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Genetic Analysis of the Hypothalamic Neurotensin System

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This study used B × D recombinant inbred mice to detect and localize genes that control the hypothalamic neurotensin (NT) system. Abundance of transcripts that encode NT and NT receptors 1, 2, and 3 (NTR1, NTR2, and NTR3) in total hypothalamic RNA was the quantitative trait measured. Analysis of transcript abundance data revealed associations with quantitative trait loci (QTL) for NT transcript abundance (NTta) on chromosome 1, 3, 6, 7, 8, and 9; for NTR1ta on chromosome 3, 8, 12, and X; for NTR2ta on chromosome 2, 4, 9, 10, 12, 13, and 17; for NTR3ta on chromosome 1, 7, 11, and 12. NTta QTL on chromosomes 3, 7, and 8 coincide with QTL previously identified that impact NT peptide content and NTR2ta QTL on chromosome 2 and 12 coincide with genes previously associated with NTR2 receptor abundance. The NTta, NTR1ta, and NTR3ta QTL were not linked to their respective structural genes, but there is a highly significant ($p < 0.001$) association for NTR2ta on chromosome 12 that includes the *Ntsr2* structural gene. There are areas of potential shared genetic regulation between NTta and NTR3ta on chromosome 1 and 7 and for all three receptors on proximal chromosome 12. The NTta QTL on chromosome 9 includes the dopamine D2 receptor (*Drd2*) gene and QTL involved in responses to dopaminergic agents (*Hts*), antipsychotics (*Hpic1*) and cocaine (*Cocrb8*), and ethanol (*Etohc3*). These results further strengthen the hypothesis that the NT system is involved in mediating the actions of antipsychotic agents and drugs of abuse. *Neuropsychopharmacology* (2006) **31,** 535–543. doi:10.1038/sj.npp.1300870; published online 10 August 2005

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INTRODUCTION

Neurotensin (NT, gene symbol *Nts*) is a tridecapeptide heterogeneously distributed in the mammalian central nervous system. There is a very high coincidence of expression of NT in dopaminergic cells, and there are significant modulatory interactions between the NT and dopamine systems (Nemeroff, 1986; Binder *et al*, 2001; Leonetti *et al*, 2004; Roubert *et al*, 2004). NT appears to impact other neurotransmitter circuits that are innervated by the dopamine system. NT-containing neural circuits have been postulated to play a role in the mechanism of action of antipsychotic agents (Nemeroff, 1980; Kinkead *et al*, 1999; Kinkead and Nemeroff, 2004). These circuits have also been implicated in the actions of ethanol and drugs of abuse and in the pathogenesis of substance abuse disorders (Erwin *et al*, 1997; Ehlers *et al*, 1999; Adams *et al*, 2001; Erwin *et al*, 2001; Dobner *et al*, 2003).

Signals in the NT system are transduced by at least three different receptors including NTR1 (gene symbol *Ntsr1*) and NTR2 (*Ntsr2*) both G-protein coupled, seven transmembrane receptors and NTR3, which is a single transmembrane Type I amino-acid receptor (Tanaka *et al*, 1990; Vita *et al*, 1993; Mazella *et al*, 1996, 1998; Sun *et al*, 2001). (The NT 3 receptor is also known as Sortilin 1 with gene symbol *Sort1,* and in this manuscript the gene product and transcript are referred to as NTR3 and the structural gene as *Sort1*). Whereas, dysfunction of the NT system may play a role in the pathophysiology of schizophrenia, the genes that encode the various components of the system (*Nts, Ntsr1, Ntsr2, and Sort1*) are most likely not the basis of this vulnerability. In contrast, genes that more globally control the system may be critical to the pathophysiology of schizophrenia and to differential responses to antipsychotic agents.

Quantitative trait analysis with panels of recombinant inbred mice has emerged as one of the principal methods to study the genetic architecture of complex neurobehavioral phenotypes. Quantitative trait loci (QTL) that control abundance of NT peptide (as measured with specific immunoreactivity (NT-IR)) and the NTR1 and NTR2 receptors (as measured with receptor-binding B_{max} values) have been detected with the LS × SS set of recombinant inbred mice (Erwin *et al*, 1997, 2001). Another level of genetic regulation of the NT system could be control of expression of NT system genes and these regulatory genes could be detected with transcript abundance as quantitative traits. Identification of those genes that control transcript

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abundance in combination with those that control peptide and receptor abundance should provide insight into the number and types of genes that control the NT system. The three goals of the present study were to determine whether abundances of the transcripts that encode NT, NTR1, NTR2, and NTR3 are controlled by QTL, to determine whether the same set of QTL control the expression of multiple NT system genes and to compare the transcript abundance QTL to those for peptide and receptor abundance.

