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ORIGINAL INVESTIGATION

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Alcohol-induced locomotor activation in C57BL/6J, A/J, and AXB/BXA recombinant inbred mice: strain distribution patterns and quantitative trait loci analysis

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Abstract *Rationale:* Quantitative trait loci (QTLs) for initial sensitivity to alcohol have been identified in a number of mouse strains (e.g. BXD); however, confirmation is required. *Objectives:* The present paper aimed to characterize the C57BL/6J, A/J, and AXB/BXA recombinant inbred (RI) strains of mice for basal and ethanol-induced locomotor activation as measured in an open field and to provide provisional location of QTLs for these phenotypes. *Methods:* A/J and C57BL/6J mice were habituated to handling and then randomly assigned to receive one of four alcohol doses (0, 0.5, 1.0, 2.0 g/kg). Subsequently, all available strains of the AXB/BXA RI were tested with the 2 g/kg dose of ethanol or vehicle control. *Results:* Simple regression and interval mapping were used initially to identify significant gene markers associated with ethanol-induced activation (calculated as total activity on alcohol day–total activity on saline day). Subsequently, composite interval mapping (CIM) was used to increase the accuracy in mapping individual loci. Genetic markers on chromosomes 2, 3, 8, 13, 16, 18 and 19 were associated with ethanol-induced activation. *Conclusions:* Three significant markers identified through CIM accounted for 86% of the genetic variance in the ethanol-induced activation. QTLs on chromosome 16 (45.6 cM) and 19 (24 cM) previously associated with alcohol consumption in the AXB/BXA RI mice were found to overlap with QTLs for ethanol-induced activation identified in the present study.

Key words Inbred mice · Recombinant mice · Ethanol · Locomotor activity

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Introduction

In recent decades, it has been demonstrated that alcohol/drug effects are influenced by genetic factors. Research has indicated an increased incidence of alcoholism in the families of alcoholics and a higher concordance for alcoholism and other substance abuse in monozygotic compared to dizygotic twins (Pickens et al. 1991). Adoption studies have shown that adopted away sons of alcoholic biological parents show a 4-fold higher rate of alcoholism than adoptees with non-alcoholic biological parents (Bohman et al. 1987). Several lines of research suggest that initial sensitivity to alcohol may influence the subsequent propensity to abuse. Individuals with decreased sensitivity to alcohol (with less subjective intoxication) following alcohol dosing have been reported to be more likely to be alcohol dependent at follow-up 10 years later (Schuckit 1994; Schuckit and Smith 1997).

In order to evaluate the influence of genetic factors on initial sensitivity to alcohol (ISA), animal studies using different genetic models and behavioural approaches have been conducted. Locomotor activation is one measure that has commonly been used to evaluate ISA, and to date there is a considerable amount of data demonstrating genetic influences (Crabbe et al. 1980; Cunningham et al. 1991; Dudek et al. 1991; Risinger et al. 1994). Locomotor activity is a commonly used, robust and reliable measure of ISA that appears to be resistant to several contextual and procedural variables (i.e. lighting conditions, testing order) making it a useful paradigm for pharmacogenetic research (Tritto and Dudek 1994).

While animal models have demonstrated the influence of genetic factors in the mediation of ethanol-induced activation (Crabbe et al. 1980; Dudek et al. 1991), specific genes remain to be identified. Strategies such as quantitative trait locus (QTL) analysis that are capable of isolating multiple-gene influences accounting for small amounts of variance, have begun to identify chromosomal regions associated with ethanol-induced activation in the mouse. QTL analysis of ethanol-in-

duced locomotor activation has been conducted in a number of recombinant inbred (RI) strains (Gora-Masalak et al. 1991; Cunningham 1995; Phillips et al. 1995, 1996; Erwin et al. 1997; Demarest et al. 1999) and F2 intercrosses (Hitzemann et al. 1998). A comparison of the existing studies reveal that overlapping loci associated with ethanol-induced activation have been identified on a number of chromosomes. It is important to note that these studies differ along several important dimensions thus accounting for the large number of identified markers. These include differences in the phenotypic measure, alcohol dose, testing procedures, and the strains of mice used. In addition, as Crabbe et al. (1999) have demonstrated, it is possible that small environmental differences across labs may result in significantly different phenotypic profiles in some inbred strains of mice.

Gora-Masalak et al. (1991) assessed ethanol-induced activity (defined as the difference between saline and ethanol administration) in BXD recombinant inbred (RI) strains. The authors identified 15 loci associated with low dose ethanol-induced locomotor activity on chromosomes 2, 4, 9 and 13. Similarly, Cunningham (1995) found significant associations between ethanol (2 g/kg)-stimulated activity in BXD RI mice and genetic markers on chromosomes 7 and 18. Phillips et al. (1995, 1996) examined responses to both acute and repeated exposures to 2 g/kg ethanol in male BXD RI mice. QTL analysis identified significant ($P<0.01$) loci on chromosomes 3, 4, 7, 11, 12, 13, 15, 17 and 18. QTLs on chromosome 5 and 13 were identified as potentially important locations for functional genes that determine variation in basal locomotor activity.

Erwin et al. (1997) used LS×SS RI strains to identify six loci for ethanol-induced locomotion at a 2 g/kg dose, and eight loci associated with low dose (e.g. 1.0–1.5 g/kg) ethanol sensitivity. Ethanol-induced locomotion following the administration of 2 g/kg ethanol was defined in this study as the total distance travelled within the test apparatus. In contrast, low dose ethanol sensitivity was based upon the slope of the line formed by a semi-log plot of activity after three low doses of ethanol (1.0, 1.25 and 1.5). Only the locus at D12Mit44 on chromosome 12 was associated with both measures of acute ethanol sensitivity.

