Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 6256–6261, May 1998
Genetics

Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities

HARUHIKO SAGO*, ELAINE J. CARLSON*, DESMOND J. SMITH†, JOSHUA KILBRIDGE‡, EDWARD M. RUBIN†, WILLIAM C. MOBLEY‡, CHARLES J. EPSTEIN*, AND TING-TING HUANG*§

Departments of *Pediatrics and ‡Neurology, University of California, Box 0546, San Francisco, CA 94143-0546; and †Human Genome Center, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Communicated by Ira Herskowitz, University of California, San Francisco, CA, March 25, 1998 (received for review December 9, 1997)

ABSTRACT **A mouse model for Down syndrome, Ts1Cje, has been developed. This model has made possible a step in the genetic dissection of the learning, behavioral, and neurological abnormalities associated with segmental trisomy for the region of mouse chromosome 16 homologous with the so-called "Down syndrome region" of human chromosome segment 21q22. Tests of learning in the Morris water maze and assessment of spontaneous locomotor activity reveal distinct learning and behavioral abnormalities, some of which are indicative of hippocampal dysfunction. The triplicated region in Ts1Cje, from *Sod1* to *Mx1*, is smaller than that in Ts65Dn, another segmental trisomy 16 mouse, and the learning deficits in Ts1Cje are less severe than those in Ts65Dn. In addition, degeneration of basal forebrain cholinergic neurons, which was observed in Ts65Dn, was absent in Ts1Cje.**

Although Down syndrome (DS) (1) is a frequent cause of mental retardation, major and minor congenital abnormalities, and, in later life, Alzheimer's disease (AD), the mechanisms by which the presence of an extra copy of chromosome 21 causes these abnormalities are still unknown. On the assumption that it will be possible to relate components of the phenotype to imbalance of a specific gene or sets of genes (2), a search for these genes is currently underway. In this regard, persons with DS resulting from segmental duplications or partial trisomies of chromosome 21 have been studied, and a so-called "DS region" in 21q22, which produces many, although probably not all, of the physical and cognitive defects of DS has been identified (3, 4).

Because the distal end of mouse chromosome (MMU) 16 corresponds genetically to most of human chromosome 21 (Mouse Genome Database; http://www.informatics.jax.org), mice with an extra whole MMU16 were the first to be used as an animal model for DS (6). Although trisomy 16 mice have several phenotypic features suggestive of DS, their value as a DS model is limited because they die *in utero* and because MMU16 contains many genes located on human chromosomes other than 21. A viable partial trisomy 16 mouse (Ts65Dn) with a translocation producing segmental trisomy of the region of MMU16 from *App* to *Mx1* has been developed (7) and extensively studied (8–10). Ts65Dn mice exhibit male sterility, developmental delay, learning and behavioral deficits, and age-related degeneration of basal forebrain cholinergic neurons (BFCNs). Degeneration of BFCNs is characteristic of elderly persons with DS and of AD patients and may contribute significantly to dementia (11). Recently, yeast artificial chromosome (YAC) transgenic mice that contain human DNA from 21q22.2 have been produced and shown to have impaired learning (12).

We now report the development of a partial trisomy 16 mouse, Ts1Cje, which carries an extra copy of the segment of MMU16 spanning from *Sod1* to *Mx1*, but functionally not including *Sod1*. This segment corresponds to human 21q22.1 to 22.3. The learning deficits of Ts1Cje are less severe than those of Ts65Dn, in which the region of MMU16 involved in the trisomy is larger than in Ts1Cje, but are more severe than those of the YAC transgenic mice in which only a small region of human 21q22.2 is present. In addition, although the Ts65Dn animals show BFCN degeneration, Ts1Cje mice do not.

MATERIALS AND METHODS

Mice. The production of the heterozygous *Sod1* mutant mice, T(12;16)1Cje (formerly known as Ts108Cje), by gene targeting has been described (13). Primers for PCR amplification of the neomycin sequence were Neo3: 5′-CTCACCTTGCTCCTGCCGAG-3′ and Neo4: 5′-CTGATGCTCTTCGTCCAGATCATC-3′. Ts1Cje animals were generated by mating Ts1Cje males on a CD1 background to (C57BL/6JEi × C3H/HeJ)F1 (JAX JR1875) females to produce a genetic background close to that of Ts65Dn for phenotype comparison. Because the trisomic segment of MMU16 contains the mutated *Sod1* gene disrupted by neomycin resistance sequence, screening of mice was performed by using multiplex PCR with primers Neo3 and Neo4, with Grik1F2: 5′-CCCCTTAGCATAACGACCAG-3′ and Grik1R2: 5′-GGCACGAGACAGACACTGAG-3′ as internal controls. PCR was performed by using the following reaction conditions: "hot start" followed by 30 cycles of 94°C, 45 s; 55°C, 60 s in a 25-μl reaction mixture containing DNA (50–100 ng), 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 0.2 mM dNTPs, 0.4 μM of each primer, and 0.7 units of AmpliTaq polymerase (Perkin–Elmer). All mice used in behavioral studies were confirmed to have intact retinas by histology because C3H mice carry the retinal degeneration (*Pdeb*^{rd1}) mutation (14).

Fluorescence *in Situ* Hybridization (FISH). Metaphase spreads were prepared as described from fetal fibroblasts (15). FISH was performed as described with some modifications (15). Probes were labeled with digoxigenin-11-dUTP (Boehringer Mannheim) by nick translation (Enzo Diagnostics). With P1 clones, bacterial artificial chromosome (BAC) clones, and sorted chromosomes, the probes were amplified by degenerate oligonucleotide-primed–PCR (16) before nick translation. Digoxigenin-labeled probes were detected with a fluorescein isothiocyanate-conjugated sheep antidigoxigenin Fab fragment (Boehringer Mannheim).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DS, Down syndrome; MMU, mouse chromosome; BFCN, basal forebrain cholinergic neuron; AD, Alzheimer's disease; YAC, yeast artificial chromosome; FISH, fluorescence *in situ* hybridization.

