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Development of the Brain Stem in the Rat. I. Thymidine-Radiographic Study of the Time of Origin of Neurons of the Lower Medulla

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ABSTRACT Groups of pregnant rats were injected with two successive daily doses of ^{3}H-thymidine from gestational days 12 and 13 (E12+13) until the day before birth (E21+22). In adult progeny of the injected rats the proportion of neurons generated on specific days was determined quantitatively in the major nuclei of the lower medulla. The earliest generated cells form two motor nuclei: the hypoglossal and dorsal vagal nuclei. The bulk of hypoglossal neurons are produced on day E12, with a small proportion earlier; the bulk of dorsal vagal neurons are produced, likewise, on day E12, with a small proportion on day E13. The neurons of the third motor nucleus of the region, the ambiguus, are generated later, with a peak on day E15. Neurons of the sensory relay nuclei, the gracilis, cuneatus, and solitarius are produced over a more extended period, with peaks on day E13; the exception was the external cuneate nucleus in which peak generation time was on day E15. In the caudal nucleus of the trigeminal complex, neurons of the subnucleus magnocellularis arise earliest, with a peak on day E14, and those of the subnucleus marginalis last, with a peak on day E15, and extending into day E16. The neurons of the nuclei raphe pallidus and obscurus, and of the dorsal and ventral portions of the caudal medullary reticular formation, are produced between days E12 and E15, without any obvious peaks. The neurons of the nucleus parasolitarius and the nucleus of Roller are produced relatively late, and the area postrema contains a germinal cell population throughout the embryonic period, presumably supplying cells to the choroid plexus of the fourth ventricle. On the basis of absolute datings, duration of neuron production, intranuclear and internuclear gradients, and other criteria, it is postulated that the neurons of the lower medulla are derived from at least eight different cytogenetic zones.[1]

This research project has descriptive as well as theoretical purposes. The first aim is to provide normative quantitative data about the chronology of neuron origin in the motor, sensory, reticular, raphe, and other nuclei of the brain stem, with respect to one another and in relation to specific structures in the rest of the nervous system with which they are affiliated. The second aim is to apply the knowledge gained from these dating studies to a reexamination of the embryological development of the brain stem, as we have done recently for the thalamus (Altman and Bayer, '79b), the hypothalamus (Altman and Bayer, '78c), and the septum (Bayer, '79). Our studies to this date have revealed a great precision in the time of origin of neurons in all brain structures examined, and an orderly temporal relationship within and between structures. Our ultimate theoretical objective is to examine whether, and to what extent, the precise chronological order in the acquisition of neurons in various functional systems is a causal factor in the orderly establishment of connections between them.

In this series of developmental studies the term "brain stem" is used to include all regions of the supraspinal central nervous system in which cranial motor nuclei are present and which, therefore, can be viewed as modified rostral extensions of the segmental neuraxis. For convenience of presentation, the brain stem is subdivided into five parts: the lower medulla, the upper medulla, the pontine

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region, the mesencephalic tegmentum, and the tectum (Fig. 1). This first paper of the series deals with thymidine-radiographic datings of the time of origin of neurons in the lower medulla. The lower medulla, as defined here, extends from the boundary of the cervical spinal cord to the rostral borders of the hypoglossal nucleus dorsally and the inferior olive ventrally. It contains three motor nuclei: the hypoglossal nucleus, the nucleus ambiguus, and the dorsal nucleus of the vagus; several second-order sensory nuclei: the nucleus gracilis, the nucleus cuneatus, the external cuneate nucleus, and the solitary nucleus; the nuclei caudalis and interpolaris of the spinal tract of the trigeminal; and the nuclei raphe pallidus and obscurus, the caudal portion of the medullary reticular formation, the lateral reticular nucleus, and the inferior olivary complex. There are also some additional nuclei present, such as the nucleus of Roller, nucleus parasolitarius, and the area postrema.

Systematic quantitative datings of cytogenesis in the lower medulla have not been published before. Taber Pierce ('73) provided a brief summary of semiquantitative datings of the time of origin of neurons of the brain stem in the mouse. We have recently described cytogenesis in the inferior olivary complex and the lateral reticular nucleus in relation to the development of the precerebellar nuclei (Altman and Bayer, '78b). In the following papers we will present data about the time of origin of neurons in the upper medulla, the pontine region, and the midbrain. A correlation of radiographic datings with the embryonic development of the brain stem will be attempted in future publications.

MATERIALS AND METHODS

Inbred Purdue-Wistar pregnant rats were injected subcutaneously with two successive daily doses of ^{3}H-thymidine (specific activity, 6.0 Ci/mM; dose, 5 μCi/g body weight) between 9:00-11:00 AM on the following gestational ages: E12+13, E13+14... E21+22. (The day of sperm positivity was counted as E1). Tbe progeny of at least two dams per injection group were killed at the constant postnatal age of 60 days by cardiac perfusion with 10% neutral formalin. The brains were embedded in paraffin, sections were cut at 6 μm serially in the three planes and every 15th section was saved. Successive sections were stained with cresyl violet and hematoxylin-eosin for examination without nuclear emulsion or were prepared for autoradiography. The latter procedure has been described elsewhere (Altman, '64). Briefly, deparaffinized sections were coated with Kodak NTB-3 emulsion in the dark, exposed for 90 days with a dessicant, developed with D-19, and stained with hematoxylin-eosin.

Coronal and sagittal sections from 6 male

Abbreviations

AM	nucleus ambiguus
AP	area postrema
CE	cerebellum
CU	cuneate nucleus (medial)
DR	dorsal raphe nucleus
EC	external cuneate nucleus (lateral)
GR	nucleus gracilis
IC	inferior colliculus
IO	inferior olive
IP	interpeduncular nucleus
LC	locus coeruleus
LR	lateral reticular nucleus
LV	ventral nucleus of lateral lemniscus
ml	medial longitudinal fasciculus
MRd	caudal medullary reticular formation, pars dorsalis
MRo	caudal medullary reticular formation, pars oralis
MRr	rostral medullary reticular formation
MRv	caudal medullary reticular formation, pars ventralis
NR	red nucleus
NT	nucleus reticularis tegmenti pontis
pd	pyramidal decussation
PN	pontine nuclei
PR	nucleus prepositus hypoglossi
PS	nucleus parasolitarius
RE	nucleus retrofacilis
RL	nucleus of Roller
RM	nucleus raphe magnus
RO	nucleus raphe obscurus
RP	nucleus raphe pallidus
SC	superior colliculus
SL	solitary nucleus
SN	substantia nigra
SO	superior olive
TD	dorsal tegmental nucleus
v4	fourth ventricle
III	oculomotor nucleus
Vi	spinal trigeminal nucleus, pars interpolaris
Vm	mesencephalic trigeminal nucleus
VM	motor trigeminal nucleus
Vsg	spinal trigeminal nucleus, pars caudalis, subnucleus gelatinosus
Vsm	spinal trigeminal nucleus, pars, caudalis, subnucleus magnocellularis
Vsz	spinal trigeminal nucleus, pars caudalis, subnucleus zonalis (marginalis)
VII	facial nucleus
VIIIl	lateral vestibular nucleus
VIIIm	medial vestibular nucleus
Xd	dorsal motor nucleus of vagus
XII	hypoglossal nucleus

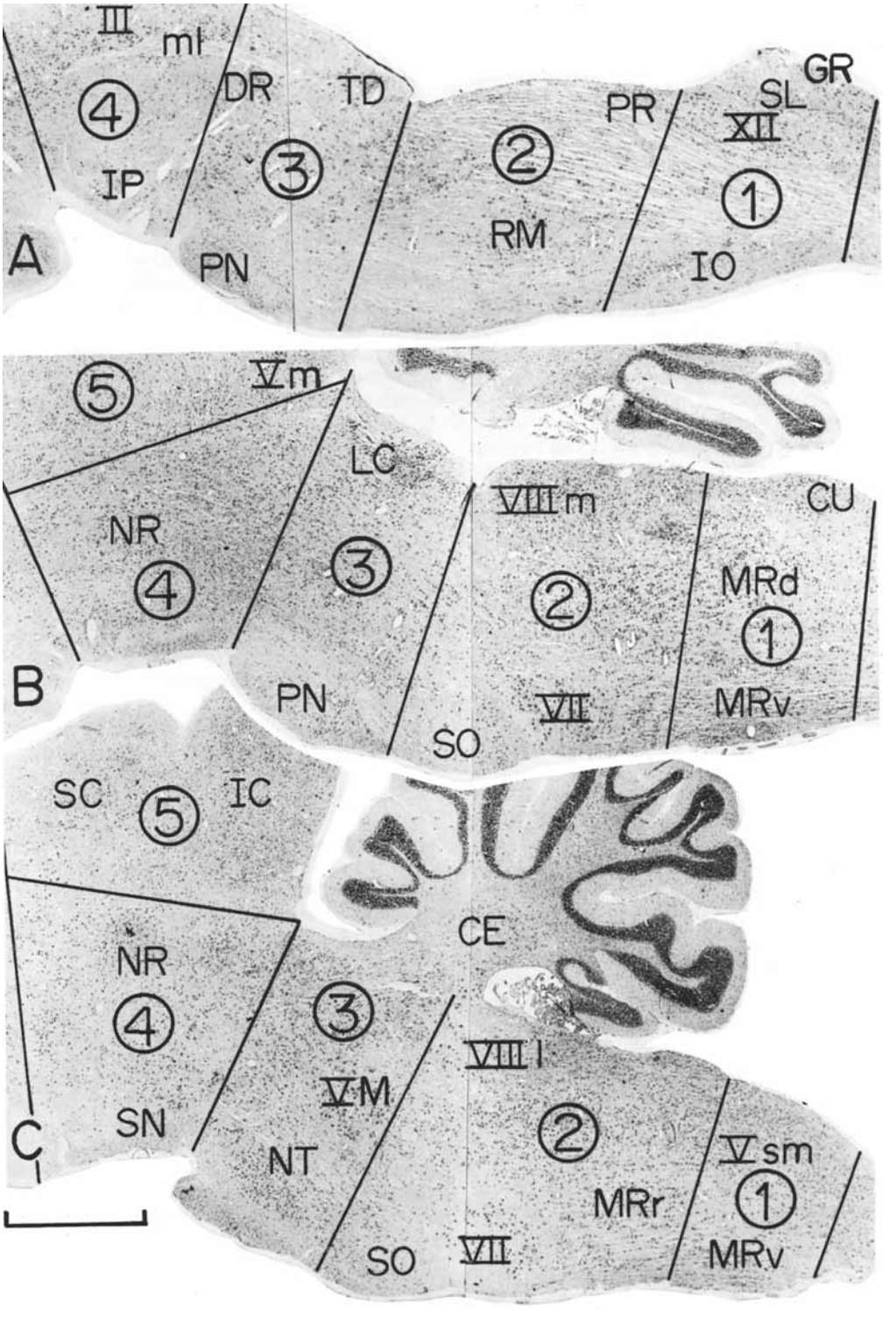

Fig. 1. Low-power thymidine-radiograms from a rat injected on days E12+13. Sagittal sections from medial (A) to lateral (C). 1, lower medulla; 2, upper medulla; 3, pontine region; 4, midbrain tegmentum; 5, midbrain tectum. Scale. 1 mm.

rats per injection group were used for quantitative purposes. Additional material was used for qualitative examinations. The proportion of labelled to unlabelled neurons was determined in 15 nuclei or subnuclei of the lower medulla. We consulted two atlases of the rat brain stem (Valverde, '62; Wünscher et al., '65) and several other atlases for other species (Berman, '68; Sidman et al., '71; Taber, '61) and made some modifications. Each nucleus or subnucleus was scanned at 625 × magnification with the aid of an ocular grid oriented to traverse strips through a given structure, at a right angle to gradients of cell labelling where such was indicated. In all instances a minimum of 100 (up to several hundred) cells were classified in each structure at a given level per animal. The estimation of the proportion of cells differentiating (ceasing to divide) on a particular day was based on the progressively delayed comprehensive labelling procedure. The rationale of this procedure is that as long as virtually all the cells of a selected brain region can be labelled (in the populations studied here nearly 100% labelling can be accomplished with two successive daily injections) all the cells are considered to be precursors that have not started to differentiate. When with delayed onset of injections all cells can no longer be tagged, the proportion of cells that can no longer be labelled as a result of a single-day-delay is taken to be the complement that differentiated on the previous day. As an example, the cells arising on day E15 are determined as follows: E15 = (E15+E16) − (E16+E17). Previous examination of our quantitative results indicated that the sequence of neuron generation in various nuclei was often more clearly indicated in the data from individual animals than in the pooled data. This apparent variability between animals might be due to differences in the exact age of the pooled individuals or their exact developmental stage (differences were often noted within littermates). Accordingly, we employed a statistical procedure, the sign test (Conover, '71), to determine the consistency of sequential neuron production in pairs of caudal medullary nuclei regardless of the chronological grouping of the individual animals. This test is based on paired comparisons (X, Y) within individual animals. The comparisons are grouped into three categories: (1) X > Y, "−" comparison; (2) X < Y, "+" comparison; (3) X = Y, "0" comparison. The zero comparisons are discarded and, depending on the total number of remaining "−" and "+" comparisons, either a binomial distribution or a normal approximation is used to calculate probabilities (p's).

RESULTS

The five subdivisions of the rat brain stem to be analyzed successively in this series of papers are indicated in three parasagittal planes (Fig. 1). Reliable surface landmarks visible in one or more of the autoradiograms shown were used to separate the lower medulla, upper medulla, and the pontine blocks; the separation of the midbrain tectum and tegmentum will follow conventional criteria. At higher magnification, the region of the lower medulla is illustrated in two representative autoradiograms from an animal injected on days E12+13 (Fig. 2A) and E13+14 (Fig. 2B); the motor neurons of the hypoglossal nucleus and the dorsal nucleus of the vagus are labelled in the former but not in the latter animal (details shown in Fig. 5).

Summary quantitative data

The nuclei that were evaluated quantitatively are outlined schematically in two coronal planes in Figure 3. The time of origin of neurons in 15 nuclei were grouped for convenience in Figure 4 into five classes: motor nuclei (Fig. 4A), secondary sensory nuclei (Fig. 4C), the nucleus caudalis (Fig. 4E) and interpolaris (Fig. 4D) of the trigeminal complex, and the raphe and reticular nuclei (Fig. 4B). Details of the results and relevant statistical data are given below together with qualitative observations.

The motor nuclei

The Hypoglossal Nucleus. Several subdivisions have been described in the mammalian hypoglossal nucleus and attempts have been made to relate them to innervation of the different muscles of the tongue (Barnard, '40). In the rat, using the cholinesterase technique, Lewis et al. ('71) distinguished between a dorsal and a ventral longitudinal column and proposed that the neurons of the dorsal subnucleus innervate the retractor muscles of the tongue (hypoglossus and styloglossus), and the neurons of ventral subnucleus innervate the protrusor muscles (gemiohyoid and gemioglossus). Similar results were obtained by Krammer et al. ('79) using the horseradish peroxidase technique. The latter authors distinguished two components in the ventral sub-

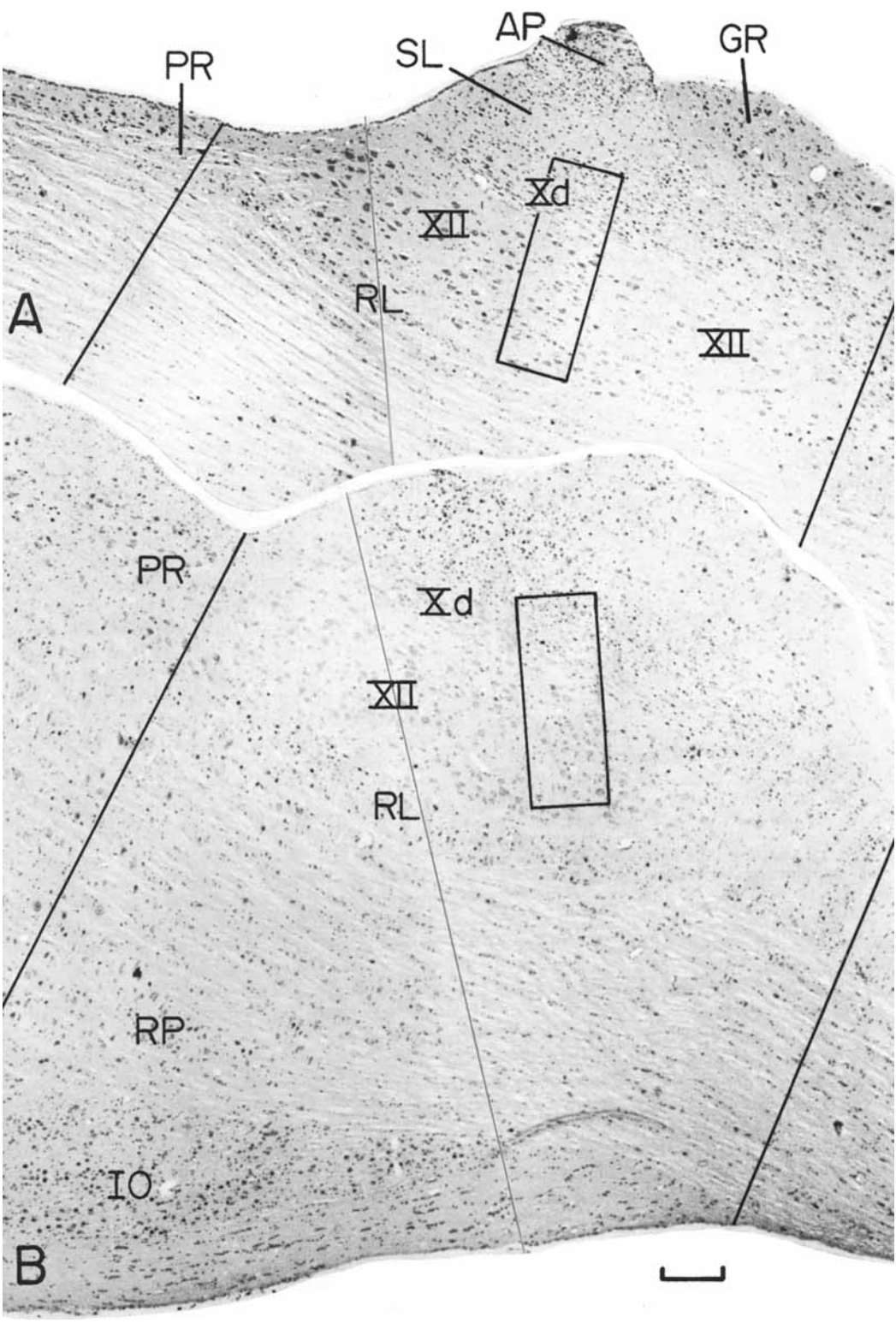

Fig. 2. Thymidine-radiograms of the lower medulla (area between oblique lines). A, injection on days E12+13; B, injection on days E13+14. Scale, 200 μm. Areas in rectangles in A and B are shown at higher magnification in Figures 5A and 5B, respectively.

