

## **Sexual dimorphism in the distribution of glial fibrillary acidic protein in the supraoptic nucleus of the hamster**

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### INTRODUCTION

Sex steroids play a significant role in modulating postnatal maturation of the brain (Toran-Allerand, 1978; MacLusky & Naftolin, 1981) and abundant receptors for sex steroid have been found in regions where neuronal sex differences have been observed (Pfaff & Keiner, 1973; Cottingham & Pfaff, 1986). The effects of sex steroids on the redistribution of glial fibrillary acidic protein (GFAP) immunoreactivity in different rat brain areas have been demonstrated (Tranque *et al.* 1987; García-Segura *et al.* 1988); however, the literature notably lacks examples of the action of circulating sex steroids on astroglial cells in hypothalamic magnocellular nuclei. In the present work we have studied GFAP-immunostained sections of the supraoptic nucleus (SON) from male and female hamsters, in order to assess possible sex differences in the GFAP immunoreactivity.

### MATERIAL AND METHODS

Eight male and 8 female hamsters, aged 1 month, were used in this study. Hamsters were anaesthetised with ether and decapitated. The brains were then quickly removed, placed in B5 fixative (mercuric chloride 30%, acetone 22% and formaldehyde 12%, buffered at pH 6.6) and trimmed into blocks containing the hypothalamus. Tissue blocks were immersed in the same fixative for 5 h at 4 °C. Blocks were dehydrated in graded concentrations of ethanol and embedded in paraffin. Serial sections of the supraoptic region were cut at a thickness of 8  $\mu$ m. Sections were deparaffinised and treated with normal serum diluted 1:30 in Tris-buffer (pH 7.6). The slides were incubated overnight in GFAP antiserum (Dakopatts), diluted 1:500 in Tris-buffer at 4 °C. Following three 5 min washes in Tris-buffer, the sections were incubated in rabbit IgG (Dakopatts), diluted 1:50 for 1 h at 20 °C, washed in Tris-buffer, and incubated in PAP complex (Dakopatts), diluted 1:200 for 1 h at 20 °C. Thereafter, sections were rinsed twice in Tris-buffer and the peroxidase activity was demonstrated with 0.03% DAB (Sigma) in 0.05 M Tris-buffer with 0.005% H<sub>2</sub>O<sub>2</sub>, washed in distilled water, counterstained with toluidine blue, dehydrated in graded concentrations of ethanol and mounted in DePeX. Some sections were incubated with normal serum at a 1:30 dilution as the primary antiserum; these control sections showed no immunoreactive product. Immunostained sections of the supraoptic nuclei were studied in a Zeiss microscope. Quantitative evaluation was performed using a

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stereological grid, according to the point-counting method of Weibel (1979), in which the ratio of the immunoreactive profile surface to the volume of a given structure (surface density,  $S_v$ ) is calculated by the formula:  $S_v = 2I/L$  ( $I$  being the number of points at which the immunopositive profiles cross the test grid lines and  $L$  being the test line length in the tissue). Mean values were compared with the unpaired Student's  $t$  test.

## RESULTS

The GFAP immunoreactivity in the SON of males presented more GFAP positive astroglial processes than those of females. GFAP immunoreactivity was virtually absent from the SON in female hamsters, especially in the dorsal part of the nucleus (Fig. 1), whereas numerous well-defined GFAP-positive processes were visible within the male SON (Fig. 2). Although the SON of females lacked GFAP-immunoreactive material, astrocytic somata were observed among the neurons (Fig. 3). In contrast, male magnocellular neurons were surrounded by GFAP-positive processes (Fig. 4). A GFAP-immunostained ventral glial lamina was also observed basally to the SON, which was thicker in males than in females (Figs 1, 2). The morphometric analysis on GFAP-immunostained sections revealed differences in the surface density ( $S_v$ ) of GFAP-immunoreactive material when the SON of males and females were compared. The highest density of GFAP-positive material was observed in males (41.2%) and the lowest in females (23.6%); these values were significantly different ( $P < 0.001$ ).

## DISCUSSION

The results of the present investigation provide evidence that there is a marked difference in GFAP immunostaining between the SON of male and female hamsters. A male-female difference in synaptic pattern in the hypothalamus has been demonstrated (Güldner, 1982; Matsumoto & Arai, 1986; Perez, Naftolin & García-Segura, 1990) and steroid hormones are implicated in the formation of new synapses (Arai & Matsumoto, 1978; Arnold & Gorski, 1984; García-Segura, Baetens & Naftolin, 1986). On the other hand, astrocytes may regulate synaptic density (Meshul, Seil & Herndon, 1987), participate in the reorganisation of hypothalamic nuclei (Suárez *et al.* 1987; García-Segura *et al.* 1988) and a reduction in the number of GFAP-positive processes in the lactating rat SON has been observed (Salm, Smithson & Hatton, 1985). The mechanisms that promote the shortening of the astrocytic processes in female hamsters or the lengthening of the astrocytic processes in the males are not known, but our present findings clearly demonstrate a sexually dimorphic SON and it would seem likely that the lengths of the GFAP-positive processes in the male and the female SON might depend on exposure to different levels of sex steroids. Two possibilities can be considered to explain the sexual dimorphism observed in the hamster SON: either testosterone might induce GFAP-immunopositive material proliferation or oestrogen might inhibit gliofilament proliferation. On the other hand, steroid receptors have been described in cultured pituitocytes (Stumpf & Sar, 1976) and GFAP proliferation has been observed in cultured astrocytes under steroid stimulation (García-Segura, Torres-Alemán & Naftolin, 1989). Whether circulating sex steroids exert a direct effect on astrocytes, or operate via some indirect mechanism, cannot yet be determined with certainty. However, in light of the present results, such an effect would implicate sex steroids as a crucial factor in astroglial plasticity.