§To whom reprint requests should be addressed. e-mail: tthuang@itsa.ucsf.edu.

Five independent λ clones covering a total 20.2 kb of the *App* gene were obtained from A. G. Reaume (Cephalon, West Chester, PA) (17). Plasmid pMx34 containing 3.2 kb of cDNA for *Mx1* (18) and plasmid mEts2S16 containing 16 kb of the gene for *Ets2* were provided by R. H. Reeves (Johns Hopkins University, Baltimore). Mouse chromosome 12-specific probes (19), which originally came from N. P. Carter (Sanger Centre, Cambridge, United Kingdom), were obtained from D. Pinkel (University of California, San Francisco). Plasmid pSod1/16 contains 16 kb of the *Sod1* gene. P1 clone Gart11313 containing 40 kb of the *Gart* gene was obtained from G. Brodsky and D. Patterson (Eleanor Roosevelt Institute, Denver). T12, a bacterial artificial chromosome (BAC) clone that covers *D12Mit41*, the most distal marker on MMU12, was provided by X.-N. Chen and J.R. Korenberg (Cedars-Sinai, Los Angeles). YAC282B10 (Research Genetics, Huntsville, AL) was shown to contain *Grik1* by PCR amplification of the expected size of fragments with two sets of primer pairs for *Grik1* (Grik1F1: 5′-CTGATGCCCAAGGCTCTATC-3′ and Grik1R1: 5′-TCATTGTCGAGCCATCTCTG-3′, 199 bp; Grik1F2–Grik1R2, 333 bp).

Morris Water Maze. The Morris water maze tests (20) were performed as described (12). The mice first were repeatedly tested for 2 days for their ability to locate a submerged platform that was marked with a flag (the visible platform test). The next week they were tested for 3 days for their ability to locate an unmarked submerged platform (the hidden platform test), followed by 2 days of testing in which the platform was switched to the opposite quadrant (the reverse hidden platform test). One hour after the final trial of each of these two tests, the platform was removed, and the times spent in each quadrant (dwell) and the number of times the mice crossed the previous site of the platform (crossings) were recorded for 1 min (as the probe and reverse probe tests, respectively). Each training block represents four different trials in which the mice were released in a pseudo-random fashion from each of four quadrants. Three blocks of trials were performed per day.

Seventeen male Ts1Cje mice and 18 male diploid litter mates were investigated twice (first test, age 12–16 weeks; second test, age 23–25 weeks). The mice were not handled before initial testing. Excluded from analysis were one Ts1Cje mouse, which died before the second test, and four diploid mice, which were found to be blind. Therefore, the data from 16 Ts1Cje (wt. 37.2 ± 0.7 g) and 14 diploid control mice (38.3 ± 1.1 g) were analyzed. To explore the effect of age on performance, eight male Ts1Cje and 10 of their diploid litter mates also were investigated for the first time at 27–28 weeks of age. Excluded from analysis were one Ts1Cje and two diploid mice that were found to be blind.

Spontaneous Locomotor Activity. Spontaneous activity was examined as described (12) 1–2 months after the first Morris water maze test. The mice (controls $n = 14$, Ts1Cje $n = 16$) were placed in a cage for 1 h in the dark during the light phase and monitored by infra-red beams. Activity in the center and periphery of the cage was measured by photocells.

Brain Analysis. Brain tissue processing and immunocytochemistry were performed as described (9). The antibody used to visualize BFCNs was REX (anti-$p75^{NGFR}$) (21). The numbers and profile areas of $p75^{NGFR}$-positive neurons were measured as described (9).

Data Analysis. Repeated measures ANOVA was used to analyze spontaneous locomotor activity and latencies in the platform tests. This statistical analysis provided tests of an overall difference between control and Ts1Cje groups, differences between blocks, and group by block interaction. For spontaneous locomotor activity, impairment could be expected to have two somewhat opposite effects: a lower initial exploratory impulse and slower learning resulting in less reduction of exploration over time. This second tendency would produce a time by group interaction that reduces overall difference between the groups, and the statistical power to detect such an interaction is low. To focus more directly on these two possible differences, two-sample *t* tests were used to compare the two groups on activity levels in the first three time blocks and on the reductions in activity between the first and last time blocks. Two-sample *t* tests also were used to compare the groups on time spent in the trained quadrant for the probe tests and on the BFCN results. One-sample *t* tests were used separately for each group to test for greater than 25% time spent in the trained quadrant in probe tests and to test for greater time spent in the trained quadrant on the repeated probe test than on the first probe test. All values in the text and figures are expressed as mean ± SEM.

RESULTS

Origin and Characterization of Ts1Cje. Ts1Cje was derived during *Sod1* gene targeting by homologous recombination using neomycin for positive and diphtheria toxin A for negative selection (13). The heterozygous *Sod1* mutant mice, identified by the presence of the *Sod1* mutant allele, a 50% reduction of CuZnSOD activity in blood, and expression of a shortened *Sod1* mRNA derived from the mutant allele, were found to have aberrant segregation when crossed to wild-type animals. In addition to the expected wild-type and heterozygous mutant mice, progeny that carried the *Sod1* mutant allele and had 100% of wild-type CuZnSOD activity also were found [201/533 (36.3%), 174/533 (31.5%), and 178/533 (32.3%), respectively]. The mice with the *Sod1* mutant allele and 100% CuZnSOD activity were found to have three copies of *Sod1* by FISH on metaphase chromosomes (Fig. 1*a*). Two of the *Sod1* alleles were on MMU16, and one was on MMU12 (Fig. 1*b*). Further characterization of the heterozygous *Sod1* mutants by FISH revealed a translocation between MMU16 proximal to *Sod1* and the very distal region of MMU12, with the MMU16 breakpoint being between *App* and *Sod1* (Fig. 1 *c* and *d*). The same translocation was observed in the original targeted *Sod1* mutant ES cell clone (data not shown), indicating that it occurred in association with the targeting of *Sod1* by homologous recombination.

