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QTL analyses of spontaneous activity by using mouse strains from Mishima battery

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Abstract. We reported previously that spontaneous activity in the home cage is highly variable among the Mishima battery of mouse strains. In that study, NJL and KJR were found to be hyperactive strains in contrast to BLG2, which showed one of the lowest activity levels. To unravel the genetic loci involved in this behavioral phenotype, we conducted QTL analyses on backcross populations of crosses between either NJL or KJR and BLG2 strains. In the backcross of NJL to BLG2, no single locus was associated with increased spontaneous activity. In the backcross of KJR to BLG2, linkage analysis showed that a locus on the most telomeric region of Chromosome (Chr) 3 was involved in the spontaneous activity, thus named *Loco1*. Further linkage analysis using selected progeny carrying the allele from KJR at the *Loco1* locus suggested the presence of another locus, *Loco2*, on Chr 17. An analysis showed that *Loco1* and *Loco2* interacted epistatically.

Introduction

Spontaneous locomotor activity is a behavior that is greatly influenced by the forces of natural selection in the process of evolution. Locomotor activity of animals in nature plays a crucial role in resource acquisition, territorial defense, and migratory behavior. When the resources are limited or the territory is large, animals have to move long distances to acquire food and to protect their territory. In the laboratory environment, mice have not been deliberately selected for differences in spontaneous locomotion, and a range of spontaneous activities has been reported across mouse strains (Toth et al. 1995). Several sources of evidence support the idea that spontaneous activity is regulated genetically. It is reported that artificial selection for a high level of voluntary wheel-running in an outbred population of mice, HSD:ICR, resulted in strains of mice that were twice as active as non-selected control lines (Koteja et al. 1999). In the inbred strains, C57BL/6J mice were significantly more active than BALB/cByJ mice when the spontaneous activity was measured telemetrically. The genetic loci with potential linkages to circadian variations in the amount of spontaneous activity were mapped to regions of Chrs 3, 8, 12, 13, and 19 by a study using 13 CXB recombinant inbred (RI) strains, generated by intercrosses between BALB/cByJ and C57BL/6J (Toth and Williams 1999).

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We previously conducted multi-phenotype behavioral characterizations using a series of inbred strains derived from wild mice in addition to the common laboratory strains, the Mishima battery of mouse strains (Koide et al. 2000; Furuse et al. 2001). The findings showed great diversity in behavioral patterns between strains in contrast to less within-strain variability. In the study of spontaneous activity in the habituated normal home cages, we found that mice of the KJR and NJL strains were hyperactive in contrast to mice of the BLG2 strain, which showed a low level of spontaneous locomotion (Koide et al. 2000). The difference in spontaneous activity observed between these strains implies a potential for mapping the genes involved in this phenotypic difference. In this study, we conducted genetic linkage mapping of the genes involved in different levels of spontaneous activity in the home cages, using backcrossed populations between NJL and BLG2, and between KJR and BLG2.

Materials and methods

Mice. All the inbred strains used in this study were maintained in the animal facility at the National Institute of Genetics (NIG), Mishima, Japan. NJL, KJR and BLG2 were established as inbred strains after 20 generations of brother-sister matings (Koide et al. 2000). For the genetic mapping, two crosses were designed. F_1 females from the cross between females of KJR and males of BLG2 were backcrossed to BLG2 to produce N2 populations, KL × L. F_1 females from the cross between females of NJL and males of BLG2 were backcrossed to BLG2 to produce N2 populations, NL × L. 376 mice of KL × L and 188 mice of NL × L were collected, and only females aged between 8 and 12 weeks were used for measuring spontaneous activity and for the subsequent genetic analyses. Because the data in the spontaneous activity test were more variable in males than in females in our preliminary study, we chose only females for the QTL analysis (Koide et al. 2000). All strains were maintained at NIG in a 12h/12h light/dark cycle with lights coming on at 08.00 a.m. and food and water available *ad libitum*.

