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DEVELOPMENT AND DIFFERENTIATION OF *NUCLEUS PARAVENTRICULARIS* AND *NUCLEUS SUPRAOPTICUS* OF THE HYPOTHALAMUS DURING THE PERINATAL PERIOD IN RATS.

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The hypothalamus plays a major role in controlling various important neuroendocrine, homeostatic and autonomic functions by releasing numerous stimulatory and inhibitory factors. *Nucleus paraventricularis* (PVN) and *nucleus supraopticus* (SON) are very complex formations localized in the anterior part of the hypothalamus. PVN is situated on each side of the third ventricle, while SON is located above the optic tract. Magnocellular and parvocellular neurons present in both nuclei start to differentiate between day 14 and 17 of fetal life. Mitosis is very intensive at the beginning of that period (Anderson 1978; Sanches *et al.* 1998).

Our investigations focus on the development, differentiation and organization of neurosecretory cells in subgroups at different levels of rat PVN and SON during the perinatal period.

Three-month-old Wistar strain rats were mated in the laboratory. The day when females were sperm-positive was considered as the first day of pregnancy. The dams, 20-day-old fetuses and 3- and 14-day-old neonatal offspring were sacrificed under ether narcosis. The hypothalamus was quickly removed, fixed in Bouin's fluid, and embedded in paraffin wax. Serial, 5μm thick, sections were stained with Gabe-Azan. Differences in the structure of the PVN and SON cells were estimated by the size of neurosecretory cell nuclei measured using a Mop-Videoplan image analysis system, by determining the maximum diameter (Dmax) and the area (Area). These analyses were made on micrographs of serial frontal hypothalamic sections from the anterior to the posterior part. The cytoarchitectonic subdivision of the PVN was determined using the classification according to Swanson and Kuypers (1980) and Armstrong *et al.* (1980). The PVN consisted of five distinct subdivisions, three magnocellular (anterior, medial and posterior) and two parvocellular (anterior and medial ones), localized in eight levels. Morphometrical analysis and histological examination were performed on 50 cells, each from all eight levels *per* animal. Parvocellular neurons in the complete PVN border were mixed with the magnocellular neurons. For this reason morphometrical data obtained for the parvocellular and magnocellular subdivision are presented together at each of the eight levels, where the dominant subdivision at each level is underlined.

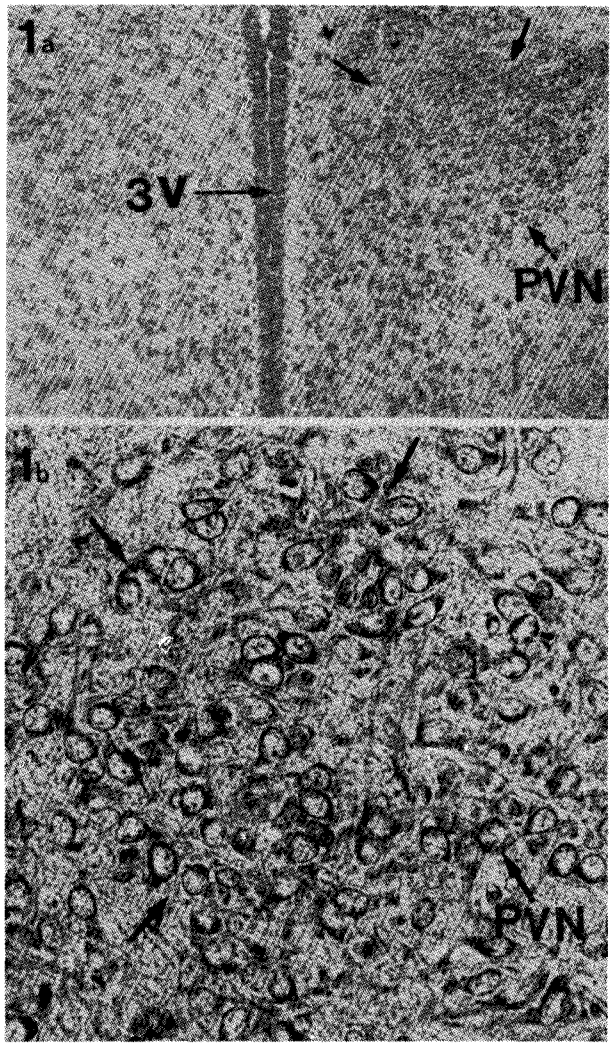
The SON is divided by the optic tract into the principal and retrochiasmatic part (Rhodes *et al.* 1981). We analyzed 50 cells in the SON division by the optic tract. The results were expressed as means for five animals in each group ± S.D. Statistical comparison of the data was made by Student's t-test.

