Aging Cell (2012) **11,** pp628–633 Doi: 10.1111/j.1474-9726.2012.00826.x

Changes in neuronal DNA content variation in the human brain during aging

Hans-Georg Fischer,[1] Markus Morawski,[1] Martina K. Brückner,[1] Anja Mittag,[2] Attila Tarnok[2] and Thomas Arendt[1]

[1]Paul Flechsig Institute for Brain Research, Universität Leipzig, [2]Department of Pediatric Cardiology, Heart Center Leipzig, Universität Leipzig, Leipzig, Germany

Summary

The human brain has been proposed to represent a genetic mosaic, containing a small but constant number of neurons with an amount of DNA exceeding the diploid level that appear to be generated through various chromosome segregation defects initially. While a portion of these cells apparently die during development, neurons with abnormal chromosomal copy number have been identified in the mature brain. This genomic alteration might to lead to chromosomal instability affecting neuronal viability and could thus contribute to age-related mental disorders. Changes in the frequency of neurons with such structural genomic variation in the adult and aging brain, however, are unknown. Here, we quantified the frequency of neurons with a more than diploid DNA content in the cerebral cortex of normal human brain and analyzed its changes between the fourth and ninth decades of life. We applied a protocol of slide-based cytometry optimized for DNA quantification of single identified neurons, which allowed to analyze the DNA content of about 500 000 neurons for each brain. On average, 11.5% of cortical neurons showed DNA content above the diploid level. The frequency of neurons with this genomic alteration was highest at younger age and declined with age. Our results indicate that the genomic variation associated with DNA content exceeding the diploid level might compromise viability of these neurons in the aging brain and might thus contribute to susceptibilities for age-related CNS disorders. Alternatively, a potential selection bias of ''healthy aging brains'' needs to be considered, assuming that DNA content variation above a certain threshold associates with Alzheimer's disease.

Key words: aneuploidy; cell cycle; cell death; chromosome; hyperploidy; slide-based cytometry.

Introduction

All somatic mammalian cells with very few exceptions have basically been assumed to contain identical genomes corresponding to a diploid set of chromosomes. Accordingly, cellular heterogeneity might largely be regulated by epigenetic mechanisms. More recent studies in the human brain, however, indicate that structural variations in the human genome owing to loss or gain of whole chromosomes or fragments thereof might be an additional mechanism to generate neuronal diversity (Rehen *et al.*, 2001, 2005; Kingsbury *et al.*, 2005; Mosch *et al.*, 2007; Yurov *et al.*, 2007; Iourov *et al.*, 2009a,b; Vorsanova *et al.*, 2010; Westra *et al.*, 2010).

A more than diploid level of neuronal DNA was first reported about 50 years ago in the rat (Brodsky & Kusc, 1962) and human brain (Müller, 1962). These findings prompted a protracted controversy as to whether the DNA content of a very large part of neurons (Lapham, 1968; Bernocchi *et al.*, 1979) or even all neurons (Bregnard *et al.*, 1975, 1979; Kuenzle *et al.*, 1978) exceeds the diploid level or not (Morselt *et al.*, 1972; Cohen *et al.*, 1973; Mann & Yates, 1973; Fujita, 1974; Fukuda *et al.*, 1978). Although consensus was reached eventually that the majority of neurons are diploid, a small but constant fraction of a few percent of neurons continuously escaped the diploid DNA amount, irrespectively of the analytical method or other confounding factors of tissue sampling and preparation (Brodsky *et al.*, 1979; Mares & van der Ploeg, 1980; Marshak *et al.*, 1985). Discrepant results were attributed to various technical limitations and cytophotometric artifacts of heterochromatin in interphase nuclei of postmitotic neurons (Fujita *et al.*, 1972; Duijndam *et al.*, 1980a,b) but also to large individual variations (Brodsky *et al.*, 1979). Against the background of these inconsistencies, however, age-dependent differences in sampling have never been considered to be of influence, and systematic studies on potential age-related changes of the single-neuron DNA amount are lacking.

More recent studies applying state-of-the-art approaches for evaluating chromosome numbers in brain cells using multicolor-fluorescence *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) with site-specific and centromeric DNA probes (Rehen *et al.*, 2001, 2005; Kingsbury *et al.*, 2005; Mosch *et al.*, 2007) or interphase high-resolution chromosome-specific multicolor banding (ICS-MCB) for the visualization of whole chromosomes (Yurov *et al.*, 2007; Iourov *et al.*, 2009a,b; Vorsanova *et al.*, 2010) constantly revealed a ''low frequency'' of chromosome number variations (CNV) (i.e., aneuploidy or gain/loss of whole chromosomes) giving rise to mosaic aneuploidy in the human brain. This CNV, which roughly amounts to about 10% of all neurons (Rehen *et al.*, 2005; Mosch *et al.*, 2007; Iourov *et al.*, 2009b), appears to be well tolerated in the normal brain, where it might contribute to genetically mosaic neuronal circuits (Kingsbury *et al.*, 2005; Morillo *et al.*, 2010).

