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# Sensitivity of AXB/BXA recombinant inbred lines of mice to the locomotor activating effects of cocaine: a quantitative trait loci analysis

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**The present study was conducted in order to characterize putative quantitative trait loci (QTL) for cocaine-induced activation in the AXB/BXA recombinant inbred (RI) lines of mice. Locomotor activity was measured in the AXB/BXA and progenitor A/J and C57BL/6J strains using a computerized open-field apparatus following saline or cocaine (0, 5, 10, 20 mg/kg) administration (i.p.). Analyses were conducted on phenotypes including both novelty (responses under initial saline conditions) and cocaine-induced locomotor activity. Significant differences were observed across RI lines on all measures. Gender differences in sensitivity to the activating effects of cocaine were not observed. The wide and continuous distributions of phenotypic responses in the AXB/BXA RI lines suggested polygenic regulation. Initial basal locomotor activity was significantly correlated with cocaine-induced activation (raw scores) ($r = 0.60$, $P = 0.0021$) but not with cocaine difference scores ($r = 0.370$, $P = 0.082$). Simple regression and interval mapping were used to initially identify significant gene markers associated with novelty and cocaine-induced activation. Subsequently, composite interval mapping was used to increase the accuracy in mapping individual loci. QTL analysis of cocaine-induced activation (difference scores – 20 mg/kg dose) identified significant loci on chromosomes 12 (23 cM), and 15 (46.8 cM). The significant QTLs were identified on chromosomes 12 and 15 map to regions in proximity to genes for the somatostatin 1 (*Smstr1* −23 cM) and 3 (*Smstr3* −46.3cM) receptors, respectively. Further research employing AcB/BcA recombinant congenic lines of mice will be employed to confirm the QTL on chromosomes 12 and 15 identified in the present study.**



*Keywords*: inbred mice, recombinant, cocaine, somatostatin, locomotor activity

## Introduction

Genetic factors are known to influence the effects of cocaine on locomotor activity (Shuster *et al.*, 1977; George *et al.*, 1990; Tolliver & Carney, 1994; Rocha *et al.*, 1998). Genetically distinct strains of rats and mice exhibit differential sensitivity to the activating effects of cocaine. For example, George *et al.* (1990) demonstrated that cocaine produced locomotor activation in Short Sleep but not Long Sleep mice. Similarly, Shuster *et al.* (1977) demonstrated increased sensitivity to cocaine's activating effects in C57BL/6J relative to A/J mice.

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While animal models have demonstrated the influence of genetic factors in the mediation of cocaine-induced activation, the specific genes which mediate this behaviour remain unknown. Strategies that can isolate multiple-genes that individually account for small amounts of variance (called quantitative-trait loci, QTL), have begun to identify chromosomal regions associated with cocaine-induced activation in the mouse. For example, QTL analyses of cocaine-induced locomotor activation have been performed using BXD recombinant inbred (RI) lines of mice. In a recent study, Jones *et al.* (1999) reported the effects of cocaine administration on several measures of activity in a panel of BXD/Ty RI strains. The activity measures included the total distance traveled, nosepokes, repeated movements and time spent in proxi-

mity to the centre of the apparatus. Most QTLs associated with locomotion were identified on chromosomes 5, 9 and 15. In contrast, nosepokes were most closely associated with loci on chromosomes 2, 3, 9, 11, 12.

Phillips *et al.* (1998) used the BXD/Ty RI lines and their progenitor C57BL/6J and DBA/2J inbred strains to estimate the genetic correlations between basal activity, acute cocaine activation (5, 10 and 40 mg/kg) and sensitized cocaine responses. Measures of cocaine activation were a function of activation corrected for both within or between group levels of basal locomotor activity (cocaine–saline). Putative QTLs associated with acute cocaine activation were identified on chromosomes 1–4, 7–10, 13–16, as well as 18 and 19.

Miner and Marley (1995) examined the influence of 10 mg/kg of cocaine on locomotor activity. Locomotor activity was defined as the distance traveled (cm). QTLs associated with cocaine difference scores (cocaine–saline) were identified on chromosomes 3, 10 and 17. Loci on chromosome 5 were highly associated with simple cocaine induced activity measures.

Finally, using 16 lines of BXD RI mice and the progenitors (C57BL/6J and DBA/2J), Tolliver *et al.* (1994) similarly demonstrated genotype-dependent effects on cocaine responsivity. QTL analysis demonstrated that the most significant correlations were identified with markers on chromosome 9 ($r = 0.74$), 11 ($r = -0.71$) 16 ($r = 0.69$) and 17 ($r = 0.84$).

