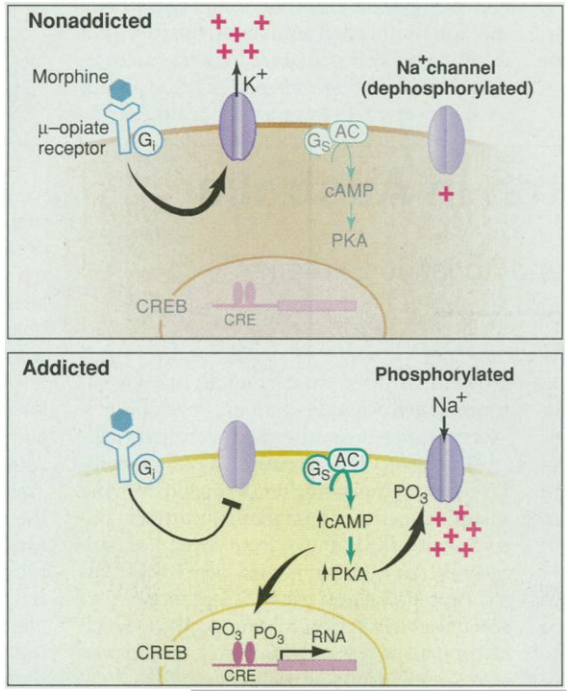
Shaking Out the Cause of Addiction

Steven E. Hyman

Repeated doses of addictive drugs—opiates, cocaine, and amphetamine—cause drug dependence and, afterward, withdrawal. These behavioral syndromes result from adaptation of the nervous system (*1*). The molecular mechanisms underlying these adaptations are just beginning to be understood. In this issue of *Science*, Maldonado and co-workers (*2*) identify one of the key elements for the withdrawal response, the transcription factor CREB (cyclic AMP–responsive element–binding protein). The new work shows that the physical effects of withdrawing opiates from addicted mice (shaking, for example) are much less severe in a mouse strain lacking two of the three types of CREB proteins.

Dependence on drugs of abuse develops only when the drug is administered in sufficiently large doses, at a high enough frequency, and over a long enough period of time. It is hypothesized that excessive bombardment of relevant receptors under these circumstances (either as a primary or secondary consequence of drug action) causes long-lived molecular adaptations in the signaling properties of neurons. These adaptations can occur in three classes of neural systems: (i) physical control systems, which mediate autonomic and other somatic functions, leading to physical dependence and subsequent physical withdrawal symptoms upon drug cessation; (ii) motivational control systems, which govern motivated behavior—especially the mesoaccumbens dopamine projection that extends from the ventral tegmental area of the midbrain to the nucleus accumbens; and (iii) associative memory systems, which produce powerful learned associations that predispose an individual to cue-dependent drug craving.

All highly addictive drugs produce adaptations in the mesoaccumbens circuitry (*1*, *3*), and CREB appears to be critical for at least some of these (*3*). Only a subset of addictive drugs—opiates and ethyl alcohol, but not cocaine or amphetamine—produce physical dependence as manifested by a physical withdrawal syndrome. Thus the physical system is a significant, but not absolutely necessary, component of drug addiction.



How opiates act in neurons of nonaddicted and addicted animals. (Top) Opiates act via their μ–opiate receptor to inhibit firing of a locus ceruleus (LC) neuron. (Bottom) With long-term opiate use, the cAMP cascade becomes up-regulated, and the excitability of LC neurons is increased, an effect that is unmasked by administration of opioid antagonists or by opiate abstinence. CREB-mediated transcription may mediate some of the key adaptations that produce this alteration in LC neurons. The critical CREB-regulated genes and their precise connection to the physiologic changes in LC excitability have not been established. AC, adenylyl cyclase; PKA, cAMP-dependent protein kinase A; +, positive charges.

The noradrenergic locus ceruleus (LC) in the dorsal pons is a critical neural substrate for opiate-induced physical dependence and withdrawal (*1*, *4*). The major opiate receptor for morphine-like opiates (including heroin) is the heterotrimeric GTP-binding protein (G protein)–linked μ–opiate receptor. This receptor is expressed on LC neurons, on neurons regulating the mesoaccumbens pathway, in the nucleus accumbens itself, and in many other brain regions. Long-term opiate administration causes a decrease in μ–opiate receptor signaling in the LC (tolerance) without causing a decrease in the number or affinity of these receptors—thus implicating post-receptor signal transduction mechanisms as the site of adaptation. Indeed, morphine-like opiates induce both tolerance and dependence in LC neurons by causing adaptations in the cyclic AMP (cAMP) signaling system (see the figure). A single dose of opiate inhibits the firing of LC neurons. Multiple opiate doses eventually cause tolerance; LC firing rates return toward normal despite the continued presence of the opiate (*1*). In addition, LC neurons exhibit dependence: Administration of opiate receptor antagonists to opiate-dependent rats results in dramatically increased LC firing rates in vivo (*1*). This increased firing of LC neurons is associated with the behavioral opioid withdrawal syndrome.

