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Mechanisms of Ageing and Development
83 (1995) 21–29

mechanisms of ageing and development

Evolution of neuronal density in the ageing thalamic reticular nucleus

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Received 16 March 1995; revision received 7 June 1995; accepted 7 June 1995

Abstract

In this paper we present an analysis of the visual sector of the Thalamic Reticular Nucleus (TRN) from 3, 6, 18, 24 and 30 month old Wistar rats using stereological methods. The volume density (Vv), the number of neurones per surface unit (Na) and the neurone numerical density (Nv) showed a progressive decrease between the 3rd and the 24th months as the animals aged, whereas a significant increase was observed between the 24th and the 30th month, the period at which these rodents have fully entered old age.

Keywords: Visual system; Thalamic reticular nucleus; Visual sector; Stereological study; Ageing

1. Introduction

The dorsocaudal region of the thalamic reticular nucleus (TRN) receives afferent fibres from layer VI of the primary visual cortex [1–3] and establishes reciprocal connections with the projection neurones of the Dorsal Lateral Geniculate Nucleus (dLGN) [2–5]. This means it has an important role as a modulating centre of the visual system [6].

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SSDI 0047-6374(95)01617-Z

During ageing, the central nervous system undergoes certain changes, those related to the visual system being of particular interest to us. Ageing in the brain is frequently associated with modifications in the number and density of neurones. However, data is often contradictory. In general, there is a consensus regarding the decrease in the various centres of the nervous system, but the degree of these changes seems to vary depending on the species and level analysed. Thus, a decline in neuronal density has been observed in the human somatosensory cortex [7], in the frontal association cortex [8], and in the nucleus basalis of Meynert [9,10]. In other species the same reduction has been equally found: in rat cerebellum [11], hippocampus [12], in the frontal cortex [13] and cerebellum [14] of the monkey. In contrast, no changes have been reported in the somatosensory cortex of the mouse [15].

As regards ageing in the visual system, a decrease in neuronal density has been observed in the human [16,17] and rat [12,18] visual cortex, whereas the findings concerning rat dLGN [19] as regards the number of neurones have revealed stability. However, up to now these parameters have not been analysed in the ageing TRN.

2. Material and methods

2.1. Material

25 albino male Wistar rats, 3, 6, 18, 24 and 30 months old, were used in this experiment, i.e., 5 in each age group. Their weight was between 275 g and 400 g (Table 1). The animals, never paired and were kept at a day/night cycle by means of artificial light and the Venice Conference guidelines [20] followed in their care. The rats were anaesthetised with chloral hydrate at 8% (0.4 ml/100 g), and perfused through the ascending aorta with a saline solution followed by buffered 10% formaldehyde.

2.2. Tissue preparation

Once decapitated, the brains were removed and immersed in the same type of fixing solution from 48 to 72 h., and finally embedded in paraffin. 8 μm thick serial coronal sections were cut at intervals from the TRN visual sector using a microtome, and following the criteria of Pellegrino [21] and Webster et al. [22] (Figs. 1.1,

Table 1
Mean weight ($\bar{x}$) and standard error of the mean ($\pm$ S.E.M.) of rats from each age group

Age (months)	Mean weight (g.)
3	275.90 $\pm$ 10.00
6	392.00 $\pm$ 21.42
18	379.61 $\pm$ 16.00
24	443.50 $\pm$ 20.51
30	360.00 $\pm$ 15.42

