Testosterone supplementation restores vasopressin innervation in the senescent rat brain

Elmer Goudsmit, Eric Fliers and Dick F. Swaab

Netherlands Institute for Brain Research, Amsterdam (The Netherlands)

(Accepted 24 May 1988)

Key words: Aging; Vasopressin innervation; Oxytocin innervation; Testosterone; Sex steroid; Plasticity in senescence; Rat

The vasopressin (AVP) innervation in the male rat brain is decreased in senescence. This decrease is particularly pronounced in brain regions where AVP fiber density is dependent on plasma levels of sex steroids. Since plasma testosterone levels decrease progressively with age in the rat, the possibility of restoring central AVP innervation by peripheral testosterone supplementation was investigated by giving senescent (33 months) Brown–Norway rats subcutaneous implants of either empty or testosterone-filled silastic tubes for the period of 1 month. Plasma testosterone levels of testosterone-treated animals were restored to values which did not differ from those of young animals. The results show that the age-related decline in AVP fiber density can indeed be reversed by testosterone supplementation. In contrast, oxytocin innervation, which was previously shown not to be testosterone-dependent, was not restored. These results show for the first time restoration of a specific innervation pattern in the senescent rat brain mediated by peripheral hormones and indicate that a considerable plasticity is retained in the aging central nervous system.

INTRODUCTION

In addition to the neurohypophyseal projections of the supraoptic (SON) and paraventricular (PVN) nuclei, from where the peptides vasopressin (AVP) and oxytocin (OXT) are released into the circulation³¹, neuronal pathways immunoreactive for AVP and OXT were demonstrated in a large number of areas in the rat brain². In these extrahypothalamic fiber systems the peptides probably act as neurotransmitters or neuromodulators^{3,42}. Extrahypothalamic AVP fibers were shown to originate from AVP cells in the PVN^{9,37}, the suprachiasmatic nucleus (SCN)²¹, the bed nucleus of the stria terminalis (BST)⁹ and the medial amygdala (MA)⁴. Extrahypothalamic OXT fibers were shown to originate in the PVN^{9,37}. Central functions of AVP and OXT may include the regulation of blood pressure³², body temperature²⁹, nociception²⁴, avoidance behavior¹ and maternal behavior⁴¹.

A sexual dimorphism in AVP fiber density, which

depends on the neonatal presence of testosterone, was demonstrated in the rat brain: males having a denser AVP innervation of several brain structures than females^{8,11}. In the adult rat, this sexual dimorphism is maintained by circulating sex steroids, since castration of adult male rats was shown to cause a decrease in AVP fiber density in a number of brain structures which could be restored through the peripheral administration of gonadal hormones^{10,12}. Dependency on sex steroids was evident in projections of the BST and the MA, whereas projections of the PVN and the SCN were not affected by castration or administration of gonadal hormones¹². OXT innervation was shown not to depend on plasma levels of sex steroids¹³.

In the senescent male rat brain a decrease in AVP fiber density was observed, which was particularly pronounced in the sex steroid-dependent areas¹⁸. Since plasma testosterone levels decrease progressively with age in the rat^{23,35}, it was proposed that the age-related decrease in central AVP innervation

might be due to decreased plasma levels of testosterone¹⁸. In the present study the hypothesis that testosterone supplementation might restore AVP innervation in the senescent rat brain was investigated. OXT innervation was also studied in order to assess the specificity of this treatment.

MATERIALS AND METHODS

Six young (2 months) and 20 aged (33 ± 0.2 months; mean $\pm$ S.E.M.) Brown-Norway rats (BN/BiRij, TNO IVEG, Rijswijk, The Netherlands) were housed under controlled temperature (21 °C) and light, and supplied with food and water ad libitum. The 50% survival of this strain is about 30 months²⁸.