The heterozygous *Sod1* mutants, which are apparently balanced carriers for the translocation designated T(12;16)1Cje, have one each of normal MMU12 and MMU16 and of 12^{16} and 16^{12} translocation chromosomes (Fig. 2*a*). Partial trisomy of the distal region of MMU16, Ts1Cje, is generated when a gamete carrying $MMU12^{16}$ and MMU16 (by adjacent-1 segregation) combines with a normal gamete carrying MMU12 and MMU16. Because the *Sod1* allele on MMU12 is disrupted by the neomycin resistance sequence, the Ts1Cje animals have 100% rather than 150% of wild-type CuZnSOD activity. Therefore, Ts1Cje is not functionally trisomic for *Sod1*.

Further FISH analysis revealed that Ts1Cje carries three copies of *Gart*, *Ets2*, and *Mx1* genes (FISH not shown) confirming the triplication of the distal region of MMU16. *Grik1*, which maps between *App* and *Sod1*, was not triplicated (Fig. 1*e*), narrowing the break point to between *Grik1* and *Sod1*. Therefore, the translocated segment of MMU16 contains, in addition to the mutant *Sod1* gene, *Gart*, *Ets2*, and *Mx1*, but not *App* or *Grik1* (Fig. 2*b*). In addition, the subtelomeric MMU12 sequence (BAC T12) was found on both MMU12s by FISH (Fig. 1*f*), indicating that few, if any, of the telomeric genes on MMU12 were missing from the 12^{16} chromosome. Therefore, Ts1Cje appears to be trisomic only for the segment of MMU16 spanning from *Sod1* to *Mx1* (but functionally not including *Sod1*).

Both male and female Ts1Cje mice are fertile and produce Ts1Cje progeny at the expected 50% segregation frequency when crossed to wild-type animals. Ts1Cje mice have survived more than 1 year and are indistinguishable from controls in

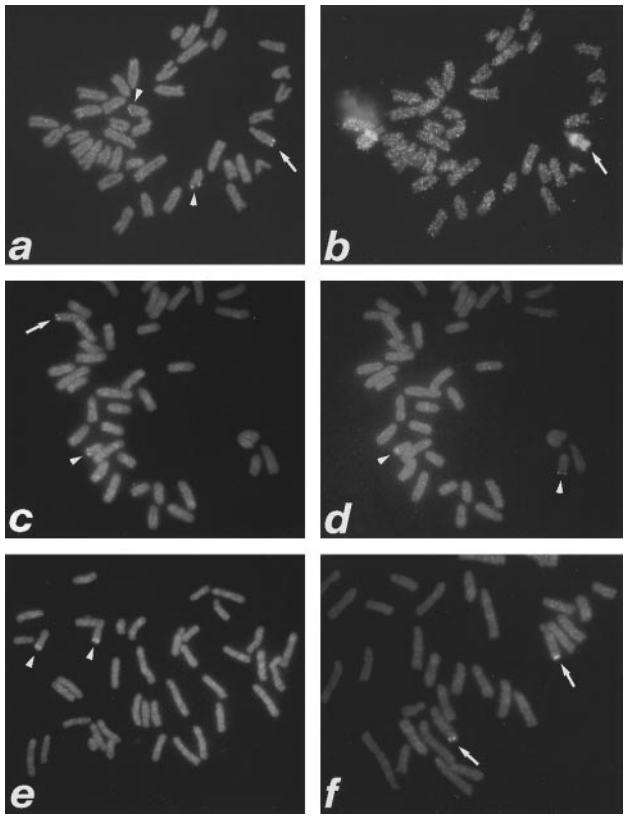


FIG. 1. Analysis of anomalous and heterozygous *Sod1* mutant mice (Ts1Cje *a*, *b*, *e*, and *f*; T(12;16)1Cje *c* and *d*) by FISH. (*a*) A *Sod1* probe hybridized to the distal parts of two MMU16 (arrowheads). In addition, the *Sod1* probe also hybridized to a third chromosome (arrow), and therefore three copies of *Sod1* gene were detectable. (*b*) The same metaphase as in *a*. The third chromosome containing the *Sod1* gene was confirmed to be MMU12 by rehybridization with a MMU12 paint probe (arrow). (*c*) The *Sod1* probe hybridized to one MMU16 (arrowhead) and to one MMU12 (arrow), indicating a 12;16 reciprocal translocation. (*d*) The same metaphase as in *c*. An *App* probe hybridized only to the two MMU16s (arrow head). (*e*) The YAC282B10 probe, which contains the *Grik1* gene, hybridized to the distal parts of two MMU16s (arrowheads), just as was observed with the *App* probe. (*f*) The most terminal MMU12 BAC probe (T12) hybridized to the distal parts of two MMU12s (arrow).

gross appearance. There are no apparent limb or facial malformations or other dysmorphic features.

Learning Deficits in the Morris Water Maze. The mice first were tested in the visible platform test. The mice need only learn to associate the flag with the platform to solve the task, and spatial information is irrelevant. Both Ts1Cje and control mice showed significant improvement in search time over successive trials, and the Ts1Cje mice performed as efficiently as control mice (Fig. 3*a*). Performance in the visible platform test is affected by factors such as vision, swimming ability, and motivation to escape from water, and Ts1Cje mice appear to be normal in these respects.