Measuring spontaneous activity. Spontaneous home-cage activity was assessed for individual mice with an infra-red sensor, AB-system 24 (Neuroscience Co. Ltd., Tokyo, Japan). The spontaneous activity was measured over a 4-day period, with the first day used for habituation. The data for activity were summed from 08.00 a.m. of the second day to 08.00 a.m. of the fifth day for each mouse. Hence, data from the first day were not used in the final data analysis.

Genotyping the microsatellite polymorphisms. Genomic DNA of each mouse was isolated from the tail by using an automatic nucleic acid isolation system, NA-2000 (KURABO, Osaka, Japan). The fol-

lowing polymorphic markers spaced around 20 cM were chosen for genome-wide scanning for 188 backcross progeny of each cross. Microsatellite markers used for KL × L backcross progeny: *D1Mit4*, *D1Mit211*, *D1Mit414*, *D1Mit7*, *D1Mit132*, *D1Mit134*, *D1Mit365*, *D1Mit386*, *D1Mit187*, *D1Mit309*, *D1Mit100*, *D1Mit265*, *D1Mit16*, *D2Mit1*, *D2Mit82*, *D2Mit9*, *D2Mit30*, *D2Mit29*, *D3Mit131*, *D3Mit55*, *D3Mit51*, *D3Mit141*, *D3Mit11*, *D3Mit216*, *D3Mit18*, *D3Mit86*, *D3Nig1*, *D3Mit128*, *D4Mit2*, *D4Mit288*, *D4Mit31*, *D4Mit127*, *D5Mit3*, *D5Mit91*, *D5Mit242*, *D5Mit101*, *D6Mit74*, *D6Mit102*, *D6Mit254*, *D6Mit15*, *D7Mit117*, *D7Mit229*, *D7Mit31*, *D7Mit9*, *D7Mit12*, *D8Mit4*, *D8Mit128*, *D8Mit113*, *D8Mit280*, *D9Mit218*, *D9Mit67*, *D9Mit22*, *D9Mit260*, *D9Mit151*, *D10Mit2*, *D10Mit15*, *D10Mit10*, *D10Mit180*, *D11Mit215*, *D11Mit28*, *D11Mit327*, *D11Mit128*, *D12Mit147*, *D12Mit5*, *D12Mit6*, *D12Mit7*, *D13Mit4*, *D13Mit9*, *D13Mit262*, *D14Mit140*, *D14Mit217*, *D14Mit69*, *D14Mit107*, *D15Mit5*, *D15Mit156*, *D15Mit159*, *D16Mit34*, *D16Mit189*, *D16Mit106*, *D17Mit22*, *D17Mit66*, *D17Mit93*, *D17Mit221*, *D18Mit14*, *D18Mit186*, *D18Mit6*, *D19Mit109*, *D19Mit19*, *D19Mit1*, *DXMit166*, DXMit114, *DXMit153*.

A new microsatellite marker, *D3Nig1*, was designed as follows from a genome sequence including the *Acadm* gene deposited in the public database (GenBank accession number: AC087184). D3Nig1-F (5′-TAGTACAACTCTACCCTCCA-3′), D3Nig1-R (5′-ATCCTCT-CTGCCCTCTTTAG-3′). It was reported that *Acadm* is situated between *D3Mit18* and *D3Mit128* according to the Mouse Genome Informatics (http://www.informatics.jax.org/). A pair of primers for a gene *Adh1* was synthesized according to the previous report (Hearne et al. 1991). Microsatellite markers used for NL × L backcross progeny: *D1Mit4*, *D1Mit414*, *D1Mit132*, *D1Mit265*, *D2Mit3*, *D2Mit8*, *D2Mit97*, *D2Mit265*, *D3Mit55*, *D3Mit51*, *D3Mit11*, *D3Mit19*, *D4Mit235*, *D4Mit153*, *D4Mit16*, *D4Mit127*, *D5Mit148*, *D5Mit6*, *D5Mit403*, *D5Mit101*, *D6Mit91*, *D6Mit316*, *D6Mit102*, *D6Mit25*, *D7Mit281*, *D7Mit100*, *D7Mit12*, *D8Mit94*, *D8Mit240*, *D8Mit13*, *D9Mit23*, *D9Mit10*, *D9Mit16*, *D10Mit51*, *D10Mit264*, *D10Mit14*, *D11Mit215*, *D11Mit28*, *D11Mit98*, *D12Mit2*, *D12Mit7*, *D12Mit4*, *D13Mit88*, *D13Mit9*, *D13Mit262*, *D14Mit2*, *D14Mit38*, *D14Mit107*, *D15Mit26*, *D15Mit159*, *D15Mit74*, *D16Mit182*, *D16Mit34*, *D16Mit125*, *D16Mit189*, *D17Mit164*, *D17Mit36*, *D17Mit38*, *D17Mit221*, *D18Mit19*, *D18Mit1*, *D19Mit19*, *D19Mit4*, *D19Mit33*, *DXMit186*, *DXMit172*, *DXMit1*.