The magnitude of the differences across laboratories in the identification of associated loci in the BXD RI lines may have resulted from confounding environmental influences as well as different phenotyping procedures. However, as the aforementioned review would suggest, common loci have been associated with cocaine-induced locomotor activation, on chromosomes 5 (Phillips *et al.*, 1998; Jones *et al.*, 1999), 9 (Tolliver *et al.*, 1994; Miner & Marley, 1995; Phillips *et al.*, 1998), 10 (Miner & Marley, 1995; Phillips *et al.*, 1998), 15 (Phillips *et al.*, 1998; Jones *et al.*, 1999) and 17 (Tolliver *et al.*, 1994; Miner & Marley, 1995).

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, $F_2$ intercrosses and congenic mice (Dudek *et al.*, 1993; Belknap *et al.*, 1996). It should also be noted that the QTLs identified with RI lines should be considered provisional due to the large number of statistical tests conducted during QTL analysis and the potential for an inflated level of type 1 error (i.e. false positives). Therefore, the present study attempted to expand upon the current database of putative QTL associated with cocaine-induced activation using the AXB/BXA RI lines of mice. The AXB/BXA RI lines were derived by inbreeding different sets of $F_2$ progeny – from reciprocal crosses between the A/J (A) and C57BL/6J (B) progenitors. AXB strains were derived from A female $\times$ B male crosses; BXA strains were derived from B female $\times$ A male crosses) (Marshall *et al.*, 1992). After more than 20 generations of inbreeding, each of the AXB/BXA RI lines has a different 'recombination' of the parental genome in a homozygous state. This RI series has not previously been phenotyped for cocaine-induced behaviours. In the present study, composite interval mapping (CIM) was used, in addition to simple interval mapping (SIM), in order to control the influence of the effects of background QTL which can contribute to the sampling variance and reduce the significance of any association (Jansen, 1994; Zeng, 1994).

## Methods

Male and female C57BL/6J, A/J and AXB/BXA recombinant inbred mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Initially, four breeder pairs/strain were established and maintained in an animal colony controlled for temperature and humidity on a 12-hour day/night cycle (lights on between 06.00 h and 18.00 h). Generally, breeder pairs were kept for 8 months, providing 4–6 litters. The mice were housed in standard show-box solid bottom cages with beta chip bedding. Pups were weaned at 4 weeks and housed with same-sex litter mates until 8 weeks of age. A total of 1207 mice were tested. Subjects used in the present study had been previously tested for locomotor activation following a single injection (i.p.) of saline or ethanol. Statistical analysis indicated that cocaine-induced locomotor activation in mice exposed to a single prior injection of ethanol did not differ from those which received a prior saline injection.

### *Locomotor activity testing*

Locomotor activity was measured in open field boxes constructed of Plexiglas, measuring 30 cm $\times$ 30 cm $\times$ 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 photo cells. Counts were automatically registered at 1-min intervals throughout 15-min sessions. A custom-designed computer software program monitored activity on the photo cells, providing measures of horizontal and stereotypic activity. Programming for the horizontal activity included routines suppressing scores from repetitive breaking of photo beams

due to grooming. Testing was performed under red light in a sound-insulated room between 15.00 h and 18.00 h 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 (i.p.) of sterile 0.9% NaCl saline solutions on two successive days. The locomotor activity scores obtained following saline administration on day 1 and 2 were considered to reflect the interaction between the subject's basal locomotor activity and their responsivity to a novel environment.

The animals were randomly assigned to one of four cocaine dose conditions (0, 5, 10 or 20 mg/kg). The subjects were injected i.p. with either a saline or cocaine solution. Activity was monitored for 15 min immediately following each injection. Measures obtained from this experimental design included responses to saline injections on saline days 1 and 2, as well as measures of (i) cocaine activation (untransformed locomotor activity scores following cocaine injections) and (ii) cocaine difference scores (activity on cocaine injection day minus activity on saline day 2).

*Statistical analysis*

Statistical analyses were performed with SPSS version 8.0 for Windows (SPSS Inc., Chicago, IL, USA). 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. Scores outside the 95% confidence interval for each strain were eliminated. In all, 65 mice randomly distributed across strains were removed. Following removal of outliers the sample size was 1135 (540 female mice and 595 male mice). A randomly selected split-half correlational analysis (corrected with Sperman-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 & 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. Heritability was defined as the proportion of phenotypic variance attributed to genetic variance (Plomin & McClearn, 1993).