Hitzemann et al. (1998) identified a significant QTL associated with ethanol-induced locomotor activation on chromosome 2 in a C57BL/6J×DBA/2J F2 intercross. The QTL on chromosome 2 was associated with a LOD score of 8.4 and accounted for 30% of the genetic variance. Using the same behavioral paradigm, Demarest et al. (1999) described a two-stage process for identifying and confirming QTLs associated with locomotor activity induced by a 1.5 g/kg challenge of ethanol. The initial stage involved the identification of QTLs associated with ethanol activation in 25 strains of BXD RI mice. Significant QTLs were identified on chromosomes 1, 2, 4, and 6. In stage 2, male C57BL/6J X DBA/2J F2 intercross animals were used to confirm the putative QTL on chromosome 2.

To date, the identification of QTLs has been a function of the use of a limited number of mouse strains (e.g. BXD RI mice). However, it is recognized that QTLs found in one genetic model need to be confirmed using a variety of models including the use of other RI sets, F2 intercrosses and congenic mice (Dudek et al. 1993; Belknap et al. 1996). Therefore, the present study was conducted in order to expand upon the current database of putative QTLs associated with ethanol-induced activation using the AXB/BXA RI mice. AXB/BXA RI mice were derived from an A/J and C57BL/6J cross. After more than 20 generations of inbreeding, each of the strains of AXB/BXA RI mice has a different "recombination" of the parental genomes in a homozygous state. This RI series of mice have not been extensively typed for ethanol-induced behaviours to date (Gill et al. 1998).

The present study utilized composite interval mapping (CIM) in addition to simple regression and interval mapping (SIM) to identify QTLs associated with ethanol-induced activation. It has been suggested that single-QTL models such as SIM are intrinsically inappropriate for multilocus systems (Jansen 1996). In the context of a multilocus model, problems associated with SIM consist of the fact that the effects of additional QTLs will contribute to the sampling variance and reduce the significance of any association (Jansen 1993, 1994; Zeng 1994). In addition, linked QTLs will cause biased estimates of association (Jansen 1994; Zeng 1994). CIM analysis reduces these limitations and enables the search for QTLs one at a time, while simultaneously accounting for the effects of other segregating QTLs.

Materials and methods

Male and female C57BL/6J, A/J, and AXB/BXA recombinant inbred mice were purchased from Jackson Laboratory (Bar Harbor, Maine, USA). Initially, four breeder pairs/strain were established and maintained in an animal colony controlled for temperature and humidity on a 12-h day/night cycle (lights on between 6 a.m. and 6 p.m.). Generally, breeder pairs were kept for 8 months providing four to five litters. The mice were housed in standard shoe-box solid bottom cages with beta chip bedding. Pups were weaned at 4 weeks and housed with same-sex littermates until 8 weeks of age.

Locomotor activity testing

Locomotor activity was measured in open field boxes constructed of Plexiglas, measuring 30 cm×30 cm×40 cm high. Six intersecting light-photocell assemblies placed inside the walls of the chambers, 3 cm above the floor, monitored the locomotor activity by automatic registration on a computer connected to the photocells. Counts were automatically registered at 1-min intervals throughout 15-min sessions. A custom-designed computer software program monitored activity on the photocells, providing measures of horizontal and stereotypic activity. Programming for the horizontal activity included routines suppressing scores from repetitive breaking of photobeams due to grooming. Testing was performed under red light in a sound-insulated room between 3 and 6 p.m. immediately prior to lights out.

Naive male and female mice were habituated to the handling and injection procedures as well as to the activity chambers by means of intraperitoneal injections (IP) of sterile 0.9% NaCl saline

solutions on 2 successive days. The locomotor activity scores obtained following saline administration on day 1 and 2 were considered to reflect the interaction between the subjects' basal locomotor activity and their responsivity to a novel environment. Two days following habituation testing animals from a subset of strains were randomly assigned to one of four alcohol dose conditions (0, 0.5, 1.0 and 2.0 g/kg). Once a dose of ethanol was identified which provided maximal phenotypic separation between progenitor A/J and C57BL/6J strains (2.0 g/kg), all available strains were then tested with the selected ethanol dose or vehicle control. The subjects were injected IP with either saline or a 10% (w/v) solution of ethanol. Activity was monitored for 15 min immediately following each injection. Measures obtained from this experimental design included responses to novelty (activity on the first saline day), habituation (change in activity from saline day 1 to saline day 2), as well as measures of ethanol-induced activation including the percent change from saline controls as well as difference scores (ethanol test day–saline day 2 activity).

Statistical analysis

Statistical analyses were performed with SPSS version 8.0 for Windows (SPSS Inc.). Comparisons between strains of mice were conducted with analysis of variance (ANOVA) techniques. The locomotor data were tested for normality as well for outliers using the SPSS explore function, and scores outside the 95% confidence interval for each strain (a total of 65 mice randomly distributed across strains) were removed from all subsequent analyses (final n=1152). A randomly selected split-half correlational analysis (corrected with Spearman-Brown formula) was performed on the range of behavioural measures in order to assess the reliability of the strain means for the entire sample (Plomin and McClearn 1993). Genetic correlations between the strain means for the various measures of locomotor activity were calculated. The RI strain means were used to obtain estimates of heritability (H^2_n) for all phenotypes.

Quantitative trait loci analysis

The phenotypic data collected on the AXB/BXA RI strains were subjected to a quantitative trait loci (QTL) analysis using Map Manager QT (Manly 1998). This analysis initially used simple regression and interval mapping procedures to test for linkage relationships between mapped marker loci and the strain distribution pattern for the range of phenotypes. A database of genetic linkage markers typed in the AXB/BXA RI strains were obtained from the Mouse Genome Database (MGD 2000). At present there are 780 markers identified in the AXB/BXA RI strains (consisting of SSLPs, restriction fragment length polymorphisms, biochemical markers, and proviral loci). Correlations between the linkage markers identified by Map Maker QT, arbitrarily scored as 1 for alleles from the A/J progenitor and 0 for the alleles from the C57BL/6J, and the distribution of strain means for the measures of locomotor activation were computed. Those associations with a P<0.01 were followed up by a stepwise multiple regression in order to estimate the total amount of genetic variance accounted for by the putative QTLs. Estimated LOD scores were calculated based upon the chi-square statistic (likelihood ratio statistic (LRS)) provided by MapManager.

