Synaptic plasticity in the dentate gyrus of aged rats is altered after chronic nimodipine application

de Jong, G.I.; Buwalda, B.; Schuurman, T.; Luiten, P.G.M.

Published in:
Brain Research

DOI:
10.1016/0006-8993(92)91570-5

Publication date:
1992

Brain Research, 596 (1992) 345–348

BRES 25446

Synaptic plasticity in the dentate gyrus of aged rats is altered after chronic nimodipine application

G.I. de Jong [a], B. Buwalda [a], T. Schuurman [b] and P.G.M. Luiten [a]

[a] *Department of Animal Physiology, University of Groningen, Haren (The Netherlands) and* [b] *Institute for Neurobiology, Troponwerke, Cologne (FRG)*

(Accepted 1 September 1992)

Key words: Aging; Synaptic plasticity; Dentate gyrus; 1,4-Dihydropyridine; Calcium antagonist; Nimodipine

We examined ultrastructural correlates of synaptic plasticity in the hippocampus of young (3 months) vs aged (30 months) Wistar rats and established the effects of the calcium antagonist nimodipine in animals chronically treated from 24 to 30 months. The effects of nimodipine was studied since this compound improves hippocampal neuronal physiology and enhances cognitive function during aging. In the supragranular layer of the dentate gyrus we found a 24% decrease in synaptic density (Nv) in aged animals, while synaptic size (S) was not significantly altered. After nimodipine treatment Nv in aged rats was not significantly different from young adults, thus being significantly increased compared to age-matched controls. The size of synapses was not significantly altered after nimodipine administration. Total synaptic surface area (Sv) in nimodipine-treated animals was significantly increased compared to aged controls, however, Sv remained significantly lower than in young adults. These data indicate that chronic administration of nimodipine enables granular cells in the dentate gyrus to maintain its number of synaptic contacts during the aging process. Furthermore, the presented influence of nimodipine on synaptic plasticity processes may underlie previously reported improved cognitive functioning of aged animals treated similarly with nimodipine.

Synaptic junctions in the central nervous system are very dynamic structures, the morphological correlates of which are continuously modified during the span of life[2,6]. Various quantitative morphological studies revealed alterations of this synaptic plasticity in aged rodents[2,6,7], as well as in aged humans and Alzheimer patients[3]. In the hippocampus, a structure that is particularly vulnerable to aging-related changes, contradictory data are available concerning alterations of synaptic density and synapse size in senescent rodents[2,5,7,17]. Most consistent are aging-related alterations in the supragranular layer of the dentate gyrus inner blade, where a decreased synaptic density has been reported in old rats[2,7].

An adequate functioning of the hippocampal formation is of crucial importance for proper cognitive performance (for review see ref. 20). Since chronic administration of the calcium antagonist nimodipine significantly reduced the occurrence of aging-related cognitive deficits[19], the aim of the present was study to examine the effects of such a long-term nimodipine treatment on quantitative synaptic morphology in the hippocampal dentate gyrus of Wistar rats.

In this study we examined the synaptic ultrastructure in young adult (3 months, $n = 5$), aged (30 months, $n = 5$) and aged, nimodipine-treated male Wistar rats (30 months, $n = 5$). All control animals were fed ad libitum with standard food pellets (Sniff; Soest, The Netherlands). The nimodipine-treated animals from the age of 24 until 30 months received identical pellets to which nimodipine (860 ppm—Bayer; Leverkusen, F.R.G.) was added.

At the end of the experiment, all animals were transcardially perfused with 0.1 M phosphate buffer containing 1% paraformaldehyde, 2% glutaraldehyde and 3% polyvinyl pyrrolidone (PVP). The dorsal hippocampus was transversely cut into 50-μm vibratome sections, which were dehydrated in up to 95% ethanol and subsequently stained in 1% phosphotungstic acid in absolute ethanol (E-PTA)[4]. Thereafter, the sections

Correspondence: G.I. de Jong, Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands. Fax: (31) (50) 635 202.