In the hidden platform test, the mice must learn the spatial relationships between objects in the room and the position of the platform to escape from the water. As expected, the control mice showed decreased latencies (time to reach the platform) over the nine blocks of training (three per day for 3 days). However, the performance of Ts1Cje mice was significantly impaired (Fig. 3*b*). The findings for path lengths closely paralleled those of escape latencies, with path length shortening as latency decreased (data not shown). After these trials were completed, the mice were then immediately tested in the probe trial. This test assesses spatial selectivity based on the premise that mice that have learned the spatial localization of

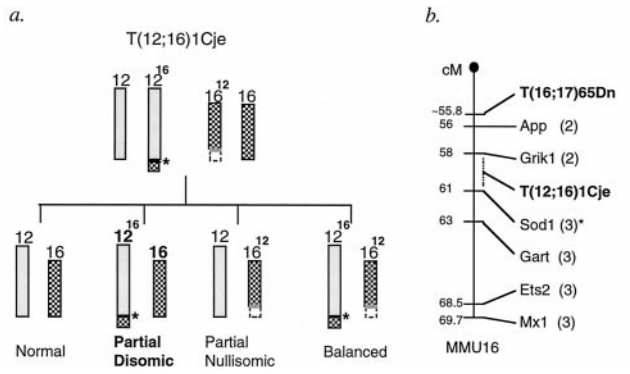


FIG. 2. (*a*) Diagram of meiosis of the 12;16 reciprocal translocation in the heterozygous *Sod1* mutants. Four types of gametes are obtained: normal, disomic, and nullisomic for the distal region of MMU16, and balanced. The partial disomic gametes produce a partial trisomy 16 (Ts1Cje). ∗ indicates the neomycin resistance marker on the 12^{16} translocation. (*b*) Diagram of the mapping of the triplicated region in Ts1Cje by FISH analysis. The numbers in the parentheses indicate the copy numbers of genes detected by FISH. There is segmental trisomy of the region from *Sod1* to *Mx1*. ∗, *Sod1* is not functionally trisomic because the *Sod1* gene in the translocated segment is inactivated by the insertion of the neomycin resistance sequence.

the platform will selectively search the place where the hidden platform had been located. The control mice preferentially searched in the quadrant in which the platform had been located during training rather than in the other three quadrants (dwell) (Fig. 3*c*). They also crossed over the site (crossings) at which the platform had been located more often than over corresponding locations in other quadrants (data not shown). In contrast, although Ts1Cje mice also spent more time in the trained quadrant than in any of the other quadrants, they spent significantly less time than controls in this quadrant (Fig. 3*c*). Ts1Cje mice failed to cross the trained site selectively and crossed significantly less frequently than controls ($P = 0.01$). Taken together, the hidden platform and probe tests demonstrate that Ts1Cje mice have substantial deficits in spatial learning.

In the reverse platform test, the mice are required to learn a novel position for the hidden platform, which has been placed in the quadrant opposite to its original location in the hidden platform test. This task is considered to be a test of cognitive flexibility in which a previously successful strategy must be inhibited and a new strategy developed to solve the new task (22). Both groups showed significant decreases in latency, indicating evidence of learning. However, Ts1Cje mice took significantly longer than control mice to locate the hidden platform (Fig. 3*d*). Similarly, in the reverse probe test, the control mice searched (dwell) preferentially in the trained quadrant in this task, whereas the Ts1Cje mice did not (Fig. 3*e*). Therefore, Ts1Cje mice show a deficit in cognitive flexibility.

Improvement in Learning 3 Months After the First Set of Tests. To explore whether the learning deficits in Ts1Cje were stable over time, the Morris water maze tests (except for the visible task) were repeated in the same test setting 3 months later. The performances in the hidden platform test of both control and Ts1Cje mice were significantly improved in comparison to 3 months earlier (Fig. 3 *b* vs. *f*). However, there still were significant differences between the two groups similar to those found in the original tests. The probe test confirmed the improvement in spatial learning in both control and Ts1Cje mice, with selectivity for the trained site becoming more pronounced (Fig. 3 *c* vs. *g*). Indeed, after 3 months there was a clear preference by Ts1Cje mice to swim in the trained quadrant. However, the reverse platform and reverse probe

