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The supraoptic nucleus: a morphological and quantitative study in control and hypophysectomised rats

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INTRODUCTION

Neurons of the supraoptic nucleus (SON) are a part of the magnocellular neurosecretory system of the hypothalamus. They synthesise either oxytocin or vasopressin, nonapeptide neurohormones, which are transported through the supraoptico-neurohypophyseal tract to the posterior lobe of the hypophysis where they are released into the bloodstream (Brownstein, Russell & Gainer, 1980; Castel, Gainer & Dellmann, 1984). SON neurohormones play a key role in the regulation of several physiological conditions, including parturition and water metabolism (North, 1987). These peptide-producing secretory neurons are a useful model for analysing the cell biology of the neuronal nucleus and cytoplasm. In this regard, a number of physiological and experimental conditions such as the diurnal cycle (Armstrong & Hatton, 1978) and dehydration (Hatton & Walters, 1973) can modify the level of neurohormone synthesis activity in SON neurons and, consequently, induce variations in the size and morphology of both cytoplasm and cell nucleus.

According to Léránth, Zaborszky, Marton & Palkovits (1975), the SON can be divided anatomically into three regions: the *pars principalis*, which consists of a well-defined mass of large neurons attached to the lateral borders of the optic chiasm and optic tracts; the *pars intraoptica*, which represents neurosecretory neurons located among the optic axons of the optic chiasm and the *pars tuberalis*, composed of neurons placed medially to the optic tracts. The *pars principalis* has been chosen for our study since it accounts for 80% of the total cell population of this nucleus (Léránth *et al.* 1975) and has very well defined boundaries. For convenience, from now on we shall refer to the *pars principalis* as the SON.

Rasmussen (1940) concluded that the SON projects massively to the neural lobe of the hypophysis since hypophysectomy causes the loss of most of the neurons in this nucleus. When hypophysectomy is performed, the severed distal end of the hypothalamo-neurohypophyseal tract eventually develops new neurohaemal contacts in the median eminence (Raisman, 1973), organising a posterior lobe-like organ which restores the release of physiological amounts of neurohormones to maintain a normal metabolism. This capacity for neurohaemal reorganisation after hypophysectomy has been related to axonal plasticity (Kawamoto & Kawashima, 1985*a*) rather than with the decline in neurohormone plasma levels because this rearrangement is progressively retarded as animals age (Kawamoto & Kawashima, 1985*b*).

During early postnatal life, these neurons develop well-structured nucleoli. This

involves the gradual transformation from relatively compact nucleoli to reticulate ones which exhibit a typical nucleolonemmal configuration with numerous nucleolar interstices and fibrillar centres (Lafarga, Villegas & Crespo, 1985). At the same time, there is a reduction in the number of neurons displaying multiple nucleoli (Crespo, Viadero, Villegas & Lafarga, 1988); the stabilisation of this number by the end of the second postnatal week coincides with the termination of the nuclear growth phase (Crespo *et al.* 1988), reflecting the attainment of the fully differentiated state of the synthesis machinery of the nucleolar and non-nucleolar RNA. The present work represents a continuation of this line of research; it presents a quantitative and morphometric analysis of the postnatal development of the SON and the effects of hypophysectomy on these neurons during the postnatal period.

MATERIALS AND METHODS

Animals

Eighteen male albino rats of the Sprague–Dawley strain (Inferfauna, Barcelona) were used in this study. They were housed in our animal house in a temperature-controlled room with photoperiods of 12 hours light/dark cycles and with free access to laboratory chow and water *ad libitum*. Three animals from each of the following age groups were employed: 1, 7, 14, 28 and 90 days old. Another group of three animals was hypophysectomised on Day 60 and studied on Day 90.

Hypophysectomy

On Day 60 deeply anaesthetised rats were hypophysectomised via the transaural space with the aid of a needle connected to a vacuum pump. After hypophysectomy, water balance alterations (polyuria and polydipsia) were the earliest manifestations observed. Total removal of the hypophysis was verified after perfusion.

Tissue preparation

Animals from Day 1 to Day 28 were anaesthetised with 3·5% chloral hydrate; older animals were anaesthetised with ether. Perfusion with Bouin's solution was performed at noon (Armstrong & Hatton, 1978) to avoid differences in the number of nucleoli resulting from diurnal variations in light exposure. The entire brains were then removed, and a block containing the optic chiasm and adjacent optic tracts was dissected out and placed in the same fixative for 24 hours. An incision in the right cortex was made to determine SON position. These blocks were rinsed in distilled water, dehydrated in ascending concentrations of ethanol, passed through xylene into several paraffin pots and embedded in Paraplast. Serial coronal sections of these blocks containing the *pars principalis* of the right SON were made through their entire length at 6 μm. The sections were then placed on albumin-coated glass slides, deparaffinised, stained with thionin and mounted under coverslips.

