Cocaine-Induced Seizure Thresholds: Quantitative Trait Loci Detection and Mapping in Two Populations Derived from the C57BL/6 and DBA/2 Mouse Strains

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ABSTRACT

Seizures are a well known consequence of human cocaine abuse, and in rodent models, sensitivity to cocaine seizures has been shown to be strongly influenced by genotype. For example, several studies have reported significant differences between the C57BL/6 (B6) and DBA/2 (D2) inbred mouse strains in their sensitivity to cocaine-induced seizures. This prompted our use of the BXD recombinant inbred (RI) strain set and an F2 population derived from the B6 and D2 progenitor strains for further genetic analyses and for gene mapping efforts in this study. Cocaine was infused into the lateral tail vein, and the doses needed to induce a running bouncing clonic seizure and a tonic hindlimb extensor seizure were recorded for each mouse. In the BXD RI set, a genome-wide search was carried out for QTLs (quantitative trait loci), which are sites on a chromosome containing genes that influence seizure susceptibility. An F2 population (B6D2F2, n = 408) was subsequently used as a second, confirmation step. Based on both RI and F2 results, three QTLs emerged as significant (P < .00005): one for clonic seizures on chromosome 9 (distal), and two for tonic seizures on chromosomes 14 (proximal to mid) and 15 (distal). Two additional QTLs emerged as suggestive (P < .0015), both associated with clonic seizures on chromosomes 9 (proximal) and 15 (distal). Both QTLs on chromosome 9 were sex-specific, with much larger effects on the phenotype seen in females than in males.

The mechanisms by which high-dose cocaine causes seizures are not well known, but several plausible hypotheses have been proposed, each with supporting evidence from studies on laboratory mice. For example, these seizures are relatively resistant to a number of classic anticonvulsants, such as diazepam, phenytoin, carbamazepine, and phenobarbital, but are readily inhibited by a variety of functional N-methyl-D-aspartate (NMDA) antagonists (Ushijima et al., 1998; Witkin et al., 1999). This suggests that the NMDA receptor complex may be involved in the cause of cocaine seizures. Agonists and antagonists at the β-carboline site modulate cocaine seizure susceptibility, suggesting another possible mechanism of action (Ushijima et al., 1998). Cocaine inhibits the dopamine and serotonin transporters, which serves to enhance dopaminergic and serotonergic transmission, respectively. These actions may also contribute to cocaine seizure activity (e.g., Ritz and George, 1997). Several other plausible mechanisms have also been proposed for cocaine seizures (reviewed in Ritz and George, 1997; Ushijima et al., 1998; Witkin et al., 1999), suggesting multiple determinants and complex mechanisms of action.

Experiments using genetic mouse models have provided evidence for an underlying genetic role in drug-induced convulsions (Marley et al., 1986; Kosobud and Crabbe, 1990, 1993; Ferraro and Berrettini, 1996) and epileptic-like convulsions (Rise et al., 1991; Frankel et al., 1995a,b; Cox et al., 1997). Sensitivity to cocaine seizures, in particular, has been studied in several inbred strains of mice and in the BXD recombinant inbred (RI) strain series (Marley et al., 1991; Miner and Marley, 1995). These responses are continuously distributed across genotypes, suggesting the traits are influenced by several to many genes, each accounting for relatively small portions of the variance, rather than one or two major genes contributing a large amount of variance.

Using quantitative trait locus (QTL) mapping methods, it is possible to detect the influence of genes that contribute

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; QTL, quantitative trait locus; LOD, logarithm of the odds; THE, tonic hindlimb extensor; RBC, running bouncing clonic; RI, recombinant inbred.

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show high thresholds. Only the threshold data were subjected to QTL and other genetic analyses described later.

**Analysis of Strain (Genetic) Differences.** Because we wanted to compare the two progenitor strains, B6 and D2, a t test was used to determine whether a difference was present; a significance level of $P < .05$ was used in this situation only. For the BXD RI strains, a one-way ANOVA by strain was performed. The proportion of the total variance for each trait due to genotype (strain), or $R^2$, was calculated as $SS_{strain}/SS_{total}$, which provides an estimate of the narrow sense (additive) heritability (Belknap, 1998). A high value indicates a high signal (genetic variation)-to-noise (environmental variation) ratio, and the phenotypic strain mean values can be regarded as an accurate predictor of genotypic value (Falconer and McKay, 1996). In contrast, if the $R^2$ value is so low that it is not significant, then the trait is not significantly genetically determined; in this case, further genetic analyses, including QTL mapping, would not be warranted.