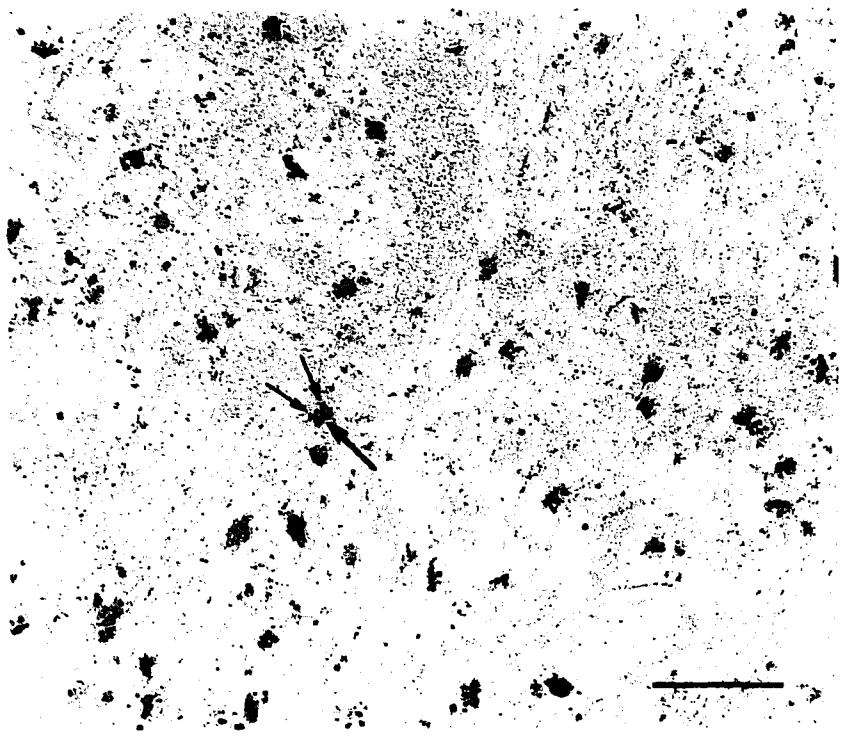

Fig. 1. Electron-microscopic photograph of the supragranular layer of the dentate gyrus stained with E-PTA. Synaptic junctions appear as a parallel dotted (small arrows) and a full line representing the pre- and postsynaptic membrane, respectively (single arrow). Scale bar = 1 μm.

were routinely embedded in Epon. Thirty electron microscopic photographs (15,360 ×) taken from the supragranular layer of the dentate gyrus inner blade (SG) were sampled per animal. The area of each picture was 102 μm^2; therefore a total area of approximately 15,300 μm^2 was investigated per group. A quadratic lattice with a 1 cm rule was printed above the photograph for use as a morphometric standard. The overall number of synapses per photograph and the number of intersection points of synaptic membranes with the test lines were determined. Weibel and Elias's morphometric formulae[21] were used to calculate the number of synapses (numerical density; Nv) and the total synaptic contact surface area (surface density; Sv) per unit tissue volume. Furthermore, the average surface area of single synapse contacts (S) was calculated per photograph by the ratio Sv/Nv^2.

All observations were performed blindly with the code broken after statistical analysis. Data were evaluated using the Student t-test corrected for the use of multiple groups, with statistical significance defined as $P < 0.05$.

After staining the sections with E-PTA synaptic membranes appear as a dotted and a full parallel line in close apposition, representing the pre- and postsynaptic membrane respectively, against a pale background (Fig. 1). In our quantifications only those contacts in which both pre- and postsynaptic membranes were visible were counted. The synaptic density (Nv) in the supragranular layer of the dentate gyrus was reduced by 24% in aged animals, when compared to young adult controls (Fig. 2, $P < 0.05$). The Nv in aged, nimodipine-treated animals was not significantly different from young controls, and was significantly higher than the Nv in age-matched controls ($P < 0.05$). The synaptic surface density (Sv) was significantly reduced by 30% in aged control animals ($P < 0.001$). In aged, nimodipine treated animals the Sv was significantly higher than in the age-matched controls ($P < 0.05$), but was significantly lower when compared to young adults ($P < 0.001$). The average surface area of single synaptic contacts (S) was not significantly altered in the aged control rats. The S of aged nimodipine-treated animals was slightly smaller, albeit not significant.

