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47. In these experiments, elimination of lamellipodial actin by CB was associated with a fairly rapid distal extension of microtubule ends. Upon removal of CB and the reappearance of actin filaments, these microtubule endings retreated to their former central locations. This phenomenon may reflect regulation of microtubule elongation by an interaction with the peripheral actin matrix, as suggested from a variety of different observations [H. C. Joshi, D. Chu, R. E. Buxbaum, S. R. Heidemann, *J. Cell Biol.* **101**, 697 (1985); P. C. Letourneau, T. A. Shattuck, A. H. Ressler, *Cell Motility* **8**, 193 (1987)].
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Cellular and Molecular Mechanisms of Drug Dependence

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The molecular and cellular actions of three classes of abused drugs—opiates, psychostimulants, and ethanol—are reviewed in the context of behavioral studies of drug dependence. The immediate effects of drugs are compared to those observed after long-term exposure. A neurobiological basis for drug dependence is proposed from the linkage between the cellular and behavioral effects of these drugs.

SUBSTANTIAL PROGRESS HAS BEEN MADE IN ANALYZING THE molecular and cellular actions of three major types of abused drugs: opiates, such as heroin and morphine; psychostimulants, such as cocaine and amphetamine; and alcoholic beverages (ethanol). The growing effects of drug addiction on society include increased criminal behavior as well as the direct consequences of drugs on health and their associated costs (1). Although our

understanding of the biology of drug addiction is improving, no effective preventative strategies have been attained. Attention and resources have been focused instead on treatment after addiction. To generate obsessive drug-seeking and drug-taking behavior, an addictive drug must act on the cells and molecules of the nervous system. However, the sites and mechanisms that participate in these effects have not been well resolved, and the basis for individual variation in addictive liability is unknown.

In this article we describe our attempts to determine whether the molecular, cellular, and behavioral data on acute and chronic effects of addictive drugs form an internally consistent sequence of events in which molecular events generate cellular effects that in turn link to behavioral phenomena to explain the common features of drug dependence (2). We discuss the basic phenomenon of drug dependence and some theories of addiction and survey recent progress in studies of the pharmacological characterization of the three proto-

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type drug classes at the behavioral, cellular, and molecular levels. Our focus is on both the acute initial effects of drugs of abuse on specific neurons and the change in these effects with continued drug exposure. We then seek to link these data into generalizable features of addictive drugs and of the systems on which they produce their effects. Finally, we propose a potential role for a specific limbic-extrapyramidal system that has been implicated in both the reinforcement and adaptive responses to all three drug classes.

Drug Dependence

Terminology. The terms drug dependence, drug abuse, and drug addiction have many colloquial meanings. In scientific studies, however, physical dependence has been defined as "an adaptive state that manifests itself by intense physical disturbances when the administration of a drug is suspended" [Eddy *et al.*, in (3), p. 723] and psychic dependence, as "a condition in which a drug produces a feeling of satisfaction and a psychic drive that requires periodic or continuous administration of the drug to produce pleasure or to avoid discomfort" [Eddy *et al.*, in (3), p. 723]. Psychic dependence has traditionally been linked to the behaviorally reinforcing properties of drugs. Both physical and psychic dependence characterize the addicted state. We maintain that these two definitions are virtually inseparable when one attempts to identify the molecular and cellular elements of drug dependence. A more recent definition of psychoactive substance dependence used for diagnostic purposes is that dependence "is a cluster of cognitive, behavioral, and physiologic symptoms that indicate that the person has impaired control of psychoactive substance use and continues use of the substance despite adverse consequences" (4, p. 166). A third component of the drug dependence picture is tolerance, that is, the progressive requirement with chronic use for higher drug doses to produce a given effect. Tolerance can be rapid or slow (5) and appears to have a major learned component (6). Tolerance can be viewed as an attempt by the body to return to homeostasis, equivalent to the drug-free condition.

Theories. Jaffe (7, p. 534) has emphasized that experimental animals with no previous drug exposure will readily self-administer psychoactive drugs and that their patterns of drug taking "are strikingly similar to those [of] human users of the same drug." He interprets this to mean that drugs can elicit their characteristic patterns of behavior on a normal biological substrate and that no preexisting psychopathology or addictive vulnerability is required for initiation of drug self-administration. This does not preclude the well-recognized individual variations in addictive vulnerability. This view, supported by data described here, raises the questions of how reinforcing effects are generated, which brain systems participate, and what the normal functions of these systems are.

Many models of addiction are based on evidence that physical dependence and tolerance generally develop and decay with a similar time course. These similar time patterns have led to the concept that as the drug user reacts to the effects of the drug, adaptive "processes" are initiated to counter the effects of the drug and these processes persist after the drug has been cleared from the brain, thereby leaving the opposing processes unopposed. Such notions of undefined opposing or adaptive processes are not new (8) and have been used at all levels of drug abuse research from the behavioral (9) to the cellular and molecular levels (10). However, many behavioral and cellular data now indicate that tolerance and dependence are separable processes with distinct spatial locations in the brain and with unique molecular mechanisms of action.

