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Research report

Quantitative trait loci for novelty/stress-induced locomotor activation in recombinant inbred (RI) and recombinant congenic (RC) strains of mice

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Abstract

The objective of the present study was to map and compare quantitative trait loci (QTLs) for an anxiety-related trait (novelty/stress-induced activation) in the AXB/BXA recombinant inbred (RI) and AcB/BcA recombinant congenic (RC) strains of mice derived from the A/J and C57BL/6J inbred progenitor strains. Activational responses to a novel open field (OF) were measured under identical stressful conditions (no prior handling or exposure to testing procedures) in both the RI and RC strains. Naive male and female mice were weighed, injected with IP saline and locomotor activity was monitored in a computerized OF apparatus for 15 min. Measures obtained from this experimental design included: (1) total activity scores, (2) time course of response (5 min time blocks over the 15 min session). Data for the RI strains were subjected to a QTL analysis using composite interval mapping. Significant loci were identified on chr 5 (*D5Mit356*, 41 cM), chr 8 (*D8Mit305*, 37 cM) and chr 14 (*D14Mit36*, 63 cM). Single locus association analysis of the AcB/BcA RC strains identified 15 putative regions, 7 of which overlapped regions independently mapped in the RI strains on chr 1 (58.5–63.1 cM), chr 4 (21.9–28.6 cM), chr 5 (19–45 & 74–86 cM), chr 6 (0.5–20.4 cM), chr 9 (15–38 cM), chr 13 (47 cM) and chr 19 (47 cM). The loci identified on chr 5 near *D5Mit356* (41 cM) in both the AXB/BXA RIS and AcB/BcA RCS maps to a region containing the genes for several $GABA_A$ receptor subunits. Additionally, the present study provides further confirmation of a frequently identified QTL on chromosome 1. The results are discussed in the context of previous QTL studies of anxiety-related traits that have used genetic crosses that include the A or B6 progenitors.
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Keywords: Anxiety; Open-field activity; Stress-induced activation; Inbred mice; Recombinant inbred; Recombinant congenic; Novelty

1. Introduction

Anxiety disorders are a highly prevalent and seriously debilitating group of disorders that produce an enormous impact on society in terms of comorbidity with medical disorders [17,52], as well as lost productivity and mental health care costs [10]. Evidence from family, twin and linkage studies has shown that genes influence the development of anxiety-related disorders [3,15,16,19,23,41,44,45]. Quantitative phenotypes have been recently defined in the human literature which describes the liability to "anxiety-proneness" in terms of early-onset, severe and comorbid anxiety disorders [43]. The mouse equivalents of anxiety-proneness include responses to novelty/stress in an open-field, elevated plus maze or light/dark box [6] as well as fear-potentiated startle [40]. When mice are introduced into a novel open-field (OF) environment, normal exploratory behavior is inhibited. Mice that are relatively inactive when exposed to a novel OF are regarded as highly anxious and show correlated responses on tests of timidity, fearfulness and avoidance [7]. The measurement of behavior in the OF has a number of advantages as a model system, including that fact that exploration it is a well-defined innate behavioral response with broad general-

Abbreviations: B6, C57BL/6J; RIS, recombinant inbred strains; RCS, recombinant congenic strains; QTL, quantitative trait locus; LRS, likelihood ratio statistic; CIM, composite interval mapping

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izability across species, and it has been shown to be heritable [13].

Trullas and Skolnick [46] ranked 16 inbred strains of mice on anxiety-related phenotypes; the A/J (A) and C57BL/6 J (B6) strains were found to be at opposite ends of the phenotypic spectrum. The A strain has been identified as one of the most anxiogenic-like or fearful strain across a number of paradigms [1,6,29,50]. Mathis et al. [28] characterized the A and B6 progenitors as well as the AXB/BXA RIS derived from them for OF activity and transitions in a light/dark paradigm. The strongest linkages for OF activity were found for markers on chr 3 (*Mpmv-9*), chr 9 (*Bgl*) and chr 11 (*Es-3*).

Flint et al. [8] used an F2 intercross of DeFries High and Low lines of mice—originally selected for high versus low activity in an open field—to map significant quantitative trait loci (QTLs) for OF activity to chr 1 (*D1Mit150*, 100 cM), chr 4 (*D4Mit81*, 38 cM), chr 12 (*D12Mit47*, 45 cM), chr 15 (*D15Mit63*, 29.2 cM), chr 17 (*D17Mit24*, 20.43 cM) and chr 18 (*D18Mit67*, 4 cM). In follow-up studies, Turri et al. [47,48] used this F2 cross and several behavioral paradigms to demonstrate that QTLs on chromosomes 1, 4 and 15 were common across multiple anxiety-related behavioral measures. The QTL on chr 4 was associated general activity levels, while the QTL on chr 1 influenced exploratory behavior and the QTL on chr 15 acted primarily on avoidance behavior. Most recently, Turri et al. [49] utilized a multivariate analysis of 23 anxiety-related traits to identify novel QTLs on chrs 3, 9, 13 and 17 in F2 mice.

Using an F2 intercross between A and B6 mice, Gershenfeld and Paul [12] mapped QTL for "fear-like" behaviors using the open-field (thigmotaxis) and light/dark (LD) transition paradigms. Significant QTL were mapped to chr 10 for LD behavior and chr 1 for thigmotaxis. Additional suggestive QTL for ambulation and rearing were mapped to chr 1 (*D1Mit116*, 100 cM), chr 10 (*D10Mit237*, 74.1 cM), chr 15 (*D15Mit107*, 41.5 cM) and chr 19 (*D19Mit46*, 24 cM) [12]. The loci on chr 1 (∼100 cM) and chr 15 (∼41 cM) independently replicated QTL found by Flint et al. [8] and Turri et al. [47] for activity in an open-field.

In a recent publication Henderson et al. [18] utilized an extensive test battery to identify anxiety-related QTLs in F2 mice derived from crosses of H1 and H2 strains (high activity) and L1 and L2 (low activity) strains. Phenotypic measures were derived from open-field, light-dark, mirror-chamber, elevated-plus maze and elevated square-maze paradigms. QTLs on chromosomes 1, 15 and 18 were associated anxiety-related behaviors.