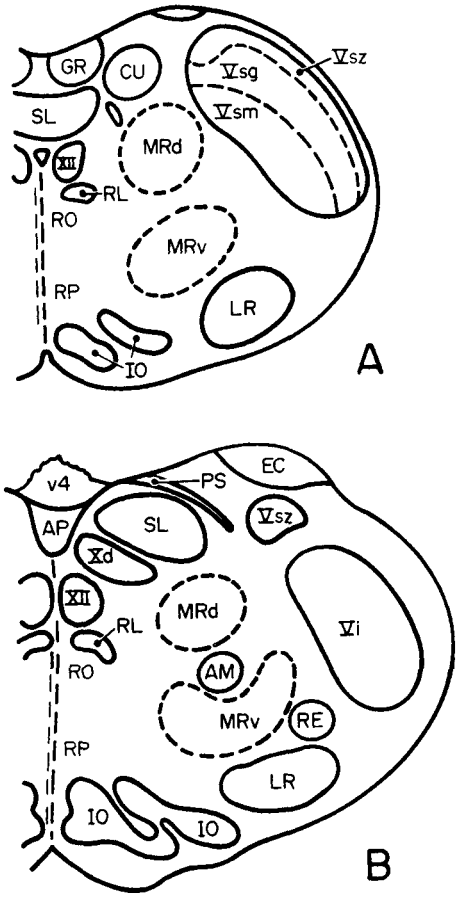

Fig.3. Schematic outline of the nuclei and regions of the lower medulla in coronal sections, from caudal (A) to rostral (B).

nucleus—a ventrolateral and a ventromedial group of cells. The caudally situated ventrolateral neurons were specifically related to the gemiohyoid muscle.

The large motor neurons of the hypoglossal nucleus (Figs. 5, 12) are the earliest elements of the caudal medulla. Nearly 90% of the cells form on day E12 (Fig. 4A). The small proportion of cells that were not labelled with injections on day E12+13, and must have left, therefore, the proliferative compartment on day 11 (or earlier), were generally situated in the posteroventral portion of the nucleus. Conversely, the few cells that had been labelled in some animals injected on days E13+14 tended to be situated anterodorsally. This is the boundary region with the magnocellular component of the prepositus nucleus, a relatively late-forming structure (Altman and Bayer, '80b). These results indicate a poster-oventral-to-anterodorsal gradient in the hypoglossal nucleus.

The Vagal Dorsal Nucleus. The dorsal nucleus of the vagus nerve is believed to be composed of preganglionic neurons of the parasympathetic system. The predominant cell type is of intermediate size and spindle-shaped (Fig. 5). But some smaller neurons are also present, resembling those of the adjacent solitary nucleus (Fig. 5). Lesion (Kerr and Preshaw, '69) and stimulation (Wyrwicka and Garcia, '79) studies have implicated the dorsal nucleus of the vagus in secretomotor functions: i.e., the facilitation of gastric acid output. It is debated whether or not the dorsal nucleus neurons are a source of cardioinhibitory fibers (Calaresu and Pearce, '65; Gunn et al., '68; Weiss and Priola, '72; Thomas and Calaresu, '74) in the cat and the dog. In the rat, two type of motor neurons were identified in the dorsal nucleus by antidromic stimulation of the cervical vagus nerve: cells with B-fiber axons and cells with C-fiber axons (Nosaka et al., '78). Stimulation of the dorsal nucleus and adjacent dorsomedial loci in the rat produced bradycardia (Nosaka et al., '79). The latter study also showed that cells in the dorsal nucleus and its vicinity were labelled when horseradish peroxidase was injected into the cardiac branch of the vagus nerve.

About 90% of the spindle-shaped cells of the dorsal nucleus originated on day E12; the rest on day E13 (Fig. 4A). No directional gradient could be detected. Some smaller neurons resembling those of the solitary nucleus were still labelled in the group injected on days E14+15; thereafter, only cells judged to be glial elements were labelled. Statistical analysis showed that the neurons of the vagal dorsal nucleus form significantly later ($P < 0.0001$) than the neurons of the hypoglossal nucleus.

The Nucleus Ambiguus. The typical motor neurons of the nucleus ambiguus form a small and irregular longitudinal column in the medullary reticular formation, extending from the rostral portion of the lower medulla (Fig. 3B) into the upper medulla. Its axons supply the striated muscles of the pharynx, larynx, and the upper part of the esophagus (Roman and Car, '67; Car and Roman, '70) mostly by way of the vagus nerve (Brodal, '69). The neurons of the ambiguus have been referred to as inspiratory cells (Lipski et al., '79), and several studies implicated them in cardiac inhibition (Gunn et al., '68; Weiss and Priola, '72;

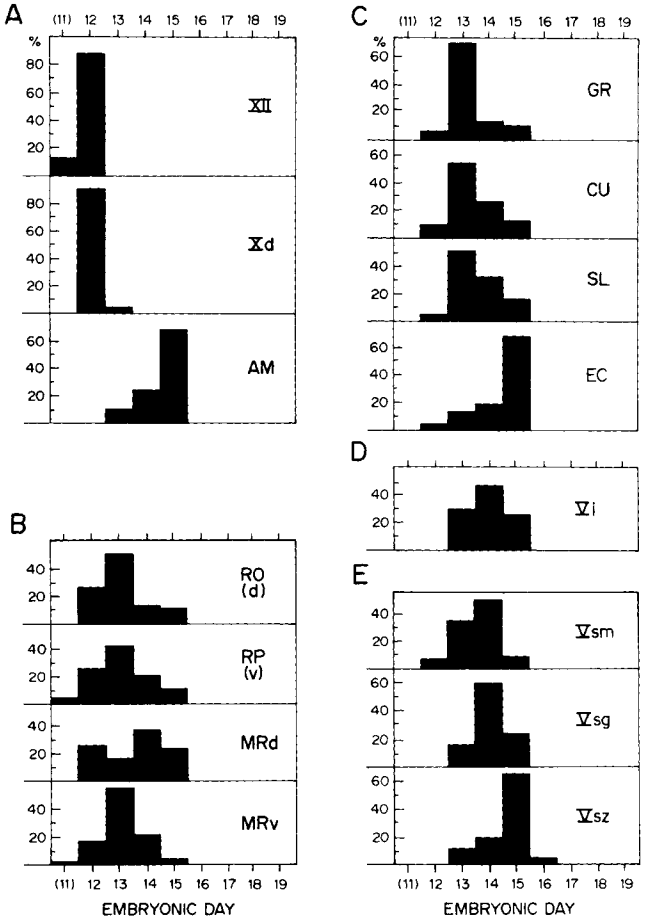

Fig. 4. Summary data of the time of origin of neurons in motor nuclei of the lower medulla (A), raphe and reticular nuclei (B), secondary sensory nuclei (C), the nucleus interpolaris of the spinal trigeminal (D), and the subnuclei of the nucleus caudalis of the spinal trigeminal (E).

Thomas and Calaresu, '74; McAllen and Spyer, '76). In the rat, the cardioinhibitory neurons were described to be located in the vicinity of the nucleus ambiguus (Nosaka et al., '79). The nucleus ambiguus receives afferents directly from the chemoreceptors of the carotid body (Davies and Edwards, '73) and indirectly by way of the solitary nucleus (Morest, '67; Cottle and Calaresu, '75).

The large motor neurons of the nucleus ambiguus (Figs. 6, 7) arise considerably later than the similar neurons of the hypoglossal nucleus ($p < 0.0001$) or the related but smaller neurons of the dorsal vagal nucleus ($p < .0001$). Nearly 70% of the ambiguus neurons leave the proliferative pool on day E15 (Fig. 4A). In the E14+15 injection group the nucleus is delineated as an island of labelled cells embedded in a field composed mostly of unlabelled cells (Fig. 6). Most of the large neurons were still labelled in the E15+16 animals, although many smaller cells within or outside of the nucleus were no longer labelled (Fig. 7). Importantly, an aggregate of similar large neurons, situated somewhat laterally and rostrally, was formed of early-arising cells; we identify the latter column as the retrofacial nucleus (Altman and Bayer, '80a). The early-forming retrofacial nucleus may have been occasionally identified as part of the nucleus ambiguus (see, for example, Nosaka et al., '79; Fig. 1). Or, more specifically, it may have been identified as that portion of the nucleus ambiguus which projects peripherally by way of the glossopharyngeal nerve rather than the vagus (Lawn, '66).

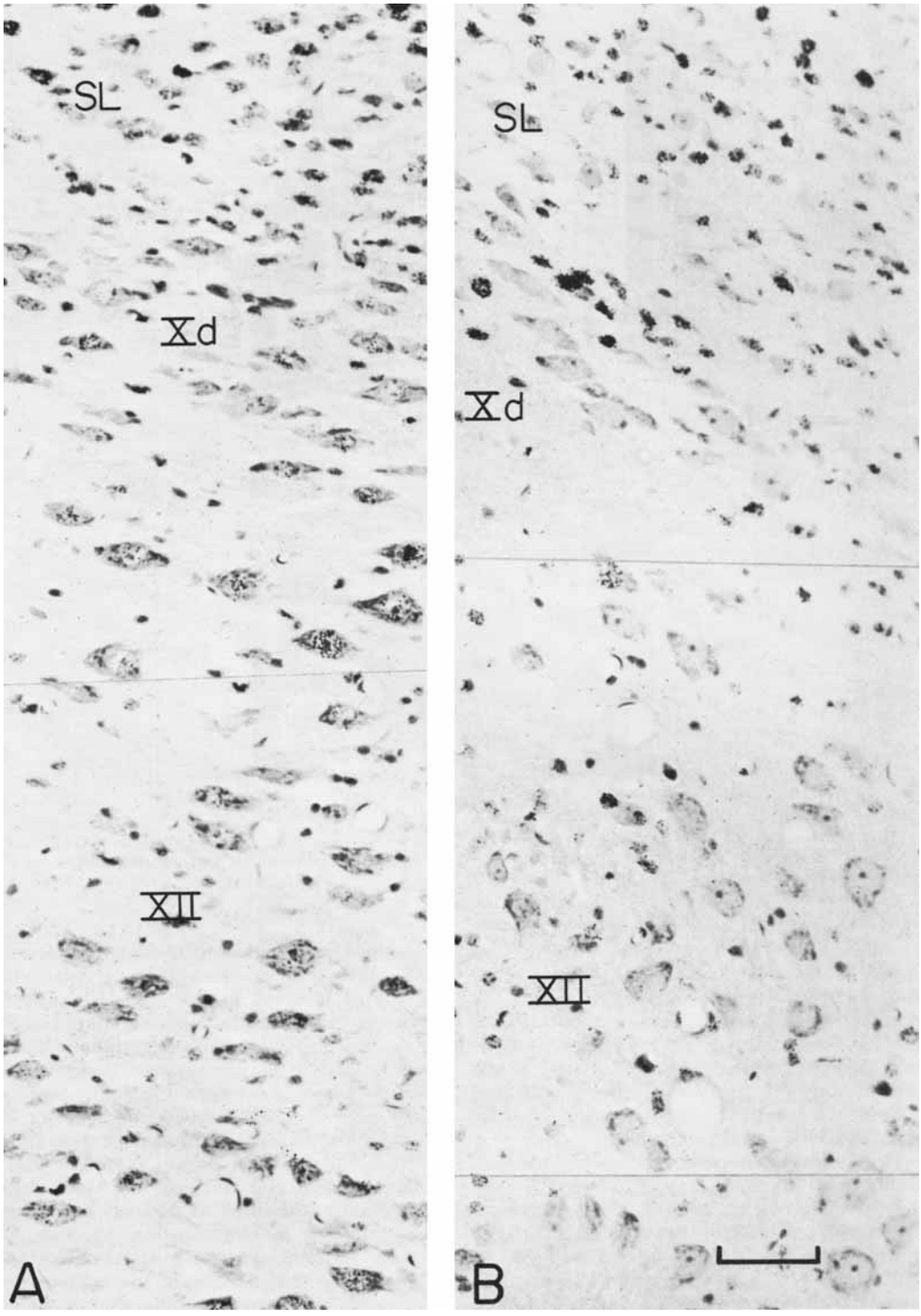

Fig. 5. The hypoglossal nucleus, the dorsal nucleus of the vagus, and the solitary nucleus in rats injected on days E12+13 (A) and in 13+14 (B). Details of the low-power micrographs shown in Figure 2. Scale, 50 μm.

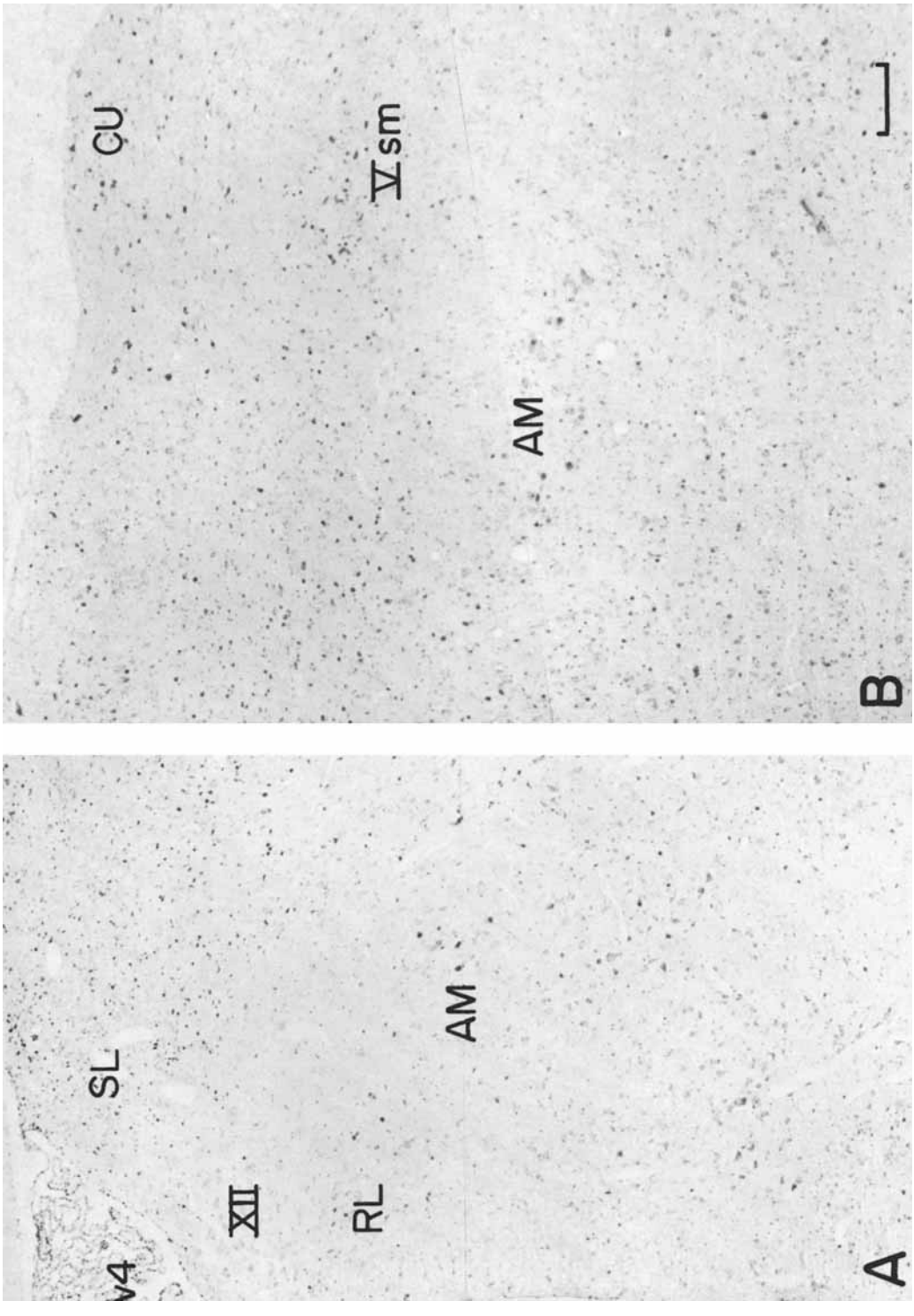

Fig. 6. Dorsal aspect of the lower medulla in rats injected on days E14+15. A, coronal section; B, sagittal section. Scale, 200 μm.

