Cell Transplantation, Vol. 20, pp. 371–379, 2011
Printed in the USA. All rights reserved.
Copyright © 2011 Cognizant Comm. Corp.

0963-6897/11 $90.00 + .00
DOI: 10.3727/096368910X528085
E-ISSN 1555-3892
www.cognizantcommunication.com

Neural Stem Cells Reduce Hippocampal Tau and Reelin Accumulation in Aged Ts65Dn Down Syndrome Mice

D. S. Kern,* K. N. Maclean,* H. Jiang,* E. Y. Synder,† J. R. Sladek, Jr.,*‡ and K. B. Bjugstad*

*Department of Pediatrics, University of Colorado Denver, Aurora, CO, USA
†Program in Stem Cell and Regenerative Biology, Burnham Institute for Medical Research, La Jolla, CA, USA
‡Department of Neurology, University of Colorado Denver, Aurora, CO, USA

Tau accumulation, in the form of neurofibrillary tangles (NFT), is an early neuropathological characteristic of Alzheimer's disease (AD) and early onset AD frequently seen in Down syndrome (DS). We investigated the presence of tau accumulation in the brains of aging DS mice using the Ts65Dn mouse model. All aged mice appeared to have substantial clusters of extracellular granules that were positive for tau and reelin, but not for amyloid-β or APP. These clusters were found primarily in CA1 of the hippocampus. In addition, the aged trisomic DS mice had a significantly greater accumulation of extracellular tau/reelin granular deposits compared to disomic littermates. These granules were similar to those described by others who also found extracelluar proteinous granules in the brains of non-DS mice engineered to model aging and/or AD. When neural stem cells (NSC) were implanted unilaterally into the hippocampus of the Ts65Dn mice, the tau/reelin-positive granules were significantly reduced in both trisomic and disomic mice. Our findings indicate that changes in tau/reelin-positive granules could be used as an index for neuropathological assessment in aging DS and AD. Furthermore, changes in granule density could be used to test the efficacy of novel treatments, such as NSC implantation. Lastly, it is speculated that the unique abilities of NSC to migrate and express growth factors might be a contributing factor to reducing tau/reelin accumulation in aging DS and AD.

Key words: Tau; Down syndrome; Alzheimer's disease; Neural stem cells; Ts65Dn mice; Reelin

INTRODUCTION

The hallmarks of Alzheimer's disease (AD), amyloid-β (Aβ) plaques, tau-based neurofibrillary tangles (NFT), and cognitive decline, are present in approximately half of the individuals with Down syndrome (DS) prior to the age of 50 (7,20,21,40,59,64). The relationship between DS and AD pathology might be related to the triplication and subsequent overexpression of certain genes on chromosome 21, including amyloid precursor protein (*APP*) and superoxide dismutase 1 (*SOD1*) (39). The distal arm of mouse chromosome 16, which contains more than 80% of gene homologues for human chromosome 21, is triplicated in the Ts65Dn mouse model of DS (1). This partially trisomic mouse recapitulates many characteristics of DS and AD, including age-specific cognitive decline, neuronal cell loss, and decreased levels of nerve growth factor (NGF) (22,24,25). These mice also have elevated expression of APP and Aβ, but plaque formation and tau accumulation have not previously been reported (24,54).

Over the years, several mouse models of AD have reported clustered granules in multiple brain areas, but specifically in the hippocampal stratum radiatum/lacunosum (Rad/LM) of CA1 (2,9,27,29,33). These "enigmatic" granules are extracellular and appear to be an accumulation of matrix proteins, dystrophic neurites, as well as tau and reelin proteins (10,34,44). Because aspects of DS appear to be closely related to AD, we examined the brains of aged Ts65Dn trisomic and disomic littermates for the presence of tau/reelin-positive granules as seen in some AD murine models.

Although neural stem cells (NSCs) are typically used as neuronal replacements, there is a growing appreciation that in the brain NSCs migrate to sites of damage, provide neuroprotection, and promote axonal growth through neurotrophin release (3,4,27,38,65). Because of these characteristics, we hypothesized that NSCs could

Received June 21, 2010; final acceptance August 2, 2010. Online prepub date: September 27, 2010.
Address correspondence to Kimberly B. Bjugstad, Ph.D., Department of Pediatrics, University of Colorado at Denver, Anschutz Medical Campus, Mail Stop 8313, 12800 E. 19th Avenue, Aurora, CO 80045, USA. Tel: 303-724-3040; Fax: 303-724-3838; E-mail: Kimberly.Bjugstad@UC Denver.edu

be a viable treatment for DS or DS with early onset of AD. We explored the possible therapeutic effects of NSCs implanted into the hippocampus on the presence of the tau/reelin-positive accumulations. We found that aged Ts65Dn trisomic mice have increased numbers of clustered extrasomatic tau/reelin-positive granules within the hippocampus compared to age-matched disomic controls. Furthermore, we present data that suggest that tau/reelin-containing granules significantly decrease in number in the presence of transplanted NSCs, indicating a possible use of NSC implantation in DS and AD to reduce the development of AD neuropathology.

MATERIALS AND METHODS

Twelve-month-old male Ts65Dn mice, both DS trisomic and their non-DS disomic littermates, were bred and maintained according to previously published reports and approved by the institute's Animal Care and Use Committee (26,54). Mice were implanted unilaterally with 500,000 viable disomic murine neural stem cells (mNSCs) into the hippocampus (coordinates: −2.0 mm AP, −2.0 mm ML, 1.5 mm DV relative to bregma). Prior to transplant, the mNSCs were labeled with a 10 μM solution of 5-bromo-2-deoxyuridine (BrdU) for postmortem identification through BrdU immunohistochemistry (BD Biosciences). As a control, some animals were sham implanted with an equal volume of saline (4 μl) rather than mNSCs. The experimental groups were: disomic/saline ($n = 6$), trisomic/saline ($n = 5$), disomic/mNSCs ($n = 6$), and trisomic/mNSCs ($n = 7$).

