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Phenotypic and Genotypic Characterization of the Indiana University Rat Lines Selectively Bred for High and Low Alcohol Preference

James M. Murphy,[1,3,6] Robert B. Stewart,[1] Richard L. Bell,[3] Nancy E. Badia-Elder,[1] Lucinda G. Carr,[2,5] William J. McBride,[3,4] Lawrence Lumeng,[2,4] and Ting-Kai Li[2,4]

The Indiana lines of selected rats, the HAD and LAD replicates and the P and NP lines, were bred for high and low alcohol preference. The P and HAD lines have met criteria for an animal model of alcoholism in that they voluntarily consume sufficient ethanol to achieve significant blood alcohol concentrations, and their alcohol-seeking behavior is reinforced by the pharmacological effects of ethanol rather than its taste, caloric content, or other properties. These lines have been characterized extensively for associated behavioral and physiological phenotypes. The P and HAD rats show an enhanced responsiveness to the stimulatory effects of ethanol and reduced sensitivity to the aversive sedative effects of ethanol. Consistent findings with the selected lines include differences in the mesolimbic dopamine reward system, as well as differences in serotonin, GABA, endogenous opioid, and neuropeptide Y systems. Genetic mapping studies have identified quantitative trait loci influencing alcohol preference on chromosomes 3, 4, and 8 in the inbred P/NP rats and on chromosomes 5, 10, 12, and 16 in the noninbred HAD1/LAD1 rats. The elucidation of the genotypes and phenotypes that result in excessive alcohol intake may lead to a better understanding of alcohol abuse and alcoholism and could guide strategies for potential treatment and prevention.

KEY WORDS: Alcohol-preferring P rats; alcohol-nonpreferring NP rats; high-alcohol-drinking (HAD) rats; low-alcohol-drinking (LAD) rats; selective breeding; phenotypes.

INTRODUCTION

There is widespread variation in alcohol consumption, suggesting that multiple factors influence drinking, including environmental and hereditary variables. The observation that people with very similar environmental backgrounds often differ considerably in alcohol consumption has lead to the hypothesis that heredity contributes to behaviors that result in some individuals drinking excessively (Cotton, 1979; Schuckit, 1986). Although this idea has been entertained for many years, scientific inquiry into inherited predispositions for alcohol abuse has not been seriously pursued until the last few decades. During the mid-1900s, correlational studies of human populations were published on the familial incidence of alcohol abuse and alcoholism (see Cotton, 1979). Concurrently, experimental investigations using animal models of genetic influences on alcohol drinking and the consequences of alcohol ingestion were also beginning to gain recognition. These two complementary avenues of investigation were instrumental in advancing an understanding of how

[1] Department of Psychology, Purdue School of Science.
[2] Department of Medicine.
[3] Department of Psychiatry.
[4] Department of Biochemistry and Molecular Biology.
[5] Department of Pharmacology, Indiana University School of Medicine, Indiana University–Purdue University Indianapolis, Indianapolis, Indiana.
[6] To whom correspondence should be addressed at Department of Psychology, LD 124, Purdue School of Science, IUPUI, 402 North Blackford Street, Indianapolis, IN 46202-1375 USA. Tel: (317) 274-6762. Fax: (317) 274-6756. e-mail: jmurphy1@iupui.edu

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hereditary differences may account for a portion of the variance influencing the development of alcohol abuse and alcoholism. Among the diverse approaches used to examine the contribution of heredity and its interaction with environmental variables on alcohol-related behaviors, the present review focuses on recent findings from one program that has investigated this question through the use of rodent models.

Development of the Models

The Indiana selection studies have been carried out to obtain lines of rats that differ widely in their preferences for ethanol solutions. The objectives were to establish high and low ethanol-drinking lines that could serve as reliable and economical models for assessing genetic predispositions to the disparate extremes of alcohol use found in human populations. Selective breeding of high vs. low ethanol-consuming animals from a heterogeneous population is accomplished through systematic mating of animals from the same extreme of the normal distribution over successive generations to obtain divergent lines that exhibit the extremes of ethanol preference. In this manner, the selectively bred lines have been developed to display high or low ethanol-drinking phenotypes based solely on selection history, without requisite environmental manipulations to induce ethanol preference or nonpreference. The selectively bred rodent lines are presumed to possess a high frequency of genes that influence the desired phenotype, while trait-irrelevant genes hypothetically remain randomly distributed. Selected lines have been used in alcohol research since the pioneering work of Williams *et al.* (1949) and Mardones and Segovia-Riquelme (1983), who demonstrated a genetic influence on ethanol self-administration in rodents through selective breeding and characterization of the selectively bred UChA (low alcohol-consuming) and UChB (high alcohol-consuming) rats. Inbred strains also have been observed to possess wide differences in ethanol preference. For example, C-57 mice display high ethanol preference, whereas DBA mice display low ethanol preference (e.g., McClearn and Rodgers, 1959, 1961). However, because inbreeding also results in fixation of trait-irrelevant genes, inbred strains may not be the best models to examine correlated traits and responses (Eriksson, 1968; Crabbe, 1989). Thus, selective bi-directional breeding for ethanol preference vs. nonpreference may serve as a more effective research tool for examining genetic factors affecting excessive ethanol intake.

To date, five separate sets of alcohol-preferring and -nonpreferring lines of rats have been developed through selective breeding programs. Three sets include the ALKO alcohol/nonalcohol (AA/ANA) lines (Eriksson, 1968), the University of Chile A and B (UChA/UchB) lines (Mardones and Segovia-Riquelme, 1983), and the Sardinian alcohol-preferring/nonpreferring (sP/sNP) lines (Colombo, 1997). Two other sets are the Indiana lines, which include the alcohol-preferring/nonpreferring (P/NP) lines (Lumeng *et al.*, 1977), and high/low alcohol-drinking (HAD/LAD) replicate lines, designated HAD_1/LAD_1 and HAD_2/LAD_2 (Li *et al.*, 1993). Although the selection criteria have differed somewhat among the various sets of lines, ethanol preference or nonpreference is defined in terms of a benchmark ethanol intake expressed in grams of absolute ethanol/kg body weight/day consumed by animals that have continuous access to an aqueous ethanol solution in the presence of *ad libitum* food and water. A second, often used, criterion measure is the percentage or ratio of ethanol intake relative to total fluid consumed, which eliminates animals with high intakes resulting from anomalous ingestive behaviors, such as polydipsia. For the Indiana lines, the general selection criteria have been that the P and HAD lines, when given free access to food, water, and a 10% (v/v) ethanol solution, should consume at least 5.0 g ethanol/kg/day with an ethanol to water ratio of at least 2:1, whereas the NP and LAD lines should drink less than 1.5 g/kg/day with an ethanol to water ratio of less than 0.5:1. At present, P/NP breeding is beyond the 50th generation, with P rats averaging approximately 6.5 g ethanol/kg/day and NP rats consuming approximately 0.5 g/kg/day. HAD/LAD breeding is now around the 35th generation, with HAD rats consuming approximately 9.5 g ethanol/kg/day and LAD rats consuming approximately 0.5 g/kg/day.

The P/NP and HAD/LAD lines have been bred from different foundation stocks in separate breeding programs, and, in the case of the HAD and LAD lines, independent replicates have been maintained. Thus, it is presumed that spurious fixations of genes unrelated to ethanol preference are unlikely to emerge in common for the separate breeding programs, whereas genes yielding phenotypes that are necessary and/or sufficient to manifest high or low ethanol preference should consistently result as a consequence of selection pressure among the different lines. The P and NP rat lines were derived by selective breeding from an outbred Wistar stock at Walter Reed Army Institute of Research (Lumeng *et al.*, 1977), whereas the HAD and LAD replicate lines were derived from the N/Nih het-

erogeneous stock rats (Hansen and Spuhler, 1984; Li *et al.,* 1993).

The goal of selective breeding for ethanol preference has been reached in at least five different breeding programs, but the theoretical and pragmatic utility of the rat lines as animal models rests partly upon whether the rats selected for high ethanol preference reasonably approximate alcohol intake levels and display distinguishing behaviors seen with human alcoholics. Thus, important criteria for an animal model of alcoholism, as initially proposed by Cicero (1979) and Lester and Freed (1973), include voluntary intake of ethanol for its pharmacological effects and not solely because of the taste, smell, or caloric properties; apparent willingness to work for ethanol through operant responding; and the development of tolerance and dependence through free-choice drinking. To date, only the P line has been thoroughly tested and found to fulfill these criteria. P rats, with free-choice drinking, attain pharmacologically relevant blood alcohol concentrations (BACs) of 50–200 mg% (Table I) by consuming 5–8 g ethanol/kg/day. This amount of ethanol, after adjusting for the greater ethanol metabolism rate in rats, is the approximate equivalent of an average adult human drinking in the range of 8–14 standard alcoholic drinks per day. P rats will perform an operant response to drink 10–40% (v/v) ethanol solutions while food and water are freely available, suggesting that ethanol can function as a reinforcer for these animals (Murphy *et al.,* 1989). Ethanol-naive P and NP rats have similar initial orofacial and behavioral responses to the taste and smell of ethanol in a taste reactivity test (Bice and Kiefer, 1990). However, P but not NP, rats will self-administer ethanol intragastrically (Waller *et al.,* 1984) as well as intracranially, directly into the ventral tegmental area (VTA; Gatto *et al.,* 1994), suggesting that the high/low intake of ethanol by P/NP rats is primarily mediated by the postingestive, presumably central nervous system (CNS), effects of ethanol rather than by its gustatory properties. Also, P rats maintain high ethanol intake even when a chocolate or saccharin solution is presented as a third-choice (Lankford *et al.,* 1991). Finally, despite the fact that ethanol-naive P and NP rats display similar levels of ethanol clearance (Lumeng *et al.,* 1982; Li and Lumeng, 1977), P rats, given chronic free-choice access to ethanol, drink sufficient amounts to develop metabolic and functional tolerance to the motor-impairing and aversive effects of ethanol (Gatto *et al.,* 1987a; Lumeng and Li, 1986; Stewart *et al.,* 1991). In addition, they develop dependence, as indicated by physical signs upon ethanol withdrawal (Kampov-Polevoy *et al.,* 2000; Waller *et al.,* 1982). In summary, the P line has been well characterized behaviorally in terms of the criteria proposed as essential for an animal model of alcoholism. However, future studies will be necessary to fully characterize the validity of the HAD replicate lines in terms of the animal model criteria.

