J. Anat. (1980), **130**, 2, pp. 341–347
With 4 figures
Printed in Great Britain

Histogenesis of the supraoptic and paraventricular neurosecretory cells of the mouse hypothalamus

M. A. KARIM,* AND J. C. SLOPER†

* *Department of Anatomy, Faculty of Medicine, University of Malaya, Malaysia and* † *Department of Experimental Pathology, Charing Cross Hospital Medical School, London, England.*

(*Accepted* 4 *April* 1979)

INTRODUCTION

This is part of a report on the histogenesis, development and maturation of the hypothalamo-neurohypophysial system (HNS) and observations on the effects of premature weaning in the mouse.

The mechanisms by which vasopressin and oxytocin are elaborated by the hypothalamo-neurohypophysial system have been fully investigated in the last few decades (Ortmann, 1960). These studies have revealed that the neurohypophysial hormones are secreted by the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus (Bargmann & Scharrer, 1951; Bargmann, 1954) rather than by the interstitial cells of the pars nervosa (Gersh, 1939). The fact that these neurons have a special secretory function raises the possibility that they may differ from other neurons of the central nervous system in the timing of their development.

Since the introduction of autoradiographic techniques (Belanger & Leblond, 1946; Endicott & Yagoda, 1947; Pelc, 1947; Doniach & Pele, 1950), it has become possible to trace labelled nuclei of dividing cells and define the approximate period of cellular proliferation in the neuronal precursors. Such techniques have been utilized to study the histogenesis in the mouse of the cerebellum (Uzman, 1960), cerebral cortex (Angevine & Sidman, 1962), brain stem (Taber, 1964), hippocampus (Angevine, 1965), dorsal and ventral cochlear nuclei (Taber-Pierce, 1967), olfactory bulb (Hinds, 1968), diencephalon (Angevine, 1970), hypothalamus (Shimada & Nakamura, 1973); and in the rabbit, that of the anterior thalamic nuclei and limbic cortex (Fernandez, 1969). Similar studies on the timing of development in the rat hypothalamus have been reported by Ifft (1972) and by Altman & Bayer (1978).

The present study was limited to determining the time of cellular proliferation in the neuronal precursors of SON and PVN with the object of discovering whether or not there were critical periods in either the prenatal or the postnatal development of HNS when stress might lead to irreversible damage, a possibility envisaged by Rodeck (1962).

METHODS

To study the timing of histogenesis of the supraoptic and paraventricular neurons, 34 pregnant mice were each given a single intraperitoneal injection of tritiated thymidine on different days of gestation from the 8th to the 18th days (Table 1). The day of vaginal plugging was taken as the first day of pregnancy, this timing being confirmed by the birth of litters on the 20th day. Litter mates of each pregnant mouse receiving tritiated thymidine on various embryonic days were killed on the 1st, 3rd,

0021-8782/80/2828-7270 $02.00 © 1980 Anat. Soc. G.B. & I.

5th and 30th day after birth, and the labelling of SON and PVN neurons was carefully studied. Where administration of tritiated thymidine did *not* produce labelling it was inferred either that proliferation in the neuronal precursors of SON and PVN had ceased, or that repeated mitoses had diluted the radioisotope. Nuclei of neurons covered by more than 25 silver grains were considered heavily labelled; those with less than 6 grains directly over them were not considered labelled.

Table 1. *Results of tritiated thymidine experiments*

Serial no. of experiments	No. of pregnant mice	Day of gestation when [^{3}H]-thymidine was injected	* No. of litter mates killed (day after birth)				Labelled nuclei of SON and PVN cells
			1st	3rd	5th	30th	
1	3	8	4	4	4	5	−
2	4	9	4	4	4	7	−
3	4	10	4	4	4	10	+
4	4	11	2	4	4	7	++
5	4	12	4	4	2	9	+++
6	4	13†	4	4	4	5	−
7	3	14†	4	6	—	3	−
8	3	15	3	3	4	6	−
9	2	16	2	1	2	6	−
10	2	17	—	1	2	7	−
11	1	18	2	—	2	2	−

−, No labelling (less than 6 grains).
+, Moderate labelling (more than 6 but less than 25 grains over the nucleus).
++, Heavy labelling (more than 25 grains over the nuclei).
+++, Heaviest labelling (difficult to count the grains).
* Litter mates of each pregnant mouse were kept separate.
† Nuclei of neurons in the adjacent lateral hypothalamic areas were found labelled.