Individual variations in addictive vulnerability have also been attributed conceptually to a variety of social and biological factors.

Experimental animals can be bred for selective sensitivity to alcohol and opiates and for preferential drug self-administration (11). Furthermore, certain patterns of alcoholism have strong familial transmittance that are independent of social and environmental effects (12). These presumptive biological components have remained largely untestable, however, because the mechanisms by which drugs could interfere with the activities of specific neurons have not been defined and because the nature of the "counteradaptive" or "opposing" mechanisms have not been well characterized.

Strategies of research. The strategies used to analyze the effect of addictive drugs on the central nervous system (CNS) may be molecular (13–15), cellular (16), or behavioral (17). With combinations of these strategies researchers can test whether a molecular mechanism operating on a specific set of neurons is necessary and sufficient to explain a behavioral effect. Early studies indicated that dopamine-containing neurons and their terminal regions are essential for the primary reinforcing effects of psychostimulants (18). In the case of opiate drugs, however, no links were found to any known transmitter or neural location before the endogenous brain opioid systems were discovered (19). That discovery then redirected both molecular and cellular research in the opiate field. Mapping the neuronal circuits containing dopamine (20) or the endogenous opioid peptides (21) provided specifiable templates for sites at which the neuronal operations of cocaine or opiate addiction could be evaluated. No comparable template has yet been formulated for the actions of ethanol, but it is also likely that more transmitters and other mechanisms of signal transduction (16) remain to be discovered.

Cocaine and Psychostimulants

Behavioral observations. Cocaine, *d*-amphetamine, and methamphetamine are readily self-administered by humans, monkeys, and rats (22), and the immediate effect of these drugs is to lower reinforcement thresholds for brain stimulation reward (23). When access to the drugs is limited to several hours per day, no evidence of physical dependence is found, and animals, as well as humans, will maintain a stable level of drug intake within a limited range of response requirements and doses (22). However, when allowed continuous access to psychostimulants, animals will self-administer the drug in long binges, and a significant number of animals will self-administer a lethal overdose after several days (24).

When a binge of psychostimulant use in humans ends, symptoms similar to an episode of major depression are observed. This "crash" is accompanied by decreases in activity and initiative, excessive drowsiness, increased appetite, dysphoria, and anhedonia (25). The dysphoria that results from chronic psychostimulant use can be lengthy and may be important for cocaine craving and recidivism (25). A neurobiological basis of psychostimulant withdrawal has been proposed because reinforcement thresholds increase after rats are withdrawn from chronic amphetamine and cocaine self-administration (26). These behavioral changes are long-lasting, specific, and may represent a model for the adaptive processes that oppose the immediate effects of cocaine (8, 26).

The neurobiological mediators of psychostimulant reinforcement appear to be the central catecholamines, most notably the neurotransmitter dopamine. Dopamine-containing neurons of the ventral tegmentum and their tracts that innervate limbic and frontal cortex are required for the acute reinforcing actions of cocaine and *d*-amphetamine (18, 27). Lesions of specific subsets of the dopamine forebrain projections have been associated with facilitated acquisition of amphetamine self-administration (28), suggesting that some specific neuropathology within the dopamine system could sensitize

individuals to the reinforcing actions of psychostimulants. No specific neurotransmitters have been implicated in the psychostimulant withdrawal symptoms (29).

Cellular actions of cocaine. The cellular basis for cocaine actions within the dopamine system of neurons has only recently begun to be elucidated. By using extracellular recording and iontophoretic drug administration, White and Wang (30) showed that dopamine normally depresses the spontaneous activity of neurons of the nucleus accumbens innervated by dopaminergic neurons of the ventral tegmental area (VTA). However, in the VTA, dopamine was three to ten times more potent in inhibiting firing than in the nucleus accumbens. Whereas the responsible receptor subtype in the accumbens was a mixture of D1 and D2 subtypes, that in the VTA was largely D2.

Intravenous administration of cocaine produced potent inhibition of VTA cell firing, but iontophoresis of cocaine directly into VTA had only modest direct effects (31). The drug did, however, significantly increase and prolong the inhibitory effects of dopamine, as do other drugs known to affect neuronal uptake of dopamine. Drugs affecting neuronal uptake of the other major brain monoamines, norepinephrine (NE) and serotonin (5HT), did not potentiate dopamine effects. Furthermore, cocaine did not potentiate NE or 5HT when these effects on dopamine action were observed. White and co-workers (31, 32) also suggested that an accumbens-to-VTA pathway, by means of γ -aminobutyric acid (GABA) as the transmitter (33), may normally coordinate this circuit through an inhibitory feedback loop. It remains to be determined whether cocaine acts only on dopaminergic neurons in the VTA or on other dopamine sources, on their synaptic targets in the accumbens or other sites, or on both sets of target structures, presynaptically and postsynaptically.

According to the currently favored molecular view, it is the ability of cocaine to inhibit dopamine reuptake (34) in sites such as the nucleus accumbens that produces its reinforcing action. Both D1 and D2 receptor subtypes have also been implicated in cocaine reinforcement (35).