Contrary to this low-frequency 'physiological' CNV, numerical chromosome aberrancies, however, might be detrimental and compromise neuronal viability, if its abundance exceeds a certain threshold. We could, thus, show recently that hyperploid neurons are rather selectively affected by cell death at very early stages of Alzheimer's disease (Arendt *et al.*, 2010). Neurodegeneration mediated by chromosome instability appears not to be unique to Alzheimer's disease and has recently also been reported for cerebellar degeneration in patients with ataxia telangiectasia (Iourov *et al.*, 2009a). These findings support previous suggestions that certain types of numerical chromosome abnormalities in the brain might contribute to neurodegenerative diseases (Potter, 1991; Geller & Potter, 1999; Iourov *et al.*, 2006; Mosch *et al.*, 2007; Boeras *et al.*, 2008; Iourov *et al.* 2008; Iourov *et al.*, 2009b; Granic *et al.*, 2010).

While the majority of neuron with chromosomal segregation defects are cleared by apoptotic cell death during development (Rehen *et al.*, 2001; Yurov *et al.*, 2005), the fate of those neurons that escape this process of developmental elimination and might relate to late-onset mental disorders remains elusive. To address questions such as the functional

Correspondence
Thomas Arendt, MD, DSc, Paul Flechsig Institute for Brain Research, Universität Leipzig, Jahnallee 59, 04109 Leipzig, Germany. Tel.: +49 341 9725721; fax: +49 341 9725729; e-mail: aret@medizin.uni-leipzig.de

Accepted for publication *7 April 2012*

significance of physiological CNV, how it can be distinguished from its pathological form, and what are the modes of its generation, more systematic studies both on its distribution throughout the brain and on its potential changes over lifetime are required.

Therefore, in this study, we quantified the frequency of neurons with a more than diploid content of neuronal DNA in different cortical areas of the normal human brain and analyzed its changes during aging. To assess changes in the frequency of neurons with an aberrant amount of DNA in space and time in a reasonably sized cohort requires a technique that allows to study changes in the DNA content efficiently in a large number of cells. To this end, we applied a protocol of slide-based cytometry (SBC) optimized for DNA quantification of identified neurons (Lenz *et al.*, 2004; Mosch *et al.*, 2006; Arendt *et al.*, 2009). As this technique provides information on the single-neuron DNA amount rather than on chromosomal copy number, we refer to neuronal DNA content variation (DCV) (Westra *et al.*, 2010) for those neurons that contained a more than diploid amount of DNA.

Results

The DNA content of single neurons was quantified by SBC in frontal, temporal, parietal, entorhinal and occipital cortices of mentally healthy individuals spanning an age range between 31 and 88 years. For each brain, we analyzed the DNA content of about 500 000 neurons. Neurons were identified by their immunoreactivity for neurofilament, and numerical neuronal density was determined as reference.

Age-related changes in numerical neuronal density

Numerical neuronal density in the cerebral cortex showed a tendency of age-related decline (Fig. 1A). There was, however, no significant correlation between decreasing neuronal number and increasing age. When individuals were split according to their age into two groups of equal size of either below or above 60 years of age, mean values differed by 17% (Fig. 1B).

When analyzed for each cortical area separately, numerical neuronal density tended to decline with age in each area (Fig. 2). Age-related changes were most pronounced in the temporal cortex and least obvious for the entorhinal cortex. Regional differences, however, were statistically not significant.

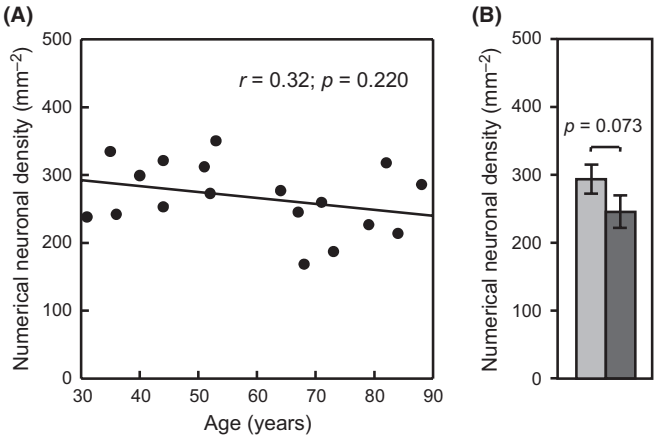

Fig. 1 Age-related changes in the numerical density of neurons in the cerebral cortex. (A) Changes in individual mean values, averaged over five cortical areas in persons between the fourth and ninth decades of life. (B) Age-related alterations are displayed as mean values for subjects below (light gray) and above (dark gray) 60 years of age (group size: nine subjects; ± SD). *r*, Pearson's product–moment correlation coefficient; *p*, level of significance (*t*-test).

