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Development of the Brain Stem in the Rat. II. Thymidine-Radiographic Study of the Time of Origin of Neurons of the Upper Medulla, Excluding the Vestibular and Auditory Nuclei

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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 radiographs from adult progeny of these rats the proportion of neurons generated on specific days was determined in the major nuclei of the upper medulla, with the exception of the vestibular and auditory nuclei. The neurons of the motor nuclei are generated over a brief period. Neurons of the retrofacial nucleus are produced first, with more than 60% of the cells arising on day E11 or earlier. Peak generation time of abducens neurons is day E12 and of the neurons of the facial nucleus is day E13. In contrast, the neurons of the superior salivatory nucleus are produced late, predominantly on day E15 and some on day E16. The generation of the (sensory relay) neurons of the nucleus oralis of the trigeminal complex takes place over an extended period between days E12 and E15; the last generated cells include the largest neurons of this nucleus. Neurons of the raphe magnus are produced between days E11 and E14, the neurons of the rostral medullary reticular formation between days E12 and E15. The latest generated neurons of the upper medulla (excluding the cochlear nuclei) belong to a structure identified as the granular layer of the raphe.

Combining these results with those of the preceding paper (Altman and Bayer, '80a) and with additional data, it is postulated that the laterally and ventrally situated motor nucleus of the trigeminal, the facial nucleus, and the nucleus ambiguus form a single longitudinal zone of branchial motor neurons with a rostral-to-caudal cytogenetic gradient. In contrast, the medially and dorsally situated (juxtaventricular) hypoglossal nucleus and abducens nucleus (together with the other nuclei of the ocular muscles) form a longitudinal somatic motor zone with a caudal-to-rostral gradient. The dorsal nucleus of the vagus and the superior salivatory nucleus may constitute a preganglionic motor zone, also with a caudal-to-rostral cytogenetic gradient.

In this series of papers on the development of the brain stem, we have divided this brain region into five parts: the lower medulla, the upper medulla, the pontine region, the mesencephalic tegmentum, and the tectum. In the first paper (Altman and Bayer, '80a), we dealt with the time of origin of neurons of the lower medulla; this paper is concerned with the time of origin of neurons of the upper medulla, excluding the vestibular and auditory nuclei. As the term is used here, the upper medulla extends from an imaginary line drawn between the anterior walls of the hypoglossal nucleus (or the posterior border of the prepositus nucleus) and inferior olive, caudally, to a line drawn between the anterior wall of the superior and lateral vestibular nuclei and the posterior wall of the pontine gray, rostrally (Fig. 1). The upper medulla contains four motor nuclei (the facial, the retrofacial, the abducens, and the salivatory nuclei); the nucleus oralis of the trigeminal complex; four vestibular nuclei (the inferior, the medial, the lateral, and the superior vestibular nuclei)

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Abbreviations

AM	nucleus ambiguus
c	caudal
CI	inferior central nucleus (raphe)
CNa	anteroventral cochlear nucleus
CNd	dorsal cochlear nucleus
CNp	posteroventral cochlear nucleus
GR	nucleus gracilis
ic	inferior cerebellar peduncle
IO	inferior olive
MRr	rostral medullary reticular formation
PN	pontine nuclei
PR	nucleus prepositus hypoglossi
r	rostral
RE	retrofacial nucleus
RG	granular layer of raphe
RM	raphe magnus
SA	salivatory nucleus (superior)
SL	solitary nucleus
SOl	lateral superior olivary nucleus
SOm	medial superior olivary nucleus
TRl	lateral trapezoid nucleus
TRm	medial trapezoid nucleus
v4	fourth ventricle
Vm	motor trigeminal nucleus
Vo	nucleus oralis of trigeminal complex
Vst	spinal tract of trigeminal
VI	abducens nucleus
VII	facial nucleus
VIIc	facial nucleus, caudal part
VIIg	genu of facial nerve
VIIl	facial nucleus, lateral part
VIIm	facial nucleus, medial part
VIIr	facial nucleus, rostral part
VIIIi	inferior vestibular nucleus
VIIIl	lateral vestibular nucleus
VIIIm	medial vestibular nucleus
VIIIs	superior vestibular nucleus
Xd	dorsal vagal nucleus
XII	hypoglossal nucleus

and the related nucleus prepositus hypoglossi; several auditory nuclei (the dorsal, anteroventral, and posteroventral cochlear nuclei; the nuclei of the trapezoid body, and the nuclei of the superior olive); the raphe magnus, the rostral portion of the medullary reticular formation, and a few smaller nuclei.