These data support the conclusion that cocaine augments dopaminergic neuron activity by prolongation of the effects of released dopamine, via blockade of the reuptake process that normally terminates local dopamine actions. Amphetamine produces a similar molecular effect (34), but it can also release dopamine and NE (13) as well as inhibit their reuptake (or transporter) site, thereby also producing both pre- and postsynaptic receptor effects. Postsynaptic effects of cocaine have not been well documented (36) nor have any changes in these catecholamine neurotransmitter actions been reported after long-term exposure to cocaine.

Opioids

Behavioral observations. The opioids, derived from the opium poppy, have been used by humans for centuries (37). Monkeys and rats will readily self-administer opioids, and single doses of opioids will lower thresholds for intracranial brain stimulation reward (38). Animals will become physically dependent with unlimited access, much like human opiate addicts; however, as with psychostimulants, human and animal users allowed only limited access to opiates will maintain a stable level and pattern of opioid intake, which—when interrupted—reveals neither tolerance nor physical dependence as measured by increases in drug intake or gross physical abstinence signs (39). In contrast, chronic unlimited access to opiates invariably produces profound tolerance, leading to increased intake and a severe withdrawal syndrome. On drug cessation (7), the symptoms that emerge range from a mild flu-like state to major physical signs

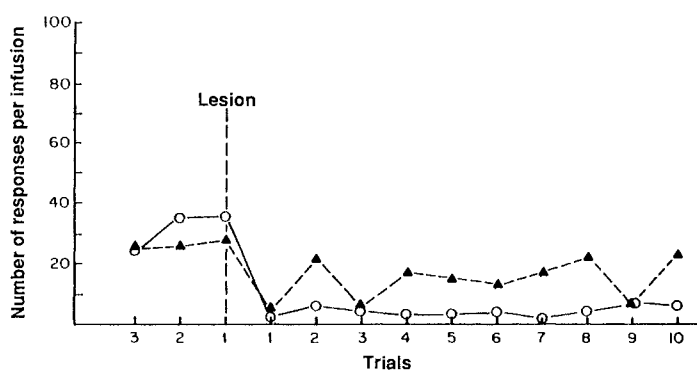


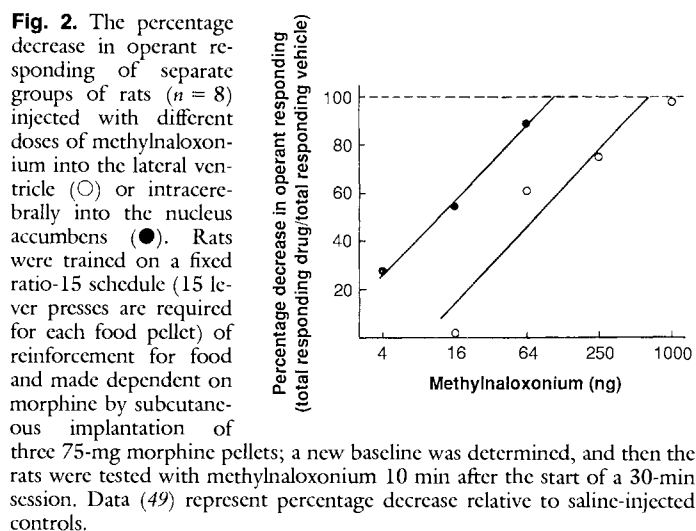
Fig. 1. Intravenous self-administration of 0.75 mg/kg of cocaine (○) and 0.06 mg/kg of heroin (▲) on alternate days before and after lesion of the nucleus accumbens with 6-hydroxydopamine in one rat [details in Pettit in (46)]. Lesion resulted in more than a 90% depletion of dopamine in the nucleus accumbens.

and discomfort including severe muscle pain, gastrointestinal cramps, and diarrhea, depending on the dose and duration of usage (3).

Although these aversive physical effects of withdrawal were thought to be a major motivating factor in opiate dependence (7, 9), more recent work has indicated that the reinforcing properties of opiates may be separated from these physical signs. Bozarth and Wise (40) showed that rats not dependent on morphine will self-administer the drug directly into the VTA with no abstinence signs of withdrawal. However, repeated administration of morphine into the periaqueductal gray, but not VTA, causes signs of withdrawal after challenge with naloxone; these data substantiate earlier observations with less precise localization of drug effects (41). Others have observed self-administration of opioids into the nucleus accumbens and lateral hypothalamus (42) in nondependent rats.

Studies in which local intracerebral injections of slowly diffusible opiate antagonists were given to animals trained to self-administer heroin intravenously have pointed to the same regions of the brain as those implicated by studies of the reinforcing properties of psychostimulants. Administration of an antagonist causes increased efforts by the animal to self-administer the drugs, presumably to restore drug effects at receptors blocked by the antagonist. Thus, administration of quaternary nalorphine into the VTA increased heroin self-administration (43), as did administration of methylaloxonium into the lateral ventricle, VTA, and nucleus accumbens, the latter being the most sensitive site (44). Similarly, methylaloxonium when injected into the nucleus accumbens was most potent in reversing the locomotor stimulation produced by heroin (45). These results suggest that opiate receptors in both the VTA and the nucleus accumbens may mediate the reinforcing actions of opiates.

