Development of the Rat Thalamus: VI. The Posterior Lobule of the Thalamic Neuroepithelium and the Time and Site of Origin and Settling Pattern of Neurons of the Lateral Geniculate and Lateral Posterior Nuclei

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ABSTRACT

Short-survival, sequential, and long-survival thymidine radiograms of rat embryos, fetuses, and young pups were analyzed in order to determine the time of origin, site of origin, migratory route, and settling pattern of neurons of the dorsal lateral geniculate (LGD), ventral lateral geniculate (LGV), and lateral posterior (LP) nuclei of the thalamus. Quantitative examination of long-survival radiograms established that the neurons of the LGD are produced on days E14 and E15. Within the LGD there is an external-to-internal neurogenic gradient; the majority (77%) of neurons of the external half are generated on day E14, while in the internal half the majority (64%) of neurons originate on day E15. The late-generated LGD neurons are located in the termination field of the uncrossed fibers of the optic tract. Examination of short-survival radiograms indicated that the neurons of the LGD originate in a discrete neuroepithelial eversion situated ventral to the pineal rudiment and dorsal to the putative neuroepithelium of the ventral nuclear complex. In sequential radiograms from rats injected with 3H-thymidine on day E15 and killed on days E16 and E17, the migration of young LGD neurons was followed in a posterolateral direction to the formative lateral geniculate body. By day E17, the day when the optic tract fibers begin to disperse over the lateral surface of the posterior diencephalon, the distribution of early and late-generated neurons of the LGD resembles that seen in young pups.

As a whole, the neurons of the LGV are produced earlier than the neurons of the LGD. The bulk of LGV neurons are generated on days E14 and E15 in a caudal-to-rostral intranuclear neurogenic gradient. Caudal LGV neurons are generated mainly on day E14 (82%), while a substantial proportion of rostral neurons (32%) are generated on day E15. Examination of short-survival and sequential radiograms suggest that the LGV neurons originate in an inverted sublobule situated beneath the putative neuroepithelium of the LGD. At anterior levels the putative inverted sublobule of the LGV merges imperceptibly with the neuroepithelium that produces the neurons of the lateral habenular nucleus. Like the neurons of the LGD and LGV, so also those of the LP are generated on days E14 and E15, but the neurogenic gradients are different. There is a lateral-to-medial gradient within the LP as a whole. Peak production of neurons is on day E14 laterally (58%) and on day E15 medially (59%). In addition, there is also a caudal-to-rostral and a dorsal-to-ventral gradient.
The major thalamic components of the mammalian visual system are the dorsal lateral geniculate and ventral lateral geniculate nuclei and the pulvinar complex, which, in the rat, is usually identified with the lateral posterior nucleus. This paper is concerned with the early development of these three functionally related thalamic structures.

The dorsal lateral geniculate nucleus (LGD) has probably received more attention by anatomists and physiologists than any other thalamic structure. This may partly be due to its role as the primary relay nucleus in the visual pathway and partly to its conspicuous appearance, particularly in primates, in which the LGD is clearly delineated from adjacent structures by its pronounced lamination (Walls, '53; Polyak, '57). Two ventrally situated magnocellular layers and four dorsally situated parvocellular layers are usually distinguished in the LGD of higher primates, different layers being innervated by optic fibers from the contralateral and ipsilateral eye. There are conspicuous differences in the lamination pattern of the LGD and in the spatial orientation of the layers in different primate species and even greater differences among other orders. In the cat only three layers can be distinguished, and in the rat the lamination is not at all obvious. However, experimental studies indicate some segregation in the termination of crossed and uncrossed optic fibers in the rat LGD (Hayhow et al., '62; Cunningham and Lud, '71; Lund et al., '74; Hickey and Spear, '76; Reese and Cowey, '83; Brauer et al., '84; Manford et al., '84). The uncrossed fibers and their terminals tend to be concentrated in a crescent-shaped zone situated medially; the more abundant crossed fibers occupy a much larger area laterally but partially overlap with the site of termination of uncrossed fibers. Two types of neurons have been distinguished in the rat LGD with the Golgi technique: a larger, multipolar cell with a tufted dendritic arbor and a myelinated, unbranched axon, presumably the geniculocortical relay cell; and a smaller cell type with a locally terminating axon-like process, possibly an interneuron (Rafols and Valverde, '73; Grossman et al., '73). The interneurons are distributed throughout the LGD (Kriebel, '75) and the ratio of relay neurons to interneurons is about 13:1 (Werner and Brauer, '84).

