

Alcohol Reinforcement and Voluntary Ethanol Consumption

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This article represents the proceedings of a symposium at the 2000 ISBRA Meeting in Yokohama, Japan. The organizer/chair was Ting-Kai Li and the co-chair was Rainer Spanagel. The presentations were (1) Genetic differences in alcohol drinking and reinforcement: The sP and sNP Rats, by Giancarlo Colombo; (2) Ventral tegmental area—Neuroanatomical substrate for alcohol reinforcement, by William J. McBride; (3) Metabolic mapping of alcohol reinforcement, by Linda J. Porrino; (4) Role of opioid receptors in the ethanol-induced place preference in rats exposed to conditioned fear stress, by Tsutomu Suzuki; and (5) Repeated deprivations enhance the reinforcing properties of ethanol in alcohol preferring (P) rats, by Zachary A. Rodd-Henricks.

Key Words: Alcohol, Genetics, Ventral Tegmental Area, Opioid Receptors, Rat.

IT IS ACCEPTED widely that both genetic and environmental factors contribute to the risk for alcohol abuse and alcoholism. Research in population-based samples also has identified genetic and environmental components of variance in alcohol drinking behavior itself in the general population. Whereas shared environmental influences largely determine the onset of drinking, addictive genetic influences become increasingly important in explaining quantity and frequency of drinking in those who have initiated drinking (Viken et al., 1999). A significant research challenge is, therefore, elucidation of the nature of biological variation in alcohol-seeking behavior and the influence of environmental factors and drinking history on this behavior. For example, is alcohol inherently more reinforcing in some individuals than others? Does tolerance develop more rapidly and to a greater degree in some individuals than others? Are some individuals more susceptible to alcohol dependence and withdrawal than others? How do these phenotypes relate? What are the neurobiological

bases of these responses to ethanol? Because of the expense and inherent limitations of this kind of research in humans (and nonhuman primates), most of our knowledge comes from studies in rodent experimental animal models.

This symposium presented recent research findings that explored the genetic and neurobiological underpinnings of alcohol drinking behavior and alcohol reinforcement in selectively bred and common stock rat models.

Rats and mice, in the aggregate, do not like to drink aqueous solutions of ethanol in concentrations greater than 5% and do not attain blood alcohol concentrations perceived to be “intoxicating.” The question had been raised in the past whether it is possible to develop a laboratory-based animal model to study alcoholism (abnormal alcohol-seeking behavior) and the effects of neuroadaptive responses to alcohol on drinking behavior (Cicero, 1979). However, there is large within-species variation in alcohol preference (Richter and Campbell, 1940) and, beginning in the 1950s, investigators from Chile, Finland, the United States, and Canada successfully developed, through selective breeding, lines of rats that voluntarily would consume large amounts of alcohol. This genetic approach led to rat and mouse models for the study of alcoholism-related endophenotypes, namely, alcohol preference (alcohol seeking), acute sensitivity to alcohol (inherent neurosensitivity and within-session tolerance), and alcohol-withdrawal reactions (Browman et al., 2000; Crabbe and Li, 1995).

Among the pairs of rodent lines that were selected for high/low alcohol preference and consumption are the University of Chile B and A rats, Alko alcohol (AA) and Alko nonalcohol (ANA) rats, alcohol-preferring (P) and alcohol-nonpreferring (NP) rats, high-alcohol-drinking (HAD) and low-alcohol-drinking (LAD) rats, Sardinian alcohol-preferring (sP) and -nonpreferring (sNP) rats, and the

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high-alcohol-preferring and low-alcohol-preferring mice. The P rats have been characterized in great detail as an animal model with abnormally intense alcohol-drinking behavior (Li and McBride, 1995), and they have the following characteristics:

1. P rats voluntarily consume 5 to 8 g ethanol/kg body weight/day and attain blood alcohol concentrations of 50 to 200 mg% with free-choice drinking.
2. P rats work by bar-pressing to obtain the ethanol orally when food and water are freely available, demonstrating that ethanol is behaviorally reinforcing. In fact, although the P rats were selected by using 10% ethanol, concentrations of ethanol as high as 35% to 40% are as reinforcing (same amount of ethanol consumed in 24 hr) as 10% ethanol.
3. P rats consume ethanol for its pharmacological effects and not because of its taste, smell, or caloric properties. It has been shown that the P and NP rats react to the taste and smell of ethanol similarly and that P rats will self-administer ethanol intragastrically. They voluntarily drink the same or greater amounts of ethanol even in the presence of other highly palatable fluids and caloric sources. Finally, recent studies have shown that the P rats will self-administer, through operant responding, nanoliter quantities of ethanol in concentrations of 50 to 200 mg% directly into the posterior ventral tegmental area (VTA).
4. P rats develop with chronic free-choice drinking metabolic as well as physiological tolerance toward the motor-impairing effects of ethanol.
5. P rats develop physical dependence with chronic free-choice drinking.