*Quantitative trait loci analysis*

The phenotypic data collected on the AXB/BXA RI lines 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 lines were obtained from the Mouse Genome Database (2000). At present, there are 780 markers identified in the AXB/BXA RI lines (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 chi-squared (likelihood ratio statistic, LRS) provided by Map Manager.

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 cocaine activation (cocaine 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 potential limit of QTL detection. 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 lines tested in the present study, it is possible that not all QTLs were detected and the strength of those that were detected may be overestimated (Manly & Olson, 1999). Therefore, it is not expected that the present study detected all relevant loci affecting cocaine-activation. This will most likely be the case for alleles of small to moderate effect.

*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 & Doerge (1994) were conducted using the Map Manager QT software (Manly, 1998). In this test, the trait values were randomly permuted among the progeny, thus destroying any relationship between the trait values and the marker loci. A regression model was fitted for the permuted data at multiple analysis points across the genome (matching the points used for detecting QTLs) and the maxi-

mum LRS was 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 were taken as critical values to establish significance (Manly, 1998). For example, the 95th percentile establishes significance corresponding to the usual criterion of $P = 0.05$. The threshold values of the permutation test labeled as suggestive or significant for linkage were taken from the guidelines of Lander & Kruglyak (1995).

## Results

A two-way (strain × gender) ANOVA was performed on total locomotor activity scores (15 min totals) obtained following the initial (day 1) saline injection. Significant strain differences were observed ($F_{24,1135} = 33.81,\ P < 0.001$) following saline injection (data not shown), however, there were no significant gender effects or strain by gender interactions ($F_{24,1135} = 1.036,\ P = 0.42$). Thus data from males and females were combined, and gender was not considered in subsequent analyses.

### *Cocaine-induced locomotor activation*

The effects of cocaine on locomotor activity in the C57BL/6J, A/J, and AXB/BXA RI mice were assessed using two distinct measures of cocaine-induced activation as discussed above in the methods section (cocaine activation and cocaine difference scores). A three-way ANOVA (strain × gender × cocaine dose) was performed on the cocaine activation scores. The results indicated that there were no significant gender effects ($F_{1,1041} = 0.759,\ P = 0.4$), strain–gender interactions ($F_{22,1041} = 0.961,\ P = 0.6$) or strain × gender–dose interactions ($F_{47,1041} = 0.990,\ P = 0.5$) on any of the measures evaluated.

The analysis of the effects of a range of cocaine doses on cocaine difference scores for those strains (14) which were tested at all three doses of cocaine (5, 10, 20 mg/kg) yielded a significant strain by cocaine dose interaction ($F_{39,737} = 4.850,\ P = 0.001$). The mean strain values across the cocaine dose–response curve are presented in Fig. 1. The results showed that maximal strain separation was observed at the 20 mg/kg dose of cocaine. On the basis of these initial dose–response data the 20 mg/kg dose of cocaine and vehicle control were selected for subsequent QTL analysis using all of the available AXB/BXA RI lines.

The mean cocaine difference scores for the C57BL/6J, A/J and AXB/BXA RI strains following administration of 20 mg/kg cocaine are presented in Fig. 2.

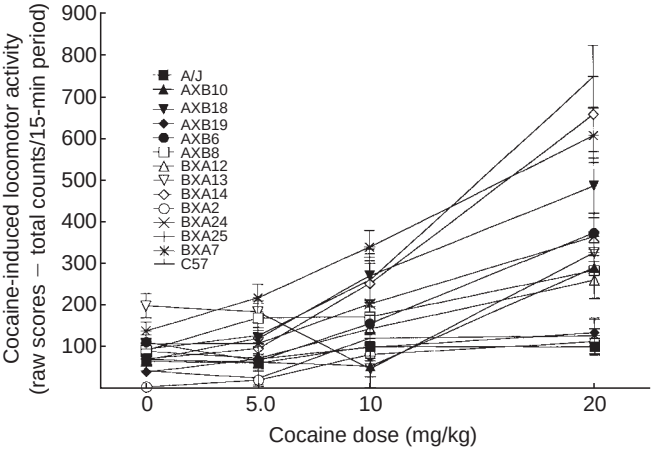


**Fig. 1.** The effects of the administration of cocaine doses (0, 5, 10, 20 mg/kg) on cocaine-induced activation (raw total locomotor activity scores) in a subset of AXB/BXA RI lines and A/J and C57BL/6J progenitors. Maximal separation in strain means was evident at the 20 mg/kg dose of cocaine.