Polymorphisms of microsatellite markers were determined by the SSLP method as described previously (Koide et al. 1998).

Detection and mapping of QTLs. All the typing data for 188 backcross progeny from both NL × L and KL × L were first analyzed by linkage analysis with MapManager QTX (Manly and Olson 1999) to detect suggestive loci associated with spontaneous activity. In the KL × L panel, the chromosomes showing a suggestive or significant score (Lander and Kruglyak 1995) for spontaneous activity were then further typed for all the backcross progeny, 376 in total. In addition to an interval mapping analysis with Map Manager QTX, a composite interval mapping analysis based on a multiple QTL model (Zeng 1993, 1994) was performed with using QTL Cartographer (Basten et al. 1999).

Statistical analysis. Data analysis was performed with the StatView software package (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was applied to determine the effects of strain or genotype within a test condition and to analyze distribution of the performance in the N2 backcross. *Post hoc* analysis utilized the Fisher's Protected Least Significant Difference (PLSD) test.

Results

Spontaneous activity of KL × L. Analysis of spontaneous activity over a 3-day period showed a great degree of difference between KJR and BLG2 (Fig. 1). ANOVA indicated that the difference between the strains was significant in KJR and BLG2, $F(1,18) = 172.02$, $p < 0.0001$. The F_1 mice performed the same high levels of spontaneous activity as KJR mice, suggesting some dominance of the hyperactive phenotype (Fig. 1). ANOVA indicated that the difference between KJR and F_1 was not significant ($F(1,18) = 0.37$, $p > 0.5$), in contrast to the