We demonstrated here a pronounced decrease of synaptic density (Nv) in the supragranular (SG) part of the inner molecular layer of the dentate gyrus of aged male Wistar rats. Previously, a decrease of the same magnitude has been reported for this region[2,7], but data concerning the synapse size in the SG of aged rats have been contradictory. Some studies showed no sig-

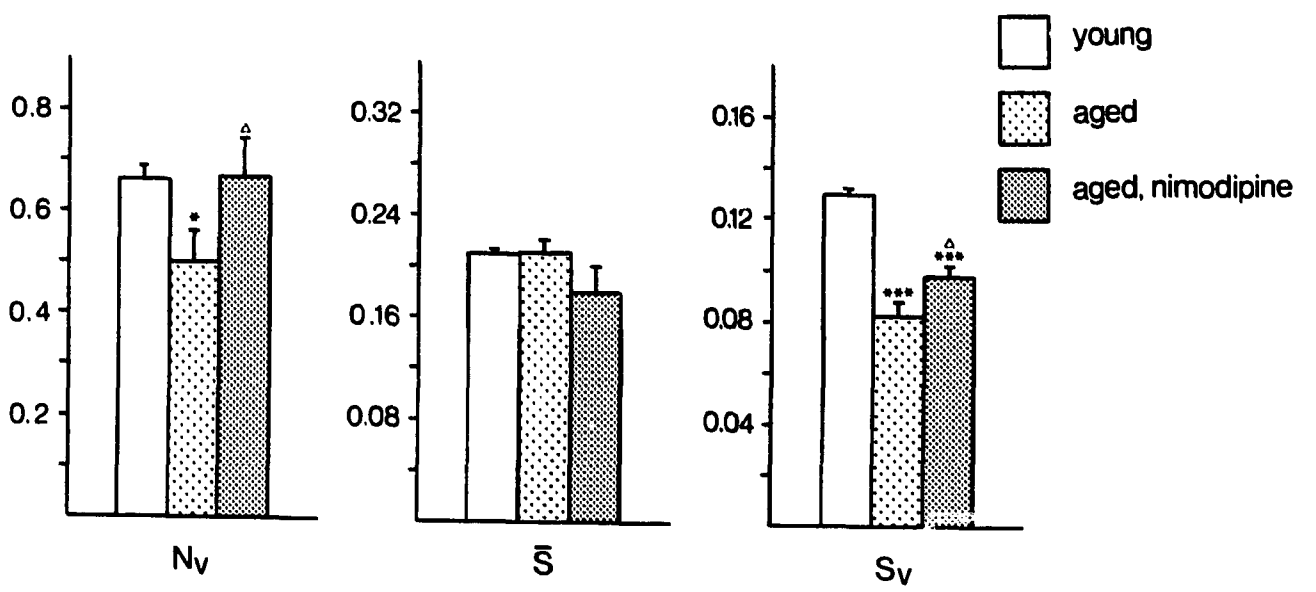

Fig. 2. The synaptic density (Nv; in synapses/μm^3), single synaptic surface area (S; in μm^2) and synaptic surface density (Sv; in $\mu m^2/\mu m^3$) are shown for young controls, aged controls and aged, nimodipine-treated animals (mean ± S.E.M.). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared to young controls; $^{\triangle}$ $P < 0.05$ when compared to age-matched controls.

nificant changes in synapse size[7], whereas other reseachers reported a significant increase of synaptic area (S) in aged rats[2]. The latter authors ascribed this increase to a growth of individual synapses as a compensatory mechanism for synaptic loss. In the present study, however, we found no such aging-related increase of S. The total synaptic surface area (Sv) was significantly decreased in the aged control animals, which is in agreement with the results of previous studies[1,2]. The values of Nv, S and Sv we found in our investigation were slightly higher than those found by others in the same hippocampal subregion, which possibly can be explained by strain differences. To our knowledge no such morphometric analyses of the synaptic population of the dentate SG are available for aged Wistar rats. The use of a conventional stereological method, biased by a number of factors, may also contribute to different values for Nv, S and Sv. However, the influences of such a bias on the estimation of Nv, S and Sv are equal for all groups studied, and cannot likely account for the differences between young, aged and aged, nimodipine-treated animals. Chronic treatment with the calcium antagonist nimodipine resulted in a complete and significant prevention of synaptic loss. Previously, Geinisman et al.[6] showed that 30% of the aged rats tested in the 8-arm radial maze did not show memory impairment. In contrast to memory-impaired rats, which showed a reduced synaptic density in the dentate gyrus, the synaptic density in the dentate gyrus of memory-intact aged rats was similar to synaptic density of young controls. Theoretically, more animals treated with nimodipine than aged controls can belong to the 30% of memory-intact aged rats in the present study. The variation (S.E.M.) of Nv in the 3 groups tested showed, however, that this possibility is unlikely to be the case. The S.E.M. values of both aged groups were much larger than the S.E.M. of the young control group, thus individual differences in synapse number were larger in our aged animals. However, the S.E.M. of the aged control group and the aged, nimodipine treated group were equal in size. Together with the strong correlation between memory impairment and synapse number in the dentate gyrus, the similar variation of Nv in both aged groups indicates that the alterations of Nv found in aged rats treated with nimodipine cannot be explained by a fortuitous distribution of animals over control and treatment groups.

