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Immunohistochemical analysis of glutamate, cholecystokinin and vasoactive intestinal polypeptide in the lateral geniculate complex of albino rat: A developmental study

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Abstract. The lateral geniculate nuclear complex of albino rats was investigated with respect to the development of neurotransmitters/neuromodulators such as glutamate, cholecystokinin and vasoactive intestinal polypeptide at gestational day 18, various postnatal age periods and in the adult using immunohistochemical methods. The study shows the unequivocal presence of and the sequential changes in the profile of glutamate while cholecystokinin and vasoactive intestinal polypeptide are not demonstrable at any of the age periods. Glutamate is seen both in the cells and fibres from 40 postnatal day onwards and immunoreactivity is more intense in the adult. The findings are discussed with relevance to the role of neurotransmitters in development.

Keywords. Neurotransmitter/neuromodulator; development; lateral geniculate nuclear complex; albino rat; immunohistochemistry.

1. Introduction

The lateral geniculate nuclear (LGN) complex in the rat receives major afferent inputs from the retina and cerebral cortex (Montero and Guillery 1968). Additonal afferents from other regions are also present (Hoover and Jacobwitz 1979; Kromer and Moore 1980; Ohara *et al* 1980; Pasquier and Villar 1982; Reese 1984). Some details of the neurochemical circuitry in this nucleus have been analysed in the mammals. Excitatory amino acids (Canzek *et al* 1981; Anderson *et al* 1987) and taurine (Pasantes Morales *et al* 1975) are said to be involved in the transmission of retinal inputs to the LGN complex. Radioimmunoassay (Duner *et al* 1954) and immunohistochemical (Brecha *et al* 1987) studies have indicated the presence of substance P (SP) immunoreactivity in the ganglion cell population in the retina as well as in the terminals in LGN and superior colliculus. Three distinct transmitter specific brain stem afferents — serotonergic from raphe nuclei (Pasquier and Villar 1982), cholinergic from pontomesencephalic tegmental field (Mesulam *et al* 1983) and noradrenergic from locus coeruleus (De Lima and Singer 1987) are known to project to the mammalian LGN. In the adult rat, however, immunocytochemical study has revealed the presence of only one neuropeptide-enkephalin (ENK) in the dorsal division (dLGN), which is concerned with visual processing while aminergic and peptidergic neurotransmitters/neuromodulators such as serotonin (SER), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP) including ENK are present in the ventral division (vLGN) and the intergeniculate leaflet (IGL), which

Abbreviations used: LGN, Lateral geniculate nuclear; SP, substance P; ENK, enkephalin; dLGN, dorsal LGN;SER, serotonin; VIP, vasoactive intestinal polypeptide; vLGN, ventral LGN; IGL, intergeniculate leaflet; CCK,cholecystokinin; DPN, days postnatal.

probably regulate the endocrine and behavioural events in response to light (Mantyh and Kemp 1983).

In the developing albino rat LGN complex, however, we have earlier studied SP, Leu-ENK and SER profiles and observed SP immunoreactivity to be present in the dLGN and found it to increase from 1 DPN to 20 DPN but decrease thereafter. SER and Leu-ENK fibres and terminals, on the other hand, seen occasionally at 1, 5 and 10 DPN were better visualised from 20 DPN and gradually increased at later age periods (Wadhwa *et al* 1990).

In the present study, we have undertaken to analyse the developmental profile of glutamte, cholecystokinin (CCK) and VIP in the albino rat LGN complex in an attempt to see if there are any changes in the neurotransmitter/neuropeptide patterns with age. No studies on developmental changes of these neurotransmitter/ neuropeptide profiles in the geniculate complex are available in primates either.

2. Materials and methods

Two to three albino rats (Wistar strain) of either sex, mostly male, from each age period of 18 day gestation, 1, 5, 10, 20 and 40 days postnatal (DPN) and 1 or 2 adults were used for each neurotransmitter/neuropeptide. The body and brain weights of the animals used in the study are given in table 1. The animals were bred and raised in appropriate environmental surroundings with *ad libitum* diet and water intake and a 12 h day and night cycle.

Table 1. Body and brain weight parameters of rats.

