Neurobiological and Neurobehavioral Mechanisms of Chronic Alcohol Drinking

Alcoholism comprises a set of complex behaviors in which an individual becomes increasingly preoccupied with obtaining alcohol. These behaviors ultimately lead to a loss of control over consumption of the drug and to the development of tolerance, dependence, and impaired social and occupational functioning.

Although valuable information regarding tolerance and dependence has been, and continues to be, gathered through human studies, much of the detailed understanding of the impact of exposure to alcohol on behavior and on the biological mechanisms underlying those behaviors has been obtained through the use of animal models for alcoholism and a variety of in vitro, or cellular, systems. Through the use of cellular systems and animal models, researchers can control the genetic background and experimental conditions under which a specific alcohol-related behavior or neurochemical change occurs.

It is difficult, if not impossible, to obtain an animal model that by itself incorporates all of the signs, symptoms, and behaviors associated with alcoholism. However, several animal models of chronic drinking reflecting the different components of alcoholism, including alcohol-seeking behaviors, have been developed. These models, which remain an integral part of the study of alcoholism, include animals that preferentially drink solutions containing alcohol, animals that self-administer alcohol during withdrawal, animals with a history of dependence that self-administer alcohol, and animals that self-administer alcohol after a period of abstinence from the drug. Genetic models for alcoholism also exist and include animals that have been bred selectively for high alcohol consumption. Studies using such models are uncovering the systemic, cellular, and molecular neurobiological mechanisms that appear to contribute to chronic alcohol consumption. The challenge of current and future studies is to understand which specific cellular and subcellular systems undergo molecular changes to influence tolerance and dependence in motivational systems that lead to chronic drinking.

Reinforcement and Reward in Chronic Drinking

Because alcoholism centers on compulsive, often excessive, use of alcohol, the concept of reinforcement or motivation is a crucial part of this syndrome. A reinforcer is defined as any

Tolerance and Dependence

Tolerance is present when, following prolonged exposure to alcohol, the brain becomes less sensitive to the acute actions of alcohol. For example, research shows that larger doses of alcohol are needed to produce an alcohol-specific effect, such as sedation, in animals that have been given alcohol for several days or weeks compared with animals given the drug the first time. Tolerance appears to occur through adaptations at the cellular and subcellular levels as the brain attempts to overcome the acute effects of alcohol intake; with prolonged alcohol abuse, these adaptations often lead to permanent adverse changes in the structure and function of neurons.

Alcohol dependence is defined as the manifestation of either physical withdrawal symptoms, such as tremors and seizures, after abrupt cessation of alcohol intake or psychological symptoms, such as a negative emotional state after intake ends. The physical and psychological symptoms that occur following termination of alcohol intake are collectively referred to as the alcohol withdrawal syndrome. Alcohol's ability to induce pleasurable feelings, reduce tension and anxiety, and ameliorate the symptoms of withdrawal make it a powerful and "attractive" drug.

event that increases the probability of a response. This explanation also can be used to define reward; in fact, "reinforcement" and "reward" frequently are used interchangeably. However, reward often also connotes an additional emotional value, such as pleasure (White and Wolf 1991). Many sources of reinforcement, such as pleasure, mood elevation, and removal of negative emotional states, contribute to compulsive alcohol use during the course of alcoholism.

The primary pharmacologic action of alcohol produces a direct effect through positive or negative reinforcement. Positive reinforcement refers to a pleasurable or otherwise positive event that increases the likelihood that additional alcohol will be sought. Alcohol itself can serve as a powerful positive reinforcing agent through its ability to induce pleasurable or mood-elevating feelings (so-called euphoric or euphorogenic effects). In contrast, negative reinforcement describes an adverse event or situation that also will lead the individual to obtain more alcohol. Examples of negative reinforcement include situations in which an individual or animal self-medicates in an attempt to overcome an existing aversive state (depression or anxiety) or to treat a drug-generated aversive state (alcohol-related withdrawal) (Wikler 1973). Both the positive and negative types of reinforcement encourage alcohol-seeking behavior and appear to contribute to chronic drinking, alcohol dependence, and a return or relapse to drinking among persons recovering from alcoholism.

The secondary pharmacologic effects of alcohol also can have powerful motivating properties. Conditioned reinforcement—when an individual learns to associate the reinforcing effects of alcohol with a previously neutral event or stimulus—results in secondary positive reinforcing effects. In practical terms, a person entering a familiar bar or pub can experience positive feelings similar to those induced by consumption of alcohol. Secondary reinforcing effects can be negative or positive; someone can also learn to associate particular stimuli with unpleasant aspects of abstinence, such as withdrawal symptoms.

However, alcohol may, under certain conditions, serve as a deterrent to the seeking or obtaining of more alcohol. Research shows that alcohol can be aversive at high doses and, in animals, can cause both place avoidance, in which animals avoid an environment where they have previously received alcohol, and taste avoidance, in which animals avoid a taste previously paired with alcohol ingestion (Cunningham et al. 1992). Alcohol-dependent individuals have what is known as elevated aversion thresholds; that is, they can consume higher levels of alcohol than non-dependent individuals before they stop drinking or avoid alcohol. This elevated aversion threshold may contribute to excessive drinking among dependent persons.

By applying these concepts, researchers can explore the neurobiological bases for the acute positive reinforcing effects of alcohol, the negative reinforcing effects imparted by the dependent state, and the conditioned reinforcing effects associated with protracted abstinence and relapse (Koob et al. 1993).

Insights Into Features of Alcoholism From Animal Models

The importance of animal studies lies in their potential for providing insight into alcoholism in humans. Although not all the factors contributing to alcoholism, including the genes responsible for alcohol-related behaviors, have been discovered, animal models of alcoholism are proving to be instrumental in identifying genetic and biological factors that confer predisposition to alcoholism. Such models enable researchers to perform controlled analyses of genetically and environmentally influenced traits and behaviors that resemble certain aspects of human alcoholism, such as alcohol consumption and preference, innate sensitivity or tolerance to alcohol, and metabolic rate of alcohol elimination. Much of the study of genetics and alcohol-related behaviors or traits is conducted through the use of selectively bred animals, such as alcohol-preferring and alcohol-nonpreferring rats and mice. Additional studies use animals that are trained or conditioned to choose alcohol over water or other usually preferable solutions.

Animal Models for Alcoholism

Validated animal models exist for many of the components of alcoholism. Validation of an animal model focuses on three factors: face validity, reliability, and predictive validity (predictability). Face validity is how similar, at least superficially, a behavioral effect in an animal is to that in humans. Face validity is a valuable starting point in alcohol research studies using animal models but is often difficult to truly achieve. Reliability refers to the consistency and stability of the variable of interest in the animal. Predictive validity refers to how closely and accurately the animal's condition mimics or predicts the condition in humans, based on the behavior of the animal model.

The *Diagnostic and Statistical Manual of Mental Disorders*, *Fourth Edition* (DSM-IV) of the American Psychiatric Association (1994) defines the criteria that provide a framework for studying the neurobiological bases for the excessive consumption associated with chronic alcohol ingestion (Koob et al. 1998). Of seven behavioral features DSM-IV uses in its criteria to define alcoholism, six have at least somewhat analogous counterparts in animal models, that is, face validity. "Tolerance" to the reinforcing effects of alcohol can be inferred from the increased consumption observed in dependent animals; more research in this area is needed, however. "Characteristic withdrawal syndrome" has both face and predictive validity in animal models. "Persistent desire" can be modeled through conditioned positive reinforcement with alcohol, which has been demonstrated in animals. "Substance taken in larger amounts than intended" could be modeled through monitoring alcohol intake during withdrawal and following periods of abstinence (the "alcohol deprivation effect"). Both of these models have some predictive validity. "Important activities reduced" is difficult to model in animals, but advances in behavioral economics, in which the "cost" of alcohol (the amount of work an animal is willing to do to obtain alcohol) is studied, would be applicable here. "A great deal of time spent in activities necessary to obtain the substance" might be modeled by a progressive ratio-type schedule in which the animal must work increasingly hard to obtain alcohol.

Animal Models for Alcohol-Seeking Behaviors

Animal models for the acute positive reinforcing effects of alcohol and for alcohol-seeking behaviors have been well established and validated (Roberts et al. 1996; Samson 1986, 1987). Two predominant approaches to the study of self-administration of alcohol are oral preference and operant behavior models. In the *oral preference model*, animals are given free access to two different solutions, alcohol and a nonalcohol reinforcer, such as a sweetened water solution; preference for alcohol versus the nonalcoholic fluid is then established by determining which solution is consumed more by the animals. Animals normally consume, at most, small amounts of alcohol, unless they have been bred for a specific genetic predisposition for alcohol preference. Thus, the results of alcohol preference tests provide researchers with information regarding an innate preference or aversion to the pharmacologic effects of alcohol or the extent to which an animal's initial aversion to the taste or smell of alcohol may be overcome to the point where the animal seeks out the alcohol solution. However, this type of task involves little work or effort to obtain alcohol.

In contrast, in *operant behavior models* of self-administration, access to alcohol requires a specific learned behavioral response (or series of responses), such as pressing a lever or running a course through a maze. The amount of alcohol consumed is related to the amount of work an animal is willing to perform. Studies that incorporate an operant behavior model not only can measure preference for alcohol, they can also provide insight into an animal's motivation or persistence to obtain alcohol under a given set of conditions.

Another model used to study alcohol-seeking behaviors in animals involves *conditioned preference tasks*. Through these tasks, animals learn to avoid or seek an environment (place preference vs. place avoidance) or a flavor (taste preference vs. taste avoidance) that previously has been paired with alcohol. Thus, using conditioned preference tasks, researchers are able to assess the reward value of alcohol in association with specific environmental cues.

Studying the Anxiolytic Effects of Alcohol

Alcohol's acute stress-reducing and sedative (anxiolytic) effects, working in concert with its mood-elevating effects, are thought to contribute to the rewarding and positive reinforcing effects of the drug. Alcohol's anxiolytic and mood-elevating effects also may contribute to continued alcohol abuse. The anxiety associated with withdrawal from alcohol use and the early phases of abstinence may motivate a person or an animal to continue to seek alcohol in an effort to relieve that stress or anxiety, thus contributing to the negative reinforcing characteristics of alcohol.

Recognizing the importance of these effects in alcohol abuse and relapse, researchers have developed a series of tests to study the anxiolytic-like properties of alcohol. One type of test involves exposing animals to a situation that generates *approach-avoidance behavior*. In such a test, animals trained operantly to respond to a stimulus such as food or alcohol will then occasionally be given an electrical shock when choosing the stimulus; with time, the animal learns to avoid responding to the food or alcohol. This avoidance behavior can be reversed by a class of drugs known as anxiolytics, such as benzodiazepines, which act as sedatives to reduce stress.

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Alcohol-associated anxiety-like behavior also may be studied using the *elevated plus maze test*, in which animals are given a choice between spending time on the enclosed versus the sideless (open) arms of a plus-shaped apparatus that is raised above the floor. Experiments show that animals treated with alcohol spend more time on the open arms than animals not exposed to alcohol. The response of the alcohol-treated animals is interpreted as a reduction in anxiety-like behavior.

Another behavioral test, the *social interaction test*, takes advantage of the observation that normal social interactions are suppressed when rats are placed in an unfamiliar and brightly lit environment. Under these conditions, anxiolytic drugs cause a marked increase in social activity.

Drug Discrimination Tests

A wide range of drugs can stimulate or inhibit specific neurotransmitters in the brain. *Drug discrimination tests* are used to determine the extent to which drugs known to activate or block specific neurotransmitter receptors in the brain are perceived as being similar to alcohol or are capable of reducing the subjective effects of alcohol, such as euphoria and anxiety. Drug discrimination tests cannot directly demonstrate the reinforcing properties of alcohol. However, they are important in providing information about neurobiological mechanisms that may contribute to the rewarding effects of alcohol.

In these tests, animals are trained to make one response in the presence of alcohol and a different response when they cannot recognize alcohol. Later, when the animal trained to respond to alcohol is given a new drug that has the same subjective effects of alcohol, the animal will respond as it would for alcohol. Conversely, the alcohol cue can be modified by concurrent administration of potential antagonists (compounds that counter the physiologic effects of alcohol). Discrimination tests and procedures have been and continue to be used to identify the neurotransmitters and receptors that modulate many of the effects of alcohol. Researchers anticipate that drug discrimination tests in particular will lead to the development of highly targeted therapies capable of altering or blocking some of the biochemical effects of alcohol and, in turn, related behaviors.

