

Genes and addiction

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Drug addiction, like all psychiatric disorders, is defined solely in behavioural terms. For example, addiction can be considered a loss of control over drug-taking, or compulsive drug-seeking and -taking despite horrendous consequences. Abnormal behaviours are a consequence of aberrant brain function, which means that it is a tangible goal to identify the biological underpinnings of addiction. The genetic basis of addiction encompasses two broad areas of enquiry. One of these is the identification of genetic variation in humans that partly determines susceptibility to addiction. The other is the use of animal models to investigate the role of specific genes in mediating the development of addiction. Whereas recent advances in this latter effort are heartening, a major challenge remains: to understand how the many genes implicated in rodent models interact to yield as complex a phenotype as addiction.

Introduction

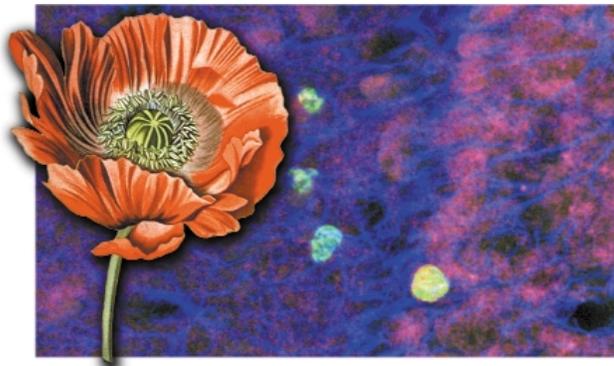
Social and psychological factors contribute to addiction, but it is clear that genetic factors also weigh in. Epidemiological studies have long established that alcoholism, for example, is familial, with estimates that genetic factors account for 40–60% of risk¹. More recent studies indicate similar rates of heritability for other drug addictions, including addiction to opiates and cocaine^{2,3}. Numerous linkage and association studies are now underway, with a view to identifying specific genetic variants that confer risk^{4,5}. Several relatively large chromosomal regions have been implicated in addiction vulnerability—although specific genetic polymorphisms have yet to be identified by this approach. It has been established, however, that some East Asian populations carry variations in enzymes (for example, the alcohol and aldehyde dehydrogenases) that metabolize alcohol. Such variants increase sensitivity to alcohol, dramatically ramping up the side effects of acute alcohol intake^{6,7}. Consequently, alcoholism is exceedingly rare in individuals, for example, that are homozygous for the *ALDH2*2* allele, which encodes a less active variant of aldehyde dehydrogenase type 2.

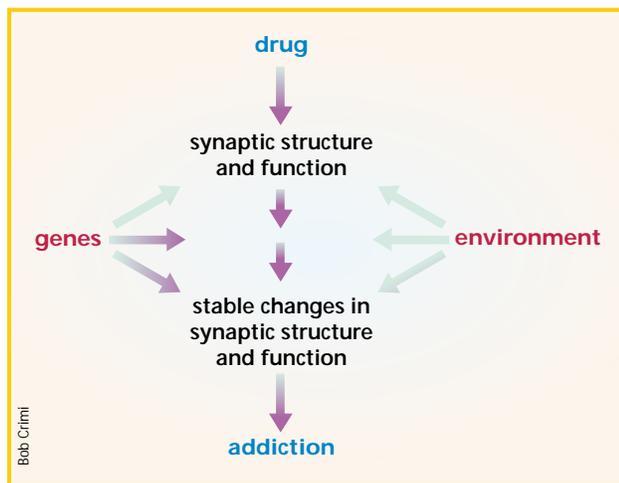
It is well established that inbred strains of mice and rats show robust differences in behavioural and biochemical responses to drugs of abuse^{8–11}. Lines of rodents have been selectively bred for increased or decreased responsiveness to alcohol or other drugs^{12,13}. They have also been bred for altered rates of alcohol drinking. Whereas genetic variations found in these models may be different from those of humans, their identification should shed light on mechanisms underlying the addiction process. Accordingly, several groups are attempting to identify the genetic basis of these behavioural differences among rodent strains and

lines by use of quantitative trait locus (QTL) analysis. In one early study, a predilection for drinking water laced with morphine was mapped to three large chromosomal regions¹¹. Several other groups have identified chromosomal regions that may contain QTLs related to particular responses to alcohol, cocaine or opiates. But as is the case with humans, no genetic polymorphism has yet been identified with certainty⁸.

