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Review

Analysis of the Mesotelencephalic Dopamine System by Quantitative-Trait Locus Introgression

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One of the significant factors that affect brain dopamine function is the activity of tyrosine hydroxylase (TH), the first and rate-limiting enzyme in catecholamine biosynthesis. For the analysis of the genetically determined role of dopamine function and TH in behavior and in the regulatory mechanisms of the mesotelencephalic dopamine system we devised a novel genetic strategy (Vadasz; Mouse Genome 88:16–18; 1990). We hypothesized that phenotypic introgression and recombinant fixation could ensure the transfer of Quantitative Trait Loci (QTL) from one strain onto the genetic background of another strain, and new, genetically very similar quasi-congenic strains could be created that would carry individual QTLs, or QTLs in various combinations. Here we summarize the construction of the first set of QTL Introgression strains, and present evidence that QTLs that are responsible for the continuous variation of mesencephalic tyrosine hydroxylase activity (TH/MES), have been transferred onto the C57BL/6By (B6) strain background from BALB/cJ (C) and CXBI (I) donor strains with high and low TH/MES, respectively. The QTL transfer was carried out in two directions by repeated backcross-intercross cycles with concomitant selection for the extreme high and low expressions of TH/MES in replicates, resulting in four QTL Introgression lines. Analysis of regional brain TH activities in the course of the QTL introgression indicated that (a) TH activity in B6.I lines showed quite limited heritability, (b) TH/MES was not highly correlated with striatal TH, and (c) the control of hypothalamic and olfactory tubercle TH activities was largely independent from that of TH/MES. Examination of the open-field (OF) behavior data demonstrated that TH activity did not correlate significantly with OF behavior. After 5 backcross-intercross cycles, TH/MES in each replicate line was still significantly different from that of the B6 background strain. A genomewide scanning of microsatellite markers in the QTL introgression lines demonstrated that about 96% of the markers were of background (B6) type. These results indicate the successful transfer of TH/MES QTLs. After the QTL transfer phase of the experiment altogether more than 100 new RQI strains were initiated in the QTL Introgression lines by strict brother × sister mating. After fixing the introgressed QTLs, ten of the inbred RQI strains were tested for TH/MES. The results showed that in one of the new RQI strains TH/MES was restored to a level that is characteristic to the C donor strain, while TH/MES values in some other strains were between those of the background and donor strains, confirming our hypothesis that phenotypic introgression and recombinant fixation can ensure a virtually complete transfer of QTLs. We con-

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tercross (b_1i_1), then by the second backcross (b_2i_1), etc. The last selection generation was b_5i_7 which reflected five backcross-intercross cycles. Offspring of b_5i_7 were designated $b_5i_7F_1$. F_n represents the number of consecutive brother x sister matings after the last backcross-intercross cycle. RQI strains are considered quasi-congenic. Accordingly, the name of an RQI strain begins with the abbreviation for the background strain followed by a period and the abbreviation for the donor strain, e.g., B6.C. This is followed by the total number of backcrosses and intercrosses experienced from F_2 through the last backcross or intercross with concomitant selection for the differential trait, and by the designation of the replicate line of origin, e.g., B6.Cb_5i_7-α. From the b_5i_7 generation B6.Cb_5i_7-α, B6.Cb_5i_7-β, B6.Ib_5i_7-α and B6.Ib_5i_7-β sets of RQI lines were derived. Finally, in each set a number from 1 through 34 was assigned to each new strain, eg., B6.Cb_5i_7-α15. Occasionally, substrains were derived (before reaching F_{16}) by attaching a upper case letter to the strain number, e.g., B6.Cb_5i_7-α15B. In addition to the b_5i_7 series, smaller replicate series, designated B6.Cb_4i_5-α and B6.Cb_4i_5-β, were derived from a cross between the b_3i_5 generation and the B6 background strain, designated $b_4i_5F_1$. Numbers from 1 through 14 were assigned to each new strain.

Development of QTL-Introgression (QI) Lines. Previous research demonstrated that among several highly inbred strains C and I had the highest and lowest TH/MES (50). To develop QI lines, we decided to use C and I as donor strains. B6 served as the background strain, because its TH/MES was intermediate between those of the donor strains, and it had already been used as a background strain for numerous congenic lines. Then, F_2 generations were derived by mating B6 females to C or to I males. α and β closed replicate lines were created by equal division of each $(B6XC)F_2$ and $(B6XI)F_2$ litter resulting in four lines: B6.C-α, B6.C-β, B6.I-α, and B6.I-β.

Brain Dissection. After the removal of the hypothalamus using the ventral approach, the mesencephalon (MES) was dissected en bloc (on ice). MES designates a mesencephalic tissue block that included all the dopaminergic cell body areas: substantia nigra, the rubral area, and the ventral tegmentum (Fig. 2). This method of dissection was used by Ross et al. (40) and Baker et al. (2) who argued that strain differences in midbrain TH activity (defined as TH activity per structure) are attributable to differences in the number of TH-positive midbrain dopaminergic neurons. For QTL introgression purposes the three mesencephalic areas were not separated, because we felt that (1) time-consuming microscopic dissection would have rendered this large-scale project intractable, and (2) if subregion-specific QTLs were operative, we could ensure the transfer of all of the QTLs, while subsequent recombination would allow us to genetically dissect TH/MES. In the first few generations tissue samples were taken from the dorsomedical caudate putamen (CS), hypothalamus (HT, excluding the preoptic area), or the olfactory tubercle (OT) to estimate correlation between regional TH activities.

Tyrosine Hydroxylase Assay. Tissue samples were sonicated, and TH activity was determined by Coyle's (8) modified (24) radioenzyme assay. Activity was expressed as nmol of [^{14}C]DOPA formed per MES per hour, or as nmol of [^{14}C]DOPA formed per mg protein per hour for striatal, hypothalamic, and olfactory tubercle samples. Dissection of MES and assaying of TH/MES were randomized to avoid line-specific bias, which could have been introduced by slight variations in dissection pattern, time of day, stress caused by cage transport, etc. Intra- and inter-assay variations were controlled by two kinds of reference samples: (1) large pools of B6 striata were homogenized and used to prepare aliquots stored in liquid nitrogen for use as reference samples, (2) mesencephalic blocks of B6 males were dissected similarly to those of test animals (B6-TH/MES). In each batch of assays, either one or both of the above references and test samples for selection were measured in duplicate or triplicate. In the course of the 13 generations of selection, B6-TH/MES references were used in F_2, b_1i_0 and b_1i_1 generations; striatal references were used in b_2i_1, b_2i_2, and b_2i_3 generations, and both kinds of references were used in all the remaining generations. Intra-assay variation was assessed by striatal reference samples assayed at the beginning and at the end of a batch. To correct assay sensitivity variations, striatal reference values were used to calculate batch correction factors. Then, corrected TH/MES values were used to calculate the ratio of test-sample TH/MES to reference B6-TH/MES. To deal with data in a familiar range, the ratio was multiplied by the constant 3.00 [i.e., final TH/MES = (3 × corrected TH/MES)/(B6-TH/MES)]. Thus, by definition, TH/MES in B6 was 3.00 nmol DOPA per MES per hr. In all generations, except for the first few crosses, TH/MES was assayed in triplicate. First, parallel assay tubes were arranged in duplicate, followed by a set of the third parallels in the same order. Differences in rank order and activity were assessed, providing an additional control of intra-assay variation.

DNA Marker Analysis. To genotype strains and congenic progeny for SSR (microsatellite) polymorphisms, PCR reactions were carried out with radioactively labeled primers and products were analyzed by polyacrylamide gel electrophoresis (56). One primer pair was selected for each of the 19 autosomal chromosomes and for the X chromosome from a murine genetic map (11). Primer pairs (MapPair™) were supplied by Research Genetics (Huntsville, AL). Preference was given to primer pairs with maximum difference in product size between donor strain C and background strain B6. DNA was prepared from spleens of male mice by standard phenol-chloroform extraction. PCR was performed with 2.0 μl DNA template (50 ng/μl) in a reaction volume of 25 μl that contained 1.5 μl kinased forward primer (6.6 μM), 0.5 μl reverse primer (6.6 μM), 8.5 μl H_2O, and 12.5 μl 2× kinased primer MAP PCR Master mix (final concentrations were: dNTPs 200 μM each; $MgCl_2$ 1.5 mM; AmpliTaq, Perkin Elmer Cetus, 0.625U/reaction). Primers were end-labeled with [γ-^{32}P] ATP (ICN, 3000 Ci/mM) using T4 polynucleotide kinase according to standard protocols. Reactions were amplified on Perkin-Elmer 9600 Thermocycler. Initial denaturation at 94°C for 3 minutes was followed by 25 cycles of 94°C for 15 seconds, 55°C for 2 minutes, and 72°C for 2 minutes. Finally, amplification was followed by a single cycle of 72°C for 7 minutes. PCR products were electrophoresed on 5% denaturing polyacrylamide gels (Long Ranger, AT Biochem) at 60 watts constant power. Gels were transferred to Whatman 3MM paper, dried, and exposed to film overnight. Exposure time was adjusted depending on results. Autoradiographs were independently evaluated twice.