Regional distribution of DCV

In all five cortical areas analyzed, the vast majority of neurons contained a diploid amount of DNA. Still, on average, 11.5% of neurons showed DNA content above the diploid level. No indications for an effect of gender were observed (mean values ± SD, men: 11.41 ± 2.95%; women: 11.59 ± 2.57%). Overall, there were no major regional differences in the abundance of neurons with DCV, varying on average between 9.9% in the parietal cortex and 13.8% in the temporal cortex (Fig. 3).

Age-related changes in DCV

Individual mean values of DCV, averaged over the five cortical areas, were highest at younger age and declined with age (Fig. 4A). This age dependency of numerical density of neurons with an elevated amount of DNA, however, was significant at the 5% level. When individuals were grouped according to their age of either below or above 60 years, mean values differed by 21% ($P < 0.05$; *t*-test) (Fig. 4B).

When age-related changes were analyzed separately for each region, there was a similar tendency for most regions (Fig. 5). This age-related decline was most pronounced in the occipital cortex while hardly present, however, in the entorhinal cortex. Regional differences in the rate of changes in the number of neurons with DCV did not reach significance.

Discussion

Previous studies have shown neuronal mosaic aneuploidy in the normal mammalian brain. Further, neuronal aneuploidy was observed at a fairly high rate during mouse cerebral cortical development and has been associated with neurodegenerative disorders such as Alzheimer's disease (for review, see Iourov *et al.*, 2011). More systematic studies on potential changes in aneuploid neurons in the adult and aging brain, however, are lacking so far.

Here, we show that in the normal adult human brain, about 11.5% of neurons contain DNA amount above the diploid level, which corresponds to the rate reported previously (Rehen *et al.*, 2005; Mosch *et al.*, 2007; Iourov *et al.*, 2009b). As our analytical technique determines the single-neuron DNA content rather than chromosomal copy numbers, the designation ''DCV'' as proposed by Westra *et al.* (2010) might be more appropriate than ''hyperploidy'' to describe this aberrancy.

We analyzed changes in the number of all neurons and neurons with DCV over a rather wide range of age from the fourth to ninth decades of life. Although we observed some tendency of an age-related decline in total neuronal density, the overall loss of neurons in the human cerebral cortex during aging might be marginal. This is in agreement with previous systematic studies on age-related changes in cortical neuron number, which, depending on the method of quantification and cortical area, reported a neuron loss of up to 10% (Haug *et al.*, 1984; Leuba & Garey, 1987; Terry *et al.*, 1987, Gómez-Isla *et al.* 1996, Pakkenberg & Gundersen, 1997; Morrison & Hof, 1997; Peters *et al.*, 1998).

Contrary to these only marginal changes in the overall number of neurons with age, we observed a significant loss of those neurons with an abnormal DNA content. This might indicate that DCV compromises neuronal viability over the lifetime of an individual. Further, it argues against the assumption that neurons with DCV are newly generated in the adult and aging brain. However, it cannot be ruled out that neurons with newly