The role of the nucleus accumbens itself in opiate dependence was strengthened by subsequent studies. Dopamine receptor blockade or dopamine denervation of the nucleus accumbens with the neurotoxin 6-hydroxydopamine can eliminate cocaine and amphetamine self-administration (17, 46), but this same treatment spares heroin self-administration in rats (Fig. 1). However, cell body lesions restricted to the nucleus accumbens block both cocaine and heroin self-administration, as do ventral pallidal lesions (46). This lesion work suggests that neurons in the region of the nucleus accumbens that project to the ventral pallidum may be critical for mediating the reinforcing properties of opiates and psychomotor stimulants. According to this view, the nucleus accumbens would be a convergent target for both psychostimulant and opiate reinforcement, with both drug classes activating a common link to the ventral pallidum.



The neural circuitry involved in opiate dependence has been explored by intracerebral injections of naloxone and methylnaloxonium (47). When a sensitive operant measure of abstinence was used (48), methylnaloxonium was substantially more potent in decreasing performance when injected into the region of the nucleus accumbens of opiate-dependent rats than when injected into the lateral ventricle, the periaqueductal gray, or the medial thalamus (49) (Fig. 2). These effects were not seen in nondependent rats except at very high doses; thus, they may be a model for the aversive stimulus effects of opiate withdrawal.

However, methylnaloxonium injected intracerebroventricularly into dependent rats can precipitate a physical withdrawal syndrome (wet dog shakes, ptosis, and jumping, for example, but not diarrhea); no similar results are achieved with injections of methylnaloxonium into the nucleus accumbens or periaqueductal gray (49). Thus, the loci for reinforcement are not identical to the loci for physical dependence.

Cellular observations on opioids. Attempts to identify the cellular mechanisms of opiate tolerance and dependence were renewed after the discoveries of multiple endogenous opioid peptides (18), their partially overlapping circuitry maps (19), and their multiple opioid receptor subtypes (50). By the late 1970s virtually all endogenous and exogenous opioids had been observed to inhibit cell firing through their specific receptors (51). Those few neurons (such as hippocampal neurons) that appear to respond to opiates by firing faster have been attributed to local disinhibitory effects, with the opioids actually inhibiting inhibitory neurons. Whereas morphine and opioid peptides, acting at the μ receptor, activate cell firing of dopamine neurons of the substantia nigra, this effect also appears to arise from disinhibition locally (52). Although the μ receptor is most frequently denoted as the critical site for opioid reinforcement (53), the overall effects of μ opioids on the firing rate of nigra neurons would be qualitatively opposite to those expected in VTA on the basis of what has been directly observed for cocaine.

One brain site examined extensively for opioid pharmacology has been the locus ceruleus (LC). This paired nuclear structure in the dorsal pons is the largest collection of noradrenergic neurons in the CNS, which have axons that project widely in the entire forebrain (54). Opioids inhibit LC firing both in vivo (55, 56) and in vitro (57). This is a μ opiate receptor response in which a membrane hyperpolarizing effect results from activation of an inwardly rectifying K^+ conductance. The μ receptor activates the K^+ conductance directly via a guanine nucleotide binding (G) protein, but with no other detectable second messenger system involved. The same K^+

conductance is also regulated independently and through a separate G protein by an α_2 adrenergic receptor (57).

Where, however, are the cellular sites responsible for withdrawal? When LC responses in rats were studied after continuous or repeated opiate exposure, the neurons showed complete tolerance (that is, no response to morphine) and, on withdrawal of the opiates, LC firing greatly increased (55). When the studies were repeated in vitro (57), only tolerance was again observed and shown not to be due to residual opioids in the tissue, opiate receptor loss, or inactivation of K^+ conductance. Furthermore, separate experiments (57) eliminated as a cause other effects such as changes in receptor number or in opioid peptide metabolism. Therefore, it would appear that opioid tolerance here arises because of a failure to couple efficiently the activated opiate receptor to the G protein that activates the K^+ conductance mediating this opioid action.

The LC neurons thus show tolerance and dependence in vivo but show only tolerance in vitro. Comparison of these data suggests that the response of the intact nervous system to abstinence cannot arise solely from the LC neurons and presumably requires their intact afferent synaptic connections. The latter idea could be tested under similar conditions by direct examination of the relatively small number of sites that connect to LC neurons (58). The actions ascribed to opiate receptors for the LC contrast with effects observed in other forebrain regions, such as hippocampus and cerebral cortex (59). These LC data also contrast with data from the cat dorsal horn neurons, which show a rapid development of dependence but no tolerance (60). Other brain sites exhibiting both tolerance and withdrawal in vivo include unidentified neurons of the cerebral cortex, medial thalamus, and striatum (61).