Recording of Behavioral Events. Placing an animal from its home cage into a novel, illuminated, enclosed open area (''open-field'') represents a mildly stressful situation. We chose the open-field test because (1) in this simple situation a wide variety of activities can easily be observed, providing a general profile of the animal's behavior, and (2) significant genetic differences have previously been reported between the donor C and the background B6 strains (53,54). Such a profile represents emotionality, stress-responsiveness, spontaneous motor activity, exploration, fear, territorial marking, and habituation to the test apparatus. Because of the relative unspecificity of this test, we felt that the probability of detecting an association between TH/MES and behavioral variables is higher than when using a highly specific behavioral test. In the course of production and testing of the 13 segregating generations of the QTL Introgression lines five observers successively collected the data, except from b_2i_3 to b_3i_5, in which period two observers worked parallel. While inter-observer reliability was good at most of the times tested, we are aware of the fact that it is very difficult to keep behavioral data collection consistent in multi-year studies and occasional inter-observer discrepancies may occur.

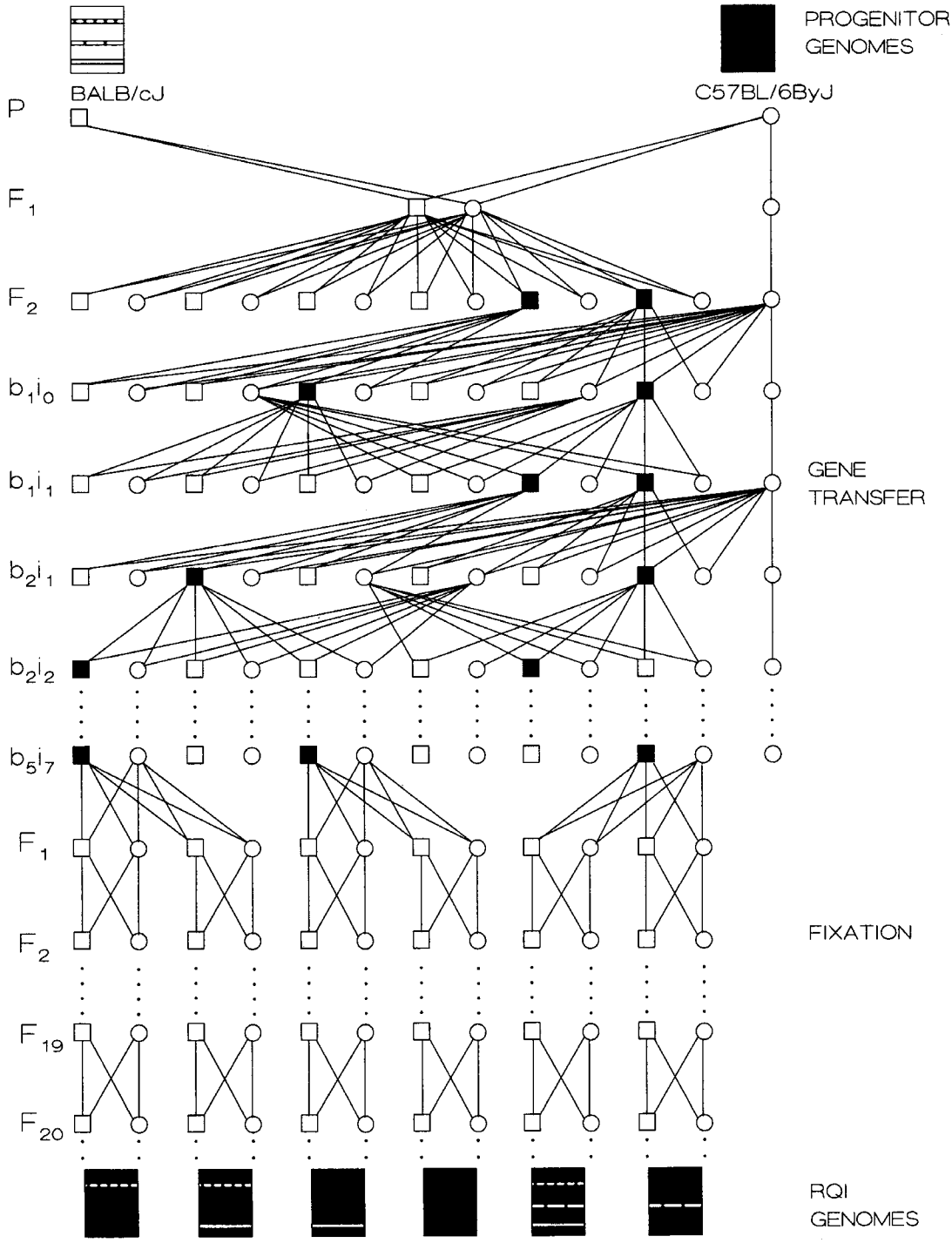

Fig. 1. The principle of the RQI strategy. Genes responsible for the strain difference in TH/MES were transferred from the partner genome to the background genome by artificial selection for high and low TH/MES with concomitant backcrosses to the background strain. (A similar procedure was applied to develop the B6.I lines, not shown.) Solid lines represent gametic contributions, squares = males, circles = females, fully filled squares = selected males). Two replicate lines were created from the high (B6.C-α and B6.C-β) and two from the low (B6.I-α and B6.I-β) preparation. The first selection was carried out in the F_2 generation and it was followed by a backcross to B6, while the last selection was done in the b_5i_7 generation. Then, using the offspring of the b_5i_7 generation, for each replicate 34 new RQI lines were established from each of the four replicates by strict brother x sister matings to drive the heterozygous genes into a homozygous condition (fixation). A similar procedure was followed to construct the b_4i_5 series using the offspring of $B6.Cb_3i_5$ males and B6 females to set up 14 new RQI lines in each replicate.

During the years, the definition of some of the behavioral categories was refined and subcategories were created; however, to make all the generations accessible for statistical analysis, we selected a standard set of behaviors that had been measured in every generations. For the first few generations behavioral check-lists were used, frequency of occurrence of each defined behavior was recorded per minute, for ten

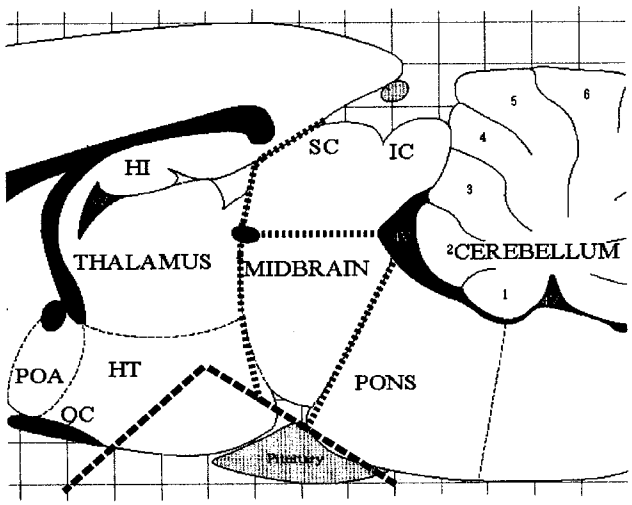

Fig. 2. After removing a part of the hypothalamus, the cerebellum, and the pons, a mesencephalic bloc of tissue that comprises the A8-A9-A10 dopaminergic cell groups was dissected using the following boundaries: caudal, the posterior edge of the inferior colliculus; rostral, the anterior edge of superior colliculus; dorsal, the cerebral aqueduct.

minutes, in a brightly lit open-field arena (51,53,54). Starting with the b_2i_2 generation, we used a computer program (ETHOGRAM, developed in our laboratory with Drs Kai Lee and Gyorgy Kobor) and a computer keyboard to record behavioral events. This recording technique allowed sophisticated analyses of sequence, latency, etc.; however, to make the data comparable across generations we elected to use only the relatively simple frequency of occurrence of a behavior, distance covered, time spent moving, and agoraphilia scores summed for the total duration of the test (10 minutes). The last three variables were collected by the same automatic video-image analyzer system for all of the 13 selection generations (VIDEOMEX, Columbus Instruments, Columbus, Ohio) and were integrated with the ETHOGRAM data by another computer program (WORKETH, developed in our laboratory with Dr. Balint Juhasz). Definitions of behaviors expressed in a 10-minute open-field test are the following (for more details see (53)): USM, release of urine droplets (frequency); ULG, release of a pool of urine (frequency); FAEC number of feces boli deposited; OHS, wiping movement on the head by one or both forelimbs (action separated from other grooming activities by at least 1 second) (frequency); PFTC, forelimb movements about chest hight or just below the mouth, may include tremor and hand shake without touching of the face (action is separated from other grooming activities by at least 1 second) (frequency); RFG, various grooming activities (licking of forepaws, strokes, head rubbing) in a repetitive manner (frequency), REAR, forepaws off the ground, back straight, action lasting longer than 1 second (frequency); LEAN, two forepaws touch the wall of the open-field apparatus (frequency); DIST, distance traveled (meters); MOVE, time spent moving (seconds); APH, preference of central area of the open-field (agoraphilia, cumulative score).