In addition to these genetically developed animal models, experimental paradigms have been developed in non-selected rats to study ethanol-reinforced operant behavior. Common stock rats (e.g., Long-Evans and Wistar) can be trained to chronically self-administer ethanol after its initial presentation in a sweet-tasting solution and the gradual replacement of the sucrose or saccharin with ethanol (Samson, 1986). Moreover, rats that have been trained in this manner and then made dependent by ethanol-vapor inhalation or by ingestion of an ethanol-containing liquid diet increase their ethanol self-administration during the withdrawal period (Schulteis et al., 1996). Rats exposed chronically to ethanol as a reinforcing substance increase their consumption of ethanol after a period of abstinence (Sinclair and Senter, 1968). This has been termed the alcohol-deprivation effect (ADE). Both the selectively bred alcohol-preferring rats (McKinzie et al., 1998; Sinclair and Li, 1989) and common stock rats (Heyser et al., 1997; Spanagel et al., 1996) exposed chronically to ethanol exhibit the ADE after varying periods of abstinence. The ADE is being explored as an animal model of relapse-like drinking behavior. In particular, studying the ADE in long-term alcohol self-administering rats after repeated periods of

abstinence has been used as a measure of relapse, craving, and loss control (Spanagel and Höltter, 1999).

Neurochemical, neuroanatomical, and neuropharmacological studies have shown that innate differences exist between the selectively bred and high-alcohol-drinking and low-alcohol-drinking rodents in various central nervous system (CNS) limbic structures. Depending on the animal model under study, differences in the mesolimbic dopamine (DA) pathway and/or the serotonin (5-HT), opioid, and γ -aminobutyric acid (GABA) systems that regulate this pathway may underlie genetic vulnerability to abnormal alcohol-seeking behavior or abnormal drinking behavior developed through experimental manipulation of drinking history (McBride and Li, 1998). In addition, the glutamatergic system may play a role in the development of alcoholism (Tsai and Coyle, 1998). In particular, alcohol-induced changes in NMDA function may underlie relapse drinking in rats (Höltter et al., 2000).

The discussants in this symposium presented data on (1) the relationship of alcohol-preference to initial sensitivity in the sP and sNP rats (Dr. Colombo), (2) the VTA as a critical site for alcohol reinforcement and the involvement of the 5-HT₃ receptor in the action of ethanol at this site rich in DA neurons (Dr. McBride), (3) the importance of behavioral context on the brain regions whose activities are affected by ethanol (Dr. Porrino), (4) the role of μ -opioid receptor function in ethanol reinforcement (Suzuki), and (5) the enhancement of the reinforcing properties of ethanol by repeated cycles of drinking and deprivation (Rodd-Henricks).

GENETIC DIFFERENCES IN ALCOHOL DRINKING AND REINFORCEMENT: THE SP AND SNP RATS

Ciancarlo Colombo

sP and sNP rats have been bred selectively for opposite alcohol preference and consumption starting from a base population of Wistar rats and by using the selection procedure previously used in breeding AA and ANA rats in Helsinki, Finland, and the P and NP rats in Indianapolis, Indiana.

When tested under the standard home-cage, two-bottle, free-choice regimen between 10% ethanol solution and water, sP rats consume approximately 6 g/kg of ethanol daily and avoid water almost completely; ethanol voluntarily consumed by sP rats has been reported to induce pharmacological effects that include motor stimulation (Colombo et al., 1998) and anxiolysis (Colombo et al., 1995). In contrast, sNP rats consume less than 1 g/kg/day of ethanol and greatly prefer water over the ethanol solution.

Recent experiments aimed at investigating the ethanol drinking behavior of sP rats have led to the hypothesis that voluntary ethanol intake in sP rats is sustained by the search for specific pharmacological effects of ethanol and is regulated by a central set-point mechanism that promotes

and limits ethanol intake on the basis of perception of those effects.

For instance, analysis of the ethanol drinking pattern revealed that sP rats tended to distribute their daily ethanol consumption in three distinct binges, rather regularly separated over the dark phase of the light/dark cycle (Agabio et al., 1996). In correspondence with these drinking bouts, ethanol intake in some rats peaked up to 1.5 g/kg/hr and gave rise to blood ethanol levels (BELs) up to 100 mg%. This trimodal pattern of ethanol intake is consistent with the pharmacokinetic characteristics of ethanol and suggests the ability of sP rats to adjust both ethanol dose and administration timing over the daily period of activity. It appears that sP rats consume ethanol to achieve specific BELs: They temporarily stop drinking once such BELs are achieved and the corresponding psychotropic effects are perceived, and they repeat the behavior when the effects have waned.

The ability of sP rats to control the amount of alcohol consumed daily is confirmed by the results of two investigations that tested ethanol intake when (a) ethanol was offered at increasing concentrations (3–60%) (Lobina et al., 1997), and (b) the ethanol solution was rendered highly palatable by addition of 1 g/liter of saccharin (Agabio et al., 2000); under both experimental conditions, the daily ethanol consumption remained constant and regular, averaging the habitually monitored amount of 6 g/kg.