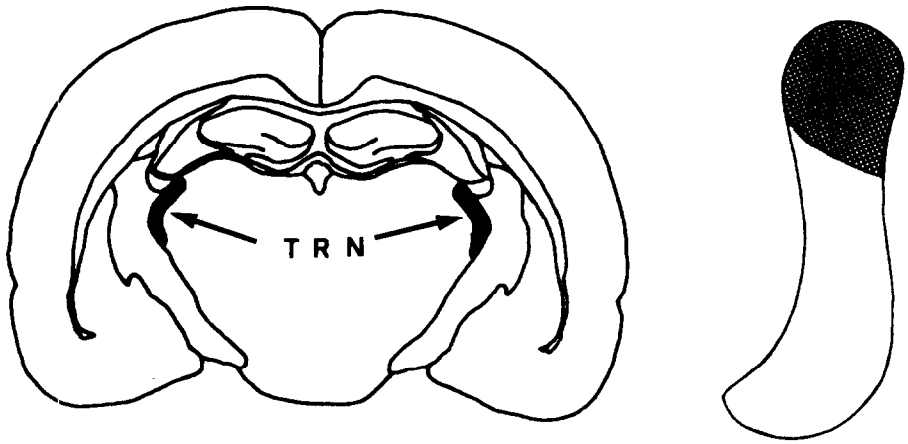

Fig. 1. (1) Diagram illustrating the location of the TRN (4.6 from the bregma). (2) Enlarged diagram illustrating the topography of the sector investigated indicated by cross-hatching.

1.2). These tissue sections were then stained according to the Einarson gallocyanin technique [23]. In order to better view the boundaries of the TRN, the intervening sections were stained using the Kluver-Barrera technique [24].

2.3. Stereological study

Volume density (Vv), number of neurones per surface unit (Na) and neuronal numerical density (Nv) were calculated for each age.

Volume density (Vv): $Vv = Vo/Vi$ (mm^3/mm^3) where Vo is the volume occupied by neuronal profiles in a given volume Vi.

To calculate this parameter we applied Hilliard and Cahn's point-counting method [25]. A grid of 225 points was used, the distance between them, 'd', being 1 cm. Each tissue section was analysed with a camera lucida at × 1.25 with a × 63 objective. Only the neuronal profiles showing evident nucleolus, according to Konigsmark's [26] criteria, were drawn when the histological field image was projected onto the grid situated on the working surface. Neurones were easily distinguished from glia by the following features: the neuronal nuclei being paler and having a granular appearance; they are larger and often have a well-developed nucleolus and a visible cytoplasmic edge [27]. The grid was applied twice on each of the twenty sections per brain analysed. In all cases the sections were taken from the left hemisphere (Figs. 2 and 3).

Number of neurones per surface unit (Na): $Na = n/At$ (mm-2) where n is the number of neurones observed in a given area At.

In our case the actual area, At, was 0.0362 mm^2

Neurone numerical density (Nv) or number of neuronal profiles per volume unit. This was calculate according to Weibel and Gomez's [28] formula: $Nv = K(Na)/\beta(Vv)$ where k is the size distribution coefficient (previously calculated as 1.0268)and, β the shape coefficient with a value of 1.4.

Fig. 2. Image of the visual sector of the TRN stained using the Kluver-Barrera technique. For each histological section the selected A and B fields are marked.(Objective 20 ×).

2.4. Statistical treatment

The Kolmogorov-Smirnov normality test was initially applied. As the distribution did not conform to normality, a non-parametric test was then applied (the Kruskall-Wallis test).In all cases, the significance level was of $P \leq 0.00001$. Finally, differences in each parameter for the various ages were analysed using a multiple comparison test at $P \leq 0.05$.

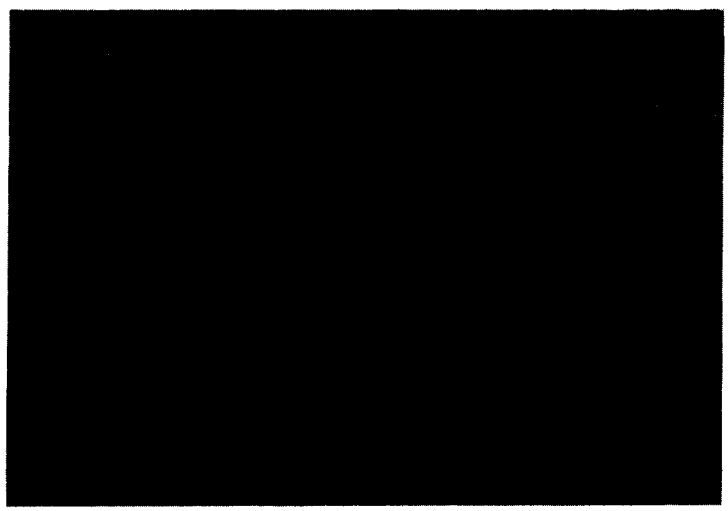

Fig. 3. Image of one of the histological fields selected, with the superimposed grid. Stained using the Einarson gallocyanin technique.(Objective 63 ×).