Statistical Analysis. Data were analyzed using SPSS 7.5 for Windows. Outliers and extremes of TH/MES data were identified by the Box plot option. Data were not transformed.

RESULTS

Construction of Replicated QTL Introgression Lines, and Differences in TH/MES. We estimated that the probability of retaining a nonselected, nonlinked donor gene in a QI line after four or five backcross-intercross cycles is about 6%, or 3%, respectively (assuming one QTL, and a probability of crossovers between passenger and the differential gene of c = 0.5; (19)). A comparison of the population means (±SE) for TH/MES in the b_5i_7 QI lines (B6.C-α: 3.49 ± 0.05; B6.C-β: 3.38 ± 0.05; B6.I-α: 2.81 ± 0.03; and B6.I-β: 2.82 ± 0.02) and in the B6 background strain (3.00 ± 0.03) indicated (1) that there were no significant differences between the replicates, but the QI lines selected for high or low TH/MES were significantly different from the B6 background strain (one-way ANOVA followed by Tukey's post hoc multiple comparison tests: B6.C-α, B6.C-β > B6 > B6.I-α, B6.I-β; HSD alpha = 0.05, df = 272; the value of the minimum significant difference was 0.157), and (2) that the original deviation of the F_2 populations from the B6 background strain was reduced by 25% in the B6.C lines, and by 57% in the B6.I lines (56). In the course of the gene transfer, coefficients of variation (CV) of TH/MES were higher in the B6.C than in the B6.I populations ($F_{[1.43]} = 10.81$; $p < 0.005$).

Mean mesencephalic TH activity levels with their strict 95% confidence intervals (2XSE) in four QI lines are shown in Figure 3. The differences in mean TH/MES originally observed between the background strain and foundation F_2 generations were somewhat reduced after the first (b_1i_0) and second (b_2i_1) backcrosses. Consequently, to increase the identifiable variation and decrease the probability of losing differential genes, backcrossing (b_2i_1 and b_3i_3) was followed by successive intercrosses (b_2i_2, b_2i_3 and b_3i_4, b_3i_5, respectively). Intercrosses were always carried out between selected males and non-littermate females within the same QI.

TH Activities in the Mesencephalon, Striatum, Olfactory Tubercle, and Hypothalamus in Segregating Generations. In the F_2 and b_1i_0 generations we collected data on TH/MES, TH/CS, and TH/HT. Multivariate and univariate analysis of variance of the pooled generations (N = 121 for B6.C, and N = 162 for B6.I) demonstrated that B6.C mice (with C-type donor genes and selection for high TH/MES) had significantly higher ($p < 0.001$) TH/CS (8.12 ± SD = 1.29 vs. 6.79 ± SD = 1.25), TH/HT (1.04 ± SD = 0.17 vs. 0.77 ± SD = 0.12), and TH/MES (3.41 ± SD = 0.46 vs. 2.78 ± SD = 0.33) when compared to B6.I mice, which carried I-type donor genes and were subjected to selection for low TH/MES. For TH/CS and TH/HT the differences were largest in the F_2 generation.

In the b_1i_1 generation TH/MES, TH/CS, and TH/OT were tested. B6.C animals had slightly higher TH/OT (p

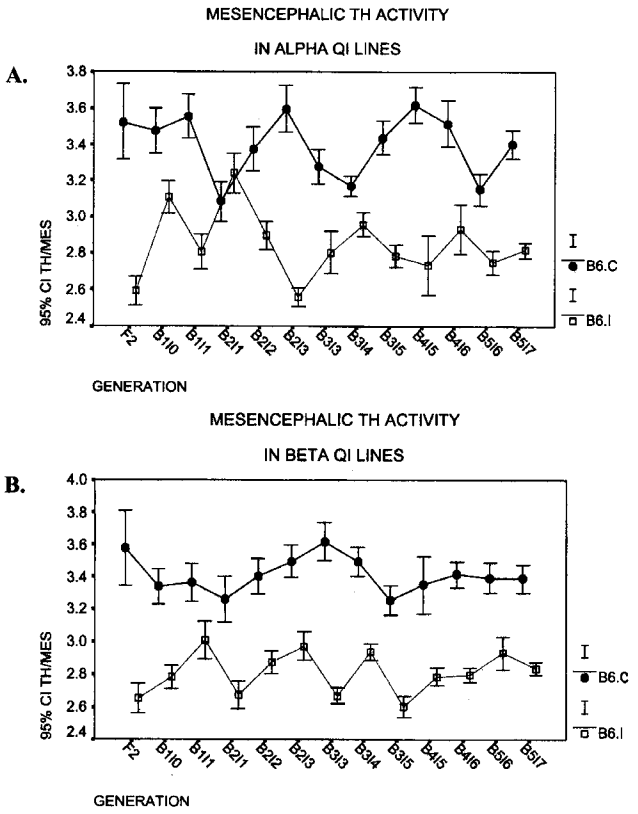

Fig. 3. Development of QI lines by repeated backcross-intercross cycles with concomitant selection for high (B6.C-α, B6.C-β) and low (B6.I-α, B6.I-β) mesencephalic TH activity. A. Alpha replicates (B6.C-α and B6.I-α). B. Beta replicates (B6.C-β and B6.I-β). These data represent observations from more than 3000 male mice, with a range of 40–90 subjects per data point. Each line had 15 or more mating pairs; their 45–90 male offspring were backcrossed to B6 females, or intercrossed with non-littermate females of the same generation; the males after having their own offspring were tested for TH/MES. At an age of 219 ± 49 days (mean ± SD, N = 3157), animals were killed by stunning, followed by quick decapitation and speedy removal of the brain. In each generation, about 1/3 of the males were selected on the basis of their TH/MES values (i.e., their offspring were used to set up the new mating pairs), and were either backcrossed to B6 females, or intercrossed within their lines, strictly avoiding brother-sister mating. In the B6.C and B6.I lines selection was directed towards the high and low values, respectively. Data shown for the B6 strain were collected simultaneously with those for the b_5i_7 generation to detect potential deviations of the developing quasi-congenic lines from the background strain.

< 0.05), while TH/CS (Table 1) and TH/MES (Fig. 7, panel K) were substantially higher in B6.C subjects ($p < 0.001$).

Multivariate Analysis. To establish whether there is a common control for regional TH activities, data from the first few generations (see Table I) were subjected to multivariate analysis. Because we did not measure every variable in every generation, the first analysis was limited to TH/MES, TH/CS, and TH/HT variables in the F_2, and b_1i_0 generations. Principal component analysis of the B6.C segregating populations yielded a correlation matrix with the following coefficients: −0.001 for TH/MES vs. TH/HT ($p > 0.05$), 0.201 for TH/MES vs. TH/CS ($p = 0.014$), and 0.033 for TH/CS vs. TH/HT ($p > 0.05$). After Varimax rotation with Kaiser normalization two components were extracted, which explained 73% of the total variance, with the following loadings (higher than 0.25). First component: 0.779 (TH/MES) and 0.771 (TH/CS). Second component: 0.995 (TH/HT). The results suggest that the mesostriatal system and the hypothalamic system are clearly separable.

Principal component analysis of the B6.I segregating populations yielded a correlation matrix with the following coefficients: 0.323 for TH/MES vs. TH/HT ($p > 0.001$), 0.178 for TH/MES vs. TH/CS ($p = 0.012$), and −0.015 for TH/CS vs. TH/HT ($p > 0.05$). After Varimax rotation with Kaiser normalization two components were extracted, which explained 79% of the total variance, with the following loadings (higher than 0.25). First component: 0.765 (TH/MES) and 0.854 (TH/HT). Second component: 0.328 (TH/MES), 0.959 (TH/CS) and −0.181 (TH/HT). These results demonstrate that depending on the presence of C-type or I-type donor genes in these segregating populations, the relationships between regional TH activities are different. In B6.I, in contrast to B6.C, significant correlation was found between TH/MES and TH/HT, while TH/CS remained uncorrelated with TH/HT.

