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Cell proliferation is reduced in the dentate gyrus of aged but not young Ts65Dn mice, a model of Down syndrome

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Abstract

Reduced number of neurons is a common feature in Down's syndrome (DS) brains. Since reduced neuronal number also occurs in the dentate gyrus of Ts65Dn mice (TS), a model for DS, hippocampal cell proliferation and survival were analyzed in young and old TS mice. For evaluating proliferation and survival, half of the mice were sacrificed 1 day, and the other half 30 days after the last bromodeoxyuridine injection, respectively. No difference was found in the number of proliferating or surviving cells of young TS and control mice. An age-associated decline in total cell number and density has been found in both genotypes, this decline being more pronounced in TS animals. Thus, aged TS mice showed reduced cell proliferation and density of surviving cells compared to CO mice. Due to the putative involvement of newborn cells in the dentate gyrus in learning processes, the reduced proliferative capacity found in TS mice could be involved in the cognitive problems found in this model of Down syndrome.

Keywords: Down's syndrome; Ts65Dn; Neurogenesis; Aging

A widely recognized feature of Down's syndrome (DS) is the reduction in the number of neurons in several brain regions, including hippocampus [9]. Ts65Dn mice (TS) are trisomic for the region of mouse chromosome 16 orthologous to human chromosome 21, spanning the region just proximal to Gabpa/App cluster to Znf295 (~23 Mb). As a genetic model for DS, the TS mouse shares many neural phenotypic characteristics of DS, including alterations in neural morphology [12,13,16], neurochemistry [5], electrophysiology [20], and cognition [7,12]. It also provides the opportunity to study underlying neurodevelopmental pathology.

Reduced hippocampal neurons have been found in the dentate gyrus of TS mice [13,16]. In this regard, it is noteworthy that the hippocampus is one of the regions where new neurons are born in adult mammalian brain [14,15,25,26]. In the dentate gyrus, stem-cell proliferation takes place in the subgranular zone (SGZ) at the border between the granule cell layer (GCL) and hilus. The multipotent progenitor cells continuously divide and give rise to neurons, astrocytes and oligodendrocytes. Newborn neurons migrate into the GCL and establish functional connexions in the dentate molecular region [26]. Therefore, it could be hypothesized that the reduction of neuronal density in the dentate gyrus of TS mice could be related to a restricted degree of neurogenesis. This would be of special relevance, as newborn neurons are likely to be involved in the establishment of long-term potentiation (LTP) and to contribute to spatial learning and memory, since hippocampal neurogenesis is important for spatial learning and memory, not only in early stages but also in adulthood and aging [19,22,24]. In fact, manipulations that enhance neurogenesis in the dentate gyrus, such as voluntary running and environmental enrichment, also enhance learning and LTP [14,15,25,26]. Interestingly, both, LTP and learning are deteriorated in TS mice [7,23].

The aim of the present study was to analyze whether neurogenesis is also present in TS mice. Since early aging is a recognized feature of DS, the study was extended to compare neurogenesis in young and adult TS mice. For this purpose, cell proliferation and survival was analyzed in the dentate

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gyrus of young and old TS mice through labeling with bromodeoxyuridine (BrdU).

Male TS mice were bred in the Faculty of Medicine colony, from Ts65Dn females and B6EiC3HF1 male breeders provided by the Robertsonian Chromosome Resources (The Jackson Laboratory, Bar Harbor, Maine, USA). Control mice (CO) were the non-trisomic littermates. In order to determine the presence of trisomy, animals were cariotyped at the age of 6–8 weeks following the method of Davisson and Akeson [5]. Cell proliferation and survival were studied in 34 young (3–5 months) and 44 old (13–15 months) mice. Half the animals were used to evaluate cell proliferation and the other half to evaluate cell survival.

BrdU was administered at the age of 3–5 months for young mice, and at 13–15 months for old mice. All mice received one daily i.p. injection of 50 μg/g of BrdU in sterile 0.9% NaCl solution for 12 days. To evaluate cell proliferation and survival, animals were sacrificed and perfused with 4% paraformaldehyde on days 1 and 30 after the last injection, respectively.

After perfusion, all brains were post-fixed overnight in paraformaldehyde at 4 °C and transferred into 30% sucrose. Coronal sections of 40 μm thickness were cut in a cryostat. Free-floating sections were used in the determination of BrdU-labeling. Every sixth 40 μm section was used.