Situations have been described where quantitative traits are influenced directly and indirectly (through interactions) by more than one QTL (Jansen 1996). Given this assumption, the influence of different unlinked QTLs may behave like additional environmental effects that act to diminish the significance of calculated associations (Zeng 1994; Jansen 1996). Therefore, in a second stage of analysis, the markers associated with ethanol activation (ethanol difference scores) were examined using composite interval mapping (CIM) with Map Manager QT (Manly 1998).

An important methodological issue in the present study is the limit of QTL detection using RI strains. The number of progeny required to detect a QTL is inversely proportional to the strength of the QTL. Thus, due to the limited number of RI strains, not all QTL will be detected and the strength of those that are detected may be overestimated (Manly and Olson 1999). Therefore it is not expected that the present study will detect all relevant loci affecting alcohol activation. This will most likely be the case for alleles of small to moderate effect. While it would be desirable to detect QTL of both small and large effect, in practice only the latter are likely to be mapped with sufficient precision given current techniques, to be useful for subsequent physical mapping and cloning. QTL of very small effect will be extremely difficult to fine map due to the inability to measure their phenotypic effects.

Criteria for QTL significance

In order to establish the significance of the associations generated by the interval mapping procedures, permutation tests taken directly from the work of Churchill and Doerge (1994) were conducted using the Map Manager QT software (Manly 1998). In this test, the trait values are randomly permuted among the progeny, thus destroying any relationship between the trait values and the marker loci. A regression model is fitted for the permuted data at multiple analysis points across the genome (matching the points used for detecting QTLs) and the maximum LRS is recorded. This procedure is repeated hundreds of times giving a distribution of LRS statistic values that would be expected if there were no QTL linked to any of the marker loci (Manly 1998). The LRS values at appropriate percentile points in the distribution are taken as critical values to establish significance (Manly 1998). For example, the 95th percentile establishes significance corresponding to the usual criterion of 0.05. The threshold values of the permutation test labeled as suggestive or significant for linkage were taken from the guidelines of Lander and Kruglyak (1995).

Results

A two-way (strain×gender) ANOVA was performed on total locomotor activity measures for saline sessions 1 and 2. The data were obtained from approximately 22 females and 24 males/strain. The analysis of the saline day 1 session failed to indicate significant gender effects [F(1,1151)=0.734, P<0.392] or gender×strain interactions [F(24,1151)=1.188, P<0.243]. Therefore, data for males and females were combined in all subsequent analyses of baseline data. Substantial strain differences were observed across phenotypes measured in terms of total locomotor activity for saline day 1 [F(24,1151)=33.814, P<0.0001] and saline day 2 [F(24,1137)=25.389, P<0.0001].

Mean locomotor activity values observed during the saline day 1 test session for A/J, C57BL/6J, and AXB/BXA RI strains are presented in Fig. 1. Strain means were presented in ascending order of activity. The reliability of strain means were computed in a random split-half correlational analysis yielding a value of r=0.968, P<0.001).

Ethanol-induced locomotor activation

The effects of ethanol administration on locomotor activity in C57BL/6J, A/J, and AXB/BXA RI mice were assessed using two distinct measures of ethanol activation.

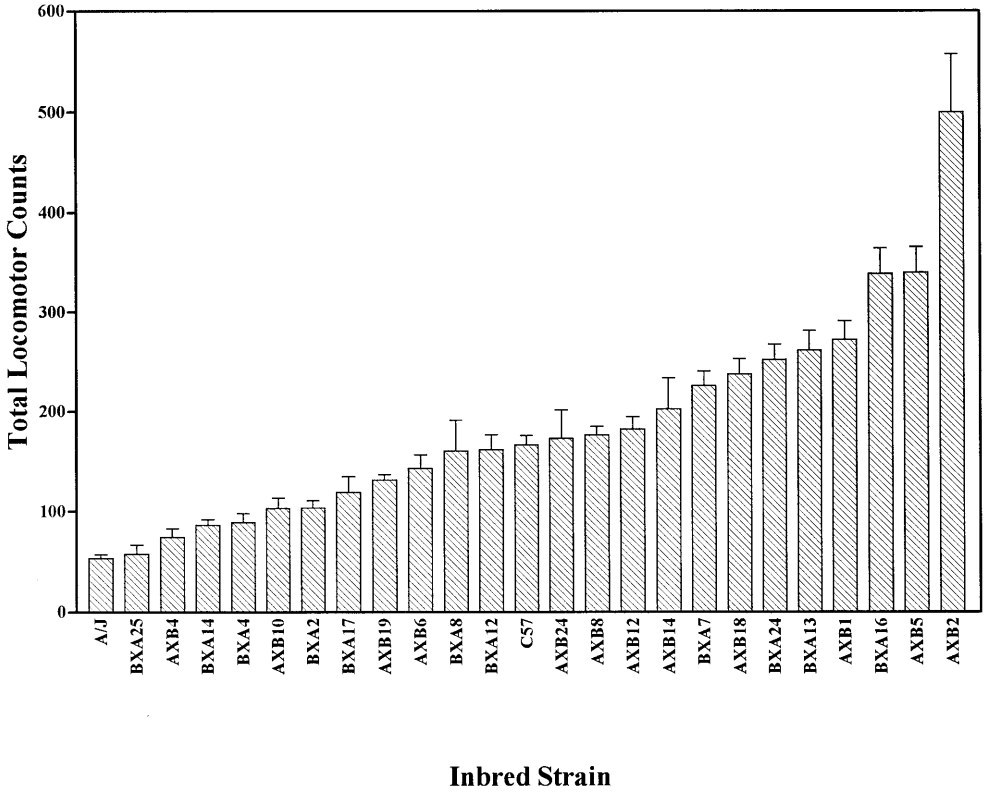

Fig. 1 Locomotor activity scores across progenitor and AXB/BXA RI strains measured following saline administration (saline day 1). Strain means are plotted in order of ascending activity scores