Age of animal	No. of animals (*n*)	Body wt (g) mean ± SD	Brain wt (g) mean ± SD
18 E	9	1·5 ± 0·01	0·20 ± 0·005
1 DPN	9	3·5 ± 0·015	0·323 ± 0·015
5 DPN	9	7·51 ± 0·008	0·603 ± 0·016
10 DPN	6	15·9 ± 0·01	1·03 ± 0·008
20 DPN	6	35·73 ± 0·008	1·45 ± 0·014
40 DPN	6	56·51 ± 0·053	1·53 ± 0·006
Adult	4	90·0 ± 2·5	1·81 ± 0·05

2.1 *Fixation*

The 18 day gestation (El8) and 1 DPN animals were decapitated after a very brief ether anaesthesia and immersion fixed. The postnatal and adult animals were anaesthetised by ether and the brain perfused through the vascular route for 30-45 min and postfixed in the same fixative overnight. For studying glutamate perfusion was done with 5% carbodiimide followed by 5% glutaraldehyde in 0·1 M PBS at pH 7·2. For localising CCK and VIP, 4% paraformaldehyde and 1% glutaraldehyde in 0·1 M PBS at pH 7·4 was followed by borate buffer at pH 11·0.

2.2 *Immunohistochemical procedure*

Following fixation the tissues were equilibrated with 30% sucrose in 0·1 M

phosphate buffer. Cryostat sections (30–35 μm) of diencephalon with lateral geniculate nuclear complex were cut coronally. The sections were incubated for immunohistochemical staining with polyclonal antibodies against CCK, VIP and monoclonal antibodies against glutamate (Incstar USA) at dilutions of 1:20, 1:20 and 1:100 respectively for a period of 48 h using avidin biotin technique and 3, 3'-diaminobenzidine (DAB) as the chromogen. In the control sections incubation with primary antibody was omitted; cervical spinal cord and habenula known to have glutamate as well as cerebral cortex known to contain VIP and CCK were taken as positive controls from the same and adult animals. Some sections were also treated with nickel II sulphate in 0·1 M imidiazole and 0·2 M acetate buffer alongwith DAB for intensification of staining.

3. Results

3.1 *Glutamate immunoreactivity*

3.1a *E18, 1, 5, 10 and 20 days:* No glutamate immunoreactivity was demonstrable at these age periods (figure 3a). However, the positive control sections of adult spinal cord and habenula processed simultaneously showed immunoreactivity (figures lb, 2). The negative control sections of spinal cord showed no immunoreactivity in the dorsal horn (figure la).

3.1b *Forty days and adult:* At both these age periods the immunopositive glutamate fibres were seen to be present in the vLGN and dLGN parts of geniculate nucleus as well as in the IGL. The immunopositive cell bodies were also observed. The cytoplasm of these neurons showed the brown precipitate of immunoreaction while the nuclear space was unstained. The immunoreactivity was more intense in the adult as compared to 40-day rats (figure 3b, c) and in the vLGN as compared to dLGN (figure 3d, e).

3.2 *VIP*

No immunoreactivity was seen in the LGN complex at any of the age periods studied (figure 4) although the positive controls from adult cerebral cortex showed VIP immunopositive neurons and their processes in layers 2 and 3 predominantly (figure 5).

3.3 *CCK immunoreactivity*

No CCK immunoreactivity was observed in the LGN complex at any of the age periods studied (figure 6a). The positive controls of adult cerebral cortex, however, showed CCK immunopositive neurons and their processes in layers 2 and 3 predominantly (figure 7a). The negative control section of LGN and cortex showed no immunoreactivity at all (figures 6b, 7b).