BrdU inmunohistochemistry was performed as described by Malberg et al. [19]. DNA denaturation was conduced by incubating sections for 2 h in 50% formamide/2× SSC at 65 °C, followed by several PBS rinses. Sections were then incubated for 30 min in 2N HCl and then 10 min in boric acid. After washing with PBS, sections were incubated for 30 min in 1% H_2O_2. After blocking with PBS-TS (PBS, 5% goat serum, 0.1% Triton X-100), cells were incubated with monoclonal mouse anti-BrdU (Roche, 1:600) overnight. Sections were then incubated for 90 min with biotinylated donkey anti-mouse IgG (Vector Laboratories, 4 μg/ml) followed by amplification with an avidin–biotin complex ABC Elite reagent (Vector Laboratories) and diaminobenzidine was used as chromogen (Vector Laboratories).

Sampling of cell proliferation was done through the whole extent of the SGZ, whereas cell survival was assayed throughout the GCL and SGZ in its rostrocaudal extension. The resulting number of BrdU-positive cells was related to the granule cell layer volume multiplying the value by 6, because every sixth section has been used.

To evaluate cell density, the GCL area was determined on sections of the tissue adjacent to those used to evaluate cell survival, stained with cresyl-violet. BrdU-positive cells were detected using an optical microscope (Axiolab, ZEISS) and analyzed with Axiovision AC 4.1 (ZEISS) software. The volume was calculated considering section thickness and number.

All animal procedures met the guidelines of the European Communities Directive 86/609/EEC regulating animal research.

ANOVAs were performed to analyze total cell number and density followed by Bonferroni post hoc tests to analyze individual differences between groups. All the analysis was done using SPSS for Windows, version 11.0.

In the young mice, the number of proliferating cells in TS mice was not significantly different from that of CO mice as shown in Figs. 1A, 2A and B. Similarly, there was no significant difference in the number of BrdU-positive cells that survived one month after treatment (survival) in young TS and CO mice [ANOVA 'genotype': $F(1,33) = 0.0$, n.s.].

In old animals, TS mice showed significantly lower cell proliferation compared to CO [$F(1,43) = 6.2$, $p < 0.05$]. However, no significant difference was found in cell survival between aged TS and CO mice (Figs. 1B, 2C and D).

During their lifespan, a significant fall in proliferation [ANOVA 'age': $F(F(1,38) = 65.52)$, $p < 0.001$] and survival [$F(1,40) = 51.65$, $p < 0.001$], was observed in all groups (Table 1). A larger number of BrdU-labeled cells were observed in young CO (Fig. 2A) and TS (Fig. 2B) animals when compared to aged mice (Fig. 2C and D). Table 1 shows that TS mice presented a larger reduction of the proliferative capacity than CO mice (87.9% in TS and 73.4% in CO mice), whereas decrease in cell survival was similar in both groups (75.7% in TS and 77.0% in CO mice).

Changes in GCL volume were found between the different groups of young and old TS and CO mice [$F(3,31) = 10.44$,

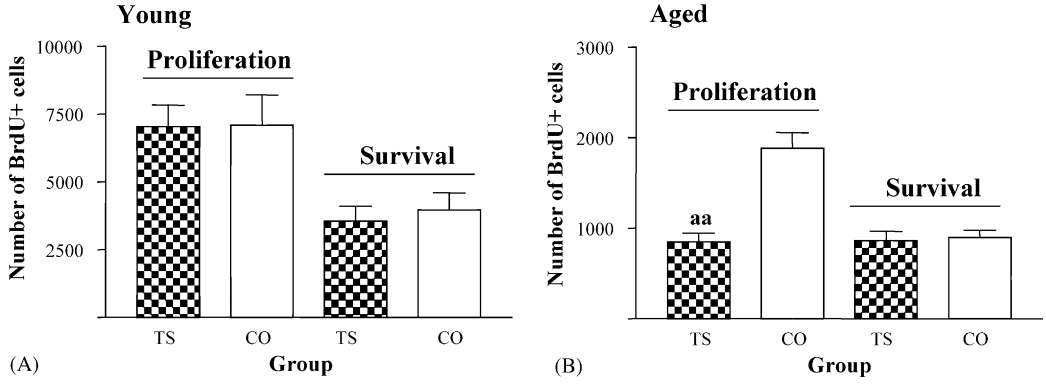

Fig. 1. Mean ± S.E.M. of the number of newborn and surviving BrdU-positive cells in the hippocampal dentate gyrus of young (A) and aged (B) TS and control mice. $^{aa}p < 0.01$ TS vs. CO, Bonferroni tests after significant ANOVA.