In summary, a number of studies have mapped QTL for traits related to anxiety, emotionality and activity under novel conditions in an open field apparatus [e.g. [8,11,12]]. Despite a number of procedural differences between studies (e.g. behavioral paradigm, strain and gender of mice, age at testing) there were several QTLs, most notably on chromosomes 1 and 15, that have been consistently identified for anxiety. Some loci were less consistently identified across studies, suggesting that further confirmation is required using a variety of methods including RI strains, F2 intercrosses or congenic mice. Therefore, the objective of the present study was to compare QTLs for novelty/stress induced activation in two series of recombinant strains of mice—the AXB/BXA recombinant inbred strains (RIS) and the AcB/BcA recombinant congenic strains (RCS)—each independently derived from the A and B6 progenitors. The entire available series of RIS and RCS strains were tested for novelty/stress-induced activation using an OF paradigm in the same laboratory under identical conditions.

2. Methods

2.1. Progenitor and RI strains

Male and female C57BL/6 J, A/J, and breeder pairs of all available AXB/BXA recombinant inbred (RI) strains were purchased from Jackson Laboratory (Bar Harbor, Maine). Four breeder pairs of each AXB and BXA RI strain were established and maintained in an animal colony controlled for temperature and humidity on a 12-h day/12-h night cycle (lights on between 6.00 a.m. and 6.00 p.m.). Breeder pairs were kept for 8 months providing 4 to 6 litters. The mice were housed in standard shoe-box solid bottom cages with beta chip bedding and nestlet pads. Pups were weaned at 4 weeks and housed with same-sex litter mates until 8 weeks of age when testing commenced. The different strains were tested in mixed squads (by strain and gender) of 24–32 mice over a two-year period, resulting in a total sample of 1159 RI mice (549 females, 604 males).

2.2. RC strains

Thirty-seven independent AcB/BcA RC inbred strains were produced from reciprocal backcrosses between the A and B6 progenitors [9] at the Research Institute of the McGill University Health Centre (MUHC) "Complex Traits Analysis Core Facility" [9]. The RC strains were produced by inbreeding independent pairs of mice from the (F1 × A) × A backcross to produce the AcB strains, and (F1 × B) × B backcross to produce the BcA strains. Each RC inbred strain carries a different set of genes from the donor strain—with the remaining percentage from the background strain [9]. For example, the BcA RC strains carry donor alleles from the A/J, with the C57BL/6 J serving as the background strain. Naive mice from 13 AcB RC strains (A/J background) and 21 BcA RC strains (B6 background) were housed in a colony controlled for temperature and humidity on a 12-h day/12-h night cycle (lights on between 6.00 a.m. and 6.00 p.m.). Pups were weaned housed with same-sex littermates until 8 weeks of age. Animals were acclimatized to the colony for at least two weeks prior to testing. The different strains were tested in mixed squads of 24 mice over a two-year period, resulting in a total sample of 667 (328 females, 339 males).

2.3. Open-field testing

Novelty/stress-induced 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 the test 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 which suppressed scores, resulting from repetitive breaking of photo beams due to grooming. Testing was performed in a sound-insulated room between 3.00 p.m. and 6.00 p.m. immediately prior to lights out.

In order to maximize both the 'novel' and 'stressful' aspects of the paradigm, mice were naive to handling, weighing and injection procedures prior to testing. On test day, male and female mice were moved to the testing room, weighed, administered intraperitoneal injections (i.p.) of sterile 0.9% saline solutions (10 ml/kg body weight), and immediately placed in the center of the open field boxes. Novelty/stress-induced activation was operationally defined as the total locomotor activity scores obtained during the 15 min exposure to the activity chambers. Measures obtained from this experimental design included: (1) total activity scores and (2) time course of activity measured in 5 min time blocks over the 15 min session.

2.4. Statistical analyses

Statistical analyses were performed with SPSS version 11.5 for Windows (SPSS Inc.). The activity data were tested for normality as well for outliers using the SPSS explore function. Scores outside the 95% confidence interval for each strain mean were eliminated. For both the RI and RC series of strains, those removed were randomly distributed across strains. A randomly selected split-half correlational analysis (corrected with Sperman–Brown formula) was performed in order to assess the reliability of the strain means for the entire sample [36]. Comparisons between strains of mice in the RI and RC series were conducted with analysis of variance (ANOVA) techniques. Post hoc comparisons for the RI strains were conducted using *t* tests with a Bonferroni correction. Post hoc Dunnett's tests were used to compare RC strain means to their corresponding background strain.

2.5. Genetic analysis—RI strains

The database of genetic linkage markers for the AXB/BXA RI lines was obtained from the Mouse Genome Database [30]. The database consisted of 780 markers of various types including SSLP's, restriction fragment length polymorphisms, biochemical markers, and proviral loci. Simple bivariate correlations between the linkage markers—arbitrarily scored as 1 for alleles from the A/J progenitor and 0 for the alleles from the C57BL/6 J—and the distribution of strain means for locomotor activation were computed. In addition, activity data collected on the AXB/BXA RI lines were subjected to a quantitative trait loci (QTL) analysis using simple interval mapping (SIM) with Map Manager QTX [26]. A preliminary analysis of the activity data for the RI strains was published earlier [2]. The present analysis expands upon the earlier work with a more detailed analysis of interactions between QTLs (using both composite interval mapping and ANOVA techniques) as well as the generation of interval maps. Interval mapping procedures tested for linkage relationships between marker loci and the strain distribution pattern (SDP) of the phenotype.

RI strain means are influenced directly and indirectly (through interactions) by more than one QTL [20]. Therefore, additional analyses were conducted using composite interval mapping (CIM) in order to evaluate linkage relationships between marker loci and the SDP of the phenotype, while including the effects of background QTL [20,53]. The background QTL included in the CIM analysis (listed as cofactors in the results) were all significantly ($p < 0.05$) associated with activity as detected by SIM [21]. Estimated LOD scores were calculated based upon the chi-square statistic (likelihood ratio statistic (LRS)) provided by MapManager QTX.

2.6. Genetic analysis—RC strains

The genomic mapping of the progenitor and all RC strains was conducted at the Research Institute of the MUHC using SSLP microsatellite markers. Oligonucleotide primer pairs for each marker were purchased from Research Genetics (Huntsville, AL, USA). The markers were typed in all strains using standard PCR-based methods [9]. To date, a total of 625 markers have been typed in each strain providing coverage of the entire genome with an average spacing of 2.6 cM. The database of genetic linkage markers was produced using map positions based the Mouse Genome Database (The Jackson Laboratory, Bar Harbor Maine) [9]. Identification of informative strains carrying donor alleles that significantly influenced the phenotype within the AcB and BcA sets was carried out using ANOVA with strain and gender as fixed factors, followed by Dunnett's post hoc analysis. In the post hoc analysis, significant overall strain effects were followed up by pair-wise comparisons between the mean phenotypic values for each RC strain and the appropriate background strain.