The difficulty in finding genes that contribute to risk for addiction parallels the difficulty in finding genes for other psychiatric disorders and, in fact, for most common diseases. There are many reasons for this difficulty^{14,15}, including the fact that addiction is a complex trait. And so any single gene might produce a relatively small effect and would therefore be difficult to detect experimentally. It is also possible that variants in different genes may contribute to addiction in different lineages. And, as epidemiological studies have shown, non-genetic factors (for example, poverty, crime and delinquency) also affect risk, although they remain vaguely defined. Animal studies also show that environmental factors such as stress can interact with an animal's genotype to determine its ultimate response to a drug of abuse¹⁶. As a result, delineating the mechanisms by which specific genetic variations and environmental factors interact (see Fig. 1) is an important focus of investigation.

Another obstacle to identifying genes that determine vulnerability to addiction is the difficulty in quantifying behavioural endpoints, which have a greater degree of variance and are perhaps more susceptible to environmental influence than many non-behavioural phenotypes. A major focus of behavioural research is to establish behavioural endpoints with the same degree of sophistication and interassay reliability as is the case for non-behavioural traits. (See the *Correspondence*¹⁷ on page 263 for discussion of this issue.)





A gene might contribute to addiction vulnerability in several ways. A mutant protein (or altered levels of a normal protein) could change the structure or functioning of specific brain circuits during development or in adulthood. These altered brain circuits could change the responsiveness of the individual to initial drug exposure or the adaptations that occur in the brain after repeated drug exposure (see Fig. 1). In a similar manner, environmental stimuli could affect addiction vulnerability by influencing these same neural circuits. Perhaps combining genetic approaches with one of these specific (and more narrowly defined) phenotypes would facilitate the identification of addiction vulnerability genes in both humans and animal models.

In contrast with the slow progress in identifying genes that affect risk for addiction in humans, great strides have been made in demonstrating the role of specific gene products as assessed in animal models. The general strategy is to modify the amount of a particular gene product or the product itself, and to characterize the consequences of such modifications in behavioural tests. Mice with constitutive mutations continue to deliver insights into drug mechanisms, and mice with inducible and tissue-specific mutations (Table 1) are increasingly used to overcome limitations of constitutive mutants¹⁸. Other genetic approaches include viral-mediated gene transfer, intra-cerebral infusions of antisense oligonucleotides and mutations in non-mammalian model organisms.

Behavioural tests

Animals with altered levels of a particular gene product in the brain are subjected to a variety of behavioural tests to assess their responses to drugs of abuse (Table 2). These include measures of locomotor activity (most drugs of abuse increase activity when given acutely), and the progressive increase in locomotor activity (locomotor sensitization) that occurs with repeated drug exposure^{19–21}. The rationale behind these tests rests on the finding that locomotor responses are mediated by the mesolimbic dopamine system, which is also implicated in reward and addiction^{22–26}, but it should be noted that the relationship between locomotor responses, drug reward and addiction is a matter of some debate.

A more direct measure of drug reward is conditioned place preference, where an animal learns to prefer an environment that is paired with drug exposure²². It, too, is mediated partly by the mesolimbic dopamine system and is thought to model some of the powerful conditioning effects of drugs of abuse that are seen in humans. As with measures of locomotor activity, place-conditioning assays are amenable to relatively high-throughput design, which explains their popularity. But neither test directly measures the behavioural abnormalities (compulsive drug-seeking

and -taking) that are the core features of human addiction. To get closer to such abnormalities, operant tests must be applied, including self-administration, intracranial self-stimulation and conditioned reinforcement paradigms^{23–26} (Table 2). These tests, however, are quite complicated. Indeed, because of their intricacy, especially in mice, they have been used on a comparatively small number of genetically altered animals^{27,28}. A major challenge is to devise schemes that make application of these behavioural tests more widely available. One approach is to use oral self-administration procedures, which are much easier to implement than intravenous methods^{11,28}.