The second principal component extraction was performed in the b_1i_1 generation with TH/MES, TH/CS, and TH/OT. When the B6.C and B6.I lines were analyzed separately interesting differences were observed: (1) correlation between TH/MES and TH/CS was significant in B6.C (0.51; $p < 0.000$) but nonsignificant in B6.I (0.11; $p > 0.05$), (2) correlations between TH/OT and TH/MES were not significant ($p > 0.05$) in neither B6.C (0.06) nor in B6.I (0.16), and (3) the correlations between TH/OT and TH/CS were negative, and low but significant in both B6.C (−0.21, $p = 0.033$) and B6.I (−0.25, $p = 0.008$). Accordingly, the rotated component matrix of B6.C yielded two components. The first component had high loadings on TH/MES (0.89) and TH/CS (0.85), while the second component had a high loading on TH/OT (0.98), suggesting an independent control for TH/OT. In B6.I, component one had the following loadings for TH/MES, TH/CS, and TH/OT: 0.02, −0.75, and 0.82, respectively, while for component two 0.92, 0.40, and 0.30 were obtained for the same variables. These relationships, summarized in Fig. 4 as component plots in rotated space, suggest that the regional control of TH is different in B6.C and B6.I. In the latter, only low or nonsignificant correlations could be detected. In previous studies (53,54) we found that the heritability of

Table I. Striatal, Hypothalamic, and Olfactory Tubercle TH Activity in F_2 and Backcross Generations

		(B6XC) F_2	(B6XI) F_2	B6.Cb_1i_0	B6.Ib_1i_0	B6.Cb_1i_1	B6.Ib_1i_1	B6.Cb_2i_1	B6.Ib_2i_1
TH/CS	MEAN	7.44	6.07	8.35	7.55	7.62	7.01	7.88	6.83
	N	79	86	88	88	82	97	94	94
	SD	1.25	0.77	1.33	1.26	1.35	1.00	0.80	0.49
95% confidence interval		7.16–7.72	5.89–6.23	8.07–8.63	7.29–7.86	7.32–7.92	6.81–7.21	7.72–8.04	6.73–6.93
TH/HT	MEAN	1.05	0.73	1.04	0.80				
	N	40	83	87	84				
	SD	0.17	0.11	0.16	0.12				
95% confidence interval		0.99–1.11	0.69–0.77	1.00–1.08	0.78–0.82				
TH/OT	MEAN					7.89	7.47		
	N					87	95		
	SD					1.29	1.35		
95% confidence interval						7.61–8.17	7.19–7.75		

Values are nmol DOPA/mg protein/hour; CS - corpus striatum, HT - hypothalamus, OT - olfactory tubercle.

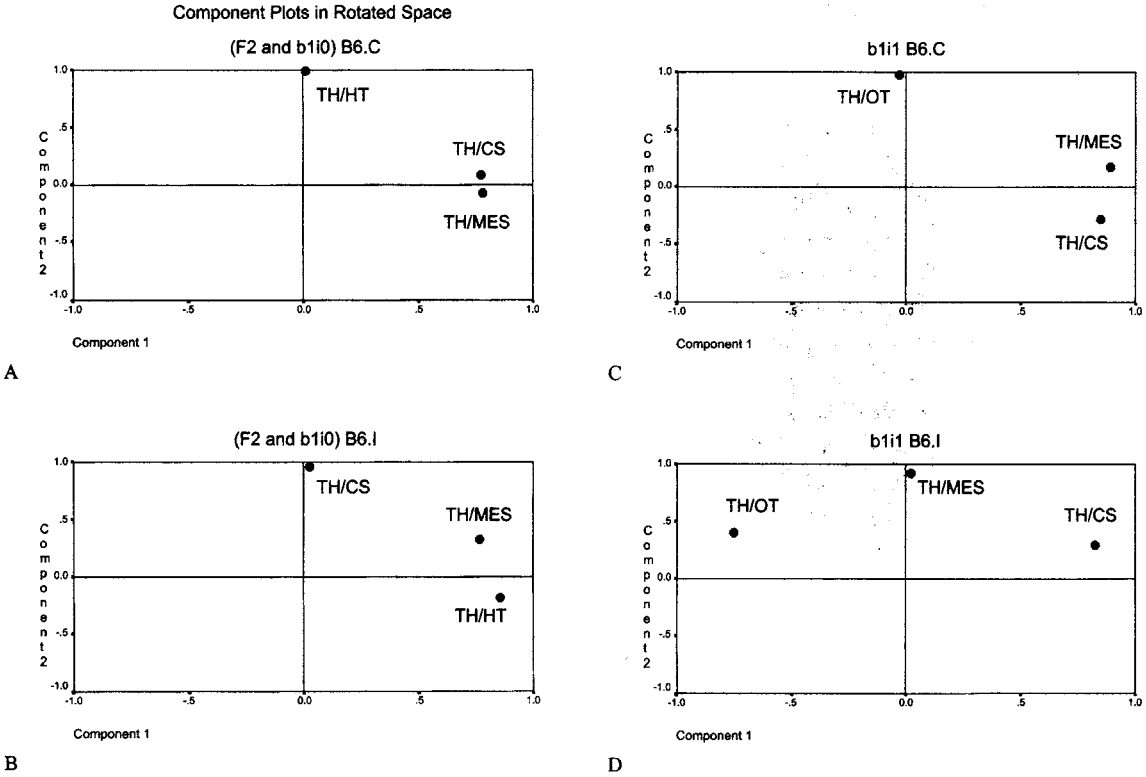

Fig. 4. Principal component plots illustrate the relationships between TH/MES, TH/CS, and TH/HT (plots A and B, pooled F_2 and b_1i_0 generations), and between TH/MES, TH/CS, and TH/OT (plots C and D, b_1i_1 generation) in B6.C and B6.I introgression-types.

TH/MES was low in B6.I suggesting that no QTLs with substantial effects on the mesencephalic cell body area are segregating in this introgression type. Accordingly, it is likely that the factor matrix pattern in B6.I represents primarily correlations of environmental origin.

Finally, we looked at the correlation between TH/MES and TH/CS in all the segregating generations (F_2, b_1i_0, b_1i_1, and b_2i_1) which were assayed parallel (Fig 5.). All correlations were significant ($p < .05 - p < .001$), except for B6.I mice in the b_1i_1 and b_2i_1 generations ($p > .05$). The Pearson correlation coefficients for the combined four generations were 0.34 for B6.C ($p < 0.001$; $N = 339$) and 0.13 for B6.I ($p < 0.05$; $N = 357$).

Gender Differences in the b_5i_7 Generation. In the last (b5i7) selection generation females were also tested

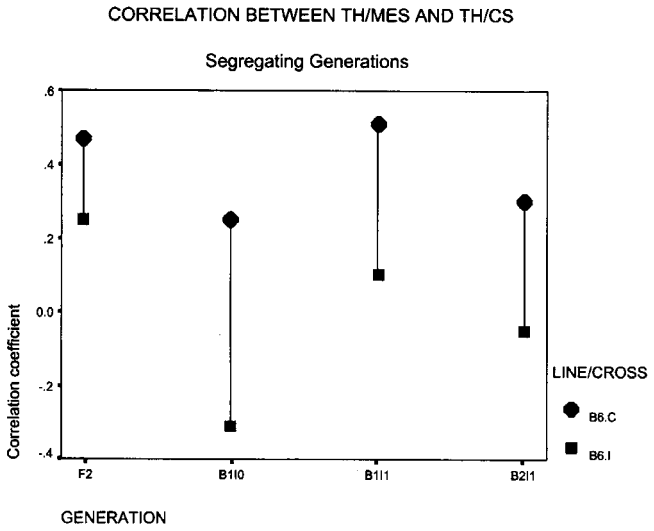

Fig. 5. Correlation between mesencephalic and striatal TH activity is consistently higher in B6.C mice than in B6.I mice in the first four generations of the QTL introgression.

in both replicate lines (α and β) of the B6.C and B6.I introgression types (IT, Table II.). Analysis of variance with three factors [IT, replicates, and sex] suggested that the effect of IT was highly significant ($p < 0.001$), the difference between replicates was not significant ($p > 0.05$), and the effect of sex was $p = 0.052$. None of the interaction effects were significant, except the replicate X sex interaction ($p < 0.05$). While in B6.I we found no significant difference between sexes, in B6.C-α the males had higher TH/MES ($p < 0.01$), and in B6.C-β the females had higher, albeit nonsignificant, TH/MES ($p > 0.05$), rendering the results inconclusive for B6.C. Taken together, these data indicate no substantial sex differences at the end of the gene transfer phase.

DNA Marker Polymorphism in b_5i_7 QI Lines in Comparison to the Background and Donor Strains. Allelic variations at polymorphic microsatellite loci were analyzed by polymerase chain reaction (PCR) in the four QI lines, the donor, and the background strains. A representative sample is shown in Fig. 6. Although the congenic lines were significantly different from the background strain in TH/MES, 212 of the tested 218 SSR genotypes in the congenic animals were of B6 background type, demonstrating that a largely homogeneous genetic background was ensured for the selectively favored TH/MES genes (Table III).

Effects of Introgression of TH/MES QTLs on Behavior. The original behavioral database contained both the sequence of behavioral events and a time stamp on each event. To analyze the gross effects of genetic manipulations, data for the appropriate variables were converted into a simpler format: total number of occurrences of the event during the 10 minutes of the OF test. Other variables, such as distance covered, time spent moving, or agoraphilia, were analyzed in their original format.