All animals received subcutaneous implants of 2.5 cm silastic tubing (Talas BV, Ommen, The Netherlands; 1.5 mm i.d.; 2.1 mm o.d.) under light ether anesthesia. Tubes were either filled with testosterone (4-androstene-17 β -ol-3-one; Steraloids Inc., Wilton NH, U.S.A.) or kept empty and sealed at both ends with silicone. The aged animals were divided at random into two equal groups, one of which received testosterone-filled implants, while the other was given empty tubes. All young animals received empty tubes. The animals were allowed to survive for 31 days with these implants and were subsequently killed by perfusion with 2.5% glutaraldehyde in 0.1 M cacodylate (pH 7.5) under Hypnorm anesthesia (0.1 ml/100 g b.wt., i.p.). Both testes were removed after perfusion and weighed together. Viscera were inspected for gross pathology. Brains were removed and left in 2.5% glutaraldehyde for 2.5 h and subsequently cut in 2 mm frontal slices and postfixed overnight in 5% glutaraldehyde at 4 °C. Brain slices were stored until sectioning at 4 °C for 5–8 weeks in 0.05 M Tris, pH 7.6, containing 0.9% NaCl (Tris-NaCl).

Radioimmunoassay

Immediately prior to perfusion 1-ml blood samples were extracted from the anesthetized animals by cardiac puncture with a 1-ml syringe prefilled with 50 μ l heparin (25 IU). Samples were kept in chilled test-tubes for approximately 1 h. The supernatant was collected after 5-min centrifugation at 1400 g and was subsequently stored at -20 °C until further processing. Total plasma testosterone levels were determined by radioimmunoassay³⁴. Plasma levels of free

testosterone were determined using a Coat-A-Count free testosterone assay kit (Diagnostic Products Corporation, Los Angeles, U.S.A.). The lower detection levels of the assays were 86 and 0.6 pg/ml for total and free testosterone, respectively. Values at detection level were used as actual data in the statistical analyses.

Immunocytochemistry

Vibratome sections (60 μ m) were taken at regular distances along the rostrocaudal axis of the brain (about 35 sections per animal) and were stained simultaneously for all animals by the following procedure: (1) washing in 0.05 M Tris, pH 7.6, containing 0.9% NaCl and 0.5% Triton X-100 (Tris-Triton) (3 $\times$ 15 min); (2) incubation with rabbit AVP-antiserum (Truus, 18-9-85) 1:1000 in Tris-Triton (overnight at 4 °C); (3) washing with Tris-Triton (3 $\times$ 15 min); (4) incubation with goat anti-rabbit IgG serum (Betsy, 16-2-82) 1:50 in Tris-Triton (90 min); (5) washing in Tris-Triton (3 $\times$ 15 min); (6) incubation with peroxidase-antiperoxidase (PAP) 1:1000 in Tris-Triton (90 min); (7) washing in Tris-NaCl (3 $\times$ 15 min); (8) incubation with 0.5 g/ml 3,3'-diaminobenzidine (DAB, Sigma) 0.05 M Tris-HCl, pH 7.6, 0.01% H₂O₂ (20 min); (9) washing in Tris-NaCl (2 $\times$ 10 min). Sections were mounted on chrome-alum coated slides, dried overnight, dehydrated and coverslipped with Entellan (Merck). The same procedure was applied using rabbit OXT antiserum (0-1-V, 4-4-75) 1:1000 in Tris-Triton.

Separate sets of free-floating sections were stained using AVP antiserum (1:450 in Tris-Triton) which had been preadsorbed with OXT-glutaraldehyde-coupled sepharose beads or using OXT antiserum (1:380 in Tris-Triton) which had been preadsorbed with AVP-glutaraldehyde-coupled sepharose beads in order to remove crossreacting components from the antisera³³. Following purification crossreactivity was reduced to the level of preimmune control serum as measured according to Pool et al.³³.

Morphological analysis

Central AVP and OXT fiber density was studied in the following areas (nomenclature and localization as the anteroposterior distance from bregma (mm) according to Paxinos and Watson³⁰): ventral limb of Broca's diagonal band (most ventral part, +0.7); lat-

eral septum (−0.3); basal nucleus of Meynert (−2.3); paraventricular thalamic nucleus (immediately adjacent to ependyma, −2.8); dorsomedial hypothalamic nucleus (−3.3); medial amygdaloid nucleus (−3.3); lateral habenular nucleus (−3.8); ventral tegmental area (−5.3); substantia nigra, pars compacta (ventromedial part, −5.8); hippocampus, ventral part (−5.8); central gray (−5.8); locus coeruleus (−9.3); nucleus of the solitary tract (−11.8) and ambiguous nucleus (−13.8). The latter 3 areas were also studied in separate sections stained with purified AVP and OXT antisera.

For each area one representative section was selected per animal and these were compared in a blind procedure using two Zeiss microscopes with $\times 25$ PLAN objectives and ranked according to fiber density by 3 separate investigators and given a corresponding rank number.