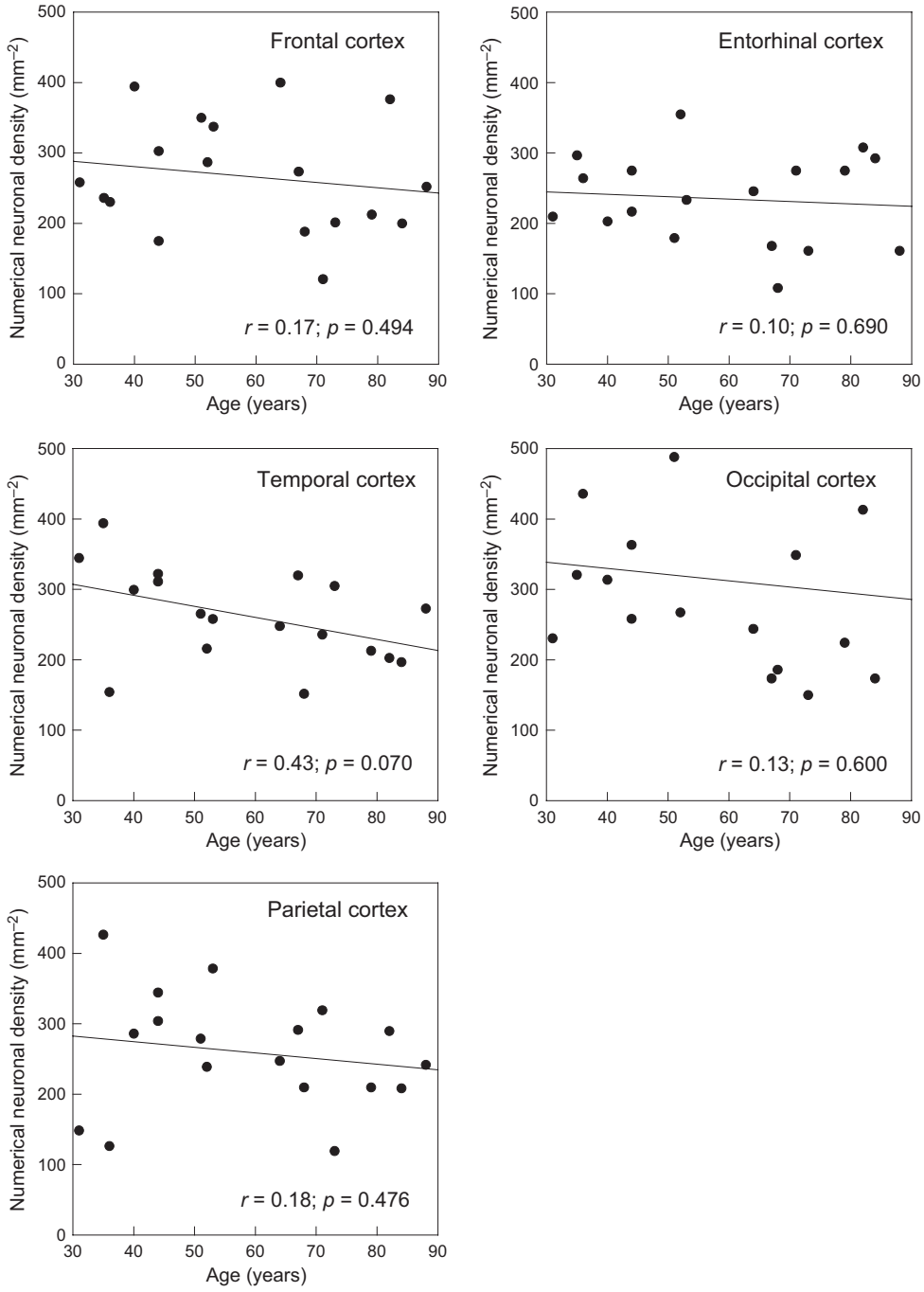

Fig. 2 Age-related changes in the numerical density of neurons in the cerebral cortex, analyzed separately for frontal (Brodmann area 10), temporal (Brodmann area 22), parietal (Brodmann area 7), entorhinal (Brodmann area 28), and occipital (Brodmann area 18) cortices. *r*, Pearson's product–moment correlation coefficient; *p*, level of significance (*t*-test).

generated DCV undergo cell death at a similar rate whereby they might escape detection. In addition, a potential selection bias of ''healthy aging brains'' needs to be considered, assuming that DCV above a certain threshold associates with Alzheimer's disease.

The majority of neuron with chromosomal segregation deficit as the most likely cause of DCV are cleared during early brain development by apoptotic cell death (Rehen *et al.*, 2001; Yurov *et al.*, 2005). While the rate of aneuploidy in proliferating neuroblasts is as high as 33% (Rehen *et al.*, 2001), it thereby decreases to about 11% in the postmitotic cells in the adult brain. This higher rate of elimination of neurons with DCV clearly indicates their higher susceptibility to cell death. Still, a considerable number of neurons can escape this clearance mechanism, apparently remaining in a state of still higher vulnerability. The age-related accumulation of factors that might additionally compromise chromosomal stability might eventually trigger a mechanism of delayed apoptotic clearance.

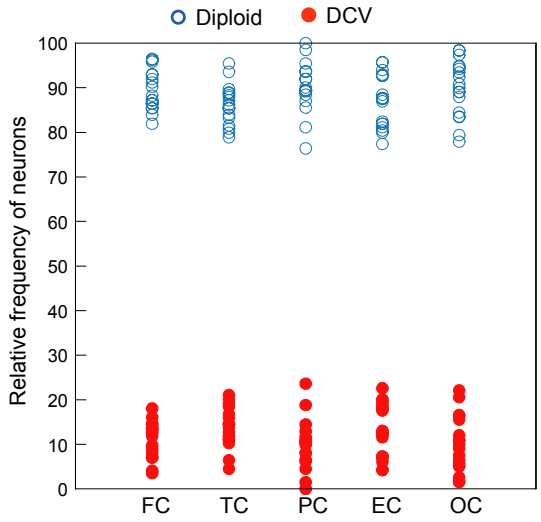

Fig. 3 Relative frequency of cortical neurons with a diploid amount of DNA (blue) and an amount of DNA exceeding the diploid level [DNA content variation (DCV); red]. FC, frontal cortex; TC, temporal cortex; PC, parietal cortex; EC, entorhinal cortex; OC, occipital cortex.