Extending the Models

In addition to evaluating the selected lines in terms of how they fulfill the basic criteria initially proposed for an animal model of alcoholism (Cicero, 1979; Lester and Freed, 1973), other lines of experimental inquiry have focused on how well these rat models parallel

Table I. Summary of BACs Attained by P Rats Under Different Conditions of Ethanol (E) Intake

Condition	BAC (mg %)	Ethanol Intake (g/kg)
24-h free access 10% E	90 ± 10 (3h, dark)[a]	6.9 ± 0.2 (24 h)
4-h limited access 10% E	120 ± 15 (after 1 h)[b]	2.1 ± 0.2 (4 h)
1-h limited access 10% E	76 ± 13 (after 1 h)[b]	1.3 ± 0.1 (1 h)
24-h free access 20% E (IG)	200 (range: 115–300)[c]	5.5 ± 0.2 (24 h)
24-h free access 40% E (IG)	230 (range: 90–415)[c]	9.4 ± 1.7 (24 h)
24-h relapse 10, 20 or 30% E	180 (range: 160–205)[d]	5.3 ± 0.6 (2 h)

Values are the means (± SEM).

[a] From Murphy *et al.* (1986). Samples taken from retro-orbital sinus at set times throughout 24-h period. Peak value attained 3 h into dark cycle.

[b] From Murphy *et al.* (1986). Samples taken from retro-orbital sinus 1 hour into the session.

[c] From Waller *et al.* (1984). Samples taken from retro-orbital sinus 30–40 min after completing an intragastric (IG) self-administration episode.

[d] From Rodd-Henricks *et al.* (2001). Trunk blood sampled 2 h after ethanol solutions were restored following 2 weeks of alcohol deprivation.

various behavioral phenotypes reported to be commonplace in children of alcoholics, alcohol abusers, and alcoholics compared with nondrinkers or light drinkers. For example, individuals prone to alcohol abuse and alcoholism typically progress through stages of increased alcohol drinking. The pattern often begins with social drinking that makes a transition into heavier drinking episodes and possibly into extended binges (Finney and Moos, 1991; McMillen, 1997; Nezlek *et al.*, 1994). Voluntary or forced abstinence may ensue upon recognition of deleterious health and social consequences resulting from continued alcohol use, but the abstinence is frequently complicated by increasingly intense craving for alcohol, which can precipitate heightened alcohol seeking, followed by relapse drinking and loss of control. Because alcoholics often go through cycles of relapse drinking and abstinence, the issue of relapse should be considered in animal models (McBride and Li, 1998), and relapse might be regarded as an additional pertinent criterion for an adequate animal model.

Toward this end, the alcohol deprivation effect (ADE), and more recently the repeated alcohol deprivation effect (RADE), have been investigated in P and HAD rats as models for loss of control and relapse behaviors seen in humans. The ADE is defined as a temporary increase in the ratio of ethanol to total fluid intake and voluntary intake of ethanol solutions over baseline drinking conditions, when ethanol is reinstated following a period of alcohol deprivation (Sinclair and Senter, 1967). Recently, the RADE, as a model of the prototypical human condition of repeated abstinence and relapse, has been investigated in P rats by employing a sequence of repeated deprivation-reinstatement cycles. In P rats given continuous free-choice between 10% (v/v) ethanol and water for 2 to 6 weeks, repeated 2-week deprivations prolonged the expression across days of the RADE, but did not alter the magnitude of the ADE (Rodd-Henricks *et al.*, 2000c). In contrast to the P line of rats, when HAD_1/HAD_2 lines were tested with a single 10% ethanol concentration and concurrent water, the expression of an ADE was dependent upon repeated deprivations, and the HAD_2 line showed a prolonged RADE with repeated deprivations (Rodd-Henricks *et al.*, 2000b). In a further extension of this model, a modification of a "loss of control" experiment (Wolfgramm and Heyne, 1995) was used in P and HAD rats tested with concurrent access to three concentrations of ethanol (10, 20, and 30% v/v) and water. Under these conditions, repeated deprivations enhanced the magnitude and prolonged the duration of the RADE and shifted preference toward the higher ethanol concentrations in P rats (Rodd-Henricks *et al.*, 2001). This model can result in ethanol intakes up to 16 g/kg/day, with 4.5–6.0 g/kg consumed in the first 2 h of reinstatement of access to ethanol. Thus, repeated deprivations yielded a drinking pattern in P rats very similar to binge drinking observed in human alcoholics (Finney and Moos, 1991; Nezlek *et al.*, 1994). After adjusting for the faster ethanol elimination rate in rats compared with humans, intake of 16 g ethanol/kg/day is approximately equivalent to consumption of 24 standard-size alcoholic drinks by a 150–160 pound person per day. Therefore, repeated alcohol deprivations and the ensuing deprivation effects, which more closely parallel the human alcohol drinking pattern (McMillen, 1997), may be a valid model for studying relapse, "loss of control," and "binge" drinking.

BEHAVIORAL PHENOTYPES OF THE SELECTED LINES

The selected lines have proved to be useful in delineating phenotypic behaviors that may be associated with the extremes of high and low alcohol drinking. Thus, various innate differences in ethanol-naive animals and differences in responses to ethanol have been compared in the selected lines with the aim of identifying associated phenotypic traits that may be relevant to neurogenetic mechanisms underlying ethanol preference or nonpreference. This process presumes that multiple genes underlie ethanol preference and that these genes also contribute to other behaviors; i.e., the associated behavioral characteristics constitute a product of the putative plieotropic influences of the gene frequencies emerging from the selection pressure for ethanol preference. Table II summarizes many of the more recent publications on behavioral phenotyping of the selected lines.

Ethanol Sensitivity

Studies of human subjects with a family history of alcoholism suggest that there is an association between a low level of response (or sensitivity) to ethanol and risk for the development of alcoholism (Schuckit 1986, 1994). When ethanol sensitivity (responsiveness to a single moderate to high dose of ethanol [$>$1.0 g/kg]) has been examined in P and NP rats, most studies found that P rats are less sensitive than NP rats (Kurtz *et al.*, 1996; Stewart *et al.* 1992; Lumeng *et al.*, 1982). When HAD and LAD rats have been compared, the results

Table II. Phenotypic Differences in Behavioral Responses in P/NP and HAD/LAD rats (1995–2001)

Correlated response	Innate difference	Differences in response to ethanol	Tolerance development
Taste reactivity: Kiefer *et al.* (1995)	HAD = LAD	HAD = LAD	Increased ingestive and less aversive responding in HAD but not in LAD following 3 weeks of free-choice alcohol drinking
Operant self-administration: Samson *et al.* (1998); Files *et al.* (1998)		FR-4: 10% EtOH (limited access): HAD1 > HAD2 = P; NP > LAD1 = LAD2 FR-4: 10% EtOH (continuous access): P/HAD1/HAD2 > NP/LAD1/LAD2 in ethanol intake. P/HAD1/HAD2 > NP/LAD1/LAD2 in number of drinking bouts/day. HAD1 has fewer bouts/day, but larger bouts than P/HAD2.	
Ontogeny of alcohol intake: McKinzie *et al.* (1998b)		P/HAD2: High ethanol intake at 3–4 weeks old. NP/LAD2: Low ethanol intake at 3–4 weeks old.	Tolerance to aversion in P rats after chronic free-choice alcohol drinking
Conditioned place avoidance: Stewart *et al.* (1996)		P < NP in avoidance of place paired with ethanol.	
Conditioned taste aversion (CTA): Badia-Elder *et al.* (1999a)		1 g/kg ethanol, less CTA in HAD	Rapid tolerance in HAD1, but not in LAD1; equal rapid tolerance in HAD2 and LAD2
Jump test to avoid foot-shock: Stewart *et al.* (1998a); Suwaki *et al.* (2001)		Faster recovery in LAD1 than HAD1; HAD2 = LAD2	Rapid tolerance in HAD1 but not LAD1; tolerance over 5 daily trials in P and NP rats (1.0, 1.25, 1.5 g/kg); P > NP (1.5 g/kg)
Oscillating bar test: Stewart *et al.* (1998a); Suwaki *et al.* (2001); Bell et al. (2001)		Faster, recovery in LAD1 than HAD1; HAD2 = LAD2; faster recovery in P than NP	Rapid tolerance in P/HAD, but not NP/LAD; sensitization in NP
Loss of righting reflex; Kurtz *et al.* (1996); Froehlich and Wand (1997)		P and HAD take longer to lose righting reflex than NP and LAD.	Persistence of greater tolerance in P than NP (2 injections of 3.5 g/kg, separated by 1 day); sensitization in NP rats (2 injections of 3.5 g/kg separated by 3 days)
Sleep time: Kurtz *et al.* (1996); Froehlich and Wand (1997)		Faster recovery in P and HAD than in NP and LAD.	
Hypothermia: Kurtz *et al.* (1996)	Elevated plus maze: HAD1/HAD2 = LAD1/LAD2	Greater temperature drop in NP than P.	
Anxiolytic effects: Stewart *et al.* (1999)	Saccharin: HAD > LAD	No effect in HAD and LAD	
Preference for flavored substances: Stewart *et al.* (1998b)	NP > immobility than P, HAD = LAD		
Forced swim test: Godfrey *et al.* (1997) Viglinskaya *et al.* (1995)	P > NP at escape attempts in modified forced swim test (four escape alleys).		
Acquisition of responding for food compared to acquisition of shock avoidance: Blackenship *et al.* (1998); Steinmetz *et al.* (2000)	P < NP on both tasks when appetitive training followed by aversive training. NP < P when order of training was reversed. Appetitive training: HAD1 = LAD1. Aversive training: HAD1 and HAD2 did not learn; LAD1 and LAD2 learned normally.		