Populations of magnocellular and parvocellular neurosecretory cells were found in the PVN (Fig. 1). The Dmax of neurosecretory cells of the PVN was between 4.5-8.05 μm in

Table 1. Changes in Dmax (μm) and Area (μm^2) of PVN neurosecretory cell nuclei (subgroups: ap-anterior parvocellular, am-anterior magnocellular, mm-medial magnocellular, pm-posterior magnocellular, mp-medial parvocellular) of 20-day-old fetuses and 3- and 14-day-old rat pups.

	levels	ap-am	am-mm	ap-mm	pm-mp	mp-pm	mp-pm	mp-pm	mp-pn
20-day-old fetuses	Dmax	5.59 ± 0.46	5.78 ± 0.50	5.24 ± 0.84	6.87 ± 0.75	5.57 ± 0.66	5.44 ± 0.47	5.35 ± 0.93	4.55 ± 1.04
	Area	22.81 ± 0.77	22.65 ± 1.04	21.03 ± 0.91	30.68 ± 2.12	21.41 ± 2.25	22.99 ± 2.07	20.19 ± 1.49	17.14 ± 0.83
3-day-old pups	Dmax	5.50 ± 0.25	5.72 ± 0.31	5.27 ± 0.26	6.90 ± 0.43	5.68 ± 0.34	5.51 ± 0.43	5.45 ± 0.46	4.65 ± 0.57
	Area	23.21 ± 3.34	23.92 ± 1.71	21.34 ± 1.91	31.32 ± 1.70	22.01 ± 1.93	23.01 ± 2.57	20.33 ± 2.43	16.4 ± 1.77
14-day-old pups	Dmax	6.73 ± 0.86	6.95 ± 1.19	6.23 ± 1.54	8.05 ± 0.86	6.81 ± 1.20	6.72 ± 0.62	6.61 ± 1.09	5.81 ± 0.47
	Area	25.92 ± 1.45	26.72±1.16*	27.01±1.37[a]**	33.01 ± 3.08	23.22 ± 2.18	24.82 ± 2.17	22.41 ± 2.96	18.63 ± 3.32

Results are expressed as means±S.D. for 5 animals in each group. * $P<0.025$ 20-day-old-fetuses *vs.* 14-day-old rats (level am-mm);
[a] $P<0.025$, 3-day-old rats *vs.* 14-day-old rats (level ap-mm); ** $P<0.005$, fetuses *vs.* 14-day-old rats (level ap-mm)



rats from the fetal period till neonatal day 14, while Area values were 16.4-33.0 μm^2 in the same period. The Area values gradually increased during the perinatal period at each level, and a significant increase was noticed at levels II and III (*am*-mm and *ap*-mm) when comparisons between fetuses, 3-day-old pups and 14-day-old pups were made (Table 1). There were no significant differences in Dmax values during the examined period. The values for Dmax of the principal part of SON neurosecretory cells were 5.7-6.9 μm during the perinatal period, while the Area was 23.6-26.4μm^2. There were no significant differences between the examined parameters, but a slight increase in Dmax and Area values was observed in 14-day-old rats.

These morphometric and histological investigations revealed that PVN subdivisions determined in adult rats are also present in 20-day-old fetuses and 3- and 14-day-old offspring. The results indicate that very intensive proliferation and differentiation occurred earlier, during day 14-17 of gestation, when the formation of recognizable nuclear groups are finished (Anderson 1978; Iqubal *et al.* 1995). Our results indicate that development of PVN subdivisions and levels and SON divisions is completed in utero.

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Fig. 1. Neurosecretory cells of posterior magnocellular and medial parvocellular (pm-mp) subdivision of PVN (level 4) in 3-day-old rats.
a) localization of PVN (3V-third ventricle; PVN-nucleus paraventricularis) (x160)
b) nuclei of PVN neurosecretory cells (PVN-nucleus paraventricularis) (x640)