For implantation, the mNSC clone C17.2 line was used and cultured as a monolayer according to previously published reports (56,57). This is a well-characterized, stable clonal population of engraftable NSCs that has been used successfully for transplantation, integration, and therapeutic benefit in many animal models of neurological disorders (35,51,52). The C17.2 line was originally generated by overexpressing *myc*, via retroviral transduction, from NSCs isolated from neonatal mouse hindbrain ventricular zone (6,32,51). The self-renewal gene *myc* preserves multipotency, self-renewal, and the undifferentiated state of the cells in vitro, but is downregulated upon contact, engraftment, and differentiation (6,14,45). The C17.2 cell line fulfills the strict operational definition of a stem cell including enhanced expression of established induced "stemness" genes (6, 45,51,52). They are nestin positive, are mitogenic to EGF (an effect that can be augmented by bFGF), and they can be maintained and proliferate in bFGF alone in place of serum (41). Thus, these cells faithfully represent and emulate the biology of endogenous NSCs, as well as the NSCs propagated using different techniques (including those used to isolate, expand, and passage NSCs as neurospheres) (51,52).

One month after implant of mNSCs, mice were euthanized by anesthetic overdose and perfusion fixed with 4% paraformaldehyde. The brains were removed, paraffin embedded, and cut into 20-μm coronal sections. Immunohistochemistry was done using primary antibodies against MAP2 (for neurons, 1:500; Millipore AB5622), GFAP (for astrocytes, 1:500; Millipore MAB360), Tau5 (1:100; InvitrogenAHB0042), reelin (1:50; Millipore MAB5364), Aβ (1:500; Millipore AB5571), APP (22c11) (1:500; Millipore MAB348), APP-Kpi domain (1:100; Millipore AB5302), and BrdU (1:50; BD Biosciences #347580). Tissues exposed to anti-APP-Kpi underwent a citrate buffer antigen retrieval step and those exposed to anti-BrdU had a 2N HCl permeablization step. Endogenous protiens, peroxidases, and biotin were blocked using appropriate blocking buffers prior to the addition of the primary antibodies. Negative control sections omitted the use of primary antibodies. Some sections were also stained using cresyl violet for contrast and Periodic acid-Schiff (PAS), which identifies extracellular matrix carbohydrate macromolecules.

The locations of protein accumulation, in clusters of extrasomatic granules, were identified from digital images taken at 20× magnification throughout the rostral–caudal extent of the hippocampus. Brain sections, 11–14 per animal, were examined bilaterally. This analysis was performed by an observer who was blinded to both the genotype and the type of transplant. Clusters of protein accumulations were counted in seven areas within the hippocampus: the three layers of CA1 and CA3 (Rad/LM, the pyramidal cell layer, and stratum oriens), and the dentate gyrus (DG). A four-way mixed-model ANOVA was used to determine statistically significant differences. Comparisons were made between genotype (trisomic vs. disomic), transplant (saline vs. mNSCs), the implanted versus unimplanted side, and lastly between the seven hippocampal areas. Significance was defined by a value of $p < 0.05$, and post hoc analyses were done using a Fisher's least significant difference test.

RESULTS AND DISCUSSION

Clusters of granulated protein accumulation were found in both aged (13 months) disomic and trisomic Ts65Dn mice (Fig. 1). Extrasomatic granules were located primarily in the hippocampus and olfactory bulb (Fig. 1A, B). Occasional clustered granules were evident within the cortex, cerebellum, and pons, but these were not quantified. Granules described here in the aged trisomic and disomic Ts65Dn mice appear to be similar in size (3.15 ± 0.17 μm diameter), appearance, and location as granules described in aged C57BL/6 mice and in murine AD models (i.e., SAM P8, AD11, SynGAP, and 3xTgAD) (2,9,28,33).