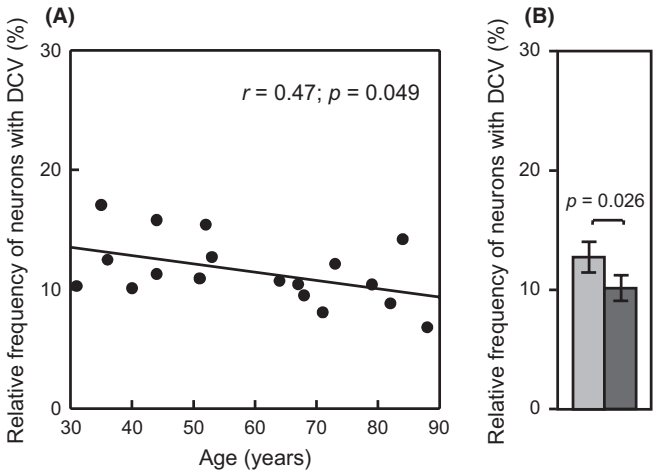

Fig. 4 Age-related changes in the relative frequency of cortical neurons with an amount of DNA exceeding the diploid level. (A) Changes in individual mean values, averaged over five cortical areas in persons between the fourth and ninth decades of life. (B) Age-related alterations are displayed as mean values for subjects below (light gray) and above (dark gray) 60 years of age (group size: nine subjects; ± SD). *r*, Pearson's product-moment correlation coefficient; *p*, level of significance (t-test).

Taken together, our results indicate that the genomic variation associated with a content of DNA exceeding the diploid level compromises viability of these neurons in the aging brain and might thus contribute to susceptibilities for age-related CNS disorders.

Experimental procedures

Case recruitment and tissue preparation

Postmortem brain tissue from 18 healthy controls dying without any history of neurological or psychiatric illness at an age range between 31 and 88 years was obtained from the Brain Bank of the Paul Flechsig Institute of Brain Research. Cases were grouped according to their age at death, and each decade between the fourth and ninth decades of life was represented by three cases. Cases were selected in a way to exclude any gender bias with respect to the age distribution (11 men, seven women; mean age ± SD; men: 59.0 ± 18.9 years; women: 59.0 ± 19.0 years).

Tissue blocks with a size of about 3 × 3 cm from five cortical areas (entorhinal cortex, Brodmann area 28; frontal cortex, Brodmann area 10; parietal cortex, Brodmann area 7; temporal cortex, Brodmann area 22; occipital cortex, Brodmann area 18) were fixed in 4% phosphate-buffered paraformaldehyde (4% PFA in PBS, pH 7.4) for 9 days and cryoprotected in 30% sucrose. Sections of 30 μm thickness were cut on a freezing microtome. For SBC, slices were processed using immunofluorescence staining as described (Mosch *et al.*, 2006). Briefly, free-floating sections were pretreated in 10 mM citrate buffer (pH 6.0) heated by microwave. After blocking of unspecific binding sites with 0.3% milk powder, 0.1% gelatine, 1% bovine serum albumin, and 0.05% Tween 20 in 10 mM Tris-buffered saline (pH 7.4), sections were incubated overnight at 4 °C with the monoclonal mouse-anti-pan-neuronal neurofilament marker (SMI-311; Sternberger Monoclonals, Baltimore, MD, USA, dilution 1:750) in blocking solution. The SMI-311 antibody was labeled with a secondary cyanine 5 (Cy5TM)-conjugated goat-anti-mouse antibody (Dianova, Hamburg, Germany, 1:300). The labeled human sections were treated with 50 μg mL^{-1} propidium iodide (PI) in Tris-buffered saline containing 100 μg mL^{-1} Ribonuclease A (Sigma, St. Louis, MO, USA) for 30 min at 37 °C. Sections were mounted onto slides using DAKO fluorescent mounting medium and stored at 4 °C in the darkness until analysis. Sections from each of the 18 subjects were processed simultaneously, and sections from three independent rounds of cutting and staining were analyzed.

Slide-based cytometry

SBC was performed using a Laser Scanning Cytometer (LSC; CompuCyte Corporation, Westwood, MA, USA) with the software WinCyte, version 3.4. Only neurofilament reactive cells were considered for SBC analysis. Each fluorescent event was recorded with respect to size, perimeter, *x-y* position on the object slide, and maximum (Max Pixel) and overall integral fluorescence intensity. The relative DNA content of the cells was determined by the integral PI fluorescence values, and these data were further analyzed using the cell cycle software ModfitLT, version 2.0 (Verity Software House Inc., Topsham, ME, USA). The entire cortical depth was scanned with 400 000–600 000 analyzed cells for each brain. The numerical neuronal density was determined using the WinCyte tool to record the *x-y*-position of each event of fluorescence. Using this tool, the number of neurons within a defined polygon field surrounding the entire cortical area was counted. Moreover, the area size of each polygon field was determined using the image processing software analySIS, version 3.00 (Soft Imaging System GmbH, Muenster, Germany). Finally, the average numerical neuronal density (amount of neurons per square mm) was calculated for each cortical area. Analyses were restricted to neurons with a DNA amount corresponding to a diploid level or above. Neurons with a reduced amount of DNA were not considered for the quantification as loss of DNA owing to partial sectioning of nuclei is difficult to control for.

Statistics

To test the strength of the linear dependence between numerical neuronal density and age, Pearson's product–moment correlation coefficient *r* was calculated and tested by *t*-test. Regional differences in age-related changes were compared by ANOVA.

