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Pubertal changes in the medio-basal hypothalamic area after neonatal suprachiasmatic nucleus lesions in the rat

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Summary. In order to get more insight into the mechanism by which the onset of puberty is controlled, a developmental study on the displacement of catalase- and dopamine-containing cells in the hypothalamic region was done in rats which received a neonatal lesion of the suprachiasmatic nucleus. The displacement of cells is delayed after these lesions. However, the time lost at the beginning of the displacement is made up at the end of the migration.
Key words. Rat hapothalamus, suprachiasmatic nucleus, neonatal lesion; pubertal changes; catalase-containing cells; dopamine-containing cells; cell displacement.

The suprachiasmatic nucleus (SCN) is a small, paired hypothalamic nucleus which is close to the midline just above the optic chiasm. It is held responsible, at least in mammals, for the generation of a number of circadian rhythms (1 for a review). The morphology and the three-dimensional extension[2] of the SCN of rats has been extensively studied. Besides its role in the control of behavioral circadian rhythms, the SCN also plays an important part in the control of endocrine rhythms. Within the SCN neurons are found that contain vasopressin, neurophysin, melatonin and LH-RH. Therefore a correlation is proposed between the SCN and the reproductive cycle[1,3-5]. Destruction of the SCN indeed leads to a loss of the estrous cycle[6].

The SCN is known to have various connections. Among these is a direct connection from the SCN to the pituitary stalk nuclei, the arcuate nucleus and the median eminence[7]. However, studies using retrograde tracers failed to demonstrate the connection from the SCN to the median eminence[8].

Histochemical studies for catalase revealed a critical period in the development of the ventrobasal hypothalamus and the pituitary stalk area around puberty, when groups of cells undergo a migration[9-13]. In adult animals the catalase activity fluctuates according to the estrous cycle, showing maximum activity at the time of the pre-estrous[14-16]. A similar shift has been found for catecholaminergic fluorescent cells, which populate the median eminence, the intermediate area and then the arcuate nucleus[17,18] following the same time sequence.

The catalase activity is related to the onset of the production of LH-RH[12,19]; the importance of the catecholaminergic cells for the function of the tubero-infundibular system in reproduction[20] has also been demonstrated. The SCN-preoptic area subserves at least the dynamic control of the estrous cycle, and as one may assume that an efferent connection exists from the SCN to the arcuate-median eminence system, we have investigated whether prepubertal ablation of the SCN affects the development of this hypothalamic system.

Material and methods. All rats used in this study (Wistar-WU, SPF, from TNO, Delft, the Netherlands) were housed under standard conditions (22 ± 1 °C, rel. hum. 65 ± 3%, light on 08.30–20.30 h) and fed ad libitum. Male newborn rats (2 days old) from several litters were stereotaxically lesioned in the SCN nucleus. Stereotaxic coordinates for the SCN were adapted from earlier data[21]: 0.9 AP, 0.6 V, 0.1 L. Coagulation was effected with a 0.1-mm needle, isolated except at its tip. A current of 0.8 mA for 5 sec was used in each nucleus. The best results were obtained by placing young males of 8–10 g in a plastic jig in a David Kopf holder and making them hypothermic with ice. All lesioned animals survived the electrocoagulation treatment and were reaccepted in the litters and by the mother. 2 animals were sacrificed at 2-day intervals from day 3 till day 55 after birth. The animals were decapitated under

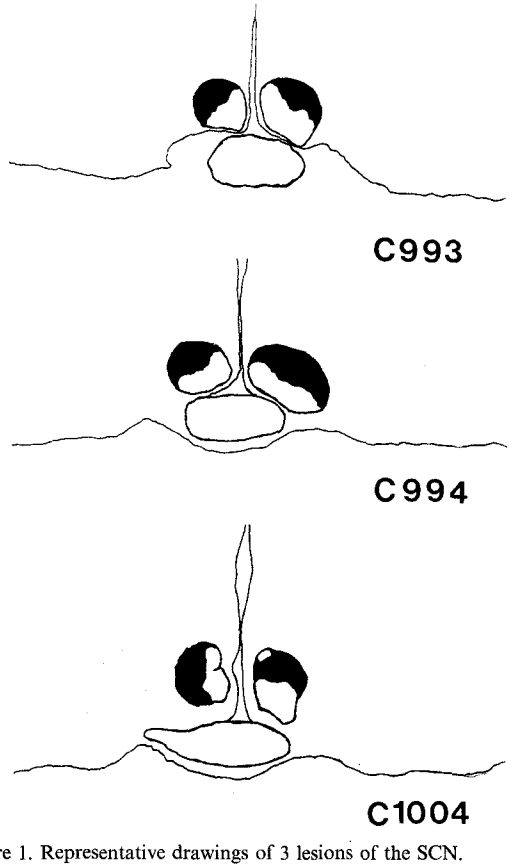

Figure 1. Representative drawings of 3 lesions of the SCN.