A. Search for Common Underlying Factors. To see an overall picture of the relationships between behavioral variables and TH/MES, all 3404 mice involved in the study were subjected to factor analysis. Components with eigenvalues over .95 were extracted. The first five components explained 69% of the total variance (Table IV). The first component had high loadings from motor activity-related variables (DIST, MOV, LEAN, REAR), the second component explained primarily eliminatory reactions (FAEC, URLG, URSM), the third component represented grooming activities (RFG, OHS), the fourth component was positively correlated with APH and negatively with LEAN, and the fifth component represented TH/MES alone (see Methods for the definitions of the abbreviations). Then, the B6.C lines and B6.I lines were analyzed separately (Tables V and VI). In the B6.C group (which included all the selection generations, N = 1395) 68% of the total variance was explained by 5 components, giving a rotated component matrix structure similar to that of the total population. In the B6.I group (N = 1325) 6 components explained 76% of the total variation. The first component represented "motor activity" (DIST, MOV, LEAN, REAR); the second component was at variance from the second component of B6.C, because it did not have high loading from URSM; the third component represented grooming; the fourth component was similar to a previous loading pattern (APH, -LEAN); and the fifth component had a high loading from URSM. Similarly to B6.C, only TH/MES had a high loading on the last extracted component. Results of the multivariate analysis suggests that there are no common underlying factors that can explain the variations in TH/MES and the recorded behaviors, either in B6.C, or in B6.I.

B. Progressive Changes in Behavior As a Function of the Increasing Proportion of the B6 Genome. A summary for all analyzed behavioral variables in all generations (mean ± SE) is shown for the B6.C and B6.I QI lines (Fig 7, Panels A through J; α and β replicates pooled). The charts indicate that if there were initial differences between B6.C and B6.I in the F_2 generation, most of these differences dissipated after five backcross-intercross cycles, and the lines became similar to B6. With the exception of FAEC, comparisons of B6.I, B6.C and B6 in the last selection generation (b_5i_7) indicated that the QI lines did not differ significantly from B6 in any of the behavioral variables (one way ANOVA with Tukey's post hoc multiple comparison test, $p > 0.05$).

Table II. Gender Differences in Mesencephalic TH Activity in the b_5i_7 Generation

			TH/MES*			
Introgression Type	Replicate	Sex	N	Mean	SE	95% Confidence Interval
B6.C	ALPHA	F	21	3.22	0.1	3.02–3.42
		M	58	3.47	0.04	3.39–3.55
	BETA	F	21	3.42	0.07	3.27–3.57
		M	59	3.38	0.05	3.27–3.49
B6.I	ALPHA	F	21	2.78	0.04	2.69–2.87
		M	59	2.83	0.02	2.78–2.88
	BETA	F	18	2.81	0.03	2.75–2.86
		M	60	2.84	0.02	2.79–2.89

* nmol DOPA/mesencephalon/hr.

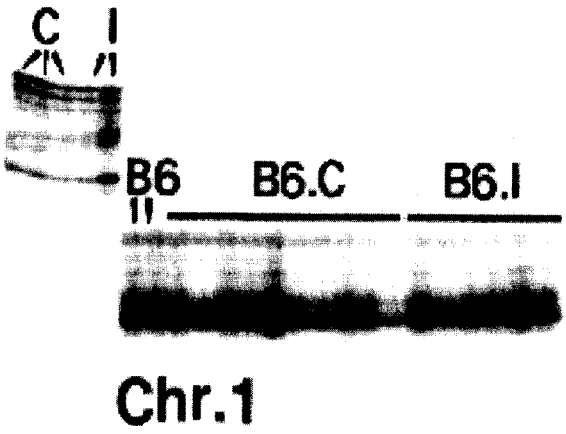

Fig. 6. Illustration of SSR polymorphisms at the D1Mit33 locus, chromosome 1, in the background (B6) and donor (C and I) strains, and four QIs (B6.C-α, B6.C-β, B6.I-α, and B6.I-β) of the b_5i_7 generation. Two allele sizes are shown: C (lanes 1–3) and I (lanes 4–5) carry the same allele (124 bp), whereas all congenic individuals (lanes 8–22) are identical to B6 (lanes 6–7) for this locus (100 bp; 8–11 = B6.C-α, 12–16 = B6.C-β, 17–21 = B6.I-α, 22 = B6.I-β).

For FAEC, Tukey's HSD test indicated a significant difference between B6 and B6.I ($p < 0.001$). B6.C and B6.I fell in the same homogeneous subset (with mean values of 1.51 and 2.03), while B6 fell in a separate subset (mean = 0.77). Because the FAEC values are higher in both B6.C and B6.I, we assume that randomly retained donor QTLs are responsible for this, in a process unrelated to the TH/MES QTL introgression. When only the two QI lines were compared, we found that REAR was significantly higher in B6.I (t test, $p < 0.001$). This difference may represent a modest inverse association between TH/MES and REAR.

C. Which Behavior Variables Best Distinguish the B6.C ("High TH/MES") and B6.I ("Low TH/MES") Selection Lines? Discriminant analysis was carried out on B6.C (N = 1395) and B6.I (N = 1325) mice. All generations from b_1i_0 through b_5i_7 were pooled, the F_1 and F_2 generations were omitted. The highest standardized canonical discriminant function coefficient was obtained for REAR (−0.689), followed by LEAN (0.546), RFG (0.441), FAEC (0.333) and OHS (0.288). Using these coefficients 57% of the original grouped cases were correctly classified. When TH/MES was added as a predictor variable, the standardized canonical discriminant function coefficients could be rank-ordered as follows: TH (0.971), LEAN (0.232), REAR (−0.175), RFG (0.118), FAEC (0.068). These variables were more useful for discriminating between the two groups, inasmuch as 78.2% of the original grouped cases were correctly classified. The results of discriminant analysis provide further support for a modest, negative association between REAR and TH/MES, and between REAR and LEAN, two dynamically related open-field movement patterns (53).

Initial Characterization of 10 New Quasi-Congenic RQI Strains and 3 Progenitor Strains for Mesencephalic TH Activity. Here we present the results of the first, preliminary characterization of inbred ($F_{n>20}$) B6.C Recombinant QTL Introgression strains of the b_4i_5 series for mesencephalic TH activity (TH/MES). Two-way ANOVA by sex and strain indicated significant sex ($F_{1,245} = 10.93$, $p < 0.01$) and strain ($F_{14,245} = 31.64$, $p < 0.001$) effects and sex × strain strain interaction ($F_{10,245} = 2.93$, $p < 0.01$). Males and females were further analyzed separately by one-way ANOVA and Scheffe's post hoc multiple comparison test (alpha = 0.05, Tables VII–VIII). Data used in the analysis have been corrected for inter-assay variability, as described in the methods. Analysis of males yielded five overlapping homogeneous subsets. The α6 RQI strain was not significantly different from the donor C (high TH/MES), indicating a successful transfer of differential QTL(s) from the donor C to the background B6. In addition, unexpectedly, we found two B6.C strains (α13 and β9),

Table III. Distribution of Polymorphic Microsatellite Markers in the B6, C, and I Progenitor Strains, and in Their Descendant QTL Introgression Lines

Chr.#	LOCUS	B6 (bb)	C (cc)	I	B6.C-α bc bb cc	B6.C-β bc bb cc	B6.I-α bb bb cc	B6.I-β bc bb cc
1	D1Mit 33	100	124	cc	5/5	5/5	5/5	1/1
2	D2Mit43	210	244	bb	1/1	1/1	1/1	1/1
3	D3Mit28	150	202	cc	1/1	1/1	1/1	1/1
4	D4Mit15	280	330	cc	5/5	5/5	5/5	3/3
5	D5Mit24	174	198	bb	1/1	1/1	1/1	1/1
6	D6Mit39	146	118	cc	3/3	5/5	5/5	3/3
8	D8Mit25	120	130	—	1/1	—	1/1	1/1
9	D9Mit27	174	188	bb	5/5	5/5	5/5	3/3
10	D10Mit10	180	128	bb	1/5 4/5	5/5	5/5	3/3
11	D11Mit41	136	178	bb	5/5	5/5	5/5	3/3
12	D12Mit27	288	306	bb	1/1	1/1	1/1	1/1
13	D13Mit21	162	180	cc	1/1	5/5	1/1	1/1
14	D14Mit37	136	94	cc	4/4	5/5	4/4	3/3
16	D16Mit5	158	134	bb	5/5	5/5	5/5	3/3
17	D17Mit11	176	150	bb	3/5 2/5	5/5	5/5	3/3
18	D18Mit17	214	190	bb	2/5 3/5	5/5	5/5	3/3
X	DXMit16	118	86	cc	5/5	4/4	5/5	3/3

In each QI line 5 animals were tested from the b_5i_7 generation. b = B6 background-type alleles, c = C donor-type alleles.

which were not significantly different in TH/MES from the other donor, I. Analysis of females yielded three overlapping subsets. As with the male data, α6 and C fell in the same homogeneous subset.