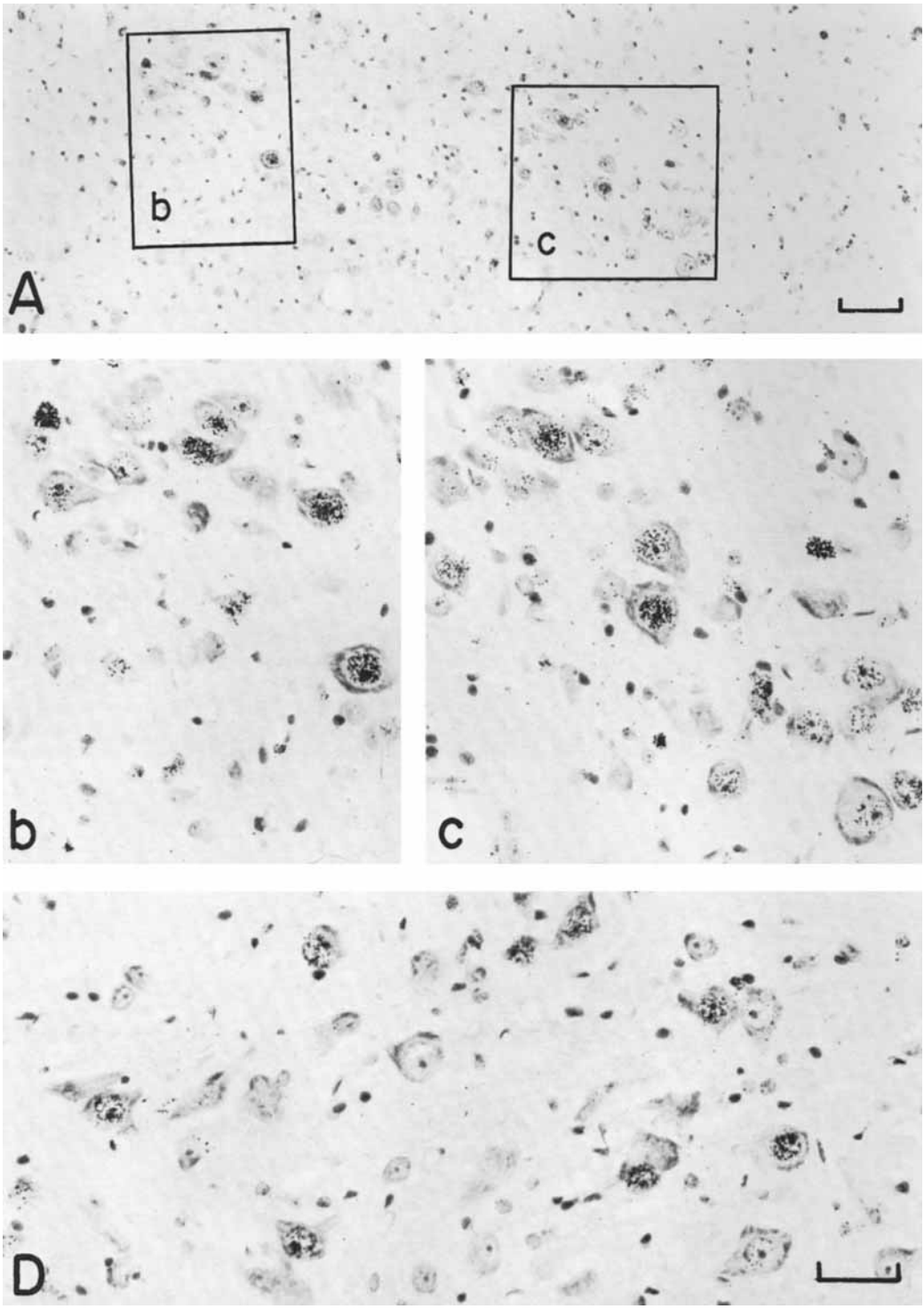

Fig. 7. A. The nucleus ambiguus in a sagittal section from a rat injected on days E14+15. Areas in rectangles (b, c) shown enlarged in b and c. D same region from a rat injected on days E15+16. Note some unlabelled neurons. Scales: A, 200 μm; b, c, and D, 100 μm.

The sensory relay nuclei

The Nucleus Gracilis and Nucleus Cuneatus. Two regions have been distinguished in the gracile nucleus (Hand, '66) and the cuneate nucleus (Kuypers and Tuerk, '64; Keller and Hand, '70): a rostral (or rostroventral) "reticular" region with diffuse dorsal column afferents, and a caudal (or caudodorsal) "cell nest" region with focal afferents. This also applies to the rat (Basbaum and Hand, '73), where in the caudal cuneate nucleus cells are said to be arranged into discrete aggregates of "slabs" or "bricks." These two regions also differ in terms of efferents. After injection of horseradish peroxidase into the cat thalamus (Blomqvist and Westman, '75; Cheek et al., '75) the round neurons in the contralateral "cell nest" region became labelled. This is in agreement with earlier studies using the retrograde degeneration technique (Kuypers and Tuerk, '64) and electrophysiology (Gordon and Jukes, '64). According to Berkley ('75), the medium-sized cells of the "cell nest" region of the cat were back-filled when horseradish peroxidase was injected into the thalamus, whereas smaller cells were preferentially labelled when injections were made into the inferior olive. In addition to a projection from the feline cuneate nucleus to the dorsal accessory olive (Hand and Van Winkle, '77), projections were also described from the rostroventral region to the following structures: inferior and superior colliculi, pars magnocellularis of the medial geniculate body, the posterior nuclear group of the thalamus, and zona incerta (Hand and Van Winkle, '77). The cells projecting to the tectal region may be fusiform-shaped neurons (Blomqvist et al., '78). Finally, physiological studies (Gordon and Horrobin, '67; Cooke et al., '71a) have indicated an ipsilateral projection from the cat dorsal column nuclei to the cerebellum. The cells of origin of this projection was traced to the rostral region, with no labelled cells seen in the "cell nest" region after injection of horseradish peroxidase into the cerebellum (Cheek et al., '75). Thalamic projection from the caudal portions of the dorsal column nuclei was also described in the rat (Lund and Webster, '67), but in this species regional differences in the projection patterns may not be as pronounced as in the cat. In all mammalian species, the segmental arrangement of fibers in the dorsal funiculi is reflected in their mode of topographic termination in the gracile and cuneate nuclei. In the rat (Basbaum and Hand, '73) cranial roots end ventrolaterally, caudal roots dorsomedially.

Three cell types (Fig. 8) were distinguished in the nucleus gracilis and the nucleus cuneatus (medialis) of the rat: 1) a small number of large multipolar cells scattered throughout the nuclei; 2) a higher proportion of small neurons; and 3) a preponderant, "spherical" cell type of intermediate size. Although in some sections a distinction could be made between the two parts of the nucleus gracilis, this proved to be difficult in most instances. Over 90% of the cells were labelled when injections were begun on day E13 (Fig. 4C). The few unlabelled cells arising on day E12 were either the large or the intermediate type (Fig. 8A). With injection delayed until day E14, an additional 70% of the cells could no longer be labelled; these cells, arising on day E13, were large and intermediate neurons (Fig. 8B). These unlabelled cells seemed to be more concentrated caudally than rostrally. The cells forming on days E14 and 15 (Fig. 4C) were predominantly the small neurons. With injections delayed until day E16 or thereafter only glia were labelled. The pattern of cell labelling was similar in the nucleus cuneatus (Fig. 4C) but with a slight, nonsignificant delay in neuron formation.

The External Cuneate Nucleus. The external (or lateral) cuneate nucleus receives dorsal root afferents from the region of the upper part of the trunk, the forelimbs and the neck, in a precise topographical manner (Liu, '56). The afferents have been identified as primary and secondary proprioceptive afferents from muscle (Cooke et al., '71b; Rosén and Sjölund, '73). In the rat (Campbell et al., '74), neck muscles are represented in the rostrolateral pole of the nucleus, arm and shoulder muscles caudomedially, and forearms and hands progressively more caudally. The efferents of the external cuneate nucleus reach the cerebellum (Brodal, '41; Grant, '62), and it has been maintained that the cuneocerebellar tract is the cervical analog of the dorsal spinocerebellar tract (Holmqvist et al., '63; Cooke et al., '71b).

The external cuneate nucleus appears to have a medial and lateral subdivision (Fig. 10). In both parts a conspicuous cell is a spindle-shaped neuron that is larger than the typical cell of the nucleus gracilis or nucleus cuneatus. The neurons of the external cuneate nucleus form appreciably later (Fig. 9) than the neurons of the dorsal column nuclei ($p < 0.0001$). The nearly 70% of the neurons that form on day E15 (Fig. 4C) include the largest

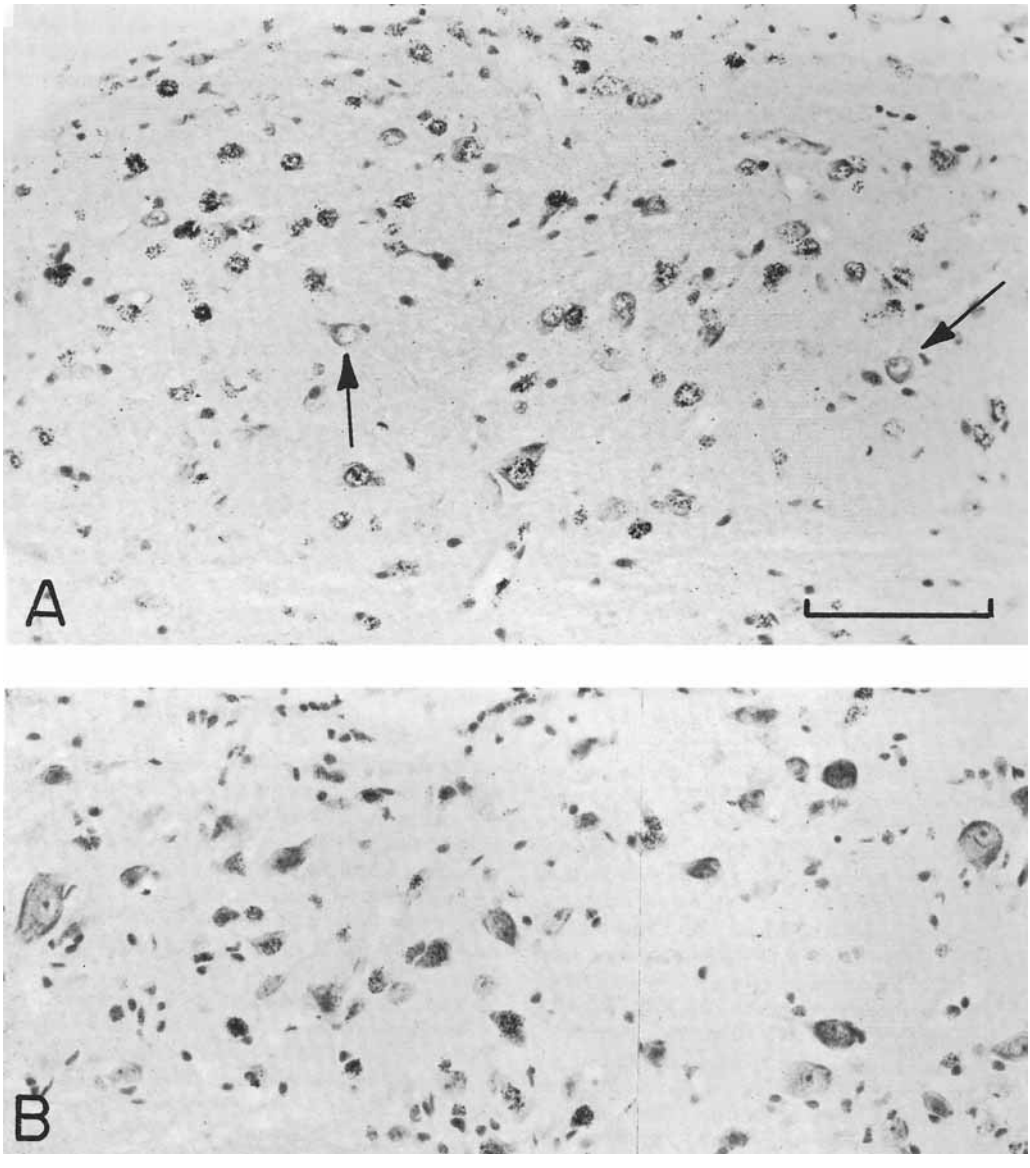

Fig. 8. Nucleus gracilis. A. Injections on days E13+14. Only a few cells are unlabelled. B. Injections on days E14+15. Large cells and many intermediate cells are unlabelled. Scale, 100 μm.

cells. In some animals injected on days E16+17 a few small labelled neurons were still encountered (Fig. 10).

The Nucleus of the Solitary Tract. The solitary nucleus (Figs. 2, 5) is composed of densely packed small cells in the dorsomedial aspect of the medulla extending from the VIIth cranial nerve to the junction of the spinal cord. Its caudal portion receives afferents, by way of the IXth and Xth cranial nerves, from the heart, lungs, and other visceral organs and structures (Torvik, '56; Loewy and Burton, '78). It may be the region implicated in the regulation of cardiovascular and respiratory functions (Euler et al., '73; Thomas and Calaresu, '74; Lipski et al., '79; Nosaka et al., '79). The efferents of the caudal portion of the solitary nucleus project widely to the dorsal vagal nucleus, the nuclei ambiguus, retrofa-

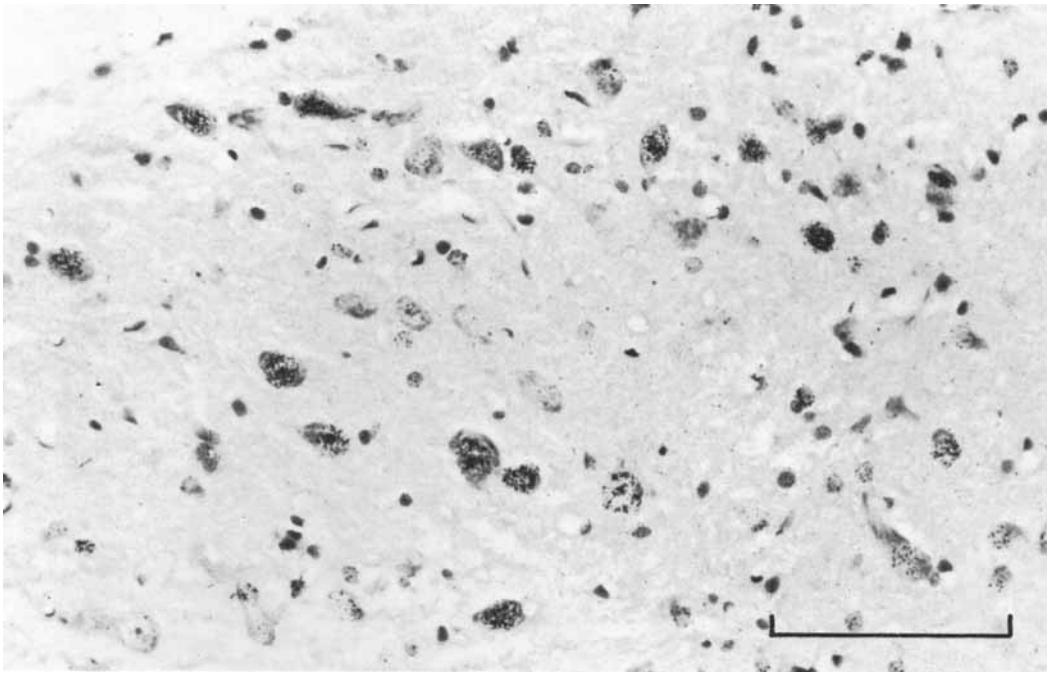

Fig. 9. Cell labelling in the external (lateral) cuneate nucleus from a rat injected on days E14+15. Scale, 100 μm.

cialis, and prepositus hypoglossi, the dorsal tegmental nucleus and parts of the reticular formation; but not to the thalamus (Morest, '67; Cottle and Calaresu, '75). This pattern of projection was confirmed in a study by Loewy and Burton ('78) in which labelled amino acids were injected into parts of the solitary nucleus in which cardiac and respiratory units were identified. In the rat there is a projection from the caudal portion of the solitary nucleus to hypothalamic and limbic structures (Ricardo and Koh, '78).