Reliability coefficients index the degree to which a given measure yields consistent or replicable results. Traits with reliability coefficients not significantly different from zero would not be suitable for QTL analysis. We used the split-half method by dividing the data for each strain (average $n = 13$) into two half-samples using the odd-even method and then calculating the strain mean values for each half sample. The correlation coefficient between the two half-samples ($r_{ch}$) was subjected to the Spearman-Brown correction (McNe- mar, 1962) for the total sample as follows: reliability $= 2r_{ch}(1 + r_{ch})$. This reliability coefficient is for the full sample. It represents an estimate of the correlation coefficient expected between the presently observed and replicated strain mean values if this entire experiment (full sample) were to be replicated exactly at a future time.

**QTL Analysis.** QTL analysis for the BXD data was carried out as described previously (Belknap et al., 1996, 1997). Briefly, the correlation coefficient, $r$, was calculated between the phenotype (strain mean values) and the genotype at each marker scored as 0 or 1 for the B6 or D2 alleles, respectively, possessed by each RI strain. A marker set of 1522 loci was used derived mostly from the database of Dr. R. W. Elliott incorporated into the Map Manager QT computer program (Manly and Olson, 1999). Because only two genotypic classes are possible per marker in the BXD strains, this point bise- rial correlation yields the same $P$ value as a $t$ test between trait mean values of strains bearing each allele. Only additive effects of a QTL were assessed by this analysis, because there were no heterozygotes that could express dominance. The positions of the markers were obtained from the 1999 Chromosome Committee reports on July 1999.

Correlations between phenotypic strain mean values and markers retaining $P < .01$, suggesting the possible presence of QTLs, were identified and are reported later. Because of the large numbers of correlations that were calculated, the number of false positives (type I errors) is problematic (Belknap et al., 1996). Therefore, the B6D2F2 population ($n = 408$) was used to determine whether the existence of each of these provisional QTLs could be independently supported.

Selective genotyping in the F2 was used to save genotyping costs (Lander and Botstein, 1989), where only the extreme ends (tail) of the trait distribution for each seizure type were genotyped, or 72 mice per trait. Thus, the 36 highest and 36 lowest threshold mice were genotyped, with almost equal sex ratios in each tail of the distribution. To further reduce costs, only the BXD-implicated QTL regions were searched in the F2, or about 15 to 20% of the genome (Belknap, 1998). For each provisional QTL, three microsatellite markers, one marker nearer the QTL and two flanking markers spaced about $\pm 10$ cM on either side, were used for confirmation testing in the B6D2F2 mice. Additional markers were added to regions where a significant QTL was found in an attempt to better estimate the region of highest association. The likelihood ratio $x^2$ test ($df = 1$) was used for the F2 population (2 × 2 table; B6 or D2 allele frequency × high or low tail) as a preliminary screen of associations.

**Materials and Methods**

**Animals.** Experimentally naïve female mice from 24 of the BXD RI strains ($n = 7$–16 mice per strain, or average of $n = 13$); their progenitor strains, B6 ($n = 18$) and D2 ($n = 17$); and both sexes of B6D2F2 mice ($n = 408$) derived from the intercross were bred at the Portland Veterans Affairs Medical Center colony. Male BXD F1 mice were committed to another experiment, leaving only females available for the present work. These populations were within three generations of breeding pairs obtained from the Jackson Laboratory (Bar Harbor, ME). All animals were maintained on a 12-h light/dark cycle (lights on at 6:00 AM) and given food and water ad libitum. After weaning at age 22 to 25 days, mice were housed in groups of two to four and were tested at age 56 to 100 days. Testing was conducted between 12:00 noon and 4:00 PM.

**Seizure Threshold.** This procedure followed one described previously in a study of other drug-induced convulsions (Kosobud and Crabbe, 1990, 1993). On the test day, mice were removed from their home cage and weighed. Mice were then loosely restrained in a 9.6-cm-long Plexiglas tube (o.d., 5.6 cm; i.d., 5.0 cm), and the mouse tail was bathed in warm water (40–45°C) for about 45 s. A 10-ml syringe containing 1 mg/ml cocaine hydrochloride dissolved in 0.9% saline was attached to the needle and placed into a syringe pump (Sage Instruments). A 27-gauge butterfly needle was inserted into the lateral tail vein, and the syringe pump was set to infuse cocaine at a rate of 0.5 mg cocaine HCl/min in a volume of 0.5 ml/min. Latency to an RBC seizure and to a tonic extensor seizure was recorded for every mouse. Infusion continued until the onset of tonic seizures. In all cases, the animals did not recover from the tonic extensor seizure. Clonic seizures invariably occurred first and ranged between 34 and 89 s after the start of infusion (mean ± S.D. = 57 ± 10 s) and tonic seizures between 69 and 216 s (mean ± S.D. = 125 ± 23 s). The threshold dose of cocaine in mg/kg was determined as the total dose infused up to the moment of seizure occurrence (both clonic and tonic) for individual mice. Thus, seizure-sensitive mice would show low thresholds, and resistant mice would have high thresholds, and only the threshold data were subjected to QTL and other genetic analyses described later.
between phenotype and genotype (Sokal and Rohlf, 1995). Those attaining a value of $P < .01$ were subjected to MapMaker QTL analysis.