Immunohistochemistry detected a strong tau and a

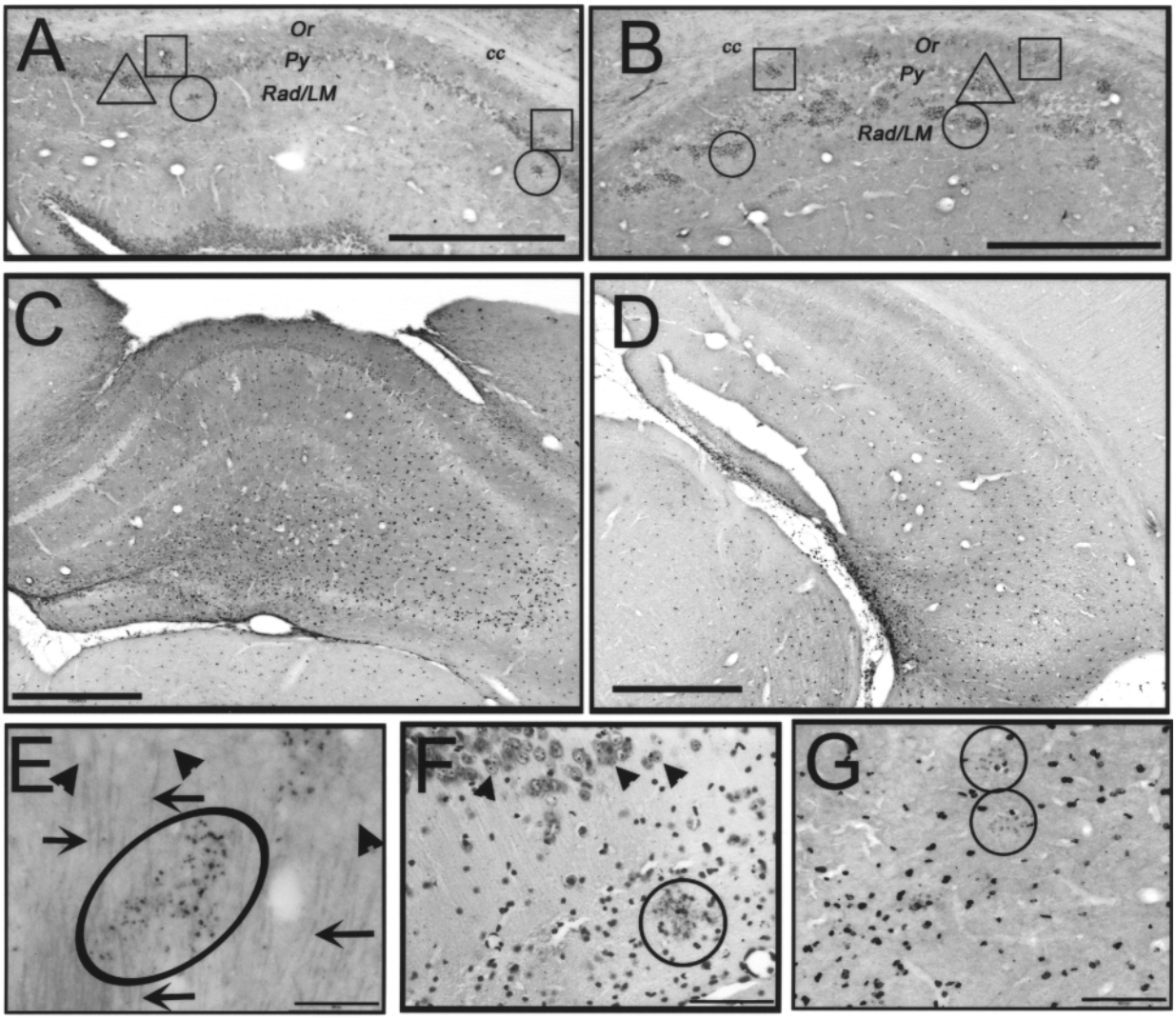


Figure 1. Protein accumulations in the form of clustered granules were present in the hippocampus of disomic and trisomic Ts65Dn mice. (A, B) Low magnification of CA1 in the hippocampus labeled with the Tau5 monoclonal antibody of 13-month-old disomic (A) and trisomic (B) Ts65Dn mice revealed tau-positive granules in clusters primarily in the Rad/LM layer of CA1 (circles), but which could also be found in Py of CA1 (triangles), and in the Or of CA1 (squares). (C, D) Double labeling of the ipsilateral hippocampus against Brdu (black), to identify the mNSC, and MAP2 (gray), to identify neurons. The mNSCs appear to migrate generously throughout all layers of the hippocampus in the disomic brain (C) and in the trisomic brain (D). (E) Higher magnification of Tau5-labeled granules (circled) interspersed in ascending dendrites (arrows) of CA1 neurons whose soma are in the pyramidal layer (arrowheads). (F) While not as intensely stained as with Tau5, granules were also positive for reelin (circled). Cresyl violet was used for contrast, revealing the pyramidal cell layer (arrowheads) and the blackish-gray reelin granules in the dendritic layer below (circled). (G) High magnification of Tau5-positive clusters (dark gray, circled) and BrdU-positive cells (black, arrows). Abbreviations: cc, corpus callosum; Or, stratum oriens; Py, pyramidal cell layer; Rad/LM, stratum radiatum and lacunosum-moleculare. Scale bars: 500 μm (A–D), 50 μm (E), 100 μm (F, G).

less strong reelin presence in the granules found in the disomic and trisomic Ts65Dn brain (Fig. 1E, F). The granules also produced a positive PAS reaction, suggesting the presence of matrix proteins, proteoglycans, and glycogen, which is consistent with an early description by Jucker et al. (28,29). The tau results, however, are in contrast with those same studies, in which tau presence was not observed; however, this variance could have resulted from different methodologies (28–30). For example, the positive granules found in the Ts65Dn brains of this present study were tagged by the monoclonal antibody Tau5, which binds both the phosphorylated and unphosphorylated states of tau (19). In the previous studies, the Tau1 antibody was used, which only binds

to unphosphorylated tau (28–30). With more than 15 potential tau phosphorylation sites it is conceivable that frequent false negatives could occur with the Tau1 antibody (58).

Recent studies from the Knuesel group (11,33,43) found reelin-positive granular accumulations in aged wild-type mice and in SynGAP and 3xTg-AD mouse models of AD. Reelin is an extracellular glycoprotein important for laminar structuring in the brain, for synaptic plasticity, and for modulating tau phosphorylation (10, 15,47); thus, it may not be surprising to find these hippocampal granules containing both reelin and tau. As such, we did find that the extracellular clusters in the disomic and trisomic brains were positive for reelin (Fig. 1F).

Immunohistochemistry against other AD markers revealed that the clustered granules did not label for Aβ or with either APP antibody. This is consistent with the findings of Jucker's group in aged mice (28,29), but inconsistent with the results from the Knuesel group, in which reelin-positive granules in their AD-transgenic mice also contained Aβ and APP (11,33,43). In the current study, Aβ immunostaining did not reveal any Aβ plaque deposition in the brains of aged Ts65Dn mice, a finding consistent with Reeves et al. (54). It is possible that the actual protein composition of these clustered granules differs between AD models engineered to express amyloid plaques, and DS models.

The tau/reelin granules in the present study were negative for MAP2 and GFAP immunoreactivity, suggesting that the granules do not contain large quantities of these proteins that are associated with neuronal and astrocytic processes, respectively. This is also consistent with previous immunohistochemical observations (28–30). However, ultrastructural analyses done by others suggest that small quantities or fractions of these proteins may still be present as dystrophic processes in and around the granules (34,44).