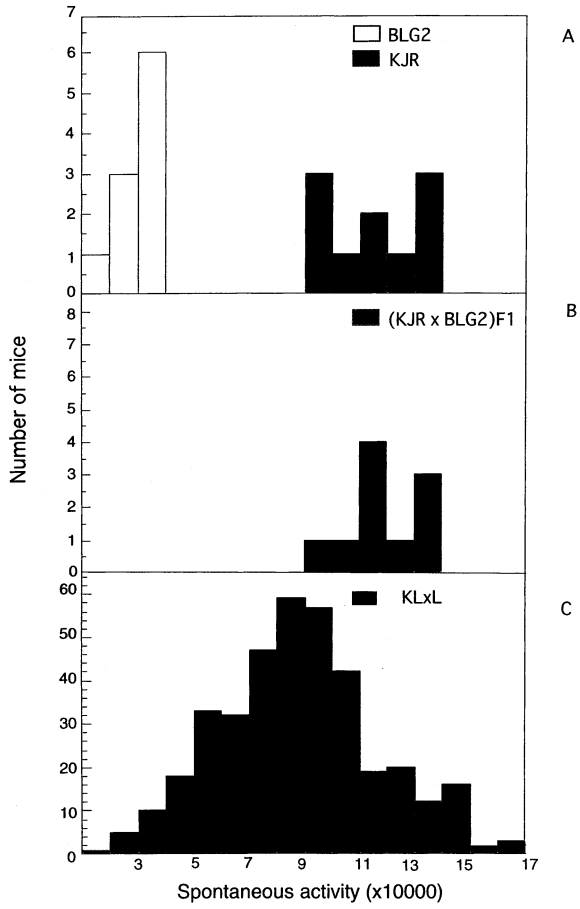

Fig. 1. Distribution of the spontaneous activity in the parental strains, F_1 and KL × L N2 backcross. Number of mice studied: BLG2, N = 10; KJR, N = 10; KJR × BLG2)F_1, N = 10; KL × L, N = 376. Data are presented as number of mice observed at each activity level. The mean spontaneous activity of each group was as follows: BLG2, 39,422; KJR, 113,925; (KJR × BLG2)F_1, 118,191.

significant difference between BLG2 and F_1 ($F(1,18) = 268.67$, $p < 0.0001$). The data in Fig. 1(C) illustrate the activity distribution for each score in the N2 backcross (N = 376). The performance distribution was normal (Kolmogorov-Smirnov statistic), indicating that multiple genes are involved in the genetic difference of spontaneous activity.

QTL analysis of KL × L. Since the differences in spontaneous activity seem to be controlled by multiple genes, QTL analysis was performed by an interval mapping method with the software MapManager QTX for KL × L. In the first survey of the KL × L panel, 188 backcross progeny were analyzed for the 93 marker loci distributed over the entire genome (Table 1). The findings showed that one locus located on a distal region of Chr 3 near *D3Mit18* and *D3Mit128* exceeded the suggestive threshold of LOD = 1.9 for BC1 (Lander and Kruglyak 1995) as a value of 2.7. No other locus was found to be suggestive (Table 1). Then, the interval mapping method was applied to Chr 3 for the 376 backcross progeny, including the additional 188 progeny (Fig. 2). In the entire sample, the maximum LOD score, 4.8, was found between the markers *D3Nig1* and *D3Mit128* and exceeded the significant threshold of LOD score 3.3 for BC1. Essentially, the same result was obtained from the composite interval mapping. We have designated this locus *locomotor activity 1* (*Loco1*).

Table 1. Result of a genome-wide scanning for 188 progeny from the KL × L backcross population.

Chr	Locus (cM)[a]	LOD score
1	*D1Mit7* (*41.5*)	1.0
1	*D1Mit132* (*43.7*)	1.4
1	*D1Mit134* (*47.0*)	1.5
1	*D1Mit365* (*57.9*)	1.3
1	*D1Mit386* (*60.1*)	1.3
1	*D1Mit187* (*64.5*)	1.1
1	*D1Mit309* (*66.7*)	1.1
1	*D1Mit100* (*74.3*)	1.5
1	*D1Mit265* (*76.5*)	1.6
2	*D2Mit9* (*38.32*)	1.3
2	*D2Mit30* (*56.8*)	1.0
3	*D3Mit11* (*37.2*)	0.8
3	*D3Mit86* (*54.6*)	1.7
3	*D3Mit18* (*54.6*)	2.0[b]
3	*D3Mit128* (*63.4*)	2.7[b]
6	*D6Mit74* (*10.9*)	0.9
7	*D7Mit9* (*45.9*)	1.0
8	*D8Mit280* (*74.3*)	1.2
11	*D11Mit28* (*36.1*)	1.3
14	*D14Mit140* (*21.9*)	0.8
16	*D16Mit34* (*12.0*)	0.9
16	*D16Mit189* (*40.4*)	1.1
17	*D17Mit22* (*9.8*)	1.6
17	*D17Mit66* (*19.7*)	1.5

Result of links report by MapManager QTX is shown.
[a] Marker positions were taken from database at http://www.genome.wi.mit.edu/.
[b] Genetic markers exceed the suggestive threshold (LOD = 1.9).