Interestingly, a recent preliminary investigation demonstrated that ethanol, at doses comparable to those usually consumed by sP rats in a single drinking episode, was capable of inducing conditioned place preference in sP but not in sNP rats (R. Ciccocioppo and M. Massi, unpublished data). These results, together with those of the present study, suggest that sP rats consume ethanol for its positively hedonic properties and regulate the amount of ethanol consumed daily; this capability is maintained even when concentration and taste of the ethanol solution are altered. These results support the hypothesis that ethanol drinking in sP rats is controlled by a hedonic set-point mechanism, based on the search of sP rats for specific pharmacological effects of ethanol.

A second line of experiments investigated whether sP and sNP rats would differ in terms of initial sensitivity to the motor-impairing and sedative-hypnotic effects of an acutely administered high dose of ethanol. This study was of some interest because recent clinical research has proposed that initial sensitivity to ethanol may be correlated negatively with levels of subsequent ethanol consumption (Schuckit, 1994; Schuckit and Smith, 1996; Schuckit et al., 2000).

Initial sensitivity to the sedative-hypnotic of ethanol was measured as the time to lose (onset) and recover (sleep time) the righting reflex after the acute, intraperitoneal administration of 3 and 3.5 g/kg of ethanol. At both ethanol doses, sP rats took a shorter time to lose the righting reflex than sNP rats; furthermore, ethanol-induced sleep time was

approximately 2-fold higher in sP than sNP rats. These results suggest that sP rats possess a genetically determined, higher initial sensitivity to the motor-impairing and sedative/hypnotic effects of ethanol than sNP rats. BELs at time of righting reflex recovery were significantly lower in sP than sNP rats, which suggested that the longer sleep time recorded in sP rats was not due to reduced rate of ethanol metabolism.

The higher sensitivity to the behavioral impairing effects of ethanol of sP compared with sNP rats subsequently was confirmed by an experiment that tested the ability of sP and sNP rats to perform a motor coordination task on the rotating drum of a roto-rod after the acute, intragastric administration of 2, 2.5 and 3 g/kg of ethanol. All the doses of ethanol induced virtually complete impairment in sP rats in terms of motor coordination on the drum; in contrast, in sNP rats only the highest dose of ethanol resulted in approximately 100% motor impairment, whereas 2 and 2.5 g/kg of ethanol induced no more than 40% and 70% impairment, respectively.

Initial sensitivity to depressant doses of ethanol has been reported to vary widely among the rat lines selectively bred for opposite ethanol preference and consumption. For instance, ethanol-preferring P rats were found to be less sensitive than ethanol-nonpreferring NP rats (Kurtz et al., 1996; Lumeng et al., 1982), whereas response to ethanol in the HAD and LAD rats was more similar to that observed in sP and sNP rats, with ethanol-preferring HAD rats being more sensitive to the motor impairing effects of ethanol than ethanol-nonpreferring LAD rats (Stewart et al., 1998). A possible explanation for this disparity may reside in the likely differences in the genetic makeup of these rat lines. Although these rat lines have been selected for the same phenotype (high ethanol preference and consumption), they have been differentiated for a number of genetically controlled neurochemical and behavioral traits, likely associated with the development of ethanol preference. These differences suggest that different genotypes are involved in the development of ethanol preference and that the contribution of each genotype may vary among these rat lines.

These differences apparently result in multiple forms of high ethanol preference in laboratory rats, which appear to reproduce the different types of alcoholics better than a theoretical single animal model. Thus, if ethanol-preferring, low sensitive P rats may represent a model for individuals with low initial intensity of response to ethanol and high likelihood of developing alcoholism (Schuckit, 1994; Schuckit and Smith, 1996; Schuckit et al., 2000), HAD and sP rats may constitute experimental models for other subsets of population at risk of alcoholism, such as those represented by sons of alcoholics who exhibit greater behavioral impairment and higher perception of intoxication after an ethanol challenge (Moss et al., 1989; Nagoshi and Wilson, 1987).

VENTRAL TEGMENTAL AREA—NEUROANATOMICAL
SUBSTRATE FOR ALCOHOL REINFORCEMENT

William J. McBride

The VTA has a key role in mediating alcohol drinking behavior and the rewarding effects of several drugs of abuse (Koob et al., 1998). Because activation of the mesolimbic DA system has been hypothesized to underlie reinforcement processes (Koob et al., 1998), the studies of Brodie et al. (1990, 1995), which indicated that local ethanol administration stimulated the firing rate of VTA DA neurons, suggested that ethanol might be reinforcing when administered directly into the VTA. The intracranial self-administration (ICSA) procedure was used to test the hypothesis that ethanol is reinforcing within the VTA. The VTA is an anatomically heterogeneous structure in the rat, and two ICSA studies indicated that there were anterior-posterior differences for the self-infusions of GABA_A agents within the VTA (Ikemoto et al., 1997, 1998). A role for 5-HT₃ receptors in mediating the excitatory actions of ethanol within the VTA was indicated by the microdialysis data of Campbell et al. (1996). Therefore, the objectives of this presentation are to review data on the (a) regional differences within the VTA for the reinforcing effects of ethanol, (b) involvement of local 5-HT₃ receptors in mediating the reinforcing actions of alcohol within the VTA, and (c) relationship between alcohol preference and the reinforcing effects of ethanol within the VTA.