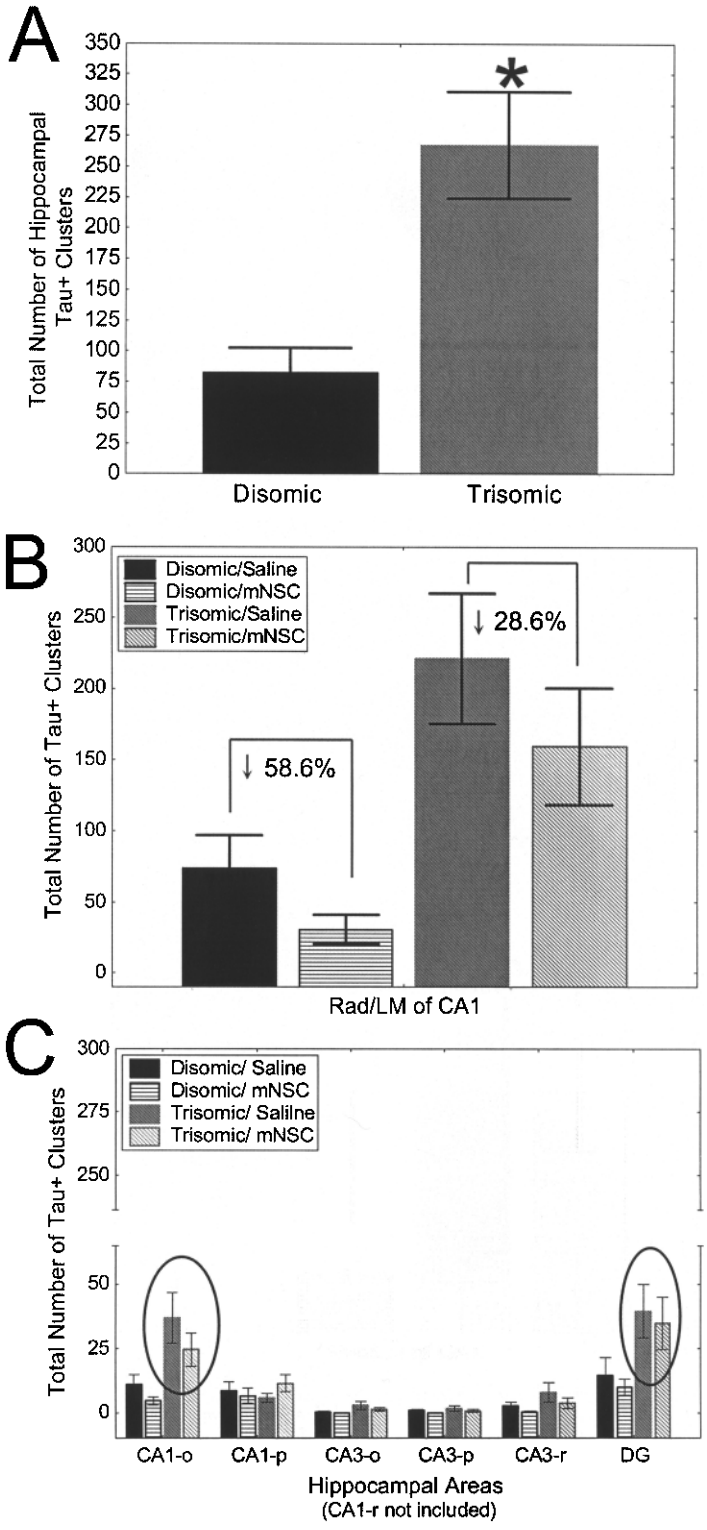
The etiology and significance of these clustered granules that are positive for tau and reelin remain unknown at this time. Electron microscopic analysis of these granules revealed a core of dense membranous material with a discontinuous outer membrane of heparin sulfate proteoglycan surrounded by degenerated axonal and dendritic processes (29,34,44). The positive PAS results found in the granules of the disomic and trisomic brains support these earlier results. Hence, the granules most likely represent a degenerative event whereby the core contains cellular debris from older cell loss surrounded by evidence of ongoing neurodegeneration.

Quantification of the clusters of tau/reelin-positive granules in the hippocampus revealed that the trisomic DS mice had as much as three times the number of clusters as their disomic littermates [genotype main effect: $F(1, 40) = 13.08$, $p < 0.01$] (Fig. 2A). The majority of hippocampal tau/reelin-positive clustered granules were located in Rad/LM of CA1 in the hippocampus ($53.97 \pm 4.99\%$ and $69.97 \pm 1.63\%$ of all clusters in the hippocampus of disomic and trisomic brains, respectively) (Fig. 1A, B). The stratum oriens and the pyramidal cell layers of CA1, the three regions of CA3, and dentate gyrus (DG) had significantly fewer tau clusters [hippocampal area main effect: $F(6, 240) = 45.31$, $p < 0.01$]. The prominent presence of granulated clusters in the Rad/LM of CA1, a dendritic layer, as opposed to layers of soma density (i.e., hippocampal pyramidal or granular cell layers) is consistent with previous reports of this phenomenon in aged and AD mouse models (2,28–30) (Fig. 1E).

Additional insight regarding the appearance of tau/reelin-positive granules is again provided by the Jucker group, who indicate that the clusters may be specific to the C57BL/6 mouse strain, as they are found infrequently in other strains (28,29,34). The Ts65Dn mouse was developed using C57BL/6 as a genetic background, which could explain their presence in the disomic littermates. In fact, many transgenic mouse models are bred onto the C57BL/6 genetic background, making it possible that the presence of these granules in other transgenic models has been underreported. Based on our data

FACING PAGE

Figure 2. Tau-positive granules were found more frequently in the trisomic brain but NSC presence induced a reduction in both disomic and trisomic brains. (A) Significantly more tau positive clusters were found in the hippocampus of the trisomic mice compared to the disomic group (mean ± SEM; $*p < 0.05$). Bars represent the mean total number of clusters found in all seven areas of the hippocampus. (B, C) The Rad/LM of CA1 was the only region to be significantly affected by the presence of mNSCs compared to the other six areas of the hippocampus (interaction between treatment and areas, $p < 0.05$). (B) In the Rad/LM of CA1, mNSCs decreased the number of clusters in both disomic and trisomic brains. While mNSCs significantly reduce the tau positive clusters, the difference as a function of genotype in the degree of reduction was similar, with a 58.6% reduction in disomic mice and a 28.6% in trisomic mice. (C) Significantly fewer tau-positive clusters were found in the remaining six areas of the hippocampus and no significant mNSC effect was found in these areas for either disomic or trisomic brains. However, one can still appreciated the elevated levels of tau positive clusters in the trisomic brain areas compared to the disomic brains, especially in CA1-o and the DG (circled bars). Abbreviations: CA1-o, stratum oriens of CA1; CA1-p, pyramidal layer of CA1, CA3-o, stratum oriens of CA3; CA3-p, pyramidal layer of CA3; CA3-r, stratum radiatum and lacunosum-moleculare (Rad/LM) of CA3; DG, dentate gyrus.