DISCUSSION

The major conclusions of this work are (1) polygenes that control mesencephalic TH activity could successfully be introgressed onto a homogeneous genetic background, (2) mesencephalic TH activity was not highly correlated with striatal TH activity, and seemed to be uncorrelated with hypothalamic and olfactory tubercle TH activity (3) the introgression process revealed that TH activity did not correlate with open-field behavior, (4) TH activity in B6.I lines showed quite limited heritability. Tyrosine hydroxylase (TH) is the first and rate limiting enzyme in catecholamine biosynthesis. Regional brain TH activity is a complex quantitative trait, which can depend on the structure of the enzyme, biochemical short- and long-term regulation, and also developmentally controlled cellular properties, such as number of neurons per structure, or density of axonal arborization. In rodents, a number of investigators reported genetic variability in whole brain (7,26), or in regional brain TH activity (2–4,38,40,41,44,45,50,52), and reported correlations between DA system properties (including TH activity) and behavior (9,21–23,25,29,34,49); however it has been difficult to identify the primary genetic and cellular factors that could explain these associations and correlations. Previous cellular studies comparing inbred mouse strains (2,40) suggested that strain differences in midbrain TH activity (expressed as activity/anatomical structure) are attributable to differences in the number of DA neurons; i.e., a strain with higher TH activity had a higher number of

Table IV. Rotated Component Matrix: Total Population (N = 3404)

	Component				
	1	2	3	4	5
APH*	.270			.864	
DIST	.873			.278	
FAEC		.764			
LEAN	.807			−.404	
OHS			.797		
REAR	.499	−.329			
RFG			.786		
TH					.970
MOV	.823			.331	
URLG		.693			
URSM		.646			

Loadings < .25 are not shown. Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
* Abbreviations (see also Methods): APH, preference of central area of the open-field (agoraphilia, cumulative score); DIST, locomotor activity, distance covered (meter); FAEC, number of feces boli deposited; LEAN, leaning, two forepaws touch the wall of the open-field apparatus (frequency); OHS, wiping movement on the head by one or both forelimbs (frequency); REAR, forepaws are off the ground (frequency); RFG, grooming activities in a repetitive manner (frequency of bouts); TH, mesencephalic TH activity; MOVE, time spent moving (second); ULG, release of a pool of urine (frequency); USM, release of urine droplets (frequency).

Table V. Rotated Component Matrix: B6.C lines (N = 1395)

	Component				
	1	2	3	4	5
APH*				.887	
DIST	.867			.275	
FAEC		.770			
LEAN	.825			−.363	
OHS			.797		
REAR	.459				.258
RFG			.789		
TH					.958
MOV	.810			.360	
URLG		.692			
URSM		.567			

Loadings < .25 are not shown. Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
* Abbreviations: see Table IV.

Table VI. Rotated Component Matrix: B6.I lines (N = 1325)

	Component					
	1	2	3	4	5	6
APH*	.259			.880		
DIST	.854			.289		
FAEC		.725			.294	
LEAN	.835			−.327		
OHS			.807			
REAR	.565				−.259	
RFG			.764			
TH						.989
MOV	.826			.306		
URLG		.861				
URSM					.939	

Loadings < .25 are not shown. Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
* Abbreviations: see Table IV.

midbrain DA neurons. Surprisingly, work on inbred rats (20) has shown the opposite relationship: the strain with a higher level of midbrain ventral tegmentum TH (assessed by blot immunolabeling) had a lower number of TH-positive DA cells. In view of the evolutionary similarity of the two species, these findings are puzzling, and indicate the need for systematic studies on the genetic control of the mesotelencephalic dopamine system.

Recently, an increasing number of human genetic studies has been focusing on the relationship between the dopamine system, TH, and complex behavioral disorders such as schizophrenia and manic-depressive illness, for example (27,29,32,33,35,46). In spite of significant efforts, no definitive, confirmed, genetically based relationship between the DA system and a complex behavioral trait, or disorder, has been reported. Limiting progress in the field has been the lack of a method for the genetic analysis of complex brain and behavioral traits. Recognition of the importance of the confounding factors (multiple genes, epistatic interactions, genotype-environment interactions, penetrance, heterogeneous genetic background, etc.) in the analysis of complex neural traits prompted us to search for the "ideal" model for QTL mapping and functional analysis. We raised the question whether it is possible to transfer genetically complex, quantitative traits from one inbred strain into another ("background") strain, in order to compare the background strain and the new model strain(s), which will be on the same homogeneous genetic background. A hypothesis was put forward that complex, quantitative traits with continuous distribution can be dissected by transferring the phenotype-specific QTLs onto a homogeneous genetic background and distributing the individual QTLs into separate, but genetically nearly identical strains, i.e., quasi-congenic Recombinant QTL Introgression (RQI) strains (47,55). Recently, we demonstrated that genetic transfer of a complex neural trait is possible by introgression of unknown QTLs into QI lines (56,57). In the following, we will discuss the consequences of manipulating the TH/MES genes on TH activities in other brain regions and on various spontaneous behaviors, and distribution of TH/MES in the new RQI strains.

Development of QTL Introgression Lines. Recently, to assess the complexity of the genetic control of TH/MES, data from the B6XC and BXI crosses were reanalyzed (56). We tested one-locus, two-locus, and polygenic hypotheses or a mixture of them. For the B6XC cross, the analysis yielded a hypothesis for two unlinked loci, equal dominance ratio, repulsion. A similar analysis of the BXI cross was inconclusive. In view of the limitations of such analyses, it seems safe to suggest that at least 2–4 QTLs affect TH/MES that may be mapped using sets of RQI strains.

The QTL introgression phase in the production of the inbred RQI strains is summarized in Fig 3. The results suggest that the larger differences between the B6.C and B6.I F_2 populations were due to nontransferrable epistatic interactions, and/or we might have lost some of the differential genes. However, this loss cannot be substantial, because one of the new RQI strains has similar TH/MES values as the C donor strain with high TH/MES (Table VII–VIII). Like the mean value deviations, variations in the F_2 populations were larger than those in the QI lines. We assume that this is partly due to the fact that in each animal the genetic interactions are different in a segregating F_2 generation, leading to higher variability in F_2s than in congenic QI lines. Co-

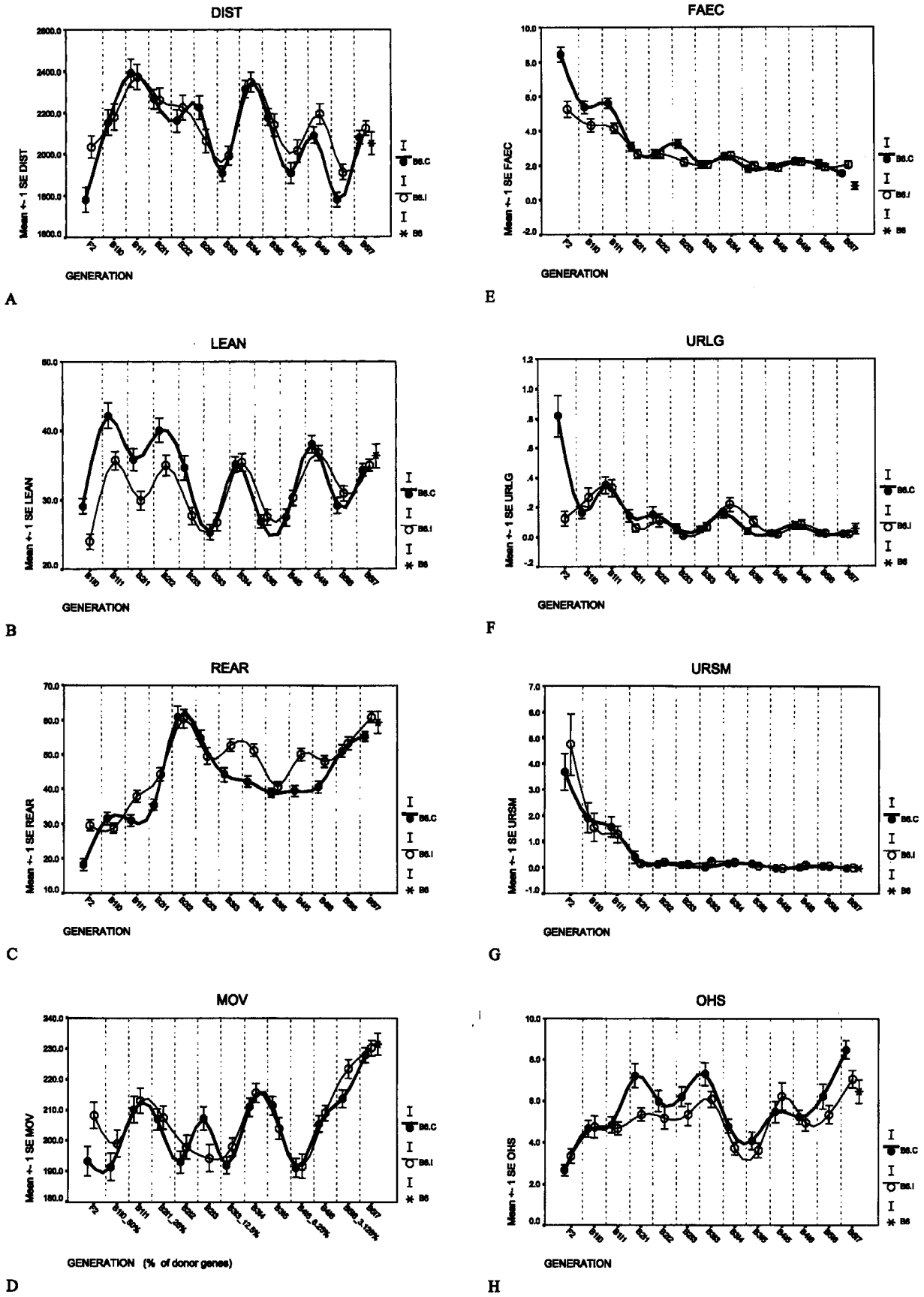

Fig. 7. Comparison of the selection variable (TH/MES, panel K) and behavioral variables (panels A–J) which were not selected for in B6.C and B6.I mice during the 13 generations of the QTL introgression.