The rostral portion of the solitary nucleus receives afferents from the tongue and its taste buds by way of the VIIth, IXth, and Xth cranial nerves. In the rat, electrical responses were recorded in the solitary nucleus following stimulation of the nerves innervating the tongue (Blomqvist and Antem, '65) or gustatory stimulation (Norgren and Leonard, '73). Efferents were traced from identified gustatory sites in this region to a small-celled region dorsal and ventral to the brachium conjunctivum, named "the pontine taste area" (Norgren and Leonard, '73).

The solitary nucleus is composed mainly of small neurons (Figs. 11, 19, 20). Most of these cells were labelled in the E13+14 animals (Fig. 12) when the great majority of the underlying neurons of the vagal dorsal nucleus could no longer be labelled. The pattern of cytogenesis in the solitary nucleus resembled that of the dorsal column nuclei (Fig. 4C) but was temporally delayed. This difference was significant with respect to the nucleus gracilis ($p < 0.0001$) and the nucleus cuneatus ($p < 0.0026$). However, the solitary nucleus neurons arose significantly ahead ($p < 0.0023$) of the neurons of the external cuneate nucleus.

The pronounced ventral-to-dorsal gradient in the upper part of the lower medulla is illustrated in Figure 12. The neurons of the dorsally situated secondary sensory nuclei (nucleus gracilis and nucleus solitarius) arise largely after the completion of the generation of motor neurons (hypoglossal and dorsal vagal nuclei).

The Nucleus Caudalis of the Trigeminal Complex. The spinal trigeminal nucleus, which receives the descending branches of the spinal tract of the trigeminal, is divided into three parts: the nucleus oralis, nucleus interpolaris, and nucleus caudalis. The nucleus caudalis extends from the obex to the first cervical root (Darian-Smith, '73) and it is subdivided into three laminar zones: the subnucleus marginalis (or zonalis), the subnu-

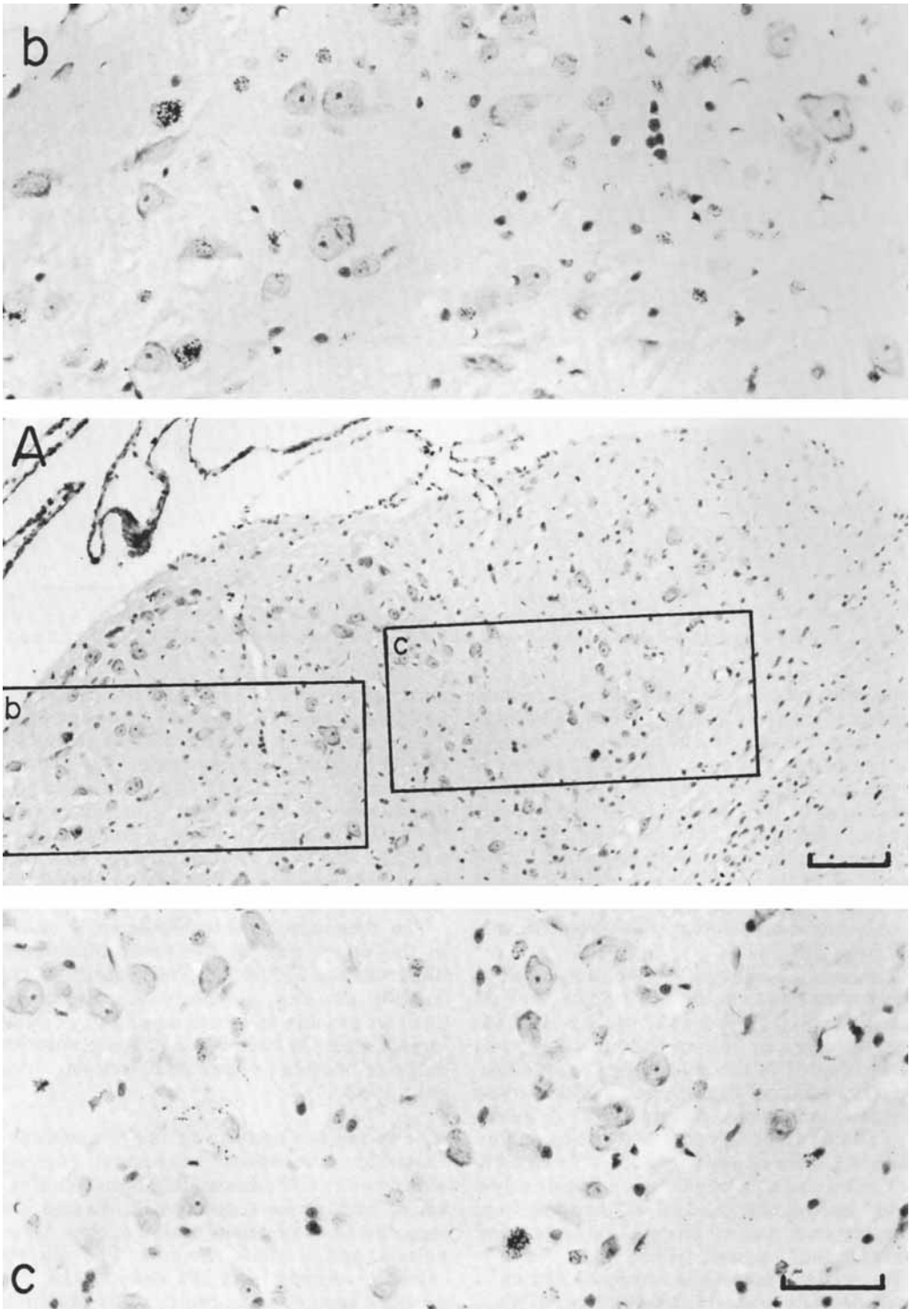

Fig. 10.A (center). Coronal section through external cuneate nucleus from a rat injected on days E16+17. Areas in rectangles (b, c), enlarged in b and c. An occasional small neuron still labelled in this animal. Scales: A, 100 μm; B, 50 μm.

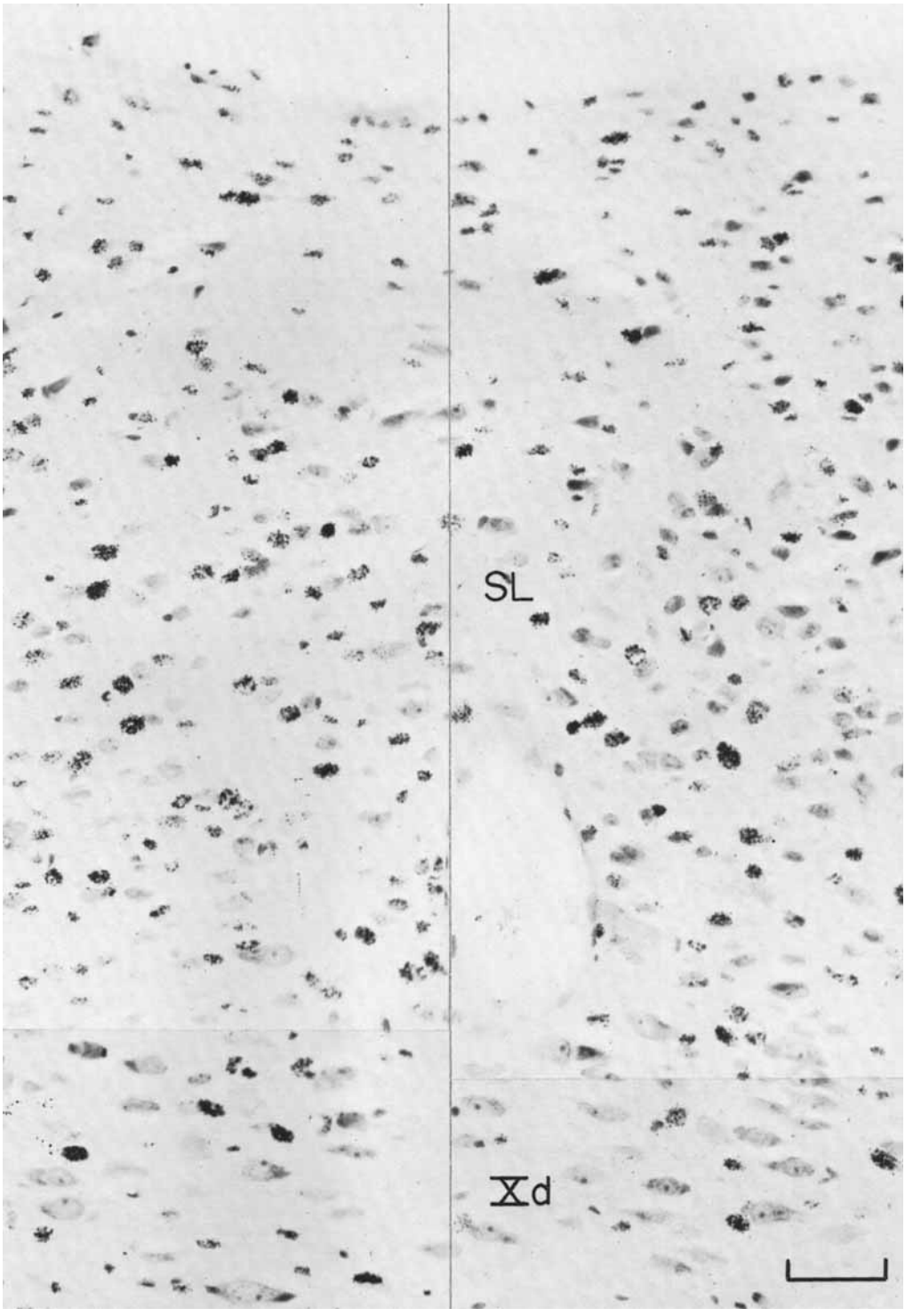

Fig. 11. Labelled and unlabelled neurons of the solitary nucleus in a rat injected on days E14+15. Note some of the labelled small neurons in the dorsal aspect of the dorsal vagal nucleus. Scale, 50 μm.

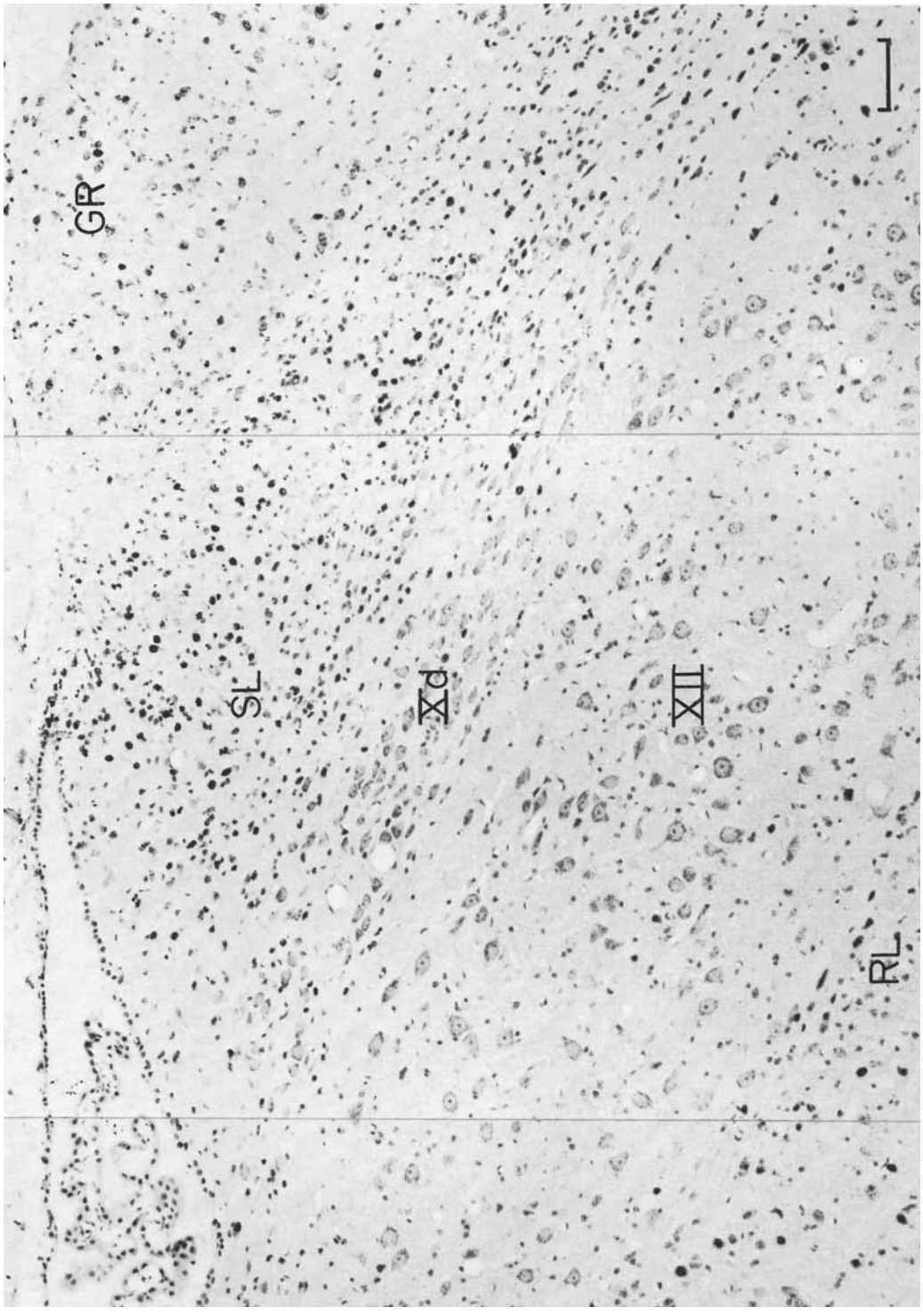

Fig. 12. Overview of the dorsal aspect of the lower medulla in a sagittal section from a rat injected on days E13+14. Anterior to the left. Note the pronounced ventral-to-dorsal gradient in the upper medulla above the nucleus of Roller. Scale, 200 μm.

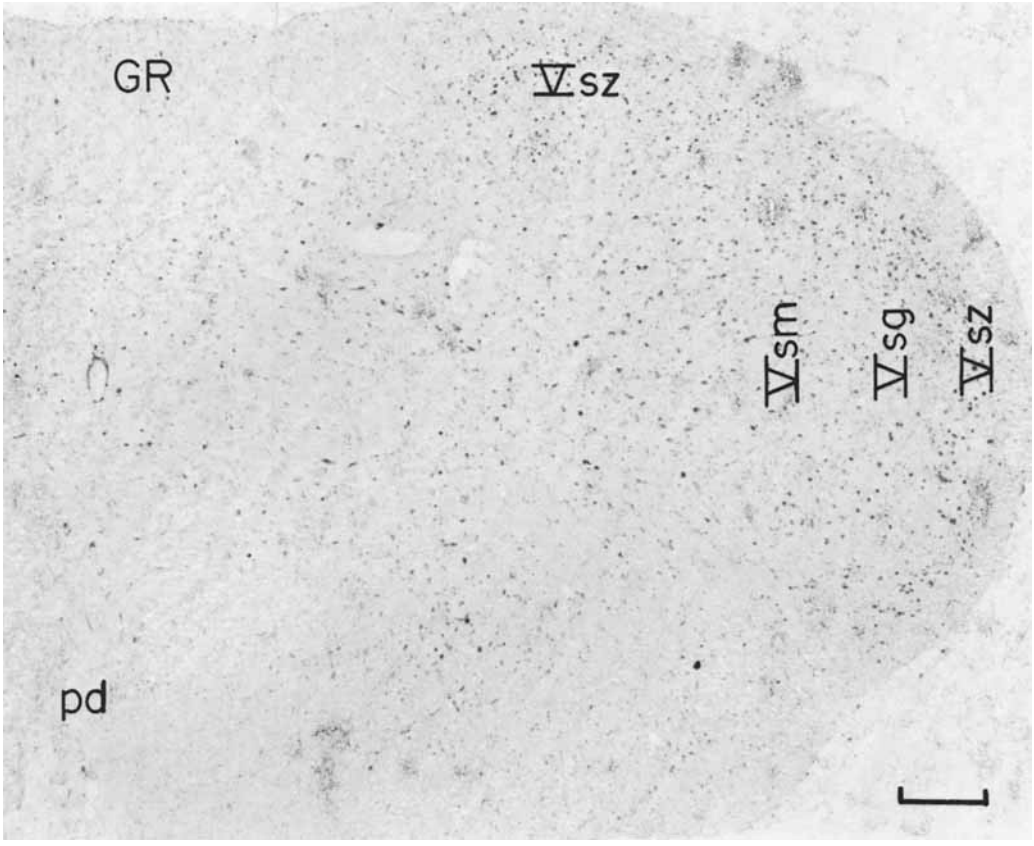

Fig. 13. The nucleus caudalis of the trigeminal complex in a coronal section of the caudal aspect of the lower medulla. From a rat injected on days E14+15. Scale, 200 μm.

cleus gelatinosus, and the subnucleus magnocellularis (Olszewski, '50). The nucleus caudalis (Fig. 13) is generally considered a rostral extension of the dorsal horn of the spinal cord, and its three subdivisions are believed to correspond to Rexed's lamina I, laminae II and III, and lamina IV, respectively (Darian-Smith, '73; Gobel et al., '77).