Quantitative analyses

Silver procedure

The left SONs of the hypophysectomised group were processed with the cyto-chemical impregnation method described by Lafarga, Gonzalez & Berciano (1986) for the selective staining of the neuronal nucleolus. Smear preparations of whole dissociated neurons were observed in a Nikon microscope using a × 100 oil objective. One hundred neurons showing a well-preserved morphology were selected and the number of nucleoli per cell counted. This allowed us to determine the mean number

of nucleoli per cell at every age. These values were subsequently used as correction factors to convert total numbers of nucleoli into cell numbers. The average number of nucleoli during normal postnatal development has been described previously (Crespo *et al.* 1988).

Number of neurons

At every stage, cells located within the right SON that showed perikarya with Nissl bodies and a defined cell nucleus were considered as SON neurons (Hatton, Johnson & Malatesta, 1972), and any spot with nucleolar appearance and size in these cell nuclei was counted. These criteria were used to differentiate neurons from both glial and endothelial cells. With the aim of determining nucleoli numbers, we examined the sections at $\times 1000$ with oil immersion; however, only every other section was studied in order to avoid overestimation due to splitting of nucleoli by the microtome knife. These estimates were then multiplied by two, to provide a figure representing the total number of nucleoli in each right SON at any stage. In the SON, each neuron may contain several nucleoli (Hatton *et al.* 1972; Crespo *et al.* 1988); thus, a correction factor has to be used in order to translate absolute numbers of nucleoli into real cell numbers (Coggeshall & Chung, 1984). The correction factor that we used was the average number of nucleoli per cell at each stage in the postnatal period as had previously been determined (Crespo *et al.* 1988).

Morphometric analysis

Thionin-stained 6 μm paraffin sections were employed for the morphometric analysis of the size of the cell soma and cell nucleus. The outlines of these structures were drawn with a camera lucida attachment using a $\times 100$ oil objective. We measured 100 neurons containing nucleoli for each SON. The data were registered and processed with a semi-automatic image analyser (Videoplan, Kontron).

Statistical analysis

The data obtained were statistically tested by means of the analysis of variance (ANOVA), and $P < 0{\cdot}05$ was the criterion of significance.

RESULTS

Control rats

In contrast with the neighbouring hypothalamic areas, the SON of one day old rats appeared as a compact mass of large neurons attached to the lateral borders of the optic chiasm and optic tracts. At this early stage, neurons stained with basic dyes had a scanty ring of heavily stained cytoplasm and large, light nuclei with typically dispersed chromatin, which allowed us to distinguish nucleoli easily. Scattered among these neurons, glial cells presented a compact nucleus with no cytoplasmic staining (Fig. 1). As postnatal development proceeded, the SON became more compact, mainly due to the increase in size of the neuronal perikarya which presented a well-defined Nissl substance located in a marginal position and a light perinuclear region. Although the cell nucleus increased in size, it showed the same pattern as in the early stages and the nucleoli were clearly observed (Fig. 2).

The quantitative study showed that the cell number in the control group remained constant from Day 1, when it was 5034 ± 348 (mean $\pm$ S.D.M.), until Day 90 when the number of cells was 5234 ± 110 (Table 1). The morphometric studies revealed that the mean nuclear area increased from an initial value of $29{\cdot}2 \pm 3{\cdot}6$ μm^2 on Day 1 to