MATERIALS AND METHODS

Animals and Tissue Preparation

Foundation stock for 26 B × D lines and the C57Bl6 and DBA2J parent lines were acquired from the Jackson Laboratory (Bar Harbor, ME) and used to establish a breeding colony. Animals were housed in a mouse-only facility. Breeding pairs consisted of one male and two female mice. Dams were individually housed while pregnant and in the postnatal period through weaning. Animals were weaned at postnatal day (PND) 21 and then group housed three per cage by same sex and strain. After weaning, animals were maintained with routine animal husbandry including regular cage changes, but were otherwise not subject to any additional stresses, manipulations or exposures. Ambient colony conditions were temperature at $23 \pm 2^{\circ}$C and relative humidity between 30 and 60%, with a 12 : 12 h light : dark cycle (lights out at 1800 h) and animals were allowed *ad libitum* access to standard laboratory chow and water. Animals were killed by cervical dislocation at PND 75–90, under low stress conditions. Brains were rapidly dissected on ice into constituent anatomical regions and stored at -80°C until utilized.

RNA Quantification

Total hypothalamic RNA was isolated with the RNeasy Mini Kit (Qiagen Inc, Valencia, CA) by manufacturer's method. Aliquots of total RNA (5 μg) from individual animals were converted to cDNA with the SuperScript™ First Strand Synthesis System for Real Time PCR (Invitrogen Life Technologies, Carlsbad, CA). Target and 18S transcript abundances were determined with specific real-time RT-PCR assays, utilizing TaqMan® detection chemistry (Applied Biosystems, Foster City, CA). Amplification and detection primers for RT-PCR assays were from ABI 'Assays-On-Demand' and included NT (accession number: NM_024435, ABI assay # Mm00481140), NTR1 (NM_018766, ABI assay # Mm00444459), NTR2 (NM_008747, ABI assay # Mm00435426), and NTR3 (*Sort1*) (NM_019972, ABI assay # Mm00490905). Aliquots of cDNA equivalent to 0.3 μg of input RNA were assayed in an ABI 7000 real-time PCR system, in the 96-well format, using the manufacturer's specified PCR cycle parameters, through 40 cycles. This is a 'relative abundance assay', and target transcript abundance is expressed as the 'detection threshold cycle' (C_t), which is in the middle of the exponential phase of the PCR amplification. As detection cycle is the readout parameter, a higher C_t value corresponds to lower transcript abundance, because more PCR cycles were required to amplify the target. A dilution series positive control and no transcript and no reverse transcriptase-negative controls were included in each assay. To assure assay stability, the entire experiment was performed in duplicate, with identical results.

Statistical Analysis and Genetic Mapping

Strain mean transcript abundances were determined from six individual male animals per line (Figure 1). Statistical analysis of transcript abundance data was conducted with the JMP-5 computer program (SAS Institute Inc, Cary, NC) on Macintosh G-4 computers. Descriptive statistics were calculated for each line, comparison of strain mean values was by ANOVA, and correlation of strain mean transcript abundance values was with Pearson's product-moment analysis as implemented by JMP-5 (JMP Statistics and Graphics Guide (2002).

Calculations of narrow sense heritability (h^2) where with the equation $h^2_{RI} = S^2_B/(S^2_B + S^2_W)$. In this equation, S^2_B is an estimate of the additive genetic variance (V_A) given by $S^2_B = (MS_B - MS_W)/n_W$, where MS_B is the mean square between strain, MS_W the mean square within strain, which is also an estimate of S^2_W and n_W is the number of mice tested for each strain (Belknap *et al*, 1996). These values are obtained from the ANOVA of the strain mean transcript abundance data. Genetic mapping was performed with the Macintosh version of QTX (http://www.mapmanager.org/mmQTX.html), run on Macintosh G-4 computers (Manly *et al*, 2001). The B × D genetic marker set used with QTX was from Dr Robert Williams at the University of Tennessee at Memphis (Williams *et al*, 2001). This genetic marker set consisted of 975 error-checked and nonredundant loci. Results of genetic mapping with QTX are expressed as Likelihood Ratio Statistic (LRS) and statistical significance is the probability of a Type I (false positive) error, or the probability of obtaining by chance an LRS value as great as that observed (Haley and Knott, 1992). Marker regression was used to detect potential QTL and these calculations were carried out at increasingly stringent point (χ^2) significance levels, from $p < 0.001$ through $p < 10^{-7}$. During the marker regression operation, QTX calculates a 95% confidence interval (CI) for the LRS peaks, expressed in centiMorgans (cM), and the width of the CIs are inversely proportional to the strength of a QTL (Darvasi and Soller, 1997). To control for the possible influence of other QTL that impact the expression of the transcript abundance phenotype, the marker with the highest LRS value from the initial marker regression was added to the trait background (Zeng, 1993, 1994). The marker regression was then repeated controlling for the effects of the background loci. Simple interval mapping was used to localize QTL detected with the initial marker regression on the relevant chromosomes, independent of the effects of other QTL (Lander and Botstein, 1989; Zeng, 1993, 1994). Critical LRS values for interval mapping, which are the genome-wide significance levels for each target transcript were empirically set with the permutation test run through 10 000 iterations at a 1 cM interval (Churchill and Doerge, 1994; Lander and Kruglyak, 1995) (Table 5). Composite interval mapping was conducted to control for the effects of other QTL, utilizing the background markers identified in the initial marker regression (Zeng, 1993, 1994). Chromosomal locations of