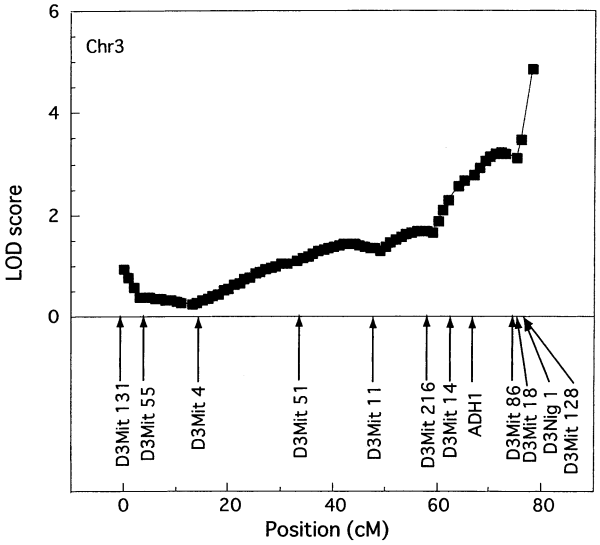

Fig. 2. Detection of QTL for spontaneous activity on Chr 3. The genetic markers used for the typing are illustrated in the figure.

Selective genotyping of KL × L. From the linkage study, the spontaneous activity QTL was linked to the marker locus *D3Mit128* on Chr 3. The levels of spontaneous activity were compared between two groups of N2 females typed directly with K/L or L/L genotypes at the locus *D3Mit128* (Table 2). The mean activity value obtained for the K/L genotype was 94,964, and for the L/L genotype it was 81,726. ANOVA indicated that the difference between the two groups is highly significant $F(1,374) = 21.34$, $p < 0.0001$. However, the difference of the mean activity value, 13238, between the groups of K/L and L/L is apparently less than the value, 74503, of parental difference. The data suggest that an additional locus or loci are essential for the hyperactive phenotype of KJR. We conducted a linkage analysis using a group selected for the K/L genotype at the *D3Mit128* locus. From the population of

Table 2. Mode of action of *Loco1* locus.

Genotype	Number of mice	Spontaneous activity	
		Mean	SEM
K/L	175	94,964.7	2,096.6
L/L	201	81,726.0	1,954.1

Genotype is determined at the *D3Mit128* locus. K, allele of *D3Mit128* from KJR strain; L, allele from BLG2 strain. SEM = standard error of the mean. ANOVA, $F(1,374) = 21.34$, $p < 0.0001$.

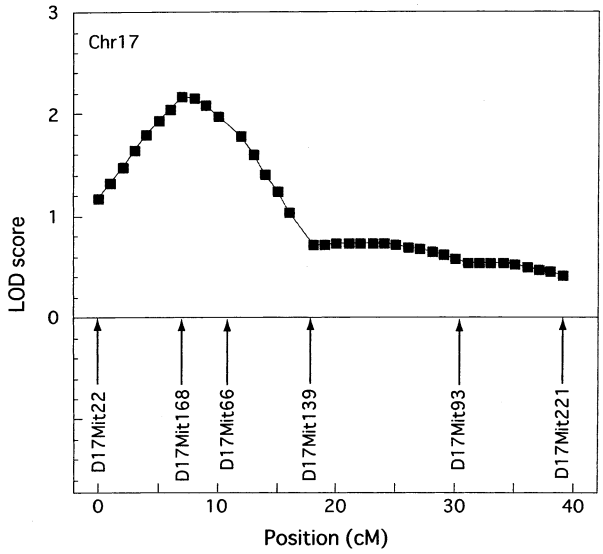

Fig. 3. Detection of a locus on Chr 17 that epistatically interacted with *Loco1*. The genetic markers used for the typing are illustrated in the figure.