Table 2
Mean ($\bar{x}$) and standard error of the mean ($\pm$ S.E.M.) corresponding to Vv, Na and Nv of the cell bodies of the TRN visual sector neurones

Age (months)	Vv (mm^3/mm^3)	Na (mm^{-2})	Nv (neurones/mm^3)
3	0.0569 ± 0.0197	417.83 ± 8.78	$26\,679.66 \pm 590.32$
6	0.0474 ± 0.0186*	333.60 ± 7.98*	$21\,129.56 \pm 565.64$*
18	0.0328 ± 0.0154*	260.75 ± 6.88*	$17\,742.40 \pm 505.02$*
24	0.0291 ± 0.0135	220.23 ± 5.93*	$14\,504.22 \pm 425.85$*
30	0.0420 ± 0.0199*	306.13 ± 7.64*	$19\,912.83 \pm 553.77$*

*represents the existence of a significant difference between the indicated value and that of the preceding age at $P \leq 0.05$

3. Results

3.1. Volume density (Vv)

Volume density was at its highest in the 3rd month (0.0569 mm^3/mm^3). It decreased in a significant way up to the 18th month and stabilised in the 24th. Finally, it underwent a significant increase from the 24th to the 30th month (Table 2).

3.2. The number of neurones per surface unit (Na)

Na was at its maximum in the 3 month-old rats and decreased significantly up to the age of 24 months. Between the 24th and 30th months a significant increase took place. (Table 2).

3.3. Neurone numerical density (Nv)

This was also at its highest in the 3 month-old animals, decreasing significantly up to the 24th month, at which point it started to increase again until it reached, at the age of 30 months, similar levels to that of 6 month-old rats (Table 2).

4. Discussion

In this paper we present our findings, as regards volume density (Vv), number of neurones per surface unit (Na) and neurone numerical density (Nv) in the visual sector of the thalamic reticular nucleus (TRN) of Wistar rats during their ageing process after following stereological methodology.

In his model Beier states [29], that the process of life development consists of two well-defined periods. The first one, fundamentally mitotic, is associated with growth; the second is the adulthood period, which is initially characterised by 'optimal functioning' or maximum vitality and simply continues into ageing or decline- 'regressive growth' [29,30].

The age of the rats used was between 3 to 30 months, as our aim was to analyse, in a sequential manner, any changes in the above-mentioned parameters during the animal's second life-period, as defined by Beier [29], (from adulthood to old age),

paying special attention to the intermediate stages, as recommended by Coleman et al. [31]. At the age of three months, (the beginning of our study), the Wistar rat can be considered a young adult since it has reached sexual maturity. From this moment onwards, and always according to Beier's model, a functional decline will have already begun, although it will not be manifested until a later age. On the other hand, 30 month old rats can certainly be considered old since the survival rate in this breed is 50% [32] between the 27th and 28th month.

During this research it was not possible to calculate the total volume of the thalamic reticular nucleus visual sector and by extension, the total number of neurones, because its ventral boundaries are not clearly established [22]. Nevertheless, the parameters analysed, namely, Vv, Na and Nv, sufficed to reveal that from adulthood to old age there are two well-defined periods. The first one includes most of the rat's life, since it extends from the 3rd to the 24th months, while the second appears from the 24th onwards.

During the first period analysed, (3–24 months), the parameters measured decreased in a progressive and significant fashion. Numerous studies of the visual system carried out in different species support this observed decrease. Thus, in area 17 of the human cerebral cortex, declines in neuronal density of up to 31% [16] and 54% [33] have been observed from the age of 18 to 95. In a similar study, but on area 18, Shefer [34] found a 14% decrease.

Ordy et al. [18], reported an 18% loss in neuronal density in the visual cortex of F344 rats between the age of 17 and 29 months, while in the same cortex area of the Wistar Kyoto rat, Knox [12] found reductions of 30% between the 3rd and the 23rd–25th month. On the other hand, Peters et al., reported that in the visual cortex of monkeys [35] and Sprague–Dawley rats [36] this parameter was stable. At a different level of the visual system, the dLGN, Satorre et al. [19], observed that the number of neurones was stable from the 3rd to the 29th month in the self-same Sprague–Dawley rats. This heterogeneity in the reported results [37] is partly explained by the fact that the organic systems and the organs of the various rodents, present different degrees of vulnerability to ageing.