Other aspects of drug exposure have been studied in genetic models. For example, withdrawal symptoms have been used as a measure of opiate dependence on many occasions²⁹. The aversive (negative emotional) effects of drug withdrawal can be measured by conditioned place-avoidance assays, in which animals learn to avoid an environment associated with withdrawal. Moreover, investigators have measured the rate and degree of tolerance (or insensitivity) to the analgesic effects of opiates^{30,31}. This is important because the molecular basis of tolerance, which limits the use of opiates in the treatment of chronic pain, is poorly understood.

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Confirming initial drug targets

A straightforward use of genetic tools in the dissection of addiction is in the confirmation of targets of drugs. Pharmacological approaches can be used to identify drug targets, but they frequently fail to identify which of several subtypes is most relevant. In contrast are studies involving 'knockout' mice, which have led to the identification of the μ -opioid receptor, the dopamine transporter, the CB₁ cannabinoid receptor and the β_2 nicotinic acetylcholine receptor as targets that mediate rewarding and other effects of opiates, stimulants, cannabinoids and nicotine, respectively^{32–34}. Such success inspires hope that knockouts will also lead to targets (of drugs of abuse) where none are presently known—for example, inhalants. In some cases, knockout of the primary drug target has revealed the existence of secondary targets that partially compensate the loss of the primary target³⁵.

The neurotransmitters

In addition to secondary targets, there are numerous neurotransmitters, their receptors and post-receptor signalling pathways that modify responses to acute and chronic drug exposure. Genetic tools have not only confirmed pharmacological studies that implicate neurotransmitter pathways, they have provided fundamentally new insights into their mechanism.

For example, several behavioural aspects of mice lacking the serotonin 5HT_{1B} receptor indicate enhanced responsiveness to cocaine and alcohol; notably, they self-administer both drugs at higher levels than wild-type controls^{27,28}. The mice also express higher levels of Δ FosB (a Fos-like transcription factor implicated in addiction) under basal conditions. These observations point to the involvement of serotonergic mechanisms in addiction. There are many other such examples. Mice deficient in the dopamine D₂ receptor or the cannabinoid CB₁ receptor have a diminished rewarding response to morphine, implicating dopaminergic systems and endogenous cannabinoid-like systems in opiate action^{34,36}. Mice lacking the β_2 subunit of nicotinic cholinergic receptors show reduced rewarding responses to cocaine³⁷.

Table 1 • Genetic tools to study addiction

Genetic tool	Advantages	Disadvantages
Constitutive mutations in mice (knockouts and overexpressors)	complete loss of gene product (in case of knockout) methods well established	for many knockouts, loss of multiple gene products developmental adaptations complicate interpretation of adult studies ubiquity of mutation complicates interpretation for particular brain regions differences in genetic background between mutants and wild types complicate interpretation of the role of a particular gene
Inducible and cell-type specific mutations in mice (knockouts and overexpressors)	time that mutation is expressed can be controlled cell type in which mutation is expressed can be controlled	methods not yet perfected longer times to generate mice multiple genes required, complicating breeding strategies
Viral-mediated gene transfer	highly circumscribed spatial and temporal resolution of mutation can be developed quickly compared with mutant mice functional knockouts possible by overexpressing dominant negative mutants	toxicity, inflammation, and other possible changes due to viral infection or intracerebral injections not all neurons in injected area are infected (incomplete effect) transient expression of mutation
Antisense oligonucleotides	highly circumscribed spatial and temporal resolution of mutation can be developed quickly compared with mutant mice	toxicity due to oligonucleotides or intracerebral injections possible sequence-specific effects of oligonucleotides not mediated via the targeted mRNA incomplete knockout of gene product cannot be used to increase expression of protein of interest
Mutations in non-mammalian model organisms	rapid, high-throughput assays potential for discovery of novel genes and biochemical pathways involved in adaptations to repeated drug exposure	relevance to addiction requires analysis in mammalian systems