175 mice, LOD 2.2, exceeding the suggestive level of 1.9, was found at the locus between *D17Mit168* and *D17Mit66* (Fig. 3). This locus was designated as *Locomotor activity 2* (*Loco2*). No suggestive level of LOD score was observed when the group of the L/L genotype at *D3Mit128* locus was analyzed for the spontaneous activity. The levels of spontaneous activity were compared among four groups of N2 females typed directly with K/L or L/L genotypes at the loci *Loco1* and *Loco2* (Fig. 4). Significant differences were found across groups [ANOVA, $F(3,372) = 10.77$, $p < 0.0001$]. No difference of activity was observed in the two groups typed at *Loco2* in the population carrying L/L allele at *Loco1* (a *post hoc* analysis, $p > 0.05$). In contrast to this population, the difference of mean activity was significant between the two groups typed directly with K/L and L/L genotypes at *Loco2* in the population carrying K/L genotype at *Loco1* (a *post hoc* analysis, $p < 0.002$). Significant interaction of *Loco2* with *Loco1* was observed [ANOVA, $F(1,372)$ for interactive effect = 8.086, $p < 0.005$].

Linkage analysis of NL × L. Analysis of spontaneous activity over a 3-day period showed a great degree of difference between NJL and BLG2 strains (Fig. 5). Analysis of variance (ANOVA) indicated that the difference between the strains was significant, $F(1,18) = 22.47$, $p < 0.0005$. The F_1 mice performed at a higher level of spontaneous activity than did NJL mice. ANOVA indicated that the difference between NJL and F_1 was significant ($F(1,18) = 4.51$, $p < 0.05$). The data of activity scores were variable within each group of NJL strain and F_1 progeny (Fig. 5A, B). A significant difference was also observed between BLG2 and F_1($F(1,18) = 89.06$, $p <$

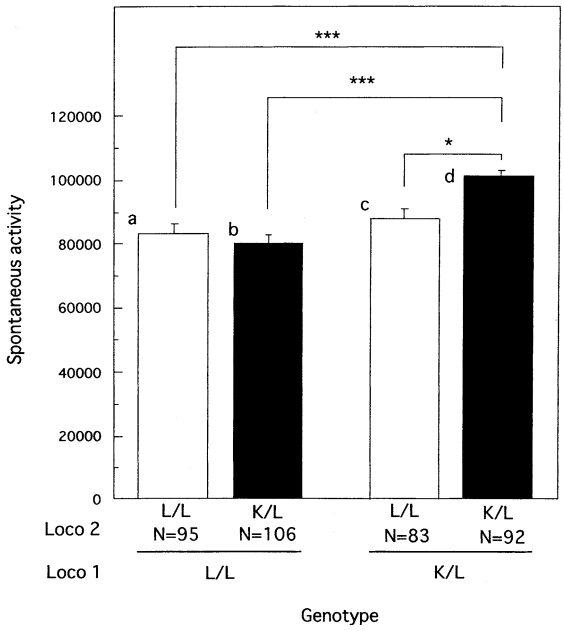

Fig. 4. Comparison of mean spontaneous activity in the groups classified for the genotypes at the *Loco1* and *Loco2* loci. Genotype at *Loco1* was typed at *D3Mit128*. Genotype at *Loco2* was typed at *D17Mit168*. K, allele from KJR strain. L, allele from BLG2 strain. The mean spontaneous activity of each group was as follows, a, 83,370; b, 80,252; c, 88,119; d, 101,140. $^{***}p < 0.0001$, $^{*}p < 0.05$. The bars indicate the SEM.