Postnatal cellular proliferation and migration in the neuronal precursors of SON and PVN were investigated in 53 mice of different ages (1–120 days), by injecting single doses of tritiated thymidine intraperitoneally and killing the animals at various time intervals from 1 to 240 hours after the administration of the radioisotope.

Serial sections of 5 μm thickness were cut in coronal and sagittal planes and mounted on specially cleaned slides. All slides were coated with G_5 nuclear emulsion by the dipping technique of Kopriwa & Leblond (1961). In all experiments, tritiated thymidine-6T(n) of the Radio-Chemical Centre, Amersham, with a specific activity of 5·0 Ci/mM, was injected into unanaesthetized animals at a dose of 5·0 μCi/g of body weight.

RESULTS

From our earlier studies it was clear that the stages in the development of the hypothalamo-neurohypophysial system of the mouse are essentially similar to those in the rat. The hypothalamus in the mouse is delineated by the 11th embryonic day, and by the 13th embryonic day the supraoptic nucleus is well defined; this is followed by the appearance of the paraventricular nucleus a day later. The neurohypophysial primordium is present as a small but distinct growth from the diencephalon on the 11th embryonic day.

None of the 53 animals which received tritiated thymidine *postnatally* showed labelling of any nuclei of secretory neurons in SON and PVN, although a few endo-

thelial cells and neuroglial cells in the region were labelled. Such labelled cells were more frequent in the younger than in the older animals. The ependymal cells lining the third and fourth ventricles were heavily labelled in mice of all groups. However, the distribution of labelled ependymal and subependymal cells at various time intervals after the administration of the radioisotope did not suggest any migration of such cells into the SON and PVN after birth.

In the litters receiving tritiated thymidine *prenatally*, labelled nuclei in the supraoptic and paraventricular neurons were seen only in those litter mates which received the radiosotope on the 10th, 11th or 12th embryonic day (Figs. 1, 2). Labelled nuclei in the neurons of SON and PVN were not observed in the litter mates which had received tritiated thymidine on the 9th embryonic day or earlier. Likewise litters receiving tritiated thymidine between the 13th and 18th embryonic days did not show any labelling in the neuronal nuclei of their SON and PVN. The neurons of SON and PVN were not readily identifiable in the litter mates of younger age groups (up to 5 days postnatally). Labelled magnocellular neurons were, however, distinct and well differentiated in the 30 days old litter mates.

Interestingly, nuclei of neurons in the lateral hypothalamic area *outside* the supraoptic and paraventricular regions were found labelled in the offspring of mice which had received tritiated thymidine either on the 13th or 14th day of pregnancy (Figs. 3, 4).

DISCUSSION

In the past, studies on the origin of neurons have been based on mitotic figures in neuronal precursors (Ramon y Cajal, 1960). It has been difficult, however, to differentiate between the mitotic figures of neuronal and neuroglial precursors in fetal brains.

From the present study it would appear that the neuronal precursors of the supraoptic and paraventricular nuclei cease to take up tritiated thymidine about the 13th embryonic day. This corroborates the findings of Shimada & Nakamura (1973), who reported a lateromedial gradient in the timing of neuron origin in the mouse hypothalamus, with most of the neurons in the suprachiasmatic and paraventricular nuclei being formed between the 11th and 14th embryonic days. In the rat (Ifft, 1972), embryonic day 14 appears to be the last day of cellular proliferation in most of the neuronal precursors of the supraoptic and paraventricular neurons. Our findings also show that the neuronal precursors in the adjacent lateral hypothalamic area continue to proliferate on the 13th and 14th embryonic days. Comparing the times of origin of the supraoptic and paraventricular neurons with those of neurons in other parts of the mouse brain, it seems that the Purkinje and Golgi Type II cells stop proliferating a day earlier (Uzman, 1960), while the neuronal precursors of the brain stem continue to divide for a day longer than the neuronal precursors of SON and PVN (Taber, 1964). Histogenesis of the SON and PVN neurons, however, ends considerably earlier than those of the hippocampus in the mouse. Angevine (1965) found that the precursors of neurons in the inner layers of the hippocampus did not cease proliferating until about the 15th embryonic day, while those in the outer layers continued proliferating up to the 20th postnatal day. In a subsequent study on the timing of neuron origin in the diencephalon of the mouse, Angevine (1970) reported a definite sequence in the times of origin of neurons in the ventral thalamus, dorsal thalamus and epithalamus between the 10th and 16th days of gestation. Shimada & Nakamura (1973) found that most of the