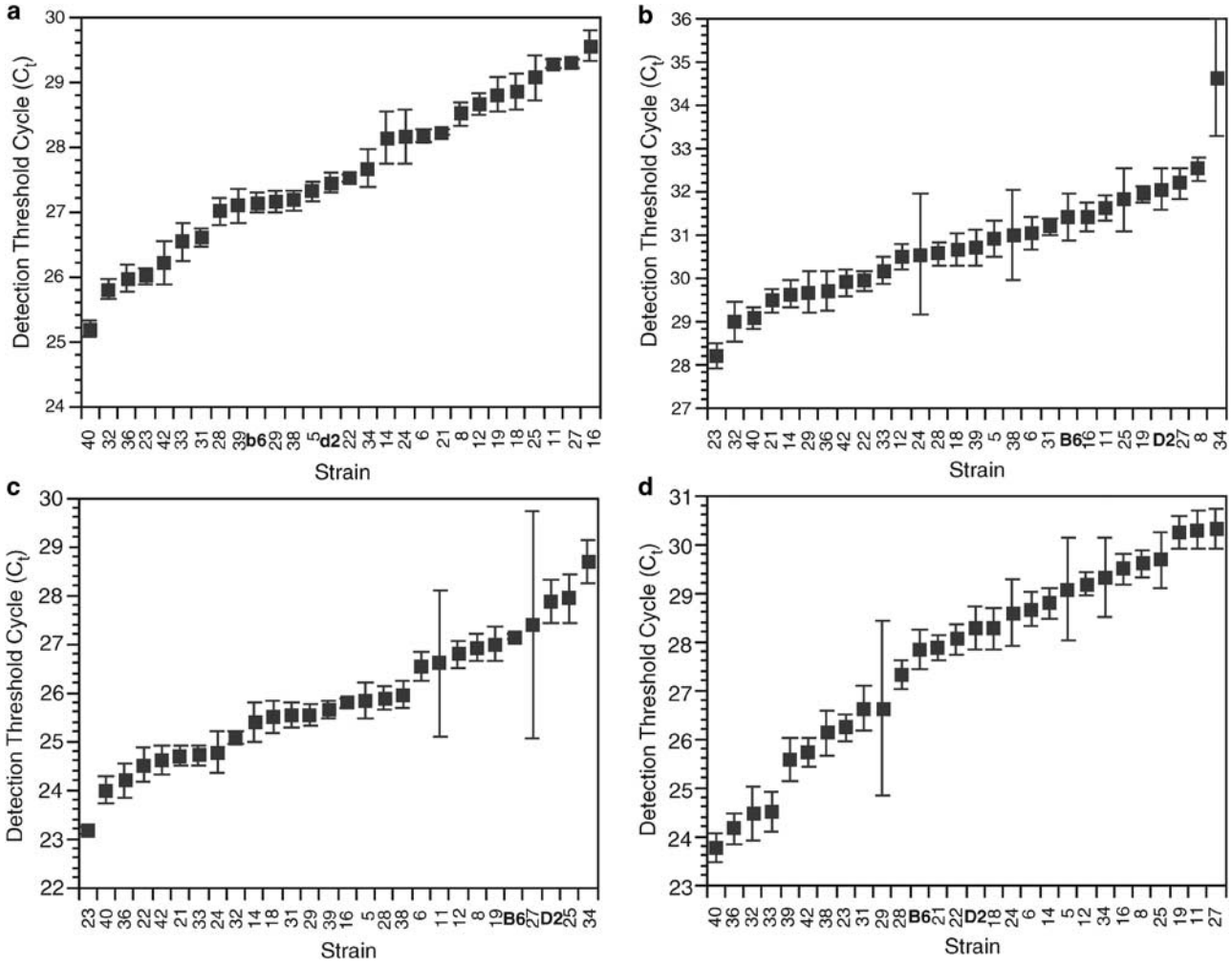

Figure 1 Strain distribution patterns for transcript abundance data for neurotensin system genes across lines of B × D mice and parent strains C57/bl6 (b6) and DBA/2J (d2). Strain mean transcript abundance values, as measured with detection threshold cycle (C_t) are calculated from six individual male animals, with SD; higher C_t values correspond to lower transcript abundances. (a) NT transcript abundance ($F = 171.455$, $df = 27, 139$, $p < 0.0001$), (b) NTR1 transcript abundance ($F = 33.445$, $df = 27, 136$, $p < 0.0001$), (c) NTR2 transcript abundance ($F = 30.786$, $df = 27, 137$, $p < 0.0001$), (d) NTR3 (*Sort1*) transcript abundance ($F = 72.095$, $df = 27, 137$, $p < 0.0001$).

candidate and target structural genes are from the Mouse Genome Database (MGD), Mouse Genome Informatics (v3.1) Website (http://www.informatics.jax.org), at the Jackson Laboratory queried on (1/20/2005) (Blake *et al*, 2003).

This research project has been reviewed and approved by the Emory University Institutional Animal Care and Use Committee (IACUC) and complied with all relevant regulations.