Statistics

Differences in hormone levels and testicular weight between groups were tested by means of the Mann–Whitney *U*-test (two-tailed, corrected for ties, 0.05 level of significance). The correlation between plasma levels of total and free testosterone was calculated using Spearman's rank correlation

(two-tailed, 0.05 level of significance). Rankings of AVP and OXT fiber density by the 3 observers were compared for each area using Friedman's test (0.01 level of significance). Differences in rank numbers of OXT and AVP fiber density between young sham-implanted and aged sham-implanted and between aged sham-implanted and aged testosterone-implanted animals were tested using the Mann–Whitney *U*-test (two-tailed, corrected for ties, 0.05 level of significance).

RESULTS

In the course of the experiment 7 aged rats died, which was in line with the survival curve of the Brown–Norway strain at 33–34 months²⁸. The following results are based on the remaining 13 aged rats, 6 of which were testosterone-implanted and 7 sham-implanted. All young rats survived until perfusion. Gross pathological examination of the aged rats revealed one pineal tumor, one liver tumor, one bladder tumor in combination with bladder stones and one soft tissue tumor of a hind paw. No macroscopic pathology was detected in any of the young rats.

The blood sample of one aged rat was too small for determination of both total and free testosterone and

Fig. 1. Plasma levels of total and free testosterone (A, B) and testicular weight (C) in young sham-implanted (Y; $n = 6$), aged sham-implanted (A; $n = 7$) and aged testosterone-implanted (A + T; $n = 6$) animals. Bars (A, B) represent median levels or (C) means $\pm$ S.E.M. Note the decrease in testosterone levels in aged rats and the restoration of these levels in testosterone-implanted aged rats. Also note the decrease in testicular weight in aged rats which is even stronger in animals which received testosterone treatment.

only total testosterone was determined in this case. Plasma levels of both total and free testosterone showed a strong positive correlation ($r = 0.96$; $P < 0.001$) and were significantly reduced in aged animals as compared with young ones ($P < 0.05$; Fig. 1). Hormone levels of testosterone-implanted aged rats were elevated as compared with sham-implanted aged rats ($P < 0.05$ and $P = 0.055$ for total and free

testosterone, respectively) to values which did not differ significantly from those of young animals ($P > 0.68$ and $P > 0.47$ for total and free testosterone, respectively; Fig. 1). Testicular weight of aged sham-implanted rats was significantly decreased as compared to young sham-implanted animals ($P < 0.01$), while testicular weight of testosterone-implanted aged rats was even lower than that of sham-im-

Fig. 2. Immunocytochemically stained vasopressin (AVP) fibers in the central gray (upper panel) and locus coeruleus (lower panel) of young sham-implanted (a, d), aged sham-implanted (b, e) and aged testosterone-implanted (c, f) rats. Note the decrease in fiber density in the aged animals which is restored by testosterone supplementation. AVP fibers in the locus coeruleus were stained with a purified antiserum in order to exclude crossreactivity with OXT fibers.

planted aged rats ($P < 0.02$; Fig. 1).

AVP immunoreactive fibers were observed in all brain structures studied, whereas extrahypothalamic fibers immunoreactive for OXT were visible in substantial numbers only in the locus coeruleus, the nucleus of the solitary tract and the ambiguous nucleus. The application of purified AVP antiserum resulted in complete absence of immunoreactivity in the nucleus of the solitary tract and the ambiguous nucleus, but not in the locus coeruleus, where fibers immunoreactive for AVP were still present (Fig. 2). Intense

staining of OXT fibers was obtained in all 3 areas with the purified OXT antiserum.

Rankings of AVP and OXT fiber density were performed in 14 different brain areas. The rankings of the 3 observers were not random according to Friedman's test ($P < 0.001$ for each area). Median rank numbers for AVP fiber density in old sham-implanted animals were lower than those of young animals in all areas studied with the exception of the vertical limb of Broca's diagonal band and the lateral habenular nucleus (Fig. 3). The decrease in AVP fiber

