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Quantitative Trait Loci (QTL) Applications to Substances of Abuse: Physical Dependence Studies with Nitrous Oxide and Ethanol in BXD Mice

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Recombinant inbred (RI) mouse strains were developed primarily as a tool to detect and provisionally map major gene loci—those with effects large enough to cause a bimodal distribution in the trait of interest. This implied that progress toward gene mapping was possible only for gene loci accounting for at least half of the genetic variance. More recently, QTL (quantitative trait loci) approaches have been advanced that do not require bimodal distributions and are thus applicable to a much wider range of phenotypes. They offer the prospect of meaningful progress toward detecting and mapping minor as well as major gene loci affecting any trait of interest, provided there is a significant degree of genetic determination among the RI strains. This paper presents a review of RI gene mapping efforts concerning phenotypes related to drug abuse and presents new data for studies now in progress for nitrous oxide and acute ethanol withdrawal intensity. These two studies exemplify several strengths and limitations of the RI QTL approach.

KEY WORDS: Quantitative trait loci (QTL); BXD; recombinant inbred strains; C57BL/6; DBA/2; nitrous oxide; ethanol; withdrawal syndromes; chromosome mapping; drug abuse; mouse.

INTRODUCTION

Recombinant inbred (RI) strains are the fully inbred descendants of an F_2 cross between two standard inbred strains. Maximal inbreeding has served to redistribute the original F_2 genetic variance, so that it now exists almost entirely between strains and is almost absent within strains (Falconer, 1989). Since an estimated four crossover events have occurred per 100-centimorgan (cM) chromosome length in the course of RI strain development (Taylor, 1978), a considerable amount of linkage disequilibrium has been fixed in these strains. Thus, each RI strain represents chance recombinations of the progenitor chromosomes in a fixed (homozygous) state (Bailey, 1981). There are presently about two dozen sets or series of RI strains, each derived from a unique pair of progenitor inbred strains (Taylor, 1989).

The RI strains were developed primarily as a tool in detecting and mapping major gene loci (Bailey, 1981). When RI strain means on a given trait are found to fall in a bimodal distribution, i.e., some RI strains resemble one progenitor and some resemble the other, and none are intermediate, this is presumptive evidence for control of that trait by a single major gene locus. Comparison of the strain distribution pattern (SDP) for that trait (i.e., which RI strains resemble one or the other progenitor strain) can be made with strain distribution patterns (SDPs) for known marker loci previously mapped to a particular chromosome region. A close match in SDPs between the unknown locus and a marker locus would suggest linkage, and thus allow provisional mapping to the chromosome region of the marker (Bailey, 1981; Taylor, 1978).

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In the 1970s, this major gene approach was attempted in a number of measures of morphine, cocaine, amphetamine, phenylethylamine, scopolamine, and ethanol sensitivity in the CXB (Bailey) RI series, comprised of seven RI strains derived from the BALB/cBy and C57BL/6By progenitor strains (reviewed by Broadhurst, 1978; Shuster, 1984, 1986, 1989; Frischknecht *et al.*, 1988; Belknap and O'Toole, 1991; Seale, 1991). Drug-induced change in activity was the primary behavior studied, but analgesia (morphine) and opioid receptor binding using ^{3}H-naloxone were also measured. These pioneering efforts clearly demonstrated a substantial degree of genetic determination of these drug response traits. However, most traits were unimodal rather than bimodal, indicating polygene (two or more minor genes) rather than major gene control. Thus, gene mapping efforts did not appear warranted. One study that was successful in gene mapping concerned a major gene influence on ethanol-induced reductions in locomotor activity (Oliverio and Eleftheriou, 1976). A bimodal distribution was seen among the CXB RI strains, suggesting a major gene locus effect. The existence of a major gene was confirmed by work with congenic strains and in a backcross population. The locus, named *Eam* (ethanol activity modifier), was mapped to chromosome 4, in a region now known as the *H-16* region. This research group also found evidence for a major locus effect on scopolamine-induced hypoactivity in the CXBs (Oliverio *et al.*, 1973), which they provisionally mapped to the *H-2* region of chromosome 17 with the aid of congenic strains. Phenylethylamine-induced hypoactivity showed a bimodal distribution of either "responders" and "nonresponders" to a high fixed dose of this amphetamine-like compound (Jeste *et al.*, 1984), but no attempt to map this presumed major gene was made at the time of the original report. Recently, we compared this SDP with the CXB markers listed by Taylor (1989). A perfect SDP match was found with the *H-27* locus on chromosome 5 ($p < .01$), indicating that this may be the site of the presumed major gene locus. This finding needs to be confirmed using a different genetic model (see below).