Female Wistar P and NP rats that were alcohol and experimentally naïve were used in these studies. Animals were stereotaxically implanted under halothane anesthesia with a 22-gauge guide cannula, aimed at the area of interest, approximately 1 week before the ICSA experiment began. ICSA sessions were conducted in standard two-lever operant chambers (Coulbourn Instruments, Allentown, PA), and an electrolytic microinfusion transducer system was used to control infusion volumes, as previously described (Gatto et al., 1994; Rodd-Henricks et al., 2000b). For most experiments, sessions were 4 hr in duration and were conducted every other day for a total of seven sessions. A fixed-ratio one (FR-1) schedule of reinforcement was used. The numbers of reinforcers and responses on the active and inactive levers were recorded. At the termination of the experiment, 1% bromophenol blue was injected into the infusion site.

The anterior VTA was defined as the region at the level of the mammillary nuclei, 4.8 to 5.2 mm posterior to bregma, and the posterior VTA as the region at the level of the interpeduncular nucleus at 5.3 to 6.3 mm posterior to bregma. For Wistar rats with placements in the posterior VTA, there was a significant ethanol dose effect [$F(7,62) = 3.5$; $p = 0.003$ (between-subjects design)], whereas with placements in the anterior VTA, there was no significant effect of ethanol [$F(2,18) = 0.2$; $p = 0.8$]. Post hoc comparisons indicated that Wistar rats received significantly more infusions of 150 to 400 mg% ethanol than artificial

cerebrospinal fluid (aCSF) in the posterior VTA (15–25 infusions/session for ethanol solutions vs. 6 infusions/session for aCSF). In addition, these rats responded significantly more on the active than inactive lever for ethanol (60 ± 11 active lever responses/session vs. 25 ± 5 inactive lever responses/session; $p < 0.05$). In the anterior VTA, the number of infusions of 200 and 400 mg% ethanol was low and similar to the number of infusions of aCSF (7 ± 2 for ethanol vs. 6 ± 2 infusions/session for aCSF).

The involvement of 5-HT₃ receptors in mediating the reinforcing effects of ethanol was examined by comparing the effects of coinfusion of a 5-HT₃ antagonist on the ICSA of 200 mg% ethanol into the posterior VTA of Wistar rats. For this experiment, four groups of rats ($n = 6$ –9/group) were given either (a) aCSF for all seven sessions; (b) 100 μ M ICS 205–930 (ICS), a 5-HT₃ antagonist, for all seven sessions; (c) 200 mg% ethanol for all seven sessions; or (d) 200 mg% ethanol for first four sessions, 200 mg% ethanol plus 100 μ M ICS for the next two sessions, followed by 200 mg% ethanol for session 7. Wistar rats given aCSF or ICS had similar low rates of reinforcements throughout (8 ± 3 infusions/session). Rats that received 200 mg% ethanol received significantly higher infusions (36 ± 5 infusions/session) compared with the aCSF or ICS group. However, when ICS was coinfused with ethanol during sessions 5 and 6, there was a significant ($p < 0.05$) reduction in the number of infusions (11 ± 4 infusions/session), which partially recovered to baseline in session 7 (22 ± 4 infusions/session) when 200 mg% ethanol alone was given.

Two separate experiments were conducted to examine the relationship between the reinforcing effects of ethanol in the VTA and alcohol preference. In the first experiment, P and NP rats were given 25, 50, 100, 150, and 200 mg% ethanol in ascending order with each concentration being presented for three consecutive sessions. P rats readily responded on the active lever for all five concentrations of ethanol (95–275 responses/session) and responded significantly more on the active lever than did NP rats at each ethanol concentration (6–18 responses/session). In the second experiment, separate groups ($n = 3$ –5/group) of P and Wistar rats were given either aCSF or 50, 75, 100, 150, or 200 mg% ethanol to self-infuse. P rats self-infused more of each ethanol solution (12 ± 3 , 20 ± 3 , 30 ± 3 , 27 ± 3 , and 28 ± 2 infusions/session for 50, 75, 100, 150, or 200 mg% ethanol, respectively) than aCSF (5 ± 2 infusions/session), whereas Wistar rats self-administered more of only the 150 (15 ± 3 infusions/session) and 200 mg% (24 ± 3 infusions/session) solutions than aCSF (5 ± 1 infusions/session).

The present results suggest that the posterior VTA supports the reinforcing effects of ethanol whereas the anterior VTA does not; that 5-HT₃ receptors are involved in mediating the reinforcing effects of ethanol within the posterior VTA; and that there is an association between alcohol preference and the reinforcing actions of ethanol within the VTA.

The differences between the anterior and posterior VTA

with regard to the self-infusion of ethanol may indicate different neuronal circuitry within the two VTA subregions, different receptor subunits that differentially react to ethanol within the two VTA subregions, and/or different functional interrelationships with other regions.