(continued)

Table II. *(Continued)*

Correlated response	Innate difference	Differences in response to ethanol	Tolerance development
Ultrasonic vocalization in response to stress: Knapp *et al.* (1997); Overstreet *et al.* (1997)	NP > P; HAD > LAD		
Pain sensitivity (hot plate): Kampov-Polevoy *et al.* (1996)	P lower threshold than NP		
Step down passive avoidance and operant responding under DRL schedule (behavioral disinhibition): Steinmetz *et al.* (2000)	Task performance: P < NP = HAD1 = LAD1		
Open field activity: Badishtov *et al.* (1995)	P > active than NP; NP > defecation in open field than P		
Acoustic startle response (ASR); fear-potentiated startle (FPS); prepulse inhibition (PPI): McKinzie *et al.* (2000); Jones *et al.* (2000)	ASR: P = NP or P > NP; FPS: P > NP; PPI: P = NP	ASR: Low dose ethanol (0.5 g/kg) reduced ASR in P but not NP; higher doses (1.0, 1.5 g/kg) reduced ASR more in NP than P. PPI: Disrupted in P but not NP (0.5 g/kg)	
Ethanol discrimination: McMillan *et al.* (1999); McMillan and Li (1999)		P > NP; HAD = LAD	
Novelty seeking: Nowak *et al.* (2000a)	Greater behavioral activation in P and HAD than in NP and LAD in response to novel odors; P = NP = HAD = LAD in nose-poking response to novel odors		
Neurophysiological response during associative learning: Slawecki *et al.* (1999)	Enhanced event-related potentials (ERPs) in P relative to NP		
Baseline EEG and ERP: Slawecki *et al.* (2000)	EEG power: HAD > LAD; ERP amplitude: LAD > HAD		

have been less consistent. In agreement with studies of P/NP rats, HAD_1 rats took longer to lose the righting reflex, regained the righting reflex more quickly and did so at higher BACs than LAD_1 rats (Froehlich and Wand 1997). However, in contrast to the studies with P/NP rats, in a test of motor impairment in which rats must jump up to a descending platform, HAD_1 rats took longer to recover to criterion performance than LAD_1 rats, but HAD_2 and LAD_2 rats did not differ (Suwaki *et al.* 2001). HAD_2 and LAD_2 rats recovered at times that were approximately midway between the recovery times of the HAD_1 and LAD_1 rats.

Ethanol Tolerance

The development of tolerance to the sedative-hypnotic and motor-impairing effects of ethanol may permit the alcoholic to drink greater amounts of alcohol over time (Kalant *et al.*, 1971). Acute tolerance, which develops during the course of a single ethanol exposure, was examined in P and NP rats using the jump-up test by administering two successive ethanol doses (Waller *et al.*, 1983). Both lines of rats developed acute tolerance, but P rats developed acute tolerance more rapidly and/or to a greater degree than NP rats.

The term rapid tolerance has come to be applied to a procedure in which two equivalent ethanol injections are administered separated by a time interval sufficiently long for the BAC from the first injection to return to zero before the second injection is administered. The selected lines of rats have been studied for development of rapid tolerance using a number of dependent measures, with tolerance defined as reduced responsiveness after the second injection compared with the first. When ethanol-induced hypothermia was used to index tolerance (Stewart *et al.,* 1992), both P and NP lines showed tolerance when 1 day separated the two injections. With 2 or 3 days separating the injections, the P rats no longer showed tolerance, whereas the NP rats showed sensitization (i.e., an increase in hypothermia after the second injection compared with the first). When sleep time or time to regain righting reflex after ethanol treatment was used to index tolerance (Kurtz *et al.,* 1996) and 1 day separated the two injections, P rats regained the righting reflex more quickly and at a higher BAC after the second injection (tolerance). However, NP rats regained the righting reflex more slowly and at a lower BAC after the second injection (sensitization). Using the jump-up test of motor impairment (Gatto *et al.,* 1987b), tolerance was observed in the P rats when the two injections were separated by as many as 10 days, whereas tolerance in the NP rats dissipated within 3 days. This was interpreted as a greater persistence of tolerance in P than in NP rats. The HAD_1/LAD_1 and HAD_2/LAD_2 lines also were examined with the jump-up test (Stewart *et al.,* 1998a). When two equivalent ethanol injections were separated by 1 day, HAD_1 rats recovered more quickly after the second injection, indicating the development of tolerance. LAD_1 rats' recovery time did not change after the first and second injection, suggesting that no tolerance had developed. However, both HAD_2 and LAD_2 rats showed similar degrees of tolerance after the second injection.

With 6 weeks of chronic free-choice drinking, P rats develop metabolic tolerance (Lumeng and Li, 1986), as evidenced by a 15% increase in ethanol elimination rate. Neuronal or functional tolerance also was seen following 14 days of free-choice drinking (Gatto *et al.,* 1987a), as evidenced by faster recovery and at a higher BAC, on a jump-up test after a single ethanol injection. Chronic tolerance to moderate doses of ethanol (injected over multiple test days) has been examined in P and NP rats using the oscillating platform task (Bell *et al.,* 2001) in which rats are trained to stay on an oscillating platform to avoid electric shock. Again, P rats demonstrated lower initial responsivity and greater tolerance compared with NP rats. Chronic tolerance has not yet been examined in the HAD and LAD lines.

The Aversive Effects of Ethanol

The initiation and acquisition of oral ethanol intake by P rats consists of a negatively accelerating increase in ethanol intake over about 30 days with stable levels of drinking thereafter (Stewart *et al.,* 1991). Such a pattern could reflect tolerance to the reinforcing effects of ethanol, but other interpretations cannot be ruled out. It also is possible that tolerance to some effect of ethanol may be occurring that normally constrains or limits ethanol intake. For example, ethanol-induced sedation or motor-impairment may interfere with ethanol drinking, and the development of tolerance to these effects may permit higher intake over time. Indeed, as described above, tolerance to the motor-impairing effects of ethanol is observed in P rats after chronic ethanol consumption (Gatto *et al.,* 1987a). It also is possible that tolerance to the aversive effects of ethanol may allow the rats to consume higher ethanol doses without suffering negative consequences.

The aversive effects of ethanol have been examined in ethanol-naive P and NP rats and in HAD_2 and LAD_2 rats using a conditioned taste aversion (CTA) procedure (Froehlich *et al.,* 1988, Badia-Elder *et al.,* 1999a). A negative relationship was found between selective breeding for high ethanol intake and CTA development, i.e., the CTA was smaller in P and HAD than in NP and LAD rats. The same results were seen testing P and NP rats with a place conditioning measure in which the aversive effects of ethanol were indexed by the avoidance of environmental cues paired with ethanol injections (Stewart *et al.,* 1996). Together, these observations suggest that the high ethanol consumption shown by P and HAD rats may result, at least in part, from a reduced sensitivity to the aversive effects of ethanol. Conversely, the low ethanol consumption shown by NP and LAD rats may result from an increased sensitivity to the aversive effects of ethanol. Indeed, P rats develop tolerance to the aversive effects of ethanol, as evidenced by a reduced ethanol-induced CTA following chronic free-choice ethanol drinking (Stewart *et al.,* 1991). In addition to the aversive CNS effects of ethanol that may be experienced when ethanol is administered, the aversive effects experienced after ethanol elimination, i.e., ethanol withdrawal, have been examined in P/NP and HAD_1/LAD_1 rat lines. Follow-

ing the same ethanol treatments, P and HAD_1 rats showed less severe behavioral signs of withdrawal than NP and LAD_1 rats (Chester *et al.,* 2002). Thus, more severe aversive withdrawal effects may constrain ethanol drinking in the alcohol-nonpreferring rat lines while less severe ethanol withdrawal may permit higher ethanol drinking in the alcohol-preferring lines.

Acoustic Startle Response

It recently has been hypothesized that alcoholics and those at risk for alcoholism may have a deficit in CNS inhibition that results in an excess of CNS excitation, and that this hyperexcitation is temporarily alleviated by ingestion of alcohol (Begleiter and Porjesz, 1999). The amplitude of the startle response to a white noise burst was used as a measure of neuronal excitation in the selectively bred lines of rats. In general, naive P and NP rats did not differ in startle threshold at lower dB levels, but P rats appear to display greater reactivity to higher dB levels. However, P rats displayed greater startle responses to startle alone and to potentiated startle stimuli after fear conditioning (McKinzie *et al.,* 2000a). Prepulse inhibition (PPI) refers to a decrement in startle response if a stimulus (e.g., a nonstartling white noise burst) precedes the startle white noise burst (Hoffman and Ison, 1980). This decrement in startle response has been attributed to sensory detection processes. P rats appeared to be more sensitive to ethanol-induced reduction of reactivity to startle and to disruption of PPI produced by low doses of ethanol (Jones *et al.,* 2000).

Anxiety Associated Behaviors

It has been proposed that ethanol has anxiolytic effects and is consumed in an attempt to reduce anxiety (Cappell and Herman, 1972; Pohorecky, 1981, 1990). Anxiety has been assessed in P/NP rats using the slip-funnel test, elevated plus maze test, and a passive avoidance paradigm. In the slip-funnel test, anxiety is determined by the amount of escape behavior (from standing in water covering the rat's hind legs) compared to passive behavior. In the elevated plus maze test, reduced time spent in open vs. closed arms is an index of increased anxiety. In the passive avoidance paradigm, greater anxiety is indexed by increased time spent on an elevated ledge to avoid foot shock. On these three tests, alcohol-preferring rats showed more anxiety-like behavior, compared with alcohol-nonpreferring rats (Salimov *et al.,* 1996; Stewart *et al.,* 1993). In an active avoidance paradigm, rats are trained to bar press to avoid a signaled footshock. Under baseline conditions, NP rats showed superior performance in comparison to P rats in learning the avoidance task, but P rats showed superior performance relative to NP rats if they learned to bar press for reinforcement in an appetitive task first (Blankenship *et al.,* 1998). These observations suggest a complex relationship between selection for divergent ethanol intake, behavioral inhibition, and sensitivity to conditioned fear. When ultrasonic vocalization (USV) was assessed as a measure of reactivity to noxious stimuli, a negative correlation was reported between number of USVs emitted to a noxious air-burst and ethanol intake (Knapp *et al.,* 1997). In general, NP rats emitted more USVs compared with P rats, and NP rats vocalized longer than P rats.

Taste Effects of Ethanol and Preference for Flavored Substances

Taste reactivity has been evaluated in P/NP and HAD/LAD rats (Bice and Kiefer, 1990; Kiefer *et al.,* 1995). Initially, ethanol-naive P and HAD rats did not differ from NP and LAD rats in their reactivity to a wide range of ethanol concentrations or to solutions of sucrose or quinine. However, after 3 weeks of free-choice ethanol drinking, P and HAD rats displayed increased ingestive and less aversive responses to ethanol, compared with NP and LAD rats. When preference for nonalcoholic flavored solutions was assessed using a two-bottle preference test, P rats showed a higher preference for, and greater intake of, solutions of saccharin (Sinclair *et al.,* 1992) or sucrose (Stewart *et al.,* 1994) than NP rats. However, P and NP rats did not differ in preference for sour or bitter flavored solutions. The association between ethanol and sweet preference has been confirmed in the HAD_1/LAD_1 and HAD_2/LAD_2 replicate lines (Stewart *et al.,* 1998b) and in the F_2 progeny of inbred P x NP crosses (Foroud *et al.,* 2002) and HAD_1 x LAD_1 crosses (Stewart *et al.,* 1998b). These findings are consistent with observations that avidity for sweet solutions is positively associated with high ethanol intake in other rodent lines and strains (Overstreet *et al.,* 1993; Belknap *et al.,* 1993) and in certain populations of human alcoholics (e.g., Kampov-Polevoy *et al.,* 1997).