Thus, opioid reinforcement may involve activation of opiate μ receptors in VTA (activating dopaminergic neurons via disinhibition, and changes in opiate receptors in nucleus accumbens and perhaps other forebrain sites). These effects would tend to inhibit the forebrain targets of the dopamine circuits. These μ opioid effects would presumably also depress neuronal activity in these forebrain locations, as well as in the LC. With continued opiate exposure, LC neurons would become tolerant and would no longer be slowed; these neurons could then serve to oppose adaptation to the actions of opiates, firing excessively in vivo when the opiate effects regress. It remains to be determined whether this alteration of LC neurons by the drugs could be a component of the learned aspect of tolerance (6). The overall immediate and long-term effects of exogenous opiates on the brain thus involve many interactive systems. Moreover, cellular and behavioral changes that result from withdrawal and that have motivational relevance to drug-seeking behavior probably involve the same neural circuits as those that participate in the positive-reinforcing effects of short-term use of opiates before dependence. However, Wise (62) has suggested an opposite hypothesis.

Ethanol

Behavioral observations. Ethanol is readily self-administered by man in virtually every culture and by selected sets of experimental animals. Ethanol has reinforcing properties, causing both mild euphoria and reduction in perceived anxiety. Those actions, generally seen on initial exposure and at lower doses, may be linked to or be independent of the resulting more prolonged motor and cognitive intoxicating effects seen at higher doses. Compared to the doses required for responses to opiates and to cocaine, ethanol is a much weaker drug (potency). However, when taken in large amounts, its profile of central depressant actions (effectiveness) broadly parallels several other CNS depressants such as barbiturates and benzodiazepines.

piners. In animals, ethanol can produce a release of punished responding (increase in behavior that was suppressed by an aversive stimulus) in conflict situations (63); recent pharmacological evidence suggests that this anticonflict behavioral effect may be mediated via the picrotoxinin site of the benzodiazepine-GABA-ionophore complex (64), but the neural location of this effect has not been established. As with opioids and psychomotor stimulants, some humans given chronic unlimited access to ethanol will self-administer it to the point of significant tolerance and dependence. Social users who voluntarily limit consumption are much less likely to become dependent or tolerant (7). The abstinence syndrome associated with ethanol withdrawal after chronic administration has also been viewed as a source of recidivism and dependency relapse (65).

Ethanol withdrawal is characterized by a syndrome that ranges from a severe hangover to profound anxiety, tremulousness, severe sympathetic hyperactivity, psychoses, seizures, and death (7, 65). This withdrawal syndrome can be readily reproduced in animals after forced administration of ethanol but is rarely observed in animals voluntarily self-administering the drug. When given continuous access to ethanol, monkeys self-administer ethanol intravenously in patterns resembling the human cycle of abuse (66).

By manipulation of reinforcement schedules, rats can be induced to ingest quantities of ethanol that result in physical dependence, but they will not sustain such levels of ingestion on termination of the schedule (67). These observations have led to questions about the validity of animal models of alcoholism and alcohol abuse (68). However, animals with limited access to food and water will readily self-administer ethanol in quantities sufficient to produce intoxication, and rats without food or water deprivation can be readily trained to self-administer ethanol to produce reliable blood ethanol levels with the use of sucrose substitution procedures (69). Furthermore, rats showing some spontaneous alcohol preference can be bred to produce offspring (11) in which this preference will progressively increase over several generations, until the rats will ingest ethanol with unlimited access in sufficient quantities to produce physical dependence.

On the basis of behavioral assays of alcohol consumption involving pharmacological treatment, several neurotransmitters, including NE, 5HT, GABA, and opioids (70), have been postulated to mediate the reinforcing actions of ethanol. Many of these drug treatments produce malaise and taste aversion, and the studies did not determine whether such treatments affected reinforcement or simply postingestional responses (71). Furthermore, few investigations have directly monitored blood alcohol levels to ensure that intake is for neuropharmacological rather than nutritional purposes.

Rats bred for alcohol preference (11) show a relative 5HT deficiency (72), which may underlie their rapid onset of tolerance to aversive actions of ethanol. This selective tolerance to aversive effects could lead to apparent "preference" by strengthening reinforcing actions. Mice fed liquid diets with ethanol show poor development of tolerance to the hypothermic and sleep-inducing effects of ethanol if central noradrenergic pathways are destroyed, but these lesions have no effect on dependence (73). A more systematic evaluation will be required before one can suggest a specific role for any brain systems in the reinforcing actions of ethanol. There is also a paucity of behavioral evidence for any ongoing opposing process during long-term intoxication, although the hyperactivity and seizures observed during ethanol withdrawal may reflect such a process and could lead to increased use of alcohol (74).

Cellular studies of ethanol. Historically, investigators studying the actions of alcohol on the brain interpreted their data as if alcohol were a lipid solvent that alters the general functions of neuronal membranes by altering the lipids (75). Such general membrane

actions seem incompatible with the neuropsychopharmacological profile of ethanol, that is, with effects on motor coordination, arousal, and cognition, as well as euphoric and anxiolytic effects. Furthermore, surprisingly selective cellular effects of ethanol have been reported (76, 77).