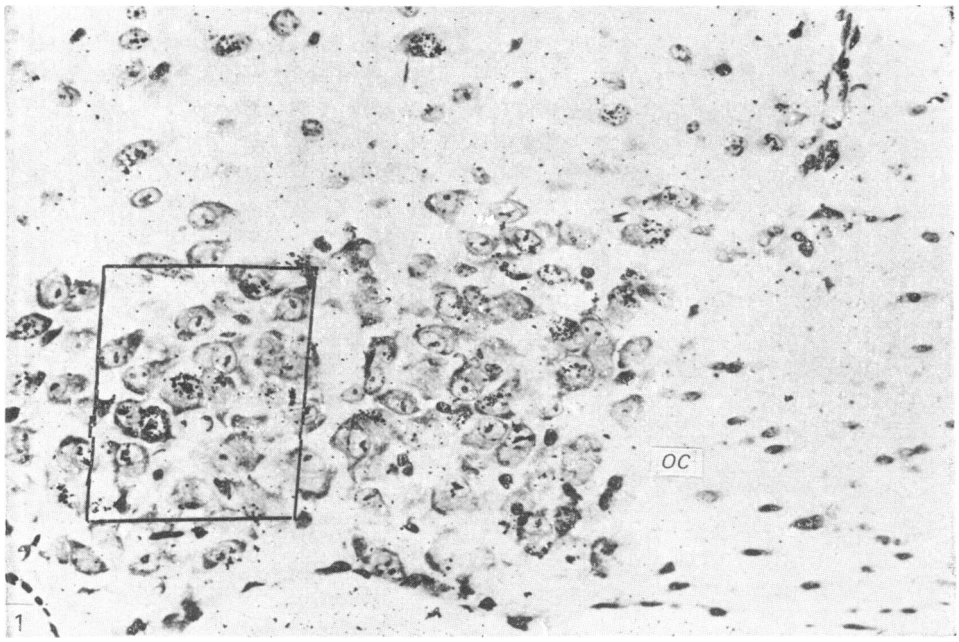

Fig. 1. Autoradiograph showing SON in a coronal section of the hypothalamus in a mouse 30 days old which received tritiated thymidine on the 12th embryonic day. Boxed area under higher magnification is seen in Fig. 2. *OC*, Optic chiasma. Cresyl violet. × 200.