The present results suggest that 5-HT₃ receptors may be involved in mediating the reinforcing actions of ethanol within the posterior VTA. If activating 5-HT₃ receptors is a main mechanism that underlies the stimulating effects of ethanol on somatodendritic DA release and the reinforcing actions of ethanol within the VTA, it is possible that 5-HT₃ receptors could be mainly located in the posterior VTA rather than in the anterior VTA, and/or their cellular location would activate DA neurons in the posterior VTA but not anterior VTA.

The finding that the VTA of the P line of rats is more sensitive to the reinforcing effects of ethanol than is the VTA of NP and Wistar rats supports the hypothesis that there is an association between alcohol preference and sensitivity to the reinforcing actions of alcohol in the VTA. Moreover, this finding suggests that the CNS reinforcing actions of ethanol play an important part in initiating and maintaining the high-alcohol-drinking behavior of the P line.

METABOLIC MAPPING OF ALCOHOL REINFORCEMENT

Linda J. Porrino

One important factor in determining the effects of any psychoactive drug is the context of its presentation. Numerous studies have shown that self-administered stimuli have different functional consequences than stimuli administered noncontingently by an experimenter (Dworkin et al., 1992; Porrino et al., 1984). More particularly, to study the reinforcing effects of a drug, self-administration paradigms are necessary.

In early studies that employed metabolic mapping methods to identify the neuroanatomical substrates of ethanol in brain, the effects of voluntary ethanol consumption were determined (Porrino et al., 1998). In these studies, rats were trained to consume ethanol by using a modified sucrose-fading procedure, and the effects of the voluntary consumption of a single dose of ethanol (0.5 g/kg) were measured. These studies identified a circuit of functional activation that included portions of the mesocorticolimbic system that is associated with drinking at this dose. Ethanol ingestion increased cerebral metabolism, as compared with rates of metabolism in rats that consumed either water or sucrose, in the rostral pole and shell of the nucleus accumbens, medial prefrontal cortex, lateral septum, basolateral and central nuclei of the amygdala, substantia nigra, and VTA. This pattern was quite distinct from the one associated with the noncontingent administration of ethanol at the same dose (Williams-Hemby and Porrino, 1994). These effects were localized to portions of the hippocampus, the anterior cingulate, and the core of the nucleus accumbens.

Clearly then, the anatomic substrates involved in mediating the effects of the self-administration of ethanol are different from those associated with the noncontingent administration. These data suggest that the circuitry that underlies the reinforcing effects of ethanol cannot be modeled by evaluating the noncontingent administration of the same dose of ethanol. Furthermore, they confirm the importance of mesocorticolimbic circuitry in mediation of the reinforcing effects of ethanol.

There are several other important findings of this study (Porrino et al., 1998) of ethanol self-administration. First, these results emphasize the involvement of brain regions, heretofore not considered, in the self-administration of ethanol. Although previous studies have demonstrated a role for the nucleus accumbens, medial prefrontal cortex, and VTA, as well as the amygdala, these results demonstrate a role for the lateral septum, substantia nigra, and specifically the basolateral and central amygdala. Second, they indicate that the increases in functional activity associated with ethanol self-administration are more robust in the rostral pole and shell than the core of the nucleus accumbens. And finally, these studies show that the simultaneous activation of an interconnected network of limbic brain regions serves as the substrate of the effects of voluntarily ingested ethanol.

One characteristic of the effects of ethanol is that they are dose-dependent. The acute administration of low and moderate doses of ethanol has a very different impact on rates of cerebral metabolism. The acute administration of a low dose such as 0.25 g/kg significantly increases functional activity, as compared with vehicle-treated controls, in structures of the mesocorticolimbic and nigrostriatal dopaminergic systems. In contrast, the administration of a moderate dose such as 1.0 g/kg of ethanol produces widespread decreases in rates of glucose utilization in brain regions involved in processing of sensory and motor information, as well as in portions of the limbic system. Within the limbic system, effects were centered in the hippocampal formation and structures related to memory acquisition and expression. Because the acute noncontingent administration of low and moderate doses of ethanol produces distinct non-overlapping patterns of functional activation (Williams-Hemby and Porrino, 1994), one important question is whether the effects of self-administered ethanol are similarly dose-dependent. The purpose of recent work was to determine if the effects of different doses of self-administered ethanol are mediated by distinct circuits as with noncontingent ethanol administration or if there are common circuits activated at all doses. In these studies, rats were allowed to drink freely just before mapping. Consumption ranged from 0.14 to 0.88 g/kg in a 15 min session. Ethanol self-administration at these doses increased functional activity in the medial prefrontal cortex, rostral nucleus accumbens, caudate, lateral septum, central amygdala, bed nucleus of stria terminalis, substantia nigra compacta, and VTA. These brain regions are similar to

those that were affected by consumption of 0.5 g/kg observed in previous studies. Activation in the medial prefrontal cortex, bed nucleus of the stria terminalis, and olfactory tubercle was present in all animals that had self-administered ethanol regardless of the dose consumed. In other structures, which included the substantia nigra, VTA, basolateral amygdala, and hippocampus, rates of glucose utilization were correlated significantly with the dose of ethanol consumed. In these areas, therefore, a strict dose-effect relationship was evident. Regardless of whether these effects on functional activity were dose-related or unrelated to dose, there were clear differences between the effects of noncontingent administration of ethanol and self-administered ethanol, which again emphasizes the importance of behavioral context in determining the functional consequences of ethanol.