RESULTS

The strain distribution patterns (SDP) for the mean hypothalamic NT system transcript abundances were calculated for 26 B × D and both parent lines. Strain mean transcript abundance for each target, as represented by detection threshold cycle (C_t), differed significantly across the panel of B × D lines (Table 1). The strain mean transcript abundances for each target are distributed continuously, which is consistent with these being quantitative traits (Figure 1). The narrow-sense heritabilities for the transcript abundance phenotypes are virtually identical to the model-corrected R^2 values for effect of strain, and suggest no meaningful epistatic interactions (Belknap *et al*,

Table 1 Statistical Analysis of Neurotensin System Transcript Abundance Data in Strains of B × D Mice

	ANOVA		
Target	**F, (df), *p***	**Adjusted R^2**	**Heritability (h^2)**
NT	171.455, (27, 139), $p < 0.0001$	0.96	0.96
NTR1	33.445, (27, 136), $p < 0.0001$	0.84	0.84
NTR2	30.786, (27, 137), $p < 0.0001$	0.83	0.83
NTR3	72.095, (27, 137), $p < 0.0001$	0.92	0.92

1996). In the SDP, for all target transcripts scrutinized in this study (NT, NTR1, NTR2, and NTR3), the B6 parent strain has a lower C_t (higher transcript abundance) than the D2 line, although the difference between the parent lines was minimal. The SDP for each of the four targets were transgressive, with high- and low-expressing strains bracketing the parent lines. Comparison of the SDP for all four targets reveals a significant correlation in strain mean transcript abundance values between NT and NTR3 and between NTR1 and NTR2 and to a lesser extent between NTR2 and NTR3 (Table 2).

Table 2 Correlation of Strain Mean Transcript Abundance Values for Each Target Across the Panel of B × D Mice, by Pearson's Product–Moment (*r*) Statistic

	NT	NTR1	NTR2	NTR3
NT	1.0			
NTR1	0.57 ($p<0.002$)	1.0		
NTR2	0.65 ($p<0.0003$)	0.89 ($p<0.0000$)	1.0	
NTR3	0.91 ($p<0.0000$)	0.645 ($p<0.0004$)	0.70 ($p<0.0001$)	1.0

Marker regression of strain mean transcript abundance was carried out for each target at increasing point significance thresholds of $p<0.001$ through $p<10^{-7}$ (Table 3). Potentially significant associations were detected for NTta on chromosome 1 at $p<10^{-5}$, and for NTR2ta on chromosome 12 at $p<10^{-7}$ and suggestive associations for NTR1ta and NTR3ta were detected at the $p<0.001$ level. Additional associations at the 10^{-6} level were detected for NTR2ta on chromosome 2, 4, 9, 10, 13, and 17. The markers in bold type in Table 3 (*D12Mit63*) and (*D12Mit222*) were added to the background for NT and NTR2, respectively. Marker regression for NTta and NTR2ta was repeated while controlling for the respective background markers (Table 4).

Table 3 Results of Marker Regression Analysis of Transcript Abundance Data for Neurotensin (NT), Neurotensin Receptor 1 (NTR1), 2 (NTR2), and 3 (NTR3)

Target transcript	Chromosome	Locus	Location (cM)	LRS	Point (χ^2) significance	95% CI (cM)	Additive regression coefficient
NT	1	*D1Mit372*	13	18.5	0.001	24	0.91
	1	***D12Mit63***	**14**[a]	**21.8**	$\mathbf{10^{-5}}$	**18**	**0.76**
	1	*D1Mit169*	15	20.6	10^{-5}	20	0.75
	1	*D1Mit211*	15	20.6	10^{-5}	20	0.75
	1	*D1Mit49*	54.5	11.8	0.001	79	−0.62
	1	*D1Mit84*	58.4	12.3	0.001	67	−0.61
	3	*D3Mit63*	22	11.4	0.001	93	0.59
	3	*D3Mit306*	22	12.7	0.001	58	0.62
	3	*D3Mit5*	25	12.7	0.001	58	0.62
	6	*D6Mit183*	26.5	12.8	0.001	58	0.72
	6	*D6Mit175*	29.88	10.8	0.001	127	0.69
	6	*D6Mit16*	30.5	11	0.001	113	0.70
	7	*S07Gnf001.580*	1[a]	11.8	0.001	80	−0.94
	8	*D8Mit360*	59	12.8	0.001	58	0.89
NTR1	3	*D3Mit221*	2.4	11.8	0.001	78	0.66
	8	*D8Mit45*	40	12.7	0.001	59	0.69
	12	*D12Mit242*	13	11.8	0.001	80	−0.66
	12	*D12Mit136*	13	11	0.001	117	−0.65
	X	*SXGnf15.425*	7[a]	14	0.001	43	0.73
	X	*SXGnf23.015*	11[a]	14	0.001	43	0.74
	X	*D0Xmsw030*	15[a]	11	0.001	113	0.65
NTR2	**12**	***D12Mit222***	**18**	**29.9**	$\mathbf{10^{-7}}$	**13**	**−1.25**
NTR3	1	*D1Mit372*	13	11.3	0.001	100	1.18
	1	*D12Mit63*	14[a]	11.4	0.001	94	1.14
	7	*S07Gnf001.580*	1[a]	12.5	0.001	62	−1.30
	11	*D11Mit179*	52	11.9	0.001	77	1.16
	12	*D12Mit235*	19	11.2	0.001	106	−1.14

[a]Approximate location.