The results indicated a wide and continuous distribution of cocaine activation values, which are consistent with a polygenic mechanism regulating the expression of cocaine activation. Significant strain differences for cocaine difference scores were observed [$F_{52,1002} = 4.188,\ P < 0.001$, and the reliability of the strain means was $r = 0.95$. A one-way ANOVA indicated significant strain differences in cocaine activation (raw total activity scores) values ($F_{52,1041} = 4.476,\ P < 0.001$ (data not shown). The reliability of the strain means was $r = 0.96$.

The two measures of cocaine-induced activation (cocaine activation and cocaine difference scores) utilized in the present study were highly correlated $r = 0.936,\ P < 0.001$). However, only the cocaine activation scores were correlated with initial (day 1) saline activity $r = 0.60,\ P < 0.0021$ [cocaine difference scores ($r = 0.370,\ P < 0.082$)].

### *Quantitative trait loci analysis (SIM and CIM)*

QTL analyses were performed on (i) total locomotor activity scores for saline day 1; (ii) the cocaine activation scores; and (iii) cocaine difference scores. Only the AXB/BXA RI lines were used in these analyses. Note that initially, separate QTL analyses were conducted for males and females on all phenotypes. However, there were no gender-specific QTLs detected. Thus, data for males and females were combined for all QTL analyses.

Total locomotor activity: in Table 1 correlations ($P < 0.01$) between the strain distribution for total locomotor activity on saline session 1 and the genetic linkage markers are reported. Map Manager QT

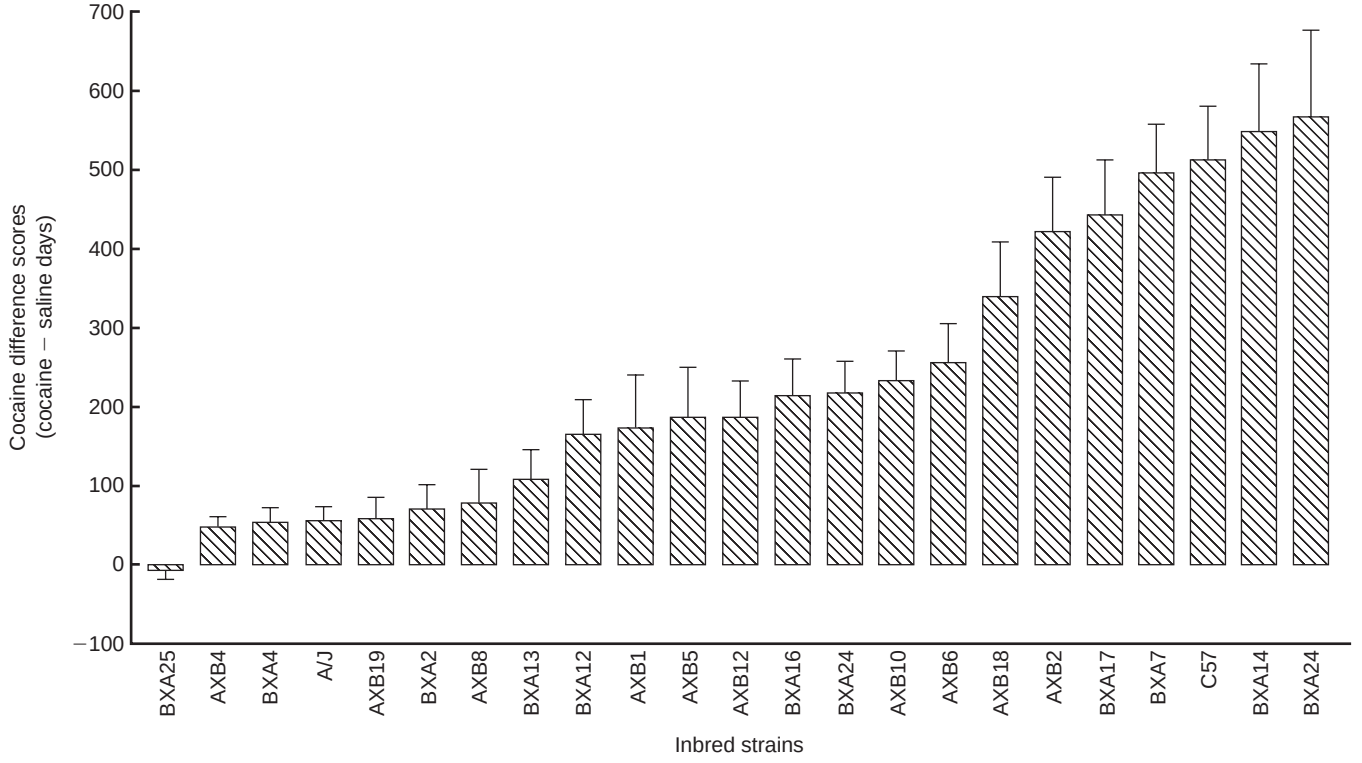


**Fig. 2.** Cocaine difference scores across progenitor and AXB/BXA RI strains. Cocaine activation scores are a function of the difference between activity following cocaine administration (20 mg/kg) and activity on the previous saline session (saline day 2). Strain means are plotted in order of ascending activity scores.