Activating Effects of Ethanol and Novelty

Low doses of ethanol reportedly are excitatory. For example, ethanol produces increases in spontaneous

motor activity in rodents (Pohorecky, 1977). It has been hypothesized that the stimulatory or behaviorally activating effects of ethanol and other drugs may be a manifestation of the reinforcing effects of the drugs (Wise and Bozarth, 1987). P rats display higher responsivity, compared with NP rats, to low dose stimulatory (locomotor activating) effects of ethanol (Waller *et al.*, 1986). Adolescent P, HAD_1, and HAD_2 rats also show greater activation than their adolescent alcohol-nonpreferring counterparts (Rodd-Henricks *et al.*, 2000a).

High novelty-seeking behavior, defined as behavioral activation in the presence of an unknown stimulus (Cloninger, 1996), appears to be predictive of current and future alcohol abuse (Andrucci *et al.*, 1989). Increases in locomotor activity in response to a novel olfactory stimulus have been used to assess novelty seeking in rats, and P and HAD rats demonstrate greater increases in activity in comparison to NP and LAD rats (Nowak *et al.*, 2000a).

The arousing/activating effects of ethanol have also been examined using autonomic and electrophysiolgical indices. During non-REM sleep, intragastric administration of a low dose of ethanol produced a persistent increase in EEG spectral power in NP rats, but P rats displayed an initial decrease in power with a return to baseline (Morzorati *et al.*, 1988). EEG spectral power increases from wakefulness to drowsiness and non-REM sleep. Therefore, the decrease in EEG spectral power seen in the P rats indicates that the ethanol was arousing for these rats. When event-related potentials (ERPs) following ethanol injections were examined, NP rats displayed a dose-dependent decline in N1 amplitude, whereas P rats showed increased N1 amplitude, particularly in the hippocampus (Ehlers *et al.*, 1991). These data suggest that ethanol produces greater stimulatory effects in P rats relative to NP rats. P rats also show increased heart rate when presented with limited access free-choice ethanol (Bell *et al.*, in press). Further, P rats displayed increased activity and heart rate during the pretest period in the third week of access. Thus, these arousing effects can be conditioned to the environment associated with ethanol availability (see also Melendez *et al.*, 2002). However, changes in heart rate have yet to be examined in low alcohol-preferring rat lines.

Operant Self-Administration of Ethanol

In the initial investigation of lever-press responding maintained by oral ethanol reinforcers, P rats self-administered greater amounts of ethanol than NP rats (Murphy *et al.*, 1989). With continuous access to ethanol solution (2–30% w/v) and water under an FR5 schedule of reinforcement (i.e., five responses required for each reinforcement), ethanol responding was greater than water responding at all ethanol concentrations for P rats, whereas with NP rats, water responding was greater than ethanol responding at concentrations of 10% and higher. Subsequent testing of P/NP and HAD/LAD rats (Samson *et al.*, 1998; Schwarz-Stevens *et al.*, 1991; Files *et al.*, 1992, 1993, 1998; Ritz *et al.*, 1994a, 1994b) confirmed that responding maintained by ethanol is consistently greater in the alcohol-preferring lines than in the alcohol-nonpreferring lines across a wide range of experimental conditions. The sucrose substitution procedure (Samson, 1986) was developed to initiate ethanol-reinforced responding in unselected rats. With the sucrose substitution procedure, increased levels of ethanol self-administration occurred in NP rats (Samson *et al.*, 1998), but this procedure was relatively ineffective in LAD_1 and LAD_2 rats (Samson *et al.*, 1998). Examination of patterns of responding with continuous access to ethanol solution and water (Files *et al.*, 1998) indicates that the higher levels of ethanol intake seen in P and HAD_2 rats are due to larger and more frequent drinking bouts per day relative to NP and LAD_2 rats. The HAD_1 rats, however, drink fewer bouts per day but have larger bouts than P and HAD_2 rats. Thus, although selection for ethanol preference and nonpreference using a two-bottle test generally is associated with high and low levels of lever-press responding maintained by ethanol, there appears to be differences among the lines in ethanol drinking patterns that are manifested when an operant procedure is used.

A robust alcohol deprivation effect (ADE) can also be demonstrated using the operant procedure (Rodd-Henricks *et al.*, 2002b; Rodd-Henricks *et al.*, 2001; McKinzie *et al.*, 1998a). When the operant procedure included repeated deprivations, ethanol reinforcement was enhanced during the first session after the second and third reinstatements of ethanol. Water responding was low and unaltered by repeated deprivations. Repeated deprivations also produced a significantly higher breakpoint (16 ± 3 for nondeprived vs. 30 ± 5 for deprived) during a modified progressive ratio procedure (Rodd-Henricks *et al.*, 2000b). Thus, the rewarding properties of ethanol appear to be enhanced in P rats that have undergone repeated alcohol deprivations. As stated above, P, but not NP, rats will intracranially self-administer nanoliter quantities of ethanol (50–200 mg%) directly into the ventral tegmental area (VTA; Gatto *et al.*, 1994). This suggested that the VTA of the

P rat is sensitive to the reinforcing effects of ethanol, whereas this is not the case for NP rats.

Development of Ethanol Self-Administration

In general, selection for divergent ethanol intake levels in adulthood holds true for periadolescent rats, as measured by ethanol intake (g/kg/day; McKinzie *et al.*, 1999, 1998b). As seen in adult rats, ethanol drinking during periadolescence results in an alcohol deprivation effect (ADE), when the deprivation manipulation is done in late adolescence/early adulthood (Rodd-Henricks *et al.*, 2001; McKinzie *et al.*, 1998a). This suggests that experimental/environmental manipulations in periadolescent rats can alter ethanol intake and possibly lead to "loss of control" drinking. These studies with periadolescent rats are in accord with observations in human clinical populations that occasional and frequent adolescent and young adult binge drinkers experience more alcohol-related problems than nonbinge drinkers (Wechsler *et al.*, 2000).

Conclusions

On the basis of studies in which P/NP and HAD/LAD replicate lines have been adequately compared under similar experimental conditions, some tentative conclusions can be made about which behavioral phenotypes are most likely to be associated with selection for high and low ethanol intake. Low sensitivity and greater tolerance development to the motor-impairing and sedative-hypnotic effects of high doses of ethanol are seen in P relative to NP rats (Waller *et al.*, 1983; Kurtz *et al.*, 1996). However, this does not appear to be the case for the HAD/LAD replicate lines, which either do not differ or differ in the opposite direction to the findings seen with the P/NP lines (Stewart *et al.*, 1998a). The phenotypes that are consistently observed across the replicate lines appear to be in measures that may be more closely related to motivational processes than ethanol-induced motor impairment and sedation. P and HAD rats show greater stimulatory responses to low ethanol doses than NP and LAD rats (Waller *et al.*, 1986; Rodd-Henricks *et al.*, 2000a), perhaps reflecting greater sensitivity to the rewarding effects of ethanol in the alcohol-preferring lines. NP and LAD rats show greater ethanol-induced CTAs than P and HAD rats (Froehlich *et al.*, 1988; Badia-Elder *et al.*, 1999a), suggesting that differential sensitivity to the aversive postingestional effects of ethanol also may contribute to differences in oral ethanol consumption. Preference for and intake of sweetened solutions are consistently associated with selection for high ethanol consumption, suggesting that common mechanisms may underlie the rewarding effects of ethanol and sweets (Sinclair *et al.*, 1992, Stewart *et al.*, 1998b).

NEUROBIOLOGICAL AND NEUROCHEMICAL PHENOTYPES IN THE SELECTED LINES

Over the past two decades, a considerable literature has accrued from studies on various aspects of CNS neurochemical and neurobiological comparisons within the Indiana selected line pairs. A previous review by McBride and Li (1998) provides a comprehensive account of these findings, and only select neurochemical systems and neurotransmitters that have received contemporary experimental attention are discussed in this review. One approach in these studies has been to compare innate differences in ethanol-naive animals defined as P, NP, HAD, or LAD based solely on breeding history, so that factors predisposing to high or low ethanol drinking behavior might be identified. A working hypothesis is that when similar differences are found in more than one line pair, then the disparate ethanol drinking characteristics between the lines are more likely to have resulted from those differences. Because the phenotype of high ethanol preference is likely mediated by multiple neurotransmitter systems and many complex interactions among the CNS systems, an alteration in one or more of these systems could yield abnormal ethanol drinking behavior. Many of the studies have focused on CNS limbic system areas thought to be involved in alcohol self-administration, and Table III updates from McBride and Li (1998) some important neurotransmitter system differences observed between lines of rats selected for high and low alcohol preference. Another approach has been to examine neurobiological changes in response to ethanol consumption or in response to ethanol injections, to determine if rats predisposed to high or low ethanol drinking might exhibit unique responses to ethanol.