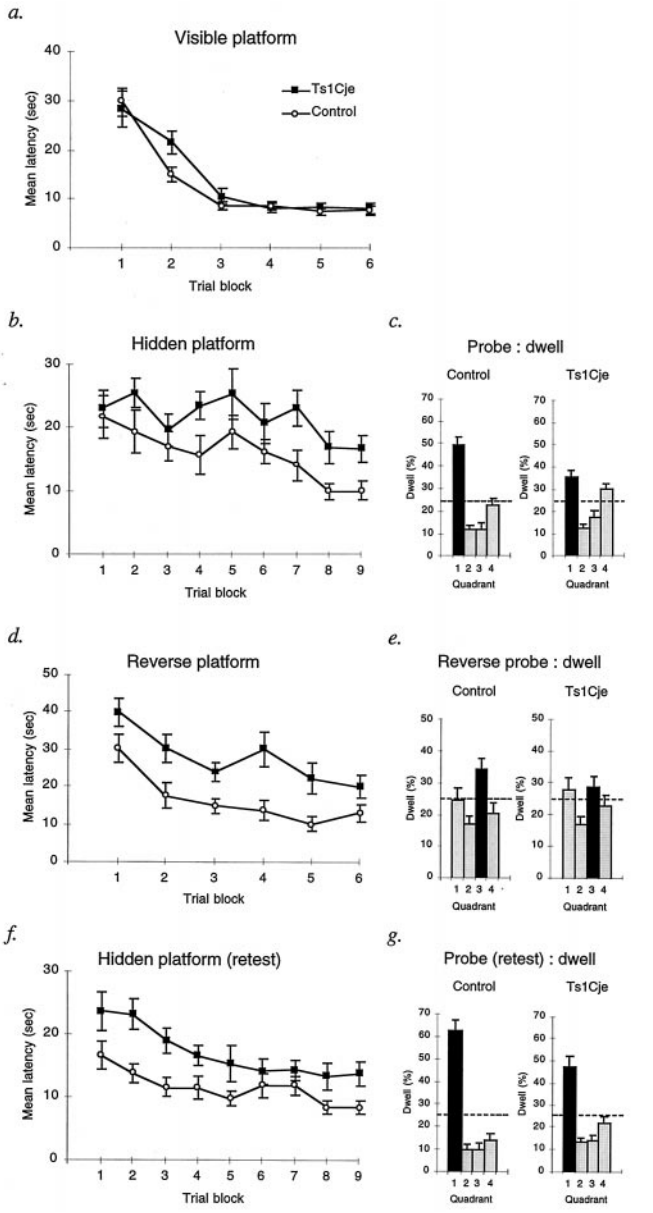


FIG. 3. Performance of control ($n = 14$) and Ts1Cje mice ($n = 16$) in the Morris water maze task (first test *a*-*e*; retest *f* and *g*). Mean latency: time to reach the platform. Dwell: the percentage time spent searching in each of the four quadrants of the pool. The trained sites were quadrants 1 and 3 in the probe and the reverse probe tests, respectively. (*a*) The visible platform test. The effect of genotype was not statistically significant ($P = 0.43$). Performance improved over blocks ($P < 0.0001$), but there was no statistically significant block by genotype interaction ($P = 0.24$). (*b*) The hidden platform test. The effect of genotype was statistically significant ($P = 0.036$). Performance improved over blocks ($P < 0.0001$), but there was no statistically significant block and genotype interaction ($P = 0.78$). (*c*) The probe test (dwell). Both control ($P < 0.0001$) and Ts1Cje ($P = 0.003$) mice spent significantly more than 25% time in the trained quadrant. The difference between the two groups in the time spent in the trained quadrant was significant ($P = 0.007$). (*d*) The reverse hidden platform test. The effect of genotype was statistically significant ($P = 0.0039$). Performance improved over blocks ($P = 0.0003$), but there was no statistically significant block by genotype interaction ($P = 0.30$). (*e*) The reverse probe test (dwell). Both groups spent more than 25% time in the trained quadrant. The preference for the trained quadrant was statistically significant for the control mice ($P = 0.023$) but not for the Ts1Cje mice ($P = 0.15$). The difference between the groups in the time spent in the trained quadrant was not statistically significant ($P = 0.35$). (*f*) The repeated hidden platform test. The effect of genotype was statistically significant ($P = 0.0013$). Performance improved over blocks ($P = 0.0006$), but there was no statistically significant block by genotype interaction. (*g*) The repeated probe test (dwell). Both control ($P < 0.0001$) and Ts1Cje mice ($P = 0.0002$) spent significantly more than 25% time in the trained quadrant, and there was a statistically significant difference between two groups ($P = 0.0074$). In addition, when compared with 3 months earlier, both control ($P = 0.011$) and Ts1Cje ($P = 0.008$) mice showed increases in time spent in the trained quadrant.

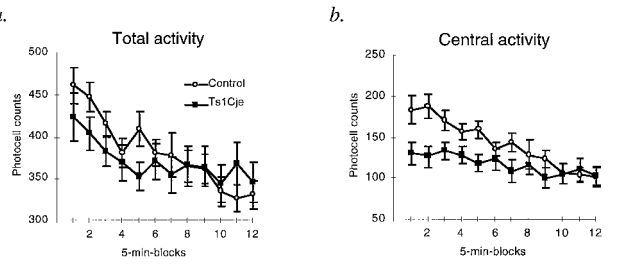


FIG. 4. Spontaneous locomotor activity of control ($n = 14$) and Ts1Cje mice ($n = 16$). The activity is shown in 12 sequential blocks of 5 min each. (*a*) Total activity. The effect of genotype was not statistically significant ($P = 0.62$). Activity decreased over blocks ($P = 0.0003$), but there was no statistically significant block by genotype interaction ($P = 0.12$). (*b*) Activity in the central region. Activity decreased over blocks ($P = 0.011$), but there was no statistically significant block by genotype interaction ($P = 0.20$). Although the effect of genotype was not statistically significant ($P = 0.06$), the activity of Ts1Cje was significantly lower than that of controls in blocks 1–3 (block 1, $P = 0.017$; block 2, $P = 0.0037$; block 3, $P = 0.0035$). In addition, the change in central activity from block 1 to block 12 was less in the Ts1Cje mice than in the controls ($P = 0.046$).

tests continued to show a deficit in learning flexibility in Ts1Cje mice (data not shown).

To investigate whether the improved performance was caused by previous training in the tests or maturation, we tested another set of control and Ts1Cje mice at age 27–28 weeks. Both control and Ts1Cje mice performed at nearly the same level as the first set of mice had at 12–16 weeks of age (data not shown). These findings suggest that previous training improved performance in spatial learning, but Ts1Cje mice still performed less well than did control mice.

Spontaneous Locomotor Activity. The total locomotor activity of both control and Ts1Cje mice significantly decreased over the period of testing, indicating habituation to a novel stimulus (Fig. 4*a*). Although Ts1Cje mice were hypoactive relative to controls in the first half of the period, the activity of the Ts1Cje mice over the entire period did not differ significantly from that of the control mice (Fig. 4*a*). Because total spontaneous locomotor activity represents gross movement of mice, activity was analyzed by the region in which the mice crossed the photo beams. Mice usually establish a home base in a corner of the cage and undertake repeat investigations of the cage from this base during the process of habituation (23). Therefore, activity in the central region of the cage is considered to reflect exploratory behavior (24). There was no significant difference between the two groups in the activity in the peripheral region of the cage ($P = 0.45$). In the central region, however, control mice showed a significant reduction in activity over the period of testing, whereas the activity of Ts1Cje mice was low during the entire test (Fig. 4*b*).