**Table 1.** QTL identified with novelty-induced locomotor activity (saline day 1) in AXB/BXA RI strains

| *Marker* | + *Cofactor* | *Chr* | *cM*[a] | *Correlation*[b] | *LOD score*[c] | P-*value* | *Candidate gene* | *Description* |
|---|---|---|---|---|---|---|---|---|
| D1Nds2 | – | 1 | 59.0 | −0.539 | 1.56 | 0.00727 | | |
| D2Mit1 | – | 2 | 1.0 | 0.587 | 1.93 | 0.00289 | | |
| D5Mit356 | – | 5 | 41.0 | −0.585 | 1.91 | 0.00299 | | |
| D8Mit305 | – | 8 | 37.5 | −0.626 | 2.26 | 0.000123 | | |
| Ets1 | – | 9 | 15.0 | −0.568 | 1.69 | 0.00523 | | |
| D13Mit10 | – | 13 | 31.0 | −0.626 | 2.26 | 0.00124 | Drd1a (32) | Dopamine receptor d1a |
| D14Mit36 | – | 14 | 3.0 | – | 1.45 | 0.00970 | | |
| | **D7nds5** | | | | **3.68** | **0.00004** | | |
| D19Mit10 | – | 19 | 47.0 | 0.565 | 1.76 | 0.00446 | | |

[a]Recombinant distance in centimorgans from centromere. [b]Correlations between SDP for marker and strain means. [c]LOD scores estimated from LRS provided by MAP MANAGER QT. Bold numbers indicate QTLs which exceed the threshold for significance.

identified eight putative markers (at the $P < 0.01$ level) on chromosomes 1, 2, 5, 8, 9, 13, 14, 19. The results of stepwise multiple regression indicated that five loci *(D8Mit305, D2Mit1, D19Mit10. D5Mit356, D13Mit10*) accounted for 59% of the genetic variance in total locomotor activity. *D8Mit305* and *D13Mit10* were identified as suggestive in that they exceeded the defined statistical threshold (see definition of permutation test in methods section). CIM analysis 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 loco-

motor activity scores were mapped while controlling for the influence of QTLs on other chromosomes. As shown in Table 1, when the background QTL *D14Mit36* was factored in, the marker *D7nds5* exceeded the LRS threshold for suggestive loci (Lander & Kruglyak, 1995) generated through the permutations test. In turn, when *D7Nds5* was used as a cofactor, the LRS value for the marker *D14Mit36* exceeded the threshold for significance.

*Cocaine activation scores*

The relationship between the strain distribution for cocaine activation and the genetic linkage markers are reported in Table 2. Putative markers (at the $P < 0.01$ level) on chromosomes 2, 6, 12, 15 and 18 were identified. The marker *Iapls2–10* (Chr 12) was identified as suggestive. The results obtained from a multiple regression analysis indicated that the markers *D2Mit117* (Chr 2), *D6Mit108* (Chr 6), *Pdgfb* (Chr 15) and *Csf1r* (Chr 18) accounted for 79% of the variance in cocaine activation. The outcome of the CIM analysis indicated that when background QTLs were factored in, the association between marker *Iapls2–10* (Chr 12) and cocaine activation exceeded the LRS threshold for significance.

*Cocaine difference scores*

The results of both SIM (without control for QTL cofactors) and CIM (with cofactors) analysis for markers ($P < 0.01$) are presented in Table 3. Four markers associated with mean cocaine difference scores were identified by Map Manager QT ($P < 0.01$) on chromosomes 5 (*D5Mit32*), 12 (*Iapls2–10*), 15 (*Pdgfb*), 18 (*Csflr*). The results of stepwise multiple regression indicated that the these markers accounted for 83% of the genetic variance in cocaine difference scores. The markers on chr 12 (*Iapls2–10*) and 15 (*Pdgfb*) were considered to be suggestive. When background QTLs were factored in, two markers (*Iapls2–10* on Chr 12 and *Pdgfb* on Chr 15) associated with the cocaine difference scores exceeded the LRS threshold for significance. Additionally, the marker *D17j2* was identified as suggestive following CIM analysis.