Physiological studies in several species indicate that neurons in the marginal layer respond to mechanical, thermal, and noxious cutaneous stimuli (Kruger et al., '61; Mosso and Kruger, '73; Yokota, '75; Price et al., '76). In the rat, marginal neurons respond to tooth pulp stimulation (Shigenaga et al., '76) and to thermal input (Dickenson et al., '79). There is a somatotopic arrangement of thermal neurons across the marginal layer of the rat: those with receptive fields in the ophthalmic division being situated most laterally and mandibular division units most medially (Dickenson et al., '79). There is evidence that, in the rat, trigeminal afferents also reach the subnucleus gelatinosus where the mandibular division is distributed most medially, the maxillary division laterally, and the ophthalmic division between the two but mostly in the caudal aspect of the nucleus and as far as C_2 (Rustioni et al., '71). Based on an extensive exploration of the response properties of medullary neurons to cutaneous stimulation in the rat, Nord ('67) suggested that the spinal trigeminal nucleus constitutes a functional unit with the dorsal column nuclei: the former responding to cutaneous input from the face and components of the buccal cavity, the latter to input from the trunk, limbs, posterior face, and pinna.

The nucleus caudalis is a relay station of cutaneous afferents to the thalamus and portions of the brain stem. Recent tracer studies have confirmed that gelatinosus neurons, but particularly marginal neurons, are sources of thalamic afferents (Trevino and Carstens, '75;

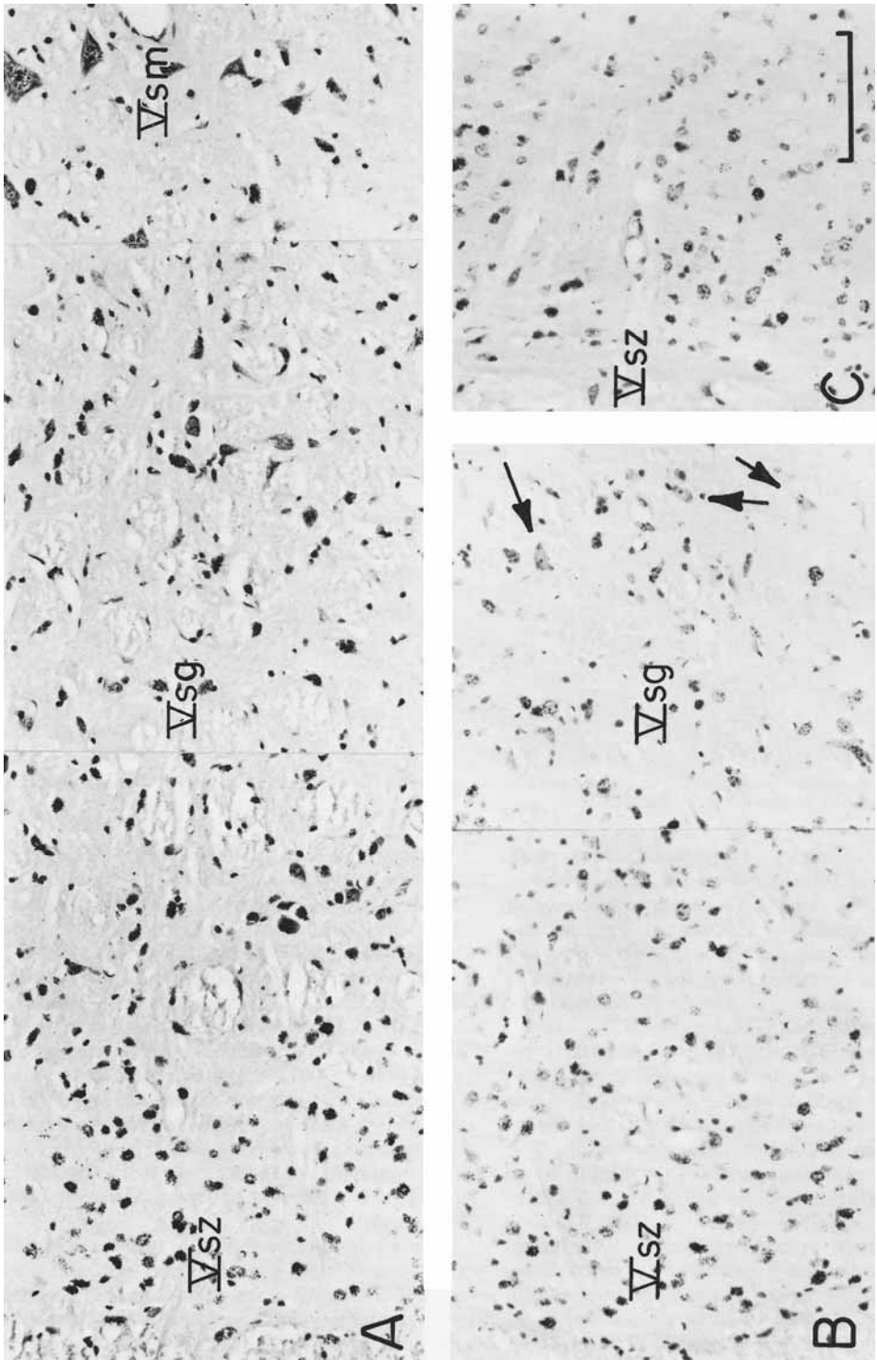

Fig. 14. Nucleus caudalis of the trigeminal complex. A. Virtually all cells are labeled in the rat injected on days E13+14. B. Many larger neurons are no longer labelled (arrows) in the medial aspect of the subnucleus gelatinosus in the rat injected on days E14+15. C. Most of the small cells are still labelled in the subnucleus marginalis (zonalis) of the rat injected on days E15+16. Scale, 100 μm.

Hockfield and Gobel, '78; Burton et al., '79). In the cat, magnocellular neurons are not labelled after horseradish peroxidase injection into the thalamus (Hockfield and Gobel, '78), but a few of them are labelled in the rat (Fukushima and Kerr, '79).

There is a medial-to-lateral ("inside-out") cytogenetic gradient in the nucleus caudalis (Fig. 13), corresponding to its laminar organization (Fig. 14). About 85% of the cells of the subnucleus magnocellularis are generated on days E13 and E14 (Fig. 4E). Only a small proportion of the cells form on day E15; these include even some large neurons. In the subnucleus gelatinosus, about 85% of the cells form on days E14 and E15. Finally, in the subnucleus marginalis (zonalis) neuron generation peaks on day E15 and extends into day E16 (Figs. 4E, 15C). The late-forming neurons of the subnucleus zonalis are all of the small type. The labelling pattern of the small cells suggests that the subnucleus marginalis changes dorsally and rostrally from its marginal position, forming a club-shaped structure or hook beneath the external cuneate nucleus (Figs 3, 15A).

Statistical analysis showed that the subnucleus magnocellularis neurons formed significantly earlier than the gelatinosus and zonalis neurons ($p < 0.0001$), and that the generation of gelatinosus neurons significantly antedated the zonalis neurons ($p < 0.023$). As sensory relay nuclei, the subnuclei zonalis were also compared with the dorsal column nuclei, the nucleus solitarius, and the external cuneate nucleus. Both the subnucleus gelatinosus and zonalis neurons form significantly later than the neurons of the nuclei gracilis, cuneatus and solitarius ($p < 0.0001$). There was no statistically significant difference between the subnucleus gelatinosus and the external cuneate nucleus. However, the subnucleus zonalis neurons were generated significantly later than the external cuneate neurons ($p < 0.0043$).

The Nucleus Interpolaris of the Trigeminal Complex. The nucleus interpolaris extends from the region of the obex to the rostral part of the inferior olive (Torvik, '56; Darian-Smith, '73). It is distinguished from the nucleus caudalis (and the dorsal horn) by an absence of a clear lamination (Olszewski, '50), although some authors (Åström, '53; Torvik, '56) distinguish a dorsomedial component from the rest of the nucleus. In the rat, a relatively high percentage of interpolaris cells receive afferents from the vibrissae (Nord, '68; Shipley, '74; Belford and Killackey, '79), but vibrissal representation is duplicated in the nucleus cardalis (Belford and Killackey, '79). The large, medium, and small cells of the nucleus provide crossed fibers to the thalamus (Fukushima and Kerr, '79). Unlike the nucleus caudalis (Ikeda, '79), the nucleus interpolaris is also a source of fibers to the ipsilateral cerebellum (Karamanlidis, '68; Ikeda, '79).

Unlike in the nucleus caudalis, there is no indication of a cytogenetic gradient in the nucleus interpolaris. The nucleus contains large, intermediate, and small neurons (Fig. 16). There were no differences in the generation time of cells of different sizes, and many of the large neurons were still labelled when smaller cells were no longer tagged (Fig. 16). The neurons of the nucleus interpolaris form between days E13 and E15 (Fig. 4D). A few labelled cells, including some of the large ones, were still labelled in some animals injected on days E16+17.

Statistical anaysis indicated that the neurons of the nucleus interpolaris are generated significantly later than the neurons of the subnucleus magnocellularis of the caudal nucleus ($p < 0.0001$); they do not differ significantly from the neurons of the subnucleus gelatinosus ($p < 0.2379$), and they antedate significantly the neurons of the subnucleus zonalis ($p < 0.0001$). Neurons of the dorsal column nuclei and the solitary nucleus are generated significantly earlier than the interpolaris neurons ($p < 0.0001$), but the difference between the latter and the external cuneate nucleus was not significant.

The raphe and reticular nuclei of the lower medulla

The Caudal Raphe Nuclei. The two midline raphe nuclei in the caudal aspect of the medulla are the dorsally situated raphe obscurus and the ventral raphe pallidus. The raphe obscurus is rich in serotonin-containing neurons, designated as group B2 (Dahlström and Fuxe, '64; Ungerstedt, '71); neurons with the same properties in and around the raphe pallidus constitute B1. Both of these nuclei are composed of neurons that range from very large to small with a preponderance of intermediate-sized cells (Fox et al., '76). The dendrites of the larger neurons extend into the adjacent reticular formation and the cells are said to have the same dendritic features as reticular neurons (Valverde, '61; Leontovich and Zhukova, '63; Fox et al., '76). The axons descend into the spinal cord (Dahlström and

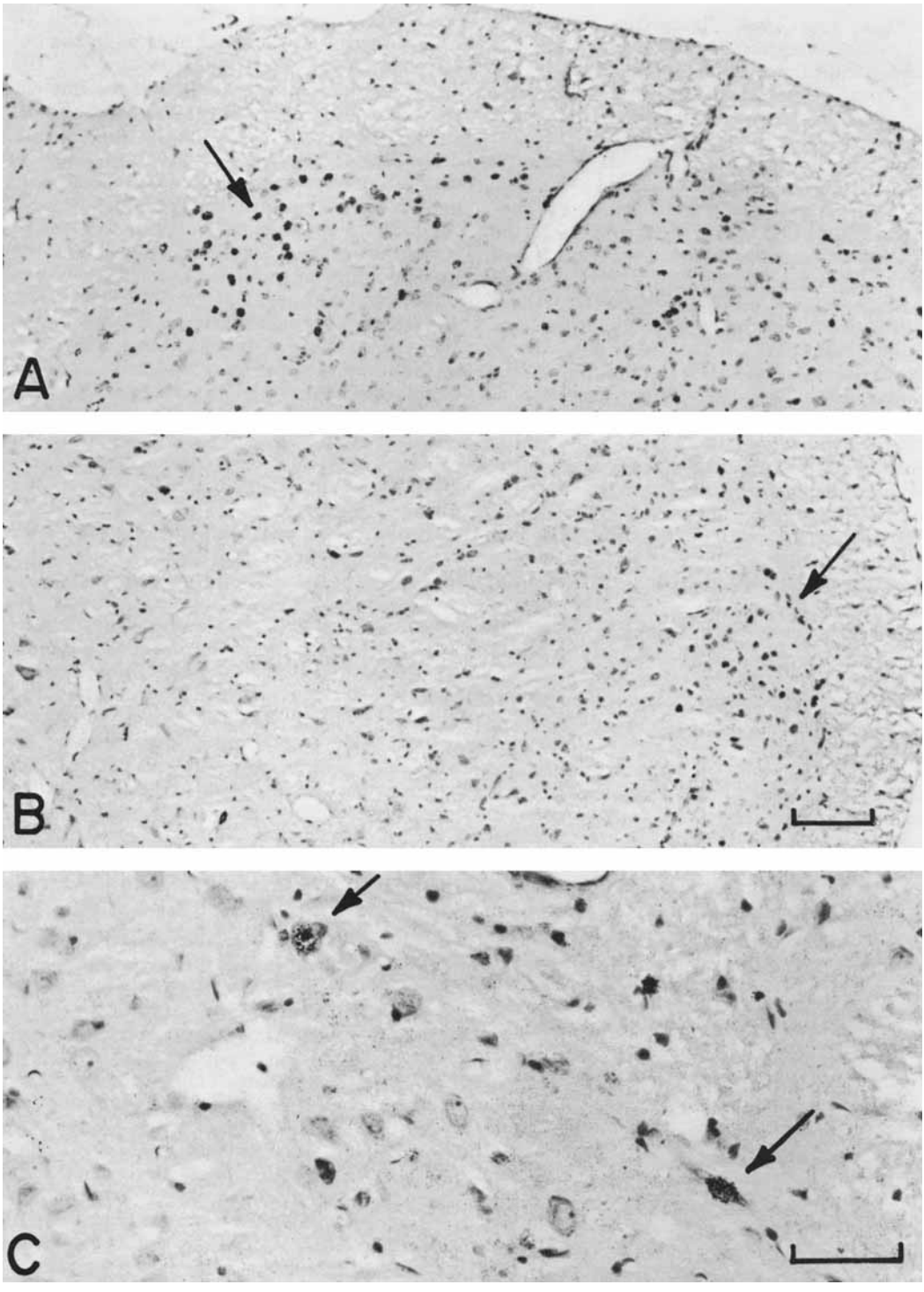

Fig. 15. Nucleus caudalis of the trigeminal complex in a rat injected on days E15 + 16 (A and B). The labelled neurons of the subnucleus zonalis (arrow) are situated dorsally in the more rostrally situated coronal section in A, but are situated laterally (arrow) in the more caudal section shown in B. In the animal injected on days E16+17 (C) a few neurons (arrows) are still labelled in the marginal aspect of the subnucleus zonalis. Scale, A and B, 100 μm; C, 50 μm.