At the moment, we cannot assess whether the decrease in neuronal density is due to a genuine loss of neurones or to something else. In principle, we assume that neuronal loss is a consequence of ageing, since this phenomenon is very frequent in other centres of the nervous system and in several species [37]. However, it is currently acknowledged that neurones maintain some degree of plasticity during most of their life span, and this could be used to compensate, at least partially, for the damage caused by the degenerative changes associated with old age like, for instance, neuronal loss. This plasticity might manifest itself either as somatic growth [38–40] or dendritic growth [37,41,42]. Dendritic proliferation has been observed in certain brain regions suffering from neuronal loss during ageing [42–44]. This is thought to be due to the diminished competition from afferent fibres encounterd by the remaining neurones, or to the existence of a trophic factor generated either by neurones about to die or by their closest glia [41]. In any case, the dendritic response persists for most of the ageing period, but it does not continue to the end [42,43].

During the second period we studied — from the 24th to the 30th month an increase in neurone density was observed. The same phenomenon has been reported by Heumann and Leuba [45] in the cerebral cortex of 24 month-old mice, a very advanced age for this rodent. Similary, Leuba and Garey [17] and Haug [46] have detected a slight increase in neuronal density in the human visual cortex after the age of 80. On the other hand, Haug [47] has also reported that the brain shrinks at an advanced age. Moreover,in other centres of the nervous system a decrease in the size of neuronal cell bodies has been observed at very advanced ages [39,46] as well as a definite decrease in the dendritic tree after a long period of expansion [42,43,48,49], which has been interpreted as an irreversible collapse of the functional compensating mechanism mentioned earlier.

This increase in neurone density — from 14 504.22 to 19 912.83 neurones/mm^3 — cannot be due to an actual increase in the number of neurones. Indeed, we have found that within this same age range the size of neurone cell bodies in the visual sector of the TRN declined in a significant way (publication in preparation); an observation which is shared by other research on different centres of the nervous system [39,46]. We consider, in agreement with several authors [42,43,48,49], that a reduction of the cell body might induce a parallel decline in dendritic branching with the consequent neuropil decrease, a fact reported in some other works [42,43,48,49]. In conclusion, cell body and neuropil reduction could partly account for the increase in neuronal density observed during the later life period of the Wistar rat in the visual sector, i.e., 24th to 30th month.

References

[1] A.J. Sefton, A. Mackay-Sim, I.A. Baur and L.J. Cottee, Cortical projection to visual centres in the rat: an HRP study. *Brain Res., 215* (1981) 1–13.

[2] M. O'Connell, P.T. O'Hara and A.R. Lieberman, Connections of the thalamic reticular nucleus in the adult rat: a study using horseradish peroxidase (HRP) techniques. *J. Anat., 134* (1982) 610–611.

[3] P.T. O'Hara and A.R. Lieberman, Thalamic reticular nucleus: anatomical evidence that cortico-reticular axons establish monosynaptic contact with reticulo-geniculate projection cells. *Brain Res., 207* (1981) 153–156.

[4] P.T. Hale, A.J. Sefton, L.A. Baur and L.J. Cottee, Interrelations of the rat's thalamic reticular and dorsal lateral geniculate nuclei. *Exp. Brain Res., 45* (1982) 217–229.

[5] J.W. Crabtree and H.P. Killackey, The topographic organisation and axis of projection within the visual sector of the rabbit's thalamic reticular nucleus. *Eur. J. Neurosci., 1* (1989) 94–108.

[6] J. Mitrofanis and R.W. Guillery, New views of the thalamic reticular nucleus in the adult and the developing brain. *Trends Neurosci., 16* (1993) 240–245.

[7] G. Henderson, B.E. Tomlinson and P.H. Gibson, Cell counts in human cerebral cortex in normal adults throughout life using an image analysing computer. *J. Neurol. Set., 46* (1980) 113–136.

[8] R.D. Terry, R. DeTeresa and L.A. Hansen, Neocortical cell counts in normal human adult aging. *Ann. Neurol., 21* (1987) 530–539.