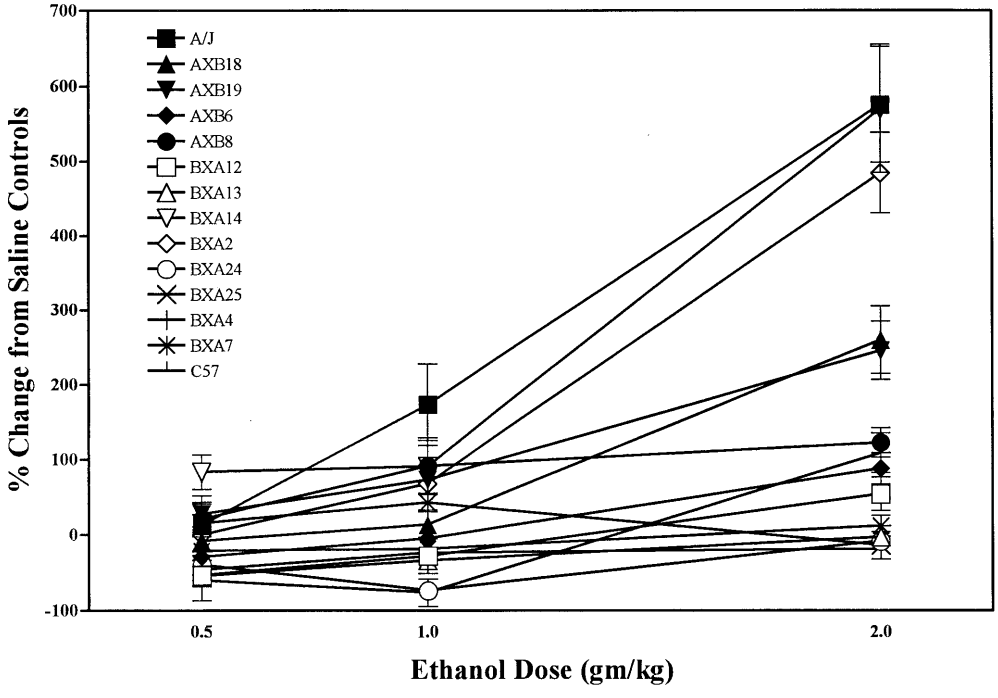

Fig. 2 The effects of the administration of a range of ethanol doses (0.5, 1.0, 2.0 g/kg) on ethanol-induced activation in the progenitor (A/J, C57BL/6J) and eight different AXB/BXA RI strains are presented. Ethanol activation scores are expressed as the percent change from saline controls. Maximal separation in strain means was evident at the 2.0 g/kg dose of ethanol

These phenotypes consisted of (1) the percent change in ethanol-induced activity relative to saline controls (ethanol percent change scores) and (2) the differences in total activity scores between ethanol and saline day 2 test sessions (ethanol difference scores). Absolute measures of ethanol activation are not presented due to the confound of strain differences and basal locomotor activity. Strain values for absolute ethanol scores (total locomotion on ethanol test day) were highly correlated ($P<0.01$) with total activity measures obtained during saline session 1 and 2 ($r=0.768$, $P<0.0001$ and 0.86, $P<0.001$, respectively).

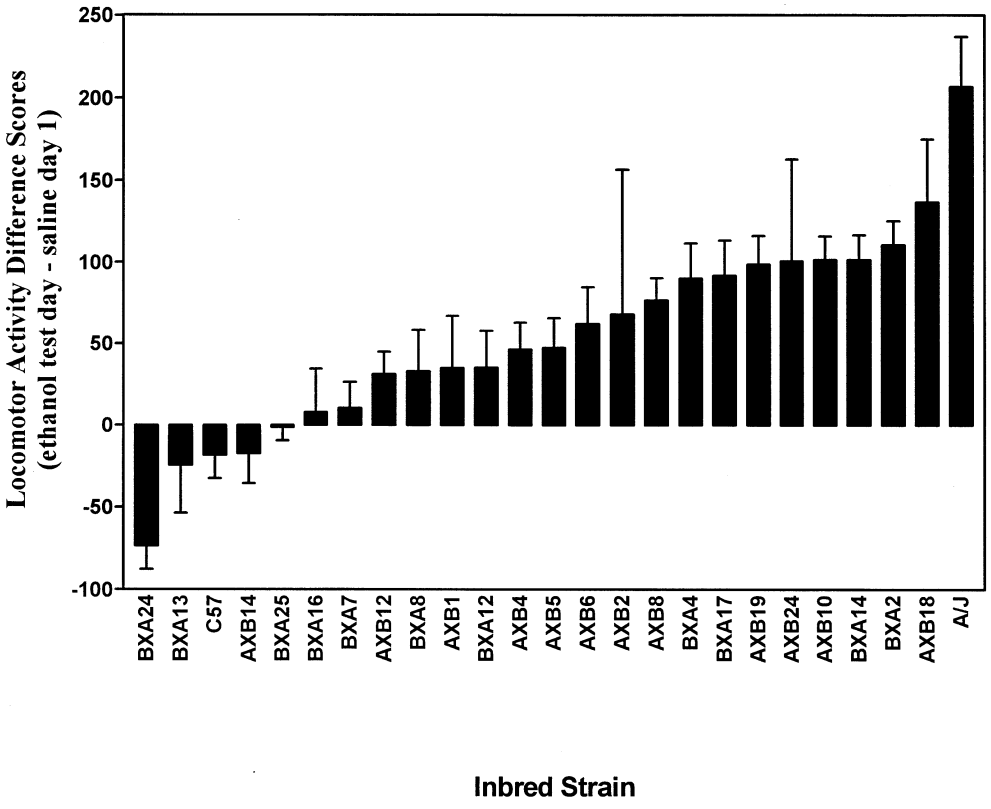

Fig. 3 Ethanol-induced activation scores across progenitor and AXB/BXA RI strains expressed as the difference between activity following ethanol administration (2 g/kg) and activity on saline day 2 (ethanol difference scores). Strain means are plotted in order of ascending activation. A wide and continuous distribution of activation scores suggest polygenic influences on the phenotype

A three-way ANOVA (strain×gender×ethanol dose) was performed on ethanol difference scores. Gender effects [$F(1,754)=2.137$, $P<0.144$] and interactions [gender×dose $F(3,754)=1.583$, $P<0.192$; strain×gender $F(13,754)=1.393$, $P<0.157$; strain×gender×dose $F(38,754)=1.002$, $P<0.468$] were not significant, therefore gender was not considered in subsequent analyses. Gender effects were not observed for total ethanol or percent change values.