For the B6D2F2 data, the MapMaker 3.0 program was used to construct the primary linkage map for at least four microsatellite markers flanking each confirmed QTL. The MapMaker/QTl 1.1 program was then used to assess the presence of a QTL within this framework (Lincoln and Lander, 1993). The latter program approximates the results of linear regression of phenotype on gene dosage (0, 1, or 2 D2 alleles) but adds several features not seen in conventional linear statistics. The most important of these are: 1) both additive and dominance effects of a QTL are assessed, 2) interval analysis using maximum likelihood estimation is used, conferring greater power to detect QTLs that may be located between markers, 3) a genotyping error check routine is built-in (Lincoln and Lander, 1992), and 4) correction for missing genotyping data. This last feature is critical in our studies because only the extreme ends of the phenotypic distribution were genotyped, leaving the middle of the distribution as “missing” (Landers and Botstein, 1989). For each QTL, if the map site of the peak log of the odds (LOD) found in the $F_2$ population was within 10 cM of the corresponding QTL site from the BXD analysis, $P$ values were combined using Fisher’s method (Sokal and Rohlf, 1995). This is justified because the 95% confidence intervals for QTL map location are at least 20 cM in each of the two populations, implying extensive overlap. The combined $P$ values were then converted to the equivalent $\chi^2/df - 1$ using the inverse $\chi^2$ distribution. This $\chi^2/df - 1$ value was divided by 4.6 to obtain LOD ($df = 1$) estimates (Landers and Kruglyak, 1995). Only $df = 1$ LOD scores are reported here for combined data.

The criteria for statistical significance were those recommended by Landers and Kruglyak (1995) for $F_2$ data (i.e., $P < .00005$ and $P < .0001$ for RI data (Belknap et al., 1996). For both RI and $F_2$ studies combined, $P < .00005$ was used for significance, which is approximately equivalent to LOD 3.8 ($df = 1$). After Landers and Kruglyak, the criterion for “suggestive” QTLs was 1.4 LOD less than this, or LOD 2.2 ($df = 1$). Suggestive implies that there will be on average one false positive, whereas significance implies that there is only a 5% chance of a false positive in a full genome search per trait. The reason such stringent criteria for significance are needed is because of the many markers required to cover the genome (all 20 chromosomes), which greatly inflates false positive errors. For example, in computer simulation studies carried out in the BXD RI strains using essentially the same marker set as used here, an average of 3.8 $\pm$ 1.9 (S.D.) false positive QTLs were seen per trait at $P < .01$ and 19 $\pm$ 4.3 (S.D.) were seen at $P < .05$ (Belknap et al., 1996). However, at $P < .0001$, only one false positive was seen for every 20 traits (approximately), or 0.05 per trait, which is by definition the genome-wide threshold for significance (Landers and Kruglyak, 1995). Thus, BXD-nominated QTLs identified at $P < .01$ must be considered provisional and require further testing in an $F_2$ or other independent population to determine whether significance can be obtained (Belknap et al., 1996, 1997).

Because both sexes were tested and genotyped in approximately equal numbers in the $F_2$, QTL analysis using the likelihood ratio $\chi^2$ analysis was carried out for each sex. QTLs were judged to be sex-specific if the LOD scores ($df = 1$) per sex differed by at least 1.3 LOD (equivalent to $P < .05$). Because only females were tested in the BXD set, we could not extend this analysis to the RI population.

Genotyping. For the B6D2F2 population, DNA was isolated from spleen and genotyped using standard methods (Buck et al., 1997). Very briefly, B6D2F2 mice were sacrificed by cervical dislocation, whereas the other half was frozen in 1.0 ml of physiological saline and stored as a backup. Genotyping using polymerase chain reaction techniques was carried out with the microsatellite marker loci developed and characterized by the group of Dr. Eric Landers at Massachusetts Institute of Technology (Dietrich et al., 1992, 1994). More than 2500 loci polymorphic in B6D2F2 intercrosses are distributed throughout the mouse genome, and all can be genotyped using the same experimental protocol with oligonucleotide primer pairs specific to each marker. The primer pairs were obtained from Research Genetics, Inc. (Huntsville, AL). Polymerase chain reaction genotyping was a modification of that described in Dietrich et al. (1992) using ethidium bromide staining and high-resolution agarose (MetaPhor; FMC) in place of $^{32}P$ radiolabeling and polyacrylamide.