The strain distribution pattern of mean phenotypic values and the chromosomal maps of SSLP markers for the AcB/BcA RCS were used to compute single locus associations with Map Manager QTX [26]. The significance of the association at each marker was tested using the LRS statistic [26,27], and the criteria used to determine suggestive and significant loci are listed in the following. This was followed up by the computation of simple bivariate correlations between the SDP of mean phenotypic values and the significant linkage markers identified by Map Manager QTX—with genotypes arbitrarily scored as 1 for the background and 2 for the donor genotypes. For any given locus, a positive correlation identified donor alleles that increased the mean phenotypic values, and negative correlations suggested the presence of a decreaser allele. Stepwise multiple regressions were performed in order to estimate the total amount of genetic variance accounted for by the putative QTLs. The AcB and BcA RCS were analyzed independently in order to examine the effect of genetic background on significant loci.

2.7. Criteria for significance

Due to the large number of statistical computations conducted in QTL analyses there is likely to be a high type I error rate. Since genetic analysis of the AcB/BcA RCS was conducted to confirm results obtained in the AXB/BXA RIS, stringent statistical criteria were adopted to reduce type I error. In some multiple comparison analyses (e.g. bivariate correlations) a Bonferroni correction was applied. With 625 linkage markers in the RCS, a nominal *p*-value of 0.00008 (0.05/625) was used to established significance. Map Manager QTX was set to detect simple locus associations at a threshold value of $p \leq 0.00001$ for the analysis of the RCS.

In addition, thresholds for significance of the LRS values generated by the interval mapping (in the RIS) and simple association (in the RCS) procedures were set using a permutation test. Per-

mutation tests [4] were conducted using the Map Manager QTX software [26] and LRS values at appropriate percentile points in the distribution were used to establish critical values for linkage [27]. The threshold values of the permutation test labeled as suggestive or significant for linkage were established using the guidelines of Lander and Kruglyak [25]. Potential candidate genes close to significant QTLs were identified using the Mouse Genome Database at http://www.informatics.jax.org.

3. Results

3.1. AXB/BXA recombinant inbred strains

Mean novelty/stress-induced locomotor responses for the A/J, C57BL/6 J and AXB/BXA RI mice are presented separately for males and females in Fig. 1. Strain differences were confirmed in a two-way ANOVA (strain × gender) using total activity scores over the entire 15 min monitoring period. The analysis yielded a significant main effect for strain [$F(24,1103) = 26.6$, $p < 0.0001$], but no main effect for gender [$F(1,1103) = 0.063$, $p = 0.80$] or a strain by gender interaction [$F(24,1103) = 1.14$, $p = 0.29$). Therefore, gender was not considered as a factor in subsequent ANOVA or QTL analyses. Overall, the mean strain phenotypic values were found to be very reliable with a random split-plot correlation of $r = 0.95$ ($p < 0.001$) (males and females combined). The strain distribution pattern for the time course of activity, analyzed in 5 min time blocks over the 15 min test session, were highly correlated with the total session values (Time block 1 (0–5 min) $r = 0.996$, $p < 0.001$; Time block 2 (5–10 min) $r = 0.993$, $p < 0.001$; Time block 3 (10–15 min) $r = 0.994$, $p < 0.001$). A two-way ANOVA with repeated measures (strain by time) was conducted to examine the time course of responses among the different strains. The analysis yielded a significant strain effect [$F(24,1122) = 28.6$, $p = 0.0001$], an effect of time [$F(2,2244) = 183.7$, $p = 0.0001$], and a strain by time interaction [$F(48,2244) = 3.92$, $p = 0.0001$] (data not shown). QTL analyses were conducted on data from each time block as described in the following.

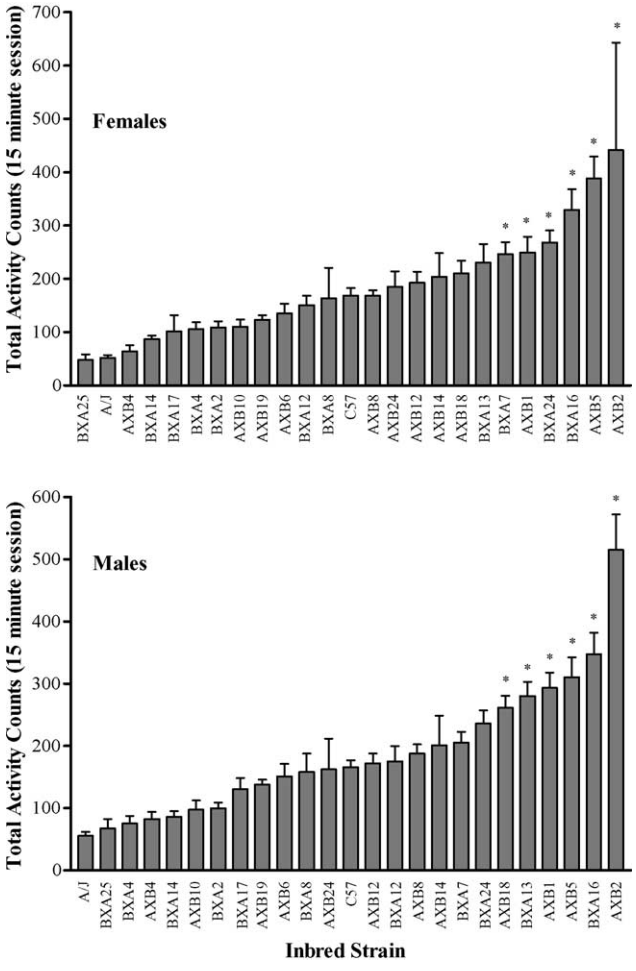

Fig. 1. Novelty/stress-induced activation scores measured on first exposure to the open field chambers. Mean values (±S.E.M.) for males and females of the progenitors as well as the AXB/BXA RI strains are presented in order of ascending activity scores for males. Asterisk (*) denotes strains that show a significant difference in mean values compared to either of the background A/J or B6 strains.

3.2. Quantitative trait loci analysis

Simple interval mapping (SIM) and composite interval mapping were conducted in order to determine the chromosomal regions of the genome associated with the activational responses in the AXB/BXA RI series. QTL analyses were performed on total activity scores, as well as the scores for each of the three time blocks. Compared to total activity scores, there were no unique QTLs identified for any of the time intervals. Thus, data are not reported for these additional phenotypes.

