glutamatergic systems. These transcription factors, in turn, regulate more delayed transcription of other genes that participate in signal transduction, synaptic plasticity and, as reported by Webb *et al.* [8], brain development. Recent experiments have also identified epigenetic modifications of histones as important regulators of changes in gene expression after exposure to drugs of abuse. When taken together, these altered patterns of gene and protein expression might serve as triggers for potentially multiple coincident and/or non-coincident switches that promote the progressive conversion from drug-exposed to drug-addicted brains.

Although these results will need to be refined further, the report by Webb *et al.* [8] should stimulate the development of systematic behavioral analyses of the molecular mechanisms, including epigenetic modifications, involved in drug dependence in the zebrafish. These experimental approaches promise to revolutionize our dissection of the molecular pathways involved in the switch to addiction that results from chronic exposure to licit and illicit drugs of abuse. This knowledge will be essential to the successful development of therapeutic approaches against amphetamine addiction.

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