The analysis of ethanol percent change scores for the 14 strains tested initially at all doses of ethanol (0, 0.5, 1.0 and 2.0) indicated a significant strain by ethanol dose interaction [$F(39,755)=11.496$, $P<0.001$]. The mean ethanol dose response values are presented in Fig. 2. In all cases, the maximal activation and separation between strains was observed at the 2.0 g/kg dose of ethanol. On the basis of this initial dose-response data, the 2.0 g/kg doses of ethanol and vehicle control were selected for further testing and subsequent QTL analysis.

The mean ethanol difference scores for all C57BL/6J, A/J, and AXB/BXA RI mice following 2 g/kg ethanol are presented in Fig. 3. The results demonstrate a wide and continuous distribution of ethanol activation values, which is consistent with a polygenic basis for the expression of ethanol activation. A one-way ANOVA yielded significant strain differences for the ethanol difference scores [$F(24,441)=7.426$, $P<0.001$]. The reliability of the strain means was $r=0.816$ ($P<0.001$). The strain distribution pattern for mean ethanol percent change values was very similar to the ethanol difference scores; therefore the data are not presented here. Analysis indicated significant strain differences in ethanol percent change scores [$F(24,432)=22.869$, $P<0.001$]. The reliability of the strain means was $r=0.80$, ($P<0.001$). Overall, the two measures of ethanol activation were highly correlated ($r=0.742$, $P<0.0001$). Heritability measures for the ethanol difference and the ethanol percent change scores were $H^2_n=0.31$ and 0.39, respectively.

Quantitative trait loci analysis

QTL analyses were performed on (1) total activity scores for saline day 1, (2) total activity scores for saline day 2, (3) the ethanol percent change scores, and (4) ethanol difference scores. Correlations ($P<0.05$) between the strain distribution for locomotor activity on saline day 1 and genetic linkage markers are reported in Table 1. Map Manager identified eight putative markers (at the $P<0.01$ level) on chromosomes 1, 2, 5, 8, 9, 13 and 19. These QTL are merely suggestive in that they failed to achieve the defined threshold for significance (see definition of permutation test in Materials and methods section). The results of multiple regression analysis indicated that four markers (D13Mit10, D8Mit305, D5Mit356, D2Mit1) accounted for 51% of the genetic variance in basal locomotor activity.

Correlations between the strain distribution for mean ethanol percent change scores and genetic linkage markers are presented in Table 2. Four loci were identified ($P<0.01$) on chromosomes 2, 8 and 13. Multiple regression indicated

Table 1 Putative quantative trait loci for basal locomotor activity in a novel environment (saline day 1 total activity scores) in AXB/BXA recombinant inbred mice

Marker	Chr	Location (cM)[a]	Correlation[b]	LOD Score[c]	*P* value
D1Nds2	1	59.0	–0.532	1.66	0.00568
D2Mit1	2	1.0	0.575	2.03	0.00235
D5Mit356	5	41.0	–0.535	2.03	0.00235
D8Mit305	8	37.0	–0.609	2.33	0.00109
Ets1	9	15.0	0.548	1.72	0.00502
D13Mit10	13	31.0	–0.612	2.35	0.00102
D13Mit146	13	51.0	0.568	1.79	0.00425
D19Mit10	19	47.0	0.547	1.79	0.00421

[a] Recombinant distance in centimorgans from centromere
[b] Correlations between SDP for marker and strain means. All correlations listed with *P* values <0.01
[c] LOD scores estimated from LRS provided by Map Manager QT

Table 2 Putative quantitative trait loci for ethanol-induced activation (ethanol percent change scores) in AXB/BXA recombinant inbred mice. Ethanol percent change scores were calculated at the percent change in ethanol-induced activity relative to saline controls run on the same test day

Marker	Chr	Location (cM)[a]	Correlation[b]	LOD score[c]	*P* value
D1Mit169	1	15.0	0.448	1.13	0.02320
D2Mit423*	2	68.9	–0.511	1.52	0.00835
D4Mit54	4	66.0	–0.413	0.93	0.03789
D5Mit356	5	41.0	0.436	1.06	0.02761
Iapls3-49	7	?	–0.427	1.00	0.03154
D8Mit3*	8	10.0	0.501	1.43	0.00997
Es1*	8	43.0	0.541	1.59	0.00703
D9Mit146	9	43.0	–0.455	1.15	0.02081
D13J1*	13	9.0	0.579	2.04	0.00219
Iapls1-30	14	48.0	–0.468	1.24	0.01717
D16Mit65	16	45.5	–0.409	0.96	0.04030
D17Mit10	17	24.5	–0.465	1.22	0.01798
Iapls3-7	18	22.0	0.478	1.30	0.01468
Xmv18	19	54.0	–0.421	0.98	0.03399

[a] Recombinant distance in centimorgans from centromere
[b] Correlations between SDP for marker and strain means. All correlations listed with *P* values <0.05 except where indicated **P*<0.01
[c] LOD scores estimated from LRS provided by Map Manager QT

Table 3 Putative quantitative trait loci for ethanol-induced activation (ethanol difference scores) in AXB/BXA recombinant inbred mice. Ethanol difference scores were calculated as the differences in total activity scores between ethanol and saline day 2 test sessions

Marker	Chr	Location (cM)[a]	Correlation[b]	LOD score[c]	*P* value
D3J1*	3	31.7	–0.521	1.59	0.00699
D5Mit32	5	80.0	0.498	1.43	0.01039
Iapls2-25	6	56	–0.395	0.85	0.04820
Iapls1-46	7	44.0	–0.479	1.30	0.01424
Es1*	8	43.0	0.529	1.50	0.00861
D12Mit37	12	1.0	0.448	1.11	0.02338
D13Mit122	13	36.0	0.444	1.11	0.02448
Iapls1-30*	14	48.0	0.541	1.74	0.00477
D16Mit47*	16	43.0	–0.619	2.41	0.00085
D16Mit203*	16	55.0	–0.572	1.98	0.00256
Iapls3-7	18	22.0	0.419	0.95	0.03504
D19Mit47	19	29.0	–0.525	1.61	0.00649