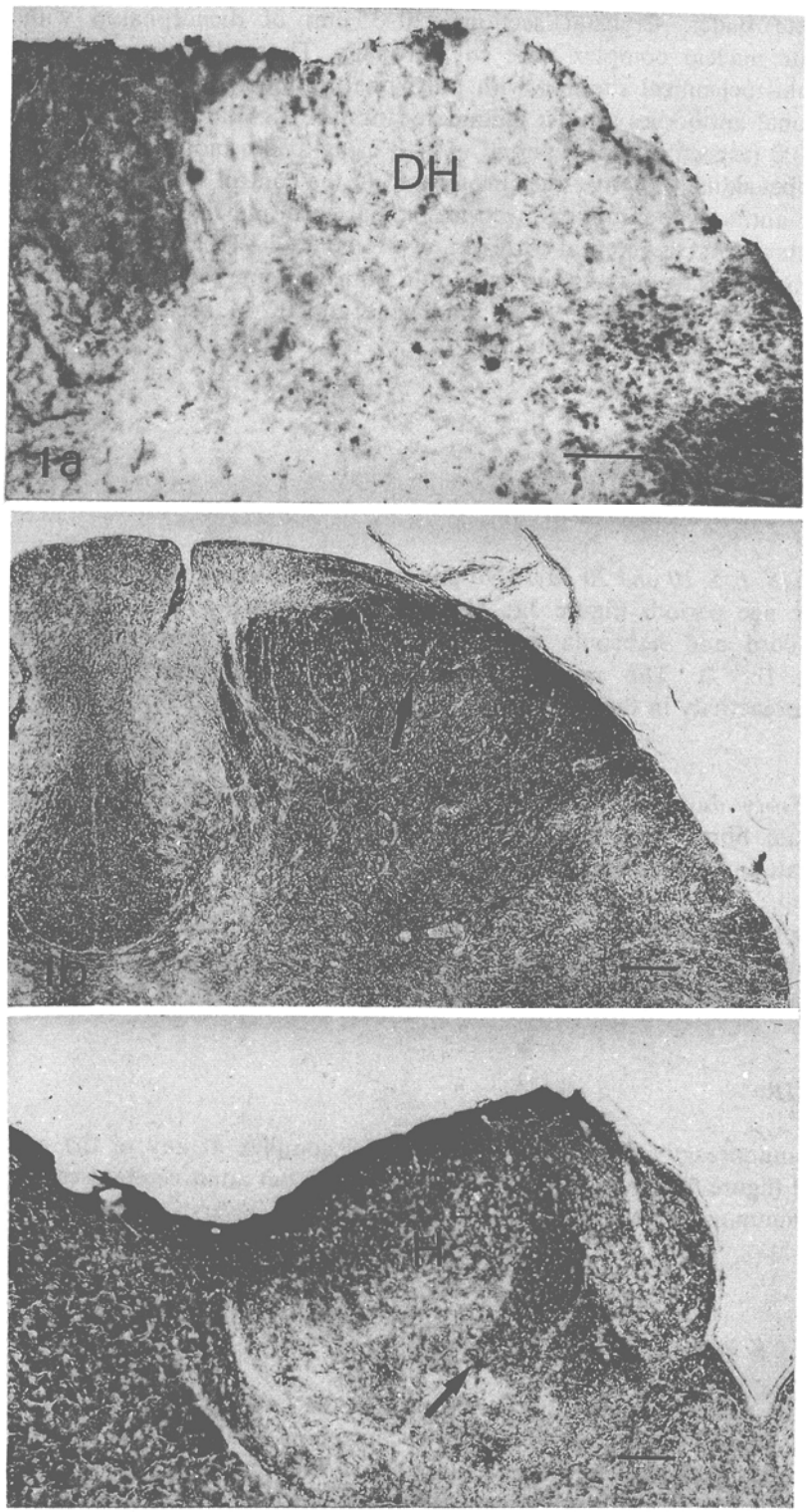

Figure 1 and **2.**

4. Discussion

In the present study we report the status of glutamate, CCK and VIP profiles in the LGN complex of albino rat during development and in the adult.

The excitatory transmitter, N-acetyl aspartyl glutamate has been identified in the rat retinal ganglion cells and their projections in the LGN complex and superior colliculi (Anderson *et al* 1987). In the present study glutamate immunoreactivity has been seen in the geniculate complex from 40 DPN and increases in intensity subsequently. It may be that the levels of glutamate in the early stages are exceedingly low and not detectable by the immunohistochemical methods. In a biochemical study on the postnatal development of neurotransmitter profiles in rat, glutamate uptake is found to be only 25 % of the adult level in the 2 day old rat, and it increases continuously and reaches adult levels of activity on day 15 (Kvale *et al* 1983). The uptake studies indicate the receptor activity and do not necessarily reflect the concentration of the neurotransmitter within the cells or fibres. The glutamate immunoreactivity in early postnatal life could be in the retinal terminals and subsequent increase probably reflects increased corticogeniculate inputs. Both retinal (Anderson *et al* 1987) and cortical (Karlssen and Fonnum 1978) terminals to LGN contain glutamate and it is also evident that retinal terminals appear earlier than cortical innervation (Poppe *et al* 1973; Frost *et al* 1979; Winkleman *et al* 1980).