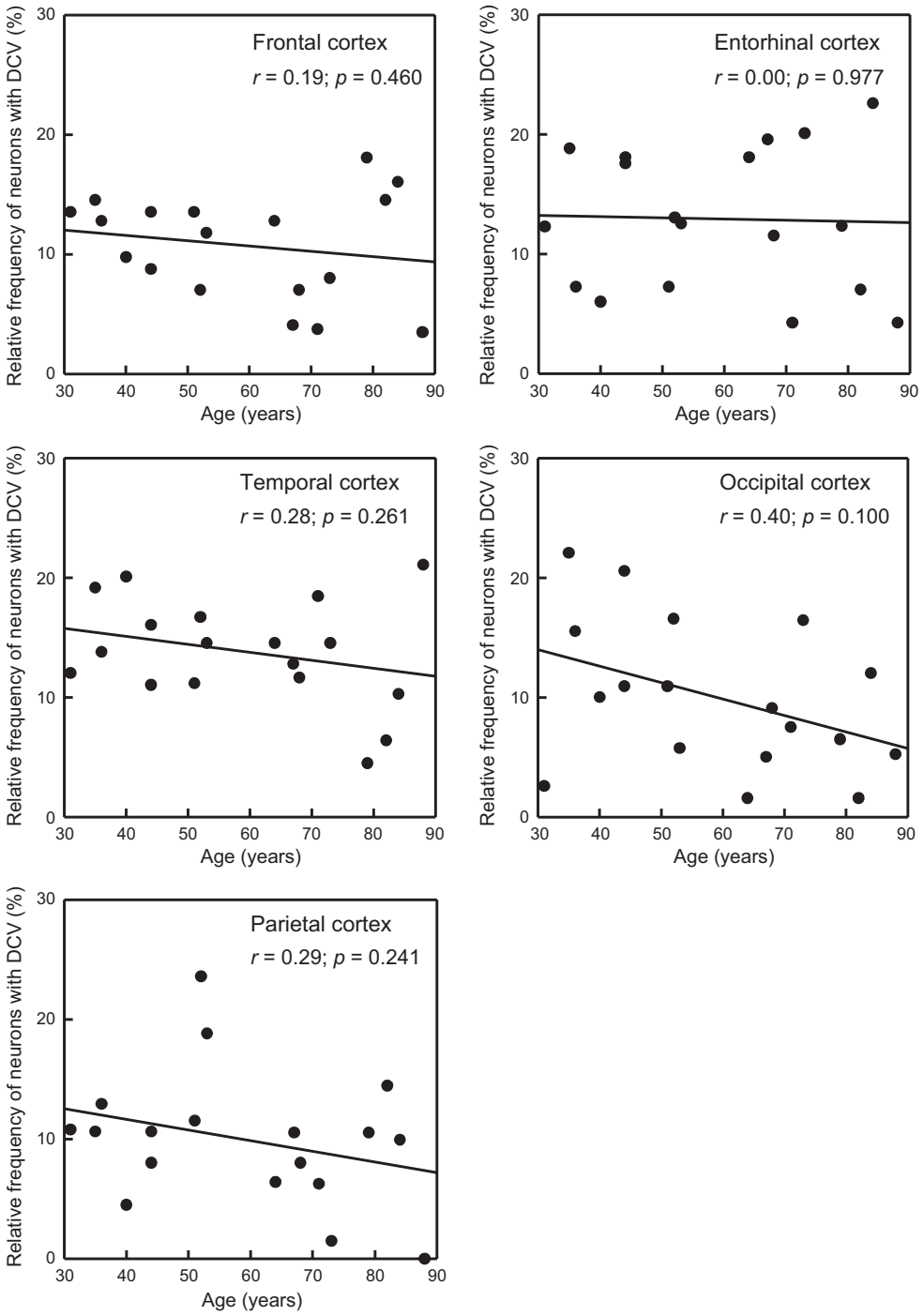

Fig. 5 Age-related changes in the relative frequency of cortical neurons with an amount of DNA exceeding the diploid level, analyzed separately for frontal (Brodmann area 10), temporal (Brodmann area 22), parietal (Brodmann area 7), entorhinal (Brodmann area 28), and occipital (Brodmann area 18) cortices. *r*, Pearson's product–moment correlation coefficient; *p*, level of significance (*t*-test).

Acknowledgments

Financial support by the BMBF (01EW0907) in the frame of ERA-Net NEURON, the European Union, and the Free State of Saxony (Grant number 14494) is gratefully acknowledged. All co-authors have seen and agreed with the contents of the manuscript, and none of the co-authors has any financial interests to disclose.

Author contributions

H.-G. Fischer, A. Tarnok, and Th. Arendt designed the experiments. H.-G. Fischer, M. Morawski, M.K. Brückner, and A. Mittag performed the experiments. Manuscript was written by Th. Arendt with the assistance of H.-G. Fischer and A. Tarnok.