In the cerebellar cortex, a brain region implicitly involved in the locomotor intoxicating actions of ethanol, altered function seems largely the result of increased activity of the inferior olivary complex. This activity results because ethanol increases climbing fiber bursts to cerebellar Purkinje cells and alters their basal mode and rate of firing (77), and perhaps thereby alters cerebellar function. The mechanism by which ethanol causes olivocerebellar activation remains unclear; one possibility is an indirect mediation by a condensation product resulting from the effects of ethanol catabolism on amines or other transmitters (78). Proof of this possibility, a variation on a recurring but never fully documented hypothesis of alcohol action, could explain the greater potency of systemic versus locally administered ethanol on several cells, including cerebellar neurons.

Locus ceruleus neurons (79) exhibit pronounced dose-dependent depression of responsiveness to sensory stimuli at doses of ethanol lower than those required for changing mean spontaneous discharge rates. Given the hypothesized role of the LC in attentional mechanisms (54), ethanol-induced disruption of LC sensory responsiveness may be expected to alter cortical information processing and may thus be a basis for ethanol-induced impairment of cognitive processes.

Dopaminergic neurons of the VTA and substantia nigra are also

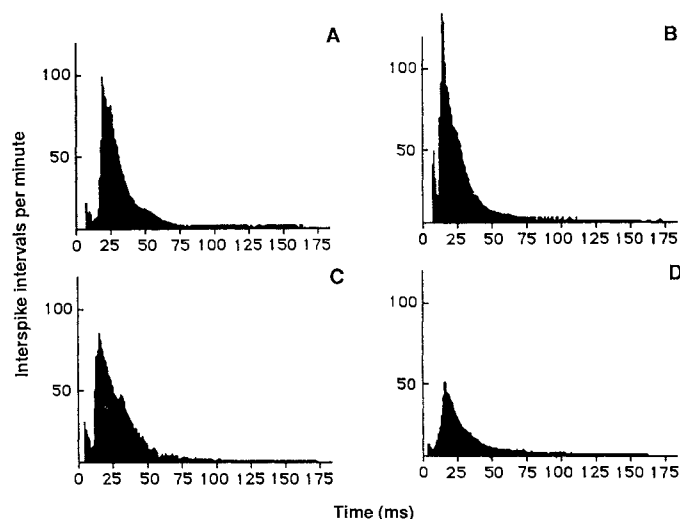


Fig. 3. The rate and patterns of firing for different groups of Purkinje neurons after various ethanol treatments. The histograms show the proportion of interspike intervals (that is, the time between two consecutive action potentials) over long sampling periods. The peak of short intervals (0.5 ms or less) reflects climbing fiber, or complex, spikes. The peak (or modal) interval at 25 to 35 ms reflects normal spontaneous firing. **(A)** Control rats, not previously exposed to ethanol (65 different cells, 19 different rats). **(B)** Effects of ethanol at 10 to 60 min after injection (22 different cells, 11 different rats; 1 to 4 g/kg); the increased short interval peak reflects increased proportions of climbing fiber bursts after ethanol treatment. **(C)** Effects of long-term (2 to 3 weeks) exposure to ethanol 0 to 3 hours after last administration (19 different cells, 4 different rats); despite the prolonged exposure to ethanol, the firing rates and patterns strongly resemble the pattern of firing of the controls **(A)** before ethanol treatment. **(D)** Withdrawal from long-term exposure to ethanol (2 to 3 weeks) 12 to 32 hours after last administration (67 different cells, 6 different rats); the cell firing patterns here show marked increases in long interspike intervals (slowing) during withdrawal, similar to the effects of LC stimulation. [Adapted from Rogers, Siggins, Schulman, and Bloom, in (77) with permission]

activated in response to systemic ethanol administration (80). The activation only occurs at relatively low doses of ethanol and the effect can be depressed by anesthesia (80). Since neurons in the adjacent substantia nigra pars reticulata are depressed by systemic ethanol, in what may be a GABA-based effect, the activation of the dopaminergic neurons by ethanol may result from their disinhibition. Within the hippocampus and cerebral cortex, systemic ethanol facilitates the depressant potency of somatostatin and enhances the excitatory effects of cholinergic muscarinic responses (81); these effects may relate to the opposing dynamic regulation of the M-current conductance by these transmitters (82). However, no direct connection to limbic effects of ethanol has been established.

Few data have appeared on the response of single brain cells to chronic exposure to ethanol. After 10 to 14 days of ethanol intoxication, cerebellar Purkinje cells show complete tolerance (77) (Fig. 3). But soon after withdrawal, Purkinje cell firing becomes depressed (77) (Fig. 3), and this depression is reversed by further exposure to ethanol. The pattern of firing of Purkinje cells changes during withdrawal and resembles their response to NE iontophoresis or LC stimulation; this result suggests that LC hyperactivity may accompany ethanol withdrawal as it does opiate tolerance and withdrawal (77).

In vitro biochemical assays of ethanol actions have focused on GABA-like increases in chloride flux (83), effects that have been difficult to replicate in studies of GABAergic pathways. Discovery of an endogenous benzodiazepine (84) and description of the detailed distribution of this peptide and its receptors may provide the starting point for an assessment of this system as a part of the ethanol neuronal substrate.