This "classical" type of RI gene mapping strategy requires that the trait of interest be bimodally distributed among the RI strain means, with one progenitor strain in each mode. Accurate construction of a binary SDP for a new trait can be achieved only if this requirement is met. An interesting question is how large a single-locus effect must be in order to cause a bimodal distribution in a trait under study. We used a computer simulation to begin to answer this question by constructing two allelic groups of strains at a single hypothetical locus, each normally distributed with a population standard deviation and variance of 1.0 and $n = 10$ strains per group. Since strain means were used, all variation between strains reflects primarily genetic variation. The variability *between* the two allelic groups reflects the effects of allelic variation at our hypothetical locus, while the variability *within* the two allelic groups reflects genetic variation at other loci. The two allelic groups were increasingly separated until a bimodal distribution was evident, in terms of both the frequency histograms and the Epanechnikov kernel densities (Silverman, 1986; Wilkinson, 1990). The results are shown in Fig. 1 for varying differences between the two allelic group means (twice the average effect of a gene substitution) and the proportion of the genetic variance accounted for by our hypothetical locus (R^2). For differences of 1 or 2 SD units, the overall distribution remained unimodal, although increasingly platykurtic. At the 3.0 SD separation, bimodality is apparent, but there is still some overlap (2 strains of 20) between the two allelic groups that would likely lead to typing errors in SDP construction. With a 4.0 SD allelic group difference, bimodality is marked, without overlap or ambiguity in typing. Thus, the threshold for apparent bimodality appears to lie between 2.0 and 3.0 SD units of separation, corresponding to 53 and 72% of the total genetic variance (R^2), respectively. Under these conditions, a single major locus would have to account for at least half of the genetic (between strain) variance in order to induce apparent bimodality. At least three-fourths of the genetic variance would have to be accounted for before the SDP can be unambiguously constructed. It is therefore no surprise that bimodal distributions are more the exception than the rule in pharmacogenetic research, especially when organismic traits, with their typically polygenic inheritance, are under study.

More recently, emphasis has shifted to the BXD RI series derived from the C57BL/6 (B6) and DBA/2 (D2) progenitor strains (Gora-Maslak *et al.*, 1991; Plomin and McClearn, 1993). Compared to the CXB RI series, there are more RI strains ($n = 24$) and a much larger set of marker loci (Taylor, 1989). Regarding drugs subject to abuse, the pioneering studies were those by Crabbe *et al.* (1983) on a

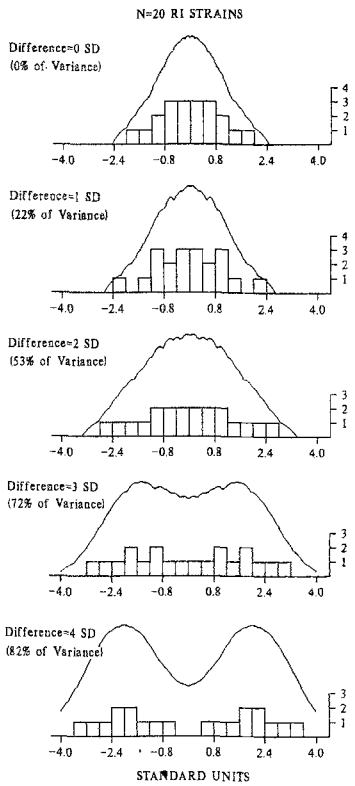

Fig. 1. Effects of increasing genetic differences between two allelic groups at a hypothetical locus in terms of SD units (within-group SD = 1.0) and the proportion of the total genetic variance (R^2) accounted for by the locus. In all panels, it was assumed that the within-allelic group variance, reflecting genetic variability at other loci, was normally distributed with a SD and variance of 1.0. Rankits were used to construct the individual strain means consistent with the normal distribution (Sokal and Rohlf, 1981). A total of 20 RI strains was simulated, 10 per allelic group. Both frequency histograms and Epanechnikov kernel densities are shown. The latter is a nonparametric continuous function that often portrays the distribution somewhat more accurately than the inherently discontinuous histogram (Silverman, 1986). A window width (tension) of 0.275 SD was assumed for the kernel densities. As can be seen, a locus would have to account for at least half of the genetic variance before a bimodal distribution would be evident.