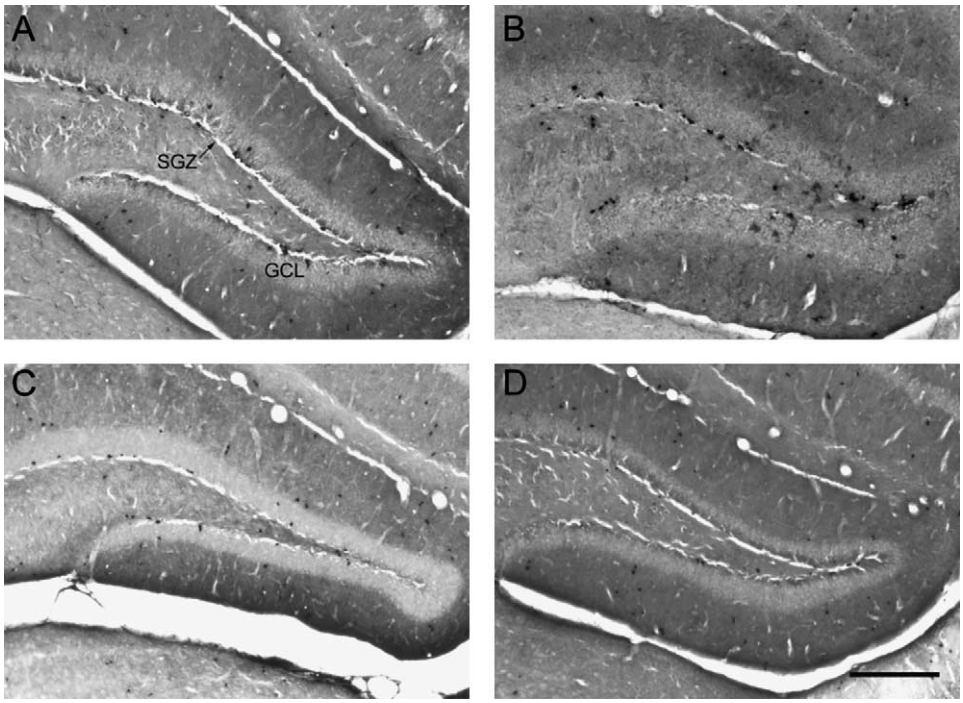

Fig. 2. Representative photomicrographs of BrdU immunohistochemistry of the dentate gyrus of young CO (A) and TS (B) and aged CO (C) and TS (D) mice. GCL, granule cell layer; SGZ, subgranular zone. Bar = 100 μm.

$p < 0.001$]. Granular cell layer volume was slightly but significantly smaller in young TS than in CO mice (TS: $0.00376 \pm 0.000103\,mm^3$; CO: $0.00453 \pm 0.000169\,mm^3$). However, no significant differences between both groups were found in old mice (TS: $0.003640 \pm 0.000287\,mm^3$; CO: $0.003060 \pm 0.000139\,mm^3$), due to the fact that age produced a decrease in volume in CO mice but not in TS mice.

As shown in Fig. 3, young TS and CO mice did not differ in BrdU-positive cell density, but aged TS mice showed a decrease in this parameter. Aging produced a pronounced fall in both genotypes [$F(3,31) = 7.29$, $p < 0.01$].

In spite of the genetic disturbance, young TS mice showed normal proliferative capacity in the dentate gyrus. This finding is consistent with the reported normal number of neurons existing in early stages of DS [9].

On the other hand, aged TS mice showed reduced proliferative capacity with respect to CO mice. This reduced proliferation might be involved in the regressive changes in the neuronal density described in the dentate gyrus of TS mice [4], since a faster degenerative process not balanced by concomitant proliferation may account for the reduced number of neurons in the dentate gyrus of TS mice previously reported [13,16]. Since newborn neurons are likely to be involved in the establishment of long-term potentiation (LTP) and to contribute to spatial learning and memory [19,22,24], this reduced proliferation may also account for the deteriorated LTP and impaired learning found in TS mice [7,23].