**Genetic Correlations with Other Convulsive Traits.** To gain some perspective on how cocaine seizures compare with other drug-induced seizures, we determined the correlation coefficient between the BXD inbred strain mean values for both seizure end points in the present study with a number of traits related to convulsions elicited by other agents or treatments also tested in these same strains. Correlation of the strain mean values for two or more traits estimates a genetic correlation, which indexes the degree to which two traits share common genetic influences (Crabbe et al., 1990). These data came from our Portland Alcohol Research Center (PARC) BXD database of more than 330 traits related to drugs of abuse that were tested on at least 18 of these strains.

**Results**

**BXD RI, B6, and D2 Strains.** As shown in Fig. 1, B6 mice were significantly more sensitive than D2 mice to tonic seizures ($P < 10^{-6}$). In contrast, no difference between these two inbred strains was found for clonic seizures ($P = .16$, NS). For both clonic and tonic seizures, a continuous distribution (Image 308x132 to 559x441)
of BXD RI strain mean values for threshold cocaine dose was seen (Fig. 1). Strains differed significantly in sensitivity to both clonic and tonic seizures (all $P < 10^{-6}$). Reliability coefficients in the RI set were $r = 0.86$ for clonic seizures and $r = 0.87$ for tonic seizures (both $P < 10^{-8}$), which indicates that both traits were sufficiently consistent and replicable for QTL analysis. We also calculated the correlation coefficient between age of the animals and the two seizure end point latencies; both $r$ values were $P > .25$, indicating that the varying ages of the animals were not significantly associated with the principal measures studied in this report.

In general, the dose infused up to the time of clonic seizures averaged about half that to induce tonic seizures. The two seizure end points were correlated: $r = 0.45$ ($P < .05$) among strain mean values and $r = 0.50$ ($P < 1 \times 10^{-5}$) among individual mice ($n = 300$). Because the correlation among strain mean values estimates a genetic correlation (Crabbe et al., 1990), this value is of particular interest for genetic analyses. When this correlation was corrected for attenuation (less-than-perfect reliability for both seizure end points), this correlation became 0.52 (McNemar, 1962). This is only slightly higher than the uncorrected $r$ value (0.45). Thus, only partial overlap in genetic determinants is indicated. Some common QTLs would be expected, but also some unique to each seizure type. The narrow sense heritability (in their BXD RI study (see Table 1, denoted by a superscript $a$), which are $r^2 = 0.21$ for clonic seizures and 0.22 for tonic seizures.).

Table 1 shows the correlation coefficients and $P$ values for marker loci associated at $P < .01$ with either clonic or tonic cocaine-induced seizures. Thirteen regions on 10 chromosomes were found to be associated at $P < .01$ with cocaine-induced seizures. Five of these provisional QTLs are in chromosomal regions reported by Miner and Marley (1995) at $P < .01$ in the BXD RI strain set, three with the B6 allele conferring a higher threshold cocaine dose in an additive manner ($d = 0$). In this case, it seems the B6 allele protects the mice against this type of seizure with greater protection in B6 homozygotes than in heterozygotes. This is opposite in direction from the other QTLs reported, which can be expected occasionally with polygenic traits.

**B6D2F2 Population.** The correlation between the two seizure end points was $r = 0.72$ ($P < 10^{-8}$), which is somewhat higher than that seen in the RI data for individual mice ($r = 0.50$). This is a phenotypic correlation reflecting both genetic and environmental sources of covariance. (There are no satisfactory methods to estimate a genetic correlation solely from F2 data.) The F2 QTL results are summarized in Table 2, and LOD plots are shown in Figs. 2 and 3.

**Combined Analysis.** Three QTLs have been confirmed as significant in the BXD RI and B6D2F2 populations after pooling both $P$ values from each population to obtain a combined $P$ value using R.A. Fisher’s method (Table 2). For the clonic seizure threshold, one significant QTL ($P = .00005$, LOD $= 3.6$) was found on distal chromosome 9 near the MvosA (formerly known as dilute) locus at 42 cM, with the D2 allele conferring a larger threshold value (greater resistance) and showing complete recessivity to the B6 allele (F2, Fig. 2). In Falconer and MacKay’s (1996) terminology, $d$ (dominance variation) was somewhat greater than $a$, the additive effect of an allele substitution. The D2 allele at this QTL in the homozygous state predisposes protection against clonic seizures induced by cocaine in these mice.