A
Total Number of Hippocampal Tau+ Clusters
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
*
Disomic
Trisomic
B
Total Number of Tau+ Clusters
300
250
200
150
100
50
0
Disomic/Saline
Disomic/mNSC
Trisomic/Saline
Trisomic/mNSC
↓ 28.6%
↓ 58.6%
Rad/LM of CA1
C
Total Number of Tau+ Clusters
300
275
250
50
25
0
Disomic/ Saline
Disomic/ mNSC
Trisomic/ Salilne
Trisomic/ mNSC
CA1-o
CA1-p
CA3-o
CA3-p
CA3-r
DG
Hippocampal Areas
(CA1-r not included)

and earlier studies by others, we know that these clustered granules, while characteristic of the C57BL/6 genetic background, are affected by certain disease states. For example, the number of clustered granules increases with age in normal C57BL/6 mice, but these granules are found with greater frequency in SAM P8 mice, leukemia model AKR mice, SynGAP mice, 3xTg-AD mice, AD11 mice, and now we find them present in the trisomic Ts65Dn mice (33,34,44).

It is tempting to suggest that the tau/reelin-positive granules are a murine homologue of human NFTs. Mice do not develop the classical intracellular "flame" of accumulated hyperphosphorylated tau indicative of NFT unless they are engineered with the human tau gene (16–18,48,55). Dense staining for hyperphosphorylated endogenous tau can be found in some hippocampal and cortical neurons of the double transgenic AD mouse which expresses the gene for the human APP mutation (*APPSw*) and a knockout of the murine gene for inducible nitric oxide synthase (*NOS2*); however, the classical NFT morphology is still absent (8,62,63).

It may also be possible that the extracellular tau accumulations found in mice are similar to the perisomatic granules found in pretangle neurons of AD patients (12, 23,53,60). The perisomatic granules appear to be proteinous aggregations juxtaposed to the soma of the pyramidal neurons in CA1, which are positive for tau but that have not developed an actual NFT. Unlike the granules found in the aged DS mice and the mouse models of AD, the perisomatic granules appear as individual granules next to the soma and not clustered within the dendritic layers, so like the NFT discussion above, it can only be speculation as to whether these granules are a murine version of what has been describe in human AD.

While the exact nature of the granules remains elusive, the currently available data strongly suggest that they are pathological, emerging as a function of age and as a consequence of disorder (33,34,44). As such, we were interested in determining if the presence of the clustered granules could be altered or reduced. To this end, aged trisomic and disomic mice were implanted with undifferentiated mNSC or with a control volume of saline.

Differences in tau/reelin clusters between mNSC- and saline-implanted brains as a function of the hippocampal area suggest that mNSCs significantly reduce tau/reelin-positive granules in the Rad/LM of CA1 in both the disomic and trisomic littermates [two-way interaction between transplant and area: $F(6, 240) = 2.28$, $p < 0.05$]. Genotype did not add any additional influence on the interaction between treatment and hippocampal area, suggesting that each group benefitted from the mNSC implants to a statistically equivalent degree [three-way interaction effect: $F(6, 240) = 0.11$, $p = 0.99$]. Trisomic mice had a 28.6% reduction in the number of tau/reelin-positive granules in the Rad/LM of CA1 and the disomic mice benefited by a 58.6% reduction (Fig. 2B). Clusters in other areas within hippocampal CA1, CA3, or DG were not significantly affected by the presence of mNSCs (Fig. 2C).

The mNSC were labeled with BrdU prior to implant and BrdU immunohistochemistry was used to identify the mNSCs postmortem. Visual analyses of BrdU immunostaining indicated that many BrdU-labeled cells were localized at the site of injection and within the implanted hippocampus 1 month postimplantation (Fig. 1C, D). Occasionally, these labeled cells were seen in the corpus callosum and contralateral hippocampus, but in very low numbers. Previous studies have indicated that NSCs take advantage of nearby white matter tracks to help them migrate to remote sites (42,49), so it is possible that as early as 1 month postimplant the NSCs migrated across the corpus callosum to gain access to the contralateral hippocampus. There were no apparent differences in the migration pattern of BrdU-labeled cells or in the apparent numbers migrating between disomic and trisomic brains. Double label immunohistochemistry with Brdu and MAP2 or Tau5 suggested that the mNSC did not differentiate into neurons because all BrdU-positive cells were MAP2 and Tau5 negative.

Because no significant difference in the number of tau/reelin clusters was found between the implanted and unimplanted sides of the hippocampus [main effect of side: $F(1, 40) = 0.008$, $p = 0.92$], it would suggest that the mNSCs induce effects in brain areas remote from the original injection site either by migration and/or through the release of soluble factors (36,37,41). Because large numbers of BrdU-labeled cells were not found in the contralateral hippocampus or encircling the clusters on the ipsilateral side (Fig. 1G), an argument for soluble factors is supported. The ability of mNSC to reduce tau/reelin-positive granules could be related to their ability to produce growth factors, which can regulate tau phosphorylation and facilitate healthy neurons (13,31,46). Decrease in neural growth factor synthesis is a prominent characteristic in DS, AD, and normal aging (5,25,50,61). The intrinsic production of growth factors by NSCs might be responsible for the lower number of tau/reelin-positive granules found in the hippocampus of both the aging trisomic and disomic Ts65Dn mice.

Because DS and AD affect multiple neural systems, they are difficult disorders to treat. Changes in the accumulation of extracellular tau and reelin could be a novel neural anatomical index for assessing potential treatments for AD in DS. As found with NSC treatment, the presence of these accumulations can be reduced in both the aged DS and aged control mice. Pursuant to this, future studies would investigate the relationship between the clusters of tau granules over time, their reduction in the presence of NSCs residing long term in the hippo-

campus, and the cognitive manifestation of these interactions.

ACKNOWLEDGMENTS: This work has been supported by funding from the Anna and John J. Sie Foundation, American Foundation for Aging Research, NIH R01 HD045224-03, P30 HD04024-36, and R01 HD45224-02S1, and by a gift from Ben and Marilee Doud of Evergreen, Colorado.