Several neuropeptides are implicated in drug responses. Mice with low levels of neuropeptide Y drink more alcohol—and those with high levels are more likely to abstain^{38,39}. Mice deficient in certain neurotrophic factors have abnormal behavioural responses to opiates and to cocaine—BDNF knockouts show reduced responsiveness, whereas GDNF knockouts are more responsive^{40,41}. These findings support the view that trophic mechanisms (for example, alterations in neural structure) may mediate some of the effects of drug administration on brain function.

Even though they may not develop the more complex aspects of addiction seen in mammals, non-mammalian model organisms can be used to identify biochemical pathways through which drugs act^{42–44}. A recent study determined that the *Drosophila melanogaster* mutant *inactive*, which is deficient in tyrosine decarboxylase and therefore cannot synthesize tyramine, remains sedate despite repeated doses of cocaine⁴³. This abnormality is reversed by administration of tyramine and raises the possibility that related biochemical pathways in mammals may modulate long-term adaptation to chronic drug exposure. In addition, the locomotor responses of flies to cocaine (acting through dopamine pathways) are remarkably similar to those of mammals, indicating that dopaminergic pathways were obligated in neuronal circuits controlling movement over one billion years ago.

Transcriptional mechanisms

The stability of the behavioural abnormalities that characterize addiction indicates that drug-induced changes in gene expression may be involved⁴⁵. As classic pharmacological agonists and antagonists to most proteins are not available, genetic tools are all the more attractive.

One demonstration of this approach is in the exploration of Δ FosB, a Fos-like transcription factor. Δ FosB accumulates in the nucleus accumbens (a target of the mesolimbic dopamine system) after chronic, but not acute, exposure to any of several drugs of abuse^{46,47}, including opiates, cocaine, amphetamine, alcohol, nicotine and phencyclidine (also known as PCP or 'angel dust'). This is in contrast with other Fos-like proteins, which are much less stable than Δ FosB and induced only transiently after acute drug administration. Consequently, Δ FosB persists in the nucleus accumbens long after drug-taking ceases. Adult mice in which Δ FosB can be induced selectively within the same subset of neurons in the nucleus accumbens as those in which it is induced by drug administration show a marked predilection for cocaine^{46–48}. It would seem that Δ FosB is a relatively sustained molecular 'switch' that contributes to a state of addiction.

The expression of another transcription factor, CREB, is also important. CREB knockout mice are less likely to develop opiate dependence²⁹—observing the effects of injecting antisense

Table 2 • Some behavioural tests commonly used to study addiction

Behavioural test	Description
Acute locomotor activation	acute increase in locomotor activity after initial administration of a drug of abuse
Locomotor sensitization	progressive increase in locomotor activity after repeated administration of a drug of abuse
Conditioned locomotor sensitization	increase in locomotor activity seen in environment (for example, testing chamber) where animals received repeated administration of a drug of abuse
Conditioned place preference	development of preference for an environment (for example, one side of testing chamber) associated with repeated administration of a drug of abuse
Oral self-administration	development of voluntary drinking of a drug of abuse in a palatable (for example, sucrose-containing) solution
Self-administration (operant-controlled)	
Acquisition	development of volitional (voluntary) administration (intravenous or intracerebral) of a drug of abuse by performing some task (for example, lever pressing)
Stable maintenance	amount of drug of abuse self-administered over a range of doses, providing a measure of the acute reinforcing value of the drug
Progressive ratio	determination of how hard an animal will work (for example, how many lever presses/unit time) to self-administer a drug of abuse
Extinction	progressive decrease in drug-associated task (for example, lever pressing) when drug is no longer available
Relapse (reinstatement)	return to drug-associated task (for example, lever pressing) even when drug of abuse is not available; this can be stimulated by acute challenge with the drug itself, a drug-associated cue (for example, light or tone), or stress
Intracranial self-stimulation	volitional (voluntary) electrical stimulation of particular brain regions by performing a task (for example, lever pressing), and potentiation of this behaviour by a drug of abuse
Conditioned reinforcement	development of volitional (voluntary) effort to receive an otherwise neutral stimulus (for example, light) associated with a reward (for example, a natural reward such as water), and potentiation of this behaviour by a drug of abuse