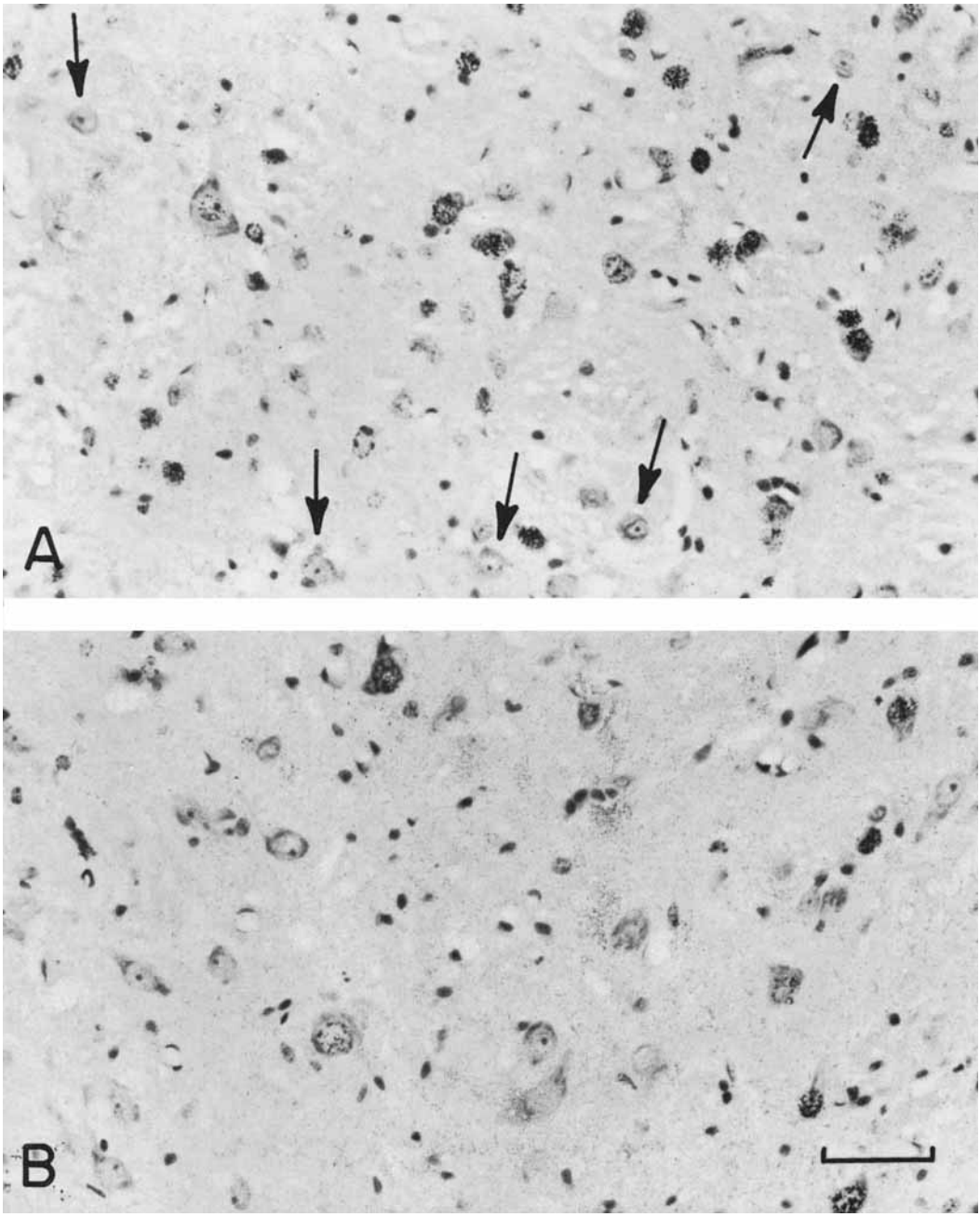

Fig. 16. Neurons of the nucleus interpolaris of the spinal trigeminal complex. A. From a rat injected on days E14+15. Note several unlabelled intermediate size cells (arrows). B. From a rat injected on days E15+16. The labelled cells include intermediate and large ones. Scale, 50 μm.

Fuxe, '64; Ungerstedt, '71) by way of the ventrolateral and ventral funiculus (Martin et al., '78).

The boundaries of the nuclei raphe obscurus and raphe pallidus are uncertain (Fig. 3) and their cell composition is quite heterogeneous. To achieve some consistency, cell counts were made for the nucleus raphe obscurus in a zone beneath the hypoglossal nucleus but not extending beyond the dorsal half of the lower

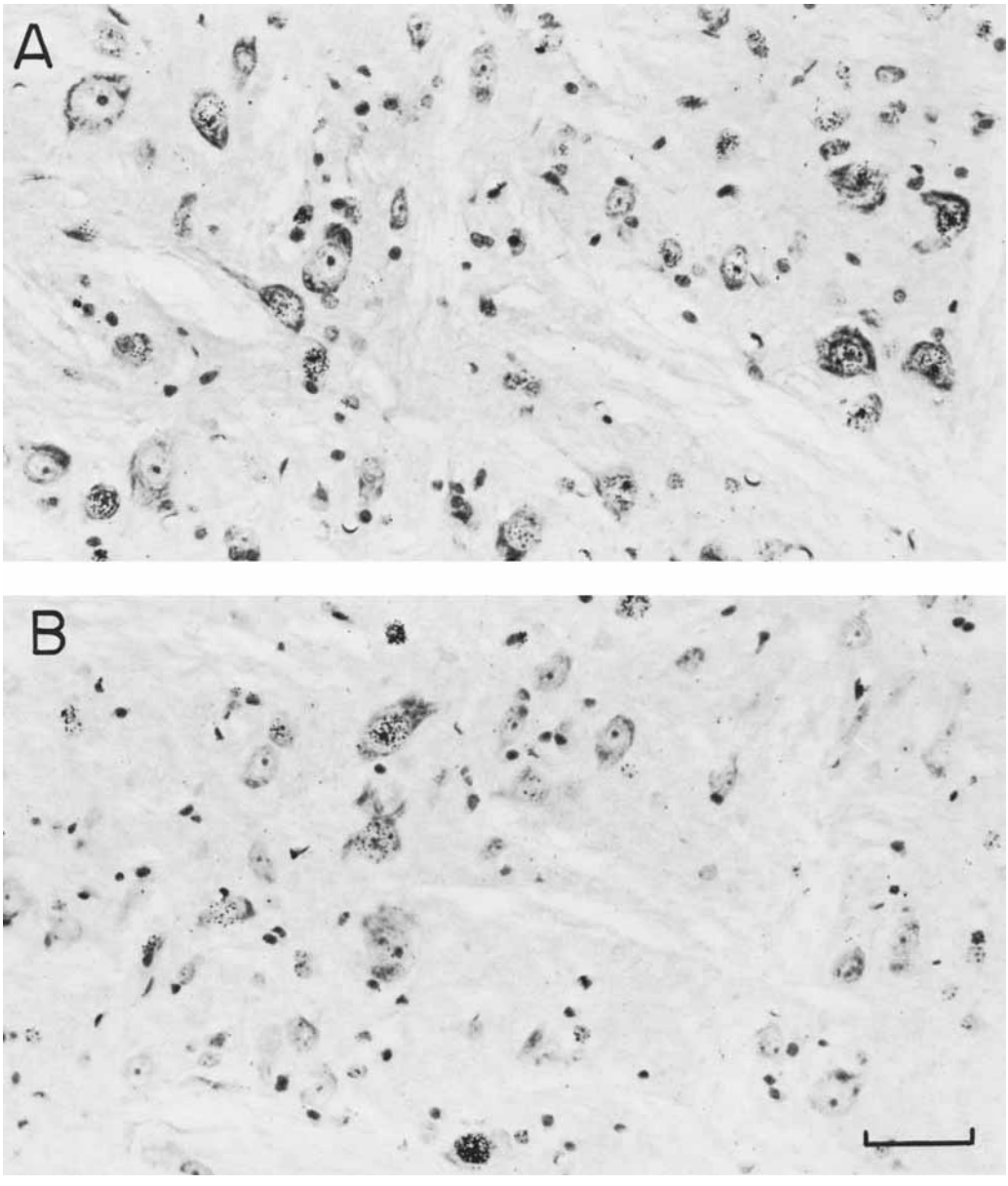

Fig. 17. Nucleus raphe pallidus. A. From a rat injected on day E13+14. There are labelled and unlabelled large and small neurons. B. From a rat injected on days E14+15. The remaining labelled cells range from small to large. Scale, 50 μm.

medulla (some of the cells that were included may have belonged to the nucleus of Roller). Cell counts for the nucleus raphe pallidus were made in a zone above the medial aspect of the inferior olive but not farther dorsally than the halfway mark of the medulla. The data indicate that neurons of both of these regions form over an extended period with peak formation time on day E13 (Fig. 4B). There was no clear difference between the generation time of neurons of different sizes (Fig. 17). Statistical analysis indicated no differences in cytogenesis between the raphe pallidus and the raphe obscurus ($p < 0.3075$).

The Caudal Medullary Reticular Forma-

tion. This portion of the reticular formation is situated between the nucleus caudalis of the trigeminal complex and the caudal raphe nuclei. The region is also known as the nucleus centralis of the medulla oblongata, pars dorsalis, and pars ventralis (Sidman et al., '71). In the ventrolateral aspect of this field is a rather discrete grouping of cells. This is the lateral reticular nucleus that we have dealt with elsewhere (Altman and Bayer, '78b). The caudal medullary reticular neurons receive afferents from the descending trigeminal tract (Torvik, '56; Clarke and Bowsher, '62) and fibers from the adjacent trigeminal nucleus caudalis (Valverde, '61). Physiological evidence, likewise, links this region to the trigeminal system, as its neurons are triggered by stimulation of the face region (Kruger and Michel, '62; Nord, '67; Nord and Kyler, '68). The reticular neurons here tend to have large receptive fields and may be involved in the mediation of facial pain (Burton, '68; Nord and Ross, '73; Yokota, '76; Shigenega et al., '76; Price et al., '76).

For purposes of cell counting the caudal medullary reticular formation was subdivided into a dorsal and a ventral component (Fig. 3). The dorsal region is situated laterally and slightly dorsally to the hypoglossal nucleus and the nucleus of Roller. This is a heterogeneous cytological region with a preponderance of small cells. The ventral region was considered to be situated dorsally and dorsolaterally to the inferior olive (Fig. 18). It proved difficult to match the regions (particularly the pars dorsalis) within and between injection groups. The data indicate (Fig. 4B) that neurons in these two regions arise between days E12 and E15; the difference between them was not significant ($p < 0.1690$). Qualitative assessment suggested that there may be three subdivisions: a relatively early-forming ventral magnocellular portion; a mixed intermediate region with a matching heterogeneity in cytogenesis; and a relatively late forming dorsal parvicellular component. However, the pattern was not consistent and in many regions large cells were still labelled where smaller cells were not and vice versa (Fig. 19).

Some additional nuclei

There are several nuclei described in the literature in the lower medulla in addition to those dealt with so far. Two of these we were not able to identify in the rat: the nucleus intercalatus and the nucleus commissuralis. The nucleus intercalatus of Staderini has been described in the cat (e.g., Brodal, '52) as a collection of small cells interposed between the hypoglossal nucleus and the dorsal nucleus of the vagus. It is illustrated in several atlases and has received experimental attention (e.g., Morest, '67; Calaresu and Henry, '70; Cottle and Calaresu, '75). We were unable to identify it in the rat between the hypoglossal and vagal nuclei. Instead, we noted in autoradiograms a conspicuous small-celled structure between the solitary nucleus and the external cuneate nucleus and identified it with the nucleus parasolitarius (Walberg et al., '62). The nucleus commissuralis is occasionally referred to, and it is illustrated (graphs 23 and 24) in the rat atlas of Wünscher et al. ('65) as being interposed medially between the solitary nucleus and hypoglossal nucleus. The nucleus is said to contain monoamine nuerons (groups A2 of Dahlström and Fuxe, '64) and has been implicated in cardiac functions (Nosaka et al., '78, '79). In our material, this nucleus could not be clearly differentiated from the solitary nucleus. In addition to the nucleus parasolitarius, we dealt with two other structures—the area postrema and the nucleus of Roller.

The Nucleus Parasolitarius. The nucleus parasolitarius is described by Walberg et al. ('62) in the cat as a slender column of densely packed small cells situated lateral to the solitary nucleus and medial to the external cuneate nucleus (their Fig. 2, section 61). They note that the nucleus is barely discernible in Nissl-stained material. Probably the same structure was described by the same name earlier by Allen ('23) in the guinea pig, and it may be identical with the nucleus parvocellularis compactus of man (Olszewszki and Baxter, '54) and cat (Taber, '61), and the lateral nucleus of the solitary tract of Berman ('68, p. 9).

In animals injected on days E14+15 (Figs. 20, 21) or days E15+16 (Fig. 22), the semilunar nucleus parasolitarius is made conspicuous by its tightly packed, labelled small cells. From a lateral position the nucleus could be traced caudally to the vicinity of the area postrema (Fig. 21) and more rostrally to the posterior dorsal aspect of the fourth ventricle (Fig. 20). In the E15+16 animals (Fig. 21) the distally located cells of the nucleus parasolitarius were no longer labelled, suggesting a lateral-to-medial gradient. Cytogenesis in the nucleus parasolitarius appeared to outlast neuron production in the solitary nucleus (Fig. 22).

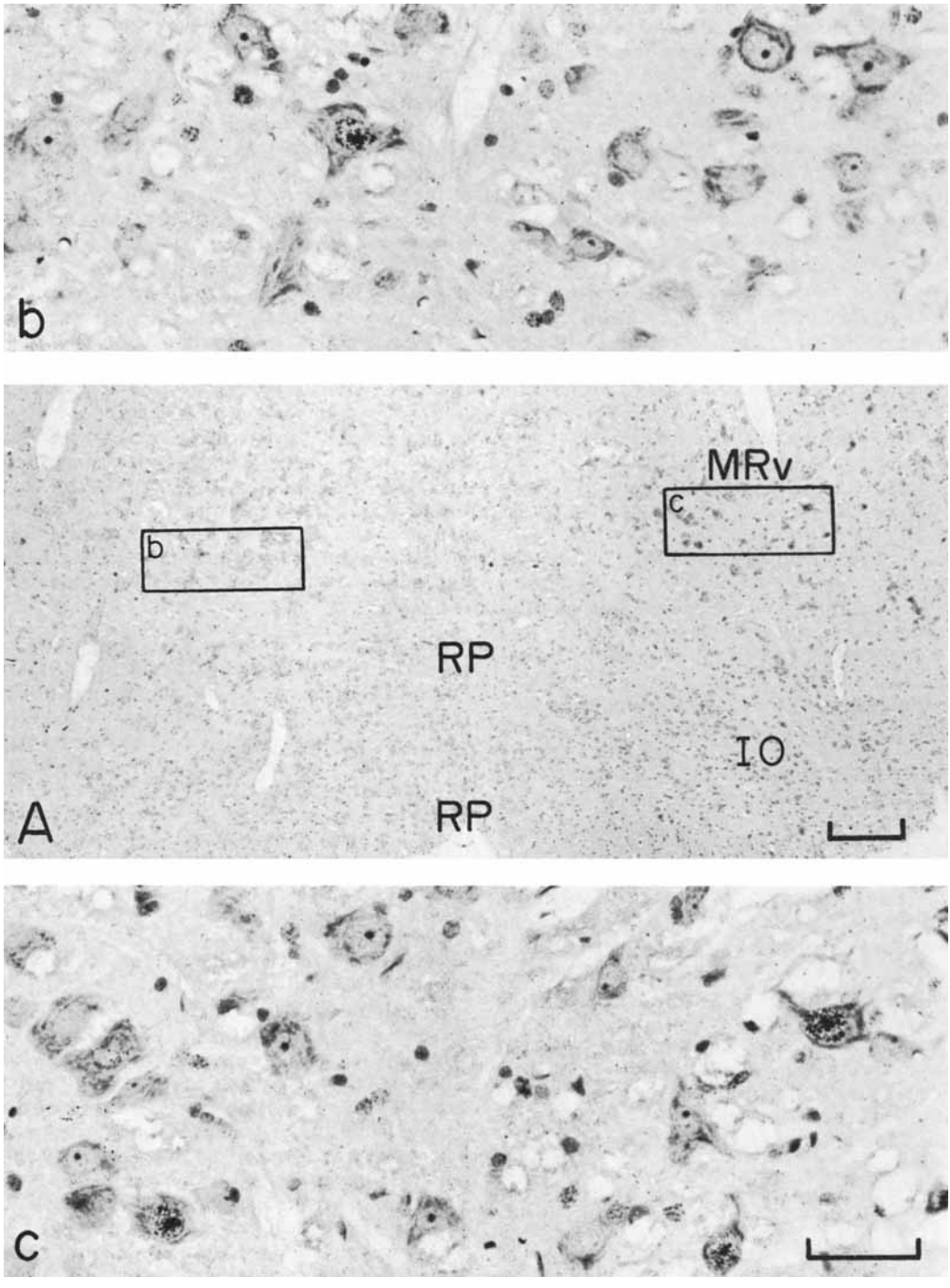

Fig. 18. Caudal medullary reticular formation, pars ventralis. A. (center) Labelled and unlabelled small and large neurons from a rat injected on days E15+16. Areas in rectangles (b and c) shown in figures b and c. Scales, A, 200 μm; b and c, 50 μm.