series of ethanol response measures and the study by Seale *et al.* (1985) on amphetamine hyperthermia. A study of morphine voluntary consumption (Phillips *et al.*, 1991; Gora-Maslak *et al.*, 1991) and a series of morphine sensitivity measures involving activity, analgesia, hypothermia, and Straub tail (Belknap and Crabbe, 1992) have been reported more recently in BXD mice. Ethanol acceptance has been retested very recently with much increased sample sizes (Plomin and McClearn, this issue). While most traits were again unimodal, ethanol acceptance (ethanol drinking), ethanol withdrawal intensity, morphine hypoactivity, and amphetamine hyperthermia did show apparent bimodal distributions indicative of possible major gene locus effects. Using QTL methods described below, the major gene affecting amphetamine hyperthermia appears to be located in the *Lamb-2* region of chromosome 1 (Gora-Maslak *et al.*, 1991) based on the data reported by Seale *et al.* (1985). The correlation between this trait and allelic variation at the *Lamb-2* locus was 0.96 ($p < .00002$). For the other three apparently bimodally distributed traits, no one chromosome region emerged as the best candidate for either of these possible major gene effects. However, several candidate regions have been identified, such as the *Ltw-4* region of chromosome 1 for ethanol acceptance (Crabbe *et al.*, 1983; Goldman *et al.*, 1987; Gora-Maslak *et al.*, 1991). Those for ethanol withdrawal and morphine activity are under investigation in our laboratories.

A relatively new RI set of 27 strains has been developed from a cross between SS (short sleep) and LS (long sleep) selectively bred mice (DeFries *et al.*, 1989; Wehner *et al.*, 1992). Work with several drugs of abuse has been carried out regarding anesthesia (loss of the righting reflex) and activity (reviewed by Erwin and Jones, this issue), but the gene mapping potential of this RI series awaits the development of a suitable number of marker loci. With the recent development of methods to detect microsatellite and other highly polymorphic DNA sequences (e.g., Dietrich *et al.*, 1992), it is likely this limitation will be only temporary (Johnson *et al.*, 1992).

QTL METHODS IN THE BXD RI STRAINS

QTL methods seek to make progress toward the detection and chromosome mapping of minor as well as major gene loci. Dramatic progress in methodology has occurred within the past 5 years (reviewed by Plomin *et al.*, 1991; Gora-Maslak *et al.*, 1991). However, only one version of QTL analysis is specifically tailored to existing mouse RI strains, particularly the BXD RI series. We used an adaptation of this approach originally advanced by the Penn State group (Plomin and McClearn, this issue) in the present work.

More powerful QTL analyses have been developed for RFLP markers in segregating F_2 and backcross populations (e.g., Lander and Botstein,

1989; Rise *et al.*, 1991). Their greater power derives largely from the greater number of genotypes available for linkage testing, which is limited only by the number of individual animals one is able to test. In contrast, the number of genotypes available in the BXD RI series is limited to 24, the number of available strains. This limitation is somewhat mitigated in RI strains because each genotype (strain) can be replicated any number of times, permitting any desired degree of accuracy in assessing the genotype based on the phenotype. In contrast, in a segregating population, each genotype is represented by an individual mouse, and measurement accuracy can be a problem if the trait of interest shows a low reliability (repeatability) of measurement or a low heritability.

In our analyses, the first step was to determine the strain means for a trait of interest and to correlate them with the allelic distributions of 352 previously mapped polymorphic marker loci with distinct SDPs (Gora-Maslak *et al.*, 1991; Plomin *et al.*, 1991). These markers were obtained from the catalog of BXD marker loci reported by Taylor (1989) and updated from a recent compilation (October, 1991) kindly provided to the authors by Dr. Benjamin Taylor of The Jackson Laboratory. For each marker gene locus, an arbitrary value of 0 was assigned to strains bearing the B6 allele, and a 1 to those bearing the D2 allele, and the product moment correlation coefficients (r) were determined between each marker locus and a trait of interest. Calculated in this way, these r values are point biserial correlations. The statistical significance of r is the same as the regression of phenotype on gene dosage for a particular locus. In this case, there are only two values for gene dosage (number of D2 alleles), 0 and 2. Moreover, for any one locus in the BXD series, the significance level (p value) of r is the same as that given by a two-tailed t test between the strains bearing the B6 allele and those bearing the D2 allele for the trait of interest.