Serotonin

One of the earliest and most robust findings with naive rats from lines selectively bred for ethanol preference or nonpreference is an association between high ethanol preference and lower serotonin (5-HT) contents in several CNS areas. Compared with NP rats, P rats have 12–26% lower levels of 5-HT and its primary metabolite 5-HIAA in the cerebral cortex, frontal

Table III. Summary of Major Innate Neurobiological Differences in Limbic Regions Between High Ethanol-Preferring and Low Ethanol-Preferring Rats

Neurotransmitter system/receptor	Differences	Selected references
Serotonin (5-HT) system		
5-HT content, innervation	NP > P	Murphy *et al.* (1982, 1987)
		Zhou *et al.* (1991a, 1991b, 1991c)
	LAD > HAD	Gongwer *et al.* (1989)
	AA ≥ ANA	Ahtee and Eriksson (1972, 1973); Korpi *et al.* (1988)
	sNP > sP (frontal cortex)	Devoto *et al.* (1998)
Postsynaptic 5-HT_{1A} receptor	P > NP	McBride *et al.* (1994, 1997b)
	HAD = LAD	McBride *et al.* (1997a)
	AA = ANA	Korpi *et al.* (1992)
5-HT_{1B} receptor	NP > P	McBride *et al.* (1997b)
5-HT_2 receptor	NP > P	McBride *et al.* (1993b)
	HAD = LAD	McBride *et al.* (1997a)
	AA = ANA	Korpi *et al.* (1992)
	sNP > sP	Ciccocioppo *et al.* (1999)
5-HT_{2C} receptor	P > NP	Pandey *et al.* (1996)
5-HT_3 receptor	P = NP	McBride *et al.* (1997b)
	AA = ANA	Ciccocioppo *et al.* (1998)
	NP > P (amygdala)	Ciccocioppo *et al.* (1998)
Dopamine (DA) system		
DA content, innervation	NP > P	Murphy *et al.* (1987); Zhou *et al.* (1995)
	LAD > HAD	Gongwer *et al.* (1989)
	AA = ANA	Korpi *et al.* (1988)
	sNP > sP	Casu *et al.* (2002)
D_1 receptor	P = NP	McBride *et al.* (1997b)
	HAD = LAD	McBride *et al.* (1997a)
	sNP > sP	DeMontis *et al.* (1993)
D_2 receptor	sNP > sP	Stefanini *et al.* (1992)
	NP > P	McBride *et al.* (1993b)
	HAD = LAD	McBride *et al.* (1997a)
	AA = ANA	Syvalahti *et al.* (1994)
D_3 receptor	P = NP	McBride *et al.* (1997b)
	HAD = LAD	McBride *et al.* (1997a)
GABA system		
Innervation within ACB	P > NP	Hwang *et al.* (1990)
	HAD > LAD	Hwang *et al.* (1990)
$GABA_A$ receptor		
response to agonist	P = NP	Thielen *et al.* (1993, 1998)
	AA = ANA	Wong *et al.* (1996)
response to BDZ	P > NP	Thielen *et al.* (1997)
	AA > ANA	Wong *et al.* (1996)
response to barbiturate	P = NP	Thielen *et al.* (1998)
Opioid system		
β-Endorphin content	ANA ≥ AA	Gianoulakis *et al.* (1992); Nylander *et al.* (1994)
enkephalin mRNA	sP > sNP	Fadda *et al.* (1999)
	P = NP	Li *et al.* (1998)
mu-opioid receptor	P > NP (opposite in HIP)	McBride *et al.* (1998)
	AA > ANA	DeWaele *et al.* (1995)
	sNP > sP	Fadda *et al.* (1999)
	LAD ≥ HAD	Gong *et al.* (1997)
delta-opioid receptor	AA ≥ ANA	DeWaele *et al.* (1995); Soini *et al.* (1998)
	NP > P	Strother *et al.* (2001)

(Continued)

Table III. *(Continued)*

Neurotransmitter system/receptor	Differences	Selected references
Neuropeptides		
Neuropeptide Y content	NP > P	Ehlers *et al.* (1998)
(CeA)	NP > P; LAD > HAD	Hwang *et al.* (1999)
(hypothalamus)	P > NP; LAD > HAD	Hwang *et al.* (1999)
Neuropeptide Y mRNA	ANA > AA	Caberlotto *et al.* (2001)
Arginine vasopressin content	P > NP	Hwang *et al.* (1998)
(hypothalamus)	LAD > HAD	Hwang *et al.* (1998)
CRF content	NP > P	Ehlers *et al.* (1992)
Substance P content	NP > P	Slawecki *et al.* (2001)
Neurokinin content	NP > P	Slawecki *et al.* (2001)
	NP > P	Ehlers *et al.* (1999)
TRH content (septum)	NP > P	Morzorati and Kubek (1993)

NP = alcohol-nonpreferring line; P = alcohol-preferring line; LAD = low alcohol-drinking line; HAD = high alcohol-drinking line; AA = ALKO alcohol line; ANA = ALKO non-alcohol line; sNP = Sardinian alcohol nonpreferring line; sP = Sardinian alcohol preferring line; BDZ = benzodiazepine; HIP = hippocampus; CeA = central nucleus of the amygdala.

cortex, whole corpus striatum, anterior striatum, nucleus accumbens, hippocampus, thalamus, and hypothalamus (Murphy *et al.,* 1982, 1987). HAD_1 rats also have lower 5-HT and/or 5-HIAA levels in the cerebral cortex, striatum, nucleus accumbens, septal nuclei, hippocampus, and hypothalamus compared with LAD_1 rats (Gongwer *et al.,* 1989). Studies of F_2 generation P x NP intercrosses further support an association between low contents of 5-HT in the nucleus accumbens and high ethanol preference (McBride *et al.,* 1995). Given the proposed role of some of these structures (e.g., nucleus accumbens and frontal cortex) in the reinforcing effects of ethanol, serotonergic deficits may be one predisposing factor to excessive ethanol drinking. These findings are similar to those from human alcohol abusers when using 5-HIAA from cerebral spinal fluid as an index of CNS 5-HT activity (Cloninger, 1987). Relatively low serotonin has not been a universal observation in all rats bred for high ethanol preference, nor has it been for human alcoholics. For example, higher levels of 5-HT were found in only certain CNS regions in AA rats compared with ANA rats (Ahtee and Eriksson, 1972, 1973; Korpi *et al.,* 1988). While some limbic structures (e.g., nucleus accumbens) have not been studied in the same way among the various models, the different findings with the AA and ANA rats may be taken to indicate that selective breeding for ethanol preference can yield a variety of phenotypes. Certain neurobiological effects, such as lower 5-HT, may not be requisite phenotypes for the manifestation of high ethanol intake but may augment ethanol preference and underlie some behavioral phenotypes consequent to 5-HT abnormalities (e.g., low impulse control) when it occurs as a product of selective breeding for high ethanol preference.

The earlier findings on 5-HT were followed up by immunocytochemical studies revealing fewer 5-HT immunostained fibers in the anterior frontal cortex, nucleus accumbens, and part of the ventral hippocampus of P rats, compared with NP rats (Zhou *et al.,* 1991a, 1991b). The dorsal and median raphe of P rats also have fewer 5-HT immunostained neurons, compared with NP rats (Zhou *et al.,* 1991c). Thus, lower 5-HT fiber density in the CNS of P rats is likely due to fewer 5-HT projection neurons from the raphe. There is also evidence for an up-regulation of post synaptic $5\text{-}HT_{1A}$ receptors in the cerebral cortex and hippocampus of P rats, compared with NP rats (Wong *et al.,* 1993; McBride *et al.,* 1994). This up-regulation might result as a consequence of the lower 5-HT innervation (Zhou *et al.,* 1991c). On the other hand, densities of $5\text{-}HT_{1B}$ receptors were moderately lower in some limbic regions (e.g., nucleus accumbens, septum, and amygdala) of the P compared to NP rats (McBride *et al.,* 1997b). Some observations with mice suggest that lower functioning of $5\text{-}HT_{1B}$ receptors is associated with high ethanol intake (Crabbe *et al.,* 1996; Risinger *et al.,* 1996). In general, densities of $5\text{-}HT_2$ receptors were also lower in several CNS regions of P compared with NP rats (McBride *et al.,* 1993a), although higher numbers of $5\text{-}HT_{2C}$ receptors were demonstrated in the hippocampus and amygdala (Pandey *et al.,* 1996). When the HAD_1 and LAD_1 lines were compared, no substantial differences in the densities of 5-HT receptors were

found in the same CNS regions as the P and NP rats (McBride *et al.,* 1997b), and no differences were found in 5-HT_1 and 5-HT_2 receptor densities in several CNS regions (e.g., brainstem, hippocampus, frontal cortex, and hypothalamus) of AA compared with ANA rats (Korpi *et al.,* 1992).

In lines of rats in which differences in 5-HT systems have been found, the potential consequence for excessive ethanol drinking has been supported by the efficacy of serotonergic pharmacological treatments on ethanol drinking. Administration of fluoxetine, a 5-HT reuptake inhibitor, significantly reduced ethanol intake of P rats under limited or continuous access conditions (Murphy *et al.,* 1985) and also reduced ethanol intake of HAD rats (McBride *et al.,* 1990, 1992). In P and HAD rats, agonists for the 5-HT_{1B} and 5-HT_{1A} receptors reduced ethanol intake (McBride *et al.,* 1990). DOI, a 5-HT_2 agonist, also reduced ethanol intake in P rats (McBride *et al.,* 1992), but caused a biphasic effect in reducing the ethanol intake of HAD rats, such that low doses increased intake and high doses decreased intake. LY53857, a 5-HT_2 receptor antagonist, also significantly reduced ethanol intake in HAD rats (McBride *et al.,* 1992). These findings suggested that the 5-HT_2 receptor may subserve ethanol reinforcement in HAD rats, such that low doses of a 5-HT_2 agonist may potentiate the reinforcing effects of ethanol, high doses of a 5-HT_2 agonist may maximally activate the receptor, and a 5-HT_2 antagonist would block the reinforcing effects of ethanol. Investigations with 5-HT_3 receptor agents have also suggested an important role of 5-HT_3 receptors in ethanol intake. McKinzie *et al.* (1998c, 2000b) found that MDL 72222, a 5-HT_3 receptor antagonist, was effective in reducing alcohol intake of P rats in conditions where an alcohol drinking solution was continuously available or when environmental cues were minimized by randomizing the time of a daily ethanol access period. However, when scheduled access to ethanol occurred at the same time each day MDL 72222 was largely ineffective.

In sum, considerable work has implicated a role for various functional aspects of 5-HT systems in alcohol preference and nonpreference of the selected lines. Studies in human alcoholic populations have also implicated altered 5-HT system functioning in alcohol abuse, and human studies have investigated aspects of 5-HT functioning, such as the 5-HT transporter, which has received comparatively little attention in studies with the selected lines (Heinz *et al.,* 2001). Clinical investigations have also found that 5-HT pharmacotherapies may be efficacious in human alcoholics. For example, ondansetron, another 5-HT_3 receptor antagonist, resulted in fewer drinks per day, compared to placebo, for presumed biologically predisposed alcoholics that had an early age of alcoholism onset, but not for a group of late-onset alcoholics (Johnson *et al.,* 2001), suggesting a difference in 5-HT systems between the these alcoholic subtypes.