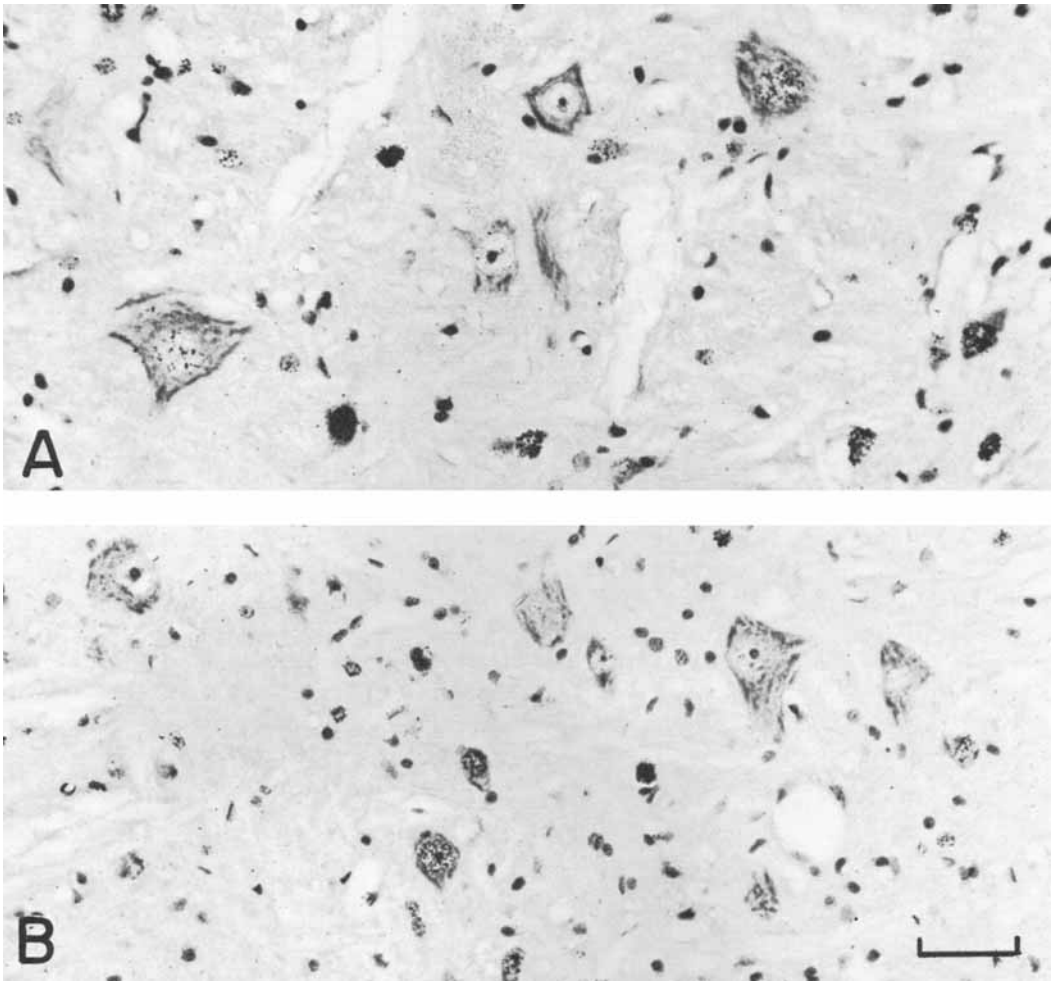

Fig. 19. Caudal medullary reticular formation. A, B. Labelled and unlabelled large and small neurons from a rat injected on days E13+14. Scale, 50 μm.

The Area Postrema. The area postrema (Figs. 2, 3B, 21) is a circumventricular organ situated beneath the caudal opening of the fourth ventrcle. It is a highly vascularized structure (Roth and Yamamoto, '68), contains neurons and glia (Brizzee and Neal, '54; Špaček and Pařizek, '69), and unciliated ependymal cells along the floor of the fourth ventricle (Dempsey, '73; Klara and Brizzee, '77). Afferents to the area postrema have been traced from the spinal cord, and its efferents reach the medial solitary nucleus (Morest, '67). Among the implicated functions are the triggering of the vomiting reflex (Borison and Brizzee, '51).

The labelling pattern in the area postrema is summarized in Figure 23. From 100% of labelled cells in the E13+14 group, the proportion fell to 44% in the E16+17 group. In terms of our analysis this means that 56% of the cells left their proliferative source by day E16, at a daily rate indicated by the histograms. But, unlike in any of the other structures so far considered, the decrement in labelled cells stopped. With injections delayed until the end of the embryonic period, approximately 44% of the cells could always be labelled. The only possible interpretation of this finding is that the tagged population of cells between days E17–E22 are germinal cells that

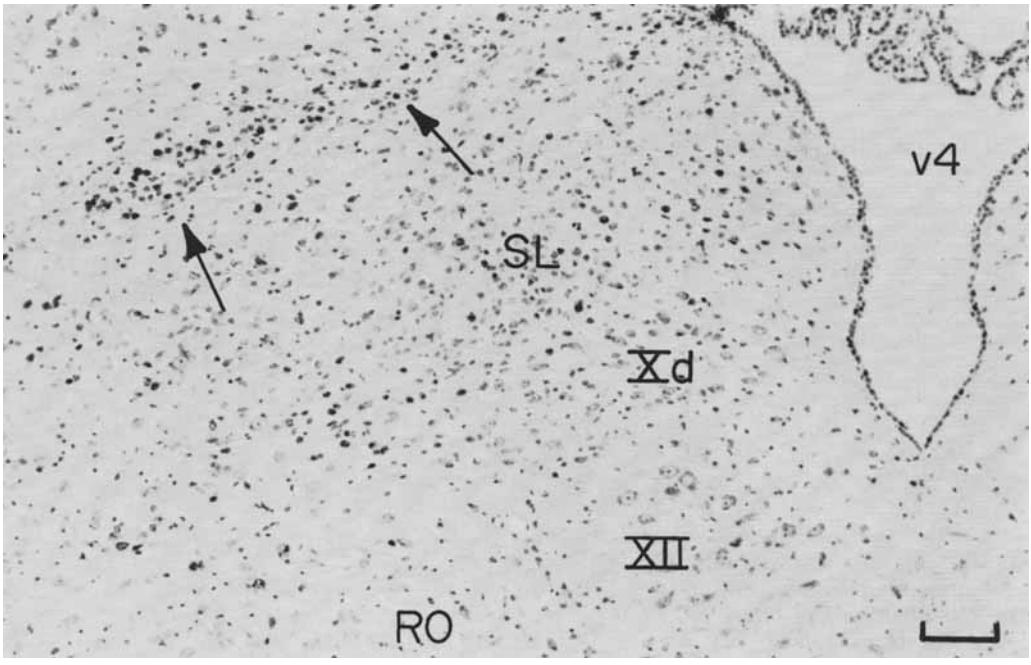

Fig. 20. Coronal section, from a rat injected on days E14+15. Arrows point to the labelled cells of the nucleus parasolitarius. Scale, 100 μm.

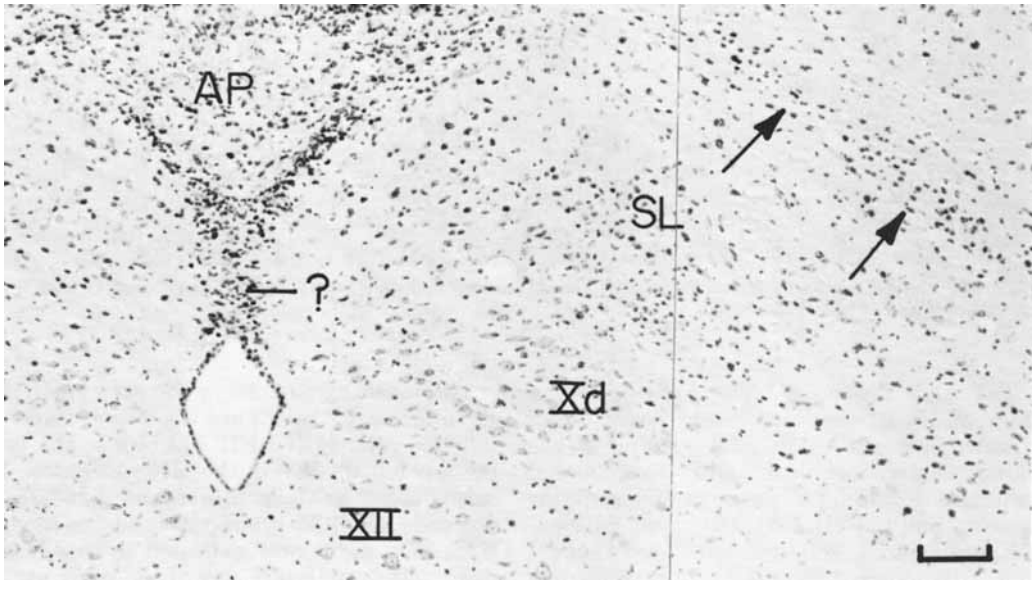

Fig. 21. Coronal section, caudal to the level shown in Figure 20, from a rat injected on days E14+15. Arrows point to the labelled cells of the nucleus parasolitarius. Scale, 100 μm.

supply daughter cells to a structure or structures outside the area postrema. Since no neurons are generated in this region after day E16 (Fig. 4), a possible target structure is the choroid plexus which is contiguous with the mediodorsal tip of the area postrema (Fig. 24). (According to our unpublished observations cell labelling continues during the early postnatal period both in the area postrema and the choroid plexus.) We conclude that the

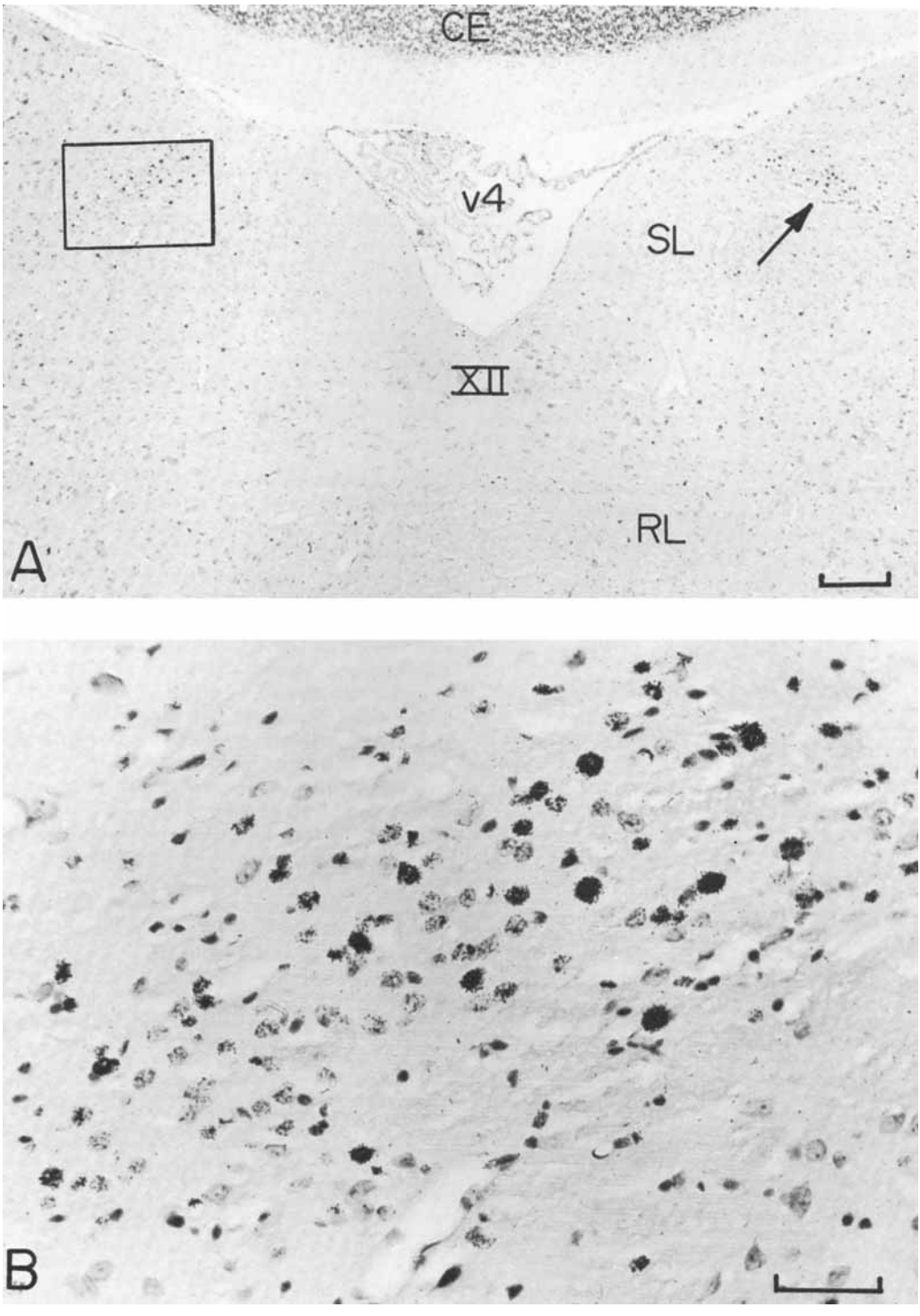

Fig. 22. A. Coronal section, corresponding to the level shown in Figure 20, from a rat injected on days E15+16. The labelled cells appear concentrated dorsomedially (arrow). Region in rectangle shown at higher magnification in *B*. *Scales:* A, 200 μm; B, 50 μm.

developing area postrema is composed of two cell populations: an early arising set of cells generated either locally or in the neuroepithelium, and another set of cells that keeps its proliferative capacity in the area postrema until sometime after birth and differentiates thereafter. What proportion of the early-generated 56% of the cells are neurons remains to be determined.

The Nucleus of Roller. A group of small cells situated beneath the hypoglossal nucleus was singled out by Roller (1881) as a separate nucleus and it has been referred to since as the nucleus of Roller. Brodal ('52) described it in the cat as a collection of large, medium, and small cells and classified the nucleus, together with the prepositus nucleus and nucleus intercalatus, as a member of the perihypoglossal group of nuclei. Experimental studies have indicated that the nucleus of Roller, like the prepositus nucleus situated more rostrally, is reciprocally connected with the cerebellum (Brodal, '52; Torvik and Brodal, '54; Walberg, '61; Alley et al., '75; Kotchabhakdi et al., '78).

In autoradiograms of rats injected on days E13+14 (Figs. 2B, 12) the nucleus of Roller stands out with its labelled cells against the unlabelled neurons of the overlying hypoglossal nucleus. The nucleus is easily traced beneath the entire length of the hypoglossal nucleus and it seems to blend imperceptibly into the nucleus prepositus dorsally (the latter will be dealt with in a later paper; Altman and Bayer, '80b). The nucleus has a reticular appearance in sagittal sections and contains cells of all sizes. Many of the cells are labelled in animals injected on days E14+15 (Fig. 25). A small proportion of cells, including the largest, are still labelled in the E15+16 group. These observations indicate that the formation time of neurons of the nucleus of Roller follows by 2–3 days the cytogenesis of the hypoglossal nucleus.

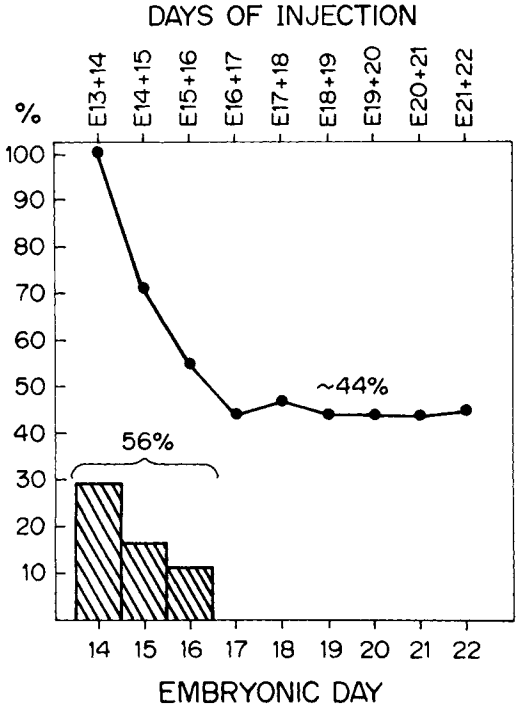

Fig. 23. Labelling pattern in the area postrema. Graph shows the percentage of labelled cells in the injection groups specified at the top. Histograms give the calculated proportion of cells forming on days E14, 15, and 16. See text for interpretation.

DISCUSSION

The discussion of the results obtained in this initial paper of the series has to be limited in scope and somewhat tentative. Several of the systems that we have described have only been partially explored; many of them have components that extend beyond the lower medulla into the upper medulla and the pons, and some into the midbrain. Examples are the subdivisions of the trigeminal complex and the rostral extensions of the raphe and reticular nuclei. Moreover, we cannot fully appreciate the possible relationships within and between sensory, motor, and other nuclear systems of the brain stem until after an examination of all of them.

Figure 26 represents an attempt to summarize the chronology of cytogenesis in the lower medulla in relation to the known structural and functional affiliations of the regions involved. We have included in the diagrams two structures of the lower medulla, the inferior olive and the lateral reticular nucleus, from a previous study (Altman and Bayer, '78b) of the same material, and use the concept of "cytogenetic zones."[1]

[1]The terms "cytogenetic zone" or "cytogenetic system" will be used interchangeably in this series of papers. The terms are based on the hypothesis that aggregates of neurons that have similar birth dates and similar temporal patterns of cell production (for instance, slow or fast) are derived from a shared germinal source. A postulated cytogenetic system (which will be designated by letters) may include several contiguous or separated structures that are distinguished from other adjacent structures (sometimes parts of a single nucleus) with different cell generation patterns. If the labelling pattern within a system shows a clear cytogenetic gradient (for instance, lateral-to-medial or ventral-to-dorsal) the gradient is used as a "directional arrow" that points towards the location of the germinal source of neurons (medial or dorsal, respectively). The validity of the postulated cytogenetic zone has to be confirmed in embryos of the appropriate ages by identifying an active germinal zone at the sites predicted. Such an identification was attempted in the embryonic third ventricle in our study of the development of the diencephalon (Altman and Bayer, '79b).