[a] Recombinant distance in centimorgans from centromere
[b] Correlations between SDP for marker and strain means. All correlations listed with *P* values <0.05 except where indicated **P*<0.01
[c] LOD scores estimated from LRS provided by Map Manager QT

that three of the suggestive markers (D8Mit3, D2Mit423, D13J1) accounted for 42% of the genetic variance in this phenotype. Six markers associated with mean ethanol difference scores were identified by Map Manager (*P*<0.01) on chromosomes 3, 8, 14, 16, and 19 (see Table 3). The results of multiple regression indicated that four markers (D3J1, Es1, Iapls1-30, D16Mit47) accounted for 70% of the genetic variance in alcohol activation. The strongest associations between ethanol-induced locomotor activation and genetic loci were observed on chromosome 16. An interval map for chromosome 16 derived from the output of Map Mana-ger is presented in Fig. 4. The interval map data indicated a suggestive LRS value of 13.9 located between loci D16Mit47 and D16Mit27.

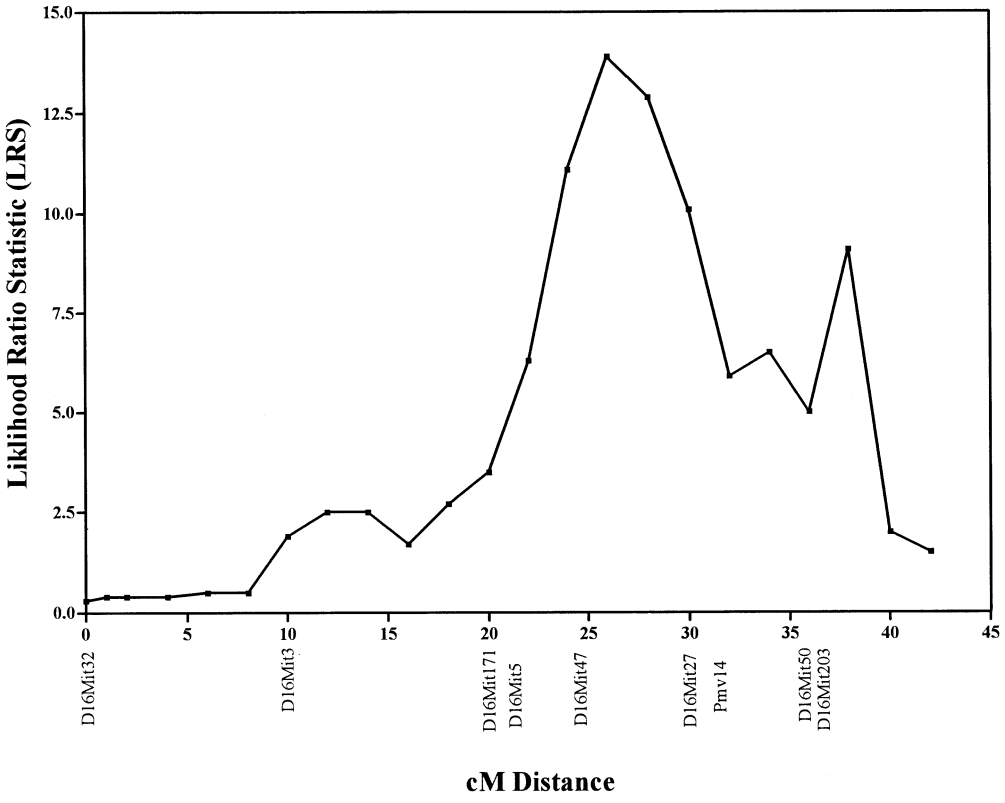

Fig. 4 Detection of putative QTL for ethanol difference scores on Chromosome 16 using simple interval mapping with Map Manager QT. The interval map data indicated a suggestive LRS value of 13.9 located between loci D16Mit47 and D16Mit27

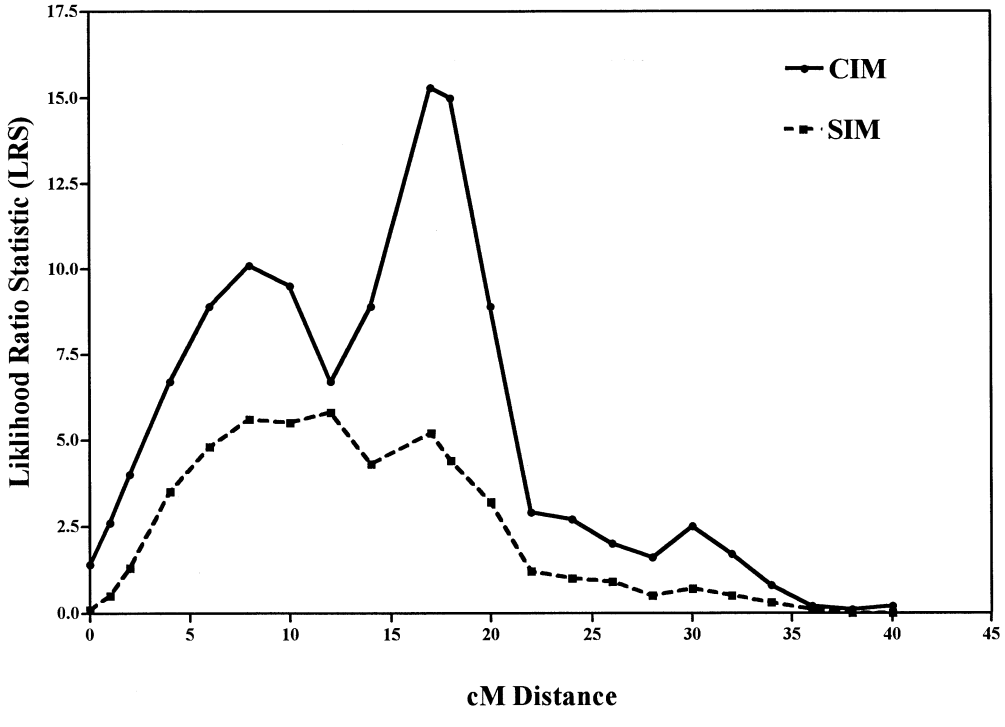

Fig. 5 Detection of putative QTL for ethanol differences scores on Chromosome 18 using composite interval mapping analysis. An interval map (1 cM steps) derived from the output of both simple (SIM) and composite interval (CIM) mapping are presented