oligonucleotides to CREB into different parts of the brain points to the locus coeruleus (a brain region important for such dependence) as one relevant site of action⁴⁹. Overexpression of CREB in the nucleus accumbens counters the rewarding properties of opiates and cocaine; overexpression of a dominant-negative CREB mutant has the opposite effect^{50,51}. These findings suggest that CREB promotes certain aspects of addiction (for example, physical dependence), while opposing others (for example, reward), and highlight that the same biochemical adaptation can have very different behavioural effects depending on the type of neuron involved.

A challenge of current research is to identify target genes through which Δ FosB, CREB and other transcription factors act. Two strategies are used. One considers candidate genes: for example, genes that contain putative response elements for the transcription factor in question or whose products are implicated in relevant mechanisms in a relevant region of the brain. This approach has led to the identification of the AMPA glutamate receptor subunit GluR2 as a mediator of Δ FosB action⁴⁶. In a similar manner, the opioid peptide dynorphin was identified as a target for CREB, and shown to partly mediate CREB-induced repression of drug reward⁵⁰.

New molecular substrates

Candidate gene approaches are limited by our rudimentary knowledge of the gene products and the complex mechanisms underlying addiction. As a result, more open-ended strategies are needed, such as those based on analysis of differential gene expression in certain brain regions under control and drug-treated conditions. Differential display, for example, enabled the identification of NAC-1, a transcription factor-like protein, which is induced in nucleus accumbens by chronic cocaine and is

now known to modulate the locomotor effects induced by cocaine⁵². The neuropeptide CART was also first identified by differential display of mRNA in the nucleus accumbens before and after drug exposure. Whereas it may have a role in addiction, its current claim to fame is as a potent anorexigenic (anti-appetite) factor⁵³ that acts in the hypothalamus.

Various types of microarray analysis have led to the identification of large numbers of drug-regulated genes; it is typical for 1–5% of the genes on an array to show consistent changes in response to drug regulation⁵⁴. But without a better means of evaluating this vast amount of information (other than exploring the function of single genes using traditional approaches), it is impossible to identify those genes that truly contribute to addiction. Strategies under current evaluation include the analysis of detailed time courses of drug action and expression changes unique to carefully characterized behavioural states.

And, in the end...

Animal models have proved to be pivotal to our understanding of neurobiological mechanisms involved in the addiction process. One drawback (and one that is not limited to the field of addiction) is that sometimes a genetic mutation is found to result in a phenotype without any plausible scheme as to how the mutation actually causes that phenotype. Fortunately, the increasing sophistication of genetic tools, together with the increasing predictive value of animal models of addiction, makes it increasingly feasible to fill in the missing pieces—to understand the cellular mechanisms and neural circuitry that ultimately connect molecular events with complex behaviour.