Dopamine

The CNS content of dopamine (DA) has been examined in P/NP (Murphy *et al.,* 1982, 1987) and HAD/LAD (Gongwer *et al.,* 1989) lines. Alcohol-preferring rats have 10–30% lower levels of DA and its metabolites (DOPAC and HVA) in the nucleus accumbens and 15–20% lower contents in the anterior striatum, compared with their alcohol-nonpreferring counterparts, suggesting a deficiency in the dopamine pathway that projects from the VTA to these regions in alcohol-preferring rats. In support of this conclusion, Zhou *et al.* (1995) found a decreased number of neuronal projections from the VTA to the nucleus accumbens in P rats relative to NP rats, indicating that the accumbens shell had fewer dopamine fibers. Additional support for these findings comes from studies on F_2 generation rats from P x NP intercrosses, such that the adult offspring with high ethanol intakes had 25% lower content of DA in the nucleus accumbens compared to low ethanol-drinking F_2 rats (McBride *et al.,* 1995). When neuronal activity of VTA DA neurons was compared in P and Wistar rats, VTA DA neurons were found to have more frequent burst firing in P rats (Morzorati, 1998). It can be speculated that this increased activity in P rats is a compensatory mechanism for the reduced DA neurons. Quantitative autoradiography revealed 20–25% lower binding of tritiated sulpiride in the caudate putamen, the medial and lateral nucleus accumbens, and the VTA of P rats, compared with NP rats (McBride *et al.,* 1993b). Research on the Sardinian sP and sNP rats has revealed similar findings (Stefanini *et al.,* 1992). These neurobiological and neurochemical findings are consistent with an association of high ethanol-drinking behavior and altered functioning within the mesolimbic DA system projecting from the VTA to the nucleus accumbens, which has been hypothesized to mediate some of the reinforcing actions of ethanol and various other drugs of abuse (Koob *et al.,* 1998).

Responses to ethanol in the DA system have been demonstrated, either as measured by tissue levels of DA and its metabolites, or by in vivo microdialysis during ongoing behavior, where DA in the microdialysate

is measured as an index of synaptic DA overflow near the site of a microdialysis probe. Oral self-administration of ethanol, contingent on operant responding, was found to increase DA overflow above baseline values to a greater extent in the nucleus accumbens of P rats than in unselected Wistar rats (Weiss *et al.*, 1993), suggesting that the VTA DA system of the P line of rats may be more sensitive to the reinforcing actions of ethanol. Anticipation for ethanol access increased extracellular nucleus accumbens DA levels in microdialysate from P rats but not Wistar rats (Katner *et al.*, 1996), and olfactory or environmental cues that were associated with anticipated access to operant responding for ethanol resulted in elevated DA levels in the microdialysate from the nucleus accumbens of Wistar or P rats (Katner *et al.*, 1999; Melendez *et al.*, 2002). In addition, the elevated extracellular accumbal DA levels occurred during operant responding for oral ethanol, but the elevated DA levels were not directly correlated with motor activity (Melendez *et al.*, 2002). P rats tested in a similar behavioral paradigm also exhibited elevated DA in microdialysates from the ventral pallidum, which receives input from the VTA, but not in the globus pallidus (Melendez *et al.*, 2001). Katner and Weiss (2001) also compared rats from the HAD_1, LAD_1, AA, ANA, and Wistar lines for the release of DA from the nucleus accumbens using a no-net-flux microdialysis procedure. Their results indicated that extracellular DA levels predicted high ethanol preference, and that rats predisposed to high ethanol intakes have a greater DA response to ethanol. That genetic factors influencing development of the mesolimbic DA system may mediate the reinforcing effects of ethanol is further supported by the observation that P rats self-administer 50–200 mg% ethanol directly into the VTA, whereas the NP rats do not (Gatto *et al.*, 1994). Overall, the findings indicate that the mesolimbic DA system is activated by ethanol and suggest that genetic factors resulting from selective breeding for ethanol preference may influence the rewarding actions of ethanol within this DA pathway.

The response of DA systems in P rats made tolerant to ethanol through chronic free-choice drinking, compared with ethanol-naive P rats, is blunted when challenged by a dose of ethanol (Murphy *et al.*, 1988). AA rats allowed free-choice access to 10% ethanol for several weeks also showed a reduced DA overflow in the nucleus accumbens to a challenge dose of ethanol compared with ethanol-naive rats (Nurmi *et al.*, 1996). In recent studies, P rats drinking ethanol during daily scheduled access for several weeks were compared with ethanol-naive P rats for DA content in dialysate from the nucleus accumbens while either a DA uptake inhibitor, GBR12909, was being delivered by reverse microdialysis (Engleman *et al.*, 2000), or while delivering the DA D_2 receptor antagonist sulpiride by reverse microdialysis (Engleman *et al.*, 2001). Compared with the naive P rats, GBR12909 caused a greater increase, and sulpiride caused a lower increase, in DA content in the dialysate from the ethanol-drinking P rats. These findings are consistent with a down-regulation of DA D_2 autoreceptors as a consequence of ethanol exposure. Taken together, these findings suggest that adaptive changes are induced in the mesolimbic DA pathway in the selectively-bred rats that drink ethanol chronically, even in the scheduled access condition when daily total ethanol consumption is relatively moderate.

Pharmacological studies also support the role of dopamine in ethanol reinforcement. GBR12909, amphetamine, "a DA releaser," and bromocriptine, a D_1 and D_2 agonist, all decreased ethanol intake in P rats (McBride *et al.*, 1990). Another study showed that bromocriptine decreased operant responding for ethanol while increasing responding for water (Weiss *et al.*, 1990). Microinjection of sulpiride, a D_2 anatagonist, into the nucleus accumbens dose-dependently increased ethanol intake in P rats (Levy *et al.*, 1991). Nowak *et al.* (2000b) found that microinjections of quinpirole or quinelorane, both D_2 agonists, in the anterior VTA decreased ethanol intake, but not saccharin solution intake, in P rats during 30 min of limited access. Microinjection of the D_2 antagonist sulpiride into the anterior VTA had no effect, but it attenuated the effects of quinpirole on ethanol intake. Posterior VTA microinjections of quinpirole nonselectively decreased both ethanol and saccharin intakes. These observations suggest only certain D_2 receptors on VTA cell bodies in P rats selectively regulate DA neurons involved in ethanol drinking behavior.

Noradrenergic Systems

Most evidence for involvement of noradrenergic systems in ethanol consumption comes from pharmacological studies. Injections of desipramine, a norepinephrine uptake inhibitor, reduced ethanol intake in P rats and reduced intake of a palatable ethanol solution in NP rats (Gatto *et al.*, 1990; McBride *et al.*, 1988; Murphy *et al.*, 1985). However, other consummatory behaviors also were decreased, indicating that the effect probably reflected a general damping of consummatory behavior. Some evidence for CNS differences

is suggested with quantitative autoradiography of [3H]-tomoxetine binding sites in the locus ceruleus, which revealed that P and HAD rats had reduced binding, compared with NP and LAD rats (Hwang *et al.,* 2000). This finding may indicate a down-regulation of norepinephrine transporters in the locus ceruleus of rats predisposed to ethanol preference.

GABA Systems

Higher densities of GABAergic terminals were identified in the nucleus accumbens of ethanol-naive P and HAD lines, compared with the low ethanol-drinking NP and LAD counterparts (Hwang *et al.,* 1990), indicating that innate differences within GABAergic inhibitory neurons of the nucleus accumbens may contribute to disparate ethanol preferences of P/HAD vs. NP/LAD lines. Thielen *et al.* (1997) used quantitative autoradiography to compare ethanol-naive P and NP rats for CNS regional densities of benzodiazepine (BDZ) recognition sites that are coupled to $GABA_A$ receptors. Significantly greater GABA-enhanced flunitrazepam binding was found in P than in NP rats in several cortical areas, whereas lower binding was found in entorhinal cortex, the mediodorsal thalamus, and the posterior hippocampus. The innate functional properties of the $GABA_A$ receptor complex may be similar in the cerebral cortex for the two lines, because GABA-mediated Cl^- influx was not generally different between the P and NP lines (Thielen *et al.,* 1998). However, GABA-stimulated Cl^- influx enhanced by flunitrazepam was significantly higher in cortical microsacs from P than from NP rats that were individually housed, but not when pair housed (Thielen *et al.,* 1993), suggesting an important differential sensitivity between the lines to environmental factors of the different housing conditions.

Systemic administration of BDZ antagonists and inverse agonists that act at the GABA-BDZ receptor complex have generally been found to reduce ethanol intake or operant responding maintained by ethanol in P rats (McBride *et al.,* 1988; June *et al.,* 1998a, 1998b). Microinjections directly into CNS sites have provided additional evidence of $GABA_A$ receptor complex involvement in high ethanol-drinking behavior. Blocking $GABA_A$ receptors in the anterior VTA with picrotoxin microinjections decreased ethanol but not saccharin intake in P rats given daily 2-h limited access to ethanol and saccharin drinking solutions (Nowak *et al.,* 1998). Co-microinjection of the $GABA_A$ agonist muscimol attenuated the picrotoxin-induced decrease in ethanol intake. An operant design was used to study activation of alpha-1 receptor subunits of the $GABA_A$-BDZ receptor complex in the anterior and medial ventral pallidum (Harvey *et al.,* 2002; June *et al.,* in press). Microinjection of a selective alpha-1 agonist produced marked reductions in ethanol-maintained responding of P and HAD_1 rats. Taken together, the findings suggest that the GABA neurons that form interconnections within the mesolimbic DA system are important in regulating ethanol consumption in these alcohol-preferring rats. However, other structures may also be involved in the control of ethanol consumption. June *et al.* (2001) examined the effects of intrahippocampal infusions of an alpha-5 subunit selective BDZ inverse agonist, RY023, on lever pressing in P rats maintained by concurrent presentation of ethanol (10% v/v) and saccharin (0.05% g/v) solutions as reinforcers. RY023 dose-dependently decreased ethanol-maintained, but not saccharin-maintained, responding. A competitive BDZ antagonist, ZK 93426, reversed the RY023-induced suppression of ethanol-maintained responding, confirming that the effect was mediated via the BDZ site on the $GABA_A$ receptor complex. The RY023 effect on ethanol-maintained responding was apparently specific to hippocampal microinjections, because no antagonism by RY023 occurred after microinjections into the nucleus accumbens or the VTA. This specificity is likely because these mesolimbic DA system structures contain high densities of $GABA_A$ receptor complex BDZ alpha-1 and alpha-2 receptor subunits, but not the alpha-5 subunit.