Alcohol as a Reinforcer

In models and tests designed to study *alcohol as a reinforcer*, alcohol is considered a reinforcer when the presentation of alcohol increases the possibility of a response. A specific pharmacologic effect is implied when intake of alcohol results in a measurable, biologically meaningful blood alcohol level (BAL) or blood alcohol concentration (BAC). (See also the box "The ABC's of BAC's" in the chapter on prevention research.) A meaningful BAC is a level known to produce alcohol-specific effects or behaviors, such as sedation. Results of studies in which the relationship between alcohol and dose has been measured and BAL's have been monitored clearly establish models, such as operant self-administration, drinking preference, and place preference, as reliable means of measuring the positive reinforcing effects of alcohol (Hyytia and Koob 1995; Koob et al. 1994*b*; Rassnick et al. 1993; Samson et al. 1993). These models have been a boon to neuropharmacologic analyses of alcohol reinforcement.

Use of Highly Palatable Solutions To Induce Alcohol Self-Administration

Animals normally have an aversion to the taste of alcohol. Thus, scientists studying the effects of alcohol consumption in laboratory animals, especially high levels of alcohol intake, need to overcome the animals' typical aversion to the drug. One method for inducing high intake of alcohol is to combine the alcohol with a highly palatable solution such as sucrose or saccharin. Numerous studies have shown this approach to be successful. For example, using an operant approach combined with the *sucrose fade-out procedure* (when the sugar sucrose is gradually replaced with alcohol in a solution), researchers showed that rats more reliably self-administered large amounts of alcohol when the sucrose was removed (Files et al. 1995; Hodge et al. 1992; Schwarz-Stevens et al. 1992).

Selective Breeding for High Alcohol Intake

Both alcohol-preferring (P) and alcohol-nonpreferring (NP) lines of rats have been selectively bred to drink high and low amounts of a 10-percent solution of alcohol, respectively, when given continuous access to alcohol in addition to free access to water and food (Li et al. 1982; Lumeng et al. 1977). The *selective breeding for alcohol preference* in P rats decreases the animal's initial sensitivity to alcohol and leads to a more rapid development of tolerance than in animals not bred for this preference (Kurtz et al. 1996). Typically, P rats drink as much as 5 grams of alcohol per kilogram of body weight each day (as opposed to less than 2 grams per kilogram in NP rats) (McBride et al. 1989) and can have BAL's as high as 200 milligrams per deciliter (0.2 percent) under conditions of continuous access with intragastric (directly into the stomach) self-administration (Waller et al. 1984). (By comparison, a BAL of 100 milligrams per deciliter (0.1 percent) is the legal limit of intoxication in most states.)

Similar increases in either initial sensitivity or more rapid development of tolerance also have been observed in the alcohol-preferring C57BL mice (Kakihana et al. 1966; Tabakoff and Ritzmann 1979) and the alcohol-preferring Finnish AA rats (Le and Kiianmaa 1988). The neurochemical differences in these alcohol-preferring animals involve neural substrates, or clusters of neurons or neural tissue in the brain, that are similar to those implicated in the neuroadaptive changes associated with chronic alcohol self-administration. These findings suggest that both environmental and genetic factors can converge to drive excessive drinking.

Self-Administration During Withdrawal: Dependence

Dependence is an important factor in the continued use of alcohol by alcoholics. It is characterized by the appearance of a withdrawal syndrome following the cessation of alcohol use. Dependence leads alcoholics to consume alcohol not simply for its mood-elevating effects but also to avoid or reverse the negative symptoms associated with withdrawal (Cappell and Le Blanc 1981; Edwards 1990). Alcohol dependence is studied experimentally by measuring either physical or motivational signs of withdrawal, some of which may reflect the desire or craving for alcohol. Researchers often use evidence of physical symptoms upon termination of alcohol consumption (physical dependence) as an index of dependence in animals. These physical symptoms, known collectively as the alcohol withdrawal syndrome, range in severity from mild tremors to massive convulsions. Similar symptoms, as well as hallucinations, are seen in humans undergoing withdrawal. The development of alcohol-dependent animal models with the goal of understanding how reward mechanisms mediating alcohol intake differ in dependent and nondependent animals is potentially important for understanding alcoholism and dependence. Measures of reward dysfunction in animals during withdrawal include brain stimulation reward thresholds (the level of brain stimulation needed to elicit a response consistent with reward), conditioned aversions, drug discrimination, and response to natural rewards. Developing animal models for the negative reinforcement associated with alcohol dependence has proven difficult, however, especially with rodents.

Attempts to develop reliable and useful models of alcohol consumption in dependent rats have taken into consideration various factors that influence dependence, such as consumption of alcohol to overcome symptoms of withdrawal. In one study, for example, rats were trained to consume 10-percent alcohol prior to continuous exposure to alcohol in a liquid diet or air vapor. During this training phase, alcohol was established as a reinforcer, and potential taste aversions were overcome. The animals were maintained at blood alcohol levels (BAL's, also called blood alcohol concentrations, or BAC's) associated with mild to moderate physical withdrawal symptoms. (See the box "The ABC's of BAC's" in the chapter on prevention research for more information.) Therefore, any withdrawal symptoms that the rats did exhibit would be predictably quite mild and would not be expected to interfere with their ability to self-administer alcohol. Results of this study showed that the dependent rats consumed more alcohol than the nondependent control rats did. Rats with BAL's higher than 100 milligrams per deciliter (0.1 percent) at the time of withdrawal from the liquid diet sustained high levels of alcohol self-administration throughout four withdrawal sessions (Schulteis et al. 1996). Thus, above and beyond the training effects, dependent rats consumed more alcohol than nondependent rats did, even when withdrawal symptoms were mild.

In a subsequent study, rats were trained to lever press for 10-percent alcohol using the saccharin fade-out procedure, in which saccharin in a water solution was gradually replaced with alcohol to increase the amount of alcohol the rats would consume (Roberts et al. 1996). Over the course of five 12-hour periods of withdrawal, the rats were allowed to respond for alcohol and water. Dependent rats, who maintained BAL's above 100 milligrams per deciliter (0.1 percent) during the entire withdrawal period, responded to a greater degree than nondependent controls did. In addition, dependent rats allowed to respond for alcohol avoided the withdrawal symptomatology present in dependent rats not allowed to respond for alcohol during the withdrawal phase. Responses across withdrawal sessions appeared to become more stable, suggesting that the rats learned to respond in a manner that controls their BAL and minimizes or avoids withdrawal.

Alcohol Self-Administration Following Periods of Abstinence in Rats With a History of Limited Access: Craving

A predominant feature of human alcohol abuse and alcoholism is a reported desire or craving to

consume alcohol that is accompanied by frequent bouts of excessive drinking following periods of abstinence. These and other factors, such as the mood-altering and anxiety-reducing effects of alcohol, may be responsible for relapse to excessive alcohol drinking. Studies in rats, mice, and monkeys have shown increases in alcohol consumption following periods of forced abstinence (Kornet et al. 1990, 1991; LeMagen 1960; Salimov and Salimova 1993; Sinclair 1979; Sinclair and Senter 1967, 1968; Spanagel et al. 1996; Wolffgramm and Heyne 1995). Two aspects of drug dependence could contribute to these increases. One reflects the negative reinforcement produced by self-medication of a withdrawal state seen in animals who have prolonged access to alcohol. The other process involves changes in the positive reinforcing properties of alcohol seen in abstinent animals and may reflect changes other than negative reinforcement. Developing a reliable model of excessive drinking has led to reevaluation of the alcohol deprivation effect in animals with limited access to alcohol and would likely be important to understanding changes in the reinforcing effects of alcohol that occur with abstinence.

For example, rats trained to lever press for 10-percent alcohol and water using the saccharin fade-out procedure established stable baseline responding for alcohol. They were then subjected to various alcohol deprivation periods (3, 5, 7, 14, or 28 days) during which no alcohol was available (Heyser et al. 1997). Responding for alcohol increased as a function of the duration of the deprivation period, compared with baseline levels. This increase was temporary and returned to baseline levels within 2 to 3 days. The shortest effective deprivation period (the shortest interval after which consumption increased) was 5 days, and the rats showed no signs of withdrawal. Thus, this transient increase in response for alcohol does not appear to be related to the manifestation of dependence and withdrawal. Rather, this increase may reflect changes in alcohol's positive reinforcement properties. These results may provide a useful tool to elucidate neuropharmacologic mechanisms underlying human alcohol-seeking behavior and relapse.

Alcohol Self-Administration Following Periods of Abstinence in Rats With a Prior History of Dependence: Relapse

Relapse, or the return to alcohol abuse following periods of abstinence, is one of the principal characteristics of dependence on alcohol. Even so, little is understood about the neurobiological factors involved in this phenomenon. Research suggests that the development of dependence plays an important role in the maintenance of compulsive use and relapse following periods of abstinence.

Dependence is, in fact, the basis of the negative reinforcement theory of alcoholism that suggests that alcoholics continue to drink to avoid withdrawal symptoms (Cappell and Le Blanc 1981; Hershon 1977). In more modern conceptualizations, this theory suggests that alcoholics drink to avoid the negative effect (emotional state) associated with withdrawal (Koob and Le Moal 1997). However, because relapse can occur even after withdrawal signs have ceased, the neurochemical changes that occur during the development of dependence may persist after the overt signs of withdrawal are no longer present. Indeed, research using animal models has shown that prior dependence lowers the dependence threshold. In other words, previously dependent animals made dependent again display more severe withdrawal symptoms than do animals receiving alcohol for the first time (Baker and Cannon 1979; Becker and Hale 1993; Becker et al. 1997; Branchey et al. 1971). This finding supports the notion that alcohol experience and, in particular, the development of dependence can lead to relatively permanent alterations in responsiveness to alcohol. Thus, a relapse model would allow research into the long-lasting changes in the alcohol reward system produced by prior dependence.

Future research should include studies in which animals previously made dependent are allowed to consume alcohol. Enhanced responding for alcohol in animals without a history of dependence that are given extended training in operant tasks also warrants further study. The possibility

that the total experience with alcohol is a major predictor of the degree to which alcohol consumption resumes after abstinence is another important question for future investigations.

Alcoholism and the Neural Structures of Reward

Research suggests that the neural substrates—the tissues and neural components changed by exposure to alcohol—and neuropharmacologic mechanisms associated with the motivational effects of alcohol withdrawal may play a role in the negative reinforcement associated with alcohol dependence. Thus, the same neural systems implicated in the positive reinforcing effects of alcohol also appear to be involved in the aversive motivational effects of alcohol withdrawal.

Acute alcohol withdrawal is characterized by symptoms that fall into four main categories: autonomic system hyperactivity, neuronal excitation and seizures, distortions of perception, and motivational effects. Autonomic system hyperactivity includes hypertension (high blood pressure) and increased heart rate. Neuronal excitation includes tremors and seizures. Distortions of perception include hallucinations, delirium, and disturbed sleep. Motivational effects include restlessness, anxiety, dysphoria (a sense of ill-being), and depression-like symptoms. Although animal models exist for many of these symptoms and physical signs have been used to explore the neural basis for alcohol withdrawal (Meert 1994), motivational measures are a more important focus.

Measurement of reward thresholds throughout the course of alcohol withdrawal has shown that these thresholds are increased following chronic administration of alcohol and all other major drugs of abuse, including opiates, psychostimulants, and nicotine. Stated another way, the amount of stimulation required to produce the same reward or effect increases when drug administration is discontinued. This effect, which can last for up to 72 hours depending on the drug and dose administered, may reflect changes in the activity of the same system—the midbrain-forebrain system—implicated in the positive reinforcing effects of alcohol and other drugs (Legault and Wise 1994; Leith and Barrett 1976; Markou and Koob 1991, 1992; Parsons et al. 1995).

Extended Amygdala

Information about the anatomy and function of the brain suggests that the neurological structures associated with the reinforcing actions of alcohol and other drugs may involve a common neural circuitry that forms a separate entity within the basal forebrain, the extended amygdala (Alheid and Heimer 1988). The term extended amygdala refers to a large structure composed of several smaller basal forebrain structures that are similar in cell structure, function, and neural connectivity (Alheid and Heimer 1988). This system has extensive connections to brain regions that play central roles in reinforcement and reward.