Table 4 Results of Marker Regression Analysis of Transcript Abundance Data for Neurotensin (NT) and Neurotensin Receptor 2 (NTR2) Controlling for Respective Background Marker (*D12Mit63*) for NT and (*D12Mit222*) for NTR2

Target transcript	Chromosome	Locus	Location (cM)	LRS	Point (χ^2) significance	Additive regression coefficient
NT	9	*D9Mit191*	26	24.6	10^{-6}	−0.57
	9	*D9Mit171*	28	24.6	10^{-6}	−0.57
	9	*D9Mit4*	29	24.6	10^{-6}	−0.57
	9	*D9Mit104*	35	24.6	10^{-6}	−0.57
NTR2	2	*D2Mit56*	41	16.7	0.0001	−0.66

In the case of NTta, the second round of marker regression returned a significant association on chromosome 9 that had not been detected in the original analysis. The second round of marker regression for NTR2ta returned a significant association on chromosome 2 in the same location as detected in the original analysis and also detected the associations identified in the original analysis (chromosome 4, 9, 10, 13, and 17) at suggestive-level ($p < 0.001$) point significance levels (data no shown).

Simple interval mapping was used to localize the linkage signals detected in the initial marker regression (Table 3) onto the respective chromosomes (Figure 2). The critical LRS values for each transcript abundance dataset were determined with the permutation test (Table 5). Simple interval mapping of NTta revealed a significant LRS peak on proximal chromosome 1 coincident with a suggestive level peak for NTR3ta (Figure 2a). There is also a suggestive LRS peak on chromosome 7 for NTta completely coincident with a suggestive NTR3ta peak (Figure 2c). There are suggestive level LRS peaks for NTta on chromosome 3 (Figure 2b) and 8 (Figure 2d) in proximity to, but not coincident with peaks for NTR1ta. There is a very strong LRS peak on proximal chromosome 12 for NTR2ta and there are suggestive level peaks for both NTR1 and NTR3 also at that location (Figure 2e). There is a LRS peak for NTR1ta on the X chromosome that is just at the edge of significance (Figure 2f). There is also a suggestive level peak for NTta on chromosome 6 and significant level peaks for NTR2ta on chromosomes 2, 4, 9, 10, 13, and 17 (data not shown).

Composite interval mapping was conducted to localize the associations detected for NTta and NTR2ta in the second round of marker regression (Table 4). The marker *D12Mit63* was added to the NT background and composite interval mapping conducted on chromosome 9 (Figure 3a) and two significant LRS peaks were detected. The marker *D12Mit222* was used as background control for NTR2 and composite interval mapping on chromosome 2 revealed a significant level LRS peak in the same location as detected with the simple interval mapping (Figure 3b). All of the other LRS peaks detected for NTR2ta (chromosome 4, 9, 10, 12, 13, and 17) with simple interval mapping were detected in the composite interval analysis at suggestive ($p < 0.63$) levels. The additional LRS peaks on chromosome 12 resulting from simple interval mapping were not detected while using *D12Mit222* as background control, indicating these most likely are artifacts of the strong *cis* acting QTL near the *Ntsr2* structural gene.

The QTL detected in this analysis that impact NT, NTR1, and NTR3 transcript abundance do not appear to be linked to the respective structural genes. The *Nts* structural gene is on chromosome 10 (55 cM), *Ntsr1* on distal chromosome 2 (107 cM), and *Sort1* on chromosome 3 (51 cM). The QTL that impacts NTR2ta on proximal chromosome 12 contains the *Ntsr2* structural gene (6 cM), though the marker with the strongest association, *D12Mit222* is slightly more distal at 18 cM. The hypothalamus is a sexually dimorphic organ, and all of the included analyses were performed in males, thus the influence of gender on these QTL cannot be ascertained until the experiment is repeated in females. This is particularly critical for the NT system, as this system is known to be responsive to estrogen and to change predictably across the estrous cycle (Watters and Dorsa, 1998; Kinkead *et al*, 2000).

DISCUSSION

The distributions of strain mean transcript abundance values for each of the four targets, NT, NTR1, NTR2, and NTR3 (*Sort1*) were continuous across the panel of B × D strains, consistent with these phenotypes being quantitative traits. The SDP were transgressive with extreme high- and low-expressing strains flanking the values of the parent lines. At least one suggestive linkage association was detected with both marker regression and simple interval mapping for all four targets and significant associations were detected for NT and NTR2 transcript abundance. The LRS peaks detected with simple interval mapping for NTta and NTR2ta were well above the significance levels set by the permutation test, so at least one QTL-controlling transcript abundance can be declared for both of these target genes and there were others for each target in the suggestive range. Composite interval mapping revealed two additional significant QTL for NT transcript abundance on chromosome 9 and another QTL for NTR2ta on chromosome 2.