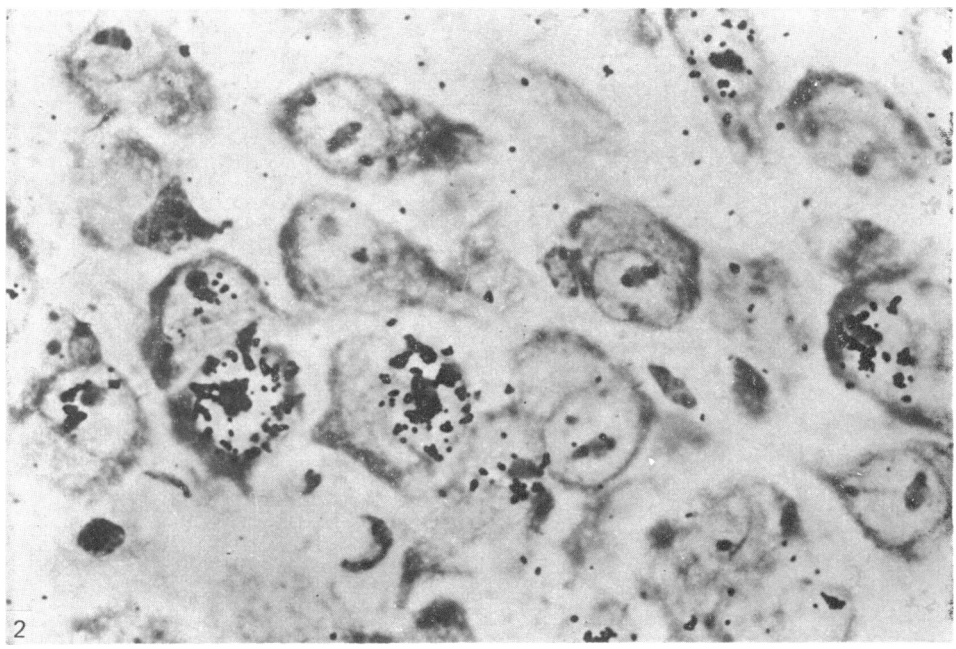

Fig. 2. Boxed area of SON in Fig. 1 seen under higher magnification. Note heavy labelling of neuronal nuclei. Cresyl violet. × 1000.

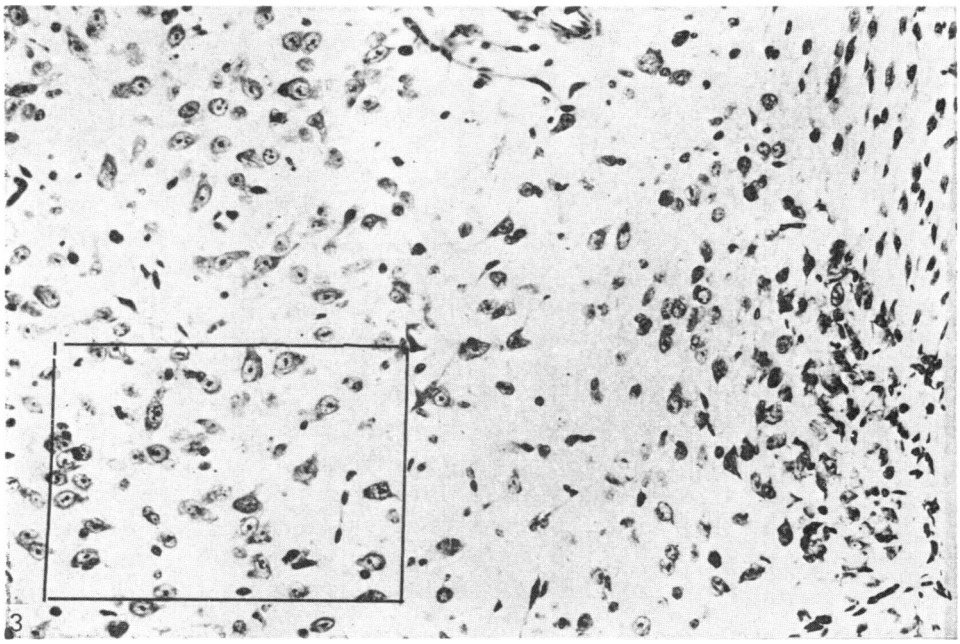

Fig. 3. Autoradiograph showing labelled neurons outside SON and PVN in the lateral hypothalamus of a mouse 5 days old which received tritiated thymidine on the 14th embryonic day. Boxed area under higher magnification seen in Fig. 4. Cresyl violet. × 160.