CONDITIONED FEAR STRESS POTENTIATES ETHANOL-
ASSOCIATED PLACE PREFERENCE THROUGH
 μ -OPIOID RECEPTOR

Tsutomu Suzuki

It has been postulated that the interaction between stress and ethanol intake may play an important role in the etiology of alcoholism (Pohorecky, 1981); ethanol intake by humans increases under conditions of psychological stress. Similarly, it has been reported that rats exposed to various types of stress, such as electric foot shock stress (Caplan and Puglisi, 1986; Wolffgramm, 1990), show increased ethanol intake. Recently, we demonstrated that conditioned fear stress, a psychological stress (Conti et al., 1990), plays an important role in the development of the rewarding effect of ethanol by using the conditioned place preference paradigm (Matsuzawa et al., 1998).

There is much evidence that the endogenous opioid system plays a critical role in the rewarding effect of ethanol through the release of opioid peptides in the mesolimbic dopamine system (Froehlich et al., 1990; Gianoulakis, 1996; Herz, 1997; Hubbell et al., 1986). The activation of the μ -opioid receptor systems by morphine and its rewarding effect can be suppressed by the selective μ -opioid receptor antagonist β -funaltrexamine (Funada et al., 1993; Suzuki et al., 1993). Therefore, it is hypothesized that the mechanism of the rewarding effect of ethanol may be linked closely to the activation of μ -opioid receptor in the mesolimbic dopamine system. In the present study, to clarify this hypothesis, we examined the involvement of the endogenous opioid system, especially μ -opioid receptor, in the development of the rewarding effect of ethanol under psychological stress in comparison with morphine reward.

Male Sprague Dawley® rats first were habituated to a shuttle box subsequently used to test conditioned place preference. The animals then were subjected to intermittent foot shock on four occasions. Twenty-four hours after the foot shock, the animals were placed in the same shock chamber for 10 min but without foot shock (conditioned

fear stress [CFS]). All animals then were injected immediately with drug (ethanol or morphine) or with saline and were confined for 30 min to the nonpreferred side of the shuttle box after injection of drug and to the preferred side after saline injection on alternate days. Subsequently, the animals were tested for conditioned place preference by scoring the time spent in the preferred and nonpreferred compartments during a 15 min session.

Ethanol (150 and 300 mg/kg intraperitoneally) failed to produce significant place preference in non-CFS rats. By contrast, the same doses produced significant ($p < 0.05$) place preference in the CFS rats. Morphine at a dose of 3 mg/kg, but not 1 mg/kg subcutaneously, produced significant ($p < 0.05$) place preference in non-CFS rats. In CFS rats, morphine produced place preference at both dose levels. Naloxone (3 mg/kg subcutaneously) and β -funaltrexamine (10 mg/kg intraperitoneally) significantly attenuated the ethanol (300 mg/kg intraperitoneally)-induced as well as the morphine (3 mg/kg subcutaneously)-induced place preference in the CFS rats. These findings are consistent with our previous report that conditioned fear stress induces ethanol-associated place preference (Matsuzawa et al., 1998). Moreover, these findings suggest that psychological stress also potentiates the development of the rewarding effects of ethanol and morphine.

There is evidence that the mesolimbic dopamine system, which contains key components of the reward pathway (VTA [cell body] to nucleus accumbens [nerve terminal]), plays a critical role in the rewarding effects of drugs of abuse. It is well known that an increase in dopamine release in the nucleus accumbens after activation of the mesolimbic dopamine system is a key mechanism in the development of the rewarding effects of drugs of abuse (Koob, 1992). Microdialysis studies have shown that both ethanol and morphine increase extracellular dopamine concentrations in the rat nucleus accumbens and activate the mesolimbic dopamine system (Bassareo et al., 1996; Di Chiara and Imperato, 1988). Conditioned fear stress also increases dopamine release in the mesolimbic dopamine system, which activates the dopamine pathway (Deutch et al., 1985; Inoue et al., 1994). Furthermore, ethanol stimulates the release of β -endorphin and enkephalins in the mesolimbic dopamine system, and conditioned fear stress stimulates the release of endogenous opioid peptides (Gianoulakis, 1989; Nabeshima et al., 1992). Thus, activation of the mesolimbic dopamine system by way of the endogenous opioid system is particularly important in the rewarding effects of several drugs of abuse, which include ethanol and morphine (Herz, 1997).

The present study also showed that ethanol- and morphine-induced place preferences were attenuated significantly by the nonselective opioid receptor antagonist naloxone and a selective μ -opioid receptor antagonist β -funaltrexamine. These findings suggest that the μ -opioid receptor may be involved in the rewarding effects of ethanol and morphine under psychological stress. Indeed, acti-

vation of μ -opioid receptor produces a rewarding effect (place preference) through activation of the mesolimbic dopamine system.