The ventral lateral geniculate nucleus (LGV) is usually divided into two components, an external (lateral) part composed of larger cells and an internal (medial) part of smaller cells (Niimi et al., '63). The LGV is better developed in rodents than in carnivores or primates (Niimi et al., '63). The lateral posterior nucleus (LP) of lower mammals is considered to be the relatively unelaborated homologue of the pulvinar of primates (Le Gros Clark, '32; Harting et al., '72) and the lateral-posterior-pulvinar complex of carnivores (Graybiel and Berson, '80; Updyke, '83). It has been assumed for some time that the pulvinar is involved in higher sensory, particularly visual, integrative functions (Walker, '38). This assumption has been based on comparative-anatomical con-

Key words: dorsal lateral geniculate nucleus, neuroembryology, pulvinar, thymidine autoradiography, ventral lateral geniculate nucleus

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Abbreviations

Abbreviations in capital letters refer to mature structures; capital letters followed by m refer to the migratory streams of a structure; letters in lower case refer to the putative cell lines of a particular structure in the neuroepithelium.

- CP: cerebral peduncle
- HB: habenular nucleus
- LGD: dorsal lateral geniculate nucleus
- LGDI: external half of dorsal lateral geniculate nucleus
- LGDm: internal half of dorsal lateral geniculate nucleus
- LGVm: caudal part of ventral lateral geniculate nucleus
- LGVr: rostral part of ventral lateral geniculate nucleus
- LP: lateral posterior nucleus
- LPm: lateral posterior migratory stream
- LPM: lateral posterior neuroepithelium
- LGDm: medial half of lateral posterior nucleus
- MG: medial geniculate nucleus
- PC: posterior commissure
- PFT: parafascicular nucleus
- PI: pretectal nucleus
- PIN: pineal nucleus
- PRT: pretectal nucleus
- PM: parafascicular migratory stream
- VB: ventrobasal nucleus
- VBM: ventrobasal migratory stream
- VL: ventrolateral nucleus
- ZI: zona incerta

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Fig. 1. The dorsal lateral geniculate, ventral lateral geniculate, and lateral posterior nuclei in a thick (50 µm) coronal section of an adult rat. Frozen section, hematoxylin and eosin. Scale: 1,000 µm.

siderations, i.e., the expansion of the pulvinar in higher mammals in relation to the evolutionary growth of the occipital, temporal, and parietal areas of the neocortex (Chalupa, '77). In the rat, the LP receives afferents from the superior colliculus (Perry, '80; Takahashi, '85) and the striate and peristriate cortices (Mason and Groos, '81; Takahashi, '85), and it projects to the retinotopically organized areas of the occipital and temporal cortices (McDaniel et al., '78; Olavarria, '79; Coleman and Clerici, '80; Mason and Groos, '81; Schober, '81).

The neurogenesis of the lateral geniculate body has been investigated in a number of species: mouse (Angevine, '70), rat (Brückner et al., '76; McAllister and Das, '77; Lund and Mustari, '77; Altman and Bayer, '78a,b), hamster (Crossland and Uchwat, '82; Crossland, '87), cat (Hickey and Hitchcock, '84), and monkey (Rakic, '77). Brückner et al. ('76) observed that with a single injection of 3H-thymidine on day E14 in the rat, 99% of the neurons of both LGD and LGV were labeled, but following injection on day E16 only 18% of the neurons of the LGV, and even fewer of the LGD (2%), were labeled. Lund and Mustari ('77) claimed that the neurons of the rat LGD are generated on gestational days 12–14. But McAllister and Das ('77) reported that neurogenesis in both the LGD and LGV of the rat peaked on day E15. All the studies referred to used the flash labeling procedure. Our quantitative studies with the cumulative labeling procedure (which is designed to specify the exact proportion of neurons generated on a particular day) indicated that the neurons of the rat LGV are produced between days E13 and E15 and those of the LGD between days E14 and E15 (Fig. 12B in Altman and Bayer, '78a). The proportion of neurons generated on day E15 was much higher in the LGD than in the LGV, indicating an internuclear gradient between the two.

Several studies have dealt with intranuclear gradients in the LGD. In the mouse, Angevine ('70) noted a lateral-to-medial gradient in the generation of LGD neurons, and in the hamster Crossland ('87) found a dorsolateral-to-ventromedial gradient. Both were interpreted as superficial-to-deep gradients. According to Rakic ('77), the earliest-produced neurons of the monkey LGD migrate to the surface of the diencephalon and the later-generated neurons accumulate in an “outside-to-inside” pattern. This gradient is initially oriented lateromedially; then it changes ventrodorsally such that the older neurons form the ventral magnocellular layers and the younger neurons form the dorsal parvocellular layers. However, not all investigators have been able to detect a neurogenetic gradient in the LGD. McAllister and Das ('77) found no clear evidence of a gradient in the rat LGD, and Hickey and Hitchcock ('84) reported the absence of intranuclear gradients in the lateral geniculate body of the cat. Our qualitative observations in the rat suggested a
Fig. 2. Coronal radiograms of the posterior thalamus from a P5 rat labeled with \( ^{3}H \)-thymidine on days E13 + E14 (A) and another rat labeled on days E15 + E16 (B). Paraffin, H&E. Scale: 200 \( \mu \)m.
Fig. 3. Coronal thalamus radigrams of the ALO, LGV, and LP, from control (A) to edentate (B), from a P5 rat labeled on days F13, F14. Broken lines indicate the approximate boundaries of the three nuclei.
Fig. 4. Coronal thymidine radiograms of the LGD, LGV, and LP, from rostral (A) to caudal (D), from a P5 rat labeled on days E15 + E16. Arrow indicates neurogenetic gradient (older to younger neurons). Paraffin, H&E. Scale: 100 μm.
lateral-to-medial gradient within both nuclei (Fig. 15A,B in Altman and Bayer, '79b) but with regional variations (Fig. 17A,B in Altman and Bayer, '79b).