The interval mapping data for chromosome 12 are presented in Fig. 3. The results indicated that the peak LRS value on chromosome 12 corresponds specifically to the marker Iapls2–10 at 23cM. Similarly, the interval mapping results for chromosome 15 following CIM (controlling for background QTL, Iapls2–10) are presented in Fig. 4. The interval map data indicated a peak LRS value of 21.8 (LOD = 4.72) located between the markers *Pdgfb* (46.8 cM) and *D15Mit105* (47.9 cM).

## Discussion

Three QTLs on chromosomes 12 (23 cM), 15 (46.8 cM), and 17 (4.1 cM) were identified with cocaine-induced locomotor activation in the AXB/BXA RI lines of inbred mice. The QTLs on chromosomes 12 and 15 were considered to be significant using conservative statistical criteria.

The QTLs identified in the present study, utilizing

**Table 2.** QTL for cocaine activation (raw scores) in AXB/BXA RI strains

| *Marker* | *+ Cofactor* | *Chr* | *cM*[a] | *Correlation*[b] | *LOD score*[c] | P-*value* | *Candidate gene* | *Description* |
|---|---|---|---|---|---|---|---|---|
| D2Mit117 | – | 2 | 5 | 0.521 | 1.45 | 0.00984 | | |
| D6Mit108* | – | 6 | 48.2 | −0.481 | 1.19 | 0.01860 | Gabt4 | Gaba-a transporter 4 |
| Iapls2–10 | – | 12 | 23 | −0.705 | 3.12 | 0.00015 | Smstr1(23) | Somatostatin receptor 1 |
| | **Pdgfb** | | | | **4.10** | **0.00001** | | |
| Pdgfb | – | 15 | 46.8 | 0.597 | 2.02 | 0.00235 | Smstr3(46.3) | Somatostatin receptor 3 |
| | Iapls2–10 | | | | 2.97 | 0.00021 | | |
| Csf1r | – | 18 | 30 | −0.623 | 1.99 | 0.00299 | Adrb2(34) | Adrenergic receptor, beta 2 |

[a]Recombinant distance in centimorgans from centromere. [b]Correlations between SDP for marker and strain means. [c]LOD scores estimated from LRS provided by MAP MANAGER QT. Bold numbers indicate QTLs which exceed the threshold for significance.

**Table 3.** QTL analysis for cocaine difference scores (cocaine–saline)

| *Marker* | *+ Cofactor* | *Chr* | *cM*[a] | *Correlation*[b] | *LOD score*[c] | P-*value* | *Candidate gene* | *Description* |
|---|---|---|---|---|---|---|---|---|
| D5Mit32 | – | 5 | 80.0 | 0.564 | 1.74 | 0.00457 | | |
| Iapls2–10 | – | 12 | 23.0 | −0.745 | 3.69 | 0.000037 | Smstr1(23) | Somatostatin receptor 1 |
| | **Pdgfb** | | | | **5.71** | **0.00000029** | | |
| Pdgfb | – | 15 | 46.8 | 0.636 | 2.36 | 0.00097 | Smstr3(46.3) | Somatostatin receptor 3 |
| | **Iapls2–10** | | | | **4.38** | **0.000007** | | |
| | **D17j2** | | | | **4.25** | **0.000006** | | |
| D17j2* | – | 17 | 4.1 | −0.449 | 1.01 | 0.02955 | | |
| | D15Mit28 | | | | 3.19 | 0.00013 | | |
| | Pdgfb | | | | 3.10 | 0.00016 | | |
| Csflr | – | 18 | 30.0 | −0.617 | 2.08 | 0.00198 | Adrb2(34) | Adrenergic receptor, beta 2 |

[a]Recombinant distance in centimorgans from centromere. [b]Correlations between SDP for marker and strain means. [c]LOD scores estimated from LRS provided by MAP MANAGER QT. Bold numbers indicate QTLs which exceed the threshold for significance.