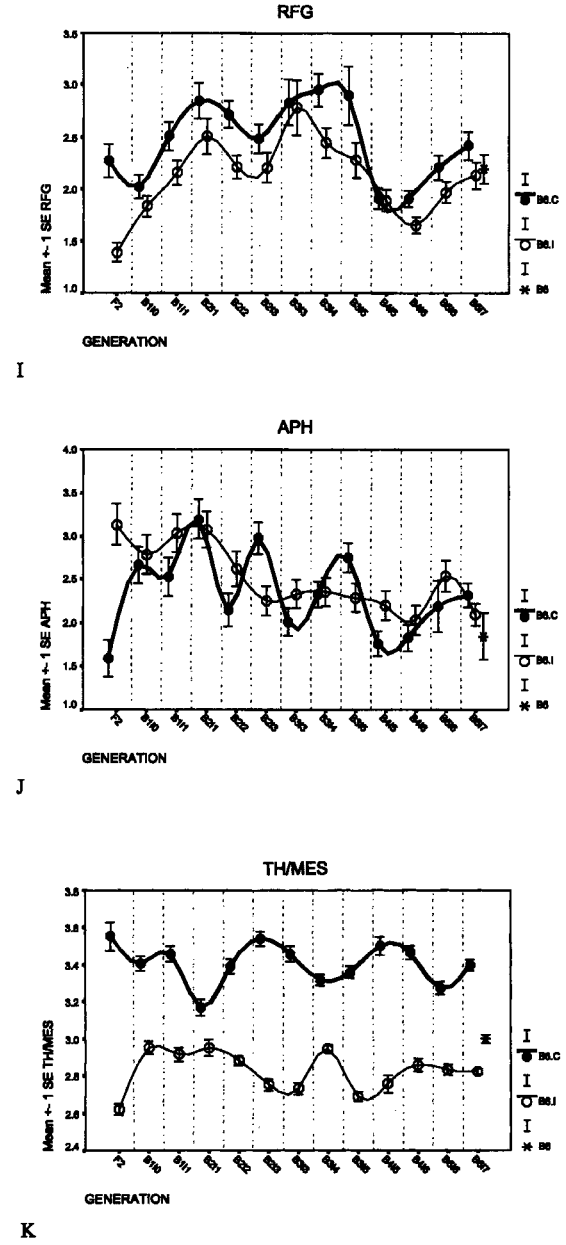

Fig. 7. Continued. Comparison of the selection variable (TH/MES, panel K) and behavioral variables (panels A–J) which were not selected for in B6.C and B6.I mice during the 13 generations of the QTL introgression.

Table VII. Mesencephalic TH Activity in Male B6.Cb$_4$i$_5$ RQI Mice

Strain	N	Mean	SEM	Subsets*
CXBI	7	2.50	.05	a
B6.Cb4i5 Beta-9	11	2.79	.03	a b
B6.Cb4i5 Alpha-13	9	2.80	.06	a b
B6.Cb4i5 Beta-13C	8	2.95	.07	b c
B6.Cb4i5 Alpha-11	8	2.99	.05	b c
C57BL/6By	44	3.00	.02	b c
B6.Cb4i5 Alpha-12A	5	3.10	.08	b c
B6.Cb4i5 Alpha-8	10	3.10	.09	b c
B6.Cb4i5 Beta-10	9	3.15	.06	b c d
B6.Cb4i5 Alpha-12	7	3.18	.09	b c d
B6.Cb4i5 Alpha-4	9	3.29	.08	c d
BALB/cJ	9	3.56	.04	d e
B6.Cb4i5 Alpha-6	11	3.71	.09	e

Values are nmol DOPA/MES/hour.
* Scheffe's multiple comparison test uses harmonic mean sample size (8.76). Strains within the same subset are not significantly different from each other.

Table VIII. Mesencephalic TH Activity in Female B6.Cb$_4$i$_5$ RQI Mice

Strain	N	Mean	SEM	Subsets*
CXBI	9	2.61	.11	a
B6.Cb4i5 Beta-9	8	2.72	.08	a
B6.Cb4i5 Beta-13C	10	2.84	.06	a
B6.Cb4i5 Alpha-11	9	2.89	.08	a b
B6.Cb4i5 Alpha-13	8	2.90	.07	a b
B6.Cb4i5 Beta-10	7	2.90	.05	a b
B6.Cb4i5 Alpha-4	7	2.93	.03	a b
B6.Cb4i5 Alpha-12	5	2.97	.12	a b
B6.Cb4i5 Alpha-10	6	3.06	.06	a b
B6.Cb4i5 Alpha-8	8	3.07	.02	a b
B6.Cb4i5 Alpha-4A	5	3.09	.03	a b
B6.Cb4i5 Alpha-6	10	3.39	.08	b c
BALB/cJ	7	3.64	.11	c

Mean values are nmol DOPA/MES/hour.
* Scheffe's multiple comparison test uses harmonic mean sample size (7.25). Strains within the same subset are not significantly different from each other.

efficients of variation (CV) of TH/MES were higher in the B6.C than in the B6.I populations ($F_{[1,43]} = 10.81$; $p < 0.005$), which suggests that the size of the effect of the differential gene(s) was larger in the B6.C lines. No consistent association could be detected between CV and the type of crossing (i.e., backcross or intercross).

Regional TH Activities. Looking beyond the goal of identifying genes that control neural function, it is of fundamental importance to understand the mechanism of common genetic control of different traits (i.e., traits are controlled by a partially overlapping set of genes). Such a control may include direct gene effects (e.g., the catalytic activity of an enzyme) and a cascade of developmental compensatory effects. Neurological mutants and knock-outs can serve as good examples for major perturbation of the developing nervous system, demonstrating powerful compensatory responses at multiple levels to a major change in a specific gene. In QTL Introgression lines and RQI strains, we can observe the effects of common genetic control in the normal range of variation that includes subtle changes in QTLs and the consequential compensatory changes. Principal component analysis of data obtained in B6.C populations of the segregating F_2 b_1i_0 and b_1i_1 generations suggests the presence of substantial common gene effect on TH ac-

tivity in the mesencephalon and striatum, but not on the hypothalamus, or the olfactory tubercle. This multivariate analysis is based on phenotypic data, accordingly, we have to be careful with conclusions about the genetic control of dopaminergic sub-systems. Such conclusions will be more reliable when between-strain (''genetic'') correlations will be compared with within-strain (''environmental'') correlations in the inbred RQI strains. If in B6.I the segregating genes have a significantly smaller size effect, then the present factor analysis may reflects primarily the underlying ''environmental'' relationships. However, if different genes segregate in B6.I and their effects was responsible for the differences between the B6.C and B6.I factor matrices, analysis of the RQI strains can be helpful in identifying these genes and their effects on dopaminergic sub-systems.

Focusing on the nigrostriatal system (TH/MES and TH/CS) and analyzing separately the two types of introgression lines (B6.C vs. B6.I) across four generations, one can notice that the correlation coefficient between TH/MES and TH/CS is dependent on the type of population: B6.C (introgressing QTLs for high TH/MES) or B6.I (introgressing QTLs for low TH/MES). In B6.C type populations the correlation is always positive, while in B6.I it seems to fluctuate around zero (Fig 5). The latter observation is consonant with the lower genetic variability of TH/MES in B6.I generations suggesting a smaller effect size for the involved QTLs and/or independent genetic control. While principal component analysis in the combined F_2 and b_1i_0 generations suggested that some of the genes that affect TH/HT, TH/MES, and TH/CS are shared, for TH/OT, TH/MES, and TH/CS data in b_1i_1 generation (Fig 4) variation in TH/OT appeared to be independent from that of TH/MES and TH/CS, raising the question of independent, or partially independent, genetic control of the mesolimbic and mesostriatal system. These issues can be better studied in inbred RQI strains, in which the genetic control of the anatomically related and functionally distinguishable pathways, such as the nigrostriatal, mesolimbic, and mesocortical systems, can be separated from the environmental effects.