As we have described in the introductory paper of this series, the caudal neuroepithelial lobe of the thalamus of day E13 rats (Figs. 1, 2 in Altman and Bayer, '88a) splits by day E14 into two components, the intermediate lobule and the posterior lobule (Figs. 4, 5 in Altman and Bayer, '88a). In the subsequent papers we sought to provide evidence that the everted or inverted sublobules into which these neuroepithelial lobules become further partitioned are sources of neurons of discrete thalamic nuclei. In the preceding paper (Altman and Bayer, '89b) we provided some evidence that one of the sublobules of the posterior lobule is the putative source of neurons of the medial geniculate body. In the present paper we will try to marshal support for the hypothesis that the other three sublobules of the posterior lobule give rise to, respectively, the neurons of the LGD, LGV, and LP.

MATERIALS AND METHODS

The material examined in this study was identical with that described in detail in the first paper of this series (Altman and Bayer, '88a). We made particular use of three collections. (1) The long-survival series was used to determine the time of origin of neurons of the LGD, LGV, and LP. This series consists of 44 paraffin-embedded brains of P5 rats whose mothers were injected with two successive daily doses of \(^{3}H\)-thymidine, with a single day delay between the groups, on gestational days E13+E14, E14+E15 ... and E18+E19. The data from six to eight pups in every relevant injection group were used for the quantification of the pro-
portion of neurons produced on particular days with special reference to internuclear and intranuclear gradients. Details of the quantification procedure and statistical method were presented in the first paper of the series (Altman and Bayer, '88a), and a shorter description is provided in the paper dealing with the ventro medial complex (Altman and Bayer, '89a). (2) Short-survival radiograms were used to locate the neuroepithelial site of origin of neurons of the LGD, LGV, and LP. This series consists of 94 paraffin- and methacrylate-embedded embryos whose mothers were injected with a single dose of ³H-thymidine on successive days extending from day E12 to E21 and who were killed 2 hours after injection. The number of specimens examined in the relevant injection groups ranged from six to 12. (3) Sequential-survival thymidine radiograms were examined to trace the migratory paths of neurons. This series consists of 254 paraffin and methacrylate embryos or fetuses that received ³H-thymidine between days E12 and 21 and were killed at daily intervals after the injection up to day P5.

RESULTS

The time of origin of neurons of the dorsal and ventral lateral geniculate nuclei and of the lateral posterior nucleus

Qualitative observations. The location and configuration of the LGD, LGV, and LP are illustrated in a coronal section from an adult rat (Fig. 1). There is no indication of lamination in the LGD but it is clearly delineated from adjacent medial structures and from the LGV ventrally and the LP dorsomedially. The LGV is quite large and its cells are stained more intensely than those of the LGD; the cells of the LP are less densely packed than those of the other two nuclei.

The changing radiographic labeling pattern in the LGD, LGV, and LP as a function of embryonic age at injection is illustrated in coronal sections from P5 rats that received two successive doses of ³H-thymidine either on day E13 + E14 (Figs. 2A, 3) or day E15 + E16 (Figs. 2B, 4, 5). In the rats that
received radioactive thymidine beginning on day E13, all the cells of the LGD, LGV, and LP are labeled (Fig. 3). In the LGD and LP the cells situated near the external wall tend to be more intensely labeled than those occupying the internal portions of the nuclei (Figs. 2A, 3). In the rats that received their first injection on day E15, the cells in the most lateral aspect of the LGD are no longer labeled, but those forming the bulk of the LGD are heavily labeled (Figs. 2B, 4). This indicates that in the LGD the cells settle in an outside-in sequence. A less consistent outside-in gradient was also indicated for the LGV and LP (Fig. 4). Both in the LGV and in the LP of E15+E16 rats there were fewer labeled cells caudally than rostrally (compare Fig. 4D and 4A), suggesting a caudal-to-rostral neurogenetic gradient. In the LGD, the cells settle in an outside-in sequence. A less consistent outside-in gradient was also indicated for the LGV and LP (Fig. 4). Both in the LGV and in the LP of E15+E16 rats there were fewer labeled cells caudally than rostrally (compare Fig. 4D and 4A), suggesting a caudal-to-rostral neurogenetic gradient. In the E15+E16 group some variability was observed among the different animals in the proportion of cells labeled in the internal components of the three nuclei (compare Figs. 4 and 5). Finally, in rats injected on days E16+E17, the cells in the three nuclei, with the exception of a few scattered ones in some animals, were no longer labeled (Fig. 6).