Opioidergic Systems

Comparisons of ethanol-naive P and NP rats revealed no line differences in preproenkephalin mRNA contents in the nucleus accumbens, striatum, amygdala, or hypothalamus, but intragastric infusion of 2.5 g/kg ethanol increased the mRNA content in the nucleus accumbens of P rats, but not in NP rats (Li *et al.,* 1998). This finding suggests that the accumbens enkephalinergic system of the P line is more sensitive to the effects of ethanol. Using quantitative autoradiographic methods, McBride *et al.* (1998) reported higher densities of μ-opioid receptors in the CNS limbic areas of the P relative to NP rats, including the nucleus accumbens shell and core. These differences are in general agreement with differences observed between the AA and ANA lines (DeWaele *et al.,* 1995). Overall, the results indicate that innate differences exist between high and low ethanol drinkers within the opioid sys-

tems of the VTA and nucleus accumbens, and that ethanol drinking can alter some of these systems, suggesting that these differences may be factors contributing to the disparate ethanol drinking behaviors of the AA and ANA rats and also the P and NP rats. The endogenous opioid system has been implicated in ethanol reinforcement with a number of studies showing that opioid antagonists reduce ethanol intake. Naltriben, a delta-2 receptor antagonist, has been shown to reduce ethanol intake (June *et al.,* 1999); but the highest dose tested also reduced saccharin intake. Naloxone, which blocks μ-opiate receptors, reduces ethanol intake in HAD (Froehlich *et al.,* 1987, 1990), P, (Badia-Elder *et al.,* 1999b; Overstreet *et al.,* 1999) and AA (Sinclair, 1990) rats, but appears to be most efficacious in reducing limited-access ethanol intake, with only a modest reduction in 24-h intake (e.g., Overstreet *et al.*, 1999). These authors also reported that P rats displaying tolerance to chronic naloxone displayed increased [^{3}H]-DAMGO binding to μ-opiate receptors. Naloxone also has been shown to reduce ethanol intake in P rats under relapse conditions (i.e., after 2 weeks of ethanol deprivation; Badia-Elder *et al.,* 1999b). Interestingly, the combination of the 5-HT_3 receptor antagonist ondansetron with the μ-opioid antagonist naltrexone has recently been reported to act synergistically to improve drinking outcomes in alcoholics characterized as having a biological predisposition to alcohol abuse and a range of impulse control disorders (Ait-Daoud *et al.,* 2001).

Corticotropin-Releasing Factor and Neuropeptide Y

Levels of corticotropin-releasing factor (CRF) were found to be lower in the amygdala, hypothalamus, prefrontal cortex, and cingulate cortex in P than in NP rats (Ehlers *et al.,* 1992). In a more recent study, CRF levels in HAD/LAD and P/NP rat lines were measured in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala, and the only significant finding was that P rats had lower CRF levels in the amygdala relative to NP rats (Hwang *et al.*, 2001). These findings are of interest because CRF levels in the amygdala are increased during restraint stress and ethanol withdrawal (Merlo-Pich *et al.,* 1995), suggesting that this neuropeptide may be involved with the aversive effects of ethanol.

Neuropeptide Y (NPY) immunoreactivity was assessed in the amygdala, hippocampus, and frontal cortex in P and NP rats, and P rats were found to have lower levels of NPY in these brain regions (Ehlers *et al.,* 1998). A second study assessed NPY immunoreactivity in the paraventricular and arcuate nuclei of the hypothalamus and central nucleus of the amygdala and found greater NPY immunoreactivity in the paraventricular nucleus of P rats, compared with NP rats, but reduced levels in HAD rats, compared with LAD rats (Hwang *et al.,* 1999). However, there were reduced levels of NPY immunoreactivity in the central amygdala of P and HAD rats, compared with NP and LAD rats, respectively, suggesting that NPY in the paraventricular nucleus may not affect ethanol intake, whereas reduced NPY in the central amygdala may contribute to the high ethanol consumption of P and HAD rats. Given the differences in NPY levels found in the selected lines combined with the identification of a quantitative trait locus (QTL) for the NPY precurser in F_2 progeny of a P x NP rat cross (Carr *et al.*, 1998; described in more detail below), research interest was stimulated for examining the role of NPY in alcohol drinking behavior. Subsequently, the effects of intracerebroventricular administration of NPY on ethanol intake were examined in P, NP, and Wistar rats (Badia-Elder *et al.,* 2001) and in HAD and LAD rats (Badia-Elder *et al.,* 2000). NPY decreased ethanol intake in P and HAD rats and to a lesser extent in LAD rats. Administration of NPY also increased ethanol-induced sedation in P rats (Badia-Elder *et al.,* 2001). These observations were consistent with those of Thiele *et al.*, (1998), who reported increased ethanol consumption and decreased sensitivity to the sedative/hypnotic effects of ethanol in NPY-deficient mice as compared to wild type mice. In contrast, transgenic mice overexpressing the NPY gene had lower ethanol preference and greater sensitivity to the sedative/hypnotic effects of ethanol as compared to wild type mice. Taken together, these findings support the contention that NPY may play a role in a genetic predisposition for excessive alcohol drinking behavior.

Local Cerebral Glucose Utilization (LCGU)

The LCGU technique utilizes a radiolabeled analogue of glucose to assess regional neuronal activity in brain. The technique has been used to compare regional CNS metabolic activity in various lines of animals that are ethanol-naive or to compare naive animals vs. animals exposed to alcohol in conditions such as prolonged drinking. Recent work comparing LCGU rates in the CNS of naive rats from the selected lines has found that LCGU rates are generally higher in P compared with NP rats, suggesting the CNS of P rats is in-

herently more active than that of NPs (Smith *et al.*, 2001a). This finding agrees with observations that the P rats are behaviorally more active than NP rats and are more reactive in a novel environment (Waller *et al.*, 1986; Nowak *et al.*, 2000a). Begleiter and Projesz (1999) have proposed that individuals with genetic vulnerability to alcohol abuse exhibit an innate hyperexcitable CNS state, and that excessive alcohol intake may attenuate this excited state toward normal control levels. In P rats, chronic ethanol intake reduces CNS functional activity as measured by LCGU (Smith *et al.*, 2001b). Recovery of LCGU rates, after abstinence for 2 weeks following chronic scheduled access (4 h/day) to ethanol, occurs in only some CNS regions, but no recovery in LCGU rates was seen in the VTA and medial prefrontal cortex (Smith *et al.*, 2001b). Overall, these results suggest that chronic ethanol drinking by P rats can produce long-lasting alterations in functional activity in several limbic structures. Contrary to the differences in LCGU rates found between P and NP rats, there was only a nonsignificant trend toward higher metabolic rates in the HAD vs. the LAD replicate lines (Learn *et al.*, 2000).

GENOTYPING THE SELECTED LINES

The Indiana selected lines and inbred rat strains provide homogeneous populations that may be used for efficient genetic determinations to isolate candidate chromosomal regions and loci. An inbred strain displaying the phenotype will be homozygous at both the loci underlying the phenotype of interest, as well as at most of the remaining genome. It is unlikely, however, that all loci underlying a particular phenotype can be identified using a single inbred strain, because some loci influencing the trait may not have segregated in the initial stock from which the rats were derived, and, in other instances, random fixation during selection may have resulted in the loss of relevant QTLs. This makes it increasingly important to develop and analyze multiple models of phenotypic traits of interest to ensure that all major loci contributing to the traits will be identified.

Since the 15th generation, the P and NP rats have been phenotypically stable. For the purpose of developing inbred strains, inbreeding was initiated after 30 generations of selection. Without selecting, the ethanol preference phenotype has remained stable in the inbred P and NP substrains. At the 19th generation of inbreeding, reciprocal crosses of the inbred P and NP animals were performed and 384 F_2 progeny were produced (Carr *et al.*, 1998). Following a genome screen that employed selective genotyping of the very high and very low drinking F_2 rats, a QTL on chromosome 4 was identified with an LOD score of 8.6 (Carr *et al.*, 1998). This QTL acted in an additive fashion, with F_2 rats homozygous for the NP genotype having the lowest drinking scores, those homozygous for the P genotype having the highest drinking scores, and those F_2 animals heterozygous at the chromosome 4 markers having intermediate drinking scores. To narrow the critical interval, eight additional markers were genotyped in this region of chromosome 4 (Bice *et al.*, 1998). The maximum LOD score increased to 9.2, with a 95% confidence interval of only 12.5 cM. The QTL mode of action remains consistent with an additive effect and accounts for 11% of the phenotypic variability. Congenic lines are currently under development, which will result in further fine mapping and localization of the chromosome 4 QTL influencing ethanol preference.

Within the candidate interval on rat chromosome 4 is the prepro-neuropeptide Y (prepro-NPY) gene. The NPY gene is an interesting candidate because NPY, which is involved in the regulation of appetitive behavior and has been shown to be anxiolytic, is being investigated for its role in modulating ethanol intake (as described above). However, sequencing of the four exons of the prepro-NPY gene in DNA from the P and NP parental rats used to create the P x NP F_2 progeny did not identify any nucleotide differences. Further sequencing of over 2000 bp of noncoding region revealed two polymorphisms. One polymorphism was in the 3rd intron (35 bp 5′ of the 4th exon), with a C in inbred NP rats and a T in inbred P rats. The other polymorphism was in the 3′-UTR (82 bp downstream of the 4th exon), with a G in NP and an A in P. Future experiments will determine whether these polymorphisms account for the differential behavior between the two lines.

QTLs identified on chromosome 8 (LOD = 2.2) and chromosome 2 (LOD = 2.5) are syntenic to mouse chromosomes where QTLs for ethanol preference have been previously identified. A QTL on mouse chromosome 9 (syntenic with rat chromosome 8), has been provisionally identified in the B x D RI mice (Phillips *et al.*, 1994). In a short-term selection, using F_2 mice derived from the same parental strains, there was suggestion for linkage with ethanol preference in the same region (Belknap *et al.*, 1997). Two receptor genes, Drd2, encoding the dopamine D_2 receptor, and Htr1b, encoding the serotonin 1b receptor, appear to be within or near this region of rat chromosome 8.

A QTL for ethanol preference has been identified on mouse chromosome 2 (Melo *et al.*, 1996; Phillips

et al., 1994; Rodriguez *et al.,* 1995) that is syntenic with rat chromosome 3. Contrary to the linkage finding in the rat (Carr *et al.,* 1998; Bice *et al.,* 1998), the QTL identified on chromosome 2 by Melo *et al.* (1996) is male-specific. The voltage-gated sodium channel Scn1a is located within this QTL region and is a possible candidate gene.