References

Arendt T, Mosch B, Morawski M (2009) Neuronal aneuploidy in health and disease: a cytomic approach to understand the molecular individuality of neurons. *Int. J. Mol. Sci.* **10**, 1609–1627.

Arendt T, Brückner MK, Mosch B, Lösche A (2010) Selective cell death of hyperploid neurons in Alzheimer's disease. *Am. J. Pathol.* **177**, 15–20.

Bernocchi G, Redi CA, Scherini E (1979) Feulgen-DNA content of the Purkinje neuron: ''diploid'' or ''tetraploid''? *Basic Appl. Histochem.* **23**, 65–70.

Boeras DI, Granic A, Padmanabhan J, Crespo NC, Rojiani AM, Potter H (2008) Alzheimer's presenilin 1 causes chromosome missegregation and aneuploidy. *Neurobiol. Aging* **29**, 319–328.

Bregnard A, Knüsel A, Kuenzle CC (1975) Are all the neuronal nuclei polyploid? *Histochemistry* **43**, 59–61.

Bregnard A, Ruch F, Lutz H, Kuenzle CC (1979) Histones and DNA increase synchronously in neurons during early postnatal development of the rat forebrain cortex. *Histochemistry* **61**, 271–279.

Brodsky VJ, Kusc AA (1962) Changes in the number of polyploid cells during postnatal development of rat tissue. *Sci. USSR.* **147**, 713–716.

Brodsky VJ, Marshak TL, Mares V, Lodin Z, Fülöp Z, Lebedev EA (1979) Constancy and variability in the content of DNA in cerebellar Purkinje cell nuclei. A cytophotometric study. *Histochemistry* **59**, 233–248.

Cohen J, Mares V, Lodin Z (1973) DNA content of purified preparations of mouse Purkinje neurons isolated by a velocity sedimentation technique. *J. Neurochem.* **20**, 651–657.

Duijndam WA, Smeulders AW, van Duijn P, Verweij AC (1980a) Optical errors in scanning stage absorbance cytophotometry. I. Procedures for correcting apparent integrated absorbance values for distributional, glare, and diffraction errors. *J. Histochem. Cytochem.* **28**, 388–394.

Duijndam WA, van Duijn P, Riddersma SH (1980b) Optical errors in scanning stage absorbance cytophotometry. II. Application of correction factors for residual distributional error, glare and diffraction error in practical cytophotometry. *J. Histochem. Cytochem.* **28**, 395–400.

Fujita S (1974) DNA constancy in neurons of the human cerebellum and spinal cord as revealed by Feulgen cytophotometry and cytofluorometry. *J. Comp. Neurol.* **155**, 195–202.

Fujita S, Fukuda M, Kitamura T, Yoshida Satoru (1972) Two-wave-length-scanning method in Feulgen cytophotometry. *Acta Histochem. Cytochem.* **5**, 146–152.

Fukuda M, Bohm N, Fujita S (1978) Cytophotometry and its biological application. *Progr. Histochem. Cytochem.* **11**, 1–119.

Geller LN, Potter H (1999) Chromosome missegregation and trisomy 21 mosaicism in Alzheimer's disease. *Neurobiol Dis.* **6**, 167–179.

Gómez-Isla T, Price JL, McKeel DW, Morris JC, Growdon JH, Hyman BT (1996) Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J. Neurosci.* **16**, 4491–4500.

Granic A, Padmanabhan J, Norden M, Potter H (2010) Alzheimer Abeta peptide induces chromosome mis-segregation and aneuploidy, including trisomy 21: requirement for tau and APP. *Mol. Biol. Cell* **21**, 511–520.

Haug H, Kühl S, Mecke E, Sass NL, Wasner K (1984) The significance of morphometric procedures in the investigation of age changes in cytoarchitectonic structures of human brain. *J. Hirnforsch.* **25**, 353–374.

Iourov IY, Vorsanova SG, Yurov YB (2006) Chromosomal variation in mammalian neuronal cells: known facts and attractive hypotheses. *Int. Rev. Cytol.* **249**, 143–191.

Iourov IY, Vorsanova SG, Yurov YB (2008) Chromosomal mosaicism goes global. *Mol. Cytogen.* **1**, 26.

Iourov IY, Vorsanova SG, Liehr T, Kolotii AD, Yurov YB (2009a) Increased chromosome instability dramatically disrupts neural genome integrity and mediates cerebellar degeneration in the ataxia-telangiectasia brain. *Human Mol. Gen.* **18**, 2656–2669.

Iourov IY, Vorsanova SG, Liehr T, Yurov YB (2009b) Aneuploidy in the normal, Alzheimer's disease and ataxia-telangiectasia brain: differential expression and pathological meaning. *Neurobiol Dis.* **34**, 212–220.

Iourov IY, Vorsanova SG, Yurov YB (2011) Genomic landscape of the Alzheimer's disease brain: chromosome instability–aneuploidy, but not tetraploidy–mediates neurodegeneration. *Neurodegen. Dis.* **8**, 35–37; discussion 38–40.