Quantitative results. The proportion of labeled and unlabeled cells in the LGD, LGV, and LP was determined in four groups of P5 rats (E13+E14, E14+E15, E15+E16, E16+E17) at three equidistant coronal levels (anterior, L1; intermediate, L2; and posterior, L3). Within each level the LGD was divided into quadrants (ventral external, dorsal external, ventral internal, dorsal internal); the LP into quadrants (ventral lateral, dorsal lateral, ventral medial, dorsal medial); and the LGV into lateral and medial halves. In Figures 7–10, the areas where neurogenesis was found to occur simultaneously were combined. LGD. There were no differences in neurogenesis between the ventral and dorsal parts of the LGD at any level, and neurogenesis was also found to be simultaneous along the rostrocaudal plane. Consequently, these data were combined to show the difference between the external and internal halves of the LGD (Fig. 7). The external cells of the LGD (LGDe, bottom graph in Fig. 7A) originate earlier (77% on day E14) than the internal cells (LGDi, top graph), where neurogenesis peaks a day later (64% on day E15). This difference was highly significant (sign test; p < .0001).

LGV. There were no chronological differences in neurogenesis between the lateral and medial halves of the LGV; the data were, therefore, combined (Fig. 8). But in contrast to the LGD, there was a caudal (older)-to-rostral (younger) neurogenetic gradient in the LGV. At L3 caudally (LGVc, bottom graph in Fig. 8A), 82% of the cells are generated on day E14 and 16% on day E15. At L1 rostrally (LGVr, top graph), 66% of the cells are generated on day E14 and 32%
on day E15. This difference is highly significant (p < .0001).

LP. Similar to the other visual thalamic nuclei, the neurons of the LP are generated on days E14 and E15. However, there are unique neurogenetic gradients in the LP, suggesting that the LP neurons have a different source than the LGD and LGV neurons. The combined data (Fig. 9A) show that neurogenesis peaks on day E14 laterally (LPL, bottom graph), and on day E15 medially (LPM, top graph), indicating a lateral (older)-to-medial (younger) gradient (p < .039). In the lateral half of LP, but not in its medial half, there are also additional gradients (Fig. 10). There is a dorsal (LPL,d)-to-ventral (LPL,v) neurogenetic gradient both at L1 rostrally (LPL,d to LPL,v in Fig. 10A) and at L3 caudally (LPL,d to LPL,v in Fig. 10B), and there are many significant (p < .001). In addition there is a neurogenetic gradient from lateral (L3, two graphs in Fig. 10B) to rostral (L1, two graphs in Fig. 10A), and these differences, too, were highly signifi-
cant (p < .0001).

The site of origin and migration of neurons of the dorsal LGN

A conspicuous neuroepithelial eversion beneath the pineal rudiment was previously identified in day E15 rats as the putative source of neurons of the LGD (Figs. 16, 17 in Altman and Bayer, '88a). This neuroepithelial eversion is illustrated in coronal sections, from rostral to caudal, from a day E15 rat that was labeled with 3H-thymidine 2 hours previously (Fig. 11). The putative neuroepithelium of the LGD (lgd) is situated ventral to the pineal recess and pineal rudiment (pir and pin in Fig. 11) and dorsal to the neuroepithelial inversion (hb in Fig. 11A-D) composed of the early generated (unlabeled) neurons of the lateral habenular nucleus (HL; work in progress). In day E15 rats that were injected with 3H-thymidine on the previous day, two waves of labeled cells surround laterally the lgd; we identify these as the young migrating neurons of the LGD (LGDm in Fig. 12). This migration is not seen at anterior levels (not shown); it is small at intermediate levels (Fig. 12A) and grows appreciably in the caudal direction (Fig. 12B–F). These labeled cells, which must have left the neuroepithelium after the morning of day E14, are apparently migrating in a posterolateral direction.