THE PROBLEM OF MULTIPLE COMPARISONS

A statistical problem arises whenever multiple comparisons are made between a trait of interest and each of a large series of marker loci, in terms of either matching SDPs, as in the original RI approach, or the correlation coefficients used here (Belknap, 1992). Because of the many comparisons (correlations) that were calculated in the BXD QTL analysis ($N = 352$), it is highly probable that at least one will be significant ($p < .05$) due to chance alone. Put in other words, the p values calculated for a single comparison (single-hypothesis test) do not provide adequate protection against Type I errors when multiple comparisons (significance tests) are made (Miller, 1981; Rice, 1989).

Two general strategies are available for dealing with this problem. First, confirmation of the BXD data can (and should) be sought by using other genetic models to determine which candidate QTLs from the BXD data can be independently supported. One suggestion (Belknap and Crabbe, 1992) is to use the BXD data as a screen to identify a handful of candidate QTL map locations; these would then be specifically tested in other RI series, standard inbred (non-RI) strains (Goldman *et al.*, 1987), congenic lines (Bailey, 1981), or F_2 or backcross populations (Lander and Botstein, 1989; Rise *et al.*, 1991; Neumann and Collins, 1991; Johnson *et al.*, 1992). If confirmation testing is to be done, then $p < .05$ (single test) may be appropriate for the BXD results, since it leads to lower Type II error rates (failing to detect important QTLs) compared to more stringent alpha values. In this case, the primary protection against Type I errors would reside in the confirmation test(s).

Second, if the BXD data are to stand alone, without confirmation from other genetic models, then a correction is needed for the multiple comparisons (correlations) calculated for the marker loci (Belknap, 1992). The simplest way is the Bonferroni correction (k), where the observed level of significance for individual marker loci (single test) is multiplied by the number of independent (orthogonal) determinations (Miller, 1981; Rice, 1989). Employing an appropriate Bonferroni correction protects against *even one* fortuitous correlation arising among the markers (Lander and Botstein, 1989). There are several approaches to estimating an appropriate value of k. We chose a value of 65 for this marker set based on the rationale presented by Belknap (1992). When $k = 65$, a $p < .0008$ (single-test) alpha becomes $p < .05$ (multiple test) after correction, i.e., $0.0008 \times 65 = 0.05$. Higher values will be needed for more complete marker sets and lower values for less complete ones, since the number of fortuitous correlations expected is a direct function of the proportion of the genome covered

by a marker set. For the published BXD marker set (Taylor, 1989), comprised of 142 mapped marker loci, $k = 40$ was suggested (Belknap, 1992).

ACUTE ETHANOL AND NITROUS OXIDE WITHDRAWAL STUDIES

Nitrous oxide (N_2O) is a colorless, nearly inert gas with analgesic, hypnotic, anesthetic, anticonvulsant, and euphorigenic properties (Eger, 1985; Smith and Wollman, 1985). Like ethanol, nitrous oxide causes loss of the righting reflex in mice, and tolerance and cross-tolerance with ethanol on this measure have been demonstrated (Koblin *et al.*, 1980). Selective breeding has successfully produced two lines of mice with either very low or very high sensitivity, respectively, to the anesthetic (loss of righting reflex) effects of nitrous oxide administered under hyperbaric conditions. These lines, named HI and LO, show similar differences in sensitivity to ethanol and to most barbiturates (Koblin *et al.*, 1982, 1984), indicating that there may be commonalities in genetic influences, and their mediating mechanisms, between anesthesia produced by nitrous oxide and these depressant drugs.

Physical dependence on nitrous oxide in mice has also been demonstrated using handling-induced convulsions (HIC) as the primary withdrawal sign (Smith *et al.*, 1979; Ruprecht *et al.*, 1983; Belknap *et al.*, 1987). While many gaseous or volatile anesthetics produce loss of the righting reflex in mice (e.g., nitrous oxide, halothane, cyclopropane, ethylene, isoflurane, enflurane, methoxyflurane), only a subset (nitrous oxide, cyclopropane, ethylene) has been observed to produce HIC upon withdrawal following short (up to 1-h) exposures to these agents (Koblin *et al.*, 1982; Smith *et al.*, 1979). Those agents that produce HIC upon withdrawal all have relatively rapid rates of elimination (Eger, 1985; Smith *et al.*, 1979; Wollman and Dripps, 1970). In clinical use, anesthetics with rapid rates of elimination tend to have relatively higher risks of causing withdrawal (emergent) hyperexcitability in surgical patients compared to those with slow rates of elimination (Smith *et al.*, 1979; Harper *et al.*, 1980; Price and Dripps, 1970).