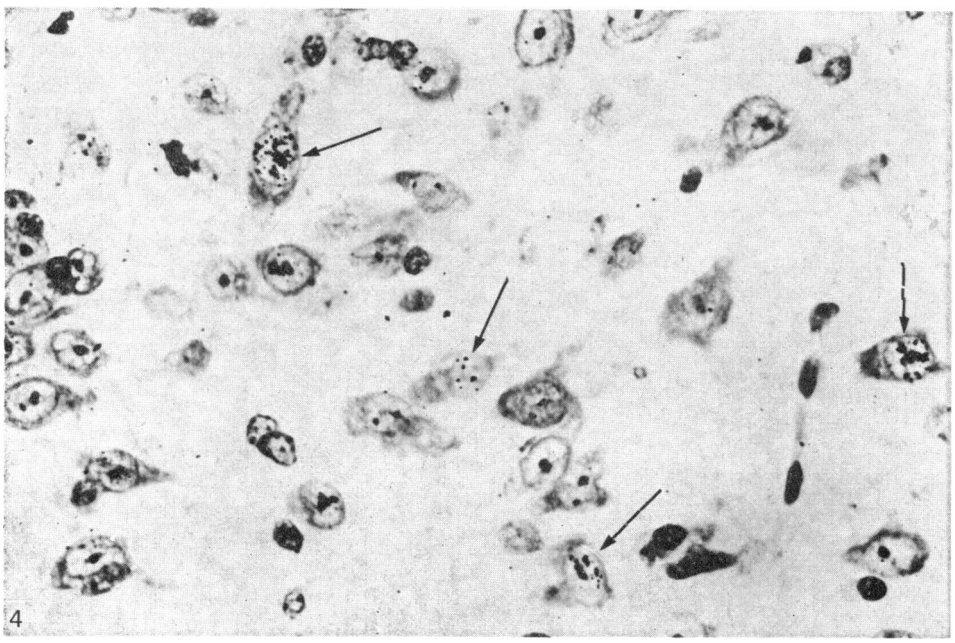

Fig. 4. Boxed area in Fig. 3 seen under higher magnification. Note heavy to moderate labelling (arrows) of neuronal nuclei within the boxed area of the lateral hypothalamus. Cresyl violet. × 500.

neurons in the mouse hypothalamus formed between the 10th and 14th embryonic days with the exception of neurons in the periventricular, arcuate and medial mammillary nuclei which continued to form until the 16th day. In a similar study in the rat hypothalamic nuclei, Ifft (1972) found the majority of neuronal precursors in SON and PVN ceased to proliferate after the 14th embryonic day, while neuronal precursors in other nuclei continued to divide until the 16th day. In the present study, although a similar sequence was found, i.e. the magnocellular neurons of SON and PVN formed earlier than the small sized neurons in the adjacent lateral hypothalamic region, it was evident that neuronal precursors ceased proliferating a day or two earlier than the times reported by Shimada & Nakamura (1973) and Ifft (1972). The assertion of Hinds (1968) that, in a given area of the brain, large neurons generally arise before small ones is confirmed in our study.

The next question arises as to whether secretory neurons of the hypothalamo-neurohypophysial system retain the *ability* to divide in postnatal life. This seems improbable but cannot be excluded, first because injury to nerves in the tractus hypophyseus does not appear always to lead to death of the neuronal cell bodies (Bodian & Maren, 1951); and second because nerve terminal regeneration can occur in the injured neurohypophysis (Sloper, 1966). The facts established in the present study that the secretory neurons of SON and PVN form early in prenatal life and that subependymal cells do not migrate into the region of the SON and PVN neurons thereafter, make it very difficult to believe that new SON and PVN neurons can be recruited in postnatal life.

Secretory activity in the hypothalamo-neurohypophysial system in the mouse, indicated by the presence of aldehyde fuchsin-positive neurosecretory material, begins on the 18th embryonic day (Enemar, 1961). It may therefore be concluded that the secretory activity of the SON and PVN on the 18th embryonic day in the mouse is preceded by proliferation of the precursors of the neurosecretory cells between the 10th and 12th embryonic days, and the organization and development of the cells of the SON and PVN on the 13th and 14th embryonic days respectively.

SUMMARY

Using tritiated thymidine in mice it was found that the precursor cells of neurons in the supraoptic and paraventricular nuclei cease proliferating by the 13th embryonic day. The significance of the early origin of these neurons is discussed.

The present investigation was carried out in the Department of Experimental Pathology and in the Research Laboratories of Charing Cross Hospital Medical School, and the authors are indebted to the members of the technical staff and the photographic department for their help.

REFERENCES

ALTMAN, J. & BAYER, S. A. (1978). Development of the diencephalon in the rat. I. Autoradiographic study of the time of origin and settling patterns of neurons of the hypothalamus. *Journal of Comparative Neurology* **182**, 945–972.

ANGEVINE, J. B. & Sidman, R. L. (1962). Autoradiographic study of histogenesis in the cerebral cortex of the mouse. *Anatomical Record* **142**, 210.