Opiates initially inhibit LC firing by regulating two types of ion channels: Opiates activate a K^+ channel and inhibit a slowly depolarizing, pacemaker-like Na^+ channel (which is activated by cAMP-dependent phosphorylation). Opiate regulation of the K^+ channels is mediated directly by the G proteins G_i and G_o, and opiate regulation of the Na^+ channel is mediated by an initial decrease in cAMP levels, presumably leading to decreased phosphorylation of the Na^+ channel (see figure, top panel).

With long-term opiate administration, levels of several proteins in the cAMP-dependent signaling cascade are increased in the LC, including adenylyl cyclase and cAMP-dependent protein kinase. Among the multiple effects of up-regulating the cAMP pathway, the slowly depolarizing Na^+ channel becomes more fully phosphorylated, making LC neurons hyperexcitable (see figure, bottom panel). Indirect evidence had suggested that CREB, the critical nuclear target of the cAMP pathway (as well as of some Ca^{2+}-dependent pathways), might regulate the amounts of these critical signaling proteins, as well as perhaps genes for biosynthetic enzymes such as tyrosine hydroxylase, which is up-regulated in the LC by opiates and binds CREB. The new work by Maldonado *et al.* (*2*) provides the most direct evidence to date that CREB is a critical actor in opiate-induced adaptations. [Because immediate early gene activation (the usual result of CREB stimulation) was not changed in the transgenic mice, and therefore the functional deficit was not localized neuroanatomically, the results of Maldonado *et al.* do not actually confirm that the critical structure in which CREB acts is the LC.]

Developmental compensations for the missing molecules make treacherous the interpretation of data from mice produced by current knockout technologies; the mice used for these experiments have diminished concentrations of CREB protein and, presumably, CREB activity in all tissues throughout

The author is director of the National Institute of Mental Health, 5600 Fishers Lane, Rockville, MD 20857, USA. E-mail: shyman@nih.gov

development. Nevertheless, in the present case these difficulties are quite significant because the investigators must implicitly assign certain adaptations to development and others to opioid drug administration. In addition, one cannot tell whether the failure of the partial CREB knockout to alter the expected induction of immediate early gene expression or of adenylyl cyclase activity in response to opioid abstinence is due to signaling pathways independent of CREB or whether the persisting CREBβ form, up-regulated in these mice, is adequate to compensate in some physiologic roles, but not others.

Despite these caveats, the work by Maldonado *et al.* provides significant new evidence that CREB is involved in physical opioid dependence. This identification of a potential role for CREB in drug-induced neural plasticity parallels recent work on memory that implicates CREB as a key molecule in converting short-term environmental stimuli into long-term changes in brain function. For example, CREB is required for the maintenance phase of long-term potentiation and for some aspects of long-term memory in *Aplysia*, *Drosophila*, and mice (5). Now, CREB is involved in another type of molecular memory—drug-induced neural plasticity that leads to behaviors associated with addiction.

References

1. E. J. Nestler, *Neuron* **16**, 897 (1996); S. E. Hyman, *ibid.*, p. 901; G. F. Koob, *ibid.*, p. 893.
2. R. Maldonado *et al*, *Science* **273**, 658 (1996).
3. R. L. Cole, C. Konradi, J. Douglass, S. E. Hyman, *Neuron* **14**, 813 (1995).
4. R. Maldonado, L. Stinus, L. H. Gold, G. F. Koob, *J. Pharmacol. Exp. Ther.* **261**, 669 (1992).
5. T. J. Carew, *ibid.* **16**, 5 (1996); R. Bourtchuladze *et al.*, *Cell* **79**, 59 (1994).

Rural Research in Australia

John C. Radcliffe and Adrienne E. Clarke

Australia's economy depends heavily on agriculture and mining, industries that provided wealth for the early European settlers. The initial driving force behind Australian research and development (R&D) began then as the settlers struggled to adapt European crops and methods of farming to food production in a very different environment.