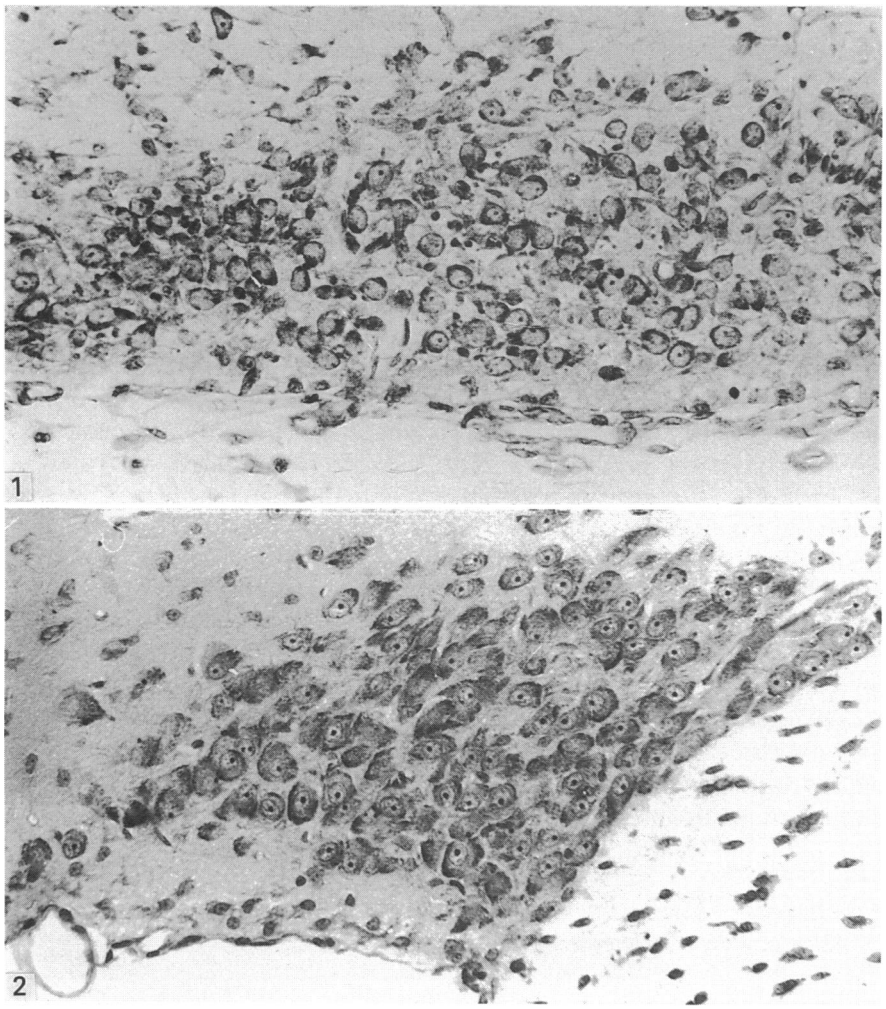

Fig. 1. Coronal 6 μm section. One day old rat. The supraoptic nucleus appears as a compact mass of neurons forming clusters separated by neuropil areas. At this early stage, the supraoptic nucleus is clearly differentiated from the neighbouring areas of the basal hypothalamus and the ventral glial lamina. $\times 300$.

Fig. 2. Coronal 6 μm section, 90 days old control rat. Supraoptic nucleus neurons show a large pale nucleus with a prominent nucleolus and a well-developed perikaryon. Note the typical marginal location of the Nissl substance in these neurosecretory neurons. $\times 250$.

$52{\cdot}1 \pm 6{\cdot}7$ μm^2 in three months old rats, which represents a 2-fold increase. The mean cell body area underwent a 3·6-fold increase from $43{\cdot}4 \pm 7{\cdot}1$ μm^2 in one day old animals to $159{\cdot}1 \pm 34{\cdot}6$ μm^2 in 90 days old rats. These results are shown in Table 2.

Hypophysectomised rats

Hypophysis extirpation induced a drastic reduction in the number of neurons of the SON (Table 1), mainly due to the disappearance of those cells located in the most ventral region near the ventral glial lamina (Fig. 3). The remaining neuronal population presented the same features as described for the control group: large cells containing a lobed nucleus with a prominent nucleolus and marginal distribution of the Nissl substance. The numbers of neurons in the SON of controls at 5 different ages

Table 1. *Number of neurons in the pars principalis of the SON at several postnatal ages.*

	Age (days)					
	1*	7*	14*	28*	90*	90**
	5301	5355	5318	5478	5263	1372
	5163	5315	4950	5029	5112	1233
	4640	5210	5336	4918	5328	1495
CF	1·59	1·60	1·32	1·33	1·34	1·32
Mean ± S.D.M.	5034 ± 348	5293 ± 74	5201 ± 217	5141 ± 296	5241 ± 110	1366 ± 131

The correction factor (CF), represents the mean number of nucleoli per cell at each age. * Control rats. ** Rats hypophysectomised on postnatal Day 60 and studied on Day 90. Note the stabilisation of the cell number from Day 1* to Day 90*. There is clearly a significant difference between the results 90* and 90**.