QTL for novelty/stress-induced activation detected by Map Manager QTX are described in Table 1. The QTL analysis identified 10 putative markers (at the $p < 0.01$ level) on chromosomes 1, 4, 5, 8, 9, 13, 14 and 19. The results of stepwise multiple regression analysis indicated that one of the QTL regions (D5Mit356) accounted for approximately 45% of the genetic variance in novelty/stress-induced activation [$R = 0.694$, $F(1,18) = 15.78$, $p < 0.001$].

CIM analysis was used in order to control for the influence of unlinked QTLs. Target loci associated with total activity scores were mapped while controlling for the influence of QTLs on other chromosomes. When background QTLs were factored in, three markers were significantly associated with novelty/stress-induced activation including *D5Mit356* (LOD = 4.3) and *D8Mit305* (LOD = 3.84) in which the B6 allele was associated with an increase in mean phenotypic values. The significant marker at *D14Mit36* (LOD = 3.69) was associated with an A/J allele that increased the phenotype. The mapping results for chromosome 8 following CIM (controlling for background QTL, *D6Mit50*) are presented in Fig. 2. The interval map data indicated a peak LRS value of 19.1 (LOD = 4.1) located between the markers *D8Mit132* (33.0 cM) and *D8Mit305* (37.0 cM). Fig. 3 presents the com-

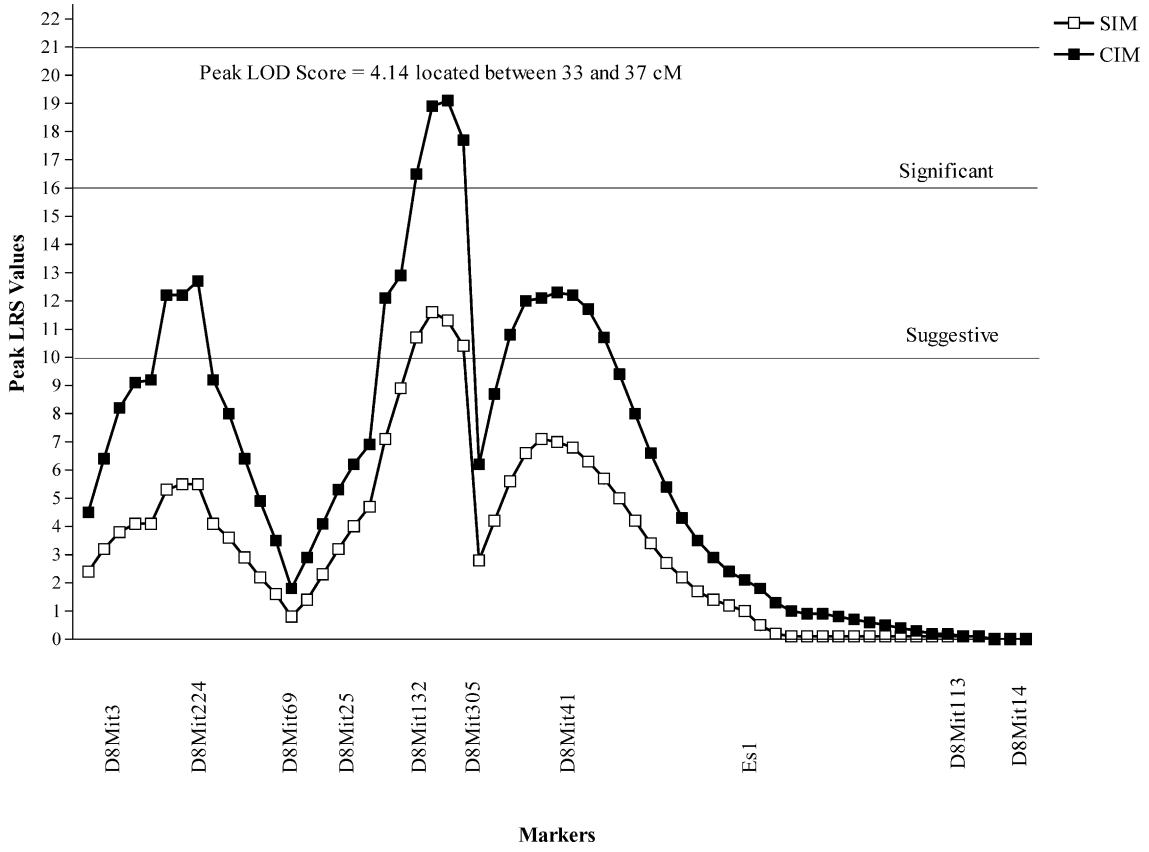

Fig. 2. A composite interval map for the QTL on chromosome 8 was derived from the output of Map Manager QTX. The interval map data indicate a peak LRS value of 19.1 located between 33 and 37 cM. The thresholds for suggestive and significant loci as determined through a permutation test are indicated.

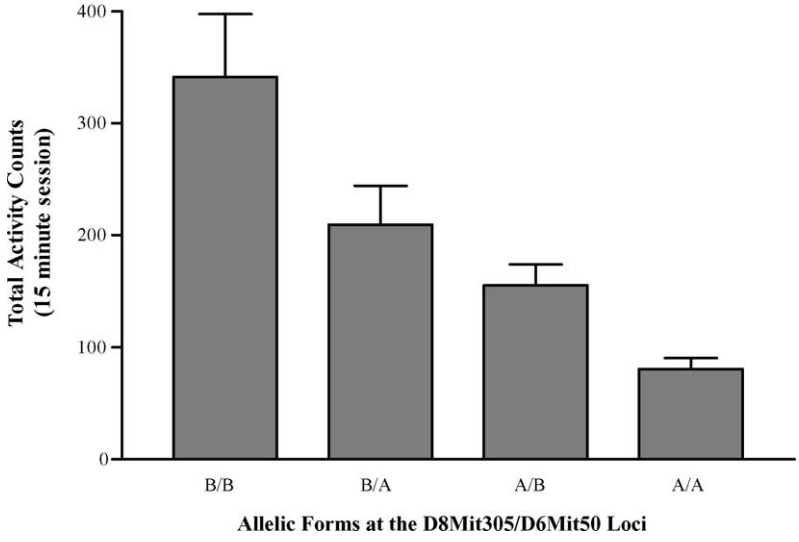

Fig. 3. The interaction between markers *D8Mit305* (chr 8) and *D6Mit50* (chr 6) in terms of novelty/stress-induced activation. The results show that a combination of B alleles at both the D8Mit305 and D6Mit50 loci significantly increased mean (±S.E.M.) activity in response to a novel open-field environment.