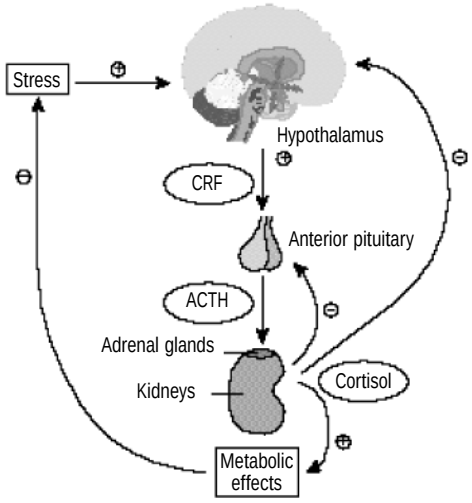
Rats trained to self-administer alcohol during withdrawal show neurochemical and neuropharmacologic changes indicative of alterations in gamma-aminobutyric acid-activating (GABAergic), dopaminergic, and serotonergic function in specific components of the extended amygdala. (Previous sections in this chapter provide background on these neurotransmitters and alcohol's impact on neurotransmitter function.) For example, inhibitory GABAergic mechanisms in the central nucleus of the amygdala have been implicated in the acute reinforcing effects of alcohol (Heyser et al. 1995; Hyytia and Koob 1995). One study showed a reduction in alcohol self-administration in nondependent rats following injections of a highly selective and potent GABA antagonist (an agent that blocks or reverses GABA's usual actions or effects) into the nucleus accumbens and central nucleus of the amygdala, with the most sensitive site being the central nucleus of the amygdala (Hyytia and Koob 1995). (The nucleus accumbens is a brain structure implicated in the reward properties of drugs of abuse—the medial nucleus accumbens is encompassed in the extended amygdala described above.) Other investigations have demonstrated selective activation of dopaminergic transmission in the shell of the nucleus accumbens in response to

acute administration of virtually all major drugs of abuse (Pontieri et al. 1995, 1996; Tanda et al. 1997).

Additional research suggests that the extended amygdala may be involved in the negative reinforcement effects associated with alcohol withdrawal. Rats lever pressing for alcohol during withdrawal showed a return toward baseline levels of dopamine and serotonin in the nucleus accumbens, in contrast with the usual decreases in these neurotransmitters during acute withdrawal (Weiss et al. 1996). A subsequent study revealed that the central nucleus of the amygdala was particularly sensitive to the suppressant effects of GABA agonists on alcohol self-administration by dependent rats (Roberts et al. 1996). (Agonists are agents that mimic the actions or effects of other agents.) Other supporting data include evidence for the activation of corticotropin-releasing factor (CRF) systems in the central nucleus of the amygdala during alcohol withdrawal (Merlo-Pich et al. 1995), and for the blocking of the anxiogenic-like responses associated with alcohol withdrawal following injection of CRF antagonists into the central nucleus. CRF is a neuropeptide that is critical to the body's response to stress.[1] It is secreted in the hypothalamus, the brain stem, and the limbic system, a network of brain structures that together function in the expression of emotional behavior (figure 1).

The research on the extended amygdala may ultimately link recent developments in the neurobiology of drug reward with existing knowledge of the substrates for emotional behavior (Davis 1997), essentially bridging what have been largely independent research pursuits. Perhaps more important, this neuronal circuit is well situated to be modeled to explore the neurobiological mechanisms associated with vulnerability to relapse and with concepts such as craving, both of which may involve secondary conditioned reinforcement.

Figure 1: The hypothalamic-pituitary-adrenal axis



In response to almost any type of stress, either physical or psychological, the hypothalamus secretes corticotropin-releasing factor (CRF), which in turn increases secretion of adrenocortotropic hormone (ACTH) by the anterior pituitary gland. In response, within minutes, the adrenal glands, located atop the two kidneys, increase secretion of cortisol. The released cortisol initiates a series of metabolic effects aimed at alleviating the harmful effects of the stress state and, through direct negative feedback to both the hypothalamus and the anterior pituitary, decreases the concentration of ACTH and cortisol in the blood once the state of stress abates. + = Excites; – = Inhibits.

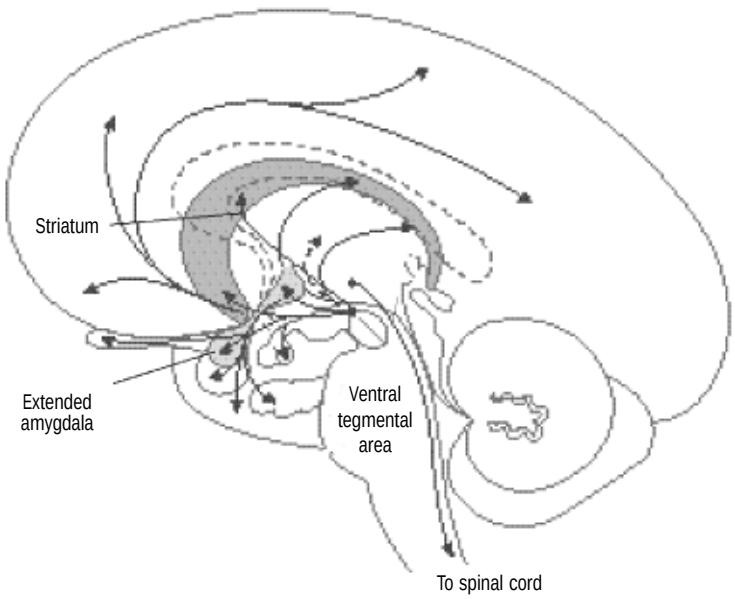
Source: Emanuele and Emanuele 1997.

Hippocampus and Ventral Tegmental Area

Researchers have hypothesized that alcohol's effects on GABAergic neurotransmission involve alteration in the expression of GABA receptor subunits in specific regions of the brain that occur with chronic treatment or alcohol. A recent study suggests that these effects are found not only in the (cerebral) cortex but also in the hippocampus, a brain structure involved in the consolidation of new memories, and the ventral tegmental area (VTA) (Charlton et al. 1997). The VTA is the source of dopamine in the mesolimbic system, a region of the brain involved in the mediation of

[1]Stress-induced physical demands, psychological distress, and adaptive changes initiate a cascade of neural and endocrine (or neuroendocrine) events that lead to the release of glucocorticoid hormones from the adrenal glands. These hormones, in turn, have widespread effects on the body's metabolic and immunologic processes. The activation of this neuroendocrine system involves the hypothalamic-pituitary-adrenal axis, which refers to the brain structures and endocrine glands in the system. (The hypothalamus is a brain structure involved in the maintenance of the internal environment and in mediating hunger, thirst, and emotional drives.) Release of CRF from the hypothalamus activates this stress-response system.

Figure 2: Dopaminergic pathways in the brain



Most dopamine-containing neurons are located within the midbrain, extending to the striatum as well as to various sites in the forebrain. Dopamine modulates such varied functions as emotion, aggression, cognition, the coordination of movement, and aspects of the development of addiction.

Source: Adapted from Heimer 1995.

alcohol reinforcement. The cell bodies of this mesolimbic dopamine system originate in the VTA and send projections to the nucleus accumbens and basal forebrain, transmitting information to the dopamine receptors in these brain areas (figure 2). Exposing laboratory animals to alcohol for 12 consecutive weeks decreased $GABA_A$ α_1 subunit activity in the VTA and hippocampus, suggesting potential changes in brain structures implicated in the rewarding and cognitive effects of alcohol, respectively. Researchers failed to detect a similar change in these regions after only 4 weeks of exposure to alcohol—a clue to the involvement of these areas specifically with chronic alcohol exposure.

Neurochemical and Molecular Adaptations to Alcohol

Research suggests that the brain attempts to overcome the acute effects of alcohol through adaptations at the cellular and subcellular levels. With prolonged alcohol abuse, these adaptations can lead to permanent adverse changes in the structure and function of neurons. Understanding the mechanisms of these adaptations may ultimately lead to therapeutic interventions to prevent the neurological abnormalities associated with protracted alcohol use and abuse.

Tolerance and withdrawal are key to the idea that neuroadaptive processes are initiated to counter the acute effects of alcohol. Historically, most models of alcoholism have emphasized the development of tolerance and withdrawal. In contrast, some more recent discussions have reduced tolerance and withdrawal to optional criteria, while other current conceptual models in animals emphasize selective aspects of tolerance and withdrawal, focusing on motivational measures rather than physical signs (Koob 1992).

Another neuroadaptive process that has been proposed as a key element in the development of

motivational aspects of alcoholism is sensitization, which is the opposite of tolerance. In brief, sensitization is the increased response to alcohol or the effects of alcohol that follows repeated intermittent exposures (Stewart and Badiani 1993). In general, sensitization is more likely to occur with intermittent, repeated exposure to alcohol (or other drugs of abuse); in contrast, tolerance is more likely to occur with continuous exposure. Some authors have suggested that sensitization may play a role in drug dependence by causing a shift toward or a progressive increase in the desire (wanting or craving) of drugs of abuse through repeated exposure to such drugs; these authors used the transition to a pathologically strong wanting or craving to define compulsive use (Robinson and Berridge 1993).

Alcohol-related neuroadaptive processes appear to persist long after the alcohol has cleared from the brain. Such neuroadaptations are under investigation at all levels of alcoholism research, from behavioral to molecular studies (Koob and Bloom 1988). Motivational hypotheses involving both sensitization (Stewart and Badiani 1993) and changes in the central nervous system that counter initial neuroadaptive alterations (Wikler 1973) have been generated; these hypotheses have particular relevance to the phenomena associated with excessive consumption of alcohol (Wikler 1973). Both neuroadaptive models incorporate the concept of change in reward function that accompanies the development of alcohol or drug dependence (sensitization and counteradaptive mechanisms) (Koob 1996).

Reinforcement and Withdrawal

Although the study of the mechanisms for the physical signs of alcohol withdrawal can provide clues to the nature of the neuroadaptive responses that chronic alcohol exposure produces, the emotional or affective aspects of alcohol withdrawal have the most motivational relevance (Koob and Le Moal 1997). The two major categories of responses that reflect motivational measures are anxiogenic-like responses (those that produce anxiety or stress) and changes in reward function. Neural substrates for the physical signs of alcohol withdrawal historically have involved substrates for central nervous system rebound hyperexcitability (the excessive brain activity seen after exposure to, and then removal of, the activity-dampening effects of alcohol); these signs reflect a decrease in function of one of the major inhibitory brain neurotransmitters, GABA, or an increase in function of one of the major excitatory brain neurotransmitters, glutamate (Grant et al. 1990; Hoffman and Tabakoff 1994; Morrisett et al. 1990). Additional research has begun to implicate other neurotransmitter/neuromodulatory systems that could contribute to a hyperexcitable state, including serotonin, dopamine, norepinephrine, adenosine, gangliosides, and neurosteroids (table 1) (Adams et al. 1995; Concas et al. 1994; Crabbe 1992; Finn et al. 1995; Grant et al. 1990; Hoffman and Tabakoff 1994; Kotlinska and Liljequist 1996; Meert 1994; Morrisett et al. 1990; Snell et al. 1996).

Anxiogenic-Like Responses in Alcoholism

Sedative-hypnotic drugs, such as barbiturates, benzodiazepines, and alcohol, all acutely produce a characteristic euphoria, disinhibition, anxiety reduction, sedation, and hypnosis. These drugs exert antianxiety or anxiety-reducing (anxiolytic) effects that reduce aggressive behavior normally exhibited by laboratory animals in conflict situations. This anticonflict effect correlates well with these drugs' ability to act as anxiolytics in humans in a clinic or treatment setting (Sepinwall and Cook 1978) and may be a major component of the reinforcing actions of these drugs.