The migration of young LGD neurons was examined in coronal sections, from caudal to rostral, in radiograms obtained from rats injected on day E15 and killed on day E16 (Figs. 13, 14) and day E17 (Figs. 15, 16). In the rat killed 24 hours after injection, the migratory stream is inconspicuous rostrally and limited to the vicinity of the neuroepithelium (LGDm in Fig. 13A). Proceeding caudally, the migratory stream fans out, forming a crescent-shaped wave front composed of heavily labeled cells (LGDm in Fig. 13B). Still more caudally, two migratory zones may be distinguished: an outer zone composed primarily of unlabeled cells, those generated before the morning of day E15 (LGDm1 in Fig. 14A,B), and an inner zone of heavily labeled cells, those generated after the morning of day E15 (LGDm2 in Fig. 14A,B). These observations suggest that the early-generated (day E14) and late-generated (day E15) LGD neurons migrate in an orderly chronological sequence in a posterolateral direction.
Fig. 11. Coronal radiograms of the region of the posterior thalamus, from rostral (A) to caudal (F), from a rat labeled on day E15 and killed 2 hours later. In this and all the subsequent figures, open circles indicate neuroepithelial eversions (concavities) and solid circles indicate inversions (convexities). Paraffin, H&E. Scale: 200 µm.
Fig. 12. Coronal radiograms of the far-posterior thalamus, from rostral (A) to caudal (F), from a rat labeled on day E14 and killed on day E15. Paraffin, H&E. Scale: 100 μm.
In the rat killed 2 days after injection on day E15, the LGD migratory wave is composed, rostrally, mostly of heavily labeled cells (LGDm2 in Fig. 15A,B), but caudally unlabeled cells predominate in what may be destined to become the early generated external half of the LGD (LGDm1 in Fig. 16A,B). This again suggests that the young LGD neurons migrate in a postero-lateral direction and that 2 days after injection many of the late-produced labeled cells are still some distance from their caudal settling site. Note also that the entire LGD migration has shifted by day E17 caudally to the level of the posterior commissure (PC in Figs. 15, 16). The neuroepithelial evagination formerly identified as the source of neurons of the LGD is still active after the cessation of LGD neurogenesis on day E17; we presume that it is producing neurons for the late-generated, medial components of the posterior thalamus (lgd in parentheses in Figs. 15, 16). The thalamus expands appreciably on the subsequent days (not shown), and in rats injected on day E15 and killed on day E22 (Fig. 17) the settled neurons of the LGD display the same chronicoroarchitectonic organization which is seen in postnatal rats injected on days E15+E16 (Figs. 4, 5).

In summary, these identifications indicate that the putative LGD neuroepithelium is situated in a dorsal area in the vicinity of the pineal rudiment, a region that is usually referred to as the epithalamus, but which we identify as a component of the posterior thalamic lobe.

The site of origin and migration of neurons of the ventral LGN

In the first paper of this series we designated a neuroepithelial inversion situated beneath the LGD neuroepithelium as the putative germinal source of neurons of the LGV (Fig. 17 in Altman and Bayer, '88a). The exact delineation of this neuroepithelium, indicated as lgv, has been made difficult by the circumstance that it is continuous rostrally with another neuroepithelium which, according to the best available current evidence, is the source of neurons of the habenular nuclei. The putative neuroepithelium of the habenular nuclei is illustrated in short-survival radiograms from a day E15 rat (hb in Fig. 11A–D). But the caudal continuation of this neuroepithelial inversion is designated as the putative germinal zone of the LGV (lgv in Fig. 11E,F). This we have done on the basis of observations in sequential radiograms. The rostral component of this neuroepithelial inversion is devoid of a laterally directed migratory stream. Our observations clearly indicate (work in progress) that this region is the source of neurons of the habenular nuclei which do not actively migrate but settle near their site of origin medially in an outside-in pattern (Figs. 7–10 in Altman and Bayer, '79b). However, a migratory stream does leave the otherwise indistinguishable neuroepithelial inversion caudally, and this migration can be traced to the LGV.

The putative neuroepithelium of the LGV (lvg) and its migration (LGVm) are illustrated in relation to the LGD migration (LGDm) in radiograms from a rat that was injected on day E14 and killed on day E15 (Fig. 12A–F) and from rats that were injected on day E15 and killed on day E16 (Figs. 13B, 14A,B) and day E17 (Figs. 15, 16). In the rat labeled on day E14 and killed on day E15, the lvg neuroepithelial inversion is distinguished from the overlying lgd eversion by its larger migratory zone of unlabeled cells (compare LGVm and LGDm in Fig. 12A–E). This is in line with the quantitative evidence that, overall, a much higher proportion of LGV cells than LGD cells are generated on day E14 (compare histograms in Figs. 7 and 8). Both in the rat labeled on day E14 and killed on day E15 (LGVm in Fig. 12A–E) and in the rat labeled on day E15 and killed on day E16 (LGVm in Figs. 13B, 14A,B), labeled cells in the LGV migratory stream are intermingled laterally with unlabeled cells. In the rat labeled on day E15 and killed on day E17 (LGVm in Figs. 15, 16) a high proportion of the labeled young neurons have reached the lateral wall of the diencephalon. As the thalamus grows on the subsequent days, the LGV is progressively displaced farther laterally.

The site of origin and migration of neurons of the LP

In the first paper of this series we have tentatively identified a neuroepithelial inversion situated beneath the pineal rudiment and above the lgd as the putative source of neurons of the LP (Figs. 15C, 16A–C in Altman and Bayer, '88a). We have distinguished this inversion from a more posteriorly situated, and earlier differentiating, obliquely oriented flattened neuroepithelium, tentatively identified as the germinal zone of the pretectal area. This neuroepithelial inversion is illustrated in radiograms from a day E15 rat killed 2 hours after injection (lp in Fig. 11A–E) and from a rat labeled on day E15 and killed on day E16 (Figs. 13, 14).