Sex Differences. In a previous study we found that in a mesencephalic tissue block, which included the A8-A9-A10 dopaminergic cell groups, TH activities for males and females in the CBA/J strain were 3.02 and 2.92, respectively, while in C the males had significantly higher TH activity (4.35) than the females (3.91), pointing to strain dependent sex differences (49). In the course of the construction of the QI lines B6 females were used as the background strain, and selection was limited to males. Accordingly, not only the majority of the donor type nonselected, nonlinked nuclear genome was lost in the gene transfer, but the cytoplasmic genetic factors of the donor strains were also not transferred to the background genome. Also, the Y chromosome is of donor origin, while the X chromosome is of background origin, in every RQI strain. Therefore, (1) the effects of sex on TH/MES can be studied without the interfering effects of heterogeneity in cytoplasmic genetic factors, or in sex chromosomes, and (2) Y chromosome-specific effects can also be detected. The replicate X sex interaction in the segregating b_5i_7 generation needs further consideration. If it represents chance variation, the nonsignificant sex differences in TH/MES suggest that when TH/MES QTLs are on B6 background sex does not modulate their expression. A more definite answer can be obtained when inbred RQI strains are tested for sex-dependent effects on TH/MES.

TH and Behavior. There are relatively specific behavioral tests that can detect certain changes in the mesotelencephalic dopamine system. However, in this study we wished to use only a simple, quick, non-specific, routinely applicable OF test to detect gross behavioral changes in response to selection and introgression. Although the genetic variability in the generations was gradually decreasing with the repeated backcrosses, and in genetic composition the QI lines were becoming more and more similar to the B6 background, we felt that pooling of all 3404 mice into one group and subjecting the data to multivariate analysis can be useful in getting an overall picture of the phenotypic relationships between open-field behaviors and TH/MES. The results of factor analysis suggested that the spontaneous behaviors observed in a mildly stressful environment (novel, brightly lit open-field arena) and TH/MES had no common underlying factors. However, we would like to point out, that it is quite conceivable, that behavioral, or pharmacological challenge of the mesotelencephalic DA system can reveal significant relationships between the genetically altered DA system and behavior (48), and such issues will be in the focus of further studies. The loading patterns of the extracted behavioral factors suggest that they may underline broader behavioral categories related to ''spontaneous activity and motor exploration'' (first factor), ''fear-induced eliminatory responses'' (second factor), ''stress-induced grooming'' (third factor), and ''agoraphilia and investigatory exploration'' (fourth factor). With habituation to the open-field arena, the ''fear-induced eliminatory responses'' might have also reflected surface marking behavior (marking with urine and feces (53,54)). Division of the total population by the type of introgression (B6.C vs. B6.I) and repetition of the factor analysis confirmed the

first results, inasmuch as similar loading patterns were obtained for "spontaneous activity and motor exploration", "stress-induced grooming", and "agoraphilia and investigatory exploration", indicating the reproducibility of factor extraction. The primary difference between B6.C and B6.I factor matrices is the emergence of an additional component in B6.I, which presumably represents a urinary marking behavior pattern (URSM, Table VI, component 5; (53,54)), in addition to a relatively small loading from REAR on the TH/MES component in the B6.C population (Table V).

We assumed that in the process of assimilating the genomes of the QI lines into that of the background B6 by repeated backcrosses, while selectively retaining the Th/MES QTLs, the routinely tested OF behaviors in the QI lines would become similar to OF behaviors in B6, unless the selectively retained donor TH/MES QTLs would also affect behavior and cause deviations from B6 towards the donor strains. In accordance with the multivariate analyses, no major deviations were found among the behavioral variables, only small differences in FAEC and REAR (Fig 7). The loading from REAR on the TH/MES component (Table V) and the results of discriminant analysis, which indicated that REAR best distinguishes B6.C from B6.I among the behavioral variables, raises the question whether TH/MES and REAR are affected by the same factor in a relatively subtle manner. It is possible that some of the new quasi-congenic RQI strains will differ in some aspects of the open-field behavior, offering the opportunity of testing the recently reported QTL map positions for open-field behavior (17). Further, systematic screening of the RQI strains is planned to establish correlation between TH/MES and dopamine-function related behaviors.

New RQI Strains: Proof of Concept. Towards the understanding the physiological mechanism of the expression of a heritable complex trait, the relevant loci have to be identified, and their relationships to the complex trait have to be established. Introgression is a method for the identification of such loci. It has been believed and explicitly stated (18) that only discrete traits, or quantitative traits with clear bimodal distribution, can be introgressed from one strain into another strain. We hypothesized that genetic transfer of complex, quantitative traits with continuous distribution from one inbred strain to another is possible by phenotypic, directional selection on segregating populations created by backcrosses, or backcross-intercross cycles. The results presented in Tables VII and VIII demonstrate that full recovery of a quantitative trait is possible by introgressing the responsible QTLs from one strain into another strain.

We predicted that fixation and recombination in the developing RQI strains will (1) force 50% of the differential loci into a homozygous condition with donor alleles, and (2) if there is more than one QTL (assuming successful transfer of the differential alleles), some strains will carry recombinant QTLs which may express the selection phenotype (TH/MES) at the level of the donor strain, while other recombinants will fall between the donor and the background strains, or will not be significantly different from the background strain. The results (Figure 3, Table VII-VIII) supported this hypothesis and clearly demonstrated the successful transfer of differential QTL(s) from the donor C to the background B6: The quasi-congenic $\alpha 6$ RQI strain was not significantly different from the donor (high TH/MES) C. Surprisingly, the multiple comparisons test also suggested that there was another statistically homogeneous set of strains, which included a donor strain and two RQI strains (CXBI, low TH/MES, $\beta 9$, and $\alpha 13$). These results were unexpected, because in the development of this set of RQI strains we selected for high TH/MES using C (high TH/MES) as donor.

There are several possible explanations of why TH/MES is lower in some RQI strains than that of the background strain, for example: (a) TH/MES is affected by a series of QTLs and the C strain carries both increaser and decreaser alleles. Assuming dispersion of genes in C and B6, a decreaser allele could be transferred from C to the B6 background by chance. After fixation in $\beta 9$ and $\alpha 13$, the effect of the decreaser alleles resulted in TH/MES lower than that of the B6 background, (b) a supressor QTL is inhibited in C, however, it is not inhibited on B6 background, and (c) in some of the RQI strains new mutation(s) arose.

Recombinant QTL Introgression is an alternative to other methods used for the identification of individual, functionally different genes which contribute to the expression of the same phenotype. Although no QTL for neurobehavioral traits has been cloned and sequenced, numerous provisional, or candidate, QTLs have been reported, using large segregating generations, recombinant inbred strains, or recombinant congenic strains, e.g., (5, 6, 12, 13, 15–17, 28, 30, 31, 36, 37, 39, 58).

What are the advantages of the RQI approach over other approaches? The RQI strategy is suitable for attacking highly complex phenotypes. First, phenotypic expression of introgressed QTLs can be measured in inbred strains by obtaining mean values for the trait. Means are far more reliable than measurements from a single individual of a segregating generation. This feature is available in recombinant inbred and recombinant congenic methods, but it is not available in F2 and back-

cross designs, or in any other designs of segregating populations. Second, complexity of the genetic system is reduced to one QTL, or the combination of a few QTLs, per strain. Third, variations in background epistatic effects are virtually eliminated by providing similar genetic background for each RQI strain (on the average, about 3% of non-selected, non-linked donor genome is present in RQI strains with 4 backcross-intercross cycles). In comparison, F2 segregating populations and sets of recombinant inbred strains are characterized by heterogeneous genetic background (50-50% of parental genome), while recombinant congenic strains have a substantially more homogeneous genetic background (12.5% of donor genome, (10)). None of the compared designs involve phenotypic selection to ensure the presence of the relevant QTLs. Fourth, creation of sets of RQI strains permits mapping of QTLs by regression analysis between abundant polymorphic DNA markers and strain means of the complex trait. Our ongoing genome scanning experiments demonstrate that many of the markers, which are polymorphic for the background and the donor strains, are non-polymorphic in the RQI strains. In comparison, in a recombinant inbred design virtually every marker is polymorphic in the RI strains if the progenitor strains carry different alleles. Therefore, corrections for multiple comparisons have to be made at a much higher level in RI strains. Fifth, individual RQI strains may serve as animal models in brain and behavioral research addressing such questions as the developmental effect of genetic manipulation of one feature of the mesencephalic dopamine cell group on various other dopaminergic properties (including neurotransmitter release, reuptake, receptor binding, cell number, axonal branching, firing pattern, etc.), or on other neurotransmitter systems, and related behaviors. Although development of the first sets of RQI strains was time consuming, recent advances in genomic technologies, like strategic use of marker-assisted selection, can significantly expedite the creation of RQI strains.

In conclusion, this paper provides experimental evidence that it is possible to transfer unknown, brain-specific genes onto a uniform genetic background. The resulting genetic constructs may facilitate (a) chromosome mapping and cloning, and (b) function oriented studies. Results from the first segregating generations of the TH/MES QTL introgression procedure suggest that there was a limited, common genetic control affecting the mesencephalic and striatal TH activity, while the control of TH activity in the hypothalamus and in the olfactory tubercle was relatively independent. Genetic manipulation of TH/MES did not significantly affect behaviors observed in a simple open-field test. However, this by no means excludes the possibility of effects on other drug-induced or natural behaviors (14).

Finally, we wish to offer collaboration to the scientific community for the use of the b_4i_5 RQI lines of mice that have been selectively bred and characterized with regard to mesencephalic tyrosine hydroxylase activity.

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