WSP (Withdrawal Seizure-Prone) and WSR (Withdrawal Seizure-Resistant) mice were selectively bred for high or low intensity, respectively, of HIC following chronic (3-day) ethanol vapor exposure. These two sets of oppositely selected lines differ by at least 10-fold in the more recent generations (Phillips and Crabbe, 1991; Crabbe and Phillips, this issue). Kosobud and Crabbe (1986) showed that WSP and WSR mice also differ similarly following withdrawal from a single acute dose of ethanol (4.0 g/kg, i.p.). This finding allowed us a model of physical dependence on ethanol that did not require the use of pyrazole, and testing could be carried out in a single day. The acute withdrawal paradigm has been shown to apply equally well to several other drugs, i.e., diazepam, pentobarbital, t-butanol, and acetaldehyde (Crabbe *et al.*, 1991), where WSP mice also show much higher acute withdrawal intensities than do WSR mice. WSP and WSR mice also differ in a parallel manner when made physically dependent on nitrous oxide (Belknap *et al.*, 1987). These findings suggest that ethanol has commonalities in the genetic determination of withdrawal intensity with a number of other agents.

An ongoing project in our laboratories is to test the BXD strains for their severity of HIC due to acute ethanol withdrawal and to compare this with HIC due to nitrous oxide withdrawal in the same animals. These studies are still in progress, but the results to date well-exemplify the value of the QTL approach. The drug administration methods have been described previously by Kosobud and Crabbe (1986) for ethanol and by Belknap *et al.* (1987) for nitrous oxide. Briefly, animals were exposed to a single acute 4.0 g/kg i.p. dose of ethanol, and withdrawal severity was monitored at frequent intervals by scoring for HIC. Peak HIC scores, indexing an acute withdrawal reaction, typically reach a maximum at about 7–10 h postinjection, when the ethanol has been largely eliminated. Two to three weeks later, these animals were exposed to a mixture of 75% nitrous oxide and 25% oxygen for 1 h and withdrawn by returning them to room air. HIC scores typically reached a peak at about 5–10 min after withdrawal. By 1 h after withdrawal, HIC scores returned essentially to baseline in most strains. The means for 19 BXD and both progenitor strains are shown in Figs. 2 and 3 for nitrous oxide and acute ethanol withdrawal, respectively. We have shown that prior testing with ethanol has a negligible effect on nitrous oxide withdrawal scores compared to saline pretreatment (unpublished).

Nitrous oxide and acute ethanol withdrawal intensity are under a significant degree of genetic determination, with heritabilities of 0.39 and 0.34,

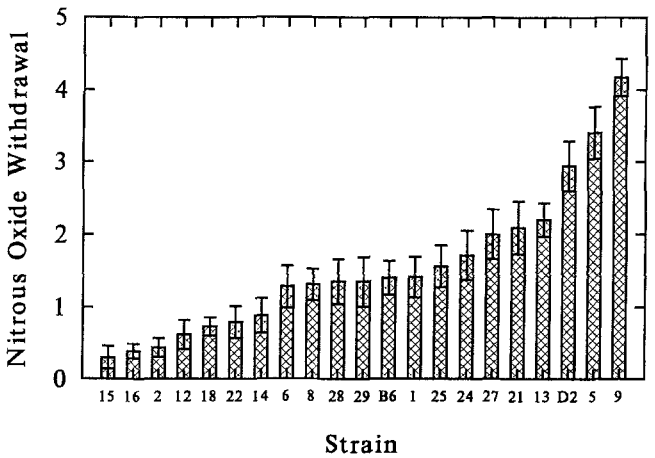

Fig. 2. The distributions of strain means (±SE) for nitrous oxide withdrawal HIC in 19 BXD strains and both progenitors, C57BL/6J (B6) and DBA/2J (D2). The withdrawal scores are based on the peak HIC seen following 1 h of nitrous oxide exposure minus the baseline (pretreatment) HIC scores. A total of 265 male mice was used in these experiments, or 6–21 per strain. The numbers at the base of each bar here and in Fig. 3 refer to the BXD strain, e.g., strain BXD-15 is listed as "15."