The sedative and anxiety-reducing effects of sedative-hypnotics are associated with facilitation of the $GABA_A$ receptor (Richards et al. 1991), but the actions of sedative-hypnotics on this receptor are complex. These drugs do not bind directly to the GABA-binding site on the $GABA_A$ receptor; instead, they appear to bind to other sites on the $GABA_A$ receptor complex, through which they facilitate activation of the receptor by GABA. Support for the role of the GABA

Table 1: Agents shown to suppress alcohol withdrawal

Neurotransmitter system	Agent	Dependent measure	Reference
		Physical Signs	
GABAergic	Diazepam	Seizures	Crabbe 1992
	Abecarnil	Seizures	Crabbe 1992
Serotonergic	Buspirone	Tremor	Meert 1994
	Mianserin	Tremor	Meert 1994
	Fluoxetine	Tremor	Meert 1994
Dopaminergic	Haloperidol	Tremor	Meert 1994
Noradrenergic	Propranolol	Tremor	Meert 1994
Glutaminergic	Nitric oxide antagonist	Tremor, rigidity	Adams et al. 1995
	MK 801	Seizures	Grant et al. 1990; Morrisett et al. 1990
	Glycine antagonists	Seizures	Hoffman and Tabakoff 1994
	Polyamine antagonists	Seizures	Kotlinska and Liljequist 1996
Adenosine	A-1 antagonist	Tremors, seizures	Concas et al. 1994
	Gangliosides	Tremors, seizures	Snell et al. 1996
Neurosteroidal	3α-Hydroxy-5α-pregnan-20-one	Seizures	Finn et al. 1995
		Motivational Signs	
GABAergic	Chlordiazepoxide	Open field	Meert 1994
	Flumazenil	Open field	Moy et al. 1997
		Social interaction	File et al. 1989, 1992
		Shuttle box avoidance	Criswell and Breese 1993
	Muscimol	Alcohol self-administration in dependence	Roberts et al. 1996
Serotonergic	Ritanserin	Open field	Meert 1994
	Mianserin	Open field	Lal et al. 1993; Meert 1994
	5-HT_3 antagonists	Plus maze	Costall et al. 1990
	Tianeptine	Social interaction	File et al. 1993
Noradrenergic	Propranolol	Open field	Meert 1994
Neuropeptidergic	Corticotropin-releasing factor antagonist	Plus maze	Baldwin et al. 1991

receptor in association with alcohol's anxiety-reducing effects is found in studies showing that the anxiogenic-like effects of alcohol withdrawal are blocked by administration of GABA agonists (Meert 1994). Numerous other neurotransmitter systems have been implicated in the anxiogenic-like effects of alcohol withdrawal, including serotonergic, noradrenergic, and neuropeptidergic

systems (see table 1) (Baldwin et al. 1991; Costall et al. 1990; Criswell and Breese 1993; File et al. 1989, 1992, 1993; Koob et al. 1994; Lal et al. 1993; Meert 1994; Moy et al. 1997; Rassnick et al. 1993; Sarnyai et al. 1995).

Compromised Reward: Clues From Other Sedative-Hypnotic Drugs

Studies of the neuropharmacologic basis for the anxiolytic properties of sedative-hypnotics provided some of the first clues to the reinforcing properties and abuse potential of these drugs (Koob and Britton 1996). Research demonstrating the ability of GABA antagonists to reverse many of the behavioral effects of alcohol led to the hypothesis that GABA has a role in the intoxicating effects of alcohol (Frye and Breese 1982; Liljequist and Engel 1982). More recent studies have shown a reduction in self-administration of alcohol among rats following microinjection of potent GABA antagonists into the brain, with the most effective area to date being the central nucleus of the amygdala (Hyytia and Koob 1995).

The antagonist actions of alcohol toward the *N*-methyl-D-aspartate (NMDA) receptor (a receptor for the excitatory neurotransmitter glutamate) also appear to contribute to the intoxicating effects of alcohol (Hoffman et al. 1989; Lovinger et al. 1989) and perhaps to the dissociative effects (antisocial and aggressive behaviors, memory and learning deficits) seen in people with high BAL's (Tsai et al. 1995). As with the effect of sedative-hypnotics on the $GABA_A$ receptor, alcohol inhibits the functioning of the NMDA receptor not by blocking the glutamate binding site but via a more complex effect on the receptor unit; this complex interaction decreases the glutamate-induced flux of sodium and calcium through the receptor channel, which, in turn, interferes with neurons' ability to transmit information (Fitzgerald and Nestler 1995). (Other sections in this chapter discuss in detail alcohol's effect on the NMDA receptor.) Whether alcohol's effect on the NMDA receptor also contributes to alcohol's reinforcing effects remains to be established. Alcohol can also exert more general inhibitory effects on voltage-gated ion channels, particularly sodium and calcium channels (Fitzgerald and Nestler 1995). These actions occur only with extremely high BAC's and do not appear to be involved in the reinforcing actions of alcohol, but they may contribute to the severe nervous system depression, even coma, that often accompanies severe intoxication.

Other Neurotransmitters

In addition to its initial effects on the $GABA_A$ and NMDA receptors, alcohol may influence several other neurotransmitter systems in the brain that are believed to be involved in alcohol's reinforcing properties. Neurochemical systems, such as the serotonergic and opioid peptide systems, likely contribute to the mediation of alcohol's reinforcing actions; in fact, researchers have suggested that multiple neurotransmitters combine to orchestrate the reward profile of alcohol (Engel et al. 1992). (Opioid peptides are endogenous compounds, naturally occurring in the body rather than externally supplied, with opiate-like activity.) A large body of evidence also implicates dopamine in the reinforcing actions of low doses of alcohol that do not induce dependence. More specifically, studies show that dopamine receptor antagonists reduce lever pressing for alcohol in nondependent rats (Pfeffer and Samson 1988). In addition, extracellular dopamine levels have been shown to increase in nondependent rats self-administering low doses of alcohol (Weiss et al. 1992).

Further research suggests that modulation of various aspects of serotonergic transmission, including increases in the synaptic availability of serotonin (5-HT), blockade of 5-HT reuptake, and blockade of certain 5-HT receptor subtypes, can decrease alcohol intake (Sellers et al. 1992). $5\text{-}HT_3$ receptor antagonists appear to decrease self-administration of alcohol (Fadda et al. 1991; Hodge et al. 1993), and $5\text{-}HT_2$ receptor antagonists, including some agents with both $5\text{-}HT_2$ receptor antagonist action and $5\text{-}HT_{1A}$ receptor agonist activity, selectively decrease acute alcohol reinforcement (Roberts et al. 1998). Several double-blind, placebo-controlled clinical studies (studies in which neither the investigator

nor the study participant knows which treatment the participant is given) have reported that selective serotonin reuptake inhibitors (SSRI's) produced modest decreases in alcohol consumption in humans (Naranjo et al. 1990). One such inhibitor, fluoxetine (Prozac), has been shown to reduce depressive symptoms and alcohol consumption in depressed alcoholics (Cornelius et al. 1997), but it may be of limited use in preventing relapse in nondepressed alcoholics (Janiri et al. 1996; Kranzler et al. 1995). The findings of clinical trials using SSRI's have been equivocal (Johnson et al 1999).

The opioid receptor antagonists, naloxone and naltrexone, also reduce alcohol self-administration in several animal models, implicating opioid peptide systems in acute alcohol reinforcement (Hubbell et al. 1991). However, some data suggest that antagonists of specific opioid receptor subtypes in certain brain regions might have more selective effects (Hyytia 1993). Of note are double-blind, placebo-controlled clinical trials in which naltrexone significantly reduced alcohol consumption, frequency of relapse, and craving for alcohol in humans (O'Malley et al. 1992; Volpicelli et al. 1992). These data suggest that alcohol's interactions with opioid neurotransmission may contribute to certain aspects of alcohol reinforcement, particularly those important to the motivation associated with relapse.

The same neurotransmitter systems implicated in the acute reinforcing effects of alcohol may be changed by withdrawal from chronic alcohol administration. The changes associated with withdrawal include decreased dopaminergic and serotonergic transmission in the nucleus accumbens (Rossetti et al. 1992; Weiss et al. 1996) and decreased GABAergic and increased NMDA glutaminergic transmission (Fitzgerald and Nestler 1995; Roberts et al. 1996; Weiss et al. 1996).

Stress-Related Systems

As mentioned above, pituitary adrenal function is also activated during dependence and acute withdrawal from alcohol and other drugs of abuse in humans (Guaza and Borrell 1984; Roberts et al. 1992). Several studies indicate that abnormal control (dysregulation) of pituitary adrenal function persists during early abstinence (Costa et al. 1996; Kreek 1987; Kreek et al. 1984; Muller et al. 1989). Both stress and repeated administration of glucocorticoids can augment the behavioral effects of psychostimulants, and some researchers have hypothesized that circulating glucocorticoids can function to maintain a sensitized state (Piazza and Le Moal 1996, 1997).

CRF function outside of the pituitary-adrenal axis (the complement of interactions between the pituitary and adrenal glands) also appears to be activated during acute withdrawal from alcohol and many other major drugs of abuse (cocaine, opiates, cannabinoids) and, thus, may mediate behavioral aspects of stress associated with abstinence (Heinrichs et al. 1995; Koob et al. 1994; Richter and Weiss 1999; Rodriguez de Fonseca et al. 1997). How this activation contributes to the decreased reward associated with acute withdrawal or prolonged abstinence remains to be determined (Koob and Bloom 1988; Koob and Le Moal 1997).

Alcoholism: Lasting Changes in the Brain

Research into the molecular and cellular mechanisms of alcohol dependence has begun to focus on changes in neurochemical systems known to be highly sensitive to the acute effects of alcohol. A large body of evidence has documented that chronic alcohol administration reduces GABAergic neurotransmission.

Prolonged alcoholism also is associated with a decreased ability of alcohol to potentiate GABA-stimulated chlorine flux, which alters GABA's normal inhibitory effects on neuronal activity and transmission of information (Frank et al. 1972; Morrow et al. 1988). However, in the absence of evidence of a decreased number of GABA receptor sites following long-term exposure to alcohol (Karobath et al. 1980), it appears that alcohol may instead alter the composition or function of GABAergic receptors. Subsequent research has demonstrated that chronic alcohol intake can decrease expression of the α_1–α_5 subunits of the GABA complex in the cerebral

cortex (Devaud et al. 1995; Mhatre et al. 1993) as well as other subunits (Devaud et al. 1997; Tabakoff and Hoffman 1996). Interestingly, chronic intermittent exposure to alcohol results in a long-lasting "kindling" effect, in which the symptoms of alcohol withdrawal increase in severity with repeated episodes of intoxication and multiple attempts to stop drinking; this effect is paralleled by an increase in the $GABA_A$ α_4 subunit (Mahmoudi et al. 1997).

Chronic alcohol consumption is also associated with increases in specific subunits (NR1 and NR2A) of NMDA receptors (Trevisan et al. 1994). For example, long-term exposure to alcohol has been shown to upregulate (stimulate) NMDA receptor function in cultures of neurons from the cortex (Hu and Ticku 1995). Another study showed that chronic alcohol treatment increased the number of NR2A and NR2B messenger ribonucleic acid subunits—a signal that the cell is synthesizing the proteins encoded by ribonucleic acid—during withdrawal but not prior to withdrawal (Follesa and Ticku 1995). Consistent with these observations, prolonged alcohol intake enhanced NMDA-stimulated nitric oxide formation without causing an increase in the number of receptors, suggesting the presence of other possible receptor sites for alcohol to enhance NMDA receptor function (Chandler et al. 1997). Research suggests that nitric oxide, a gas with neurotransmitter and neurotoxic actions, is the chemical mediator linking excitatory neurotransmission, a process that leads to significant increases in intracellular calcium, and cell death. Alcohol withdrawal also results in increased extracellular concentrations of glutamate in the striatum, a part of the brain where up-regulation of the NR1 and GluR1 subunits of the glutamate receptor complex has been observed following long-term exposure to alcohol (Rossetti and Carboni 1995).

Such findings link the neuroadaptive changes in the glutamate complex to the motivational systems implicated by pharmacologic and neurochemical studies (Ortiz et al. 1995). Defining the relationship between the molecular changes in alcohol-receptive elements such as the $GABA_A$ subunits and the specific aspects of the motivation for excessive alcohol consumption outlined above provides a challenge for future research.

Other modifications in receptor function following protracted alcohol exposure include changes in calcium ion channels. In animals, calcium ion channel antagonists have been shown to attenuate alcohol withdrawal symptoms, particularly those associated with physical signs and seizures (Colombo et al. 1995; Watson and Little 1997). Histologic (tissue and cell) studies indicate that alcohol withdrawal excitability in the hippocampus involves increased activity of calcium ion channels (Shindou et al. 1994). The ability of chronic alcohol exposure to increase protein kinase C activity could, in turn, regulate calcium ion channels and the expression of genes for these channels (Messing et al. 1990, 1991). Finally, alcohol withdrawal results in decreases in the firing rate and firing pattern of dopaminergic cells in the VTA area of the mesolimbic dopamine system (Diana et al. 1995). Although calcium ion channel antagonists have shown promise in animal studies, additional research is needed to establish the potential of these agents in humans (Johnson et al 1999).