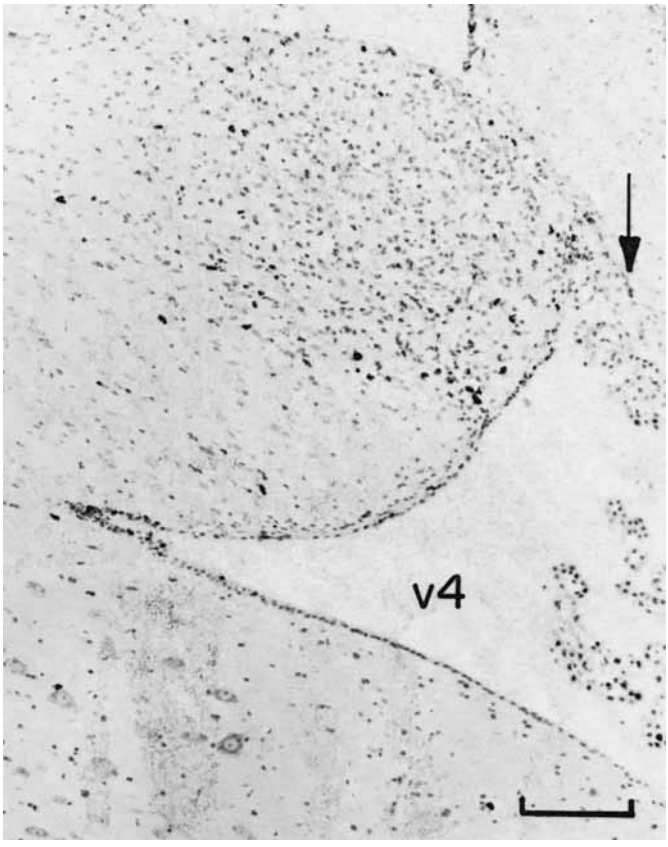

Fig. 24. The area postrema in sagittal section from a rat injected on days E16+17. Arrow points to the attachment of the choroid plexus. Scale, 50 μm.

The motor nuclei

The earliest arising structures of the lower medulla are two cranial motor nuclei: the hypoglossal nucleus and the dorsal vagal nucleus. In both structures over 80% of the cell population is acquired on day E12. The two nuclei are also unique in the rapid generation of their cells (Fig. 4). There was, however, a slight (but stastically significant) difference between the two. A small proportion of hypoglossal neurons is generated before day E12, whereas a small proportion of the dorsal vagal neurons are generated after day E12. The hypoglossal neurons innervate striated muscles whereas the dorsal vagal neurons are preganglionic motor elements. The slight gradient may indicate a sequence in the generation of somatic and autonomic motor neurons. However, we have tentatively included these nuclei in one cytogenetic system (designated as zone MS; Fig. 26) because of their many similarities (time of origin, rapid generation time, and close proximity to the ventricular system). Their similarities are highlighted when we compare the cytogenesis of the dorsal vagal nucleus with the nucleus ambiguus, both of which are components of the vagal efferent system. (But compare with Altman and Bayer, '80a; Fig. 15).

The neurons of the nucleus ambiguus are generated over a longer period and with a peak that occurs 3 days after the other two motor nuclei. The nucleus ambiguus has been classified as part of the branchial system (the pharynx, larynx, and the upper part of the esophagus which it innervates being considered derivatives of the fourth and fifth branchial arches). It is possible, therefore, that the nucleus ambiguus, which is situated far from

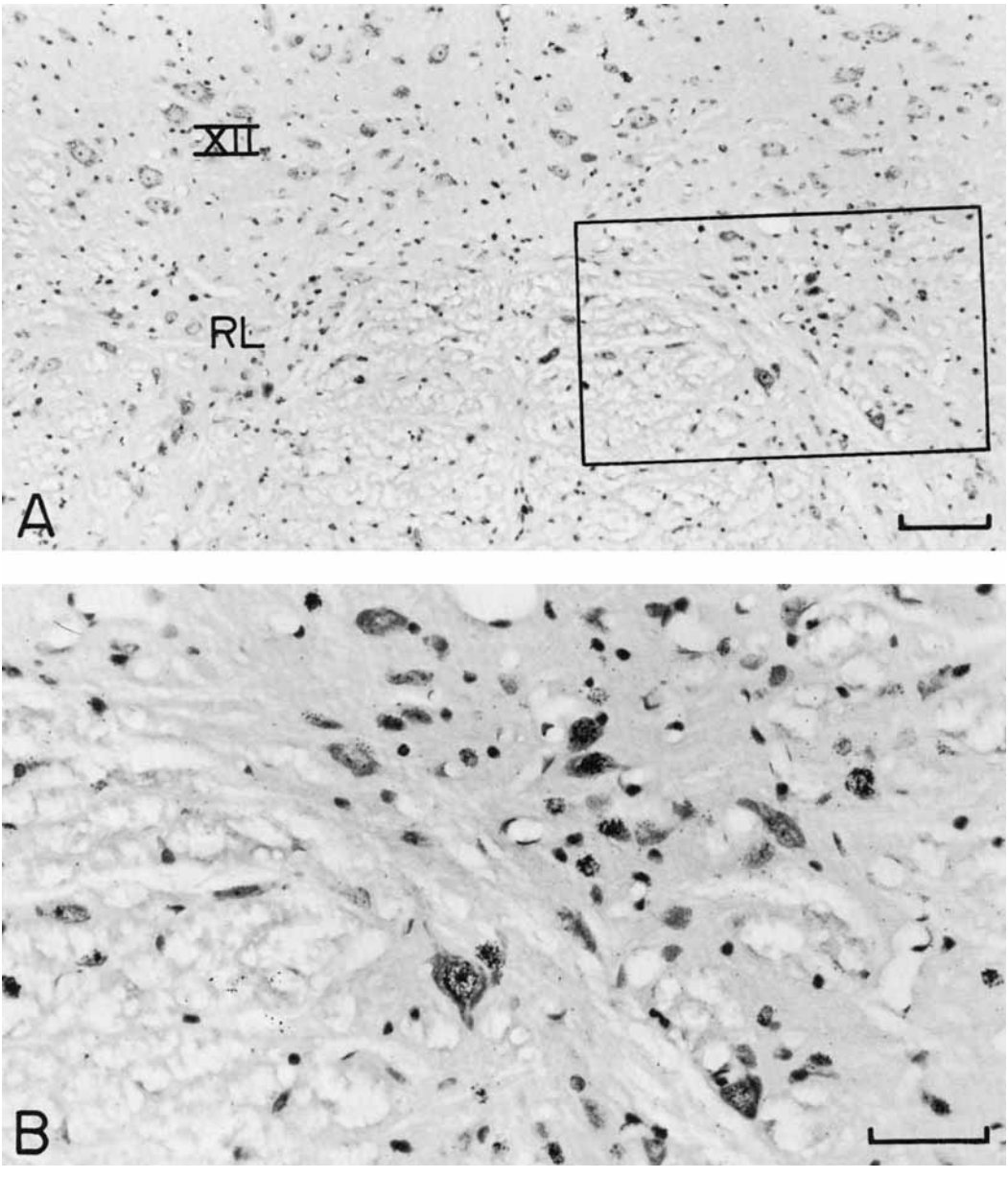

Fig. 25. A. The nucleus of Roller in coronal section, from a rat injected on days E14+15. Area in rectangle shown at higher magnification in B. Scales: A, 100 μm; B, 50, μm.

the ventricular system, is of a different cytogenetic origin. We shall present evidence in the succeeding paper (Altman and Bayer, '80a) that the ambiguus nucleus, together with the facial nucleus and the motor nucleus of the trigeminal (the other components of the branchial system) may constitute a single cytogenetic unit with a specific rostral-to-caudal gradient. We designate this region as cytogenetic zone MB (Fig. 26A).

The sensory relay nuclei

The times of origin of neurons of the dorsal column nuclei and the solitary nucleus are strikingly alike, and they differ from the ventrally situated motor nuclei both in terms of

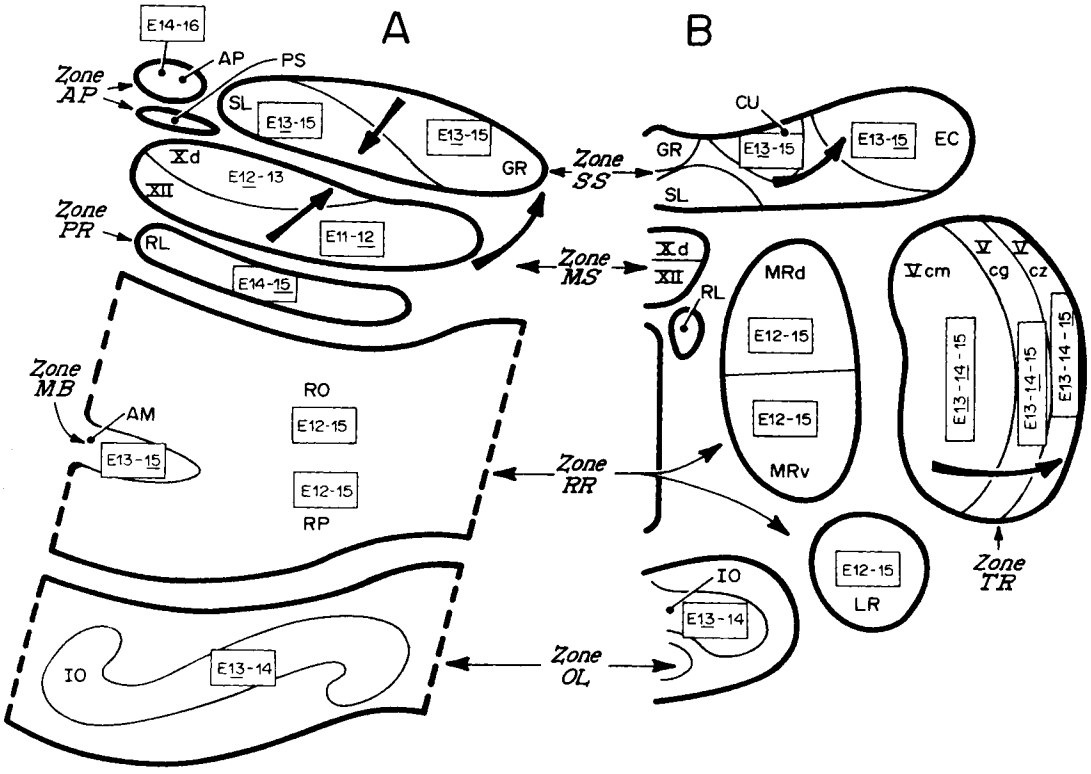

Fig. 26. Summary diagrams of intranuclear and internuclear gradients, and postulated cytogenetic zones in the lower medulla, in sagittal (A) and coronal (B) sections. Numbers in rectangles, appropriate dates of neuron production, with peak days underlined. Heavy arrows indicate cytogenetic gradients. The letters designating the cytogenetic zones are not identical with the abbreviations designating special structures.

a later origin of their neurons and a longer generation period (Fig. 4). There was no statistically significant difference in the birth dates of neurons of the nucleus gracilis and the nucleus cuneatus, which matches the absence of a gradient in their target nucleus in the thalamus (Altman and Bayer, '79a). However, the generation of dorsal column nuclei neurons antedated slightly, but significantly, the solitary nucleus neurons. This suggests a slight precedence of the somatic afferent (somesthetic) relay neurons over the visceral afferent (gustatory) relay neurons; this is similar to the order obtained in the adjacent motor nuclei. The slight gradients observed in the motor and sensory relay nuclei result in mirror images (Fig. 26A), possibly reflecting an "inside-out" (Angevine, '70) settling pattern with relation to the basal and alar plates of the neuroepithelium of the lower medulla.

A striking temporal difference has been obtained between two components of a related system, namely, the dorsal column nuclei and the external (lateral) cuneate nucleus. The peak production time of neurons of the external cuneate nucleus occurred 2 days after the cuneate nucleus. This suggests that the medullary relay neurons of the peoprioceptive afferents are generated later than those of the exteroceptive afferents. Furthermore, there is a correlation between the sequential generation of these two relay systems and their target structures: The maturation of the thalamus (Altman and Bayer, '79a), where the dorsal column fibers terminate, antedates the maturation of the cerebellum (Altman and Bayer, '78a), where the external cuneate fibers are distributed. In reference to the cerebellum, we found in our study of four precerebellar nuclei (Altman and Bayer, '78b; Fig. 3) the following sequence of production: inferior olive (peak on day E13), lateral reticular nucleus (peak on day E14), nucleus reticularis tegmenti pontis (peak on day E16), and the pontine gray (peak on day E17). The external cuneate nucleus, with its peak generation

time on day E15, thus fits between the lateral reticular nucleus and the pontine gray. The afferents of the earliest generated neurons of the inferior olive terminate as climbing fibers (Eccles et al., '67) directly on the relatively early-produced Purkinje cells, whereas the afferents of all the other later-forming nuclei, including the external cuneate nucleus, terminate as mossy fibers and synapse with late-arising granule cells.

If the external cuneate nucleus is part of the cytogenetic system that generates the dorsally situated relay nuclei, then there is a pronounced medial-to-lateral internuclear gradient present in this region. We shall tentatively designate this sensory plate as zone SS (Fig. 26A).

It is somewhat difficult to compare the cytogenesis of the caudal nucleus of the trigeminal complex with the other sensory relay nuclei because of its more complex organization and the pronounced gradient within its subnuclei. The gradient is a medial-to-lateral one and it corresponds to the inside-out gradients that we have encountered so far. Insofar as the subnucleus zonalis and subnucleus gelatinosus seem to contain the neurons that project to the thalamus (Trevino and Carstens, '75; Hockfield and Gobel, '78; Burton et al., '79), these two subnuclei may be justly compared with the other thalamic relay nuclei of the lower medulla. The peak generation time of gelatinosus neurons occurs 1 day later than in the dorsal column nuclei, and the peak of zonalis neurons is delayed by 2 days (Fig. 4). One way of expressing this is that the relay nuclei of somesthetic afferents from the face area are produced later than those of somesthetic afferents from the trunk and limbs. It will be possible to further examine this interpretation when data will be available on the time of origin of primary afferent neurons in the cranial and spinal sensory ganglia (work in progress). An alternative interpretation would be that exteroceptive relay neurons implicated in nociceptive functions (trigeminal system) arise later than those mediating innocuous stimuli (dorsal column nuclei). But, as we saw earlier, this proposition is controversial. It is, moreover, counterindicated by our data regarding the time of origin of neurons of the nucleus interpolaris of the trigeminal complex. This nucleus has been implicated in receiving afferents from the vibrissae (Nord, '68; Shipley, '74; Belford and Killackey, '79) rather than pain fibers; nevertheless, neuron production lags here with respect to the dorsal column nuclei (Fig. 4).

The medial-to-lateral gradient seen in the nucleus caudalis of the trigeminal resembles that reported in the spinal cord (Nornes and Das, '73) where the late-generated neurons of the substantia gelatinosa migrate past earlier-produced neurons and settle externally. We shall tentatively classify the laterally placed caudal trigeminal nucleus as a separate cytogenetic system and designate it as zone TR (Fig. 26B). Because this nuclear complex extends into the pontine region we shall return to its discussion in subsequent publications.

Other components of the lower medulla

A general discussion of the raphe and reticular nuclei will be postponed until we have presented data about the rostral components of these regions. On account of the long generation time of their neurons and their many similarities to each other we shall distinguish them from the sensory relay and motor nuclei and consider them a separate cytogenetic unit (zone RR). At least three other regions of the lower medulla differ from those so far considered and from each other: the nucleus of Roller, the area postrema, and the inferior olive. The nucleus of Roller neurons are generated much later than the motor nuclei neurons situated dorsally. The nucleus of Roller, like the prepositus nucleus with which it is contiguous rostrally, has intimate connections with the cerebellum (Brodal, '52; Torvik and Brodal, '54; Walberg, '61; Alley et al., '75; Kotchabhakdi et al., '78). It resembles the prepositus nucleus also in terms of the late formation of neurons (Altman and Bayer, '80b). We shall tentatively designate this region as zone PR and entertain the possibility that it is a caudal, tongue-like extension of the prepositus system.

A clearly unique cytogenetic system is the area postrema (zone AP; Fig. 26B). As we saw, it is singular among all regions of the lower medulla in having a fixed population of cells that can be relabelled with any injection schedule between days E17 and E22. This is possible only if the cells are germinal elements and their daughter cells migrate elsewhere. We postulated that the migrating cells are contributed to the contiguous choroid plexus. The nucleus parasolitarius, with its late-forming small neurons, may belong to this cytogenetic system. Finally, we shall designate the inferior olive as zone I0. In agreement with previous reports (His, '1890; Harkmark, '54; Ellenberger et al., '69) we have obtained experimental evidence (Altman and Bayer, '78b) that the neurons of the inferior olive are

generated far from their settling site in the dorsal recess of the fourth ventricle in the upper medulla. It is such embryological evidence, which pinpoints the germinal source of a specific brain structure, that justifies the identification of a brain region or group of nuclei as a cytogenetic system. The embryological identification of the germinal sites of the other postulated cytogenetic systems will be attempted in the future.

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