One significant QTL for the tonic seizure threshold trait was located on chromosome 15 near the D15Mit48 marker (60 cM, $P = .000015$, LOD $= 4.1$, Fig. 3), with the B6 allele conferring a higher threshold cocaine dose in an additive manner ($d = 0$). In this case, it seems the B6 allele protects the mice against this type of seizure with greater protection in B6 homozygotes than in heterozygotes. This is opposite in direction from the other QTLs reported, which can be expected occasionally with polygenic traits.

A third QTL was found to be significant on chromosome 14 for tonic seizures in the vicinity of the D14Mit39 marker (30 cM, $P = .0000012$, LOD $= 5.1$, Fig. 2), with the D2 allele associated with higher thresholds in an additive manner ($d = 0$). LOD plots of F2 data were constructed using information from MapMaker QTL for each chromosome containing a significant QTL, and the results are shown in Figs. 2 and 3.

The QTL analyses pooled over both populations (Table 2) show that of the 13 provisional QTLs found for both clonic and tonic seizures at $P < .01$ in the BXD RI strain set, three were confirmed as significant, and two additional QTLs were confirmed at the suggestive level. Because “suggestive” implies that there will be an average of one false positive in a full genome search per trait (Lander and Kruglyak, 1995), we can roughly surmise that one of the two suggestive QTLs is probably a true positive, bringing our estimate of the number of “true” QTLs to roughly 4 of the original 13 BXD-nominated QTLs. Of the remaining nine provisional QTLs, we surmise that some of them reflect true QTLs with effects too small to

### Table 1
Provisional QTLs ($P < .01$) identified by QTL analysis of BXD RI data

<table>
<thead>
<tr>
<th>Marker Locus</th>
<th>Range at $P &lt; .05$</th>
<th>Clonic SZ</th>
<th>Tonic SZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChromosomeM</td>
<td>70–98</td>
<td>$-0.61 (.002)$</td>
<td></td>
</tr>
<tr>
<td>D2Bir1 (2:73)$^b$</td>
<td>11–12</td>
<td>0.53 (.008)</td>
<td>0.52 (.009)</td>
</tr>
<tr>
<td>D4Bir4 (4:11)$^b$</td>
<td>35–45</td>
<td>$-0.67 (.002)$</td>
<td></td>
</tr>
<tr>
<td>Ptprd (4:38)</td>
<td>60–83</td>
<td>$-0.56 (.008)$</td>
<td></td>
</tr>
<tr>
<td>Nhel (4:65)</td>
<td>30–38</td>
<td>0.60 (.006)</td>
<td></td>
</tr>
<tr>
<td>D6Ncvs37$^a$ (6:30)$^b$</td>
<td>9–15</td>
<td>$-0.59 (.003)$</td>
<td></td>
</tr>
<tr>
<td>Mag$^a$ (7:11)$^b$</td>
<td>45–50</td>
<td>$-0.62 (.006)$</td>
<td></td>
</tr>
<tr>
<td>Hbb-y$^a$ (7:50)$^b$</td>
<td>58–61</td>
<td>0.56 (.007)</td>
<td></td>
</tr>
<tr>
<td>Fv2 (9:61)</td>
<td>13–50</td>
<td>$-0.54 (.006)$</td>
<td></td>
</tr>
<tr>
<td>D11Ncvs70 (11:25)</td>
<td>0</td>
<td>$-0.53 (.007)$</td>
<td></td>
</tr>
<tr>
<td>Mod1r (12:0)</td>
<td>38–58</td>
<td>$-0.54 (.007)$</td>
<td></td>
</tr>
<tr>
<td>Tstop 198 (13:48)</td>
<td>14–43</td>
<td>0.62 (.001)</td>
<td></td>
</tr>
<tr>
<td>D14Byu5 (14:33)</td>
<td>18–59</td>
<td>$-0.59 (.003)$</td>
<td></td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Marker Locus</th>
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<th>Clonic SZ</th>
<th>Tonic SZ</th>
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<td></td>
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</tbody>
</table>

$^a$ Range in cM for markers attaining $P < .05$ association with the seizure traits.

$^b$ Markers in the same chromosomal region as those found by Miner and Marley (1995) at $P < .05$. Because of procedural differences (see Discussion), a higher level of agreement between the present study and their study was not expected.
be reliably detected with our present sample size. However, a
good proportion of the nine are probably false positives in the
ultimate sense (i.e., they represent purely random variation;
Belknap et al., 1996). This underscores the desirability of
subjecting BXD RI QTL data to further testing in an addi-
tional independent mapping population: in our case, an F2.