The persistent changes in alcohol reinforcement mechanisms that characterize addiction suggest that the underlying molecular mechanisms are long lasting. Indeed, considerable research is focused on drug-related regulation of gene expression. For example, researchers have hypothesized that two types of transcription factors, CREB and novel Fos-like proteins (termed chronic Fos-related antigens), may be possible mediators of chronic drug action (Hope et al. 1994; Hyman 1996; Widnell et al. 1996). Transcription factors alter the expression of other genes that may contribute to the long-lasting effects of neurotransmitters on alcohol tolerance (Hoffman 1994; Szabo et al. 1996). Alcohol can induce changes in c-*fos* in limbic structures in the brain (Costa et al. 1996; Muller et al. 1989). The challenge for the future will be to relate regulation of a specific transcription factor, such as c-*fos*, to specific features of drug reinforcement in

connection with specific histories of drug administration.

Tolerance

Tolerance to the reinforcing actions of alcohol also may contribute to excessive drinking. As with most studies of withdrawal, until recently, studies of tolerance have focused largely on physical measures, such as loss of the righting reflex and impairment of motor coordination. Evaluation of the neural substrates associated with motivational measures of tolerance suggests that these mechanisms may differ from the neural substrates linked with the physical signs of tolerance.

Researchers have hypothesized that the neural substrates for alcohol tolerance may overlap significantly with those associated with acute withdrawal because tolerance and withdrawal sometimes appear to be components of the same neuroadaptive process. Tolerance also depends on learning processes, as has been well documented in the context of alcohol (Young and Goudie 1995). Molecular mechanisms for tolerance that appear to overlap with those for dependence (Nestler et al. 1993) include increases in intracellular calcium and protein kinase activity that occur in the presence of alcohol and also appear to produce increases in transcription factors, such as c-*fos* and c-*jun*. Acute moderate doses of alcohol also induce the expression of c-*fos* in the extended amygdala, resulting in apparent tolerance with repeated dosing (Ryabinin et al. 1997). Mechanisms for these learning processes may involve several neurotransmitters independent of their role in acute withdrawal, including norepinephrine and 5-HT (Tabakoff and Hoffman 1992), glutamate (Collingridge and Singer 1990; Khanna et al. 1992, 1994), and arginine vasopressin (AVP) (Hoffman 1994). (Of note is that mice with a disrupted subtype of the 5-HT receptor [5-HT_{1B} knockout mice] developed less tolerance than mice with the intact receptor but developed the same level of physical dependence [Crabbe et al. 1996].)

The neurotransmitter AVP is localized in the hypothalamus and basal forebrain; alteration of vasopressin systems influences learning and memory. Administration of a selective AVP antagonist to alcohol-tolerant mice produced an increased rate of loss of tolerance, which is opposite the effect of exogenously (externally) administered AVP (Szabo et al. 1988). One more recent study found that an antagonist to the transcription factor c-*fos* blocked the ability of AVP to maintain alcohol tolerance (Szabo et al. 1996).

Another possible mechanism of tolerance, hypothesized from a cell culture model, involves alcohol-induced changes in the cyclic adenosine monophosphate (cAMP) signaling system, with roles for the enzymes protein kinase A, protein phosphatase, and protein kinase C. (See the sections “Setting the Stage: The Structure and Function of Neurons” and “From Cell Membrane to Nucleus: The Effects of Alcohol on Brain Neurons” earlier in this chapter for additional information about these signaling systems and proteins.) For example, the inhibition of adenosine uptake by certain cell cultures exposed to alcohol (Krauss et al. 1993; Nagy et al. 1990) requires cAMP-dependent protein kinase activity. (Adenosine is a compound with numerous functions, among them that of an inhibitory neurotransmitter.) Inhibition of protein kinase A activity, in turn, mimics alcohol tolerance (in which alcohol no longer inhibits adenosine uptake), which can be prevented by inhibiting protein phosphatase activity (Coe et al. 1996*a*).

In another study, activation of protein kinase C also was shown to produce the characteristics of tolerance in naive cells (cells not previously exposed to alcohol), while inhibition of protein kinase C activity during chronic exposure to alcohol prevented the development of tolerance (Coe et al. 1996*b*). Again, the challenge for future studies will be to identify and understand how these specific cellular systems undergo the changes that are responsible for tolerance to motivational effects of alcohol.

Sensitization

The repeated administration of drugs, including alcohol, can result in an enhancement of their behavioral effects, particularly if the treatment regimen involves intermittent, noncontinuous administration (Phillips et al. 1989). Sensitization has been observed in association with the locomotor stimulant effects of alcohol in mice but not rats; this association is also highly dependent on the strain of mouse being studied, suggesting a strong genetic component to sensitization (Phillips et al. 1997). Studies of the neurochemical substrates for sensitization have focused primarily on increased activity in the mesocorticolimbic dopamine system (Stewart and Badiani 1993; Wise and Leeb 1993). Research suggests a time-dependent chain of neurobiological changes within this system that lead to sensitization (Henry and White 1991; Kalivas and Stewart 1991; White and Wolf 1991), with the likely site of action identified as the dopamine-producing cells in the VTA. One of these studies showed that repeated administration of cocaine produced a decrease in the sensitivity of dopamine D2 autoreceptors (dopamine receptors on a cell that itself releases dopamine); dopaminergic function was enhanced with subsequent injections (White and Wolf 1991). Although the time course of dopaminergic subsensitivity was only 4 to 8 days, behavioral sensitization persisted for weeks. More prolonged effects that last for weeks include changes in the nucleus accumbens, such as supersensitivity of D1 receptors and changes in second-messenger systems (internal cell signaling) (Koob and Nestler 1997), suggesting that the initial events triggered in the VTA are followed by more prolonged neurochemical adaptations. In addition, increased release of dopamine in the nucleus accumbens accompanies the increased behavioral responsivity to psychomotor stimulants such as cocaine or alcohol (Kalivas and Stewart 1991).

Stressors can also cause sensitization to stimulant drugs; research suggests an important role for the hypothalamic-pituitary-adrenal stress axis and the extrahypothalamic CRF system in stress-induced sensitization to psychostimulant drugs (Koob and Cador 1993). In addition, a role for brain glutamate systems in sensitization has been hypothesized from results of studies showing that administration of NMDA receptor antagonists blocks the development of sensitization to psychomotor stimulants (Karler et al. 1989; Wise 1988). The locomotor activation produced by acute doses of alcohol in mice does appear to depend on dopaminergic mechanisms (Koechling et al. 1990). How the neuropharmacologic changes observed with intermittent exposure to stimulants relate to sensitization of the motor-activating effects of alcohol and the potential sensitization to the rewarding effects of alcohol remains a challenge for future studies.

Another form of sensitization that has gained significant clinical interest and that may contribute to excessive drinking and vulnerability to relapse is the enhanced withdrawal responses observed during repeated intoxication and withdrawal, known as a "kindling" effect because of its similarity to the kindling of brain seizures (Ballenger and Post 1978; Becker et al. 1997; Kokka et al. 1993). Mice exposed chronically to alcohol vapors (to produce dependence) and then subjected to repeated withdrawal episodes showed progressive increases in the intensity of withdrawal seizures (Becker and Hale 1993; Becker et al. 1997). Rats subjected to repeated withdrawal from chronic alcohol also showed a kindling effect on seizure activity. This kindling effect subsequently was blocked by administration of diazepam, a drug that enhances GABA activity (Ulrichsen et al. 1995), and has been linked to decreases in $GABA_A$ receptor-mediated inhibition (Kang et al. 1996). The challenge for future research will be to test the hypothesis that these kindling phenomena extend to motivational measures of alcohol-seeking behaviors.

Relapse

The study of neurobiological mechanisms associated with relapse has been limited. Animal models for the study of alcohol relapse are under development (Koob 1995). Neuropharmacologic agents that activate the mesocorticolimbic dopamine system can rapidly reinstate drug self-administration in trained animals, but this activation can be extinguished through intravenous self-administration of alcohol (de Wit

and Stewart 1981; Stewart and de Wit 1987). Chronic alcohol administration with a liquid diet to induce dependence has been shown to produce increases in amphetamine- and cocaine-induced locomotor activity up to 2 months after exposure to alcohol (Manley and Little 1997). These findings suggest that a history of dependence may produce a sensitization of the mesolimbic dopamine system. Consistent with this conclusion is the observation that psychostimulant drugs can potentiate conditioned reinforcing effects produced by alcohol (Slawecki et al. 1998).

Research using other animal models, cell systems, and drugs is limited but shows some promise. Acamprosate, a drug being marketed in Europe to prevent relapse in alcoholics, blocks the increase in drinking observed in nondependent rodents after a forced abstinence (Heyser et al. 1996, 1997; Holter et al. 1997; Spanagel and Zieglgansberger 1997; see also the section "Treatment of Alcohol Dependence With Medications" in the chapter on treatment research). Acamprosate may modulate glutamate activity, possibly by enhancing the effects of glutamate under certain situations (Madamba et al. 1996) and inhibiting glutamate activation in other situations (Spanagel and Zieglgansberger 1997; Zeise et al. 1993).

Similarly, opioid antagonists have been shown to prevent an increase in drinking of alcohol by animals following their exposure to certain stressors (Volpicelli et al. 1986). Subsequent studies demonstrated naltrexone's efficacy in preventing relapse in alcoholics who had undergone detoxification (O'Malley et al. 1992; Volpicelli et al. 1992). Naltrexone may act by modulating some aspect of the mesolimbic dopamine reward circuitry, either presynaptically or postsynaptically (Spanagel and Zieglgansberger 1997). For example, some studies have reported that naloxone administered through a microdialysis probe in the nucleus accumbens inhibits alcohol-induced dopamine release (Benjamin et al. 1993; Widdowson and Holman 1992). Other studies have shown that naloxone blocks the inhibitory effect of endogenous opioids on GABA-releasing neurons in the dopaminergic VTA, resulting ultimately in disinhibition (Spanagel and Zieglgansberger 1997).

Identifying and understanding the neurological substrates and the biochemical and molecular mechanisms underlying relapse following abstinence from alcohol should facilitate the development of treatments and/or therapeutic agents that will reduce or eliminate the likelihood of relapse.

References

Adams, M.L.; Sewing, B.N.; Chen, J.; Meyer, E.R.; and Cicero, T.J. Nitric oxide-related agents alter alcohol withdrawal in male rats. *Alcohol Clin Exp Res* 19(1):195–199, 1995.

Alheid, G.F., and Heimer, L. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: The striatopallidal, amygdaloid, and corticopetal components of substantia innominata. *Neuroscience* 27(1):1–39, 1988.

American Psychological Association. *The Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. Washington, DC: American Psychological Association, 1994.

Baker, T.B., and Cannon, D.S. Potentiation of ethanol withdrawal by prior dependence. *Psychopharmacology* 60(2):105–110, 1979.

Baldwin, H.A.; Rassnick, S.; Rivier, J.; Koob, G.F.; and Britton, K.T. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. *Psychopharmacology* 103(2):227–232, 1991.

Ballenger, J.C., and Post, R.M. Kindling as a model for alcohol withdrawal syndromes. *Br J Psychiatry* 133:1–14, 1978.

Becker, H.C.; Diaz-Granados, J.L.; and Weathersby, R.T. Repeated ethanol withdrawal experience increases the severity and duration of subsequent withdrawal seizures in mice. *Alcohol* 14(4):319–326, 1997.

Becker, H.C., and Hale, R.L. Repeated episodes of ethanol withdrawal potentiate the severity of subsequent withdrawal seizures: An animal model of alcohol withdrawal "kindling." *Alcohol Clin Exp Res* 17:94–98, 1993.

Benjamin, D.; Grant, E.R.; and Pohorecky, L.A. Naltrexone reverses ethanol-induced dopamine release in the nucleus accumbens in awake, freely moving rats. *Brain Res* 621(1):137–140, 1993.

Branchey, M.; Rauscher, G.; and Kissin, B. Modifications in the response to alcohol following the establishment of physical dependence. *Psychopharmacologia* 22(4):314–322, 1971.

Cappell, H., and Le Blanc, A.E. Tolerance and physical dependence: Do they play a role in alcohol and drug self-administration? *Recent Adv Alcohol Drug Probl* 6:159–196, 1981.