NT system transcript abundances were highly heritable quantitative traits. The phenotype quantified in this investigation, transcript abundance in total RNA is a measure of the basal level of regulation of the NT system, as these animals were not subject to any manipulations or challenges. This suggests a very strong genetic influence on establishing the basal levels of expression in this system. These heritabilities are also a reflection of the controlled

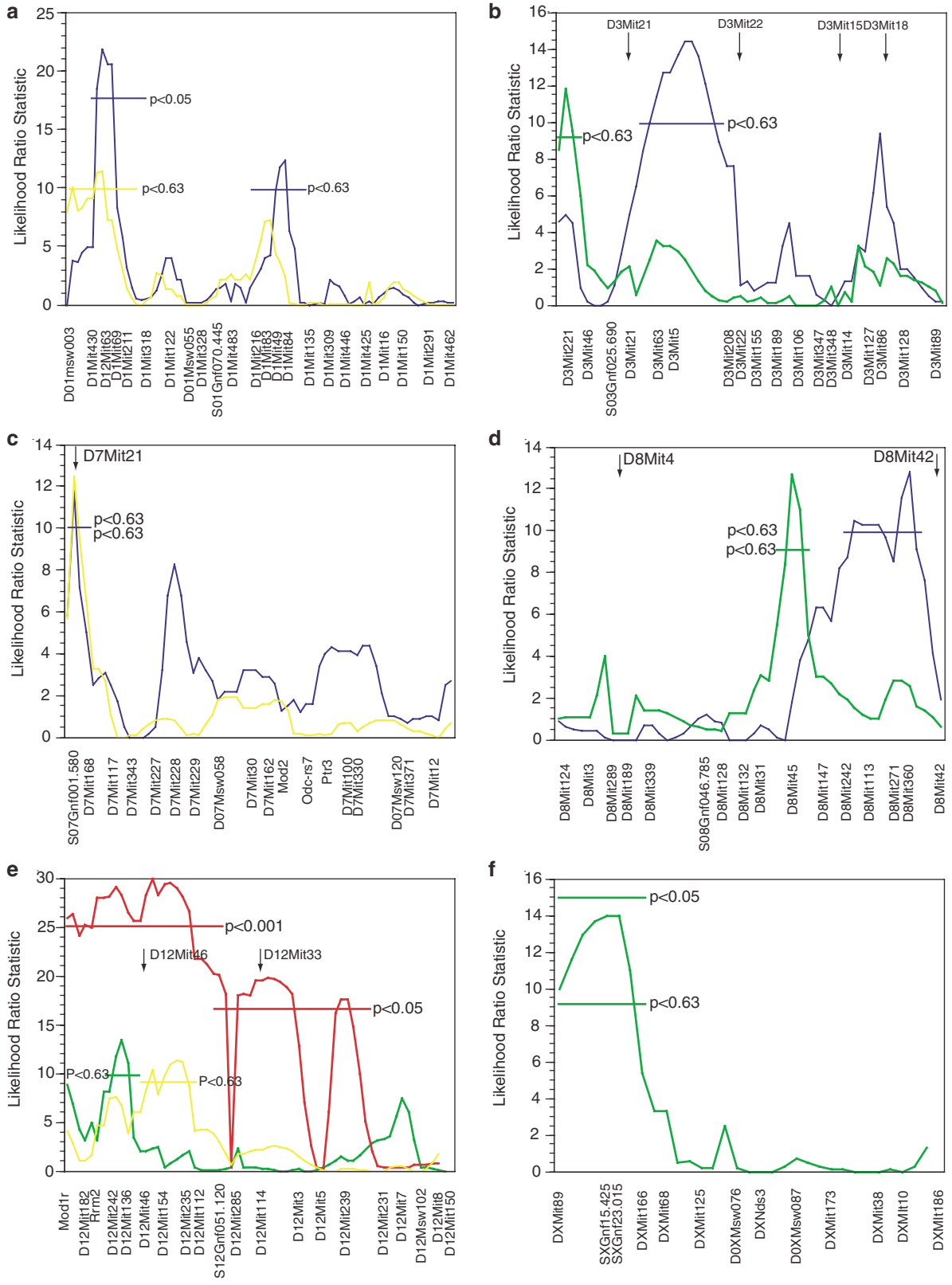

Figure 2 Results of simple interval mapping data of NT system transcript abundance data on six mouse chromosomes. Critical likelihood ratio statistic (LRS) values for mapping were determined with permutation test run through 10 000 iterations for each target (Table 5); NT (blue), NTR1 (green), NTR2 (red), NTR3 (yellow); downward pointing arrows mark locations and identities of genetic markers previously associated with NT system QTL (Erwin *et al*, 1997). (a) chromosome 1, (b) chromosome 3, (c) chromosome 7, (d) chromosome 8, (e) chromosome 12, and (f) X chromosome.

Table 5 Critical Likelihood Ratio Statistic (LRS) Values for Interval Mapping Determined by Permutation Test Run Through 10 000 Iterations for Each Set of Target Data

Target	Suggestive LRS ($p<0.63$)	Significant LRS ($p<0.05$)	Highly significant LRS ($p<0.001$)
NT	9.9	17.5	28
NTR1	9.1	15	20.8
NTR2	9.7	16.7	25.2
NTR3	9.9	17.7	28.9