In conclusion, the data presented here suggest that psychological stress may act as an important motivating factor in the development of the rewarding effects of ethanol and morphine. Furthermore, the endogenous opioid system (especially μ -opioid receptor) may constitute one of the functional neurochemical links that underlie the rewarding effect of ethanol especially during psychological stress.

REPEATED DEPRIVATIONS ENHANCE THE REINFORCING PROPERTIES OF ETHANOL IN ALCOHOL-PREFERRING (P) RATS

Zachary A. Rodd-Henricks

The ADE is defined as a temporary increase in the ratio of ethanol/total fluid intake and voluntary intake of ethanol solutions over baseline drinking conditions, when ethanol is reinstated after a period of alcohol deprivation. The ADE is a robust phenomenon observed in rats (McKinzie et al., 1998) and humans (Mello and Mendelson, 1972). Although the underlying physiological basis that mediates the ADE is unknown, it has been suggested that the ADE reflects craving for alcohol and contributes to alcohol relapse (Sinclair and Li, 1989).

The ADE phenomenon has been examined to some extent in the P rat. Adult male P rats exposed to 10% (v/v) ethanol under continuous access displayed a robust ADE on ethanol reexposure (Rodd-Henricks et al., 2000a; Sinclair and Li, 1989). Under 4 hr operant conditions, adult P males exhibited an increase in responding for ethanol compared with baseline responding after 2 weeks of ethanol (McKinzie et al., 1998). Furthermore, repeated deprivations prolonged and/or enhanced the expression of the ADE and shifted drinking preferences for solution with higher ethanol concentration in P rats (Rodd-Henricks et al., 2000a,d). The current study was conducted to examine the effects of repeated deprivations on operant responding in P rats. Additionally, the current study quantitatively assessed the effects of repeated deprivations on the reinforcing properties of ethanol by observing operant responding for ethanol under a progressive ratio procedure.

Adult, male P rats (45th generation; $n = 30$) were subjected to 48 hr of forced ethanol in their home cages (10% v/v) followed by 2 weeks of 24 hr free-choice and 3 weeks of 4 hr access. Water was always available in the home cage. Operant testing was conducted in standard two-lever operant chambers. The levers activated a dipper cup (0.1 ml) that rose through a trough to deliver response-contingent fluids. All operant testing was conducted in the dark phase of the animal's cycle. During the last 2 weeks of 4 hr access to ethanol, rats were trained to respond for saccharin or water (two levers) on an FR-1 schedule during a 1 hr session. Rats then were placed on an FR-1 schedule for water and 15% v/v ethanol. The FR schedule for ethanol

was gradually increased to FR-5. The rats were maintained on the 1 hr sessions for 6 weeks. Levers were counterbalanced among rats but were constant for each animal. During this 6 week period, ethanol was available only during the 1 hr operant session. Baseline values were the average of the last three sessions before deprivation. Rats were assigned randomly to one of four groups that were deprived for 0, 2, 5, or 8 weeks. During deprivation, rats were not placed in the operant chambers. After deprivation, rats were given access to ethanol for 2 weeks during 1-hr operant sessions every day. After this period, all previously deprived rats were again deprived for 2 weeks. This cycle of 2 weeks of ethanol access and 2 weeks of ethanol deprivation was repeated for a total of four deprivations. After the third deprivation period, deprived rats were allowed access to the 1 hr operant ethanol sessions for an additional 2 weeks and then were deprived again for a fourth time. After the fourth deprivation period, all rats were exposed to a modified progressive ratio procedure. An arithmetic series that started at FR-2 was incremented by two following three reinforcers at each level. Breakpoint was defined as a 7 min period without a reinforcer.

Baseline ethanol lever responding before the deprivation did not differ between groups [ethanol range of responses/session: 296 ± 44 to 309 ± 44 ; $F(3,28) = 0.3, p = 0.9$]. After the first deprivation period, only P rats deprived for 5 weeks displayed an increase in responding (462 ± 42) during the initial reinstatement session. Repeated deprivations increased the number of ethanol lever responses and reinforcers received [$F(12,126) = 11.3, p < 0.00001$] during the initial reinstatement session (588 ± 53 and 638 ± 71 , second and third deprivation, respectively) and prolonged this increase into the third and fourth reinstatement sessions. Additionally, all groups of deprived rats displayed a higher breakpoint ratio than nondeprived controls (32 ± 3 vs. 17 ± 2 ; $p < 0.01$).

In agreement with previous reports, the present findings indicate that the ADE is a robust phenomenon that readily can be observed under operant paradigms after a single deprivation (Heyser et al., 1998, McKinzie et al., 1998). The present results, with operant conditions, extend and confirm the previous findings that repeated deprivations can enhance and prolong the ADE (Rodd-Henricks et al., 2000a,d). Additionally, repeated deprivations increased the breakpoint to termination of responding under the progressive ratio component of the experiment, which suggested that repeated deprivations increased the reinforcing properties of ethanol.