A systematic study of the time of origin of neurons of the upper medulla has not been published before. Some information is available about cytogenesis in the cochlear nuclei; it will be discussed in the succeeding paper (Altman and Bayer, '80b) which deals with the time of origin of neurons of the medullary vestibular and auditory nuclei. Cytogenesis in the pontine region will be the subject of the fourth paper (Altman and Bayer, '80c), together with a comparison of components of those systems (in particular the trigeminal and the auditory) that extend rostrally into and beyond the pontine region. A correlation of radiographic datings obtained in adults with the embryonic development of the brain stem will be attempted in future publications.

MATERIALS AND METHODS

The autoradiographic material used in this study was identical with that used and described in the preceding paper (Altman and Bayer, '80a). The proportion of labelled cells was quantified in seven regions of the upper medulla in six animals each in all relevant injection groups from days E12 + 13 to days E21 + 22. Statistical statements are based on Conover's sign test, as described previously (Altman and Bayer, '80a).

RESULTS

Summary quantitative data

The region of the upper medulla is illustrated in a sagittal autoradiogram in Figure 1. The locations of the nuclei or regions in which labelled cells were counted are indicated schematically in two coronal planes (Figs. 2A,B), together with quantitative data on the proportion of cells formed on specific days in three motor nuclei (Fig. 2C), the raphe and reticular region of this level (Fig. 2D), and the nucleus oralis of the trigeminal complex (Fig. 2E). The observations made are described below under three corresponding headings. The nucleus prepositus hypoglossi will be dealt with in the succeeding paper (Altman and Bayer, '80b) in association with the vestibular nuclei.

The motor nuclei

The facial nerve nucleus. The nucleus of the seventh cranial nerve is composed of typical multipolar neurons (Figs. 3,4B). From four to six subdivisions of the nucleus have been described in carnivores (Papez, '27; Vraa-Jensen, '42; Courville, '66; Kitai et al., '72) and the rat (Papez, '27; Hogg, '28; Nishi, '65; Martin and Lodge, '77). With minor differences, the medial cell groups of the nucleus have been related to the auricular and stapedius branches of the nerve; the dorsal and intermediate cell groups to the ramus temporalis and ramus zygometico-orbitalis; and the lateral cell groups to the inferior and superior buccolabialis.