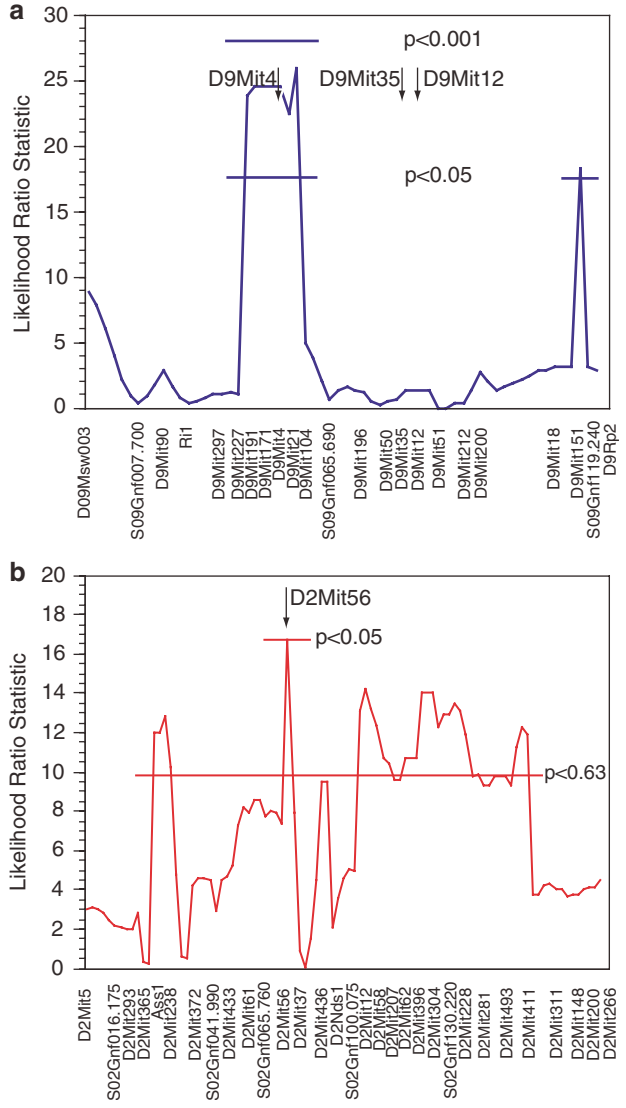

Figure 3 Results of composite interval mapping data of NT and NTR2 transcript abundance data on two mouse chromosomes; NT (blue) and NTR2 (red); downward pointing arrows mark locations and identities of genetic markers previously associated with NT system QTL (Erwin *et al*, 1997). (a) chromosome 9, utilizing marker *D12Mit63* as background control, (b) chromosome 2, utilizing marker *D12Mit222* as background control.

and stable environment in which the animals were produced and that all included animals were reared and killed during the same 3-month interval. This also minimized the potential for seasonal influences of NT expression (Bissette *et al*, 1995).

There are QTL that apparently control expression of only one target and others that potentially impact expression of more than one target gene. Inspection of the QTL detection (Table 3) and localization (Figure 2) results suggests that expression of NT and NTR3 are tightly connected. The SDP for these two transcripts were highly correlated (Table 2) and the LRS peaks on chromosome 1 and chromosome 7 are essentially completely coincident with each other. There are other QTL that control NTta separate from NTR3 and NTR3 distinct from NT, so this shared genetic control is not absolute. All three NT receptors appear to share elements of genetic regulation. The SDP for NTR1 and NTR2 transcript abundance are highly correlated (Table 2) and there is a cluster of QTL on proximal chromosome 12 that regulate all three transcripts. There are QTL for NT and NTR1 transcript abundance on both chromosomes 3 and 8 although these peaks do not appear to overlap and the strain distributions are only modestly correlated, so the existence of shared genetic regulation of these two targets is less certain.

Previously, the NT system was subject to QTL analysis, utilizing the LS × SS panel of RI mice with peptide immunoreactive material (NT-IR) and NTR1 and NTR2 abundance by receptor B_{max} value as the quantitative traits (Erwin *et al*, 1997). Some of the QTL identified in the previous investigation appear to coincide with those detected in the present analysis of transcript abundance. The locations and identity of these coincidental associations are marked in Figures 2 and 3 with downward pointing arrows. The markers *D3Mit21*, *D3Mit22*, and *D3Mit18* on chromosome 3 were associated with QTL controlling NT-IR and *D3Mit21*, *D3Mit22*, and *D3Mit15* were associated with QTL impacting NTR1 B_{max}. These associations are close to QTL for NT and NTR1 transcript abundance on chromosome 3 (Figure 2b). The marker *D7Mit21* was associated with a QTL that controls NT-IR and is centered directly over a QTL that appears to regulate NT and NTR3 transcript abundance (Figure 2c). Two markers on chromosome 8, *D8Mit4*, and *D8Mit42* were associated with NT-IR, but only *D8Mit42* could be related to NT transcript abundance (Figure 2d). *D8Mit4* and a more proximal marker *D8Mit18* (not shown) were associated with NTR1 B_{max}, but appear quite distant from the chromosome 8 QTL-controlling NTR1ta. Two marker associations for NT-IR were reported on chromosome 9 at *D9Mit35* and *D9Mit12* that do not appear to coincide with the QTL detected with composite interval mapping of NTta on this chromosome (Figure 3a). Interestingly, the marker *D9Mit4* at 29 cM, which is within the strong NTta QTL on chromosome 9, was reported to be associated with QTL controlling both NTR1 and NTR2 B_{max} values. The markers *D12Mit46* and *D12Mit33* were associated with QTL-controlling NTR2 B_{max} values and are contained within the strong chromosome 12 QTL that impacts NTR2 transcript abundance (Figure 2e). The LRS peak for the NTR2 QTL on chromosome 2 revealed in the composite interval mapping is at *D2Mit56*, which was also detected with NTR2 B_{max} value (Figure 3b).