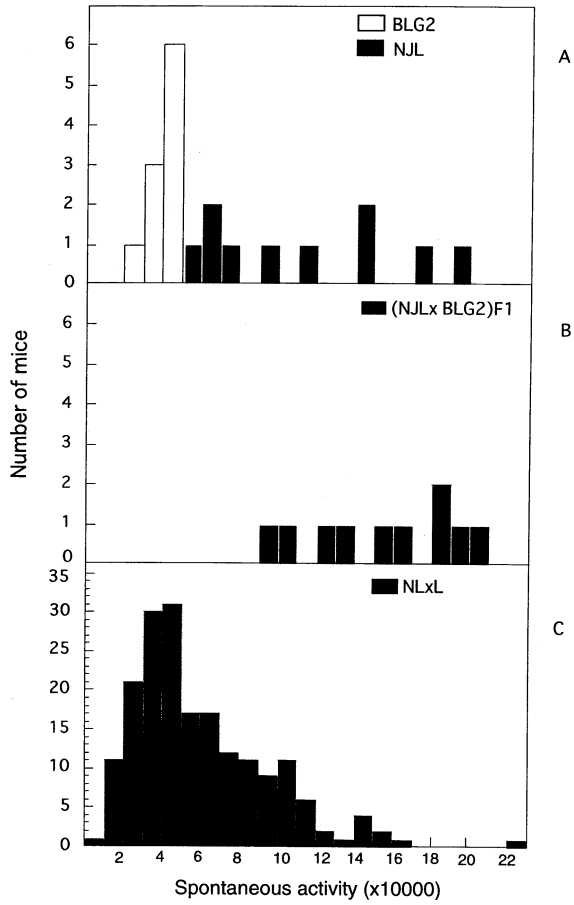

Fig. 5. Distribution of the spontaneous activity in the parental strains, F_1 and NL × L N2 backcross. Number of mice studied: BLG2, N = 10; NJL, N = 10; (NJL × BLG2)F_1, N = 10; NL × L, N = 188. Data are presented as number of mice observed at each activity level.

0.0001). The data in Fig. 5(C) illustrate the activity distribution for each score in the N2 backcross progeny (N = 188). The performance distribution was normal (Kolmogorov-Smirnov statistic), indicating that multiple genes are involved in the difference of spontaneous activity. In the NL × L panel, 188 backcross progeny were analyzed for the 67 marker loci distributed over the entire genome. In contrast to the data from KL × L, no single locus was found to be significantly associated with the hyperactive phenotype of NJL (Table 3).

Discussion

In our previous study of the Mishima battery of mouse strains, two wild strains (KJR and NJL) were found to be hyperactive, while another three (BFM/2, BLG2, and HMI) were hypoactive (Koide et al. 2000). The present study demonstrates that *Loco1* on Chr 3 is associated with increased spontaneous activity of KJR. *Loco1* is a new locus since the previous report of a locus influencing spontaneous activity on Chr 3 in CXB recombinant inbred strains (Toth and Williams 1999) maps proximal to *Loco1*. In contrast to the successful mapping in the KL × L cross between KJR and BLG2, we were unable to find any locus suggesting the presence of a gene involved in spontaneous activity in another backcross panel, NL × L. In a linkage analysis for the NL × L panel after a genome-wide scan with 67 microsatellite markers for 188 progeny, no single locus was found to be associated with increased activity of NJL mice (Table 3). These findings suggest that a different set of genes is associated with increased activity in the KJR and NJL strains. An analysis of actual behavior of KJR and NJL during the dark period via a video recorder showed that the two strains behave differently, as KJR hangs upside down and walks around the top cover of the cage in contrast to NJL, which shows continuous somersaulting for almost the entire dark period (data not shown). Different types of behavior exhibited by KJR and NJL might explain why the *Loco1* locus is not associated with a high level of spontaneous activity in NJL in our genetic analysis. Several possibilities may explain why we were unable to detect a locus associated with increased locomotion of NJL mice. First, multiple loci, each with a weak effect for the spontaneous activity, may be involved in this phenotype. Second, complex genetic interactions between multiple loci may be required for the hyperactive phenotype. Finally, it is possible that the large variation of activity scores observed in NJL and F_1 mice (Fig. 5) reduced the sensitivity of detection for mapping QTL. It is hard to conclude which is the actual reason for failing to detect a locus involved in the hyperactive phenotype of NJL from our current data. In this study, we used a backcross design for the genetic mapping, and one possibility is that we would have found the loci if we had used an intercross F_2 design.