Composite interval mapping

CIM was used in order to control for the influence of unlinked QTLs on the loci being mapped. In a systematic fashion, target loci associated with ethanol activation (difference scores) were mapped while controlling for the influence of QTLs on other chromosomes. The results of both SIM (without control for QTL cofactors) and CIM (with cofactors) analysis for markers ($P<0.01$) are presented in Table 4 . The results indicated that when background QTLs are factored in, one marker associated with the ethanol difference scores exceeded the LRS

Table 4 QTLs associated with ethanol-induced activation (ethanol difference scores) following SIM (without control for QTL cofactors) and CIM (with cofactors) analysis. $P<0.05$ in original SIM mapping analysis

Marker	Chr (cM)	Cofactor	LRS	Correlation	LOD score	*P*-value
D3J1	3 (31.7)	–	7.3	–0.521	1.59	0.00699
Es1	8 (43)	–	6.9	0.529	1.50	0.008610
		D16Mit203	14.9		3.23	00011
Iapls1–30	14 (48)	–	8.0	0.541	1.74	0.00470
D16Mit47	16 (43)	–	11.1	–0.619	2.41	0.00085
D16Mit203	16 (55)	–	9.11	–0.572	1.98	0.00256
		Iapls3–7	19.6		**4.21**	**0.0000095**
		Es1	5.3		3.32	0.000093
Iapls3-7	18 (22)	–	4.4	0.419	0.95	0.03504
		D16Mit203	15.0		3.25	0.00011

Table 5 Summary of QTLs (confirmed or overlapping) for ethanol-induced locomotor activation in mice. Note that bolded characters indicate confirmed or overlapping loci

Marker	Chr	Location (cM)	Reference	Comment
D2Mit17	2	53–72	Demarest et al. (1999)	BXD RI
D2Mit340	**2**	**68.9**	**Gill et al.** (1998)	**AXB/BXA RI mice**
Iapls2.4	2	86	Demarest et al. (1999)	BXD RI
D3ncvs24	3	22–24	Phillips et al. (1996)	BXD/TY RI
D3J1	3	31.7	Gill et al. (1998)	AXB/BXA RI mice
Rnu1B1	3	43	Phillips et al. (1996)	BXD/TY RI
D4Mc2	4	41–43	Phillips et al. (1996)	BXD/TY RI
Ly-20	4	62–67	Gora-Maslak et al. (1991)	BXD/TY RI
D4Mit54	**4**	**66.0**	**Gill et al.** (1998)	**AXB/BXA RI mice**
Ly31	4	66–78	Demarest et al. (1999)	BXD RI
D6ncvs35	6	30	Phillips et al. (1996)	BXD/TY RI
Iapls2-25	6	56	Gill et al. (1998)	AXB/BXA RI mice
Ly49c	6	62	Demarest et al. (1999)	BXD RI
D6Mit371	6	72	Hitzemann et al (1998)	C57BL/6J X DBA/2J F2 Intercross
D7ncvs52	7	6	Phillips et al. (1996)	BXD/TY RI
D7rp2	7	10–16	Cunningham (1995)	BXD/TY RI (Initial ethanol activation)
Iapls1-46	7	44.0	Gill et al. (1998)	AXB/BXA RI mice
Es1	8	43.0	Gill et al. (1998)	AXB/BXA RI mice
Xmmv-2	9	27	Gora-Maslak et al. (1991)	BXD/TY RI
D9Mit146	9	43.0	Gill et al. (1998)	AXB/BXA RI mice
D9Mit18	9	71	Erwin et al. (1997)	LSxSS RI
Hmg1-rs4	12	0–7	Phillips et al. (1996)	BXD/TY RI
D12Mit37	**12**	**1.0**	**Gill et al.** (1998)	**AXB/BXA RI mice**
D12Mit44	12	4	Erwin et al. (1997)	LSxSS RI
D16Mit27	16	45.5	Gill et al. (1998)	AXB/BXA RI mice
D16Mit203	16	55.0	Gill et al. (1998)	AXB/BXA RI mice
D17tu36	17	10	Phillips et al. (1996)	BXD/TY RI
D17Mit10	**17**	**25**	**Gill et al.** (1998)	**AXB/BXA RI mice**
D17Mit10	17	25	Erwin et al. (1997)	LSxSS RI
Iapls3-7	18	22.0	Gill et al. (1998)	AXB/BXA RI mice
Xmv-29	18	36–56	Cunningham (1995)	BXD/TY RI (Initial ethanol activation)
D18Mit8	18	48	Phillips et al. (1995)	BXD RI (acute ethanol exposure)
D19Mit47	19	29	Gill et al. (1998)	AXB/BXA RI mice

threshold for significance (guidelines of Lander and Kruglyak 1995) generated through the permutations test. Specifically, D16Mit203 (chr 16) exceeded the threshold value of 17.4. Stepwise, multiple regression analysis indicated that these markers D16Mit203 (chr 16), Es1 (chr 8), and Iapls3-7 (chr18) accounted for 86% of the variance in the mean ethanol difference scores. The genetic marker Iapls3-7 (chr18) was not identified when simple interval mapping was applied. However, when background QTLs were controlled using CIM, an LRS value of 15.0 was obtained. The interval mapping results for chromosome 18 following SIM and CIM (controlling for background QTLs) are presented in Fig. 5.