Basal Forebrain Cholinergic System. The brains of adult Ts1Cje mice were grossly normal. To determine whether Ts1Cje mice have BFCN abnormalities similar to those seen in Ts65Dn, in which the number and size of $p75^{NGFR}$-positive neurons is decreased at 6 months (9), the number and sizes of $p75^{NGFR}$-positive neurons in the medial septal nucleus of control and Ts1Cje mice were measured. At 6 months of age, there was no significant difference in number [Ts1Cje, 958 ±

76 ($n = 5$) versus control, 985 ± 68 ($n = 5$), $P = 0.798$]. Also, the mean cross-sectional profile area of $p75^{NGFR}$-positive neurons of Ts1Cje mice did not differ significantly from that of control neurons [Ts1Cje, 163.7 ± 2.23 μm^2 ($n = 5, n = 400$) versus control, 160.3 ± 2.13 μm^2 ($n = 5, n = 400$), $P = 0.274$].

DISCUSSION

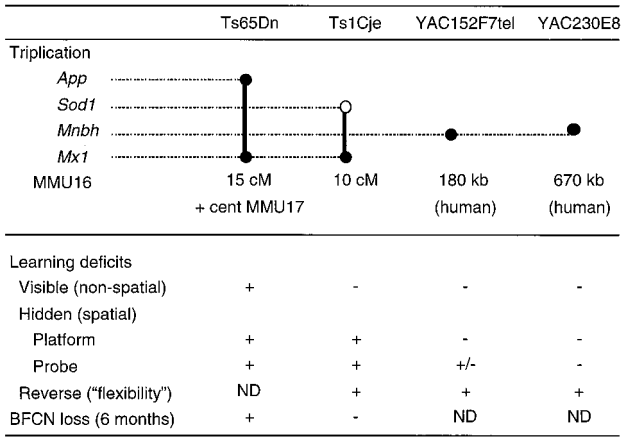
Ts1Cje, a mouse model for Down syndrome, is the result of a fortuitous translocation that occurred during the targeting of *Sod1*. To our knowledge, there is no precedent for such a translocation in association with gene targeting by homologous recombination. There is, however, a report of a reciprocal translocation that is thought to have occurred during the construction of a transgenic mouse (25). Although the precise mechanism is not known, it seems reasonable to infer that the chromosomal rearrangement was mediated by the homologous recombination event induced by exogenous DNA.

Ts1Cje mice have distinct learning and behavioral abnormalities. As adults, Ts1Cje mice show low levels of locomotor activity in the center squares of the activity box, suggesting decreased exploratory behavior. In the Morris water maze, they are able to perform normally in the nonspatial learning (visible platform) task but exhibit an impairment of spatial learning in the hidden platform and probe tasks that can be improved by previous training. They also show a deficit in learning flexibility in the reverse hidden platform task.

Rats and mice with hippocampal lesions or in which the septohippocampal pathway is disrupted are impaired in the spatial (invisible platform) version, but not in the cued (visible platform) version of the test (20). Hippocampal lesions also affect exploratory locomotion (26). These findings suggest that the deficits exhibited by Ts1Cje mice are caused by abnormalities within the hippocampus that result in abnormal hippocampal function.

Ts1Cje mice differ significantly from Ts65Dn mice, the other viable partial trisomy 16. The triplicated region in Ts1Cje is smaller than that in Ts65Dn, which is segmentally trisomic for the region from *App* to *Mx1* (8). This region corresponds to most of the long arm of human chromosome 21 except for the region distal to *MX1*. Unlike Ts1Cje males, which are fertile, Ts65Dn males are sterile (7). This sterility may be a result of the extra minute chromosome in Ts65Dn, which has 41 chromosomes and is also segmentally trisomic for a small region of proximal MMU17. Ts65Dn mice exhibit developmental delay in neonatal life (9), impaired performance in complex learning tasks (8, 9), and behavioral abnormalities (8–10). In the Morris water maze, Ts65Dn mice show learning impairment in the nonspatial (visible platform) test that ranges from mild to severe, depending on the report. They also show a severe impairment in spatial learning as revealed by the hidden platform and probe tests (8, 9). These findings are in contrast to those with Ts1Cje mice that have no deficits in the nonspatial version of the task and moderate to severe impairment of spatial learning (Fig. 5). Locomotor hyperactivity is seen in Ts65Dn and is thought to be caused by deficits in controlling and inhibiting behavior, possibly in association with deficits in prefrontal cortical function (10). Learning deficits in the visible platform task have been seen in decorticate animals with superior colliculus or basal ganglia ablations but not in decorticate animals from whom the hippocampus has been removed (27). The more widespread learning and behavioral impairments of Ts65Dn likely result from deficits in the function of the hippocampus and its afferents as well as other brain regions.

One hallmark neuropathological abnormality in Ts65Dn is an age-related loss of immunoreactive BFCNs (9), which mimics the age-related dysfunction in BFCNs seen in DS and AD (28). BFCNs play an important role in attention, memory, and learning, and their degeneration may contribute to the dementia of DS and AD (11). Unlike Ts65Dn mice, which at 6 months of age have a significant loss in number of immunoreactive BFCNs and a modest decrease in their size, in Ts1Cje these neurons were normal in number and size. Although these findings define a clear difference between Ts65Dn and Ts1Cje, they do not exclude the possibility that dysfunction of BFCN synapses in the Ts1Cje hippocampus contributes to the learning deficits.