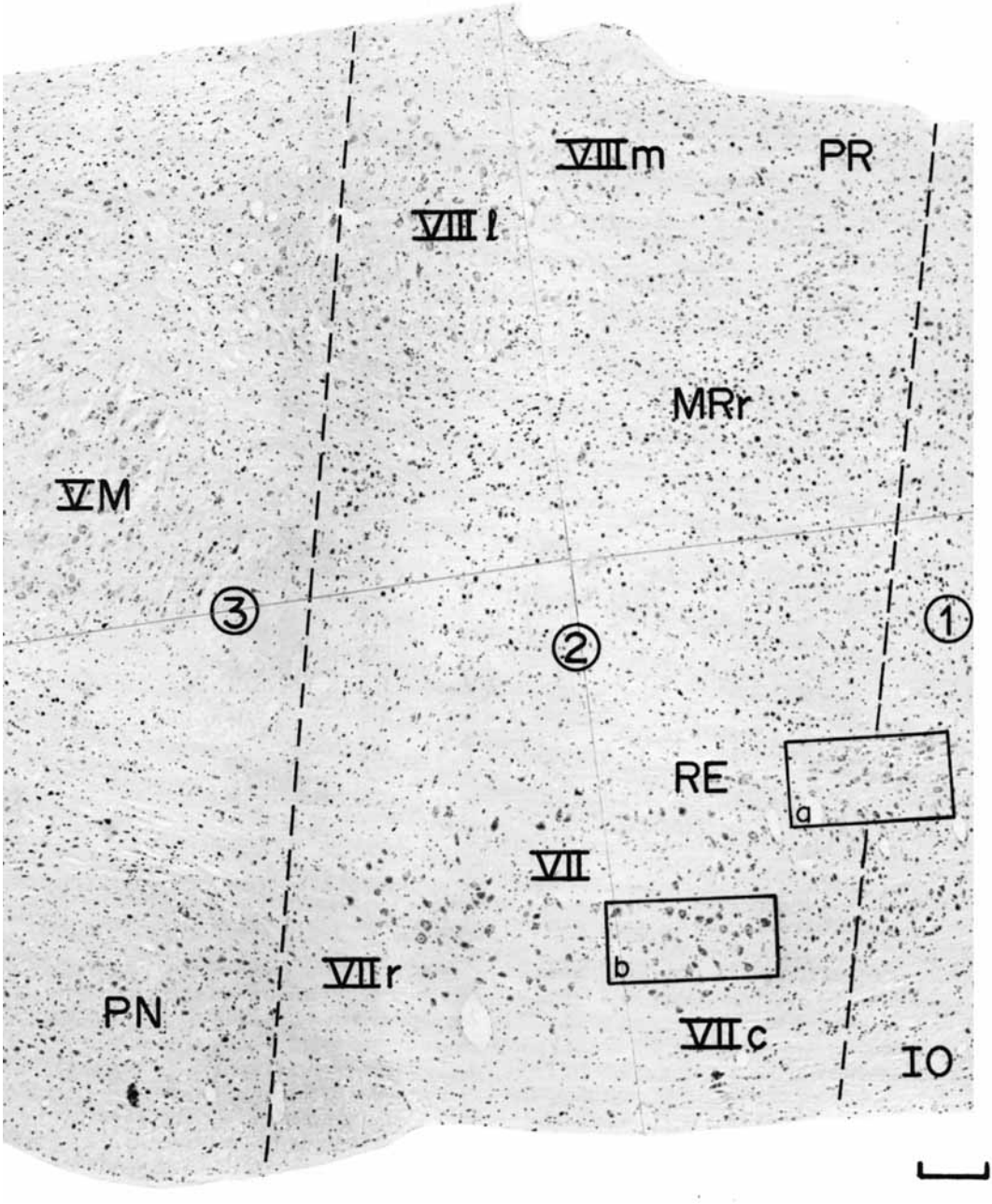


Fig. 1. Thymidine-radiogram of a sagittal section of the upper medulla (2) from a rat injected on days E13 + 14. Regions of the retrofacial nucleus in rectangle a, and of the caudal facial nucleus in rectangle b, are shown at higher magnification in Figures 4A and 4B, respectively. Scale, 200 μm.