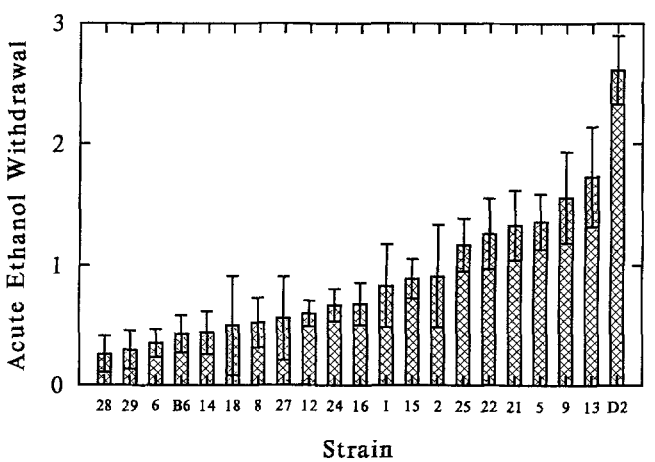

Fig. 3. The distributions of strain means (±SE) for acute ethanol withdrawal HIC in 19 BXD strains and both progenitors, C57BL/6J (B6) and DBA/2J (D2). The withdrawal scores are based on the peak HIC seen following a 4.0 g/kg i.p. dose of ethanol minus the baseline (pretreatment) HIC scores. A total of 232 male mice was used in these experiments, or 6–17 per strain.

respectively, based on R^2 values. Moreover, both traits are genetically correlated ($r = .60$, $p < .004$) among the strain means (Fig. 4). These two withdrawal states do share some degree of common genetic determination, but it is by no means complete. Nitrous oxide withdrawal appears not to

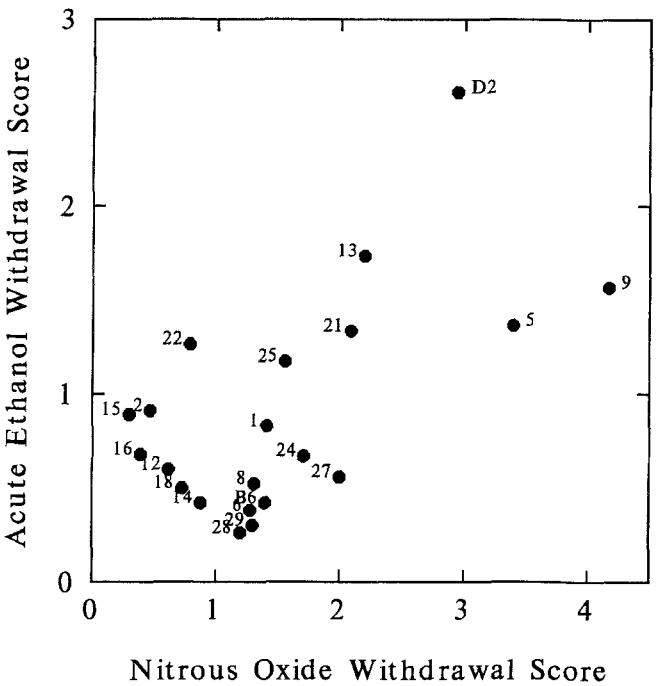

Fig. 4. Scatterplot of strain means for withdrawal-induced HIC from either acute ethanol injection (ordinate) or 60-min nitrous oxide exposure (abscissa) in 19 BXD and both progenitor strains, C57BL/6J (B6) and DBA/2J (D2). The numbers by the symbols refer to the BXD strain, e.g., "16" refers to strain BXD-16. The correlation between ethanol and nitrous oxide withdrawal scores was 0.60 ($p < .004$), indicating a substantial degree of common genetic determination of the two withdrawal states. However, when the effects of the *Pmv-7* locus were removed, the partial correlation between the two withdrawal states was 0.19 (n.s.).

be a carbon copy of acute ethanol withdrawal in its genetic underpinnings, although there are similarities.

Both sets of HIC scores were subjected to QTL (quantitative trait loci) analysis to identify candidate QTL sites affecting these two withdrawal states. These are shown in Table I. For each chromosome region listed, only the marker with the strongest association is shown. Six chromosome regions met the $p < .05$ (single-test) criterion for each trait. For nitrous oxide, the strongest association ($r = 0.71$, $p < .0006$) was with the *Pmv-7* region of chromosome 2 (Fig. 5). The strongest association for the acute ethanol withdrawal data was the *Ly-20* region of chromosome 4 ($r = -.74, p < .002$). However, the second strongest association ($r = .64, p < .003$) was with the *Pmv-7* region of chromosome 2, similar to the nitrous oxide results. Figure 6 shows the results for all markers on chromosome 2 for both

Table I. Marker Loci Significantly Associated ($p < .05$, Single Test) with Handling-Induced Convulsion Intensity Following Nitrous Oxide Withdrawal (Upper Half) or Acute Ethanol Withdrawal (Lower Half) in 19 BXD strains[a]