REFERENCES

1. Akeson, E. C.; Lambert, J. P.; Narayanswami, S.; Gardiner, K. L. J.; Bechtel, L. J.; Davisson, M. T. Ts65Dn localization of the translocation breakpoint and trisomic gene content in a mouse model for Down syndrome. Cytogenet. Cell Genet. 93:270–276; 2001.
2. Akiyama, H.; Kameyama, M.; Akiguchi, I.; Sugiyama, H.; Kawamata, T.; Fukuyama, H.; Kimura, H.; Matsushita, M.; Takeda, T. Periodic acid-Schiff (PAS)-positive, granular structures increase in the brain of senescence accelerated mouse (SAM). Acta Neuropathol. 72:124–129; 1986.
3. Bjugstad, K. B.; Redmond, Jr., D. E.; Teng, Y. D.; Elsworth, J. D.; Roth, R. H.; Blanchard, B. C.; Snyder, E. Y.; Sladek, Jr., J. R. Neural stem cells implanted into MPTP-treated monkeys increase the size of endogenous tyrosine hydroxylase-positive cells found in the striatum: A return to control measures. Cell Transplant. 14:183–192; 2005.
4. Bjugstad, K. B.; Teng, Y. D.; Redmond, Jr., D. E.; Elsworth, J. D.; Roth, R. H.; Cornelius, S. K.; Snyder, E. Y.; Sladek, Jr., J. R. Human neural stem cells migrate along the nigrostriatal pathway in a primate model of Parkinson's disease. Exp. Neurol. 211:362–369; 2008.
5. Capsoni, S.; Ugolini, G.; Comparini, A.; Ruberti, F.; Berardi, N.; Cattaneo, A. Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. Proc. Natl. Acad. Sci. USA 97:6826–6831; 2000.
6. Cartwright, P.; McLean, C.; Sheppard, A.; Rivett, D.; Jones, K.; Dalton, S. LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. Development 132:885–896; 2005.
7. Chapman, R. S.; Hesketh, L. J. Behavioral phenotype of individuals with Down syndrome. Ment. Retard. Dev. Disabil. Res. Rev. 6:84–95; 2000.
8. Colton, C. A.; Vitek, M. P.; Wink, D. A.; Xu, Q.; Cantillana, V.; Previti, M. L.; Van Nostrand, W. E.; Weinberg, J. B.; Dawson, H. NO synthase 2 (NOS2) deletion promotes multiple pathologies in a mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA 103:12867–12872; 2006.
9. Corsetti, V.; Amadoro, G.; Gentile, A.; Capsoni, S.; Ciotti, M. T.; Cencioni, M. T.; Atlante, A.; Canu, N.; Rohn, T. T.; Cattaneo, A.; Calissano, P. Identification of a caspase-derived N-terminal tau fragment in cellular and animal Alzheimer's disease models. Mol. Cell. Neurosci. 38: 381–392; 2008.
10. Deutsch, S. I.; Rosse, R. B.; Lakshman, R. M. Dysregulation of tau phosphorylation is a hypothesized point of convergence in the pathogenesis of Alzheimer's disease, frontotemporal dementia and schizophrenia with therapeutic implications. Prog. Neuropsychopharmacol. Biol. Psychiatry 30:1369–1380; 2006.
11. Doehner, J.; Madhusudan, A.; Konietzko, U.; Fritschy, J. M.; Knuesel, I. Co-localization of reelin and proteolytic AbetaPP fragments in hippocampal plaques in aged wild-type mice. J. Alzheimers Dis. 19:1339–1357; 2010.
12. Duyckaerts, C.; Delatour, B.; Potier, M. C. Classification and basic pathology of Alzheimer disease. Acta Neuropathol. 118:5–36; 2009.
13. Elliott, E.; Ginzburg, I. The role of neurotrophins and insulin on tau pathology in Alzheimer's disease. Rev. Neurosci. 17:635–642; 2006.
14. Flax, J. D.; Aurora, S.; Yang, C.; Simonin, C.; Wills, A. M.; Billinghurst, L. L.; Jendoubi, M.; Sidman, R. L.; Wolfe, J. H.; Kim, S. U.; Snyder, E. Y. Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. Nat. Biotechnol. 16: 1033–1039; 1998.
15. Frotscher, M. Dual role of Cajal-Retzius cells and reelin in cortical development. Cell Tissue Res. 290:315–322; 1997.
16. Gotz, J. Tau and transgenic animal models. Brain Res. Brain Res. Rev. 35:266–286; 2001.
17. Gotz, J.; Deters, N.; Doldissen, A.; Bokhari, L.; Ke, Y.; Wiesner, A.; Schonrock, N.; Ittner, L. M. A decade of tau transgenic animal models and beyond. Brain Pathol. 17: 91–103; 2007.
18. Gotz, J.; Streffer, J. R.; David, D.; Schild, A.; Hoerndli, F.; Pennanen, L.; Kurosinski, P.; Chen, F. Transgenic animal models of Alzheimer's disease and related disorders: Histopathology, behavior and therapy. Mol. Psychiatry 9: 664–683; 2004.
19. Guillozet-Bongaarts, A. L.; Garcia-Sierra, F.; Reynolds, M. R.; Horowitz, P. M.; Fu, Y.; Wang, T.; Cahill, M. E.; Bigio, E. H.; Berry, R. W.; Binder, L. I. Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease. Neurobiol. Aging 26:1015–1022; 2005.
20. Harman, D. Alzheimer's disease pathogenesis: Role of aging. Ann. NY Acad. Sci. 1067:454–460; 2006.
21. Head, E.; Lott, I. T.; Patterson, D.; Doran, E.; Haier, R. J. Possible compensatory events in adult Down syndrome brain prior to the development of Alzheimer disease neuropathology: Targets for nonpharmacological intervention. J. Alzheimers Dis. 11:61–76; 2007.
22. Holtzman, D. M.; Santucci, D.; Kilbridge, J.; Chua-Couzens, J.; Fontana, D. J.; Johnson, R. M.; Chen, K.; Sun, Y.; Carlson, E.; Alleva, E.; Epstein, C. J.; Mobley, W. C. Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. Proc. Natl. Acad. Sci. USA 93:13333–13338; 1996.
23. Hoozemans, J. J. M.; van Haastert, E. S.; Nijholt, D. A.; Rozemuller, A. J.; Eikelenboom, P.; Scheper, W. The unfolded protein response is activated in pretangle neurons in Alzheimer's disease hippocampus. Am. J. Pathol. 174: 1241–1251; 2009.
24. Hunter, C. L.; Bimonte-Nelson, H. A.; Nelson, M.; Eckman, C. B.; Granholm, A. C. Behavioral and neurobiological markers of Alzheimer's disease in Ts65Dn mice: Effects of estrogen. Neurobiol. Aging 25:873–884; 2004.
25. Hunter, C. L.; Isacson, O.; Nelson, M.; Bimonte-Nelson, H.; Seo, H.; Lin, L.; Ford, K.; Kindy, M. S.; Granholm, A. C. Regional alterations in amyloid precursor protein and nerve growth factor across age in a mouse model of Down's syndrome. Neurosci. Res. 45:437–445; 2003.
26. Hyde, L. A.; Crnic, L. S. Age-related deficits in context discrimination learning in Ts65Dn mice that model Down syndrome and Alzheimer's disease. Behav. Neurosci. 115: 1239–1246; 2001.
27. Imitola, J.; Raddassi, K.; Park, K. I.; Mueller, F. J.; Nieto, M.; Teng, Y. D.; Frenkel, D.; Li, J.; Sidman, R. L.; Walsh, C. A.; Snyder, E. Y.; Khoury, S. J. Directed migration of neural stem cells to sites of CNS injury by the stromal

cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc. Natl. Acad. Sci. USA 101:18117–18122; 2004.
28. Jucker, M.; Ingram, D. K. Age-related fibrillar material in mouse brain. Assessing its potential as a biomarker of aging and as a model of human neurodegenerative disease. Ann. NY Acad. Sci. 719:238–247; 1994.
29. Jucker, M.; Walker, L. C.; Kuo, H.; Tian, M.; Ingram, D. K. Age-related fibrillar deposits in brains of C57BL/6 mice. A review of localization, staining characteristics, and strain specificity. Mol. Neurobiol. 9:125–133; 1994.
30. Jucker, M.; Walker, L. C.; Schwarb, P.; Hengemihle, J.; Kuo, H.; Snow, A. D.; Bamert, F.; Ingram, D. K. Age-related deposition of glia-associated fibrillar material in brains of C57BL/6 mice. Neuroscience 60:875–889; 1994.
31. Kamei, N.; Tanaka, N.; Oishi, Y.; Hamasaki, T.; Nakanishi, K.; Sakai, N.; Ochi, M. BDNF, NT-3, and NGF released from transplanted neural progenitor cells promote corticospinal axon growth in organotypic cocultures. Spine 32:1272–1278; 2007
32. Kitchens, D. L.; Snyder, E. Y.; Gottlieb, D. I. FGF and EGF are mitogens for immortalized neural progenitors. J. Neurobiol. 25:797–807; 1994.
33. Knuesel, I.; Nyffeler, M.; Morm?de, C.; Muhia, M.; Meyer, U.; Pietropaolo, S.; Yee, B. K.; Pryce, C. R.; LaFerla, F. M.; Marighetto, A.; Feldon, J. Age-related accumulation of reelin in amyloid-like deposits. Neurobiol. Aging 30:697–716; 2009.
34. Kuo, H.; Ingram, D. K.; Walker, L. C.; Tian, M.; Hengemihle, J. M.; Jucker, M. Similarities in the age-related hippocampal deposition of periodic acid-schiff-positive granules in the senescence-accelerated mouse P8 and C57BL/6 mouse strains. Neuroscience 74:733–740; 1996.
35. Lee, P.; Jeyakumar, M.; Gonzalez, R.; Takahashi, H.; Lee, P. J.; Baek, R. C.; Clark, D.; Rose, H.; Fu, G.; Clarke, J.; McKercher, S.; Meerloo, J.; Muller, F. J.; Park, K. I.; Butters, T. D.; Dwek, R. A.; Schwartz, P.; Tong, G.; Wenger, D.; Lipton, S. A.; Seyfried, T. N.; Platt, F. M.; Snyder, E. Y. Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. Nat. Med. 13:439–447; 2007.
36. Li, J.; Imitola, J.; Snyder, E. Y.; Sidman, R. L. Neural stem cells rescue nervous Purkinje neurons by restoring molecular homeostasis of tissue plasminogen activator and downstream targets. J. Neurosci. 26:7839–7848; 2006.
37. Li, K.; Lee, S. M.; Su, R. J.; Zhang, X. B.; Yuen, P. M.; Li, C. K.; Yang, M.; Tsang, K. S.; James, A. E.; Tse, Y. H.; Ng, L. Y.; Fok, T. F. Multipotent neural precursors express neural and hematopoietic factors, and enhance ex vivo expansion of cord blood CD34+ cells, colony forming units and NOD/SCID-repopulating cells in contact and noncontact cultures. Leukemia 19:91–97; 2005.
38. Lladó, J.; Haenggeli, C.; Maragakis, N. J.; Snyder, E. Y.; Rothstein, J. D. Neural stem cells protect against glutamate-induced excitotoxicity and promote survival of injured motor neurons through the secretion of neurotrophic factors. Mol. Cell. Neurosci. 27:322–331; 2004.
39. Lott, I. T.; Head, E. Alzheimer disease and Down syndrome: Factors in pathogenesis. Neurobiol. Aging 26: 383–389; 2005.
40. Lott, I. T.; Head, E.; Doran, E.; Busciglio, J. Beta-amyloid, oxidative stress and down syndrome. Curr. Alzheimer Res. 3:521–528; 2006.
41. Lu, P.; Jones, L. L.; Snyder, E. Y.; Tuszynski, M. H. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. Exp. Neurol. 181:115–129; 2003.
42. Maciaczyk, J.; Singec, I.; Maciaczyk, D.; Klein, A.; Nikkhah, G. Restricted spontaneous in vitro differentiation and region-specific migration of long-term expanded fetal human neural precursor cells after transplantation into the adult rat brain. Stem Cells Dev. 18:1043–1058; 2009.
43. Madhusudan, A.; Sidler, C.; Knuesel, I. Accumulation of reelin-positive plaques is accompanied by a decline in basal forebrain projection neurons during normal aging. Eur. J. Neurosci. 30:1064–1076; 2009.
44. Mitsuno, S.; Takahashi, M.; Gondo, T.; Hoshii, Y.; Hanai, N.; Ishihara, T.; Yamada, M. Immunohistochemical, conventional and immunoelectron microscopical characteristics of periodic acid-Schiff-positive granules in the mouse brain. Acta Neuropathol. 98:31–38; 1999.
45. Murphy, M. J.; Wilson, A.; Trumpp, A. More than just proliferation: Myc function in stem cells. Trends Cell Biol. 15:128–137; 2005.
46. Niles, L. P.; Armstrong, K. J.; Rincón Castro, L. M.; Dao, C. V.; Sharma, R.; McMillan, C. R.; Doering, L. C.; Kirkham, D. L. Neural stem cells express melatonin receptors and neurotrophic factors: Colocalization of the MT1 receptor with neuronal and glial markers. BMC Neurosci. 5: 41–49; 2004.
47. Niu, S.; Yabut, O.; D'Arcangelo, G. J. The reelin signaling pathway promotes dendritic spine development in hippocampal neurons. J. Neurosci. 28:10339–10348; 2008.
48. Oddo, S.; Caccamo, A.; Shepherd, J. D.; Murphy, M. P.; Golde, T. E.; Kayed, R.; Metherate, R.; Mattson, M. P.; Akbari, Y.; LaFerla, F. M. Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular Abeta and synaptic dysfunction. Neuron 39:409–421; 2003.
49. Olstorn, H.; Moe, M. C.; Røste, G. K.; Bueters, T.; Langmoen, I. A. Transplantation of stem cells from the adult human brain to the adult rat brain. Neurosurgery 60:1089–1098; 2007.
50. Pallas, M.; Camins, A.; Smith, M. A.; Perry, G.; Lee, H. G.; Casadesus, G. From aging to Alzheimer's disease: Unveiling "the switch" with the senescence-accelerated mouse model (SAMP8). J. Alzheimers Dis. 15:615–624; 2008.
51. Park, K. I.; Hack, M. A.; Ourednik, J.; Yandava, B.; Flax, J. D.; Hack, M. A.; Ourednik, J.; Yandava, B.; Flax, J. D.; Stieg, P. E.; Gullans, S.; Jensen, F. E.; Sidman, R. L.; Ourednik, V.; Snyder, E. Y. Acute injury directs the migration, proliferation, and differentiation of solid organ stem cells: Evidence from the effect of hypoxia-ischemia in the CNS on clonal "reporter" neural stem cells. Exp. Neurol. 199:156–178; 2006.
52. Parker, M. A.; Anderson, J. K.; Corliss, D. A.; Abraria, V. E.; Sidman, R. L.; Park, K. I.; Teng, Y. D.; Cotanche, D. A.; Snyder, E. Y. Expression profile of an operationally-defined neural stem cell clone. Exp. Neurol. 194: 320–332; 2005.
53. Probst, A.; Herzig, M. C.; Mistl, C.; Ipsen, S.; Tolnay, M. Perisomatic granules (non-plaque dystrophic dendrites) of hippocampal CA1 neurons in Alzheimer's disease and Pick's disease: A lesion distinct from granulovacuolar degeneration. Acta Neuropathol. 102:636–644; 2001.
54. Reeves, R. H.; Irving, N. G.; Moran, T. H.; Wohn, A.; Kitt, C.; Sisodia, S. S.; Schmidt, C.; Bronson, R. T.; Davisson, M. T. A mouse model for Down syndrome exhibits learning and behaviour deficits. Nat. Genet. 11: 177–184; 1995.