Chandler, L.J.; Sutton, G.; Norwood, D.; Sumners, C.; and Crews, F.T. Chronic ethanol increases *N*-methyl-D-aspartate-stimulated nitric oxide formation but not receptor density in cultured cortical neurons. *Mol Pharmacol* 51(5):733–740, 1997.

Charlton, M.E.; Sweetnam, P.M.; Fitzgerald, L.W.; Terwilliger, R.Z.; Nestler, E.J.; and Duman, R.S. Chronic ethanol administration regulates the expression of $GABA_A$ receptor α_1 and α_5 subunits in the ventral tegmental area and hippocampus. *J Neurochem* 68(1):121–127, 1997.

Coe, I.R.; Dohrman, D.P.; Constantinescu, A.; Diamond, I.; and Gordon, A.S. Activation of cyclic AMP-dependent protein kinase reverses tolerance of a nucleoside transporter to ethanol. *J Pharmacol Exp Ther* 276(2):365–369, 1996*a*.

Coe, I.R.; Yao, L.; Diamond, I.; and Gordon, A.S. The role of protein kinase C in cellular tolerance to ethanol. *J Biol Chem* 271(46): 29468–29472, 1996*b*.

Collingridge, G.L., and Singer, W. Excitatory amino acid receptors and synaptic plasticity. *Trends Pharmacol Sci* 11(7):290–296, 1990.

Colombo, G.; Agabio, R.; Lobina, C.; Reali, R.; Melis, F.; Fadda, F.; and Gessa, G.L. Effects of the calcium channel antagonist darodipine on ethanol withdrawal in rats. *Alcohol Alcohol* 30(1): 125–131, 1995.

Concas, A.; Cuccheddu, T.; Floris, S.; Mascia, M.P.; and Biggio, G. 2-Chloro-N^6-cyclopentyl adenosine (CCPA), an adenosine A_1 receptor agonist, suppresses ethanol withdrawal syndrome in rats. *Alcohol Alcohol* 29(3):261–264, 1994.

Cornelius, J.R.; Salloum, I.M.; Ehler, J.G.; Jarrett, P.J.; Cornelius, M.D.; Perel, J.M.; Thase, M.E.; and Black, A. Fluoxetine in depressed alcoholics: A double-blind, placebo-controlled trial. *Arch Gen Psychiatry* 54(8):700–705, 1997.

Costa, A.; Bono, G.; Martignoni, E.; Merlo, P.; Sances, G.; and Nappi, G. An assessment of hypothalamo-pituitary-adrenal axis functioning in non-depressed, early abstinent alcoholics. *Psychoneuroendocrinology* 21(3):263–275, 1996.

Costall, B.; Naylor, R.J.; and Tyers, M.B. The psychopharmacology of $5\text{-}HT_3$ receptors. *Pharmacol Ther* 47(2):181–202, 1990.

Crabbe, J.C. Antagonism of ethanol withdrawal convulsions in withdrawal seizure prone mice by diazepam and abecarnil. *Eur J Pharmacol* 221(1): 85–90, 1992.

Crabbe, J.C.; Phillips, T.J.; Feller, D.J.; Hen, R.; Wenger, C.D.; Lessov, C.N.; and Schafer, G.L. Elevated alcohol consumption in null mutant mice lacking $5\text{-}HT_{1B}$ serotonin receptors. *Nat Genet* 14(1):98–101, 1996.

Criswell, H.E., and Breese, G.R. Similar effects of ethanol and flumazenil on acquisition of a shuttle-box avoidance response during withdrawal from chronic ethanol treatment. *Br J Pharmacol* 110(2):753–760, 1993.

Cunningham, C.L.; Niehus, J.S.; and Bachtold, J.F. Ambient temperature effects on taste aversion conditioned by ethanol: Contribution of ethanol-induced hypothermia. *Alcohol Clin Exp Res* 16(6):1117–1124, 1992.

Davis, M. Neurobiology of fear responses: The role of the amygdala. *J Neuropsychiatry Clin Neurosci* 9(3):382–402, 1997.

Devaud, L.L.; Fritschy, J.M.; Sieghart, W.; and Morrow, A.L. Bidirectional alterations of $GABA_A$ receptor subunit peptide levels in rat cortex during chronic ethanol consumption and withdrawal. *J Neurochem* 69(1):126–130, 1997.

Devaud, L.L.; Smith, F.D.; Grayson, D.R.; and Morrow, A.L. Chronic ethanol consumption differentially alters the expression of gamma-aminobutyric $acid_A$ receptor subunit mRNAs in rat cerebral cortex: Competitive, quantitative reverse transcriptase-polymerase chain reaction analysis. *Mol Pharmacol* 48(5):861–868, 1995.

de Wit, H., and Stewart, J. Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology* 75(2):134–143, 1981.

Diana, M.; Pistis, M.; Muntoni, A.L.; and Gessa, G.L. Ethanol withdrawal does not induce a reduction in the number of spontaneously active dopaminergic neurons in the mesolimbic system. *Brain Res* 682(1–2):29–34, 1995.

Edwards, G. Withdrawal symptoms and alcohol dependence: Fruitful mysteries. *Br J Addict* 85(4):447–461, 1990.

Emanuele N., and Emanuele, M.A. The endocrine system: Alcohol alters critical hormonal balance. *Alcohol Health Res World* 21(1):53–64, 1997.

Engel, J.A.; Enerback, C.; Fahlke, C.; Hulthe, P.; Hard, E.; Johannessen, K.; Svensson, L.; and Soderpalm, B. Serotonergic and dopaminergic involvement in ethanol intake. In: Naranjo, C.A., and Sellers, E.M., eds. *Novel Pharmacological Interventions for Alcoholism.* New York, NY: Springer, 1992. pp. 68–82.

Fadda, F.; Garau, B.; Marchei, F.; Colombo, G.; and Gessa, G.L. MDL 72222, a selective $5\text{-}HT_3$ receptor antagonist, suppresses voluntary ethanol consumption in alcohol-preferring rats. *Alcohol Alcohol* 26(2):107–110, 1991.

File, S.E.; Andrews, N.; and Al-Farhan, M. Anxiogenic responses of rats on withdrawal from chronic treatment: Effects of tianeptine. *Alcohol Alcohol* 28(3):281–286, 1993.

File, S.E.; Baldwin, H.A.; and Hitchcott, P.K. Flumazenil but not nitrendipine reverses the increased anxiety during ethanol withdrawal in the rat. *Psychopharmacology* 98(2):262–264, 1989.

File, S.E.; Zharkovsky, A.; and Hitchcott, P.K. Effects of nitrendipine, chlordiazepoxide, flumazenil and baclofen on the increased anxiety resulting from alcohol withdrawal. *Prog Neuropsycopharmacol Biol Psychiatry* 16(1):87–93, 1992.

Finn, D.A.; Roberts, A.J.; and Crabbe, J.C. Neuroactive steroid sensitivity in withdrawal seizure-prone and -resistant mice. *Alcohol Clin Exp Res* 19(2):410–415, 1995.

Fitzgerald, L.W., and Nestler, E.J. Molecular and cellular adaptations in signal transduction pathways following ethanol exposure. *Clin Neurosci* 3(3):165–173, 1995.

Follesa, P., and Ticku, M.K. Chronic ethanol treatment differentially regulates NMDA receptor subunit mRNA expression in rat brain. *Mol Brain Res* 29(1):99–106, 1995.

Frank, M.M.; Sergent, J.S.; Kane, M.A.; and Alling, D.W. Epsilon aminocaproic acid therapy of hereditary angioneurotic edema: A double-blind study. *N Engl J Med* 286(15):808–812, 1972.

Frye, G.D., and Breese, G.R. GABAergic modulation of ethanol-induced motor impairment. *J Pharmacol Exp Ther* 223(3):750–756, 1982.

Grant, K.A.; Valverius, P.; Hudspith, M.; and Tabakoff, B. Ethanol withdrawal seizures and the NMDA receptor complex. *Eur J Pharmacol* 176(3):289–296, 1990.

Guaza, C., and Borrell, S. Effect of naloxone administration upon responses of adrenal hormones to withdrawal from ethanol. *Psychopharmacology* 82(3):181–184, 1984.

Heimer, L. *The Human Brain and Spinal Cord: Functional Neuroanatomy and Dissection Guide*, 2nd ed. New York, NY: Springer-Verlag, 1995.

Heimer, L., and Alheid, G. Piecing together the puzzle of basal forebrain anatomy. In: Napier, T.C., Kalivas, P.W., and Hanin, I., eds. *The Basal Forebrain: Anatomy to Function. Vol. 295. Advances in Experimental Medicine and Biology.* New York, NY: Plenum Press, 1991. pp. 1–42.

Heinrichs, S.C.; Menzaghi, F.; Schulteis, G.; Koob, G.F.; and Stinus, L. Suppression of corticotropin-releasing factor in the amygdala attenuates aversive consequences of morphine withdrawal. *Behav Pharmacol* 6:74–80, 1995.

Henry, D.J., and White, F.J. Repeated cocaine administration causes persistent enhancement of D1 dopamine receptor sensitivity within the rat nucleus accumbens. *J Pharmacol Exp Ther* 258(3):882–890, 1991.

Hershon, H.I. Alcohol withdrawal symptoms and drinking behavior. *J Stud Alcohol* 38(5):953–971, 1977.

Heyser, C.J.; Roberts, A.J.; Schulteis, G.; Hyytia, P.; and Koob, G.F. Central administration of an opiate antagonist decreases oral ethanol self-administration in rats. *Neurosci Abstr* 21:1698, 1995.

Heyser, C.J.; Schulteis, G.; Durbin, P.; and Koob, G.F. Chronic acamprosate decreases deprivation-induced ethanol self-administration in rats. *Alcohol Clin Exp Res* 20:15A, 1996.

Heyser, C.L.; Schulteis, G.; and Koob, G.F. Increased ethanol self-administration after a period of imposed ethanol deprivation in rats trained in a limited access paradigm. *Alcohol Clin Exp Res* 21(5):784–791, 1997.

Hodge, C.W.; Samson, H.H.; Lewis, R.S.; and Erickson, H.L. Specific decreases in ethanol- but not water-reinforced responding produced by the 5-HT_3 antagonist ICS 205-930. *Alcohol* 10(3): 191–196, 1993.

Hoffman, P.L. Neuroadaptive functions of the neuropeptide arginine vasopressin: Ethanol tolerance. In: Strand, F.L.; Beckwith, B.; and Chronwall, B., eds. *Models of Neuropeptide Action. Vol. 739. Annals of the New York Academy of Sciences.* New York, NY: New York Academy of Sciences, 1994. pp. 168–175.

Hoffman, P.L.; Rabe, C.S.; Moses, F.; and Tabakoff, B. *N*-methyl-D-aspartate receptors and ethanol: Inhibition of calcium flux and cyclic GMP production. *J Neurochem* 52(6):1937–1940, 1989.

Hoffman, P.L., and Tabakoff, B. The role of the NMDA receptor in ethanol withdrawal. In: Jansson, B.; Jornvall, H.; Rydberg, U.; Terenius, L.; and Vallee, B.L., eds. *Toward a Molecular Basis of Alcohol Use and Abuse.* Boston, MA: Birkhauser-Verlag, 1994. pp. 61–70.

Holter, S.M.; Landgraf, R.; Zieglgansberger, W.; and Spanagel, R. Time course of acamprosate action on operant ethanol self-administration after ethanol deprivation. *Alcohol Clin Exp Res* 21(5):862–868, 1997.

Hope, B.T.; Nye, H.E.; Kelz, M.B.; Self, D.W.; Iadarola, M.J.; Nakabeppu, Y.; Duman, R.S.; and Nestler, E.J. Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* 13(5):1235–1244, 1994.

Hu, X.J., and Ticku, M.K. Chronic ethanol treatment upregulates the NMDA receptor function and binding in mammalian cortical neurons. *Mol Brain Res* 30(2):347–356, 1995.

Hubbell, C.L.; Marglin, S.H.; Spitalnic, S.J.; Abelson, M.L.; Wild, K.D.; and Reid, L.D. Opioidergic, serotonergic, and dopaminergic manipulations and rats' intake of a sweetened alcoholic beverage. *Alcohol* 8(5):355–367, 1991.

Hyman, S.E. Addiction to cocaine and amphetamine. *Neuron* 16(5):901–904, 1996.