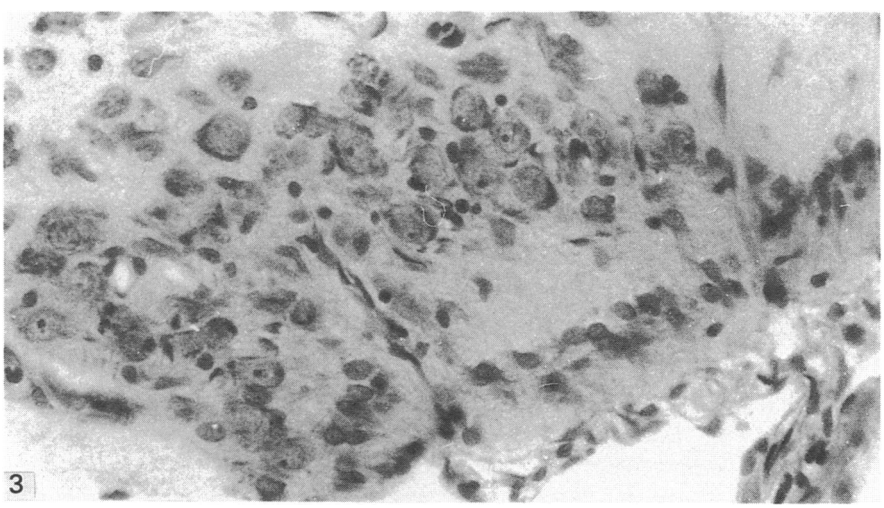

Fig. 3. Coronal 6 μm section, 90 days old rat hypophysectomised on Day 60. The cells exhibit the typical morphology of neurosecretory neurons. Note the reduction in the number of cells. × 300.

and in that of hypophysectomised rats at Day 90 are shown in Table 1. Note that there were no significant differences in the number of neurons during the postnatal period.

The morphometric comparisons between three months old control animals and those of the same age that had been hypophysectomised on Day 60 revealed no significant differences between soma areas in the two groups ($159{\cdot}1 \pm 34{\cdot}6\ \mu m^2$ in controls and $162{\cdot}5 \pm 32{\cdot}4\ \mu m^2$ in hypophysectomised animals). On the other hand, differences were found in nuclear area: $52{\cdot}1 \pm 6{\cdot}7\ \mu m^2$ for controls and $57{\cdot}3 \pm 6{\cdot}8\ \mu m^2$ for the experimental group. These data are presented in Table 2.

DISCUSSION

An accurate technique for estimating neuron numbers from nucleoli counts must satisfy two requirements. First, a reliable correction factor must be obtained, which in our case is the average number of nucleoli per cell at any given stage. For this purpose, cytochemical nucleolar staining of whole-mounted SON neurons has several

Table 2. *Mean soma and nuclear areas (S.D.M.) in the control supraoptic nucleus on Day 1 and Day 90*

	Age (days)		
	1	90	90*
Soma area (μm^2)	43·4 ± 7·1	159·1 ± 34·6	162·5 ± 32·4
Nuclear area (μm^2)	29·2 ± 3·6	52·1 ± 6·7	57·3 ± 6·8

Animals hypophysectomised on Day 60 and studied on Day 90. The mean nuclear area in control animals at Day 90 is significantly different from the mean nuclear area at Day 90* in animals hypophysectomised on Day 60 ($P < 0.05$).

advantages (Crespo *et al.* 1988). Secondly, an appropriate procedure must be used (Cooper, Payne & Horobin, 1988) to count the total number of nucleoli. We chose 6 μm thick paraffin sections stained with thionin solution. Adult SON neurons have an average nucleolar diameter of 0·75 μm, so that in 6 μm sections a given nucleolus can be observed in a maximum of two consecutive sections. Thus, in order to avoid double counting, only every other section was studied. Error could be introduced into our estimates by the inclusion of the small interneurons of the SON (Léránth *et al.* 1975), which represent 6% of the total neuronal population of the SON. To avoid this, we have used the usual criteria, reported by Itoh, Iijima & Kowada (1986), for distinguishing these interneurons from SON neurosecretory neurons: the interneurons are small, pale neurons, mainly located in the most dorsal region of the SON and displaying an ellipsoid eccentrically located nucleus.

Our results evidence a non-significant variation in the numbers of SON neurons from Day 1 (5034 ± 348) to Day 90 (5234 ± 110). Previous reports (Rasmussen, 1940; Bandaranayake, 1971; Ifft, 1972; Kawamoto & Kawashima, 1987) have dealt with the number of SON neurons in postnatal and adult rats. However, there are great discrepancies among them, with neuron numbers ranging from a minimum of 4353 (Léránth *et al.* 1975) to a maximum of 6787 (Bandaranayake, 1971), which represents a 35% variation. Some of this variability may be due to real differences in cell numbers in different strains (Hatton *et al.* 1972) or to sexual dimorphism (McEwen, 1981).