55. Schwab, C.; Hosokawa, M.; McGeer, P. L. Transgenic mice overexpressing amyloid beta protein are an incomplete model of Alzheimer disease. Exp. Neurol. 188:52–64; 2004.
56. Snyder, E. Y.; Deitcher, D. L.; Walsh, C.; Arnold-Aldea, S.; Hartwieg, E. A.; Cepko, C. L. Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. Cell 68:33–51; 1992.
57. Snyder, E. Y.; Yoon, C.; Flax, J. D.; Macklis, J. D. Multipotent neural precursors can differentiate toward replacement of neurons undergoing targeted apoptotic degeneration in adult mouse neocortex. Proc. Natl. Acad. Sci. USA 94:11663–11668; 1997.
58. Szendrei, G. I.; Lee, V. M.; Otvos, Jr., L. Recognition of the minimal epitope of monoclonal antibody Tau depends upon the presence of a phosphate group but not its location. J. Neurosci. Res. 34:243–249; 1993.
59. Teipel, S. J.; Hampel, H. Neuroanatomy of Down syndrome in vivo: A model of preclinical Alzheimer's disease. Behav. Genet. 36:405–415; 2006.
60. Tolnay, M.; Probst, A. Tau protein pathology in Alzheimer's disease and related disorders. Neuropathol. Appl. Neurobiol. 25:171–187; 1999
61. Tomobe, K.; Nomura, Y. Neurochemistry, neuropathology, and heredity in SAMP8: A mouse model of senescence. Neurochem. Res. 34:660–669; 2009.
62. Wilcock, D. M.; Gharkholonarehe, N.; Van Nostrand, W. E.; Davis, J.; Vitek, M. P.; Colton, C. A. Amyloid reduction by amyloid-beta vaccination also reduces mouse tau pathology and protects from neuron loss in two mouse models of Alzheimer's disease. J. Neurosci. 29:7957–7965; 2009.
63. Wilcock, D. M.; Lewis, M. R.; Van Nostrand, W. E.; Davis, J.; Previti, M. L.; Gharkholonarehe, N.; Vitek, M. P.; Colton, C. A. Progression of amyloid pathology to Alzheimer's disease pathology in an amyloid precursor protein transgenic mouse model by removal of nitric oxide synthase 2. J. Neurosci. 28:1537–1545; 2008
64. Wisniewski, K. E.; Wisniewski, H. M.; Wen, G. Y. Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. Ann. Neurol. 17:278–282; 1985.
65. Yan, J.; Welsh, A. M.; Bora, S. H.; Snyder, E. Y.; Koliatsos, V. E. Differentiation and tropic/trophic effects of exogenous neural precursors in the adult spinal cord. J. Comp. Neurol. 480:101–114; 2004.