Of the five QTLs attaining either significant or suggestive
status, two showed clear and significant sex specificity. Both
were on chromosome 9, influencing clonic seizures, with the
females showing the larger effect on the phenotype in each
case. For proximal chromosome 9, as indexed by the marker
D9Mit205 (18 cM), females showed an LOD score of 3.7
compared with an LOD score 0.5 for males (df = 1, P = .0002
for the sex difference). When the female F2 data were pooled
with the female BXD data, this QTL becomes LOD 2.7 (P = 2
× 10^{-4}), which is still only suggestive. For distal chromo-
some 9, as indexed by the marker D9Mit51 (61 cM), females
showed an LOD score of 2.4 versus 0.5 for the males (df = 1,
P = .002 for the sex difference). The combination of the

### TABLE 2

Combined RI and F2 QTL results for suggestive (LOD > 2.3) and significant (LOD > 3.6) QTLs for both types of cocaine-induced seizures

<table>
<thead>
<tr>
<th>Chrom. (RI, F2 cM)</th>
<th>RI P Value</th>
<th>F2 P Value</th>
<th>Combined P (LOD, sign)</th>
<th>Mode in F2 (h2QTL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonic SZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:(24, 21)</td>
<td>.40</td>
<td>4 × 10^{-4}</td>
<td>1.6 × 10^{-3} (2.2, D2+)</td>
<td>Additive (.06)</td>
</tr>
<tr>
<td>9:(61, 55)</td>
<td>.007</td>
<td>5 × 10^{-4}</td>
<td>5 × 10^{-5} (3.6, D2+)</td>
<td>B6 dominance (.06)</td>
</tr>
<tr>
<td>15:(58, 61)</td>
<td>.15</td>
<td>6 × 10^{-4}</td>
<td>8 × 10^{-4} (2.4, B6+)</td>
<td>Additive (.07)</td>
</tr>
<tr>
<td>Tonic SZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:(32, 22)</td>
<td>.0014</td>
<td>5 × 10^{-5}</td>
<td>1.2 × 10^{-6} (5.1, D2+)</td>
<td>Additive (.13)</td>
</tr>
<tr>
<td>15:(48, 60)</td>
<td>.0025</td>
<td>4 × 10^{-4}</td>
<td>1.5 × 10^{-5} (4.1, B6+)</td>
<td>Additive (.05)</td>
</tr>
</tbody>
</table>

* Location (cM) of minimum P (peak LOD) for RI and for F2 QTL analyses, respectively.
* Direction of effect: the allele that is associated with higher threshold values (less sensitivity to seizures).
* Mode of inheritance (F2 data): if the dominance deviation (d) was less than one fourth of the additive effect (a, also called “weight” in MapMaker QTL), then the QTL was judged to be essentially additive. Dominance here implies complete dominance, or d ≥ a. The heritability of a QTL (h2QTL), or the proportion of the trait variance accounted for by a QTL, was taken from F2 MapMaker QTL output.

Fig. 2. MapMaker QTL results for the F2 data expressed as LOD scores (df = 2) plotted against map distance along chromosome 9 for clonic seizures (top) or chromosome 14 for tonic seizures (bottom). The “free” model from MapMaker QTL output refers to no constraints placed on the mode of inheritance (i.e., a dominance, recessive, or additive model was not imposed). Chromosome 9 results suggest two QTLs: one proximal with a strictly additive mode of inheritance (d = 0) and the other distal with a completely dominant (d ≥ a) mode of inheritance for the B6 (decreasing) allele. This difference provides further evidence that the proximal and distal regions reflect distinct QTLs. The plotted LOD values for chromosome 9 predominantly reflect the contribution of females, because the males contributed relatively little to either of the two QTLs. In contrast, both sexes contributed equally to the chromosome 14 QTL (bottom).

Fig. 3. MapMaker QTL results for the F2 data expressed as LOD scores (df = 2) plotted against map distance along chromosome 15 for clonic seizures (top) or tonic seizures (bottom) for the “free” model. Both QTLs were essentially additive (d = 0) and equal in the two sexes.