Kingsbury MA, Friedman B, McConnell MJ, Rehen SK, Yang AH, Kaushal D, Chun J (2005) Aneuploid neurons are functionally active and integrated into brain circuitry. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6143–6147.

Kuenzle CC, Bregnard A, Hübscher U, Ruch F (1978) Extra DNA in forebrain cortical neurons. *Exp. Cell Res.* **113**, 151–160.

Lapham LW (1968) Tetraploid DNA content of Purkinje neurons of human cerebellar cortex. *Science* **159**, 310–312.

Lenz D, Mosch B, Bocsi J, Arendt T, Tarnok A (2004) Assessment of DNA replication in central nervous system by laser scanning cytometry. in *Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues II* (Nicolau DV, ed.). San Jose, CA; Bellingham, WA, USA: SPIE, pp. 146–156.

Leuba G, Garey LJ (1987) Evolution of neuronal numerical density in the developing and aging human visual cortex. *Human Neurobiol.* **6**, 11–18.

Mann DM, Yates PO (1973) Polyploidy in the human nervous system: part 1. The DNA content of neurones and glia of the cerebellum. *J. Neurol. Sci.* **18**, 183–196.

Mares V, van der Ploeg M (1980) Cytophotometric re-investigation of DNA content in Purkinje cells of the rat cerebellum. *Histochemistry* **69**, 161–167.

Marshak TL, Mares V, Brodsky VY (1985) An attempt to influence DNA content in postmitotic Purkinje cells of the cerebellum. *Acta Histochem.* **76**, 193–200.

Morillo SM, Escoll P, de La Hera A, Frade JM (2010) Somatic tetraploidy in specific chick retinal ganglion cells induced by nerve growth factor. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 109–114.

Morrison JH, Hof PR (1997) Life and death of neurons in the aging brain. *Science* **278**, 412–419.

Morselt AF, Braakman DJ, James J (1972) Feulgen-DNA and fast-green histone estimations in individual cell nuclei of the cerebellum of young and old rats. *Acta Histochem.* **43**, 281–286.

Mosch B, Mittag A, Lenz D, Arendt T, Tárnok A (2006) Laser scanning cytometry in human brain slices. *Cytometry A: J. Int. Soc. Analytic Cytol.* **69**, 135–138.

Mosch B, Morawski M, Mittag A, Lenz D, Tarnok A, Arendt T (2007) Aneuploidy and DNA replication in the normal human brain and Alzheimer's disease. *J. Neurosci.* **27**, 6859–6867.

Müller H (1962) Cytophotometrische DNS-Messungen an Ganglienzellkernen des Nucleus dentatus beim Menschen. *Naturwissenschaften* **49**, 243.

Pakkenberg B, Gundersen HJ (1997) Neocortical neuron number in humans: effect of sex and age. *J. Comp. Neurol.* **384**, 312–320.

Peters A, Morrison JH, Rosene DL, Hyman BT (1998) Feature article: are neurons lost from the primate cerebral cortex during normal aging? *Cereb. Cortex* **8**, 295–300.

Potter H (1991) Review and hypothesis: alzheimer disease and Down syndrome–chromosome 21 nondisjunction may underlie both disorders. *Am. J. Human Gen.* **48**, 1192–1200.

Rehen SK, McConnell MJ, Kaushal D, Kingsbury MA, Yang AH, Chun J (2001) Chromosomal variation in neurons of the developing and adult mammalian nervous system. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 13361–13366.

Rehen SK, Yung YC, McCreight MP, Kaushal D, Yang AH, Almeida BSV, Kingsbury MA, Cabral KMS, McConnell MJ, Anliker B, Fontanoz M, Chun J (2005) Constitutional aneuploidy in the normal human brain. *J. Neurosci.* **25**, 2176–2180.

Terry RD, DeTeresa R, Hansen LA (1987) Neocortical cell counts in normal human adult aging. *Ann. Neurol.* **21**, 530–539.

Vorsanova SG, Yurov YB, Iourov IY (2010) Human interphase chromosomes: a review of available molecular cytogenetic technologies. *Mol. Cytogen.* **3**, 1.

Westra JW, Rivera RR, Bushman DM, Yung YC, Peterson SE, Barral S, Chun J (2010) Neuronal DNA content variation (DCV) with regional and individual differences in the human brain. *J. Comp. Neurol.* **518**, 3981–4000.

Yurov YB, Iourov IY, Monakhov VV, Soloviev IV, Vostrikov VM, Vorsanova SG (2005) The variation of aneuploidy frequency in the developing and adult human brain revealed by an interphase FISH study. *J. Histochem. Cytochem.* **53**, 385–390.

Yurov YB, Iourov IY, Vorsanova SG, Liehr T, Kolotii AD, Kutsev SI, Pellestor F, Beresheva AK, Demidova IA, Kravets VS, Monakhov VV, Soloviev IV (2007) Aneuploidy and confined chromosomal mosaicism in the developing human brain. *PLoS ONE* **2**, e558.