The area of the individual synaptic contacts (S) tended to be smaller in the nimodipine-treated animals. Possibly, the synaptic morphology resembles those of intermediate-aged animals, since others reported smaller synapses in animals aged 12 months when compared to animals 3 months of age[2]. The Sv significantly decreases during aging. The Sv in aged rats was significantly increased after chronic nimodipine treatment; however, this increase did not entirely compensate for the aging-related decrease. The values of Nv and Sv are estimations of synaptic numerical and surface density per unit volume. The reference volume of the SG was not determined. However, increases in brain weight of 8% and 12% in aged controls and aged, nimodipine-treated animals, respectively (own unpublished observations), and an increase of dentate gyrus area (5%) in other aged animals treated in a similar fashion with nimodipine (own unpublished observations) cannot account for the differences observed in the present study.

The effects of nimodipine treatment on the observed aging-related synaptic plasticity may be explained via cerebrovascular and/or neuronal mechanisms. Nimodipine is a calcium antagonist of the 1,4-dihydropyridine type, which specifically blocks the L-type calcium channels[15]. A relatively high density of nimodipine binding sites is present especially in the molecular layers of the hippocampus[16]. Nimodipine readily crosses the blood–brain barrier[12], and exerts a preferentially central action[11]. Nimodipine acts as a vasodilator, thereby increasing cerebral blood flow[11], which is known to be impaired during aging[8]. Furthermore, we showed that the aging-related occurrence of microvascular aberrations, thought to hamper a proper nutrient supply to the neuropil, is significantly reduced after long-term nimodipine treatment[9,10].

Besides its vascular effects, nimodipine directly influences calcium influx in neurons. In aged rats an increased calcium influx through L-type calcium channels is thought to underlie aging-related alterations of hippocampal pyramidal cell function[14]. Application of nimodipine prevents this increased calcium influx[13], which results in an economized cell metabolism. The beneficial effects of nimodipine on neuronal calcium homeostasis in the hippocampus could lead to a more balanced maintenance of synaptic terminals in aged rats. Together with a sufficient blood and nutrient supply, this would explain the improved capacity of granular cells to retain the number of synaptic contacts during aging after nimodipine treatment.

Others previously demonstrated that synaptic plasticity in aged rats can be influenced by drug treatment[1]. We now show that chronic treatment with the calcium antagonist nimodipine also enables the hippocampal granular cells to keep the number of synapses constant during the aging process. Furthermore, the effects of nimodipine on synaptic plasticity shown in this study may underlie the previously reported beneficial effects

of this drug on preventing the aging-related deterioration of cognitive performance in aged Wistar rats[19].

1 Bertoni-Freddari, C., Giuli, C., Pieri, C., Paci, D. and Dravid, A., A modulating effect of hydergine on the synaptic plasticity of old rats, *J. Pharmacol.*, 16 (SII) (1985) 33–38.

2 Bertoni-Freddari, C., Giuli, C., Pieri, C. and Paci, D., Quantitative investigation of the morphological plasticity of synaptic junctions in rat dentate gyrus during aging, *Brain Res.*, 366 (1986) 187–192.