Marker	r	Location
Nitrous oxide		
Pmv-7	.71 ($p < .0006$)*	Chr. 2, 34 cM
Qui	.64 ($p < .01$)	Chr. 6, 60 cM
Spt-2	.59 ($p < .01$)	Chr. 15, ? cM
Ltw-4	.57 ($p < .01$)	Chr. 1, 60 cM
Zpf-4	.52 ($p < .03$)	Chr. 8, 45 cM
Mtv-1	−.49 ($p < .05$)	Chr. 7, 31 cM
Acute ethanol		
Ly-20	−.74 ($p < .002$)	Chr. 4, 55 cM
Pmv-7	.64 ($p < .003$)	Chr. 2, 34 cM
Aox-1	−.53 ($p < .03$)	Chr. 1, 20 cM
Lyb-2	.51 ($p < .03$)	Chr. 4, 23 cM
B	.48 ($p < .04$)	Chr. 7, ?
Abpa	−.47 ($p < .04$)	Chr. 7, 10 cM

[a] Only the marker showing the highest correlation among several closely linked markers is shown.

* $p < .0008$ (single test), which becomes $p < .05$ (multiple test) after a making a Bonferroni correction (k) of 65, as suggested for this marker set by Belknap (1992).

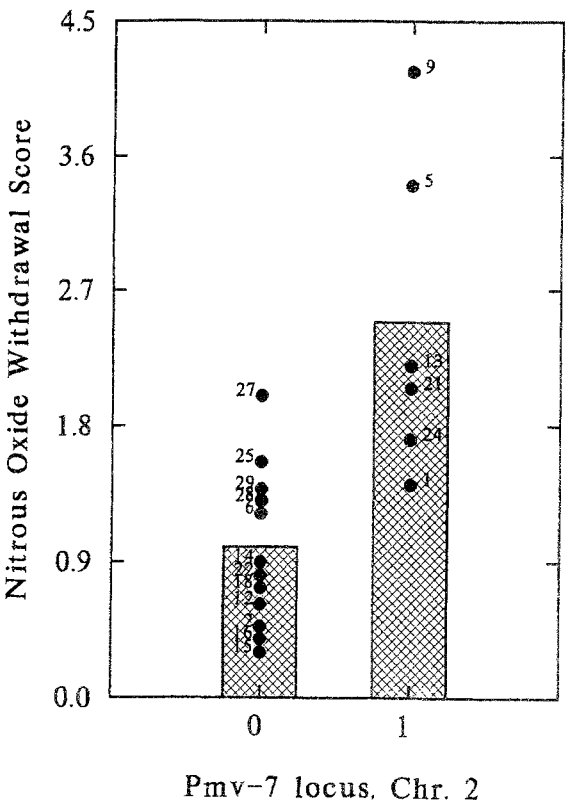

Fig. 5. Bar graph showing the mean nitrous oxide withdrawal HIC score of those strains possessing the C57BL/6J allele (left bar) and those possessing the DBA/2J allele (right bar) at the *Pmv-7* locus on chromosome 2. Superimposed on the bars is a plot of the individual strain means. Each number refers to the strain numbers, e.g., strain BXD-27 is symbolized as "27." The correlation (r) between nitrous oxide HIC and the two alleles at the *Pmv-7* locus, scored as a 0 (C57BL/6 allele) or a 1 (DBA/2 allele), was 0.71 ($p < .0006$, $n = 19$ strains). The p value for r is the same as that obtained from a two-tailed t test between the two allelic groups of strains, which in this case was $t = 4.2$, $p < .0006$.

withdrawal studies. Also shown in Fig. 6 are the QTL results for the same chromosome for chronic ethanol withdrawal from 3 days of vapor inhalation (Crabbe *et al.*, 1983) and high-pressure tonic convulsions induced by compression in helium (McCall and Frierson, 1981). A frequently advanced hypothesis in the hyperbaric literature is that high pressure leads to increased rigidity of the neural membranes, which in turn leads to convulsive activity as part of the high-pressure nervous syndrome, or HPNS (reviewed by Akers and Belknap, 1988; Halsey, 1982). A similar hypothesis was current in the ethanol withdrawal literature not too long ago (reviewed by Hunt, 1985). As shown in Fig. 6, all four convulsive measures show associations to this same chromosome region. While the match is not perfect, these results indicate that there may be a gene locus in this region affecting all four convulsive measures. There is a fifth convulsive measure in the BXD literature of which we are aware, namely, audiogenic seizures (Neumann and Seyfried, 1990). This trait did not show a significant association with any marker on chromosome 2 and was not significantly correlated with either withdrawal measure reported here.