Mantyh and Kemp (1983) demonstrated few fibres of VIP in the lateral part of vLGN and none in the remaining portions of LGN complex in the adult rat. Similarly CCK is also absent. Since neuropeptide SP (Wadhwa *et al* 1990) is present in postnatal period in the rat with increasing density up to 20 DPN and later decreases at 40 DPN as well as in the adult, there was a possibility of VIP and CCK also being present in early postnatal period. However, the present study shows complete absence of these two neuropeptides in the rat LGN complex during postnatal development as well as in the adult.

The neurotransmitters and neuropeptides during development have been seen to have a trophic role and influence the cell division, morphogenetic cell movements (McMohan 1974), morphology and growth of neurites (Lipton and Kater 1989) and synaptogenesis (Wolff *et al* 1979). Glutamate receptor antagonists have been shown to enhance dendrite outgrowth in hippocampal neurons when co-cultured with entorhinal cortex suggesting that glutamate acts to stabilize target cell outgrowth and thereby provide opportunity for prolonged axodendritic interactions leading to

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Figures 1 and 2. (1) Negative **(a)** and positive **(b)** control sections of adult rat spinal cord. Note the absence **(a)** and black stippling (arrow) **(b)** of glutamate immunoreactivity in the dorsal horn (DH). Scale bar= 100 μm. **(2)** Positive control section of the adult habenula region (H). Note the black stippled fibres and some glutamate positive cells (arrow) in the habenula. Scale bar= 100 μm.

Figure 3. (a–c) Lateral geniculate complex of 20 DPN **(a),** 40 DPN **(b)** and adult **(c)** rat. **(a)** Note the absence of black stippled glutamate immunoreactive fibres in both the dLGN and vLGN nuclei, **(b)** Showing the greater intensity of glutamate positive staining in the vLGN as compared to dLGN. **(c)** Showing the immunopositive black stippled glutamate fibres, both in the dLGN as well as in the ventrally placed vLGN. cc, Crus cerebri. Scale bar=100 μm. **(d and e)** Higher magnification of **(b).** Note the greater number of black immunopositive varicosities (arrows) in the vLGN **(d)** as compared to dLGN **(e)** and the immunopositive neurons (arrowhead). Scale bar = 50 μm.