Hyytia, P. Involvement of μ-opioid receptors in alcohol drinking by alcohol-preferring AA rats. *Pharmacol Biochem Behav* 45(3):697–701, 1993.

Hyytia, P., and Koob, G.F. $GABA_A$ receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. *Eur J Pharmacol* 283(1–3):151–159, 1995.

Janiri, L.; Gobbi, G.; Mannelli, P.; Pozzi, G.; Serretti, A.; and Tempesta, E. Effects of fluoxetine at antidepressant doses on short-term outcome of detoxified alcoholics. *Int Clin Psychopharmacol* 11(2):109–117, 1996.

Johnson, B.A., and Ait-Daoud, N. Medications to treat alcoholism. *Alcohol Health Res* 23:99–105, 1999.

Kakihana, R.; Brown, D.R.; McClearn, G.E.; and Tabershaw, I.R. Brain sensitivity to alcohol in inbred mouse strains. *Science* 154(756): 1574–1575, 1966.

Kalivas, P.W., and Stewart, J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Rev* 16(3):223–244, 1991.

Kang, M.; Spigelman, I.; Sapp, D.W.; and Olsen, R.W. Persistent reduction of $GABA_A$ receptor-mediated inhibition in rat hippocampus after chronic intermittent ethanol treatment. *Brain Res* 709(2):221–228, 1996.

Karler, R.; Calder, L.D.; Chaudhry, I.A.; and Turkanis, S.A. Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. *Life Sci* 45(7):599–606, 1989.

Karobath, M.; Rogers, J.; and Bloom, F.E. Benzodiazepine receptors remain unchanged after chronic ethanol administration. *Neuropharmacology* 19(1):125–128, 1980.

Khanna, J.M.; Kalant, H.; Shah, G.; and Chau, A. Effect of (+)MK-801 and ketamine on rapid tolerance to ethanol. *Brain Res Bull* 28(2): 311–314, 1992.

Khanna, J.M.; Morato, G.S.; Chau, A.; Shah, G.; and Kalant, H. Effect of NMDA antagonists on rapid and chronic tolerance to ethanol: Importance of intoxicated practice. *Pharmacol Biochem Behav* 48(3):755–763, 1994.

Koechling, U.M.; Smith, B.R.; and Amit, Z. Differential effects of catecholamine antagonists on ethanol-induced excitation in mice. *Psychopharmacology* 102(2):234–238, 1990.

Kokka, N.; Sapp, D.W.; Taylor, A.M.; and Olsen, R.W. The kindling model of alcohol dependence: Similar persistent reduction in seizure threshold to pentylenetetrazol in animals receiving chronic ethanol or chronic pentylenetetrazol. *Alcohol Clin Exp Res* 17(3):525–531, 1993.

Koob, G.F. Dopamine, addiction and reward. *Semin Neurosci* 4:139–148, 1992.

Koob, G.F. Animal models of drug addiction. In: Bloom, F.E. and Kupfer, D.J., eds. *Psychopharmacology: The Fourth Generation of Progress.* New York, NY: Raven Press, 1995. pp. 759–772.

Koob, G.F. Drug addiction: The yin and yang of hedonic homeostasis. *Neuron* 16(5):893–896, 1996.

Koob, G.F., and Bloom, F.E. Cellular and molecular mechanisms of drug dependence. *Science* 242(4879):715–723, 1988.

Koob, G.F., and Britton, K.T. Neurobiological substrates for the anti-anxiety effects of ethanol. In: Begleiter, H., and Kissin, B., eds. *Pharmacology of Alcohol and Alcohol Dependence. Vol. 2. Alcohol and Alcoholism.* New York, NY: Oxford University Press, 1996. pp. 477–506.

Koob, G.F., and Cador, M. Psychomotor stimulant sensitization: The corticotropin-releasing factor-steroid connection. *Behav Pharmacol* 4:351–354, 1993.

Koob, G.F.; Heinrichs, S.C.; Menzaghi, F.; Merlo-Pich, E.; and Britton, K.T. Corticotropin releasing factor, stress and behavior. *Semin Neurosci* 6:221–229, 1994.

Koob, G.F., and Le Moal, M. Drug abuse: Hedonic homeostatic dysregulation. *Science* 278(5353):52–58, 1997.

Koob, G.F.; Markou, A.; Weiss, F.; Schulteis, G. Opponent process and drug dependence: Neurobiological mechanisms. *Semin Neurosci* 5(5):351–358, 1993.

Koob, G.F., and Nestler, E.J. The neurobiology of drug addiction. *J Neuropsychiatry Clin Neurosci* 9(3):482–497, 1997.

Kornet, M.; Goosen, C.; and Van Ree, J.M. The effect of interrupted alcohol supply on spontaneous alcohol consumption by rhesus monkeys. *Alcohol Alcohol* 25(4):407–412, 1990.

Kornet, M.; Goosen, C.; and Van Ree, J.M. Effect of naltrexone on alcohol consumption during chronic alcohol drinking and after a period of imposed abstinence in free-choice drinking rhesus monkeys. *Psychopharmacology* 104(3):367–376, 1991.

Kotlinska, J., and Liljequist, S. Oral administration of glycine and polyamine receptor antagonists blocks ethanol withdrawal seizures. *Psychopharmacology* 127(3):238–244, 1996.

Kranzler, H.R.; Burleson, J.A.; Korner, P.; Del Boca, F.K.; Bohn, M.J.; Brown, J.; and Liebowitz, N. Placebo-controlled trial of fluoxetine as an adjunct to relapse prevention in alcoholics. *Am J Psychiatry* 152(3):391–397, 1995.

Krauss, S.W.; Ghirnikar, R.B.; Diamond, I.; and Gordon, A.S. Inhibition of adenosine uptake by ethanol is specific for one class of nucleoside transporters. *Mol Pharmacol* 44(5):1021–1026, 1993.

Kreek, M.J. Multiple drug abuse patterns and medical consequences. In: Meltzer, H.Y., ed. *Psychopharmacology: The Third Generation of Progress.* New York, NY: Raven Press, 1987. pp. 1597–1604.

Kreek, M.J.; Ragunath, J.; Plevy, S.; Hamer, D.; Schneider, B.; and Hartman, N. ACTH, cortisol and beta-endorphin response to metyrapone testing during chronic methadone maintenance treatment in humans. *Neuropeptides* 5(1–3): 277–278, 1984.

Lal, H.; Prather, P.L.; and Rezazadeh, S.M. Potential role of $5HT_{1C}$ and/or $5HT_2$ receptors in the mianserin-induced prevention of anxiogenic behaviors occurring during ethanol withdrawal. *Alcohol Clin Exp Res* 17(2):411–417, 1993.

Legault, M., and Wise, R.A. Effects of withdrawal from nicotine on intracranial self-stimulation. *Neurosci Abstr* 20:1032, 1994.

Leith, N.J., and Barrett, R.J. Amphetamine and the reward system: Evidence for tolerance and post-drug depression. *Psychopharmacologia* 46(1):19–25, 1976.

LeMagen, J. Etude de quelques facteurs associé à des modification de la consommation spontanée d'alcool éthylique par le rat. *J Physiol Paris* 52:873–884, 1960.

Liljequist, S., and Engel, J. Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. *Psychopharmacology* 78(1):71–75, 1982.

Lovinger, D.M.; White, G.; and Weight, F.F. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science* 243(4899): 1721–1724, 1989.

Madamba, S.G.; Schweitzer, P.; Zieglgansberger, W.; and Siggins, G.R. Acamprosate (calcium acetylhomortaurinate) enhances the *N*-methyl-D-aspartate component of excitatory neurotransmission in rat hippocampal CA1 neurons in vitro. *Alcohol Clin Exp Res* 20(4):651–658, 1996.

Mahmoudi, M.; Kang, M.H.; Tillakaratne, N.; Tobin, A.J.; and Olsen, R.W. Chronic intermittent ethanol treatment in rats increases $GABA_A$ receptor α_4-subunit expression: Possible relevance to alcohol dependence. *J Neurochem* 68(6):2485–2492, 1997.

Manley, S.J., and Little, H.J. Enhancement of amphetamine- and cocaine-induced locomotor activity after chronic ethanol administration. *J Pharmacol Exp Ther* 281(3):1330–1339, 1997.

Markou, A., and Koob, G.F. Post cocaine anhedonia: An animal model of cocaine withdrawal. *Neuropsychopharmacology* 4(1):17–26, 1991.

Markou, A., and Koob, G.F. Construct validity of a self-stimulation threshold paradigm: Effects of reward and performance manipulations. *Physiol Behav* 51(1):111–119, 1992.

Meert, T.F. Pharmacological evaluation of alcohol withdrawal-induced inhibition of exploratory behaviour and supersensitivity to harmine-induced tremor. *Alcohol Alcohol* 29(1):91–102, 1994.

Merlo-Pich, E.; Lorang, M.; Yeganeh, M.: Rodriguez de Fonseca, F.; Raber, J.; Koob, G.F.; and Weiss, F. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci* 15:5439–5447, 1995.

Messing, R.O.; Petersen, P.J.; and Henrich, C.J. Chronic ethanol exposure increases levels of protein kinase C delta and epsilon and protein kinase C-mediated phosphorylation in cultured neural cells. *J Biol Chem* 266(34):23428–23432, 1991.

Messing, R.O.; Sneade, A.B.; and Savidge, B. Protein kinase C participates in upregulation of dihydropyridine-sensitive calcium channels by ethanol. *J Neurochem* 55(4):1383–1389, 1990.

Mhatre, M.C.; Pena, G.; Sieghart, W.; and Ticku, M.K. Antibodies specific for $GABA_A$ receptor alpha subunits reveal that chronic alcohol treatment down-regulates alpha-subunit expression in rat brain regions. *J Neurochem* 61(5):1620–1625, 1993.

Morrisett, R.A.; Rezvani, A.H.; Overstreet, D.; Janowsky, D.S.; Wilson, W.A.; and Swartzwelder, H.S. MK-801 potently inhibits alcohol withdrawal seizures in rats. *Eur J Pharmacol* 176(1): 103–105, 1990.

Morrow, A.L.; Suzdak, P.D.; Karanian, J.W.; and Paul, S.M. Chronic ethanol administration alters gamma-aminobutyric acid, pentobarbital and ethanol-mediated $^{36}Cl^-$ uptake in cerebral cortical synaptoneurosomes. *J Pharmacol Exp Ther* 246(1):158–164, 1988.

Moy, S.S.; Knapp, D.J.; Criswell, H.E.; and Breese, G.R. Flumazenil blockade of anxiety following ethanol withdrawal in rats. *Psychopharmacology* 131(4):354–360, 1997.

Muller, N.; Hoehe, M.; Klein, H.E.; Nieberle, G.; Kapfhammer, H.P.; May, F.; Muller, O.A.; and Fichter, M. Endocrinological studies in alcoholics during withdrawal and after abstinence. *Psychoneuroendocrinology* 14(1–2):113–123, 1989.

Nagy, L.E.; Diamond, I.; Casso, D.J.; Franklin, C.; and Gordon, A.S. Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. *J Biol Chem* 265:1946–1951, 1990.

Naranjo, C.A.; Kadlec, K.E.; Sanhueza, P.; Woodley-Remus, D.; and Sellers, E.M. Fluoxetine differentially alters alcohol intake and other consummatory behaviors in problem drinkers. *Clin Pharmacol Ther* 47(4):490–498, 1990.

Nestler, E.J.; Hope, B.T.; and Widnell, K.L. Drug addiction: A model for the molecular basis of neural plasticity. *Neuron* 11(6):995–1006, 1993.

O'Malley, S.S.; Jaffe, A.J.; Chang, G.; Schottenfeld, R.S.; Meyer, R.E.; and Rounsaville, B. Naltrexone and coping skills therapy for alcohol dependence: A controlled study. *Arch Gen Psychiatry* 49(11):881–887, 1992.

Ortiz, J.; Fitzgerald, L.W.; Charlton, M.; Lane, S.; Trevisan, L.; Guitart, X.; Shoemaker, W.; Duman, R.S.; and Nestler, E.J. Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. *Synapse* 21(4): 289–298, 1995.

Parsons, L.H.; Koob, G.F.; and Weiss, F. Serotonin dysfunction in the nucleus accumbens of rats during withdrawal after unlimited access to intravenous cocaine. *J Pharmacol Exp Ther* 274(3):1182–1191, 1995.