Table 1
Results of QTL analysis for total novelty/stress-induced activation in the AXB/BXA RI strains

Chr	Peak marker[a]	(+) Cofactor[b]	cM[c]	R[d]	LRS[e]	*p*-value	Gene candidate[f]	Description
1	*D1Mit26*	–	62.1	−.539	7.2	0.007	*Lrnx1 (60.8)*	QTL-contextual fear conditioning 1
		Iapls3–9 (5:82)			10.5*	0.001	*Htr5b (63)*	Serotonin receptor 5B
							Actd1 (65)	QTL-activity distance traveled 1
							Lrnx12 (65)	QTL-contextual fear conditioning 12
4	*Mup1*	–	27.8	+.509	5.4	0.02		
		D13Mit10 (13:31)			12.4*	0.0004		
5	*D5Mit356*	–	41	−.585	8.8	0.003	*Gabra2 (40)*	GABA-A receptor, alpha2 subunit
		Iapls3–9 (5:82)			13.0*	0.0003	*Gabrb1 (40)*	GABA-A receptor, beta1 subunit
		D18Mit20 (18:5)			14.5*	0.0001	*Gabrg1 (40)*	GABA-A receptor, gamma1subunit
		1 + 2			**19.8****	0.0001	*Clock (43)*	Circadian locomotor output
8	*D8Mit305*	–	37	−.626	10.4*	0.001	*Npy1r (33)*	Neuropeptide Y receptor Y1
		D18Mit20 (18:5)			**17.4****	0.00003	*Slc181a (33)*	Vesicular monoamine carrier 18
		D6Mit50 (6:3.3)			**17.7****	0.00003		
9	*Ets1*	–	15	+.568	7.8	0.005		Note: cofactor D7j4 is close to a QTL for anxiety in an open-field (*Axtofd2*, 44*cM*), as well as the albino (c) locus (*Tyr*, 44*cM*)
		D7j4 (7:45)			11.4*	0.0007		
13	*D13Mit10*	–	31	−.626	10.4*	0.001	*Drd1a (32)*	Dopamine receptor 1a
		D6Mit50 (6:3.3)			14.6*	0.0001		
13	*D13Mit110*	–	47	+.493	5.1	0.015	*Crhbp (52)*	Corticotrophin releasing hormone-binding protein
		Mup1 (4:27.8)			12.9*	0.0003		
14	*Il3ra*	–	1.5	+.537	7.1	0.008		
		Iapls3–9 (5:82)			14.8*	0.0001		
14	*D14Mit36*	–	63	+.522	6.7	0.01		Note: cofactor D7nds5 is close to *Grin2d* (23.5 cM) the epsilon4 subunit of the NMDA receptor
		D7nds5 (7:23)			**17.0****	0.00004		
19	*D19Mit10*	–	47	+.565	8.1	0.004	*Lrnx6 (45)*	QTL-contextual fear conditioning 6
		D1J2 (1:15)			13.1*	0.0003	*Adra2a (50)*	Adrenergic receptor alpha 2a
							Adrab1 (51)	Adrenergic receptor beta 1

[a] Peak marker identified by Map Manager QTX.
[b] Background QTL at significant markers identified by SIM analysis ($p < 0.05$) are listed as cofactors (chromosome: cM position) as described in the text.
[c] Recombinant distance in centimorgans from centromere.
[d] Correlations between SDP for marker and strain means. For these computations, the A/J was allele was scored as a 1 and C57BL/6 J scored as 0, thus negative correlations indicate that the B6 allele increased the phenotype and positive correlations indicate that the A allele increased the phenotypic values.
[e] LRS values provided by Map Manager QTX. *Suggestive or **significant generated through the permutations test as described in the text, following the guidelines of Lander and Kruglyak (1995).
[f] Potential candidate genes and position (cM) identified using the Mouse Genome Database (2004).

bined effects of genotypes at markers *D8Mit305* (chr 8) and *D6Mit50* (chr 6) for total novelty/stress-induced activity. Comparison of the four genotypes by one-way ANOVA indicated that there was a significant effect of genotype [$F(3,20) = 9.5$, $p = 0.001$]. Post hoc analysis (Tukey HSD) indicated that the combination of B alleles at *D8Mit305* and *D6Mit50* (B/B genotype) resulted in significantly ($p < 0.01$) higher activity compared to all other genotypes.

3.3. AcB/BcA recombinant congenic strains

A two-way (strain × gender) ANOVA was performed on total activity scores using all strains. There were no significant gender main effects ($F(1,667) = 1.654$, $p = 0.199$) or interactions ($F(33,667) = 0.950$, $p = 0.551$). Therefore, gender was not considered in subsequent analyses. The RC strain means were found to be reliable with random split plot correlations of $r = 0.88$ ($p < 0.01$) (males and females combined). Similar to the RIS, the strain distribution for the RCS time course data (analyzed in 5 min time blocks over the 15 min test session) were highly correlated with the total session values (Time block 1 (0–5 min) $r = 0.98$, $p < 0.001$; Time block 2 (5–10 min) $r = 0.994$, $p < 0.001$; Time block 3 (10–15 min) $r = 0.984$, $p < 0.001$). A two-way ANOVA with repeated measures (strain by time) was conducted to examine the time course of responses among the different strains. The analysis yielded a significant strain effect [$F(35,631) = 10.09$, $p = 0.001$], an effect

of time [$F(2,1262) = 157.95$, $p = 0.0001$], and a strain by time interaction [$F(70,1262) = 2.04$, $p = 0.0001$] (data not shown).

Mean total novelty/stress-induced activational responses for the A/J, C57BL/6 J, and AcB/BcA strains are presented in Fig. 4. AcB and BcA strains are independently presented in ascending order of activity. Significant strain differences were noted for the AcB strains [$F(15,335) = 7.5$, $p = 0.01$] as well as the BcA strains [$F(21,457) = 5.1$, $p = 0.01$]. Dunnett's post hoc tests identified five informative strains among the AcB set (compared to the background A/J) and ten among the BcA set (compared to the background C57BL/6 J). The informative strains are denoted in Fig. 4 with asterisks.