	Ts65Dn	Ts1Cje	YAC152F7tel	YAC230E8
Learning deficits				
Visible (non-spatial)	+	-	-	-
Hidden (spatial)				
Platform	+	+	-	-
Probe	+	+	+/-	-
Reverse ("flexibility")	ND	+	+	+
BFCN loss (6 months)	+	-	ND	ND

FIG. 5. Genotype-phenotype correlations in Ts65Dn, Ts1Cje, and YAC152F7tel and YAC230E8 transgenic mice. + indicates deficits, ± indicates mild deficits, and − indicates no deficits.

There are about 20 identified genes in the extra segment of MMU16 present in Ts1Cje (Mouse Genome Database; http://www.informatics.jax.org). Among these genes, a few of the human homologues of *Drosophila* genes, such as *simple minded*, *SIM2* (29) and *minibrain*, *MNBH* (30), have been highlighted as possible candidate genes for the neurological abnormalities of DS. Recently, a series of YAC transgenic mice containing DNA from 21q22.2 has been developed, and two strains of YAC transgenic animals, YAC152F7tel and YAC230E8, are of particular interest (12). YAC152F7tel, which encompasses the human *minibrain* region, is a telomeric fragment of YAC152F7; YAC230E8 is proximal to YAC152F7 and encompasses the *carbonyl reductase* region. In the Morris water maze, YAC152F7tel transgenic mice are normal in the visible and hidden platform tests, mildly impaired in probe test crossings, and severely impaired in the reverse versions of the tasks that assess learning flexibility. YAC230E8 transgenic mice are normal in both nonspatial and spatial learning but have deficits in learning flexibility.

Overall, the learning deficits of YAC152F7tel transgenic mice in the Morris water maze appear to be greater than those of YAC230E8 transgenic mice but less than those of Ts1Cje mice (Fig. 5). Ts1Cje mice, in turn, are less severely impaired than are Ts65Dn (Fig. 5). Therefore, although imbalance of the *Mnbh* region has an impact on cognitive function, the learning and behavioral abnormalities of Ts1Cje cannot be attributed solely to the presence of an extra copy of *Mnbh*. Other genes in the region from below *Sod1* to *Mx1* must contribute to the spatial learning deficits.

Ts1Cje does not demonstrate the neuronal atrophy found in Ts65Dn, suggesting that the region from *App* to *Sod1* is required for this pathology to develop, as well as for the impaired performance in the visible platform task. Two interesting genes are located in this region. One is *App*, which is thought to be associated with AD pathology (31), and the other is *Grik1*, the product of which, the GluR5 kainate receptor, regulates inhibitory synaptic transmission (32).

Although the inferences just drawn from the comparison of the various partial trisomics and transgenics are consistent with the data, they must be viewed with caution. The different

strains of mice were on different genetic backgrounds and were tested at different ages. The YAC transgenic animals expressed human rather than mouse genes, and the transgenic regions were not in their normal locations in the genome. The apparatus and paradigms used to test the Ts65Dn animals are different from those used to test Ts1Cje and the YAC transgenic mice, although the latter two were, in fact, tested with the same apparatus and paradigms. To make definitive conclusions about the differences among the trisomic, transgenic, and control strains under discussion, they will have to be bred on the same genetic background and to be tested at the same age with the same protocols and apparatus. Furthermore, to assess the specific contributions of genes in the *App* to *Sod1* region to the phenotype of Ts65Dn, it will be necessary to generate animals trisomic for just this region and to test them in parallel with Ts65Dn and Ts1Cje (2). This can be done by breeding Ts65Dn females with balanced T(12;16)1Cje males, which will generate all of the desired genotypes, including Ts65Dn, Ts1Cje, segmental trisomy for *App* to *Sod1*, and diploid controls.

As the mouse models for DS have progressed from the original trisomy 16, with its severe abnormalities affecting many organs and systems, to the partial trisomies Ts65Dn and Ts1Cje and the 21q22.2 YAC transgenics, attention has focused principally on the brain as the chief site of pathology. How relevant then are the neuronal and behavioral abnormalities in these animal models to the situation in DS? We believe that it would be premature, at this time, to attempt to draw any strict parallels between the cognitive deficits in human being with DS and the learning, behavioral, and structural abnormalities present in trisomic and transgenic mice. More has to be done in both species to define precisely the nature of the deficits that are present. Nevertheless, the animal models will permit us to gain insight into the chromosomal regions and the genes they contain (which can be considered as candidate genes) that have the capability of perturbing neuronal structure and function when present in an extra dose, and the mechanisms by which these perturbations occur are likely to be relevant to the human situation. Furthermore, as has been demonstrated by the reversal of the atrophy of BFCNs in trisomy 16 by the treatment with nerve growth factor (5) and the improvement of the spatial learning deficits in Ts1Cje by previous training in this study, these animal models can be used to assess potential pharmacological agents and various forms of environmental enrichment for their ability to improve learning and behavior.

We thank R. Gacayan and M. Doan for excellent animal care, A. M. Gillespie for technical assistance, A. G. Reaume, R. H. Reeves, N. P. Carter, G. Brodsky, D. Patterson, X.-N. Chen, and J. R. Korenberg for DNA probes, L. F. Reichardt for the REX antibody, and C. Chen, M. Schonberg, G. Mohapatra, D. Pinkel, J. W. Gray, L. H. Tecot, and L. S. Crnic for advice, and P. Bacchetti for statistical analysis. This work was supported by National Institutes of Health Grants AG08938, HD31498, and HL18574, and by grants from the Adler Foundation and the McGowan Charitable Trust.