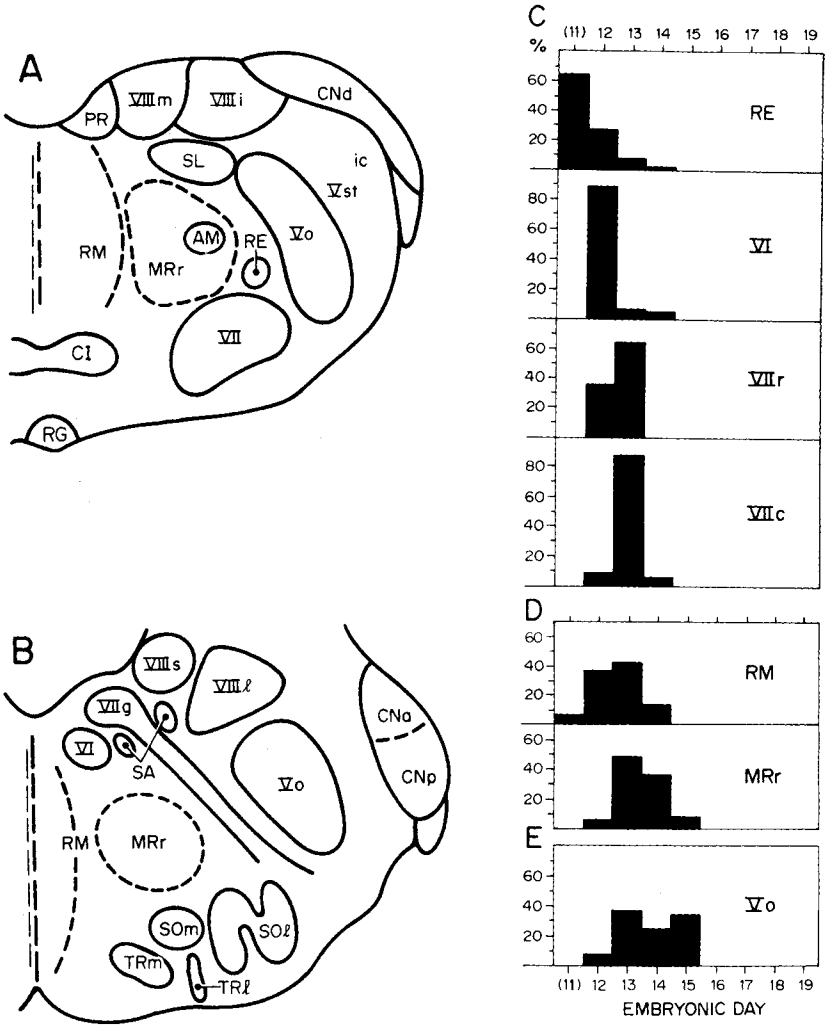


Fig. 2. Schematic outlines of the caudal (A) and rostral (B) aspects of the upper medulla in coronal sections. Quantitative data of the time of origin of neurons in this region are shown for the motor nuclei (C), the raphe magnus and medullary reticular formation (D), and the nucleus oralis of the trigeminal complex (E).

Ascending afferents to the facial nucleus originate in the cervical spinal cord (Hazlett et al., '72; Dom et al., '73; Tanaka et al., '78) and are distributed mainly to the medial portion of the nucleus. Descending afferents have been traced from the superior coliculus, the red nucleus, and midbrain tegmentum (Courville, '66; Martin and Dom, '70; Edwards, '72; Harting et al., '73; Dom et al., '73). In the opossum (Dom et al., '73), an extensive projection was traced to the buccolabial portion of the facial nucleus from the spinal trigeminal nucleus and the parvicellular reticular formation.

Neurons of the facial nucleus form clusters but their boundaries were difficult to delineate for quantitative purposes. It proved easy, because of the great length of the nucleus (Fig. 1), to make separate counts in the rostral and caudal poles of the nucleus (Fig. 2c). Rostrally 35% of the neurons are generated on day E12, and the rest on day E13. Caudally, 87% of the cells are generated on day E13 and 5% of the neurons were still labelled in animals injected on days E14 + 15. The difference was significant at the $p < 0.0225$ level. In addition to this rostral-to-caudal cytogenetic gradient, a medial-to-lateral gradient was also consist-