3.4. Genetic analysis in the RC strains

Chromosomal regions potentially carrying donor alleles modifying novelty/stress-induced activation were identified through the calculation of single locus associations with Map Manger QTX [26] and confirmed through the calculation of bivariate correlations. Marker regions that were significantly associated with novelty/stress-induced activation at the $p \leq 0.00001$ level are listed in Tables 2 and 4. In many cases, multiple markers in a given region were identified by Map Manager QTX, however, only the peak markers (with the largest correlations) were included in the tables. The minimum interval (in centimorgans) containing the donor QTL were identified as the common region among informative strains that possessed donor alleles at the significant markers identified by Map Manager QTX. Table 2 presents the putative chromosomal regions containing B6 donor alleles associated with novelty/stress-induced activation in the AcB RCS. Analysis of total activity scores in the AcB strains resulted in the identification of seven putative regions on chromosomes 3, 6, 8, 13, 14, 17, 18, and 19. Examples of the intervals containing B6 increaser alleles on chr 3 and 6 are presented in Table 3. The allelic status at each locus is represented by A for A/J and B for the B6 allele. The strains are ordered on the basis of increasing phenotypic values. Those strains possessing the B6 alleles on chromosomes 3 and 6 were observed to exhibit significantly greater activity scores than strains that possessed the A/J allele at these loci. Stepwise multiple regression analysis demonstrated that a subset of two markers including *D3Mit185* (29.5 cM) and *D8Mit186* (59 cM) accounted for 84% of the genetic variance in novelty/stress-induced activation scores [$R = 0.918$; $F(2,12) = 29.96$, $p < 0.0001$]. Similarly, the putative chromosomal regions containing A/J alleles associated with total novelty/stress-induced activation in the BcA RCS are presented in Table 4. Analysis of the data resulted in the identification of eight regions on chromosomes 1, 3, 5, 9, 11 and 17. Stepwise multiple regression analysis demonstrated that a subset of four markers including *D11Mit167* (71 cM), *D5Mit356* (41 cM), *D9Mit297* (15 cM) and *D17Mit139* (30.2 cM) accounted for 71% of the genetic variance in novelty/stress-induced activation scores [$R = 0.844$; $F(4,20) = 9.91$, $p < 0.0001$].

4. Discussion

A wide, continuous range of novelty/stress-induced activation was displayed by the AXB/BXA RIS and AcB/BcA RCS, findings that are consistent with a quantitative trait involving additive effects of several genes. A large number of strains in both the RIS and RCS show significantly higher activational responses than either the A/J or B6 progenitors, suggesting potential gene interactions. In this context, it should be noted that correlations between novelty/stress-induced activation and the SDP for linkage markers in the AXB/BXA RIS were both positive and negative (see Table 1). In the RI series, the genotype at each linkage marker was arbitrarily scored as 1 for alleles from the A/J progenitor and 0 for alleles from the B6. Thus, negative correlations for any given marker indicated that higher activity was associated with the B6 allele. Positive correlations (e.g., at D14Mit36) demonstrated that while the inbred A/J are generally inhibited in novel environments, recombination of progenitor alleles in the AXB/BXA RI strains revealed alleles coming from the A/J that were capable of increasing the mean phenotypic values.

The tendency for A/J and B6 alleles to both increase and decrease mean phenotypic values was also noted among the AcB and BcA RCS. The AcB strains inherited B6 donor alleles that were expected to increase the mean strain phenotypic values relative to the A/J background. However, as shown in Table 2, correlations at the peak markers were both positive and negative, indicating that in some strains the B6 donor alleles decreased mean phenotypic values (e.g. at D8Mit186). Similarly, some BcA strains inherited A/J donor alleles that increased mean phenotypic values (e.g. at D9Mit297) relative to the B6 background, rather than decreased as expected (see Table 4). Thus, the phenotypic level exhibited by any given RIS or RCS can be attributed to the particular combinations of QTLs inherited from the A/J and C57BL/6 J progenitors at a number of sites.

The potential importance of the combination of QTLs is reflected in the results of interval mapping in the AXB/BXA RIS. QTL analyses identified a number of suggestive QTL and three highly significant QTL on chr 5 (*D5Mit356*, 41 cM), chr 8 (*D8Mit305*, 37 cM) and chr 14 (*D14Mit36*, 63 cM). All significant QTL were identified using CIM analysis with cofactors on several chromosomes including chr 5 (*Iapls3–9*, 82 cM), chr 6 (*D6Mit50*, 3.3 cM), chr 7 (*D7nds5*, 23 cM) and chr 18 (*D18Mit20*, 5 cM). CIM analyses were performed in order to control for interactions with other QTL in the genome. In this case, it is notable that two of the cofactors (*Iapls3–9* and *D6Mit50*) mapped very close to QTL independently identified in the AcB/BcA RCS as shown in Table 5. The cofactors on chromosome 7 at markers *D7nds5* (23 cM) and *D7j4* (45 cM) are also worth noting. The marker at *D7nds5* is very close to the gene coding for the epsilon 4 subunit of the NMDA receptor (*Grin2d*, 23.5 cM). Epsilon 4 mutant mice lacking this subunit have been shown to exhibit abnormal novelty-induced locomotor activity in

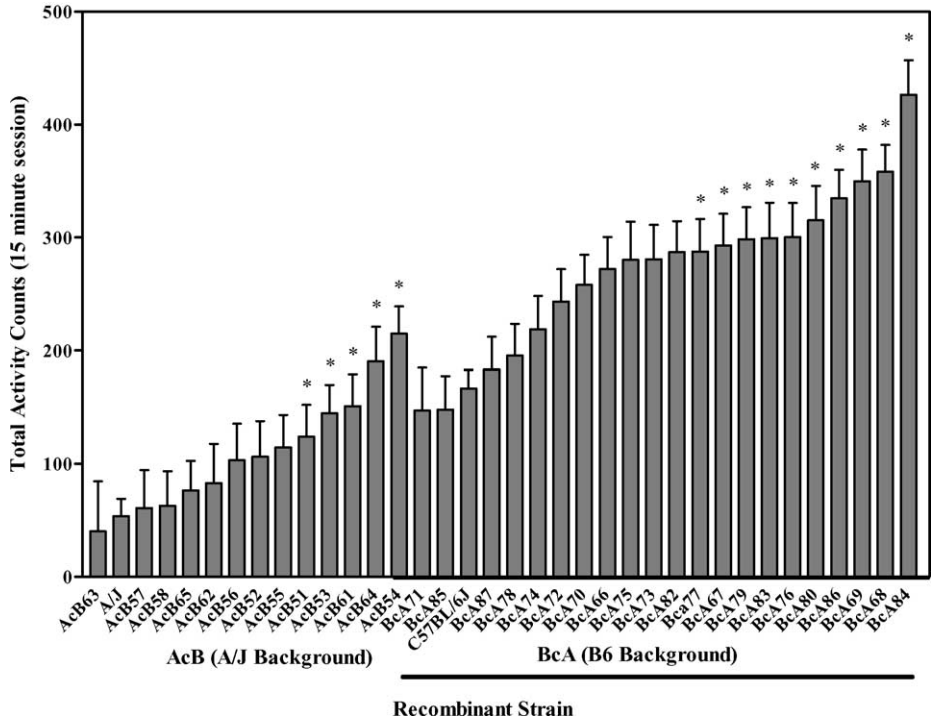

Fig. 4. Mean novelty/stress-induced locomotor activity (±S.E.M.) for the A/J, C57BL/6J, and AcB/BcA series of strains. The AcB and BcA strains are independently presented in ascending order of activity. Asterisk (*) denotes RCS that have a significantly different mean phenotype compared to the background strain.

an OF. The authors suggested that the epsilon4 mutants have dysfunctional NMDA receptors and displayed altered emotional behavior in general [31]. Similarly, the marker *D7j4* is close to a QTL (*Axtofd2*, 44 cM) for an anxiety-related trait (defecation) in an open-field [47] as well as the albino locus (*Tyr*, 44 cM). The albino locus is known to affect locomotor activity in an OF, and thus it was not surprising to find that it served as a cofactor in the QTL analysis.