The migration of cells from the putative neuroepithelium of the LP is illustrated in sequential radiograms from rats labeled on day E15 and killed on days E16 (LPM1, LPM2 in Fig. 14A,B) and E17 (LPM1, LPM2 in Figs. 15A,B, 16A). In rats killed on day E16, a migration is not evident around the lp at rostral levels where the pineal rudiment is quite large in cross sections (Fig. 13A,B). But somewhat more caudally, where the pineal rudiment diminishes in size, a migratory zone is present with two components: a wave front of unlabeled cells, those generated before the injection on the morning of day E15 (LPM1 in Fig. 14A,B), and a trailing component of heavily labeled cells, those generated after the injection (LPM2 in Fig. 14A,B). In rats injected on day E15 and killed on day E17, the LP migration has become translocated in a posterior direction (note the presence of the posterior commissure, PC, in Figs. 15, 16). At rostral levels the second wave of heavily labeled cells has reached the dorsolateral wall of the diencephalon (LPM2 in Fig. 15A). More caudally (Figs. 15B, 16A) two zones may be distinguished: an outer zone composed of unlabeled (early generated) cells (LPM1) and an inner zone composed of labeled (late generated) cells (LPM2). The final settling position of LP neurons is illustrated in radiograms from rats labeled on day E15 and killed on day E22 (LP in Fig. 17). In summary, these observations suggest that the neurons of the LP are generated in a discrete neuroepithelial inversion sandwiched between the germinal sources of the pineal gland and the putative neuroepithelium of the LGD; that they migrate in a posterior direction as they fan out dorsally and dorsolaterally; and that they settle in a combined caudal-to-rostral and outside-in pattern.

DISCUSSION

The chronicoroarchitectonics of the dorsal LGN

This study confirms earlier results (McAllister and Das, '77; Altman and Bayer, '79a) to the effect that the neurons of the rat LGD are generated on days E14 and E15 and it supplements previous observations by providing both qualitative and quantitative evidence for the presence of a neuro-
Fig. 13. Coronal radiograms, from rostral (A) to caudal (B), of the region of the LGD and LGV migrations from a rat labeled on day E15 and killed on day E16. Paraffin, H&E. Scale: 100 µm.
Fig. 14. Continuation of the series shown in Figure 13. Paraffin, H&E. Scale: 100 μm.
genetic gradient within the LGD. The quantitative results show that the majority of neurons situated in the external half of the nucleus are generated on day E14, whereas the majority of neurons situated in the internal half of the nucleus are generated on day E15 (Fig. 7). Interestingly, the pattern of cell distribution, as seen in radiograms from P5 rats injected with $^3$H-thymidine beginning on day E15 (Figs. 4, 5), indicates that the late-generated (or labeled) neurons are situated in a region of the rat LGD where the uncrossed optic nerve fibers terminate (Hayhow et al., '62; Cunningham and Lund, '71). However, our current finding of such a gradient in fetuses and P5 pups contrasts with our earlier failure to detect a clear neurogenetic gradient in the LGD of P60 rats following injection of $^3$H-thymidine begin-
ning on day E15, even though we did note that the distribution of unlabeled and labeled cells is not random in the LGD of adults (Figs. 15, 17 in Altman and Bayer, '79b). Of possible relevance in this context is the finding by Manford et al. ('84) that the distribution of ipsilateral optic nerve fibers to the internal half of the LGD is extensive in the newborn rat, shrinks on subsequent days, and reaches the more circumscribed adult pattern by days P9–P12. Conceivably, there is a reorganization of the rat LGD at about the time of eye opening. The hypothesis that LGD neurons receiving contralateral projection are generated earlier than LGD neurons receiving ipsilateral projection will have to be tested by...
Fig. 17. Coronal radiograms of the LP, LGDi, LGDe, and LGV, from rostral (A) to caudal (D), from a rat labeled on day E15 and killed on day E22. Paraffin, H&E. Scale: 100 μm.
double labeling with 3H-thymidine and an anterograde axoplasmic tracer injected into one eye.

As we noted in the beginning of this paper, a neurogenetic gradient has been observed in the LGD of the monkey (Rakic, '77) and hamster (Crossland, '87) though not of the cat (Hickey and Hitchcock, '84). The exact orientation of this gradient in these two species differs from that observed in the rat but conforms to the principle of being an outside-in pattern.