The results of the past and present investigations tend to be concordant in that QTL were detected with both measurements at some of the same locations in the genome.

However, genetic analysis of both types of trait, protein abundance and transcript abundance, also revealed QTL apparently unique to that particular phenotype. Thus there are genes that regulate transcript abundance; genes that regulate protein abundance and some that impact both phenotypes simultaneously. The QTL that were detected with both protein and transcript abundance are possibly genes that globally regulate the growth and differentiation of the NT system, while those detected at only one phenotype level may control biological processes more closely linked to that level of gene expression.

The NT system is involved in a number of processes including sensorimotor gating, nociception, and in the pharmacological responses to both therapeutic agents and drugs of abuse, and therefore QTL that impact these types of phenotypes could be detecting the same underlying genes as the QTL controlling the NT system. Four QTL impacting responses to intoxicating substances, *Alcp4, Alcp25, Morph1*, and *Cocia4* reside on proximal chromosome 1 within the NTta QTL located there (Whatley *et al*, 1999; Bergeson *et al*, 2001; Gill and Boyle, 2003). The *Morph1* QTL is related to morphine-induced antinociception, the *Alcp* QTL are involved in gender-specific alcohol preference and *Cocia4* impacts psychomotor stimulant effects of cocaine. The NT system has been implicated in both nociception and response to alcohol and stimulants; either of these phenotypes could be detecting the same QTL as was detected with NTta. There are other interesting QTL in this region that could be NT system related including genes that control body weight (adiposity 1, *Adip1*, obesity 2, *Obq2*) and circadian activity patterns (circadian period of locomotor activity 4, *Cplaq4*). The NTta and NTR3ta QTL on proximal chromosome 7 are of particular interest as this is one of the loci that coincide between the present study and the previous investigation of NT-IR. The protein kinase C gamma (*Prkcc*) subunit resides at 2 cM of chromosome 7 and this gene product has been implicated in various neurobehavioral processes including anxiety behaviors, spatial and contextual learning, ethanol consumption and impulsive behaviors (Abeliovich *et al*, 1993a, b; Bowers *et al*, 2000; Bowers and Wehner, 2001). There are a number of intriguing genes within the NTta LRS peak on chromosome 9, between 25 and 35 cM including the dopamine 2 receptor structural gene (*Drd2*) at 28 cM (O'Dowd *et al*, 1990), hypothermia sensitivity (*Hts*) which is in response to D2-selective agent quinpirole (Buck *et al*, 2000), cocaine-related behavior 8 (*Cocrb8*) at 28 cM, ethanol consumption 3 (*Etohc3*) at 29 cM (Phillips *et al*, 1998; Jones *et al*, 1999), and haloperidol-induced catalepsy 1 (*Hpic1*) at 35 cM (Patel and Hitzemann, 1999). There are also QTL in this region that impact body weight/composition (abdominal fat weight 4, *Afw4*, body weight *Bwnd2wk6*, *Bwnd6wk4*, *Bwnd5wk5*) and activity patterns (activity–distance traveled 4, *Actd4*). Remarkably, a QTL impacting NTR1, and NTR2 B_{max} values was mapped at 29 cM of chromosome 9, within the same region covered by the NTta LRS peak. The QTL on chromosome 2 for NTR2ta and NTR2 B_{max} at *D2Mit56* contains the QTL nitrous oxide antinociception 1 (*Noan1*) that impacts nociceptive responses in the presence of nitrous oxide, again pointing to a potential relationship of the genetic regulation of the NT system to that of nocioceptive pathways (Mueller *et al*, 2004).

There are several limitations to this study. The first is that it was conducted in one set of segregating animals, that being the B × D RI set, and therefore the linkage findings need to be confirmed in a separate, independent analysis. These experiments are ongoing, but the statistical significance of some of the associations, in particular for NTta on chromosomes 1 and 9 and for NTR2ta on chromosomes 2 and 12 argues against these being false-positive findings. That different targets had unique and shared regions of linkage argues in favor of these QTL being elements of the genetic system that controls the hypothalamic NT system. That the QTL detected with the transcript abundance phenotype corresponded to those previously described for NT system proteins further bolsters the strength of the present findings. Another limitation is that all of the animals in this experiment were male, so no conclusions can be drawn as to the genetic regulation of the NT system in females. The resolution of the mapping algorithms employed, and the density of recombinations in the strain set are not sufficient to engage in fine mapping, so linkages in smaller candidate intervals cannot be detected, thus limiting the resolution of the mapping results.

In conclusion, we present evidence that the hypothalamic NT system is controlled by a series of QTL, some of which are specific to only one target gene and some may exert a more global influence on multiple genes in the NT system and may impact both transcript and protein abundances. Transcript abundances of NT, NTR1, NTR2, and NTR3 (*Sort1*) in total hypothalamic RNA are quantitative traits and we have detected at least one QTL that controls the abundance of each of these transcripts in total hypothalamic RNA.

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