1. Epstein, C. J. (1995) in *The Metabolic and Molecular Bases of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), 7th Ed., pp. 749–794.
2. Epstein, C. J. (1986) *The Consequence of Chromosome Imbalance: Principles, Mechanisms, and Models* (Cambridge Univ. Press, New York).
3. McCormick, M. K., Schinzel, A., Petersen, M. B., Stetten, G., Driscoll, D. J., Cantu, E. S., Tranebjaerg, L., Mikkelsen, M., Watkins, P. C. & Antonarakis, S. E. (1989) *Genomics* **5,** 325–331.
4. Korenberg, J. R., Chen, X. N., Schipper, R., Sun, Z., Gonsky, R., Gerwehr, S., Carpenter, N., Daumer, C., Dignan, P., Disteche, C., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4997–5001.
5. Holtzman, D. M., Li, Y., Chen, K., Gage, F. H., Epstein, C. J. & Mobley, W. C. (1993) *Neurology* **43,** 2668–2673.
6. Cox, D. R., Smith, S. A., Epstein, L. B. & Epstein, C. J. (1984) *Dev. Biol.* **101,** 416–424.
7. Davisson, M. T., Schmidt, C., Reeves, R. H., Irving, N. G., Akeson, E. C., Harris, B. S. & Bronson, R. T. (1993) *Prog. Clin. Biol. Res.* **384,** 117–133.
8. Reeves, R. H., Irving, N. G., Moran, T. H., Wohn, A., Kitt, C., Sisodia, S. S., Schmidt, C., Bronson, R. T. & Davisson, M. T. (1995) *Nat. Genet.* **11,** 177–184.
9. Holtzman, D. M., Santucci, D., Kilbridge, J., Chua-Couzens, J., Fontana, D. J., Daniels, S. E., Johnson, R. M., Chen, K., Sun, Y., Carlson, E., *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93,** 13333–13338.
10. Coussons-Read, M. E. & Crnic, L. S. (1996) *Behav. Genet.* **26,** 7–13.
11. Coyle, J. T., Oster-Granite, M. L., Reeves, R. H. & Gearhart, J. D. (1988) *Trends Neurosci.* **11,** 390–394.
12. Smith, D. J., Stevens, M. E., Sudanagunta, S. P., Bronson, R. T., Makhinson, M., Watabe, A. M., O'Dell, T. J., Fung, J., Weier, H. U. G., Chen, J. F. & Rubin, E. M. (1997) *Nat. Genet.* **16,** 28–36.
13. Huang, T. T., Yasunami, M., Carlson, E. J., Gillespie, A. M., Reaume, A. G., Hoffman, E. K., Chan, P. H., Scott, R. W. & Epstein, C. J. (1997) *Arch. Biochem. Biophys.* **344,** 424–432.
14. Pittler, S. J. & Baehr, W. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8322–8326.
15. Shi, Y. P., Huang, T. T., Carlson, E. J. & Epstein, C. J. (1994) *Mamm. Genome* **5,** 337–341.
16. Telenius, H., Carter, N. P., Bebb, C. E., Nordenskjold, M., Ponder, B. A. J. & Tunnacliffe, A. (1992) *Genomics* **13,** 718–725.
17. Reaume, A. G., Howland, D. S., Trusko, S. P., Savage, M. J., Lang, D. M., Greenberg, B. D., Siman, R. & Scott, R. W. (1996) *J. Biol. Chem.* **271,** 23380–23388.
18. Staeheli, P., Haller, O., Boll, W., Lindenmann, J. & Weissmann, C. (1986) *Cell* **44,** 147–158.
19. Rabbitts, P., Impey, H., Heppell-Parton, A., Langford, C., Tease, C., Lowe, N., Bailey, D., Ferguson-Smith, M. & Carter, N. (1995) *Nat. Genet.* **9,** 369–375.
20. Morris, R. G. M., Garrud, P., Rawlins, J. N. P. & O'Keefe, J. (1982) *Nature (London)* **297,** 681–683.
21. Weskamp, G. & Reichardt, L. F. (1991) *Neuron* **6,** 649–663.
22. Nasir, J., Floresco, S. B., O'Kusky, J. R., Diewert, V. M., Richman, J. M., Zeisler, J., Borowski, A., Marth, J. D., Phillips, A. G. & Hayden, M. R. (1995) *Cell* **81,** 811–823.
23. Eilam, D. & Golani, I. (1989) *Behav. Brain Res.* **34,** 199–211.
24. Geyer, M. A., Russo, P. V. & Masten, V. L. (1986) *Pharmacol. Biochem. Behav.* **25,** 277–288.
25. Mahon, K. A., Overbeek, P. A. & Westphal, H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1165–1168.
26. Sandin, J., Georgieva, J., Schött, P. A., Ogren, S. O. & Terenius, L. (1997) *Eur. J. Neurosci.* **9,** 194–197.
27. Whishaw, I. Q. & Kolb, B. (1984) *Behav. Brain Res.* **11,** 123–134.
28. Yates, C. M., Simpson, J., Gordon, A., Maloney, A. F. J., Allison, Y., Ritchie, I. M. & Urquhart, A. (1983) *Brain Res.* **280,** 119–126.
29. Chen, H., Chrast, R., Rossier, C., Gos, A., Antonarakis, S. E., Kudoh, J., Yamaki, A., Shindoh, N., Maeda, H., Minoshima, S. & Shimizu, N. (1995) *Nat. Genet.* **10,** 9–10.
30. Song, W. J., Sternberg, L. R., Kasten-Sportes, C., Van Keuren, M. L., Chung, S. H., Slack, A. C., Miller, D. E., Glover, T. W., Chiang, P. W., Lou, L. & Kurnit, D. M. (1996) *Genomics* **38,** 331–339.
31. Selkoe, D. J. (1996) *J. Biol. Chem.* **271,** 18295–18298.
32. Clarke, V. R. J., Ballyk, B. A., Hoo, K. H., Mandelzyz, A., Pellizzari, A., Bath, C. P., Thomas, J., Sharpe, E. F., Davies, C. H., Ornstein, P. L., *et al.* (1997) *Nature (London)* **389,** 599–603.