Furthermore, an age-associated decline has been found in total cell number and density in both groups of mice that was far more severe in TS mice. It is well-known that hippocampal adult neurogenesis decreases with age [14]. Several other changes have been described in the aging hippocampus such as neuronal cell loss [8], reduced synaptic density [3], and

Table 1
Changes in proliferative and surviving capacity of cells in the dentate gyrus of TS and CO mice throughout the lifespan

	Young	Aged	Percent reduction	t, p
Proliferation				
TS	7025.7 ± 795.7	847.2 ± 99.34	87.94	7.7, <0.001
CO	7083.9 ± 1112	1882.9 ± 173.9	73.41	4.62, <0.01
Survival				
TS	3555.3 ± 544.1	863.0 ± 103.8	75.72	4.86, <0.01
CO	3960.1 ± 628.2	898.6 ± 77.09	77.03	4.83, <0.001

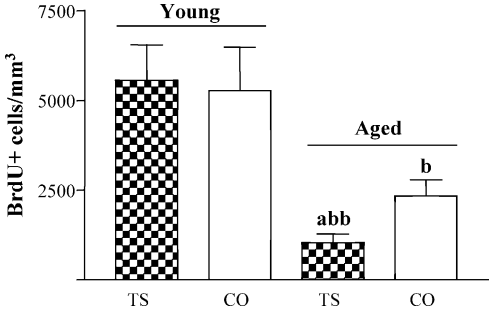

Fig. 3. Mean ± S.E.M. of the density of BrdU-positive cells in the hippocampal dentate gyrus of young and aged TS and control mice. $^{a}p < 0.05$; TS vs. CO; $^{b}p < 0.05$; $^{bb}p < 0.01$ young vs. aged, Bonferroni tests after significant ANOVA.

decreased glucose utilization [10] that may account for the age-related functional decline in the dentate gyrus.

Several factors have been implicated in the control of adult mammalian neurogenesis, including growth factors, hormones and neurotransmitters [18]. Among those, the positive role of BDNF, through specific TrkB receptors and subsequent activation of MAP kinases pathway, as well as the involvement of the cAMP cascade/phosphorylated cAMP response element (pCREB) in the regulation and survival of newborn hippocampal neurons should be considered [17,21], because substantial deficits in the adenylyl cyclase production in response to neurotransmitters and forskolin, and failure in BDNF signaling have been demonstrated in TS mice [1,6].

Although the physiologic significance of hippocampal neurogenesis is not known, it has been proposed that it is implicated in continuous learning and memory processes. Manipulations that enhance the number of BrdU-labeled cells in the dentate gyrus such as running and enriched environment, are associated with improved performance of hippocampal-dependent learning tasks [15,25], whereas factors that inhibit new cell production such as adrenal steroids, stress and aging, are associated with impaired performance on hippocampal-dependent tasks [2,11]. It is conceivable that the reduced proliferative capacity in TS mice might account for their hippocampal-dependent learning deficits [7,12,20].

The total number of cells persisting one month after treatment did not differ statistically between CO and TS mice, regardless of age. In spite of the reduced GCL volume described in young TS mice, cell density did not differ between young TS and CO mice. However, the density of surviving BrdU-labeled cells was reduced in aged TS mice, probably due to the slight but not significant increase in GCL volume. Aging produced a significant decrease in GCL volume in CO but not in TS mice. Interestingly, while in aged CO mice only 50% of the newborn cells survived one month later, in the aged TS mice the number of proliferating cells was very similar to the number of surviving cells. This effect might be due to differences in cell migration processes between TS and CO mice; the newborn cells in CO mice might migrate sooner to adjacent zones, whilst in TS mice they might stay longer in the GCL of the dentate gyrus. In this regard, the existence of a low number of total newborn cells in the hippocampus of TS aged mice could also limit the migration process in these animals. Changes in other processes such as a reduced apoptosis or increased gliosis in TS mice should also be considered. However, since the phenotype of these mature cells was not identified, it is not possible to ascertain possible variations among the different groups of mice in cellular survival of neurons, glia and astrocytes. A correct interpretation of ongoing processes will require the precise identification of the maturating cells, a goal that goes clearly beyond the scope of the present study.

In conclusion, no difference was found in cell proliferation or survival in the dentate gyrus of young male TS mice, compared to CO littermates; however, aged TS mice showed less proliferative capacity than CO mice and less density of surviving cells. Future work should analyze the proportion of these cells that differentiate into neurons or astrocytes.

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