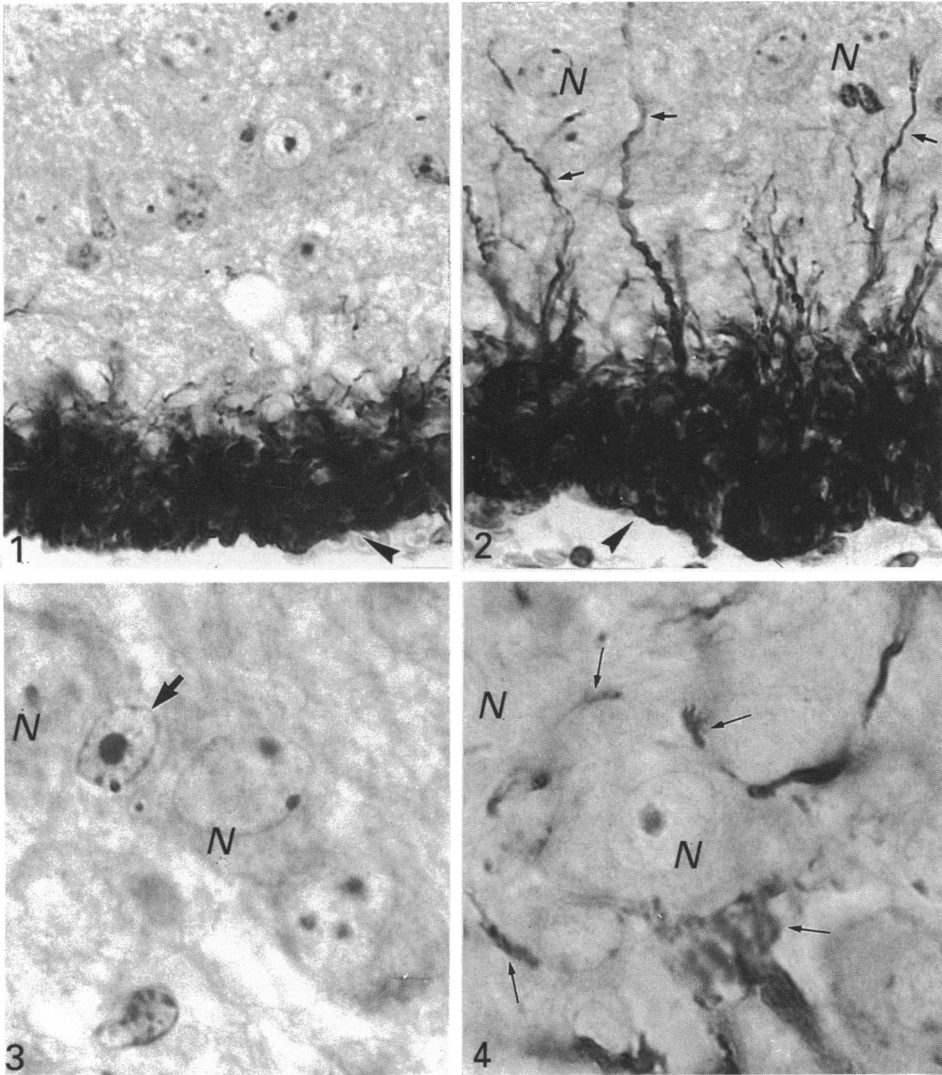


Fig. 1. Immunohistochemical localisation of GFAP in the female supraoptic nucleus showing GFAP-positive staining only in the subpial glial limiting membrane (arrowhead).  $\times 550$ .

Fig. 2. Immunohistochemical localisation of GFAP in the male supraoptic nucleus, showing increased GFAP-positive material in the subpial glial limiting membrane (arrowhead). GFAP immunopositive processes (arrows) are interposed among the neurosecretory neurons (*N*).  $\times 550$ .

Fig. 3. Dorsal zone of the female supraoptic nucleus. No GFAP immunoreactivity is observed. *N*, neurosecretory neurons. Astrocytic nucleus (arrow).  $\times 1400$ .

Fig. 4. Dorsal zone of the male supraoptic nucleus. GFAP immunoreactive processes (arrows) are located among the neurons (*N*). These astrocytic processes present different density and thickness in their immunoreactive material.  $\times 1400$ .