[9] D.M. Mann, P.O. Yates and B. Marcyniuk, Changes in nerve cells of the nucleus basalis of Meynert in Alzheimer's disease and their relationship to ageing and to the accumulation of lipofucsin pigment. *Mech. Ageing Dev., 25* (1984) 189–204.

[10] P.L. McGeer, E.G. McGeer, J.S. Suzuki, C.E. Dolman and T. Nagai, Aging, Alzheimer's disease and the cholinergic system of the basal forebrain. *Neurology, 34* (1984) 741–745.

[11] J. Rogers, M.A. Silver, W.J. Shoemaker and F.E. Bloom, Senescent changes in a neurobiological model system: cerebellar Purkinje cell electrophysiology and correlative anatomy. *Neurobiol. Aging*, *1* (1980) 3–11.
[12] C.A. Knox, Effects of aging and chronic arterial hypertension on the cell populations in the neocortex and archicortex of the rat. *Acta Neuropathol.*, *56* (1982) 139–145.
[13] K.R. Brizzee, J.M. Ordy and R.T. Bartus, Localization of cellular changes within multimodal sensory regions in aged monkey brain: possible implications for aged-related cognitive loss. *Aging*, *1* (1980) 45–52.
[14] R. Nandy, Morphological changes in the cerebellar cortex of aging *Macaca nemestrina*. *Neurobiol. Aging*, *2* (1981) 61–64.
[15] C.A. Curcio and P.D. Coleman, Stability of neuron number in cortical barrels of aging mice. *J. Comp. Neurol.*, *212* (1982) 158–172.
[16] H. Brody, Organization of the cerebral cortex. III. A study of aging in the human cerebral cortex. *J. Comp. Neurol.*, *102* (1955) 511–556.
[17] G. Leuba and L.J. Garey, Evolution of neuronal numerical density in the developing and aging human visual cortex. *Human Neurobiol.*, *6* (1987) 11–18.
[18] J.M. Ordy, K.R. Brizzee, B. Kaack and J. Hansche, Age differences in short-term memory and cell loss in the cortex of the rat. *Gerontology*, *24* (1978), 276–285.
[19] J. Satorre, J. Cano and F. Reinoso-Suarez, Stability of the neuronal population of the dorsal lateral geniculate nucleus (LGNd) of aged rats. *Brain Res.*, *339* (1985), 375–377.
[20] J. Rogers, Summary and recommendations of the Venice conference on animal models for aging research. *Neurobiol. Aging*, *12* (1991) 699–701.
[21] L.J. Pellegrino, A.S. Pellegrino and A.J. Cushman, *A stereotaxic atlas of the rat brain*. In L.J. Pellegrino (ed.), Plenum Press. New York. 1979.
[22] K.E. Webster, B.J. Arzymanow, P.T. O'Hara and A.R. Lieberman, Transneuronal labelling of the visual portion of the thalamic reticular nucleus in the rat. *J. Anat.*, *133* (1981) 654.
[23] L. Einarson, On the theory of gallocyanin-chromalum staining and its application for quantitative estimation of basophilia. A selective staining for exquisite progressivity. *Acta Pathol. Microbiol. Scand.*, *28* (1951) 82–102.
[24] H. Kluver and E. Barrera, A method for the combined staining of cell and fibers in the nervous system. *J. Neuropath. Exp. Neurol.*, *12* (1953) 400–403.
[25] J.E. Hilliard and J.W. Cahn, An evaluation of procedures in quantitative metallography for volume fraction analysis. *Transactions of the Metallurgical Society of the American Engineers*, 1961, pp. 221–344.
[26] B.W. Konigsmark, Methods for the counting of neurons. In W.J.H. Nauta and E.O.E. Ebbersson (eds.), *Contemporary Research Methods in Neuroanatomy*. Springer. New York, 1970, pp. 315–340.
[27] G. Leuba and R. Kraftsik, Visual cortex in Alzheimer's disease: Occurrence of neuronal death and glial proliferation, and correlation with pathological hallmarks. *Neurobiol. Aging*, *15* (1994) 23–49.
[28] E.R. Weibel and D.M. Gomez, A principle for counting tissue structures on random sections. *J. Appl. Physiol.*, *17* (1962) 343–348.
[29] W. Beier, Vitalitätskonzept und biologishcer Index nach Ries. *Z. Z. Gerontpl.*, *18* (1985) 353–357.
[30] A. Ruiz-Torres and E. Corpas, Anthropometrical changes in aging: Their relationship with GH secretion and its possible metabolic consequences. In Z. Laron, O. Butenand (eds.), *Growth Hormone Replacement Therapy in Adults: Pros and Cons.*, Freund Publishing Houe Ltd, London, 1983, pp. 123–136.
[31] P. Coleman, C. Finch and J. Joseph, The need for multiple time points in aging studies. *Neurobiol. Aging*, *11* (1990) 1–2.
[32] B. Janicke, D. Wrobel and G. Schulze. The effects of various drugs on performance capacity of old rats. *Pharmacopsychiatr.*, *18* (1985) 136–137.
[33] K.O. Devaney and H.A. Johnson, Neuron loss in the aging visual cortex of man. *J. Gerontol.*, *35* (1980) 836–841.
[34] V.F. Shefer, Absolute number of neurons and thickness of the cerebral cortex during aging, senile and vascular dementia, and Pick's and Alzheimer's diseases. *Neurosci. Behav. Physiol.*, *6* (1973), 319–324.