The early farmers identified problems needing solutions and soon petitioned the government to invest in R&D. A modern system of rural R&D has subsequently evolved in which rural producers, processors, and the research community together secure budgets and set research agendas. The system encourages teamwork and cooperation within the R&D community, which is small on a world scale. Producer and scientist peer-review is a cornerstone of the system, and Australian farmers are avid users of resulting technology.

Almost all Australian rural R&D is performed in the public sector within 10 federally funded universities, by state and territory governments, or by national agencies. The principal national research body is the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Traditionally, CSIRO undertakes investigations of a more strategic nature. Locally relevant and applied research is undertaken by the state agencies.

Since 1936, agricultural industries have created research councils by collecting levies on production (similar to the "check-offs" collected in some American rural industries). The federal government has provided dollar-for-dollar matching for the levies collected from growers. In 1989, these councils became autonomous R&D corporations, developing 5-year strategic plans and annual operating plans.

Much corporation research is now commissioned by competitive tendering on cost and scientific merit, criteria that may attract consortia with skills drawn from several organizations. An example is the Dairy R&D Corporation project on the development of persistent legumes in tropical dairy pastures, which encompasses researchers from two separate states' agricultural agencies and CSIRO.

Is rural R&D worth the cost? A 1995 Industries Commission inquiry (*1*) examined a range of benefit:cost studies as part of their evidence. One of these studies included research projects (with their benefit:cost ratios) on control of "take-all" disease in wheat (92.2), nematode-tolerant grape root stocks (25.5), new cotton varieties (18.4), new tropical pastures based on disease-resistant *Stylosanthes* spp. (4.7), and replacement of asbestos fiber reinforcing with wood fibers in cement sheet (72.2). In all, the CSIRO research cost $AUD 161 million with estimated returns of $AUD 2371 million (*2*). The Industries Commission concluded that "the returns to society from investing in rural R&D are high."

Technologies derived from R&D have benefited the nation enormously. Natural resources have been protected through biological control methods, including the elimination of large areas of prickly pear (*Opuntia*) by *Cactoblastis cactorum*, the development of *myxomatosis* to control rabbits, and the control of skeleton weed (*Chondrilla juncea*) with a rust fungus and of sirex wasp (*Sirex noctilio*) in forests with parasitic nematodes.

An effective Australian timber industry has developed from the establishment of major plantations based on the scientific management and improvement of *Pinus radiata*; the development of high-temperature softwood drying technologies; and the development of low-cost, readily-available chemical preservatives for plantation timbers lacking natural durability. Hardwood eucalypts have been adapted for paper-making; their fine fibers complementing the larger fibers of softwoods to produce a smooth, fine paper sheet.

Australia's crop and livestock industries have also benefited greatly from research. Examples include the development of rhizobial nitrogen and phosphate fertilizers to exploit Australia's low-fertility soils; recognition of the role of hard seededness in maintaining annual medics and clovers in cereal-livestock rotations; and modeling the cropping potential of Australia's cereal lands by defining the mathematical relation between rainfall, its seasonal distribution, and grain yield. Decision-support systems based on previous research results are now becoming available for farmers.

Australia's wine industry has recently become internationally competitive through research that allowed adaptation of Australian vineyards to mechanized pruning and picking, and the development of a scientific understanding and technical reproducibility of the cold-fermentation processes brought from Germany in the 19th century. New industries since 1970 include cotton, now based on Australian-bred varieties; tropical beef based on *Stylosanthes* pastures, currently expanding at the rate of 100,000 hectares per annum; grain-lupines, into which high-methionine genes are now being incorporated; and the adaptation of canola to Australian conditions to form a new oilseed industry.

A National Agricultural Research Strategy has recently been developed for government and industry to maximize complementarity between researchers and R&D investors. When adopted, Australia should retain its competitiveness through producer-driven, government-encouraged R&D.

References

1. Industries Commission, *Research and Development* [Australian Government Publishing Service, Canberra, Australian Capital Territory (ACT), 1995].
2. B. Johnston *et al.*, "Rural Research—The Pay-Off," *CSIRO Occas. Pap. no. 7* (CSIRO, ACT, 1992).

J. C. Radcliffe is at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Private Bag 2, Glen Osmond, South Australia 5064, Australia. E-mail: john.radcliffe@exec.csiro.au. A. E. Clarke is in the School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia. E-mail: adrienne_clarke@muwayf.unimelb.edu.au