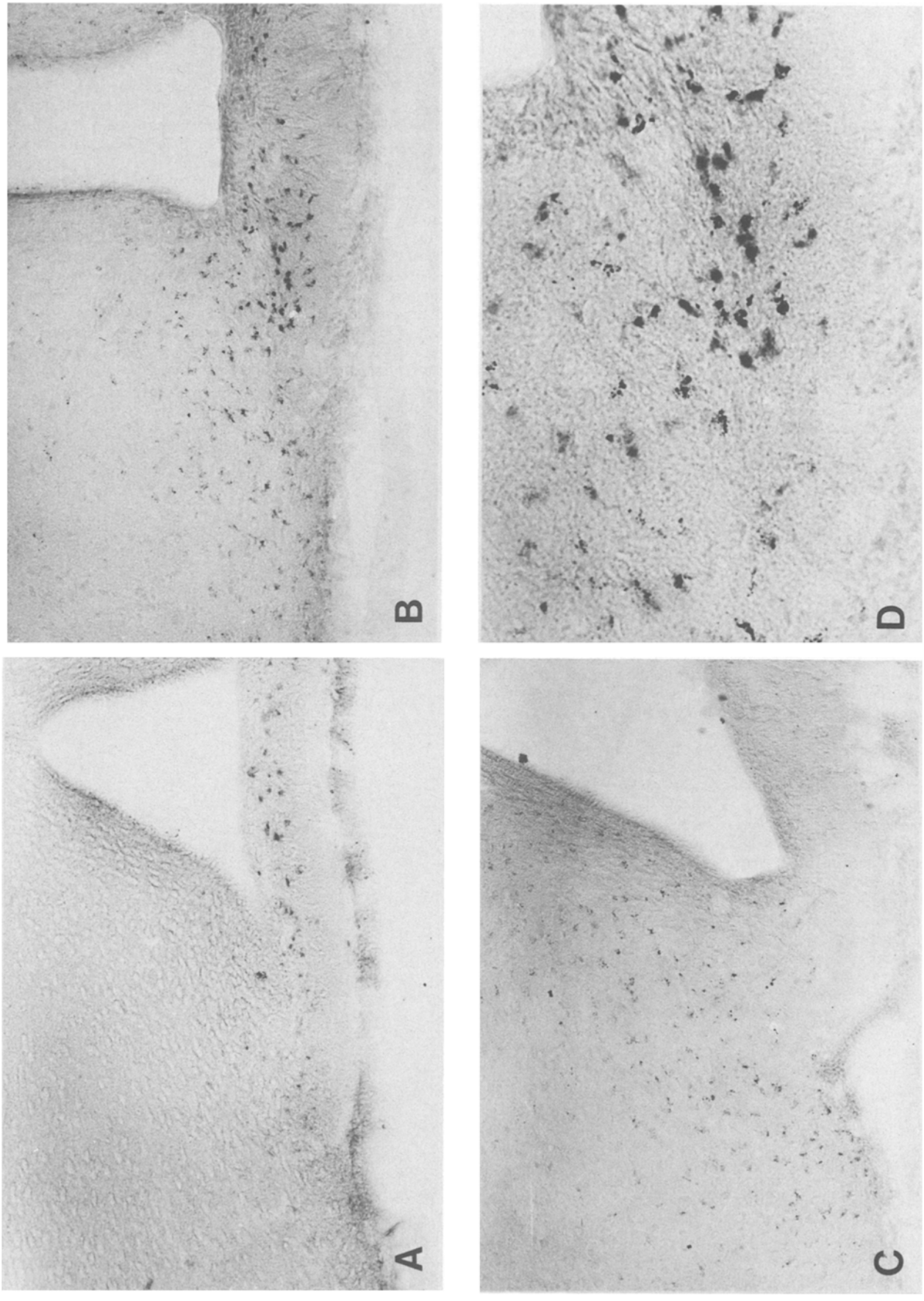

Figure 2. *A* Normal appearance of catalase reaction products at day 22 after birth, *B* at day 37 after birth, *C* at day 55 after birth. *D* contains a detail of day 37 after birth.

chloroform anesthesia, and the brains were removed and prepared for cryostat sectioning[22]. Sections were cut from the hypothalamic area under study, and 3 slices were taken out of every 10 sections. The 3 series thus obtained were used for Nissl[23], fluorescence[24] and catalase staining[25]. Lesioned areas were studied in Nissl series and camera lucida drawings were made of all SCN sections. Representative sections of several series are depicted in this article. Control series with the Nissl, catalase and fluorescence stain were obtained from unlesioned animals in addition to materials that have been described in previous studies[9,10,12-15,17,18]. The spread of the dendritic arborizations of the SCN cells was studied in Golgi series, which have already been described elsewhere[26]. Some SCN-lesioned rats remained and were studied using an automatic food-intake control system[27,28].