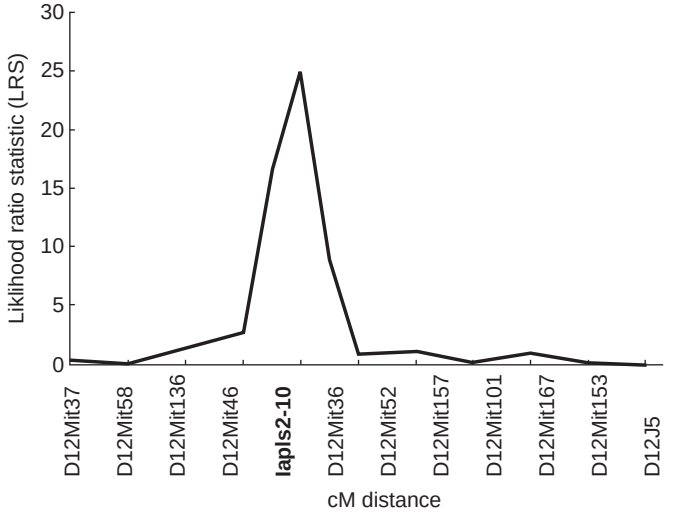


**Fig. 3.** Detection of a QTL for cocaine difference scores on Chromosome 12 using composite interval mapping with Map Manager QT. The interval map data indicated a significant peak LRS value of 26.3 (LOD = 5.71) located at marker *Iapls2–10* (23 cM).

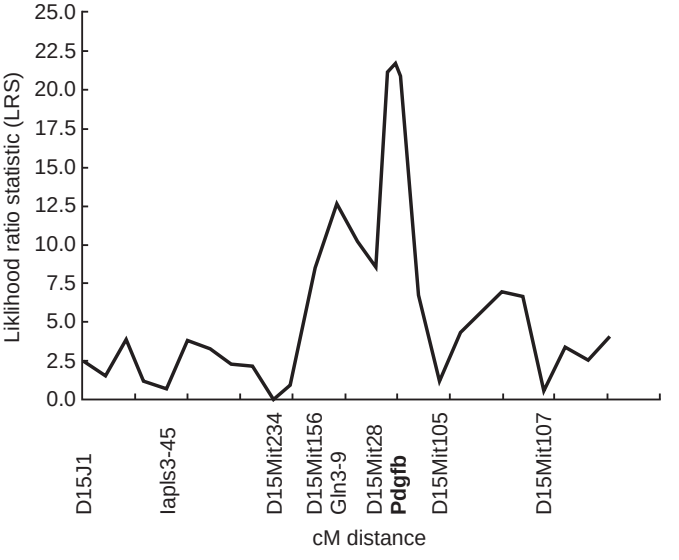


**Fig. 4.** Detection of a QTL for cocaine difference scores on Chromosome 15 using composite interval mapping with Map Manager QT. The interval map data indicated a significant peak LRS value of 21.8 (LOD = 4.72) located between the *Pdgfb* (46.8 cM) and *D15Mit105* (47.9 cM).

AXB/BXA RI mice, confirm several loci previously identified using the BXD RI lines of mice (Miner & Marley, 1995; Phillips *et al.*, 1998; Jones *et al.*, 1999). Table 4 presents an abbreviated list of potentially overlapping QTLs mapped for cocaine-induced activation to date. 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. In particular, interest is drawn to the contiguous region

**Table 4.** Summary of QTLs (confirmed or overlapping) for cocaine-induced locomotor activation in mice

| *Marker* | *Chr* | *Location (cM)* | *Reference* |
|---|---|---|---|
| Nfe2u | 5 | 68–82 | Phillips *et al.* (1998) |
| D5Mit10 | 5 | 54 | Jones *et al.* (1999) |
| D5Mit32 | 5 | 80.0 | Boyle and Gill (present study) |
| Lapls2–10 | 12 | 23.0 | Boyle and Gill (present study) |
| Cbg | 12 | 51–52 | Phillips *et al.* (1998) |
| D15ncvs26 | 15 | 34–48 | Phillips *et al.* (1998) |
| Pdgfb | 15 | 46.8 | Boyle and Gill (present study) |
| D15Mit3 | 15 | 39.6 | Jones *et al.* (1999) |
| D17j2 | 17 | 4.10 | Boyle and Gill (present study) |
| D17ncvs32 | 17 | 20.5 | Miner and Marley (1995) |
| D17Mit7 | 17 | 27.0 | Tolliver *et al.* (1994) |
| Lapls3–5 | 18 | 1.0 | Phillips *et al.* (1998) |
| D18Mit15 | 18 | 16 | Miner and Marley (1995) |
| Csflr | 18 | 30.0 | Boyle and Gill (present study) |

between 39.1 and 47.9cM on chromosome 15 which encompasses the loci *Pdgfb* (chromosome 15) and surrounding suggestive loci (Fig. 4). This region overlapped loci independently identified by Phillips *et al.* (1998) at 34–48 cM and Jones *et al.* (1999) at 39.6 cM. An additional locus identified in the present study on chromosome 5 (80 cM) is consistent with a report in the literature by Phillips *et al.* (1998). Interestingly, the region on chromosome 15 identified in the present study has also been previously associated with amphetamine (Chr 15–47.5) and Phencyclidine (Chr 15–40 cM)-induced locomotor activation (Alexander *et al.*, 1996; Grisel *et al.*, 1997). Thus, the QTL identified on chromosome 15 may be associated with the expression of strain differences in psychomotor stimulants in general and not simply cocaine.