An important objective of the present study was to compare QTLs mapped in the RIS and RCS. A comparison of the mapping results in the RIS and RCS indicate a num-

Table 2
Chromosomal regions containing B6 donor alleles associated with total novelty/stress-induced activation in the AcB

Chr	Peak marker[a]	cM[b]	Correlation[c]	Region containing donor QTL (cM)	Gene candidate	Description
3	*D3Mit185***	29.5	+0.769	29.5		
6	*D6Mit264***	3.2	+0.764	0.5–20.4		
8	*D8Mit186***	59	−0.649	47–73	*Siafq1 (56)*	QTL-stress-induced analgesia 1 QTL-acoustic startle [22]
13	*D13Mit110*	47	+0.723	47		See Table 1-same region in AXB/BXA RI
14	*D14Mit239*	42.5	−0.556	25–44.3	*Htr2a (41.5)*	Serotonin receptor 2A QTL-acoustic startle [22]
17	*D17Mit160*	42	+0.564	42		
18	*D18Mit120*	16	+0.779	16		
19	*D19Mit10**	51	+0.600	24–55.7	*Lrnx6 (45)* *Adra2a (50)* *Adrab1 (51)*	QTL-contextual fear conditioning 6 Adrenergic receptor alpha 2a Adrenergic receptor beta 1

(−) decreaser allele from B6 donor; (+) increaser allele from B6 donor.

[a] Peak marker in significant region identified by Map Manager QTX, $p < 0.00001$. *Suggestive or **significant loci were determined in follow-up analyses using LRS (likelihood ratio statistic) and permutations tests, following guidelines of Lander and Kruglyak [25].

[b] Recombinant distance in centimorgans from centromere.

[c] Correlations between SDP for marker and AcB strain means. It should be noted that in many cases multiple markers in a given region were identified by Map Manager QTX, however, only the peak marker (with the largest correlation) was included in the table. The genetic maps were used to determine the interval containing the QTL by identifying all strains which possessed donor alleles at the significant markers.

Table 3
Haplotypes of the AcB recombinant congenic strains at significant marker loci highlighted on chromosomes 3 and 6

		AcB Recombinant Congenic Strain														
		AcB63	AcB57	AcB58	AcB65	AcB59	AcB62	AcB56	AcB60	AcB52	AcB55	AcB51	AcB53	AcB61	AcB64	AcB54
Marker	cM															
D3Mit224	22.00	A	A	A	A	A	A	B	A	A	A	A	A	A	A	B
D3Mit185	29.50	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B
D3Mit335	29.50	A	A	0	A	A	A	A	0	A	0	A	0	A	A	B
D6Mit166	0.60	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
D6Mit264	3.20	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B
D6Mit159	7.00	A	A	A	A	A	A	A	A	A	B	A	B	A	B	B
D6Mit268	15.60	A	A	A	A	A	A	A	A	A	B	A	B	A	B	B
D6Mit223	19.00	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B
D6Mit351	20.40	A	A	A	A	A	A	A	A	A	A	A	B	A	A	B
D6Mit184	26.35	A	A	A	A	A	A	A	A	A	B	A	A	A	A	B

Informative strains (those with a significantly higher mean phenotypic value compared to the background A/J are highlighted. Donor alleles inherited from the B6 strain are designated with a B.

ber of overlapping QTL as shown in Table 5. Note that the AXB/BXA RIS and the AcB/BcA RCS were independently derived from the same progenitors, but were tested under identical conditions in the same laboratory. Thus, the shared QTL collectively provide very strong evidence for QTL in the specified regions as follows:

- Chromosome 1—The QTL shared by the RIS and RCS in the present study at 58.5–63.1 cM is consistent with an A allele that decreased and B6 allele that increased mean phenotypic values. This region is close to QTLs that have been previously mapped for contextual fear conditioning (*Lrnx1*, 60.2 cM; *Lrnx12*, 65 cM) [35], autonomic reactivity (such as open field urination and defecation) (58–84 cM) [18] and open-field activity (*Actd1*, 65 cM) [8,24]. The consistency of the findings in this region make chr 1 the strongest candidate QTL region for anxiety-related traits across a variety of strains and crosses that share a B6 ancestor.
- Chromosome 5—The identical marker (*D5Mit356*) was identified as the peak marker (41 cM) in both the RIS and RCS. The RCS strains carrying the A/J donor allele at this site in the BcA RCS showed a lower phenotype compared to those strains with the B6 allele. The QTL on chr 5 near the marker *Iapls3–9* (82 cM) was a significant cofactor at three sites in the CIM analysis of the RIS, and in the same region (74–86 cM) as a highly significant QTL in the BcA RCS.
- Chromosome 9—The peak marker identified in the RIS and RCS mapped to 15 cM on chr 9. This QTL was asso-

Table 4
Chromosomal regions containing A/J donor alleles associated with total novelty/stress-induced activation in the BcA

Chr	Peak marker[a]	cM[b]	Correlation[c]	Region containing donor QTL (cM)	Candidate gene	Description
1	*D1Mit136*	59.6	−0.770	58.5–63.1		See Table 1–same region in AXB/BXA RI
3	*D3Mit110***	64.1	−0.754	64.1–76.2	*Hn1 (63.7)*	Hypothalamic norepinephrine level
5	*D5Mit356*	41	−0.612	19–45		See Table 1–same region in AXB/BXA RI
5	*D5Mit99*	80	−0.819	74–86	*Elmaz2 (76)*	QTL-Elevated plus maze behavior 2
9	*D9Mit297**	15	+0.550	15–38	*Grik4 (23)*	Glutamate receptor, kainate 4
					Drd2 (28)	Dopamine receptor 2
11	*D11Mit119*	47.67	−0.536	47.6–54	*Lrnx4 (53)*	QTL-contextual fear conditioning 4
11	*D11Mit167***	71	−0.655	70–74.5	*Galr2 (70)*	Galanin receptor 2
17	*D17Mit139*	30.2	+0.572	24.5–32.3		

(−) decreaser allele from A/J donor; (+) increaser allele from A/J donor.