The site of origin and settling pattern of neurons of the dorsal LGN

In the first paper of this series (Figs. 16, 17 in Altman and Bayer, '88a) we identified a conspicuous neuroepithelial eversion beneath the pineal rudiment as the putative source of neurons of the LGD. Examining sequential radiograms from rats injected with 3H-thymidine on day E15 and killed on subsequent days (Figs. 12–16), we were able to trace from this neuroepithelial eversion (Fig. 11) a stream of unlabeled and labeled cells apparently moving in the direction of the formative LGD. By day E16 (Figs. 13, 14), the labeled cells (those forming after the morning of day E15) have become translocated some distance in the posterolateral direction and, by day E17, the unlabeled cells (those generated before the morning of day E15) and the labeled cells (those generated after the morning of day E15) have reached the formative LGD, where they are arranged in an outside-in pattern (Fig. 16). The possibility that the late-generated cells of the LGD are its smaller neurons is counterindicated by the report that these are scattered throughout the LGD (Krikel, '75).

The arrival of the bulk of LGD neurons by day E17 in their final location appears to be synchronized with the ingrowth of optic nerve fibers. According to our observations, few if any optic nerve fibers are present in the future region of the optic chiasma on day E16, but the chiasma is clearly recognizable by day E17 (e.g., Fig. 14 in Altman and Bayer, '79b). This is in agreement with the observations of Lund and Bunt ('76). (Although Lund and Bunt state that the chiasma first becomes visible on day E16, this is not a discrepancy because they designate the morning following mating on the previous night as gestational day 0 whereas we designate it as day E1.) Moreover, Lund and Bunt have traced optic tract fibers on the same day (our day E17) to what they refer to as the epithalamus.

Rakic ('77), in his study of the embryonic development of the monkey LGD, proposed that the neurons of the LGD are generated in two germinal areas, the ventricular and subventricular zones of the ventral aspect of the dorsal thalamus (Figs. 6A–D, 9A–C in Rakic, '77). Our studies indicate that, in the rat, the latter region is the source of neurons of the ventral nuclear complex, not the LGD (Altman and Bayer, '89a). Our hypothetical identification of the LGD neuroepithelium (as well as LGV and LP neuroepithelia) more dorsally within a region usually thought of as the epithalamus warrants further consideration as does also the implication that these central optic nuclei of the thalamus derive from a neuroepithelial region which is contiguous with the pineal rudiment.

The term epithalamus has been widely used since the end of the last century (e.g., Edinger, 1896). It was Herrick ('10) who popularized the notion that the epithalamus, like the ventral thalamus and the hypothalamus, is not part of the thalamus proper. Most current textbooks describe the epithalamus as a separate diencephalic region composed of the habenular nuclei, the stria medullaris, and the pineal body. However, Rose ('42) argued from a developmental perspective that "any basis is lacking for the assumption that the habenular complex as such should be a special division of the thalamus. ... The habenula does not ... by any means represent the largest group of this region. That is formed by the prehippocampal complex" (Rose, '42; p. 93). Although the idea that the epithalamus is a distinct diencephalic region is supported by observations in the maturing and adult brain (the conspicuous habenular nuclei are segregated dorsally from the rest of the thalamus) our preliminary developmental observations (work in progress) place the putative source of neurons of the habenular nuclei in a conspicuous neuroepithelial inversion situated not at the roof of the third ventricle but beneath the putative neuroepithelium of the LGD and LP. However, instead of arguing that the LGD and LP neurons, like those of the habenular nuclei, derive from the epithalamus, it seems more parsimonious to follow Rose's suggestion that the epithalamus is not a distinct germinal region. In line with our original observations, it appears reasonable to consider the putative LGD neuroepithelium—together with that of the LP and LGV, and with the medial geniculate neuroepithelium previously considered (Altman and Bayer, '88b)—as a component of another distinct morphogenetic region, i.e., the posterior lobe of the thalamic neuroepithelium (Figs. 4B, 7B in Altman and Bayer, '88a).

The other matter worthy of consideration is the contiguity of the putative LP and LGD neuroepithelium with the germinal source of the pineal body, a structure implicated in optic functions. The pineal body is phylogenetically related to the dorsal, or parietal, eye of extinct ostracoderms, bony fish, amphibians, and reptiles of the Devonian period (Oksche, '85; Romer, '70). Moreover, in lampreys and some bony fishes, amphibia, and lizards (though not in mammals), the pineal body contains photoreceptor cells (Kappers, '65; Oksche and Hartwig, '79). Like the pineal body, so also the LGD, or its homologue, is present in lower vertebrates (Ebbeson, '72), suggesting an ancient ancestry. The same applies to the LP, or pulvinar, which is thought to be represented in submammalian vertebrates, ranging from elasmobranchs to birds, by the nucleus rotundus (Ebbeson, '72). Thus, our present finding of the contiguity of the putative LP and LGD neuroepithelium with the pineal rudiment raises the possibility that these two visual processing nuclei of the thalamus have evolved early in the phylogeny of vertebrates in relation to the dorsal eye rather than the paired lateral eyes.