While the identifications of specific genes associated with cocaine activation remain to be positively identified, there are a number of interesting candidate genes that invite some speculation. Genes regulating the expression of somatostatin receptors are situated in close proximity to both significant QTLs identified in the present study. Specifically, the genetic marker Iapls2–10 (chr12–23 cM) maps very close to the region containing the somatostatin receptor 1 gene (*Smstr1*) located at 23 cM on chromosome 12. An examination of the CIM interval data indicated that the peak LRS value of 26.3 obtained for marker Iapls2–10 (23cM) is not exceeded in any of the intervals surrounding the marker. Similarly, the marker *Pdgfb* (Chr 15–46.8 cM) maps to the region containing the somatostatin receptor 3 gene (*Smstr3*), located at 46.3 cM. Polymorphisms in *Smstr3* have been identified in both A/J and C57BL/6J inbred strains of mice (AXB/BXA progenitors), and polymorphisms in *Smstr1* have been identified in the C57BL/6J inbred strain (Mouse Genome Database, 2000).

A number of lines of research indicate that somatostatin has significant effects on both the dopamine system (an important mediator of cocaine's activating effects) and the expression locomotor activation (Beal & Martin, 1984; Vecsei & Widerlov, 1988; Tatsuoka *et al.*, 1987; Vecsei *et al.*, 1990; Raynor *et al.*, 1993; Thermos *et al.*, 1996; Hathaway, 1998). For example, Raynor *et al.* (1993) demonstrated that the administration of somatostatin into the nucleus accumbens increased locomotor activity by 228%, comparable to that obtained by 3–10 μg of amphetamine. These stimulatory effects of somatostatin were suggested to be a function of the somatostatin 1 receptor (Raynor *et al.*, 1993), via an interaction with the dopamine system (Beal & Martin, 1984; Vecsei *et al.*, 1990; Thermos *et al.*, 1996; Hathaway, 1998). Somatostatin administration produced dose-dependent increases in dopamine levels in the striatum (Thermos *et al.*, 1996). Hathaway *et al.* (1998) similarly demonstrated that somatostatin increased striatal concentrations of dopamine by as much as 28-fold, in addition to increasing glutamate and GABA levels. As noted above, the potential involvement of the somatostatin receptor system in the expression of cocaine-induced locomotor activation is highly speculative at this time, requiring considerable additional research for confirmation.

In contrast to the potential concordance observed for QTLs identified with cocaine, amphetamine and phencyclidine-induced activation, the QTLs identified in the present study were not consistent with those previously identified for ethanol-induced activation (loci on chromosomes 8, 16, and 18) (Gill *et al.*, 2000). The absence of shared loci would suggest that different genes may mediate the expression of cocaine and ethanol-induced locomotor activation.

Similarly, the results of the present study indicated that different loci were associated with novelty and cocaine-induced locomotor activation. Novelty in the present study was operationally defined as the influence of first exposure to the open field chambers and handling procedures on day 1. Measures of basal

locomotor activity have been shown to be highly heritable, and animals that are relatively inactive when exposed to a novel environment (e.g. the A/J) are regarded as highly anxious and show correlated responses on tests of timidity, fearfulness and avoidance (Flint & Corley, 1996). A number of studies have identified the A/J strain as the most anxious strain in the elevated plus maze or open field tests (Crawley *et al.*, 1997; Gershenfeld *et al.*, 1997). A suggestive QTL for basal (or novelty-induced) total locomotor activity was mapped to chromosome 14 (LOD score 3.68). The absence of shared loci for novelty and cocaine-induced activation is consistent with the failure, in the present study, to demonstrate a genetic correlation between these phenotypes. The absence of a correlation between initial and cocaine-induced activation replicates those reported by Miner and Marley (1995). However, contrary results have been reported in the literature (Phillips *et al.*, 1998).

Overall, the results of the present study using AXB/BXA RI mice have confirmed the association between a number of genetic linkage markers identified in the literature and the expression of cocaine-induced activation. In particular, the contiguous chromosomal region encompassing the suggestive and significant loci identified on chromosome 15, in the present study, are consistent with putative QTLs identified previously (Phillips *et al.*, 1998; Jones *et al.*, 1999). In addition, new loci on chromosomes 12, 17 and 18 were identified.

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