Discussion

A wide range of basal and ethanol-induced locomotor activity was displayed by the A/J, C57BL/6J and AXB/BXA RI strains of mice over a continuous distribution, indicating polygenic influences. This finding was confirmed in the QTL analysis where putative loci ($P<0.01$) on a number of chromosomes were associated with basal locomotor activity. However, these QTLs were suggestive and did not exceed the LRS thresholds for significance. Similarly, multiple genetic linkage markers on chromosomes 2, 3, 8, 13, 14, 16 and 19 were associated with ethanol-induced activation.

In AXB/BXA RI mice, basal locomotor and ethanol-induced activation did not share common loci, consistent with the lack of genetic correlation between these phenotypes. This finding would suggest that basal locomotor activity (typically associated with emotional reactivity) and ethanol-induced activation were not mediated by a common genetic component. The absence of a relationship between basal activity levels and ethanol-induced activation has been similarly reported in BXD RI mice by Phillips et al. (1996) and Cunningham (1995). The association of genetic linkage markers on chromosome 5 (D5Mit356) and 13 (D13Mit10) with basal locomotor activity is consistent with findings reported in the literature (e.g. Phillips et al. 1995, 1996).

A number of unique marker regions associated with ethanol-induced locomotor activation were identified in AXB/BXA RI mice. Specifically, QTLs on chromosomes 8 (Es1-43 cM), 16 (D16Mit203-55 cM), 18 (Iapls3-7-22 cM) and 19 were detected. A potential candidate gene Adra2a (50 cM) overlaps the marker region identified ($P<0.05$) on chromosome 19 (Xmv18-54 cM). Research has suggested that alterations in $alpha_{2a}$ adrenergic receptor expression (mis-sense changes) may be associated with the expression of alcohol/drug dependence (Feng et al. 1998). $Alpha_{2a}$ adrenergic receptors are widely distributed both pre- and postsynaptically, and have been demonstrated to mediate noradrenaline release in the locus coeruleus. It has been suggested that the $alpha_{2a}$ adrenoreceptor subtype may be involved in the mediation anaesthetic sedative/hypnotic effects of dexmedetomidine (Mizobe et al. 1996).

A quantitative genetic trait can be influenced by multiple QTLs with substantial individual effects, that are masked by their involvement in genetic interactions (Fijneman et al. 1998). CIM analysis in the present study confirms that the identification of QTLs using SIM may underestimate or fail to identify significant loci. In the present study, a suggestive locus associated with ethanol difference scores (Iapls3-7) following CIM (LRS=15.0) was not detected when only SIM (LRS=4.4) analysis was employed. Similarly, D16Mit203 identified as suggestive using SIM (LRS=9.1) was subsequently identified as significant following CIM (LRS=19.6) analysis. These findings suggest that composite interval mapping may complement and enhance the efficacy of QTL identification. An appropriate strategy would be to identify the important markers using simple regression and interval mapping, and subsequently use the identified loci as cofactors (Jansen 1994).

Table 5 presents a list of potentially overlapping QTLs mapped for ethanol-induced activation to date. QTLs mapped in the present study, as well as those that potentially overlap (±5 cM) are highlighted in bold. While different genetic models have been used, there is a degree concordance between the genetic linkage markers identified in the present study and those reported in the literature. For example, the marker D17Mit10 on chromosome 17 identified in the present study was independently identified by Erwin et al. (1997) using LS×SS RI mice. Loci identified on chromosomes 2 (D2Mit340-68.9 cM), 4 (D4Mit54-66 cM) and 12 (D12Mit37-1 cM) overlap with independently identified markers (Gora-Masalak et al. 1991; Phillips et al. 1996; Erwin et al. 1997; Demarest et al. 1999).

It is of interest that the two different ethanol activation phenotypes utilized in the present study differed with regards to their associated markers. While the phenotypes were highly correlated ($r=0.742$ $P<0.001$), only one marker identified by Map Manager at the $P<0.01$ level (Es1 on chromosome 8; $P<0.01$) was shared. When loci with weaker associations ($P<0.05$) were considered together (e.g. all QTL reported on Table 2 and Table 3) a greater degree of commonality was observed. Similarly, Erwin et al. (1997) reported that ethanol activation phenotypes that were highly correlated ($r=0.73$) only shared one common QTL out of 11. Thus, it would appear that variations in the behavioural definition of ethanol-induced locomotor activity across studies may be an important source of variation in the identification of associated QTL.

A number of the QTL associated with ethanol activation in the present study overlap with those previously identified with ethanol drinking in AXB/BXA RI mice (Gill et al. 1998). Putative QTLs on chromosomes 16 for both males and females (Pmv14–45.6 cM) and chromosome 19 in females (D19Mit46–24 cM) overlap the genetic linkage markers identified in the present study (D16Mit27–45.5 cM and D19Mit47–29 cM, respectively). Furthermore, an analysis of the genetic correlations indicate that ethanol-induced activation (present study) and ethanol drinking (Gill et al. 1998) are inversely correlated ($r=-0.486$, $P<0.02$). This would suggest that in the AXB/BXA RI mice, ethanol-reward and ethanol activation share a common genetic component. It is interesting to note that the inverse correlation (lower locomotor sensitivity predicts higher alcohol consumption) is similar to that reported in human alcoholics (Schuckit 1994; Schuckit and Smith 1997).

In contrast, an examination of the data failed to identify any significant overlap among QTL for the basal locomotor activity scores in the present study and those identified previously for ethanol self-administration (Gill et al. 1998). This finding is consistent with a report by Cunningham (1995) in which the author failed to find any genetic correlations or overlap of strong provisional QTL between ethanol-induced conditioned place preference and habituation session activity in BXD RI strains. These data fail to support the notion that novelty-induced locomotor activation and sensitivity to the rewarding effects of ethanol are mediated by a common genetic mechanism.

Overall, the results of the present study using AXB/BXA RI mice have confirmed the association between a number of genetic linkage markers (chromosomes 2, 4, 12 and 17) and the expression of ethanol-induced activation. In addition, new loci on chromosomes 8, 16 and 19 were identified. The results suggest that the identification of significant loci associated with

ethanol activation may be enhanced through the use of composite interval mapping.

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