Autoradiographic studies (Ifft, 1972; Altman & Bayer, 1978; Anderson, 1987) have demonstrated that rat SON neurons originate from the lateral ependymal wall of the third ventricle between embryonic Days 13 and 15, and that no further proliferation takes place, either in this ependymal layer or in the SON after this period of neurogenesis. The postmitotic immature neurons migrate ventrolaterally towards the borders of the developing optic chiasm and optic tracts, where they aggregate to form the SON. A number of these migrating neurons fail to reach their definitive location and become displaced neurosecretory cells, forming the accessory SON. The total number of accessory neurons has been found to be inversely proportional to the total number of SON neurons. Quantitative studies by Bandaranayake (1971) have established that it contains about 450 cells. Even if no accessory SON is formed, due to the fact that all the neurons migrate properly to their SON location, this number is too small to account for great differences.

As Cowan, Fawcett, O'Leary & Stanfield (1984) have pointed out, the final number of cells in any region of the developing nervous system is determined by a combination of progressive and regressive processes, such as cell proliferation and naturally occurring cell death. This last event is a phenomenon that takes place in most regions

of the nervous system and in only a few locations has it been found that there is no postnatal cell death (Oppenheim, 1986). Our results confirm that the magnocellular neurons of the SON constitute a stable cell population during the early postnatal period; in fact, this number remains constant even in older rats as has previously been reported by McNeill, Clayton & Sladek (1980) who found no variations in cell counts of these neurons in 3 to 30 months old rats.

The reduction in the number of neurons in the SON after hypophysectomy is a consequence of retrograde degeneration; the maintenance of the remaining neuronal population is probably due to the fact that these neurons present axon collaterals before the median eminence or to the fact that they project directly to another extraneurohypophyseal system (Silverman & Zimmerman, 1983). This remaining neuronal population shows a significant increase in nuclear size in comparison with control animals. Similar increases in nuclear area have been observed in these neurosecretory neurons during pregnancy and lactation, which produce an increased demand of oxytocin (Russell, 1980) and, in rat drinking hypertonic solutions, to create a chronic increase in plasma osmolality, which results in an increase in vasopressin production (Enestrom, 1967; Paterson & Leblond, 1977). Lin, Peng, Peng & Tseng, (1976) reported a decrease in neuronal nuclear size in the SON between 5 and 22 months of age but Davies & Fotheringham (1980) found no change in nuclear size between 6 and 28 months of age in the mouse SON. Lin *et al.* (1976) reported a 31–35% decrease in nuclear volume in female rats and 11% reduction in male rats with ageing, but such a decrease with ageing has not been found in mice (Davies & Fotheringham, 1980). In the magnocellular division of the paraventricular nucleus, which is a functionally similar cell population, Ferres-Torres, Perez-Delgado, Castañeyra-Perdomo & Gonzalez-Hernandez (1986) have induced an increase in nuclear size by thyroid hormone deprivation.

In adult neurons, most protein synthesis activity is carried out in order to renew those proteins that have lost biological activity in maintaining cell morphology and function (Jones & LaVelle, 1986) but in young neurons this activity is aimed at ensuring normal cell growth. Thus, during postnatal development, structural proteins are predominantly synthesised to ensure adequate neuronal maturation and growth (Adams & Fox, 1969). As nuclear size is an index of neuronal protein synthesis activity, the increment observed in nuclear size after hypophysectomy, in comparison with control animals, could represent an increase in the nuclear mRNA synthesis activity of neurohormone precursors which could be necessary to maintain the minimum plasma threshold level of these neurohormones, given the drastic reduction in the number of neurosecretory cells resulting from neurohypophysis removal.

SUMMARY

Several quantitative and morphometric parameters were analysed in the *pars principalis* of the supraoptic nucleus (SON) of the hypothalamus in control and hypophysectomised rats at several postnatal ages. The cell number in the control group remained approximately constant from Day 1, when it was 5034 ± 348 (mean $\pm$ S.D.M.), until Day 90 when the number of cells was 5234 ± 110. In the group that was hypophysectomised on Day 60, the number of neurons on Day 90 was 1366 ± 131, which represents a loss of 74% of the neurons. Morphometric parameters on Day 90 indicated a significant difference in nuclear size, $52{\cdot}1 \pm 6{\cdot}7\ \mu m^2$ in controls and $57{\cdot}3 \pm 6{\cdot}8\ \mu m^2$ in the hypophysectomised group. Based on these results, it is concluded that there is no postnatal loss of neurons in the *pars principalis* of the SON

in normal rats. As a consequence of the stress induced by hypophysectomy, the remaining cell population undergoes adaptive changes in the nuclear RNA synthesis machinery of neurohypophyseal hormone precursors in order to compensate for the reduction in the number of neurosecretory neurons.

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