ANGEVINE, J. B. (1965). Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. *Experimental Neurology*, Suppl. **2**, 1–72.

ANGEVINE, J. B. (1970). Time of neuron origin in the diencephalon of the mouse. An autoradiographic study. *Journal of Comparative Neurology* **139**, 129–188.

BARGMANN, W. & SCHARRER, E. (1951). The site of origin of the hormone of the posterior pituitary. *American Science* **39**, 255–259.

BARGMANN, W. (1954). Neurosecretion und hypothalamisch-hypophysares System. *Verhandlungen der Anatomischen Gesellschaft; Ergänzungsheft zum Anatomischer Anzeiger* **51**, 30–45.

BELANGER, L. F. & LEBLOND, C. P. (1946). A method of locating radioactive elements by covering histological sections with a photographic emulsion. *Endocrinology* **39**, 8–13.

BODIAN, D. & MAREN, T. H. (1951). Effect of neuro- and adenohypophysectomy on retrograde degeneration in the hypothalamic nuclei of the rat. *Journal of Comparative Neurology* **94**, 485–511.

DONIACH, I. & PELE, S. R. (1950). Autoradiographic technique. *British Journal of Radiology* **23**, 184–192.

ENDICOTT, K. M. & YAGODA, H. (1947). Microscopic technic for locating and quantitating radioactive elements in tissue. *Proceedings of the Society for Experimental Biology* **64**, 170–172.

ENEMAR, A. (1961). Notes on the histogenesis of the hypophysis of the laboratory mouse, with special reference to its relation to the development of the hypophysial portal system. *Acta Universitatis lundensis* **2**, 1–37.

FERNANDEZ, V. (1969). An autoradiographic study of the development of the anterior thalamic group and limbic cortex in the rabbit. *Journal of Comparative Neurology* **136**, 423–452.

GERSH, I. (1939). The structure and function of the parenchymatous glandular cells in the neurohypophysis in the rat. *American Journal of Anatomy* **64**, 407–443.

HINDS, J. W. (1968). Autoradiographic study of histogenesis in the mouse olfactory bulb. I. Time of origin of neurons and neuroglia. *Journal of Comparative Neurology* **134**, 289–304.

IFFT, J. D. (1972). An autoradiographic study of the time of final division of neurons in rat hypothalamic nuclei. *Journal of Comparative Neurology* **144**, 193–204.

KOPRIWA, B. M. & LEBLOND, C. P. (1961). Improvements in the coating technique of radioautography, *Journal of Histochemistry and Cytochemistry* **10**, 269–284.

ORTMANN, R. (1960). In *Handbook of Physiology*, Section I, *Neurophysiology*, vol. II (ed. J. Fields, H. W. Magoun & V. E. Hall), pp. 1039–1065. Washington: American Physiological Society.

PELC, S. R. (1947). Autoradiographic technique. *Nature* **160**, 749–750.

RAMON Y CAJAL, S. (1960). In *Studies on Vertebrate Neurogenesis* (translated into English by L. Guth). Springfield: Thomas.

Rodeck, H. (1962). In *Neurosecretion* (ed. H. Heller & R. B. Clark), pp. 247–254. London: Academic Press.

SHIMADA, M. & NAKAMURA, T. (1973). Time of neuron origin in mouse hypothalamic nuclei. *Experimental Neurology* **41**, 163–173.

SLOPER, J. C. (1966). The experimental and cytopathological investigation of neurosecretion in the hypothalamus and pituitary. In *The Pituitary Gland*, vol. 3 (ed. G. W. Harris & B. T. Donovan), pp 131–239. London: Butterworths.

TABER, B. (1964). Histogenesis of brain stem neurons studied autoradiographically with thymidine-H^3 in the mouse. *Anatomical Record* **148**, 344.

TABER-PIERCE, E. (1967). Histogenesis of the dorsal and ventral cochlear nuclei in the mouse. An autoradiographic study. *Journal of Comparative Neurology* **131**, 27–54.

UZMAN, L. L. (1960). The histogenesis of the mouse cerebellum as studied by its tritiated thymidine uptake. *Journal of Comparative Neurology* **114**, 137–159.