Fig. 3. Median rank numbers of AVP and OXT fiber density in 14 brain areas in young sham-implanted (dark-gray bars; $n = 6$), aged sham-implanted (light-gray bars; $n = 7$) and aged testosterone-implanted (hatched bars; $n = 6$) rats. Note the general tendency for a decrease in AVP and OXT fiber density with aging, which reached statistical significance in the central gray and the locus coeruleus (o). Also note the general tendency for an increase in AVP fiber density following testosterone supplementation, which reached statistical significance in the ventral tegmental area, the substantia nigra pars compacta, the hippocampus, the central gray and the locus coeruleus (*). In contrast, OXT fiber density was not enhanced in testosterone-implanted aged rats. o, $P < 0.05$; *, $P < 0.05$; **, $P < 0.01$. Amb, ambiguous nucleus; B, basal nucleus of Meynert; CG, central gray; DM, dorsomedial hypothalamic nucleus; Hi, hippocampus; LC, locus coeruleus; LH, lateral habenular nucleus; LS, lateral septum; MA, medial amygdaloid nucleus; PV, paraventricular thalamic nucleus; SNC, substantia nigra pars compacta; Sol, nucleus of the solitary tract; VDB, ventral limb of Broca's diagonal band; VTA, ventral tegmental area.

density reached statistical significance in the central gray and the locus coeruleus ($P < 0.05$; Figs. 2 and 3). OXT innervation was also diminished in the sham-implanted aged animals (Fig. 3), the decrease being statistically significant in the locus coeruleus ($P < 0.05$). Testosterone-implanted aged rats showed an increase in AVP fiber density as compared with sham-implanted aged animals in all areas (Fig. 3). This increase was statistically significant in the ventral tegmental area, substantia nigra pars compacta, ventral hippocampus, central gray and locus coeruleus ($P < 0.05$). In contrast to the AVP innervation, OXT innervation was not enhanced by testosterone supplementation in any of the areas studied (Fig. 3).

DISCUSSION

The present results confirm the age-related decrease in plasma testosterone levels and testicular weight reported in various rat strains, including the Brown-Norway strain^{23,35}. The additional decrease in testicular weight in testosterone-implanted aged rats suggests testicular atrophy due to inhibition of gonadotropin release³⁶. The highly significant correlation between plasma levels of free and total testosterone excludes age-related changes in the bio-availability of this hormone due to altered binding to plasma hormones as seen in aging rhesus monkey⁵ and man³⁸.

The decrease in central AVP fiber density observed in the untreated aged rats is in line with observations by Fliers et al. in the same strain¹⁸, although the changes reported by these authors reached statistical significance in a larger number of areas. The differences may in part be explained by variability in plasma testosterone levels and AVP fiber density between individual senescent animals. In addition, the AVP antisera used in the two studies were different, the present staining being more intense. The present data also agree with those presented by Dorsa and Bottemiller¹⁵, who reported a decrease in AVP content determined by radioimmunoassay in the locus coeruleus in 26-month-old Long-Evans rats. However, the age-related decrease in AVP content in the dorsal septum as reported by these authors was not reflected by a statistically significant decrease in AVP fiber density in that area.

In the present study no AVP immunoreactivity

was detected in the nucleus of the solitary tract and in the ambiguous nucleus when purified antisera were used, indicating that these two nuclei only contain OXT fibers. This finding seems to be at variance with the demonstration of AVP-immunoreactive terminals in the nucleus of the solitary tract in the Wistar rat using a purified antiserum⁴² and with the detection of AVP in the nucleus of the solitary tract and the ambiguous nucleus in that strain by radioimmunoassay¹⁴. In addition to strain differences, this discrepancy might be due to differences in fixation, antiserum or purification procedure. The presence of both AVP and OXT fibers in the locus coeruleus^{12,18} was confirmed by the present results.

Testosterone supplementation restored total and free plasma testosterone levels in senescent rats to levels observed in young animals. In the testosterone-supplemented aged rats a significant increase in AVP fiber density was observed in the ventral hippocampus, central gray, substantia nigra pars compacta and locus coeruleus; areas where AVP innervation was previously shown to be testosterone-dependent^{10,12,13}. These fiber systems probably originate in the bed nucleus of the stria terminalis (BST) and the medial amygdala (MA)^{4,9}. High concentrations of androgen- and estrogen-concentrating neurons were demonstrated in the BST and MA³⁹ and testosterone was shown to enhance AVP immunoreactivity in these nuclei, probably by stimulating AVP synthesis^{12,40}. This might cause efferent fibers to 'fill up' again with AVP, thus explaining the enhanced detection of AVP-immunoreactive fibers observed in testosterone-treated senescent rats. Data of Jolkkonen et al.²², who showed that CSF levels of AVP are higher in male than in female rats and decrease with age, are in line with a stimulatory role of testosterone on central AVP synthesis and release. An alternative mechanism for the observed increase in AVP fiber density in testosterone-supplemented aged rats would be proliferation of AVP fibers, analogous to the dendritic growth shown in motoneurons of castrated adult male rats and in neurons of the song control system of ovariectomized adult female canaries following 4 weeks of testosterone supplementation^{7,25}.