[a] Peak marker in significant region identified by Map Manager QTX, $p < 0.00001$. *Suggestive or **significant loci were determined in follow-up analyses using LRS (likelihood ratio statistic) and permutations tests, following guidelines of Lander and Kruglyak [25].

[b] Recombinant distance in centimorgans from centromere.

[c] Correlations between SDP for marker and the BcA strain means. It should be noted that in many cases multiple markers in a given region were identified by Map Manager QTX, however, only the peak marker (with the largest correlation) was included in the table. The genetic maps were used to determine the interval containing the QTL by identifying all strains which possessed donor alleles at the significant markers.

Table 5
Common QTL's for open-field activity/emotionality in the literature, compared to the AXB/BXA RIS and AcB/BcA RCS

RI strains			RC strains					Literature	
Chr	Peak marker in RI strains	Peak cM	Chr	Peak marker in RC strains	Peak cM	Range cM	RCS	Chr	Includes research using recombinant inbred, F2 or other gentic crosses with C57BL/6 J or A/J ancestors
1	*D1Mit26*	62.1	1	*D1Mit136*	59.6	58.5–63.1	BcA	1	Contextual fear conditioning [35]; OF activity [8,24]; exploratory behavior [12]; acoustic startle [22]; OF/light/dark [42]
			3	*D3Mit185*	29.5	0.5–20.4	AcB	3	OF activity [39]
			3	*D3Mit110*	64.1	64.1–76.2		3	OF activity [28]; OF activity [38]; acoustic startle [22]
4	*Mup1*	27.8	4	*D4Mit288***	28.6	21.9–28.6	AcB/BcA	4	OF activity [8,48]
5	***D5Mit356***	**41**	**5**	***D5Mit356***	**41**	**19–45**	**BcA**	5	OF activity [37]
5	*Iapls3–9**	82	5	*D5Mit99*	80	74–86	BcA	5	Anxiety-related trait [5]; OF activity [39]
6	*D6Mit50**	3.3	6	*D6Mit264*	3.2	0.5–20.4	AcB	6	OF activity [39]; exploratory behavior [12]; OF/light/dark [42]
			8	*D8Mit186*	59	47–73	AcB	8	Locomotor activity and crosses into a novel environment [33]; acoustic startle [22]
9	*Ets1*	15	9	*D9Mit297*	15	15–38	BcA	9	OF activity [37]
			11	*D11Mit167*	71	70–74.5	BcA	11	OF activity [28]
13	***D13Mit110***	**47**	**13**	***D13Mit110***	**47**	**47**	**AcB**		
			17	*D17Mit139*	30.2	24.5–32.3	AcB	17	OF activity [8]; OF activity [39]; OF/light/dark [42]
				D17Mit160	42	42	BcA		
19	*D19Mit10*	47	19	*D19Mit123*	51	24–55.7	AcB	19	OF [12]

* Identified by SIM analysis ($p < 0.05$) in the RI strains, and used as cofactor in CIM analysis as reported in Table 1.
** Note that this marker was identified at $p < 0.0001$ in both the AcB and BcA RCS. It did not meet statistical criteria for inclusion in Tables 2 and 4.

ciated with an A/J allele that increased mean phenotypic values in the RIS and RCS.

- Chromosome 13—The identical marker (*D13Mit110*) was identified as the peak marker (47 cM) in both the RIS and RCS. The A/J allele was associated with an increased mean phenotypic value in the RIS, while the B6 allele was associated with the same effect among the AcB RCS. Thus, both the A and B6 alleles increased the phenotype depending on the cross and background strain.
- Chromosome 19—The identical marker (*D19Mit10*) was identified as the peak marker (47 cM) in both the RIS and RCS. The A/J allele was associated with an increased mean phenotypic value in the RIS, while the B6 allele was associated with the same effect among the AcB RCS. Thus, both the A and B6 alleles increased the phenotype depending on the cross and background strain.

As shown in Tables 1, 2 and 4, there are a large number of potential candidates for the common QTL mapped in both the RIS and RCS that are related to general locomotor behavior (dopamine D1 and D2 receptors, albino locus) and other anxiety-related traits (contextual fear conditioning, acoustic startle, elevated plus maze). It is acknowledged that selection of candidate genes is highly speculative and that considerable research will have to be undertaken in order to narrow the intervals containing significant QTL, and to explore the involvement of particular candidates.

In this context, preliminary data have been reported which provide insight in to the nature of the specific genes regulating the expression of anxiety in the mouse. Specifically, Yalcin et al. [51] utilized a combined genetic and functional approach to dissect the commonly identified chromosome 1 QTL. The authors identified the gene Rgs2, which encodes a regulator of G protein signaling, as a mediator of variations in the ex-

pression of anxiety. Interestingly, this QTL is syntenic with minor linkage peak (1q) identified in a recent genome-wide linkage analysis of neuroticism and mood-related scale [32].

In summary, measurement of novelty/stress induced activation on an open-field paradigm yielded evidence for a quantitative trait influenced by several QTL. Among the AXB/BXA RIS significant loci were identified on chr 5 (*D5Mit356*, 41 cM), chr 8 (*D8Mit305*, 37 cM) and chr 14 (*D14Mit36*, 63 cM). In the AcB/BcA RCS, donor alleles from the A/J and C57BL/6 J that significantly modified phenotypic values relative to the background were identified in 15 regions on chromosomes 1, 3, 5, 6, 8, 9, 11, 13, 14, 17, and 18. Comparison of QTLs identified in both the AXB/BXA RIS and AcB/BcA RCS resulted in the identification of 7 common regions on chr 1 (58.5–63.1 cM), chr 4 (21.9–28.6 cM), chr 5 (19–45 and 74–86 cM), chr 6 (0.5–20.4 cM), chr 9 (15–38 cM), chr 13 (47 cM) and chr 19 (47 cM). The peak marker identified on chr 5 near *D5Mit356* (41 cM) in both the AXB/BXA RIS and AcB/BcA RCS maps to a region containing the genes for several $GABA_A$ receptor subunits. Future research will attempt to narrow the QTL intervals identified in the present study, and examine the relationship between the $GABA_A$ receptor system and the expression of anxiety-related traits in the A, B6, AXB/BXA RIS and AcB/BcA RCS.

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