As noted above, nitrous oxide and acute ethanol withdrawal are genetically correlated ($r = .60$, $p < .004$). It is of interest to calculate the partial correlation between the two withdrawal states with the effects of the *Pmv-7* locus removed (held constant). This partial correlation of 0.19 was not significant. This result indicates that much of the positive genetic correlation between these two traits may be due to the common influence of a locus in the *Pmv-7* region of chromosome 2.

We plan to test our BXD chromosome mapping results in a segregating F_2 population from a C57BL/6 × DBA/2 cross. Until this is done, the present BXD results should be regarded as provisional. PCR primers specific to these chromosome regions will be used to genotype individual mice previously tested for withdrawal severity. Emphasis will be given to the primer sequences for micro-

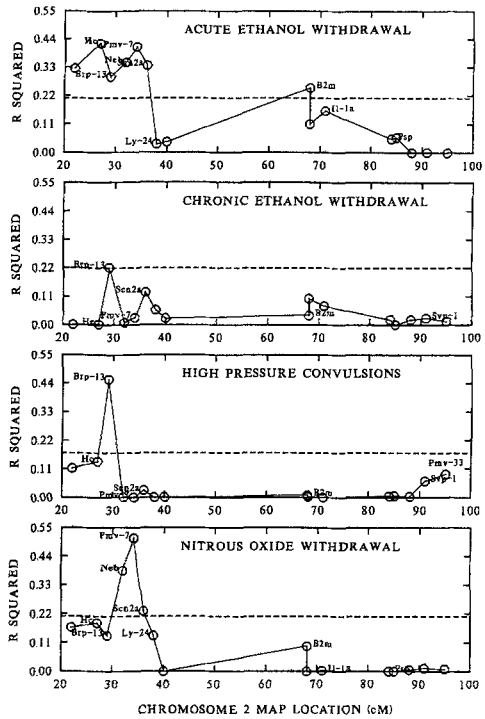

Fig. 6. Plot of the associations (R^2) of each marker locus on chromosome 2 with four measures of convulsive activity in the BXD strains. The known linear order of mapped marker loci is shown along the *X* axis, expressed as centimorgans (cM) of map distance from the centromere. The four measures were (first, top) acute ethanol withdrawal HIC scores, (second) chronic ethanol withdrawal HIC scores after 3 days of ethanol vapor inhalation reported by Crabbe *et al.* (1983), (third) high-pressure Type I (clonic) convulsions induced by high pressure in helium (McCall and Frierson, 1981), and (fourth) nitrous oxide withdrawal HIC scores. Each correlation coefficient (r) was calculated using the strain means for each measure and scores of 0 (C57BL/6 allele) or 1 (DBA/2 allele) for the BXD strains at each marker locus. Squaring the correlation coefficient (R^2) yields the proportion of the total genetic variance "accounted for" by each marker locus. The horizontal dashed line represents the $p<.05$ (single test) significance threshold. Map locations were obtained from the linkage map of Davisson and Roderick (1989). For all four measures, the associations meeting the $p<.05$ criterion were with one or more markers in the *Hc* to *Pmv-7* region near the proximal end of chromosome 2.

satellite markers recently published by Dietrich *et al.* (1992) for the BXD strains.

CONCLUSIONS

For gene mapping purposes, the RI strains are often of great value in generating a small number of candidate QTL for further testing using other linkage or association methods. Thus, the RI approach offers the prospect of meaningful progress toward QTL detection and mapping. The best developed RI series at present for gene mapping purposes is the BXD series developed by Taylor (1989). Because the study of 24 RI strains represents only 24 genotypes, the BXD results alone can map only loci with major effects. For loci with smaller effects, such as those typically observed for most drug responses, other genetic models must be employed for verification purposes, as discussed above. The data presented also illustrate the utility of RI strains for estimating genetic correlations and for discerning the possible QTL basis for these correlations.

The present results also illustrate a major strength of the RI approach—the almost limitless replicability of the genotypes involved. This allows the genotype to be measured with any desired degree of accuracy from the phenotype. In addition, knowledge gained in the future can be directly compared and accumulated with the wealth of data collected in the past on what is essentially the same genotypes, excepting new mutations (Plomin *et al.*, 1991; Gora-Maslak *et al.*, 1991). This is especially important given the rapid advances expected for drug abuse-related phenotypes in the foreseeable future.

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