Of the five QTLs attaining either significant or suggestive status, two showed clear and significant sex specificity. Both were on chromosome 9, influencing clonic seizures, with the females showing the larger effect on the phenotype in each case. For proximal chromosome 9, as indexed by the marker D9Mit205 (18 cM), females showed an LOD score of 3.7 compared with an LOD score 0.5 for males (df = 1, P = .0002 for the sex difference). When the female F2 data were pooled with the female BXD data, this QTL becomes LOD 2.7 (P = 2 × 10^{-4}), which is still only suggestive. For distal chromosome 9, as indexed by the marker D9Mit51 (61 cM), females showed an LOD score of 2.4 versus 0.5 for the males (df = 1, P = .002 for the sex difference). The combination of the
female F2 and female BXD data yielded an LOD value of 3.4 (P = 8 × 10⁻⁵), which is just below the significance threshold. Thus, it is clear that for chromosome 9 alone, the females contributed much more than the males to the LOD plots for both QTLs shown in Fig. 1 (top) for both sexes combined. For the remaining QTLs, no sign of sex specificity was evident (all P > .05).

A comparison of the BXD RI strain mean values for the two seizure phenotypes with other convulsive traits in the PARC database reveals that neither clonic nor tonic seizures due to cocaine are significantly genetically correlated (all r < 0.43, all P > .05) with either handling-induced withdrawal convulsions due to acute (Buck et al., 1997) or chronic (Crabbe, 1998) alcohol, acute nitrous oxide (Belknap et al., 1997), and acute pentobarbital (Buck et al., 1999), nor are tonic or clonic convulsions induced by high pressure (McCall and Frierson, 1981), loud sound (audiogenic seizures, Neumann and Seyfried, 1991), or i.v. NMDA infusion (B. Martin, in preparation). (This last is particularly interesting because the NMDA receptor complex is one of the most commonly hypothesized mechanisms for cocaine seizures.)

**Discussion**

The B6 and D2 strains are the two most studied mouse strains in genetic research involving cocaine. This has resulted in sizeable literature concerning these two strains (Seale, 1991; Marley et al., 1992; Morse et al., 1995). For example, B6 mice, compared with D2 mice, voluntarily consume more cocaine in the drinking fluid versus water, are less activated in an open field, are more resistant to hepatotoxicity, and are more sensitive to seizures. The B6 and D2 strains have also been studied with regard to sensitivity to a wide variety of convulsant drugs other than cocaine, and the general finding has been that the D2 strain is more sensitive to the vast majority of them (reviewed in Kosobud and Crabbe, 1990). Cocaine-induced tonic seizures are thus an exception in that the B6 strain is the more sensitive. Other exceptions are strychnine-induced myoclonus (but not THF seizures) and picrotoxin-induced THF (but not myoclonus). This suggests that the mechanisms of action of cocaine-induced tonic seizures may not correspond closely to those of the majority of classic convulsive agents. [For clonic cocaine seizures, no significant difference was seen (P = .16, NS), although the trend was in the same direction as tonic seizures.]

Much stronger evidence for the relationships among convulsants comes from studies on 20 or more of the BXD inbred strains, where genetic correlations can be estimated (Crabbe et al., 1990). As listed in Results, no significant genetic correlations were observed between the two cocaine seizure end points and a number of other drug-induced convulsions. To summarize, cocaine appears not to be a “carbon copy” of any of the other convulsant agents or treatments studied genetically. Therefore, it is likely that its QTL underpinnings will differ substantially from the other convulsants studied.

The one previous report on cocaine seizures in the BXD RI set (Miner and Marley, 1995) identified provisional QTLs that partially matched our BXD results. They used males, whereas we used females, which appears to be an important difference given the sex-specific QTLs found in the present work. In their experiments, a fixed i.p. dose of cocaine (60 mg/kg) was used and scoring was based on whether each mouse did or did not have a clonic seizure (a quantal measure). They identified nine provisional QTLs at P < .05, five of which were found in our BXD study at P < .01, as listed in Table 1. The correlation among strain mean values between our study and theirs was r = 0.52 (P = .007) for clonic seizures and r = 0.59 (P = .002) for tonic seizures, showing moderate agreement between the two BXD studies. However, about half of the 24 RI strains in their sample showed zero or near-zero scores, which imposed a “floor” effect, tending to artifactually reduce variability across strains (truncated genetic variance). Because no such floor effect was seen in our study, we surmise that this factor may have been an important reason why better agreement between the two studies was not seen.