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1. Kendler, K.S. *et al.* A twin-family study of alcoholism in women. *Am. J. Psychiatry* **151**, 707–715 (1994).
2. Tsuang, M.T. *et al.* Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch. Gen. Psychiatry* **55**, 967–972 (1998).
3. Kendler, K.S., Karkowski, L.M., Neale, M.C. & Prescott, C.A. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Arch. Gen. Psychiatry* **57**, 261–269 (2000).
4. Reich, T. *et al.* Genome-wide search for genes affecting the risk for alcohol dependence. *Am. J. Med. Genet.* **81**, 207–215 (1998).
5. Long, J.C. *et al.* Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *Am. J. Med. Genet.* **81**, 216–221 (1998).
6. Thomasson, H.R. *et al.* Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am. J. Hum. Genet.* **48**, 677–681 (1991).
7. Chen, Y.-C. *et al.* Alcohol metabolism and cardiovascular response in an alcoholic patient homozygous for the ALDH2*2 variant gene allele. *Alcohol. Clin. Exp. Res.* **23**, 1853–1860 (1999).
8. Crabbe, J.C., Phillips, T.J., Buck, K.J., Cunningham, C.L. & Belknap, J.K. Identifying genes for alcohol and drug sensitivity: recent progress and future directions. *Trends Neurosci.* **22**, 173–179 (1999).
9. Crawley, J.N. *et al.* Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology* **132**, 107–124 (1997).
10. Brodtkin, E.S. *et al.* Genetic analysis of behavioral, neuroendocrine, and biochemical parameters in inbred rodents: initial studies in Lewis and Fischer 344 rats and in *AJ* and *C57BL/6J* mice. *Brain Res.* **805**, 55–68 (1998).
11. Berrettini, W.H., Ferraro, T.N., Alexander, R.C., Buchberg, A.M. & Vogel, W.H. Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. *Nature Genet.* **7**, 54–58 (1994).
12. McBride, W.J. & Li, T.K. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit. Rev. Neurobiol.* **12**, 339–369 (1998).
13. Mogil, J.S. *et al.* The genetics of pain and pain inhibition. *Proc. Natl. Acad. Sci. USA* **93**, 3048–3055 (1996).
14. Weiss, K.M. & Terwilliger, J.D. How many diseases does it take to map a gene with SNPs? *Nature Genet.* **26**, 151–157 (2000).
15. Burmeister, M. Basic concepts in the study of diseases with complex genetics. *Biol. Psychiatry* **45**, 522–532 (1999).
16. Nestler, E.J., Berhow, M.T. & Brodtkin, E.S. Molecular mechanisms of drug addiction: adaptations in signal transduction pathways. *Mol. Psychiatry* **1**, 190–199 (1996).
17. Würbel, H. Behaviour and the standardization fallacy. *Nature Genet.* **26**, 263 (2000).
18. Gerlai, R. Molecular genetic analysis of mammalian behavior and brain processes: caveats and perspectives. *Sem. Neurosci.* **8**, 153–161 (1996).
19. Berridge, K.C. & Robinson, T.E. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Rev.* **28**, 309–369 (1998).
20. Wolf, M.E. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog. Neurobiol.* **54**, 679–720 (1998).
21. Kalivas, P.W. & Nakamura, M. Neural systems for behavioral activation and reward. *Curr. Opin. Neurobiol.* **9**, 223–227 (1999).
22. Tzschentke, T.M. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog. Neurobiol.* **56**, 613–672 (1998).
23. Koob, G.F., Sanna, P.P. & Bloom, F.E. Neuroscience of addiction. *Neuron* **21**, 467–476 (1998).
24. Wise, R.A. Drug-activation of brain reward pathways. *Drug Alcohol Depend.* **51**, 13–22 (1998).
25. Everitt, B.J. *et al.* Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. *Ann. NY Acad. Sci.* **877**, 412–438 (1999).
26. Shaham, Y., Erb, S. & Stewart, J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Res. Rev.* **33**, 13–33 (2000).
27. Rocha, B.A. *et al.* Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. *Nature* **393**, 175–178 (1998).
28. Crabbe, J.C. Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. *Nature Genet.* **14**, 98–101 (1996).