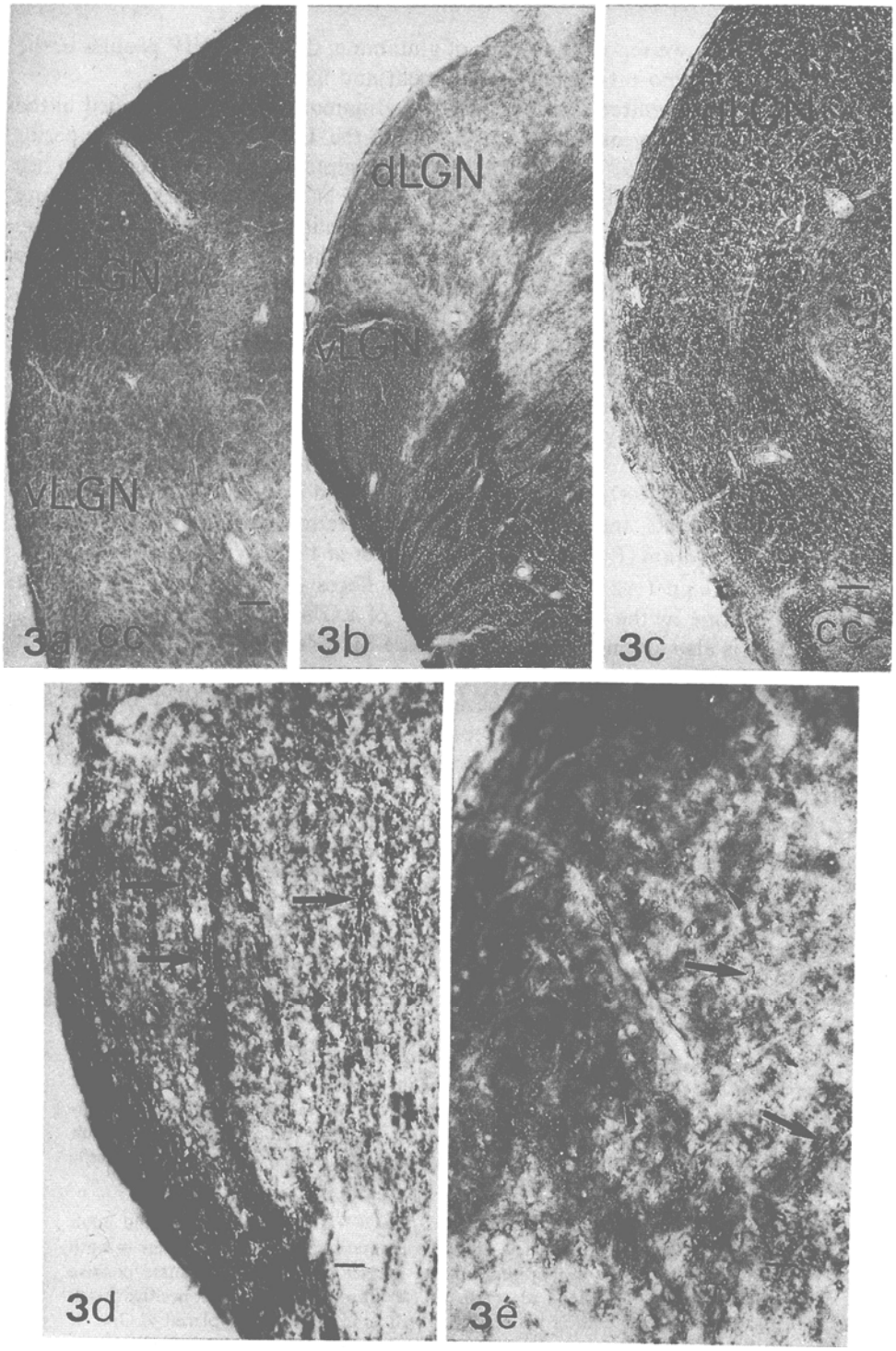
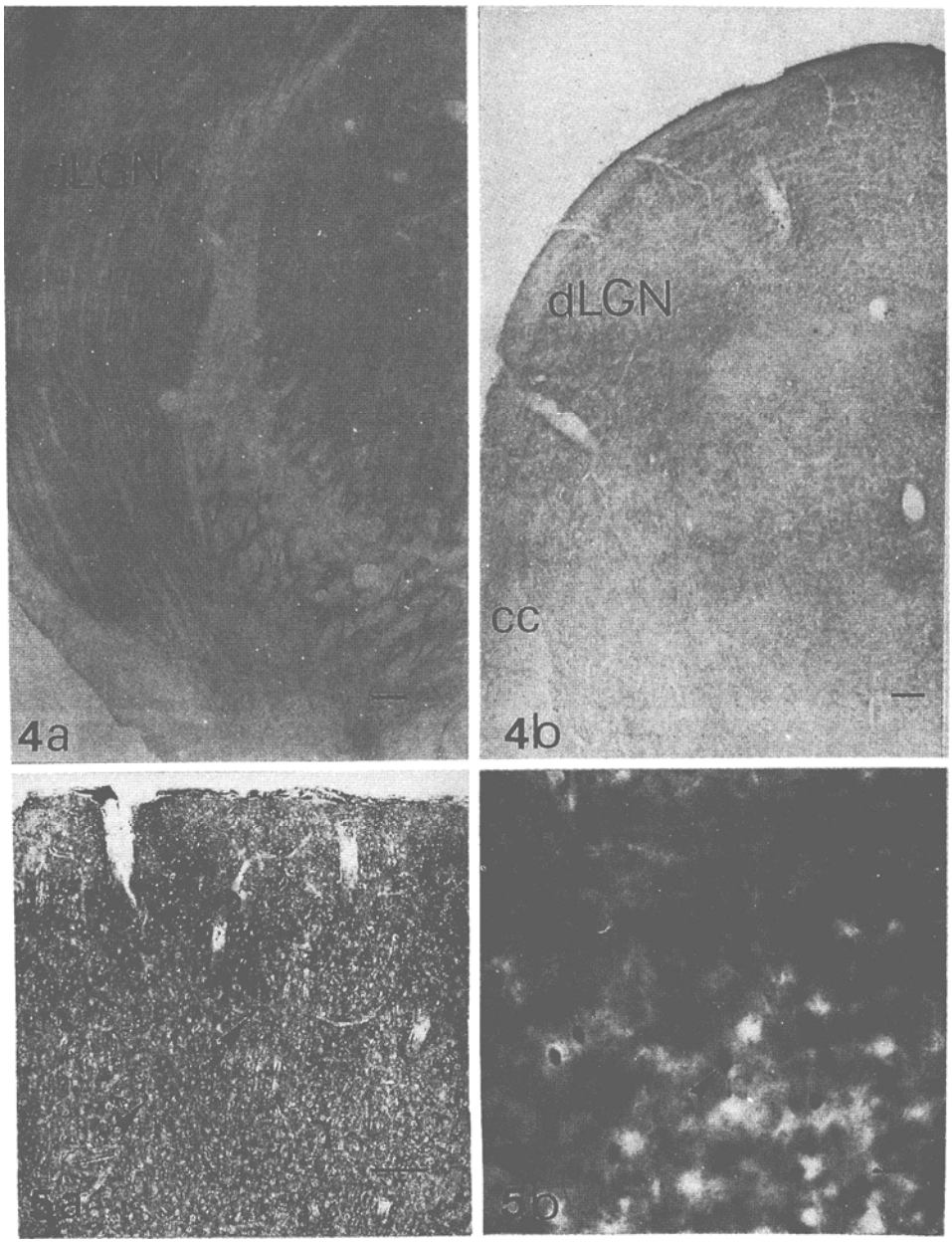