The present observation that OXT innervation, which was not found to be testosterone-dependent¹³, is not restored in senescence by testosterone supple-

mentation emphasizes the marked specificity in the effects of testosterone on AVP fibers in the senescent rat brain.

The functional implications of restoration of AVP innervation in specific areas in the senescent rat brain are unknown at present. However, one might speculate on a role in cognitive functioning with regard to the locus coeruleus and the hippocampus¹.

The present results are in line with studies which demonstrate that a marked degree of plasticity is retained in the central nervous system to a very old age. Dendritic proliferation, which might be compensatory for neuronal loss, was demonstrated in the aging human brain⁶ and recovery of lesion-induced synapse loss was shown in the brain of 2–2.5-year-old Sprague–Dawley rats²⁰. In addition, an increase in cortical thickness and a restoration of sleep patterns were demonstrated in senescent rats following exposure to an enriched environment²⁷. A role of gonadal hormones in plasticity of the senescent rat brain, as indicated by the present results, is supported by the demonstration of enhanced synapse formation in the arcuate nucleus of estrogen-treated senescent female rats²⁶. In addition, a restoration of axoplasmic transport was shown in motoneuronal axons of senescent rats treated with sex steroids¹⁹.

The presently reported restoration of central AVP innervation in the senescent rat brain following restoration of peripheral hormone levels might be rele-

vant for the development of therapeutic strategies in some age-related disorders of the central nervous system. Recent reports on decreased plasma estrogen levels in postmenopausal women suffering from Alzheimer's disease as compared with age-matched controls¹⁶ and on the positive effects of estradiol therapy on both affective and cognitive test scores in these patients¹⁷ might be based on comparable mechanisms. In this regard it is of interest that estradiol was also shown to restore AVP innervation in castrated adult male rats, although less effectively than testosterone¹³. However, the ways in which sex steroids may affect the senescent *human* brain are at present unknown.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Tj. van der Woude and Ms. E.M. van der Beek for technical assistance and Mr. N. Groenestein for performing the radioimmunoassays. We also thank Mr. H. Stoffels and Mr. G. van der Meulen for preparing the illustrations and Dr. R.A. Baker for correcting the English. This work is published within the framework of the Commission of the European Communities Concerted Action on Cellular Aging and Disease (EURAGE) and has been supported by the Foundation for Medical and Health Research (MEDIGON; Grant 900-552-056).

REFERENCES

- 1 Bohus, B., Conti, L., Kovács, G.L. and Versteeg, D.H.G., Modulation of memory processes by neuropeptides: interaction with neurotransmitter systems. In C.A. Marsan and H. Matthies (Eds.), *Neuronal Plasticity and Memory Formation*, Raven, New York, 1982, pp. 75–87.
- 2 Buijs, R.M., Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat: pathways to the limbic system, medulla oblongata and spinal cord, *Cell Tissue Res.*, 192 (1978) 423–435.
- 3 Buijs, R.M. and Van Heerikhuizen, J.J., Vasopressin and oxytocin release in the brain — a synaptic event, *Brain Research*, 252 (1982) 71–76.
- 4 Caffé, A.R., Van Leeuwen, F.W. and Luiten, P.G.M., Vasopressin cells in the medial amygdala of the rat project to the lateral septum and ventral hippocampus, *J. Comp. Neurol.*, 261 (1987) 237–252.
- 5 Chambers, K.C., Hess, D.L. and Phoenix, C.H., Relationship of free and bound testosterone to sexual behavior in old rhesus males, *Physiol. Behav.*, 27 (1981) 615–620.
- 6 Coleman, P.D. and Flood, D.G., 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.), *Aging of the Brain and Alzheimer's Disease, Progress in Brain Research, Vol. 70*, Elsevier, Amsterdam, 1986, pp. 227–237.
- 7 DeVoogd, T. and Nottebohm, F., Gonadal hormones induce dendritic growth in the adult avian brain, *Science*, 214 (1981) 202–204.
- 8 De Vries, G.J., Best, W. and Sluiter, A.A., The influence of gonadal steroids on the development of a sex difference in the vasopressinergic innervation of the brain, *Dev. Brain Res.*, 8 (1983) 377–380.
- 9 De Vries, G.J. and Buijs, R.M., The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum, *Brain Research*, 273 (1983) 307–317.
- 10 De Vries, G.J., Buijs, R.M. and Sluiter, A.A., Gonadal hormone actions on the morphology of the vasopressinergic innervation of the adult rat brain, *Brain Research*, 298 (1984) 141–145.
- 11 De Vries, G.J., Buijs, R.M. and Swaab, D.F., Ontogeny of the vasopressinergic neurons of the suprachiasmatic nucleus and their extrahypothalamic projections in the rat brain — presence of a sex difference in the lateral septum,