Central nervous system-relevant genes located in the same chromosomal region as a QTL can be considered as candidate genes that may be the basis for the QTL. By identifying candidate genes, additional studies can examine whether these genes in fact affect seizure threshold in the BXD RI, B6, D2, and B6D2F2 populations, and possibly other mouse populations. In the region on chromosome 9 in which one of the suggestive QTLs was mapped lies the Drd2 (29 cM) gene, which encodes the dopamine D2 receptor, and the more distal significant chromosome 9 QTL lies in the general region of the Htr1b gene (46 cM), which encodes the serotonin1B receptor. Other investigators have found a link between the regions on chromosome 9 associated with cocaine seizures and other seizure phenotypes. Two QTLs for a mouse model of partial epilepsy, El15 (at 55 cm) and El4 (at 23 cM), have been mapped to chromosome 9 near the presently reported QTLs for cocaine (Rise et al., 1991; Frankel et al., 1995b). Grik4 (23 cM), a glutamate (kainate) receptor gene, maps very near El4. The Bis3 locus (~42 cM) is a QTL on chromosome 9 influencing seizures induced by methyl β-carboline-3-carboxylate, an inverse agonist at the γ-aminobutyric acid, benzodiazepine site (Clément et al., 1996). This is particularly interesting in view of recent findings that agonists and antagonists at this β-carboline site potently modulate sensitivity to cocaine-induced seizures in mice (Ushijima et al., 1998). These findings suggest the possibility that the gene or genes responsible for the chromosome 9 cocaine QTL may be related to specific other seizure phenotypes, but more work is needed to determine whether the same gene is involved in each case. High-resolution mapping efforts will be especially useful here.

A plausible candidate gene for the chromosome 14 QTL influencing cocaine-induced tonic seizures is Grid1 (14 cm), the glutamate receptor δ1 subunit gene. Also in this region is El15 (28 cM), a QTL influencing a partial epilepsy seizure model (Frankel et al., 1995b). For the chromosome 15 QTL affecting both clonic and tonic seizures, two candidate genes are evident: the Scn8a gene (60 cm), which encodes a voltage-gated sodium channel, and the Cehb3 gene (60 cm), which encodes the B3 subunit of a calcium channel. Both sodium and calcium channels have been implicated in seizure occurrence. Again, this region appears to be associated with seizure phenotypes that may not be specific to cocaine-induced seizures.
Finally, it is important to note that the thresholds used to determine the phenotype for genetic analysis may be in part influenced by pharmacokinetic factors (i.e., those that determine the concentration of cocaine reaching sensitive central nervous system sites). Azar et al. (1998) have shown that after i.p. cocaine injections of 30 mg/kg in D2 and B6 mice, brain concentrations were significantly higher in D2 mice, especially at the earliest (5 and 15 min) time points. Jones et al. (1993) also found higher brain cocaine concentrations in D2 versus B6 mice, but statistically significant was not attained (P < .07). Womer et al. (1994) and Tolliver et al. (1994), however, reported no significant differences between these two strains in brain cocaine levels at 5 or 15 min, respectively, after i.p. administration of cocaine. We do not know whether these findings apply to i.v. infusions, but the large bolus, no absorption, and short infusion times (0.5–3.5 min) in our study would be expected to produce smaller pharmacokinetic effects than the i.p. route. Nevertheless, it is possible that some of the five QTLs we have identified influence seizures through their effects on cocaine kinetics. Another consideration is that active metabolites, particularly norcocaine, may contribute to the seizures we observed, and if so, the rate at which norcocaine is generated from cocaine may be important. However, all seizures seen in the present study occurred at 0.5 to 3.5 min after the start of infusion, which does not allow much time for biotransformation of appreciable amounts of norcocaine to take place.

In summary, three significant QTLs (each P < .00005) associated with cocaine-induced seizures were discovered in a two-step QTL analysis using the BXD RI strains and a B6D2F2 population. One QTL was associated with chronic seizures on chromosome 9 (distal), and two QTLs were associated with tonic seizures on proximal to mid chromosome 14 and distal chromosome 15, respectively. In addition, two more QTLs were found to be suggestive by Lander and Kruglyak (1995) criteria, with both involving clonic seizures. These were located on chromosomes 9 (proximal) and 15 (distal). Of the five QTLs identified as either suggestive or significant, two of them appear to be largely specific to females in their effects on the phenotype. Both of these involve clonic seizures, and both are located on chromosome 9 based on F2 data. Unfortunately, we did not have BXD data for both sexes that would have allowed us to confirm this, but confirmation tests are planned using congenic mice now under development.

The QTL on chromosome 15 appears to influence both seizure traits and thus may contribute to common mechanisms underlying both seizure types. Because the other QTLs did not overlap, however, this indicates likely differences in these seizure types as well. Several promising candidate genes were proposed that can be studied to try to elucidate the mechanistic actions of cocaine-induced seizures. To aid this effort, we plan to develop congenic strains for each QTL and to use them to attain much higher map resolution, down from our present 20 to 30 cM (95% confidence intervals) to 1 to 2 cM using the interval-specific congenic strain strategy (Crabbe et al., 1999). These new strains should greatly facilitate efforts to identify the gene or genes underlying each QTL and to characterize QTL actions at all levels from the molecular to the organismic.

References


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