3 Bertoni-Freddari, C., Fattoretti, P., Casoli, T., Meier-Ruge, W. and Ulrich, J., Morphological adaptive response of the synaptic junctional zones in the human dentate gyrus during aging and Alzheimer's disease, *Brain Res.*, 517 (1990) 69–75.

4 Bloom, F.E. and Aghajanian, G.K., Fine structural and cytochemical analysis of the staining of synaptic junctions with phosphotungstic acid, *J. Ultrastructure Res.*, 22 (1968) 361–375.

5 Curcio, C.A. and Hinds, J.W., Stability of synaptic density and spine volume in dentate gyrus of aged rats, *Neurobiol. Aging*, 4 (1983) 77–87.

6 DeToledo-Morrell, L., Geinisman, Y. and Morrell, F., Age-dependent alterations in hippocampal synaptic plasticity: relation to memory disorders, *Neurobiol. Aging*, 9 (1988) 581–590.

7 Geinisman, Y., Bondareff, W. and Dodge, J.T., Partial deafferentation of neurons in the dentate gyrus of senescent rats, *Brain Res.*, 134 (1977) 541–545.

8 Goldman, H., Berman, R.F., Gershon, S., Murphy, S.L. and Altman, H.J., Correlation of behavioral and cerebrovascular functions in the aging rat, *Neurobiol. Aging*, 8 (1987) 409–416.

9 Jong, G.I., de, Jansen, A.S.P., Horvath, E., Gispen, W.H. and Luiten, P.G.M., Nimodipine effects on cerebral microvessels and sciatic nerve in aging rats, *Neurobiol. Aging*, 13 (1992) 73–81.

10 Jong, G.I., de, Weerd, H., de, Schuurman, T., Traber, J. and Luiten, P.G.M., Microvascular changes in aged rat forebrain. Effects of chronic nimodipine treatment, *Neurobiol. Aging*, 11 (1990) 381–389.

11 Kazda, S. and Towart, R., Nimodipine, a new calcium antagonist drug with a preferential cerebrovascular action, *Acta Neurochir.*, 63 (1982) 259–265.

12 Kerkhoff, W., van den and Drewes, L.R., Transfer of nimodipine and other calcium antagonists across the blood-brain barrier and their regional distribution in vivo. In M. Bergener and B. Reisberg (Eds.), *Diagnosis and Treatment of Senile Dementia*, Springer, Berlin, 1989, pp. 308–321.

13 Landfield, P.W., Increased calcium currents in rat hippocampal neurones during aging. In M. Morad, W. Nayler, S. Kazda, and M. Schramm (Eds.), *The Calcium Channel: Structure, Function and Implications*, Springer, Berlin, 1988, pp. 465–477.

14 Landfield, P.W. and Pitler, T.A., Prolonged Ca^{2+}-dependent afterhyperpolarizations in hippocampal neurons of aged rats, *Science*, 226 (1984) 1089–1092.

15 Miller, R.J., Multiple calcium channels and neuronal function, *Science*, 235 (1987) 46–52.

16 Quirion, R., Lal, S., Vasavan Hair, N.P. Stratford, J.G. Ford, R.M. Olivier, A., Comparative autoradiographic distribution of calcium channel antagonist binding sites for 1,4-dihydropyridine and phenylalkylamine in rat, guinea pig and human brain, *Prog. Neuropsychopharmacol. Biol. Psychiatr.*, 9 (1985) 643–649.

17 Scheff, S.W., Anderson, K.J. and DeKosky, S.T., Strain comparison of synaptic density in hippocampal CA1 of aged rats, *Neurbiol Aging*, 6 (1985) 29–34.

18 Schuurman, T., Klein, H., Beneke, M. and Traber, J., Nimodipine and motor deficits in the aged rat, *Neurosci. Res. Commun.*, 1 (1987) 9–15.

19 Scriabine, A., Schuurman, T. and Traber, J., Pharmacological basis for the use of nimodipine in central nervous system disorders, *Faseb. J.*, 3 (1989) 1799–1806.

20 Squire, L.W., *Memory and Brain*, Oxford University Press, New York, 1987.

21 Weibel, E.R. and Elias, H., Introduction to stereological principles. In E.R. Weibel and H. Elias (Eds.), *Quantitative Methods in Morphology*, Springer, Berlin, 1967, pp. 89–98.