- Brain Research*, 218 (1981) 67–78.
- 12 De Vries, G.J., Buijs, R.M., Van Leeuwen, F.W., Caffé, A.R. and Swaab, D.F., The vasopressinergic innervation of the brain in normal and castrated rats, *J. Comp. Neurol.*, 233 (1985) 1–19.
 - 13 De Vries, G.J., Duetz, W., Buijs, R.M., Van Heerikhuijsen, J. and Vreeburg, J.T.M., Effects of androgen and estrogen on the vasopressin and oxytocin innervation of the adult rat brain, *Brain Research*, 399 (1986) 296–302.
 - 14 Dogterom, J. and Buijs, R.M., Vasopressin and oxytocin distribution in rat brain: radioimmunoassay and immunocytochemical studies. In C.A. Marsan and W.Z. Traczyk (Eds.), *Neuropeptides and Neural Transmission*, Raven, New York, 1980, pp. 307–314.
 - 15 Dorsa, D.M. and Bottemiller, L., Age-related changes of vasopressin content of microdissected areas of the rat brain, *Brain Research*, 242 (1982) 151–156.
 - 16 Fillit, H.M., Ashby, D., Weinreb, H., Zabriskie, J.B., Luine, V.N. and McEwen, B.S., Estrogen levels in postmenopausal women with senile dementia-Alzheimer's type (SDAT) are significantly lower than matched controls, *Soc. Neurosci. Abstr.*, 12 (1986) 259.11.
 - 17 Fillit, H., Weinreb, H., Cholt, I., Luine, V., McEwen, B., Amador, R. and Zabriskie, J., Observations in a preliminary open trial of estradiol therapy for senile dementia-Alzheimer's type, *Psychoneuroendocrinology*, 11 (1986) 337–345.
 - 18 Fliers, E., De Vries, G.J. and Swaab, D.F., Changes with aging in the vasopressin and oxytocin innervation of the rat brain, *Brain Research*, 348 (1985) 1–8.
 - 19 Frolkis, V.V., Tanin, S.A., Marcinko, V.I., Kulchitsky, O.K. and Yasechko, A.V., Axoplasmic transport of substances in motoneuronal axons of the spinal cord in old age, *Mech. Ageing Dev.*, 29 (1985) 19–28.
 - 20 Hoff, S.F., Scheff, S.W., Benardo, L.S. and Cotman, C.W., Lesion-induced synaptogenesis in the dentate gyrus of aged rats: I. Loss and reacquisition of normal synaptic density, *J. Comp. Neurol.*, 205 (1982) 246–252.
 - 21 Hoorneman, E.M.D. and Buijs, R.M., Vasopressin fiber pathways in the rat brain following suprachiasmatic nucleus lesioning, *Brain Research*, 243 (1982) 235–241.
 - 22 Jolkonen, J., Tuomisto, L., Van Wimersma Greidanus, T.B., Läärä, E. and Riekkinen, P.J., Vasopressin levels in cerebrospinal fluid in rats of different age and sex, *Neuroendocrinology*, 44 (1986) 163–167.
 - 23 Kaler, L.W. and Neaves, W.B., The androgen status of the aging male rat, *Endocrinology*, 108 (1981) 712–719.
 - 24 Kordower, J.H., Sikorsky, V. and Bodnar, R.J., Central antinociceptive effects of lysine-vasopressin and an analogue, *Peptides*, 3 (1982) 613–617.
 - 25 Kurz, E.M., Sengelaub, D.R. and Arnold, A.P., Androgens regulate the dendritic length of mammalian motoneurons in adulthood, *Science*, 232 (1986) 395–398.
 - 26 Matsumoto, A., Arai, Y. and Osanai, M., Estrogen stimulates neuronal plasticity in the deafferented hypothalamic arcuate nucleus in aged female rats, *Neurosci. Res.*, 2 (1985) 412–418.