The site of origin and settling pattern of neurons of the ventral LGN and LP

Neither the embryonic development of the LGV nor of the LP has, to our knowledge, ever received much attention. Papez ('40) maintained that, together with the reticular nucleus, the LGV is a derivative of the reticular component of the ventral thalamus; a view with which Rose ('42) appears to have concurred. Indeed, the connectivities of the LGV and its uncertain boundary with the zona incerta do suggest affinities with ventral diencephalic structures. But our identification of the site of origin of the reticular nucleus at a more anterior diencephalic level (Altman and Bayer, '88c) argues against the common origin of the reticular
nuclei and the LGV. Our observations suggest that the LGV neurons originate from a neuroepithelial complex which is the source of the two other visual components of the thalamus, the LGD and LP. We did stress the tentative nature of our identification of the LGV neuroepithelium with the inverted sublobule situated beneath the everted sublobule identified as a putative source of LGD neurons. Our inference that the LGV neurons derive from a different germinal source than the LGD neurons is supported by the observation that there is a caudal-to-rostral gradient in the LGV (Fig. 8) but no such gradient in the LGD. We noted in sequential radiograms from rats labeled on day E15 (Figs. 13, 14) that the ventrally situated neuroepithelial inversion (the putative LGV neuroepithelium) is surrounded by a migratory zone containing a higher proportion of unlabeled cells than the migratory zone surrounding the dorsally situated neuroepithelial inversion (the putative LGD neuroepithelium). This feature in young embryos is relatable to the quantitative results in P5 rats where peak production of neurons throughout the LGV neurons is on day E14 (Fig. 8), whereas in the internal half of the LGD (LGDi) peak production is on day E15 (Fig. 7).

The rat LP is often linked to the lateral dorsal (or lateral anterior) nucleus, and the two are referred to collectively as the lateral nuclear group (e.g., Faull and Mehler, '85). But we have presented observational and quantitative evidence a decade ago (Figs. 10C, 11B in Altman and Bayer, '79a; Fig. 1 in Altman and Bayer, '88b), the neurogenetic gradient in the lateral half of the early generated LP is from caudal to rostral (Fig. 10). Indeed, we presented some evidence that the neurons of the lateral dorsal nucleus derive from the anterior lobule of the thalamic neuroepithelium (Altman and Bayer, '88b) in contrast to the neurons of the LP which we tentatively traced in the present study to the posterior lobule of the thalamic neuroepithelium.

Concluding remarks

Our current analysis of the time and site of origin and settling pattern of thalamic neurons has so far been limited to early generated structures that derive from the first outflow of differentiating neurons from the thalamic neuroepithelium. Indeed, even this task has yet to be completed since at least one component of this system, the lateral habenular nucleus, needs to be reinvestigated from our new perspective. Nevertheless, a picture is already emerging that allows a tentative correlation between the morphogenetic organization of the thalamic neuroepithelium and the functional organization of the mature thalamus. We have distinguished in day E13 rat embryos two components of the thalamic neuroepithelium, the rostral lobe and the caudal lobe, and we could follow, on the subsequent days, the partitioning of the rostral lobe into the anterior and reticular lobules and of the caudal lobe into the intermediate and posterior lobules (Altman and Bayer, '88a). We have provided some evidence that the anterodorsal, anteroventral, anteromedial, and lateral dorsal nuclei are derivatives of the anterior lobe. These are thalamic structures that are intimately associated with the limbic system (reviewed in Altman and Bayer, '88b). As yet we cannot say much about the structures that appear to derive from the second wave of outflow from the anterior neuroepithelial lobule, such as the central lateral and mediodorsal nuclei, but the available evidence does support the inference that the neurons derived from the anterior neuroepithelial lobule form the limbic thalamus within the traditional Papez circuit. The second derivative of the rostral neuroepithelial lobe, the reticular lobe, together with the reticular protuberance (Altman and Bayer, '88c), constitutes a unique thalamic system the functional significance of which remains to be elucidated. We can say more about the two derivatives of the caudal neuroepithelial lobe, the intermediate lobule and the posterior lobule, as they appear to be sources of neurons of the thalamic relay nuclei. The intermediate lobule, which is distinguished from the others by the presence of a subependymal germinal zone, is the putative source of neurons that relay direct and indirect input from the proximal receptors of the body, the somesthetic and proprioceptive (cerebellar) systems, to the cerebral cortex (Altman and Bayer, '89a). The posterior lobule, in contrast, is the putative source of neurons of several thalamic nuclei that relay information from the distal receptors of the body (olfaction excepted), i.e., the auditory system of the medial geniculate body (Altman and Bayer, '88b), and, as the present study suggests, the three thalamic nuclei implicated in visual functions, the LGD, the LGV, and the LP (pulvinar). The relation of the latter structures to the putative habenular neuroepithelium (with which the putative neuroepithelium of the LGV is contiguous) and to the neuroepithelium of the pretectal system (with which the putative LP neuroepithelium is contiguous) remains to be elucidated. Another important task that remains to be accomplished is the clarification of the fate of neurons that derive from the later outflow of cells from the posterior neuroepithelial lobule and which settle medial to the principal relay nuclei.

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