#### SUMMARY

The supraoptic nuclei (SON) of the hypothalamus of male and female hamsters were examined immunohistochemically at 1 month of age for possible sex differences in astroglial organisation. The morphometric analysis revealed the presence of more glial fibrillary acidic protein (GFAP) immunostaining in the SON of males as

compared with females. GFAP-positive processes were located among the neurosecretory neurons in the males, but were quite scarce among these neurons in the female SON. These results indicate the existence of sexual dimorphism in the SON which could be mediated by sex steroids.

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## REFERENCES

- ARAI, Y. & MATSUMOTO, A. (1978). Synapse formation of the hypothalamic arcuate nucleus during post-natal development in the female rat and its modification by neonatal oestrogen treatment. *Psychoneuroendocrinology* **3**, 31–45.
- ARNOLD, A. P. & GORSKI, R. A. (1984). Gonadal steroid induction of structural sex differences in the central nervous system. *Annual Review of Neuroscience* **7**, 413–442.
- COTTINGHAM, S. L. & PFAFF, D. (1986). Interconnectedness of steroid hormone-binding neurons: existence and implications. In *Current Topics in Neuroendocrinology*, vol. 7. *Morphology of Hypothalamus and its Connections* (ed. D. Ganten & D. Pfaff), pp. 223–250. Berlin: Springer.
- GARCÍA-SEGURA, L. M., BAETENS, D. & NAFTOLIN, F. (1986). Synaptic remodelling in arcuate nucleus after injection of estradiol valerate in adult female rats. *Brain Research* **366**, 131–136.
- GARCÍA-SEGURA, L. M., SUÁREZ, I., SEGOVIA, S., TRANQUE, P. A., CALES, J. M., AGUILERA, P., OLMOS, G. & GUILLAMON, A. (1988). The distribution of glial fibrillary acidic protein in the adult rat brain is influenced by the neonatal levels of sex steroids. *Brain Research* **456**, 357–363.
- GARCÍA-SEGURA, L. M., TORRES-ALEMÁN, I. & NAFTOLIN, F. (1989). Astrocytic shape and glial fibrillary acidic protein immunoreactivity are modified by estradiol in primary rat hypothalamic cultures. *Developmental Brain Research* **47**, 298–302.
- GÜLDNER, F. H. (1982). Sexual dimorphisms of axo-spine synapses and postsynaptic density material in the suprachiasmatic nucleus of the rat. *Neuroscience Letters* **28**, 145–150.
- MACLUSKY, N. J. & NAFTOLIN, F. (1981). Sexual differentiation of the central nervous system. *Science* **211**, 1294–1303.
- MATSUMOTO, A. & ARAI, Y. (1986). Development of sexual dimorphism in synaptic organization in the ventromedial nucleus of the hypothalamus in rats. *Neuroscience Letters* **68**, 165–168.
- MESHUL, C. K., SEIL, F. J. & HERNDON, R. M. (1987). Astrocytes play a role in regulation of synaptic density. *Brain Research* **402**, 139–145.
- PEREZ, J., NAFTOLIN, F. & GARCÍA-SEGURA, L. M. (1990). Sexual differentiation of synaptic connectivity and neuronal plasma membrane in the arcuate nucleus of the rat hypothalamus. *Brain Research* **527**, 116–122.
- PFAFF, D. W. & KEINER, M. (1973). Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *Journal of Comparative Neurology* **151**, 121–158.
- SALM, A. K., SMITHSON, K. G. & HATTON, G. I. (1985). Lactation-associated redistribution of the glial fibrillary acidic protein within the supraoptic nucleus. An immunocytochemical study. *Cell and Tissue Research* **242**, 9–15.
- STUMPF, W. E. & SAR, M. (1976). Autoradiographic localization of oestrogen, androgen, progesterin and glucocorticosteroid in 'target tissues' and 'non-target tissues'. In *Receptors and Mechanism of Action of Steroid Hormones* (ed. J. Pascualini), pp. 41–84. New York: Marcel Dekker.
- SUÁREZ, I., FERNANDEZ, B., BODEGA, G., TRANQUE, P., OLMOS, G. & GARCÍA-SEGURA, L. M. (1987). Postnatal development of glial fibrillary acidic protein immunoreactivity in the hamster arcuate nucleus. *Developmental Brain Research* **37**, 85–95.
- TORAN-ALLERAND, C. D. (1978). Gonadal hormones and brain development: cellular aspects of sexual differentiation. *American Zoology* **18**, 553–565.
- TRANQUE, P. A., SUÁREZ, I., OLMOS, G., FERNANDEZ, B. & GARCÍA-SEGURA, L. M. (1987). Estradiol-induced redistribution of glial fibrillary acidic protein immunoreactivity in the rat brain. *Brain Research* **406**, 348–351.
- WEIBEL, E. R. (1979). *Stereological Methods*, vol. 1. *Practical Methods for Biological Morphometry*. London: Academic Press.