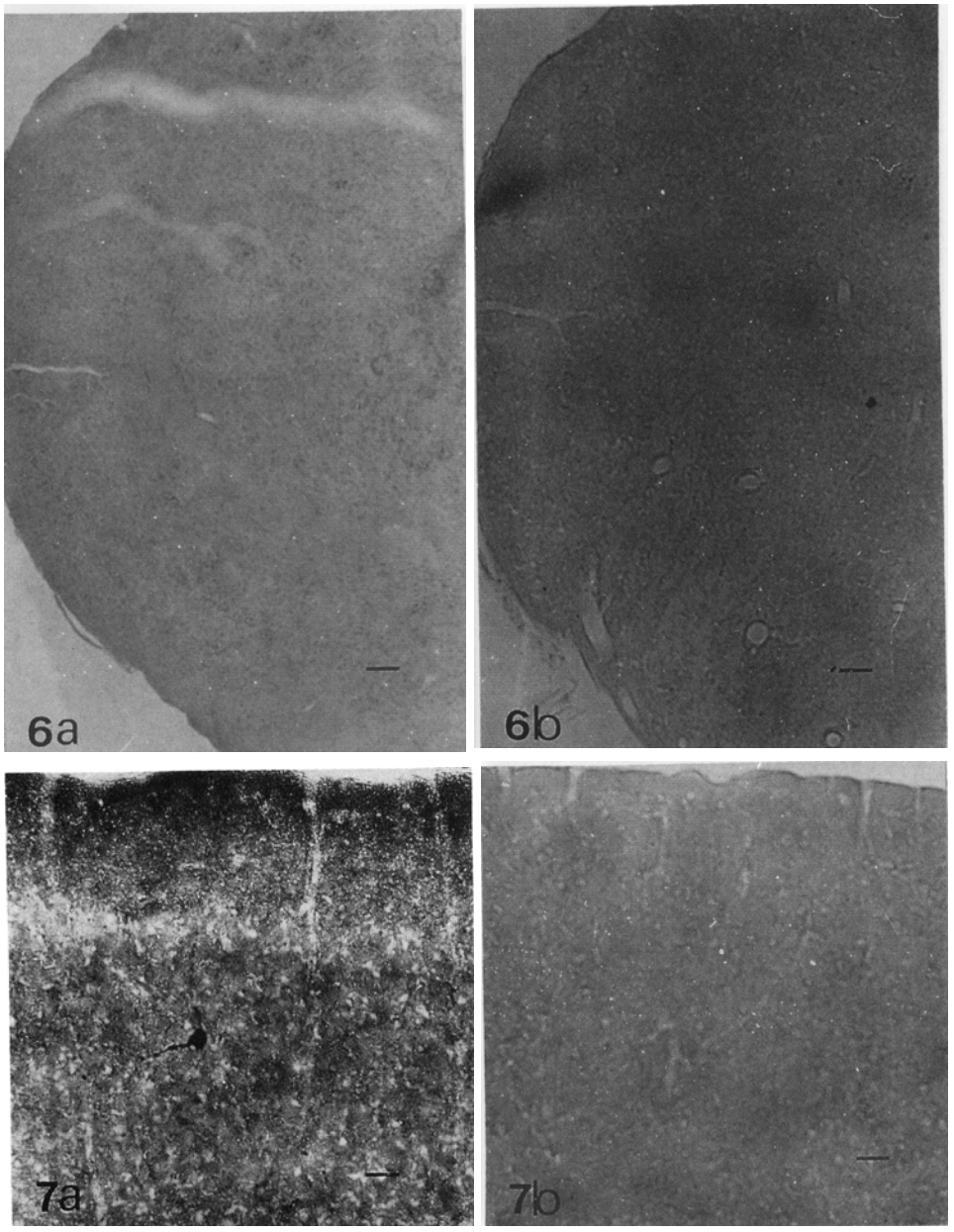