In the KL × L panel, the analysis of activity distribution in the N2 backcross progeny from the cross between KJR and BLG2 indicates the involvement of multiple genes. However, we were unable to find other loci involved in the increased activity of KJR on different chromosomes from the whole-genome scanning. When the mean activity value was compared between two groups directly typed by a marker genotype at *D3Mit128*, the difference was significant but not marked like that between the parental strains. This finding suggests that other genes as well as *Loco1* are required for inducing elevated spontaneous activity. When the progeny carrying the allele of

Table 3. Result of a genome-wide scanning for 188 progeny from the NL × L backcross population.

Chr	Locus (cM)[a]	LOD score
Chr4	*D4Mit16* (56.8)	1.0
Chr8	*D8Mit240* (42.6)	1.1
Chr10	*D10Mit14* (69.9)	0.9
Chr19	*D19Mit19* (26.2)	1.4

Result of links report by MapManager QTX is shown.
[a] Marker positions were taken from database at http://www.genome.wi.mit.edu/.

D3Mit128 from KJR were analyzed for linkage to the increased spontaneous activity, the existence of another locus, *Loco2*, on Chr 17 was suggested. It is assumed that the epistatic interaction between *Loco1* and *Loco2* induced the high level of spontaneous activity in KJR mice. Indeed, there was no difference of mean spontaneous activity between two groups of K/L and L/L genotypes at the *Loco2* locus when the population of the two groups was selected for the ones not carrying the *Loco1* allele from KJR strain. However, the difference of mean activity was significant between two groups of K/L and L/L genotypes at the *Loco2* locus when the population of both groups was selected for the ones carrying the allele of the *Loco1* from KJR (Fig. 4). The result indicates that KJR alleles at *Loco1* and *Loco2* loci interact epistatically. The difference in the spontaneous activity between K/L; K/L and L/L; L/L at *Loco1*; *Loco2* loci was 17,770 (= 101,140 − 83,370), which was about one-quarter (23%) of the difference between the parental (BLG2 × KJR)F_1 and BLG2, 78,769 (= 118,191 − 39,422). If we neglect the interaction effect between the *Loco1* and *Loco2* loci, the *Loco1* locus effect maybe estimated to be 13,239 (= 94,965 − 81,726) from Table 2, and the additional effect of *Loco2* was not significant at all (data not shown). Thus, the magnitude of the interactive effect could be comparable to the additive effect. The relative contribution of gene-by-gene interaction effect could be even larger in the remaining three-quarters of the difference between the parental (BLG2 × KJR) F_1 and BLG2 mice.

It was reported previously that disruption of the dopamine transporter gene causes hyperactive phenotype in both dark and light phases (Giros et al. 1996). Dopamine is the principal neurotransmitter influencing motivated behavior (Graybiel et al. 1994). The dopamine transporter is believed to control the temporal and spatial activity of released dopamine by rapid uptake of the neurotransmitter into presynaptic terminals. However, no gene that is directly involved in the dopamine signaling has been mapped to the telomeric region of Chr 3 to date. However, the telomeric region of Chr 3 carries alcohol dehydrogenase genes, *Adh1* and *Adh3*. It is reported that *ADH1* in humans is expressed mainly in the liver and involved in the degradation of dopamine (Mardh and Vallee 1986). We mapped *Adh1* in the KL × L backcross panel using a polymorphic marker reported by Hearne et al. (1991). A linkage analysis revealed that *Adh1* is located between *D3Mit14* and *D3Mit86*, more proximal to *Loco1*, outside of the candidate region (Fig. 2).

This study showed that mapped genetic loci are associated with the increased spontaneous activity observed in one of the Mishima battery of mouse strains. As we reported previously, a high frequency of genetic polymorphism is observed among strains of Mishima battery (Koide et al. 1998, 2000). The genetic diversity found in these wild-derived strains offers a great advantage for finding behavioral differences among strains and for subsequent genetic mapping with polymorphic microsatellite markers. This study on genetic mapping of spontaneous activity demonstrates that the Mishima battery of mouse strains is highly useful for a wide variety of biobehavioral studies.

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