[35] A. Peters. The absence of significant neuronal loss from cerebral cortex with age. *Neurobiol. Aging, 14* (1993) 657–658.

[36] A. Peters, M.L. Feldman and D.W. Vaughan, The effect of aging on the neuronal population within area 17 of adult rat cerebral cortex. *Neurobiol. Aging, 4* (1983) 273–282.

[37] P.D. Coleman and D.G. Flood, Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. *Neurobiol. Aging, 8* (1987) 521–545.

[38] G.M. Gilad, J.M. Rabey, Y. Tizabi and V.H. Gilad, Age-dependent loss and compensatory changes of septohippocampal cholinergic neurons in two rat strains differing in longevity and response to stress. *Brain Res., 436* (1987) 311–322.

[39] S. De Lacalle, I. Iraizoz and L.M. Gonzalo, Differential changes in cell size and number in topographic subdivisions of human basal nucleus in normal aging. *Neuroscience, 43* (1991) 445–456.

[40] T. Yoshinaga, Morphometric study of the human locus coeruleus changes with ageing and degenerative neurological diseases. *Fukuoka Acta Med., 77* (1986) 293–308.

[41] P.D. Coleman and D.G. Flood, Dendritic proliferation in the aging brain as a compensatory repair mechanism. In D.F. Swaab, E. Fliers, M. Mirmiran, W.A. Van Gool and F. Van Haaren (eds.), *Progress in Brain Research*, Vol.70. Elsevier, Amsterdam, 1986, pp. 227–237.

[42] D.G. Flood, S.J. Buell, C.H. DeFiore, G.J. Horwitz and P.D. Coleman, Age-related dendritic growth in dentate gyrus of human brain is followed by regression in the 'oldest old'. *Brain Res., 345* (1985) 366–368.

[43] J.W. Hinds and N.A. McNelly, Aging of the rat olfactory bulb: growth and atrophy of constituent layers and changes in size and number of mitral cells. *J. Comp. Neurol., 171* (1977) 345–368.

[44] S.J. Buell and P.D Coleman, Quantitative evidence for selective dendritic growth in normal human aging but not in senile dementia. *Brain Res., 214* (1981) 23–41.

[45] D. Heumann and G. Leuba, Neuronal death in the development and aging of the cerebral cortex of the mouse. *Neuropathol. Appl. Neurobiol., 9* (1983), 297–311.

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

[47] H. Haug, Are neurons of the human cerebral cortex really lost during aging? A morphometric examination. In J. Traber and W.H. Gispen, (eds.), *Senile Dementia of the Alzheimer Type*. Springer, Berlin-Heidelberg-New York, 1985, pp 150–163.

[48] J.A. Rafols, H.W. Cheng and T.H. McNeill, Golgi study of the mouse striatum: age-related dendritic changes in different neuronal populations. *J. Comp. Neurol., 279* (1989) 212–227.

[49] R.E.J. Dyball and L.L. Garten, Stimulus-related changes in the dendrites of magnocellular neurons. *Brain Res. Bull., 20* (1988) 675–680.