Long-Term Adaptive Phenomena Associated with Dependence

Long-term changes in subjects previously dependent on drugs have until recently been difficult to assess, particularly changes in long-term physiological markers. However, extensive studies with opiate addicts (85) and more recent work with cocaine addicts (25) have revealed long-term changes in mood, particularly associated with environmental conditions that signal various aspects of drug-seeking behavior. With detoxified opiate addicts, stimuli previously associated with obtaining an opiate can produce a significant conditioned withdrawal syndrome combined with craving for the drug. Similarly, data indicate that detoxified cocaine users experience a prolonged dysphoric state that, combined with stimuli associated with obtaining cocaine, can produce a significant craving for the drug (25). If craving is defined as a memory for the pleasant aspects of the drug, then these results suggest that various external and internal signals can act as discriminative stimuli for eliciting the memory of drug experiences and these memories may serve as motivational factors in drug recidivism. Animal models do exist for conditioned withdrawal responses. Repeated exposure of monkeys to stimuli paired with abstinence will produce a conditioned abstinence response (86), and recent work has shown that rats will avoid an environment previously paired with naloxone-precipitated withdrawal (87). However, the neurobiological bases for these effects are unknown.

Other associative phenomena that may have a role in drug dependence, particularly during the early stages in dependence, are those of sensitization and conditioning. The psychomotor stimulant effects of cocaine increase with repeated injection, and this sensitization can be enhanced by the drug state being paired with a particular environment (88). Similarly, a conditioned locomotor response can be observed in the absence of drug when the animal is exposed to

stimuli previously paired to the drug state (89). For psychomotor stimulants, both sensitization and conditioning may depend on intact dopamine function (88, 89). The exact significance of this sensitization for drug-seeking behavior is not yet clear, but it may be important for the lack of tolerance observed with limited access to drugs. The conditioning of previously neutral stimuli with drug effects may also contribute to the motivational properties of stimuli associated with craving (90).

Concordance of Behavioral, Cellular, and Molecular Studies

Two possible mechanisms can be hypothesized to link the molecular and cellular data with the behavioral observations on tolerance and withdrawal: a within-systems adaptation and a between-systems adaptation. In a within-systems opposing process, the primary cellular response element to the drug would itself adapt to neutralize the drug's effects; persistence of the opposing effects after the drug disappears would produce the withdrawal response. For example, one could hypothesize that the immediate effect of ethanol on motor behavior may in part reflect an action of ethanol on inferior olivary nucleus cells projecting to the cerebellum and that the manifestation of tremor during ethanol withdrawal may represent a rebound effect after cellular adaptation (or overadaptation) of these same cells to ethanol.

Similarly, tolerance to opiates has been postulated in terms of opiate receptor changes (91). Although the concept of receptor number regulation has been well established within the endocrine system and for certain components of the peripheral autonomic and central catecholaminergic neurons (92), its application to opioid tolerance has been disappointing. No reproducible changes in opioid binding site number or binding site affinity or peptide content (93) have been found after chronic treatment with opiates sufficient to produce marked dependence and withdrawal.

In the between-systems opposing process, a different cellular system and separable molecular apparatus would be triggered by the changes in the primary drug response neurons and would produce the adaptation and tolerance. Thus, the immediate effects of cocaine are postulated to increase release and duration of dopamine actions, thereby inhibiting GABAergic circuits that normally feed back to inhibit dopamine neurons. Tolerance to the primary euphorogenic effects of cocaine has been reported anecdotally in humans as depression appears after drug withdrawal. A between-systems adaptation could derive from the counterbalancing effect of the overactive GABAergic interneurons regaining control over the dopamine neurons.

This within- versus between-systems distinction could also be conceived at the molecular level of analysis between the guanine nucleotides that allow different receptor systems to regulate ion conductances dynamically, but such effects require additional data to link them to the behavioral phenomena. Although speculative at this point, the contrasting features of within- and between-systems adaptation may explain why tolerance and adaptation can frequently be separated spatially, temporally, and presumably mechanistically.

Thus, we can show that a conceptual framework based on systematic observation of drug-seeking behavior in animals can be extended to a neurophysiological analysis of drug-dependence mechanisms. Even though only a few brain locations and transmitter-specific mechanisms have been analyzed, we hypothesize that some form of opponent process in the CNS is critical for the development of tolerance and of dependence. At the molecular and cellular levels this appears to be a between-systems adaptation.