Figure 3. For caption, see page no. 233.



Figures 4 and 5. (4) Lateral geniculate complex showing the ventral and dorsal (dLGN) parts from the adult rat **(a)** and 40 DPN rat **(b).** Note the absence of VIP immunopositive fibres and cells in both age periods and in both the regions of the geniculate complex, cc, Crus cerebri. Scale bar=100 μm. **(5)** VIP immunopositive cells and fibres in the cerebral cortex of adult rat at low magnification **(a)** (scale bar= 100 μm) and at high magnification **(b)** (scale bar = 50 μm). Note the black stained cells indicated by arrowheads.



Figures 6 and 7. (6) Adult rat LGN complex showing the absence of staining for CCK in the stained **(a)** and negative control section **(b).** Scale bar=100 μm. **(7)** Adult rat cerebral cortex showing the presence of CCK immunopositive neuron (black) and fibres in the stained section **(a)** and absence of staining in the negative control section of the cortex **(b).** Scale bar = 50 μm.

synaptic differentiation (Mattson *et al* 1988). It is also known that exposure of developing tadpole to NMDA receptor antagonists prevents normal segregation of retinotectal synapses (Cline and Constantine-Paton 1989) and addition of

exogenous NMDA to the tadpoles sharpens the retinotectal map (Cline and Constantine-Paton 1990). In the rat LGN, the level of glutamate before eye opening (14 DPN), when segregation of retinal inputs occurs (Land and Lund 1979; Jeffery 1984), is probably inactivated or balanced by GABA. GABA is shown to increase continuously from birth to 24 DPN and decrease thereafter to adult levels in the rat LGN (Kvale *et al* 1983). This seems to be corroborated by the reduction seen in dendritic outgrowth inhibition and neurotoxic actions of glutamate when GABA receptors are simultaneously activated in the hippocampal-entorhinal co-cultures (Mattson and Kater 1989).

It is evident from the present study and our earlier work (Wadhwa *et al* 1990) that SP, SER, Leu-ENK and glutamate appear sequentially in the rat LGN. For the neurotransmitters to play roles in neural development, it is essential that both neurotransmitters and their receptors are properly paired in time and space. While data pertaining to the neurotransmitter development in rat LGN is substantially increasing, our knowledge regarding receptor expression is still quite limited. Increasing evidence is being provided for spatial and temporal integration of neurotransmitter signals in the development of neural circuitry. However, there is also strong evidence that different receptors and transmitters are not expressed simultaneously and may be expressed transiently and their unique presence very likely plays a crucial role in functional neuronal circuits (Mattson and Hauser 1991).

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