SIGNIFICANCE

The sP rats have been selectively bred for high alcohol consumption and preference for over 20 years. Here, Dr. Colombo describes the response to an acute alcohol injection of sP and sNP rats. Studies in humans have shown that a low level of response (LR) or relative insensitivity to the

acute effects of alcohol predicts alcohol abuse and dependence (Schuckit and Smith, 1996). Nonalcoholic sons and daughters of alcoholic probands, identified from the Collaborative Study on the Genetics of Alcoholics (COGA) study, represent a genetically vulnerable, high-risk group of subjects and exhibit low LR on oral ethanol challenge (Schuckit et al., 2000). These observations suggest a genetic correlation between LR and alcohol-drinking behavior that can be tested in the rat lines selected for high and low alcohol consumption. Previous studies that compared P and NP rats found such a relationship; namely, LR was inversely related to alcohol preference. The relationship was observed also in the F2 cross of the P and NP rats, which suggested that the association indeed might be genetic. An inverse correlation between LR and alcohol preference also has been observed in heterogeneous stock mice and rats and in the AA and ANA rats. However, as here reported, the sP rats are actually more sensitive to CNS-depressant doses of ethanol as measured by the loss of righting reflex and motor impairment on the roto-rod. A recent report indicates that the replicate HAD and LAD lines of rats also behave differently in relative responses to the acute effects of ethanol (Stewart et al., 2000). Only the HAD1/LAD1 comparison was similar to the P/NP findings, but the HAD2/LAD2 lines did not differ in tolerance development. Thus, the acute response of ethanol-naïve animals to a first-ever dose of ethanol does not correlate uniquely with high voluntary ethanol consumption. As Dr. Colombo pointed out, the disparate findings may be due to the different genetic backgrounds of the selected lines; however, the HAD/LAD replicate lines are from the same genetic background. Another possible explanation may be drinking history. Because the human subjects studied by Schuckit and coworkers were not ethanol-naïve, it would be important to examine how LR relates to alcohol preference in alcohol-experienced animals from the different selected pairs of high- and low-alcohol-drinking lines.

Dr. McBride showed that ethanol is reinforcing at lower concentrations to the P rats than to Wistar and NP rats, as demonstrated by the dose-response of ethanol operantly self-administered directly into the posterior VTA. It is interesting that many of the alcohol-preferring rat lines exhibit locomotor activation with low doses of ethanol (Rodd-Henricks et al., 2000c). Locomotor activation is considered by some investigators to reflect the reinforcing effects of drugs, which include ethanol. It would be of interest to examine whether other ethanol-preferring lines are also more sensitive than their nonpreferring counterparts in intracranial ethanol self-administration.

The studies reported by Drs. McBride, Porrino, and Suzuki emphasize that, although the mesolimbic dopaminergic pathway serves as perhaps a common final pathway for ethanol reward (as it does for other substances of abuse such as cocaine, amphetamine opioids, and nicotine), self-administered alcohol also activates certain corticomesolimbic and nigrostriatal regions of brain that are not involved

if ethanol is administered noncontingently. This anatomic pattern of response is consistent with neurochemical/neuropharmacological findings that activation and inhibition of the mesolimbic dopaminergic pathway by ethanol involve multiple neurotransmitter systems, which include the 5-HT, GABA, glutamate, and opioid systems. The pursuit of the neurocircuitry of ethanol reinforcement that integrates anatomic, electrophysiological, neurochemical, pharmacological, and molecular biological approaches of discovery is key to our understanding of the nature of alcohol abuse and dependence. Furthermore, the use of the electrolytic microinfusion transducer system (described by Dr. McBride) will allow researchers to microinfuse ethanol in the nanoliter range into defined brain areas to further study putative brain sites involved in ethanol reinforcement.

One of the domains of alcohol dependence most refractory to experimental dissection in animal models has been loss of control and relapse to drinking. Recently, a couple of experimental paradigms have offered promising results: one is the extinction of reinforced responding, followed by subsequent reinstatement of responding by stress (Lê et al., 1998). In this regard, the report by Dr. Suzuki of the role of stress in the development of a liking for ethanol as demonstrated by ethanol-conditioned place preference is instructive of an opioid-receptor indicating mechanism. The other paradigm is the ADE and the effect of repeated bouts of ADE on drinking. The studies presented by Dr. Rodd-Henricks demonstrate the dramatic effect of repeated deprivation episodes on increasing the amount of ethanol consumed and the duration of the heightened ethanol consumption. Furthermore, preliminary operant studies demonstrate that repeated deprivations increase the reinforcing efficacy of ethanol. These data are in line with other findings derived from a new rat model of long-term alcohol self-administration with repeated deprivation periods (Spanagel and Höltter, 1999). In this model, the motivation to work for alcohol was tested under various progressive ratio schedules. Under all conditions, the breaking point was significantly higher after alcohol deprivation than during baseline drinking, and repeated alcohol deprivation periods further increased the motivation to drink ethanol (Spanagel and Höltter, 2000). These experimental designs provide a means to elucidating the neurobiological substrates that underlie loss of control drinking and relapse that would lead to the rational design of medications to treat alcoholism and prevent relapse.

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