Across all levels of analysis, molecular and cellular mechanisms of

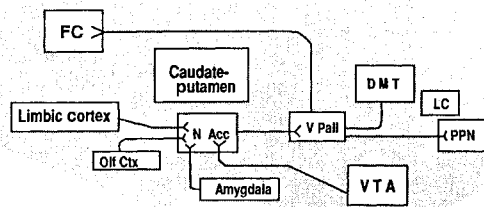


Fig. 4. Schematic model of brain locations and interconnecting circuitry that participate in the reinforcing and adaptive opposing actions of opiates, psychostimulants, and ethanol. The region of the nucleus accumbens (N Acc) is the target of dopaminergic links from the ventral tegmental area (VTA) and of afferents from olfactory cortex (Olf Ctx) and from limbic cortex, some of which may furnish afferents to the accumbens that account for its opiate receptors. The nucleus accumbens, although part of the basal ganglia, is independent of the extrapyramidal motor regulation system in the caudate-putamen; the nucleus accumbens in turn projects, among other targets, to the ventral pallidum (V Pall), and also sends a reciprocal connection, believed to be GABA-mediated, back to the VTA. From the ventral pallidum, connections project to the pedunculopontine nucleus (PPN) and to the dorsal medial thalamus (DMT), which have been proposed as being functionally important in motor activation in the rat (97). The ventral pallidum may also regulate responsiveness of neurons in the frontal cortex (FC), a site from which psychostimulant reinforcement has also been observed. Also illustrated as potentially important, particularly for the implementation of the adaptive opposing responses to the reinforcing effects of these drugs, is the locus coeruleus (LC); although its connections are not shown, the LC projects to the amygdala and to olfactory, frontal, and limbic cortices.

the nervous system react to addictive drugs to initiate and maintain patterns of drug-seeking behavior. We hypothesize that these patterns of behavior emerge primarily because the drugs are able to usurp the crucial reinforcement systems and the small finite number of transmitters and response sites that operate normally to shape survival of the organism (94).

We further hypothesize that the same neurobiological circuits involved in the acute hedonic or "positive reinforcing" actions of drugs may become modified through chronic use as the self-corrective homeostatic responses of the brain adapt to the drug actions. The opposing process may neutralize reinforcing effects and on withdrawal produce the aversive stimulus effects of the abstinence syndrome. We speculate that these "negative reinforcing" effects (for example, malaise, dysphoria, and anhedonia) are a major etiological and motivational factor in maintaining drug dependence. Thus in this conceptual framework, physical signs of abstinence per se are not necessary or sufficient for dependence but the "negative reinforcing" effects are necessary and sufficient for dependence.

Two of the classes of drugs reviewed here, opioids and psychostimulants, have specific endogenous ligands on which they act at specific places to produce distinct patterns of behavior relevant to dependence. In contrast, ethanol appears to act at many sites in the brain, each with its own dose threshold, to produce anxiety reduction, euphoria, motor incoordination, and cognitive depression. Not all of these effects need be considered as critical to the reinforcing aspect of alcohol consumption, because the cellular site and mechanisms of ethanol's action and ethanol's endogenous ligand, if any, are unknown.

Despite the diversity in their primary sites and mechanisms of action, all three classes of drugs may elicit their initial arousing-reinforcing effects via neurons that are components of the VTA-nucleus accumbens-ventral pallidal extrapyramidal motor regulatory system (Fig. 4). How the brain locations within the VTA-accumbens-pallidal-forebrain circuit register a reinforcement effect from

this series of cellular actions remains unclear, as does its molecular mechanism.

This same circuitry may be responsible for the aversive properties of drug withdrawal. Cellular studies of dependence in model systems, such as the LC-cortical forebrain noradrenergic circuitry, have revealed, for example, that chronic drug use with opiates, and perhaps with ethanol, may induce both tolerance and signs of withdrawal in vivo that are not detectable in vitro. Such distinctions directly support the concept of a between-systems adaptational response that underlies dependence and operates through intercellular interactions. This concept is fully compatible with the total lack of within-systems changes, in terms of opioid peptide amount, synthesis rates, or receptor properties, during development of opiate tolerance and dependence or during withdrawal. These data suggest that the opposing process may well involve separate neuronal systems at the cellular level from those directly altered by the acute effects of the drug.

Finally, there is a need for more integration of dependence studies across all levels of investigation (95). Studies of behavior have already benefited immeasurably from cellular studies that have identified drug-sensitive neurotransmission systems and molecular tools for their analysis. It is time for the cellular and molecular analyses to sample and exploit the spoils of these studies of behavior to identify the potentially critical cell types, locations, and molecular mechanisms for reinforcing effects of dependent drugs (96).

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 17. Research at the behavioral level centers on the integrative phenomena that link populations of neurons into the extended specialized circuits, ensembles, or more pervasively distributed systems that accomplish tasks, such as sensing, moving, or reinforcing. Such work includes the complex but still poorly understood mechanisms of learning, reward, and reinforcement. To some experts, work on the affective qualities of drugs may only be properly pursued in humans. The general field has been advanced by the application of noninvasive brain imaging methods that permit identification of the sites in normal and patient samples that are altered in prolonged states of abnormal functional activity and that have also pointed toward specific sites at which addictive drugs may act. L. R. Baxter, Jr., J. M. Schwartz, M. E. Phelps, J. C. Mazziotta, J. Barrio, *J. Clin. Psychiatry* **49** (suppl.), 23 (1988); M. I. Posner, S. E. Petersen, P. T. Fox, M. E. Raichle, *Science* **240**, 1627 (1988); S. E. Petersen, P. T. Fox, M. I. Posner, M. Minton, M. E. Raichle, *Nature* **331**, 585 (1988).
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