To identify additional QTLs contributing to ethanol consumption that were not segregating in the P/NP rats, a genome screen was performed in the selectively bred HAD and LAD lines. Six reciprocal crosses of HAD_1 and LAD_1 rats, from the 26th generation of selection were performed. F_1 progeny from each of the reciprocal crosses were intercrossed to create 459 F_2 rats (Foroud *et al.,* 2000). This is the first published study to utilize noninbred animal models for the identification of QTLs underlying ethanol consumption. In the selected, noninbred HAD and LAD lines, it is likely that the rats are homozygous for the major QTLs influencing ethanol preference. However, because the original parental lines were not inbred, they should still be largely heterozygous at the microsatellite markers tested.

The segregation of markers and QTLs were studied in several generations and across several matings to identify the QTLs in the noninbred HAD/LAD rats. The HAD and LAD founders were crossed to create F_1 progeny, which were then intercrossed to generate the F_2 sample consisting of six families. Because the parental generation is heterozygous for the microsatellite markers tested, the F_1 rats are not genetically identical as they are in the inbred study design. This study design is completely analogous with the collection of human pedigree data, in which markers are segregating in all generations and individuals are never homozygous for all marker loci. One difference between the human data and the noninbred HAD/LAD model is the assumption regarding QTL fixation. Whereas it is extremely unlikely that a human population could be found in which all or even most of the relevant QTLs for a phenotype have been fixed, selection for ethanol preference in the HAD/LAD model is likely to have fixed most of the QTLs of major effect, increasing the power for QTL detection.

To date, five chromosomal regions (1, 5, 10, 12, and 16) were identified with LOD scores greater than 2.0 in the selectively genotyped sample. Genotyping of the entire sample of 459 F_2 progeny produced maximum LOD scores of 3.5 on chromosome 5, 2.4 on chromosome 10, 4.7 on chromosome 12, and 2.9 on chromosome 16 (Foroud *et al.,* 2000). The effect of gender was examined for each QTL region. When male and female F_2 rats were analyzed separately for the QTL on chromosome 12, there was greater evidence of linkage in the males (LOD = 5.0) compared to the females (LOD = 1.6). For all other QTLs, there was no evidence of gender-specific effects. Pedigree effects were also examined for each QTL, and it was determined that its effect was not equal in all families (Foroud *et al.,* 2000).

Rat chromosome 12 is homologous to the telomeric portion of murine chromosome 5, beginning at approximately position 65 cM. There is a promising candidate gene in this region, neuronal nitric oxide synthase 1 (NOS1). Studies have shown that drugs that inhibit nitric oxide synthase produce a reduction in ethanol intake (Calapai *et al.,* 1996; Lallemand and DeWitte, 1997). The QTL on chromosome 12 also appears to correspond to human chromosome 12q24.2-24.3; however, no evidence of an alcoholism susceptibility locus has been reported on this chromosome in human studies. The QTL on rat chromosome 5 also exceeded an LOD score of 3.0. This region of rat chromosome 5 corresponds to murine chromosome 4 and human chromosome 8q23-q24. Within the murine region, an interesting candidate gene is proenkephalin, which is part of the opioid pathway. Evidence suggests that enhanced responsiveness of the enkephalinergic system to ethanol is associated with, and may be functionally involved in, mediating high ethanol-drinking behavior (Li *et al.,* 1998; see section above).

Evidence of linkage to chromosome 4, identified in the P x NP F_2 sample (Carr *et al.,* 1998; Bice *et al.,* 1998), was not observed in the HAD_1 x LAD_1 F_2 progeny. One likely explanation for this discordance is the different origin of the stock rats from which the P/NP and HAD_1/LAD_1 lines were derived. The P and NP lines were derived from a randomly bred closed colony of Wistar rats (Wrm: WRC(WI)BR) (Li *et al.,* 1981), whereas the HAD_1 and LAD_1 lines were derived from the N/Nih heterogeneous stock (Li *et al.,* 1993). Although the N/Nih stock did have Wistar-derived rats within the initial 8 strain cross (WN/N, WKY/N and MR/N), these Wistar rats were of quite varied ancestry and were already inbred (Hansen and Spuhler, 1984). Thus, they already had randomly fixed ethanol-related QTLs that had been segregating in their outbred Wistar ancestors. Therefore, the founders from which the P/NP and HAD_1/LAD_1 lines were selected likely segregated unique alcohol-related QTLs, and consequently it is not unexpected that novel QTLs would be identified in the P x NP and HAD_1 x LAD_1 F_2 crosses.

A genome screen for saccharin consumption was performed using the selectively genotyped P x NP F_2 rats, chosen for extreme ethanol consumption (Foroud *et al.,* 2002). Evidence for a possible QTL on chromosome 16 was observed in the selectively genotyped sample. Genotyping of the full sample resulted in an LOD score of 4.0 and a QTL that acts in a dominant manner (NP allele dominant), with rats homozygous for the P allele having significantly higher saccharin consumption compared to those that are either heterozygous or homozygous for the NP allele. This QTL is not in the same region of chromosome 16 as the QTL identified in the HAD_1/LAD_1 for ethanol consumption.

To improve the power to detect QTLs influencing saccharin preference, 73 markers were genotyped in the 90 P x NP F_2 with extreme saccharin preference to complete a genome screen to identify additional saccharin QTLs. Additional LOD scores greater than 2.0 were found on chromosomes 3, 15, and 18 in the sample selectively genotyped for extreme saccharin intake. On chromosome 3, the maximum LOD score in the full sample was 2.8 with saccharin preference. This QTL acts in a dominant fashion, with rats homozgyous for the P allele consuming significantly more saccharin than either the heterozygotes or NP homozygotes. This QTL appears to overlap with a QTL identified for ethanol consumption in the P and NP lines. Of interest, this region of rat chromosome 3 is syntenic with mouse chromosome 2, where a QTL influencing ethanol preference has been previously reported. Additional analyses for pleiotropic effects have been performed using QTL Cartographer (Basten *et al.,* 1994) to examine the effect of the chromosome 3 QTL using both the ethanol and saccharin phenotypes simultaneously. These analyses support a locus with pleiotropic effects on both phenotypes. The QTL on chromosome 15 (maximum LOD = 2.8) also acts in a dominant fashion, with rats homozygous for the P allele drinking significantly more than the heterozygous and homozygous NP rats. The QTL on chromosome 18 (maximum LOD = 2.7) has the opposite QTL action, with rats homozygous for the NP allele consuming significantly more saccharin than the heterozygous or homozygous P rats (Foroud *et al.,* 2002).

The 459 HAD_1 x LAD_1 F_2 animals were phenotyped for saccharin consumption, and a highly significant correlation was found between ethanol and saccharin consumption. The same 151 rats used in the ethanol preference genome screen were also used to perform a genome screen for saccharin preference and consumption. A LOD score of 3.2 for saccharin preference was obtained in the full sample near the marker D4Mit7, where a major QTL for ethanol consumption was mapped in the P and NP rats (Bice *et al.,* 2000).

In summary, the analytic and molecular techniques have now been developed to make it feasible to begin mapping genes influencing alcohol preference and other related phenotypes in animal lines selectively bred for alcohol preference. QTLs influencing alcohol preference have been identified on chromosomes 3, 4, and 8 in the inbred iP/iNP rats and on chromosomes 5, 10, 12, and 16 in the noninbred HAD1/LAD1 rats. QTL analysis in multiple selected inbred strains and noninbred lines originally selected from two different stocks will increase the probability of identifying and confirming chromosomal regions influencing alcohol preference in the rat. The genes identified within these QTLs will be excellent candidates for human studies.

SUMMARY AND CONCLUSIONS

Selectively bred rats have served as valuable tools and models in basic research on alcohol abuse and alcoholism. Work with the Indiana selected lines has provided significant insight into behavioral and neurobiological phenotypes that may result in abnormal ethanol-seeking behavior. One possible shortcoming in the overall body of research may be the issue of whether differences observed between alcohol preferring and nonpreferring lines of rats reflect a divergence from the foundation stock mostly in the direction of preference or in the direction of nonpreference. For various reasons, most studies have not compared the selected lines against the appropriate foundation stock or unselected control line. For example, it has consistently been observed that alcohol-preferring and -nonpreferring lines differ in sensitivity to the aversive effects of alcohol, but it is unclear whether this difference promotes high alcohol drinking due to low sensitivity or, conversely, promotes low alcohol drinking due to enhanced sensitivity.

McBride and Li (1998) point out that a consistent finding with selected lines has been differences in the mesolimbic DA system, a system believed by many to, at least partly, mediate excessive drug use. Among the rat lines selectively bred for disparate voluntary ethanol consumption, some consistent differences in the CNS have been reported for three different pairs of rat lines, the P and NP lines, the HAD and LAD lines, and the sP and sNP lines. Thus, discovered phenotypic differences in neurochemical systems, such as apparent imbalances within serotonin or dopamine systems, may indicate

primary contributors to excessive ethanol consumption, especially because many human studies have also implicated these same systems (Cloninger, 1987; 1996). Experimental evidence accrued via comparisons between high and low drinking lines, and common attributes that appear to occur with high ethanol-drinking behavior that are discovered by comparisons between different selected line pairs, add credence to the importance of these common systems and behaviors. This is a fundamental approach in which hypotheses regarding associations with excessive ethanol intake are generated and tested. Excessive ethanol intake and abnormal ethanol-seeking behavior presumably involves multiple genetic determinants leading to multifaceted phenotypic substrates, including various protein products, multiple neurotransmitter systems in different CNS regions, and ultimately a variety of behavioral phenotypes. The frequency with which the identified genetics and phenotypes are associated with the ethanol preference and excessive ethanol consumption likely depends on a complex interaction of the specific inherited variables, as well as an interaction with the prevailing environment. Conceivably, lines of rats with high ethanol preference can emerge from selective breeding and can serve as an adequate model, without the particular line exhibiting all genotypes and resultant phenotypes that could contribute to a predisposition for ethanol preference and excessive ethanol intake. Over the past few years, genotyping of the selected lines, the derived inbred lines, and F_2 generations has expanded, but a clear future direction is to continue documenting the genetic profiles that apparently lead to a propensity for abnormal ethanol-seeking behavior.

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