29. Maldonado, R. *et al.* Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. *Science* **273**, 657–659 (1996).
30. Bohn, L.M. *et al.* Enhanced morphine analgesia in mice lacking β -arrestin 2. *Science* **286**, 2495–2498 (1999).
31. Zhu, Y. *et al.* Retention of supraspinal d-like analgesia and loss of morphinemetolerance in d opioid receptor knockout mice. *Neuron* **24**, 243–252 (1999).
32. Matthes, H.W. *et al.* Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the m-opioid receptor gene. *Nature* **383**, 819–823 (1996).
33. Giros, B., Jaber, M., Jones, S.R., Wightman, R.M. & Caron, M.G. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* **379**, 606–612 (1996).
34. Ledent, C. *et al.* Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* **283**, 401–404 (1999).
35. Rocha, B.A. *et al.* Cocaine self-administration in dopamine-transporter knockout mice. *Nature Neurosci.* **1**, 132–137 (1998).
36. Maldonado, R. *et al.* Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. *Nature* **388**, 586–589 (1997).
37. Zachariou, V., Caldarone, B.J., Weathers-Lowin, A. & Picciotto, M.R. Nicotine receptor inactivation decreases sensitivity to cocaine. *Neuropsychopharmacology* (in press).
38. Thiele, T.E., Marsh, D.J., Ste Marie, L., Bernstein, I.L. & Palmiter, R.D. Ethanol consumption and resistance are inversely related to neuropeptide Y levels. *Nature* **396**, 366–369 (1998).
39. Murtra, P., Sheasby, A.M., Hunt, S.P. & De Felipe, C. Rewarding effects of opiates are absent in mice lacking the receptor for substance P. *Nature* **405**, 180–183 (2000).
40. Horger, B.A. *et al.* Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J. Neurosci.* **19**, 4110–4122 (1999).
41. Messer, C.J. *et al.* Role of GDNF in biochemical and behavioral adaptations to drugs of abuse. *Neuron* **26**, 247–257 (2000).
42. Bainton, R.J. *et al.* Dopamine modulates acute responses to cocaine, nicotine and ethanol in *Drosophila*. *Curr. Biol.* **10**, 187–194 (2000).
43. McClung, C. & Hirsch, J. The trace amine tyramine is essential for normal sensitization to cocaine in *Drosophila*. *Curr. Biol.* **9**, 853–860 (1999).
44. Andretic, R., Chaney, S. & Hirsch, J. A role for circadian genes in cocaine sensitization in *Drosophila melanogaster*. *Science* **285**, 1066–1068 (1999).
45. Nestler, E.J. & Aghajanian, G.K. Molecular and cellular basis of addiction. *Science* **278**, 58–63 (1997).
46. Kelz, M.B. *et al.* Expression of the transcription factor D FosB in the brain controls sensitivity to cocaine. *Nature* **401**, 272–276 (1999).
47. Kelz, M.B. & Nestler, E.J. D FosB: A mediator of long-term neural plasticity. *Curr. Opin. Neurol.* (in press).
48. Whisler, K. *et al.* Effects of conditional over-expression of D FosB in nucleus accumbens on cocaine-seeking behavior. *Soc. Neurosci. Abs.* **25**, 811 (1999).
49. Lane-Ladd, S.B. *et al.* CREB (cAMP response element-binding protein) in the locus coeruleus: biochemical, physiological, and behavioral evidence for a role in opiate dependence. *J. Neurosci.* **17**, 7890–7901 (1997).
50. Carlezon, W.A. Jr *et al.* Regulation of cocaine reward by CREB. *Science* **282**, 2272–2275 (1998).
51. Barrot, M., Neve, R.L. & Nestler, E.J. Influence of CREB activity in nucleus accumbens shell on psychomotor and rewarding properties of morphine. *Soc. Neurosci. Abs.* **25**, 35 (1999).
52. Kalivas, P.W., Duffy, P. & Mackler, S.A. Interrupted expression of NAC-1 augments the behavioral responses to cocaine. *Synapse* **33**, 153–159 (1999).
53. Kuhar, M.J. & Dall Vechia, S.E. CART peptides: novel addiction- and feeding-related neuropeptides. *Trends Neurosci.* **22**, 316–320 (1999).
54. Chen, J.S. *et al.* Induction of cyclin-dependent kinase 5 in hippocampus by chronic electroconvulsive seizures: role of D FosB. *J. Neurosci.* (in press).