Results. Histology and body weight. The SCN has only a small dendritic halo around its nucleus, to which the dendritic arborization of the cells extends. This means that Nissl preparations can be used to indicate the lesion damage in this study. Most of the dendrites are directed towards the inner part of this nucleus. The nucleus is encapsulated in glial and fiber stuctures, which demarcate the border of this nucleus both in Nissl- and Golgi studies.

The SCN lesions all destroyed the upper parts of the nuclei on both sides of the ventricle, thus avoiding optic chiasm damage (fig. 1).

Within the lesioned area gliosis was developed in older specimens. The extension of the lesion was studied in all series. The damage was mainly superficial at the rostral side and seldom reached the center of the nucleus. The remaining lesioned animals were studied using an automatic food-intake control system. It appeared that all rats exhibited arhythmicity of eating activity[1] indicating a loss of functional activity of the SCN. All lesioned animals showed a normal growth pattern. The body weight of lesioned male rats was normal, indicating that arhythmicity did not influence growth in terms of body weight.

Histochemistry (figs 2 and 3). The control series showed the normal shifts as described in the introduction. Catalase activity was first found at day 22 after birth in the median eminence and shifted via the ventral part of the ventromedial hypothalamus (the intermediate area) towards the arcuate nucleus. It entered the arcuate nucleus at day 45 after birth and totally filled the ventromedial part of this nucleus by day 55 after birth. The catecholaminergic fluorescence also underwent a migration, which terminated around the same time in the dorsolateral part of this nucleus.

In the series of SCN lesioned animals there were definite changes in the sequence, both for the presence of catalase and for catecholaminergic fluorescence. Catalase activity could not be demonstrated in the median eminence till day 32 after birth. Activity was then absent in the intermediate area and arcuate nucleus. However, although the onset of catalase activity was later, its final development took place more quickly. The invasion of catalase activity in the intermediate area was seen at day 36, and the shift towards the arcuate nucleus started at day 40 after birth. By day 55 the catalase activity was present (as normal) in the ventromedial part of the arcuate nucleus. The sequence for catecholamine fluorescence, studied using the method of de la Torre[24], was affected similarly. The normal brilliant fluorescence did not appear in the median eminence till day 32 after birth in the SCN lesioned rats, although sparse fluorescent varicosities were present in the pituitary stalk. Normally heavy fluorescence is already seen in the median eminence at day 18 after birth. Subsequently displacement of fluorescent perikarya[13,14] took place via the intermediate area, and was present again in this area at day 36. This process appeared to be accelerated. At day 55 after birth the dorsolateral part of the arcuate nucleus contained fluorescent perikarya.

Discussion. A hypothesis concerning the onset of puberty was proposed[12] using a simple neuronal circuit involving the median eminence and the arcuate nucleus. It is based on the likelihood that the SCN is the pace-maker of the estrous cycle[1]. The suprachiasmatic nucleus can govern the mature arcuate nucleus and it was proposed that this action is exerted via the dorsolateral part of the arcuate nucleus. The dopaminergic, monosodium glutamate sensitive cells[18] of this part of the arcuate nucleus activate in their turn the ventromedial, catalase containing cells[11-13]. The catalase part of the arcuate nucleus was found to be LH-RF positive[19] in mature animals. At the onset of puberty, the displacement of the nucleus and perikaryal cytoplasm of the cell, located at the median eminence, into the arcuate nucleus makes it possible for the suprachiasmatic nucleus to exert its influence, resulting in a periodic LH-RF release. Golgi studies confirm the presence of bipolar cells[26] with protrusions towards the median eminence.