 - 27 Mirmiran, M., Van Gool, W.A., Van Haaren, F. and Polak, C.E., Environmental influences on brain and behavior in aging and Alzheimer's disease. In D.F. Swaab, E. Fliers, M. Mirmiran, W.A. Van Gool and F. Van Haaren (Eds.), *Aging of the Brain and Alzheimer's Disease, Progress in Brain Research*, Vol. 70, Elsevier, Amsterdam, 1986, pp. 443–459.
 - 28 Mos, J. and Hollander, C.F., Analysis of survival data on aging rat cohorts: pitfalls and some practical considerations, *Mech. Ageing Dev.*, 38 (1987) 89–105.
 - 29 Naylor, A.M., Ruwe, W.D. and Veale, W.L., Thermoregulatory actions of centrally administered vasopressin in the rat, *Neuropharmacology*, 25 (1986) 787–794.
 - 30 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic, Sidney, 1982.
 - 31 Pickering, B.T., The molecules of neurosecretion: their formation, transport and release. In M.A. Corner and D.F. Swaab (Eds.), *Perspectives in Brain Research, Progress in Brain Research*, Vol. 45, Elsevier, Amsterdam, 1976, pp. 161–179.
 - 32 Pittman, Q.L., Lawrence, D. and McLean, L., Central effects of arginine vasopressin on blood pressure in rats, *Endocrinology*, 110 (1982) 1058–1060.
 - 33 Pool, Chr.W., Madlener, S., Diegenbach, P.C., Sluiter, A.A. and Van der Sluis, P., Quantification of antiserum reactivity in immunocytochemistry. Two new methods for measuring peroxidase activity on antigen-coupled beads incubated according to an immunoperoxidase method, *J. Histochem. Cytochem.*, 32 (1984) 921–928.
 - 34 Pratt, J.J., Wiegman, T., Lappöhn, R.E. and Woldring, M.G., Estimation of plasma testosterone without extraction and chromatography, *Clin. Chim. Acta*, 59 (1975) 337–346.
 - 35 Ravid, R., Fliers, E., Swaab, D.F. and Zurcher, C., Changes in vasopressin and testosterone in the senescent Brown-Norway (BN/BiRij) rat, *Gerontology*, 33 (1987) 87–98.
 - 36 Sartin, J.L. and Lamperti, A.A., FSH and LH response to testosterone and gonadotropin-releasing hormone in aging male rats, *Exp. Aging Res.*, 10 (1984) 183–187.
 - 37 Sawchenko, P.E. and Swanson, L.W., Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or the spinal cord in the rat, *J. Comp. Neurol.*, 205 (1982) 260–272.
 - 38 Semmens, J.B., Rouse, I.L., Beilin, L.J. and Masarei, J.R.L., Relationships between age, body weight, physical fitness and sex-hormone-binding globulin capacity, *Clin. Chim. Acta*, 113 (1983) 295–300.
 - 39 Stumpf, W.E. and Sar, M., Steroid hormone target sites in the brain: the differential distribution of estrogen, progesterone, androgen and glucocorticosteroid, *J. Steroid Biochem.*, 7 (1976) 1163–1170.
 - 40 Van Leeuwen, F.W., Caffé, A.R. and De Vries, G.J., Vasopressin cells in the bed nucleus of the stria terminalis of the rat: sex differences and the influence of androgens, *Brain Research*, 325 (1985) 391–394.
 - 41 Van Leengoed, E., Kerker, E. and Swanson, H.H., Inhibition of post-partum maternal behaviour in the rat by injecting oxytocin antagonist into the cerebral ventricles, *J. Endocrinol.*, 112 (1987) 275–282.
 - 42 Voorn, P. and Buijs, R.M., An immuno-electronmicroscopical study comparing vasopressin, oxytocin, substance P and enkephalin containing nerve terminals in the nucleus of the solitary tract in the rat, *Brain Research*, 270 (1983) 169–173.