Pfeffer, A.O., and Samson, H.H. Haloperidol and apomorphine effects on ethanol reinforcement in free-feeding rats. *Pharmacol Biochem Behav* 29(2):343–350, 1988.

Phillips, T.J.; Feller, D.J.; and Crabbe, J.C. Selected mouse lines, alcohol, and behavior. *Experientia* 45(9):805–827, 1989.

Phillips, T.J.; Roberts, A.J.; and Lessov, C.N. Behavorial sensitization to ethanol: Genetics and the effects of stress. *Pharmacol Biochem Behav* 57(3):487–493, 1997.

Piazza, P.V., and Le Moal, M.L. Pathophysiological basis of vulnerability to drug abuse: Role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu Rev Pharmacol Toxicol* 36:359–378, 1996.

Piazza, P.V., and Le Moal, M. Glucocorticoids as a biological substrate of reward: Physiological and pathophysiological implications. *Brain Res Rev* 25(3):359–372, 1997.

Pontieri, F.E.; Tanda, G.; and Di Chiara, G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. *Proc Natl Acad Sci USA* 92(26):12304–12308, 1995.

Pontieri, F.E.; Tanda, G.; Orzi, F.; and Di Chiara, G. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature* 382(6588):255–257, 1996.

Rassnick, S.; D'Amico, E.; Riley, E.; and Koob, G.F. GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. *Alcohol Clin Exp Res* 17(1):124–130, 1993*a*.

Rassnick, S.; Heinrichs, S.C.; Britton, K.T.; and Koob, G.F. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res* 605(1):25–32, 1993*b*.

Richards, G.; Schoch, P.; and Haefely, W. Benzodiazepine receptors: New vistas. *Semin Neurosci* 3:191–203, 1991.

Richter, R.M., and Weiss, F. In vivo CRF release in rat amygdala is increased during cocaine withdrawal in self-administering rats. *Synapse* 32(4):254–261, 1999.

Roberts, A.J.; Cole, M.; and Koob, G.F. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. *Alcohol Clin Exp Res* 20(7):1289–1298, 1996.

Roberts, A.J.; Crabbe, J.C.; and Keith, L.D. Genetic differences in hypothalamic-pituitary-adrenal axis responsiveness to acute ethanol and acute ethanol withdrawal. *Brain Res* 579(2): 296–302, 1992.

Roberts, A.J.; McArthur, R.A.; Hull, E.E.; Post; C.; and Koob, G.F. Effects of amperozide, 8-OH-DPAT, and FG 5974 on operant responding for ethanol. *Psychopharmacology* 137(1):25–32, 1998.

Robinson, T.E., and Berridge, K.C. The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Res Rev* 18(3): 247–291, 1993.

Rodriguez de Fonseca, F.; Carrera, M.R.A.; Navarro, M.; Koob, G.F.; and Weiss, F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* 276(5321):2050–2054, 1997.

Rossetti, Z.L., and Carboni, S. Ethanol withdrawal is associated with increased extracellular glutamate in the rat striatum. *Eur J Pharmacol* 283(1–3):177–183, 1995.

Rossetti, Z.L.; Hmaidan, Y.; and Gessa, G.L. Marked inhibition of mesolimbic dopamine release: A common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. *Eur J Pharmacol* 221(2–3):227–234, 1992.

Roberts, A.J.; McArthur, R.A.; Hall, E.G.; Post, C.; and Koob, G.F. Effects of amperocide, 8-OH-DPAT, and FG 5974 on operant responding for ethanol. *Psychoparmacology* 137(1):25–32. 1998.

Ryabinin, A.E.; Criado, J.R.; Henriksen, S.J.; Bloom, F.E.; and Wilson, M.C. Differential sensitivity of c-Fos expression in hippocampus and other brain regions to moderate and low doses of alcohol. *Mol Psychiatry* 2(1):32–43, 1997.

Salimov, R.M., and Salimova, N.B. The alcohol-deprivation effect in hybrid mice. *Drug Alcohol Depend* 32(2):187–191, 1993.

Sarnyai, Z.; Biro, E.; Gardi, J.; Vecsernyes, M.; Julesz, J.; and Telegdy, G. Brain corticotropin-releasing factor mediates "anxiety-like" behavior induced by cocaine withdrawal in rats. *Brain Res* 675(1–2):89–97, 1995.

Schulteis, G.; Hyytia, P.; Heinrichs, S.C.; and Koob, G.F. Effects of chronic ethanol exposure on oral self-administration of ethanol or saccharin by Wistar rats. *Alcohol Clin Exp Res* 20(1):164–171, 1996.

Sellers, E.M.; Higgins, G.A.; and Sobell, M.B. 5-HT and alcohol abuse. *Trends Pharmacol Sci* 13(2):69–75, 1992.

Sepinwall, J., and Cook, L. Behavioral pharmacology of anti-anxiety drugs. In: Iversen, L.L.; Iversen, S.D.; and Snyder, S.H., eds. *Handbook of Psychopharmacology.* Vol. 13. New York, NY: Plenum Press, 1978. pp. 345–393.

Shindou, T.; Watanabe, S.; Kamata, O.; Yamamoto, K.; and Nakanishi, H. Calcium-dependent hyperexcitability of hippocampal CA1 pyramidal cells in an in vitro slice after ethanol withdrawal of the rat. *Brain Res* 656(2):432–436, 1994.

Sinclair, J.D. Alcohol-deprivation effect in rats genetically selected for their ethanol preference. *Pharmacol Biochem Behav* 10(4):597–602, 1979.

Sinclair, J.D., and Senter, R.J. Increased preference for ethanol in rats following alcohol deprivation. *Psychonomic Sci* 8(1):11–12, 1967.

Sinclair, J.D., and Senter, R.J. Development of an alcohol-deprivation effect in rats. *Q J Stud Alcohol* 29(4):863–867, 1968.

Slawecki, C.J.; Samson, H.H.; and Chappelle, A. Intra-nucleus accumbens amphetamine infusions enhance responding maintained by a stimulus complex paired with oral ethanol self-administration. *Pharmacol Biochem Behav* 58:1065–1073, 1998.

Snell, L.D.; Szabo, G.; Tabakoff, B.; and Hoffman, P.L. Gangliosides reduce the development of ethanol dependence without affecting ethanol tolerance. *J Pharmacol Exp Ther* 279(1):128–136, 1996.

Spanagel, R.; Holter, S.M.; Allingham, K.; Landgraf, R.; and Zieglgansberger, W. Acamprosate and alcohol. I. Effects on alcohol intake following alcohol deprivation in the rat. *Eur J Pharmacol* 305(1–3):39–44, 1996.

Spanagel, R., and Zieglgansberger, W. Anti-craving compounds for ethanol: New pharmacological tools to study addictive processes. *Trends Pharmacol Sci* 18(2):54–59, 1997.

Stewart, J., and Badiani, A. Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol* 4(4):289–312, 1993.

Stewart, J., and de Wit, H. Reinstatement of drug-taking behavior as a method of assessing incentive motivational properties of drugs. In: Bozarth, M.A., ed. *Assessing the Reinforcing Properties of Abused Drugs.* New York: Springer-Verlag, 1987. pp. 211–227.

Szabo, G.; Nunley, K.R.; and Hoffman, P.L. Antisense oligonucleotide to c-*fos* blocks the ability of arginine vasopressin to maintain ethanol tolerance. *Eur J Pharmacol* 306(1–3):67–72, 1996.

Szabo, G.; Tabakoff, B.; and Hoffman, P.L. Receptors with V_1 characteristics mediate the maintenance of ethanol tolerance by vasopressin. *J Pharmacol Exp Ther* 247(2):536–541, 1988.

Tabakoff, B., and Hoffman, P.L. Alcohol: Neurobiology. In: Lowinson, J.H.; Ruiz, P.; Millman, R.B., and Langrod, J.G., eds. *Substance Abuse: A Comprehensive Textbook*, 2nd ed. Baltimore, MD: Williams & Wilkins, 1992. pp. 152–185.

Tabakoff, B., and Hoffman, P.L. Alcohol addiction: An enigma among us. *Neuron* 16(5): 909–912, 1996.

Tabakoff, B., and Ritzmann, R.F. Acute tolerance in inbred and selected lines of mice. *Drug Alcohol Depend* 4(1–2):87–90, 1979.

Tanda, G.; Pontieri, F.E.; and Di Chiara, G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. *Science* 276(5231): 2048–2050, 1997.

Trevisan, L.; Fitzgerald, L.W.; Brose, N.; Gasic, G.P.; Heinemann, S.F.; Duman, R.S.; and Nestler, E.J. Chronic ingestion of ethanol up-regulates NMDA-R1 receptor subunit immunoreactivity in rat hippocampus. *J Neurochem* 62(4):1635–1638, 1994.

Tsai, G.; Gastfriend, D.R.; and Coyle, J.T. The glutametergic basis of human alcoholism. *Am J Psychiatry* 152(3):332–340, 1995.

Ulrichsen, J.; Bech, B.; Allerup, P.; and Hemmingsen, R. Diazepam prevents progression of kindled alcohol withdrawal behaviour. *Psychopharmacology* 121(4):451–460, 1995.

Volpicelli, J.R.; Alterman, A.I.; Hayashida, M.; and O'Brien, C.P. Naltrexone in the treatment of alcohol dependence. *Arch Gen Psychiatry* 49(11): 876–880, 1992.

Volpicelli, J.R.; Davis, M.A.; and Olgin, J.E. Naltrexone blocks the post-shock increase of ethanol consumption. *Life Sci* 38(9):841–847, 1986.

Watson, W.P., and Little, J.J. Effects of dihydropyridines on the components of the ethanol withdrawal syndrome: Possible evidence for involvement of potassium, as well as calcium? *Alcohol Clin Exp Res* 21(3):409–416, 1997.

Weiss, F.; Hurd, Y.L.; Ungerstedt, U.; Markou, A.; Plotsky, P.M.; and Koob, G.F. Neurochemical correlates of cocaine and ethanol self-administration. In: Kalivas, P.W., and Samson, H.H., eds. *Annals of the New York Academy of Sciences. Vol. 654. The Neurobiology of Drug and Alcohol Addiction.* New York, NY: New York Academy of Sciences, 1992. pp. 220–241.

Weiss, F.; Parsons, L.H.; Schulteis, G.; Hyytia, P.; Lorang, M.T.; Bloom, F.E.; and Koob, G.F. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *J Neurosci* 16(10):3474–3485, 1996.

White, F.J., and Wolf, M.E. Psychomotor stimulants. In: Pratt, J.A., ed. *The Biological Bases of Drug Tolerance and Dependence.* London, UK: Academic Press, 1991. pp. 153–197.

Widdowson, P.S., and Holman, R.B. Ethanol-induced increase in endogenous dopamine release may involve endogenous opiates. *J Neurochem* 59(1):157–163, 1992.

Widnell, K.L.; Self, D.W.; Lane, S.B.; Russell, D.S.; Vaidya, V.A.; Miserendino, M.J.; Rubin, C.S.; Duman, R.S.; and Nestler, E.J. Regulation of CREB expression: In vivo evidence for a functional role in morphine action in the nucleus accumbens. *J Pharmacol Exp Ther* 276(1): 306–315, 1996.

Wikler, A. Dynamics of drug dependence: Implications of a conditioning theory for research and treatment. *Arch Gen Psychiatry* 28(5):611–616, 1973.

Wise, R.A. The neurobiology of craving: Implications for the understanding and treatment of addiction. *J Abnorm Psychol* 97(2):118–132, 1988.

Wise, R.A., and Leeb, K. Psychomotor-stimulant sensitization: A unitary phenomenon? *Behav Pharmacol* 4:339–349, 1993.

Wolffgramm, J., and Heyne, A. From controlled drug intake to loss of control: The irreversible development of drug addiction in the rat. *Behav Brain Res* 70(1):77–94, 1995.

Young, A.M., and Goudie, A.J. Adaptive processes regulating tolerance to the behavioral effects of drugs. In: Bloom, F.E., and Kupfer, D.J., eds. *Psychopharmacology: The Fourth Generation of Progress.* New York, NY: Raven Press, 1995. pp. 733–742.

Zeise, M.L.; Kasparov, S.; Capogna, M.; and Zieglgansberger, W. Acamprosate (calcium acetylhomotaurinate) decreases postsynaptic potentials in the rat neocortex: Possible involvement of excitatory amino acid receptors. *Eur J Pharmacol* 231(1):47–52, 1993.