The experimental setups to provoke changes in the migration of catalase and dopaminergic cells indicate for monosodium glutamate that the loss of fluorescent cells in the arcuate nucleus has to be considered as a secondary effect. The primary effect of monosodium glutamate administered postnatally is the acceleration of the perikaryal migration due to the stimulation or deinhibition of the normal processes before puberty[18]. The effect of castration on the onset of puberty[13] in testosterone-treated rats demonstrated that the catalase activity ap-

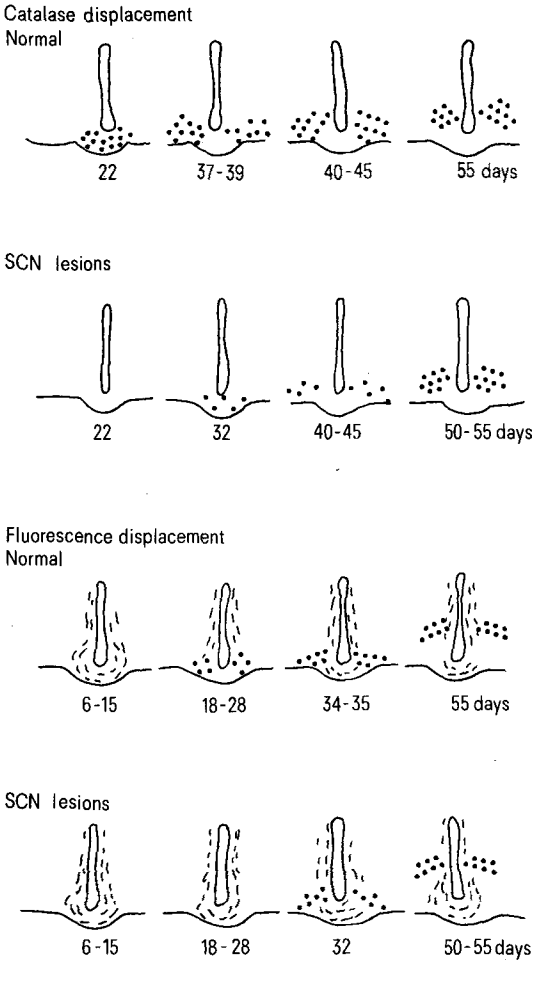

Figure 3. This schematic drawing summarizes the results of SCN lesioned – and untreated animals for the distributions of catalase granules (2 upper rows, dark dots) and for the topography of dopaminergic cells (2 lower rows, the dark dots) and catecholaminergic fibers.

peared earlier in the migration areas, but the catalase activity lingers longer in the intermediate area as compared to untreated rats.

The histochemical changes reported in this study showed a delay in the appearance of catalase-containing and catecholaminergic fluorescent cells in the mediobasal hypothalamus. The time lost at the beginning of their pubertal development is made up by a faster displacement from the median eminence towards the arcuate nucleus. This is in contrast to the results of prolactin administration[29], which delayed total fluorescence of catecholaminergic cells, but also the expression of catalase activity during the displacement.

In terms of the model proposed by Tresquerres et al.[20] these results could be explained by the presence of a tonic center which has an effect on hypothalamic development. Although the damaging of the cyclic center causes a delay in the maturation of the hypothalamus it can in fact be overcome by the plasticity of the system and by the action of the tonic center.

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Dense core vesicles during photoreceptor development

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Summary. The presence of dense core vesicles in the terminal expansions of photoreceptors in development is decribed in the chick embryo retina, from the 16th to the 18th day of incubation.
Key words. Chick embryo; retina; photoreceptor; dense core vesicles.

In the course of our studies on the histogenesis of the chick retina, we have detected various vesicle populations at the synaptic terminals of developing photoreceptors. Although these vesicles (coated vesicles, dense core vesicles and clear vesicles), have been described in the adult retina[2-4], the simultaneous existence of clear and dense core vesicles, together in the same synaptic terminal, has never been observed in normal animals.

We have used 168 retinas of chick embryos at various days of incubation (10th–20th) (table 1). After enucleation, the retina was dissected and fixed by immersion in 3% glutaraldehyde, 1% formaldehyde and 0.5% acrolein in 0.1 M sodium cacodylate buffer (pH 7.2), and postfixed in 2% osmium tetroxide, dehydrated in a graded series of acetones, and stained in block (0.5% uranyl acetate in 70% acetone for 1 or 2 h). The pieces of retina were embedded in Araldyte CY 212 (Durcupan). Ultrathin sections (600 A) were stained with uranyl acetate and lead citrate. A Jeol 100C electron microscope was used for observation at 80kV.

Table 1. Relationships of the embryos and retinas of various stages used

Day of incubation	Stage	No. embryos	No. retinas
10	HH-36	11	13
11	HH-37	6	9
12	HH-38	7	10
13	HH-39	7	14
14	HH-40	10	16
15	HH-41	10	20
16	HH-42	10	20
17	HH-43	12	20
18	HH-44	10	20
19	HH-45	8	16
20	HH-46	5	10
		96	168