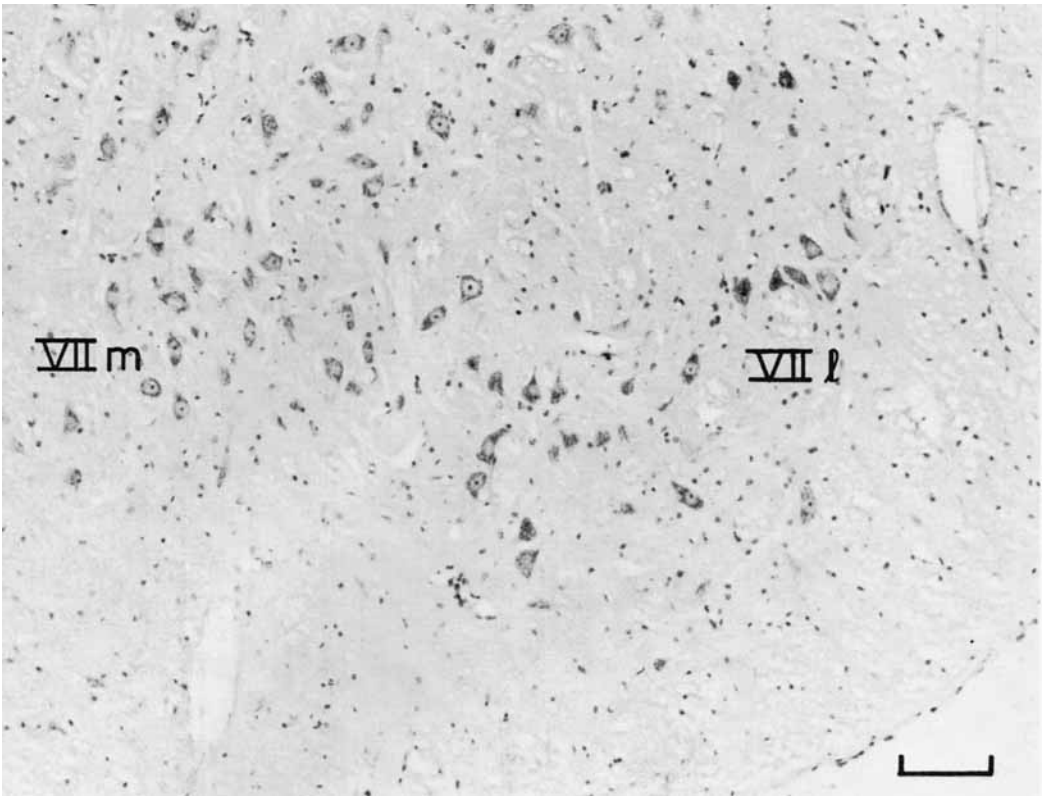


Fig. 3. The facial nucleus in coronal section from a rat injected on days E14 + 15. Note the presence of labelled cells laterally and ventrolaterally. Scale, 100 μm.

ently seen; the motor neurons labelled in the E14 + 15 rats were typically located in the lateral and lateroventral aspects of the caudal facial nucleus (Fig. 3). These late-forming cells may be identical with those innervating the inferior and superior buccolabialis branches of the seventh nerve implicated in mimetic functions.

The retrofacial nucleus. The retrofacial nucleus is not referred to in most textbooks of neuroanatomy but is illustrated in brain atlases of the mouse (Sidman ae al., '71), the rat (Wünscher et al., '65), and the cat (Taber, '61). It is a compact longitudinal column of motor neurons (Fig. 1) situated caudal to the facial nerve nucleus and ventrolateral and somewhat rostral to the nucleus ambiguus (Altman and Bayer, '80a). It appears to have been considered a portion of the nucleus ambiguus by some observers (for instance, Valverde, '62, p. 29). The retrofacial nucleus may be identical with that component of the rabbit ambiguus which is described to be situated ventrolateral to the rostral tip of the principal column of the ambiguus (Lawn, '66). According to Lawn this is the region which supplies fibers to the glossophryngeal nerve, whereas the rest of the nucleus projects peripherally by way of the vagus. The region is labelled as the retrotrigeminal nucleus by Berman ('68, p. 24), who discusses some of the earlier identifications of this nucleus.

In animals injected on days E13 + 14, in which the majority of neurons of the caudal facial nerve nucleus are labelled (Figs. 1, 4B), the neuons of the retrofacial nucleus (Figs. 1, 4A) are rendered conspicuous by an absence of labelling. In the preceding paper (Altman and Bayer, '80a) we used this early cessation of labelling in the retrofacial nucleus to distinguish it from the late forming nucleus ambiguus. Two-thirds of the neurons of the retrofacial nucleus form before day E12 (Fig. 2C);

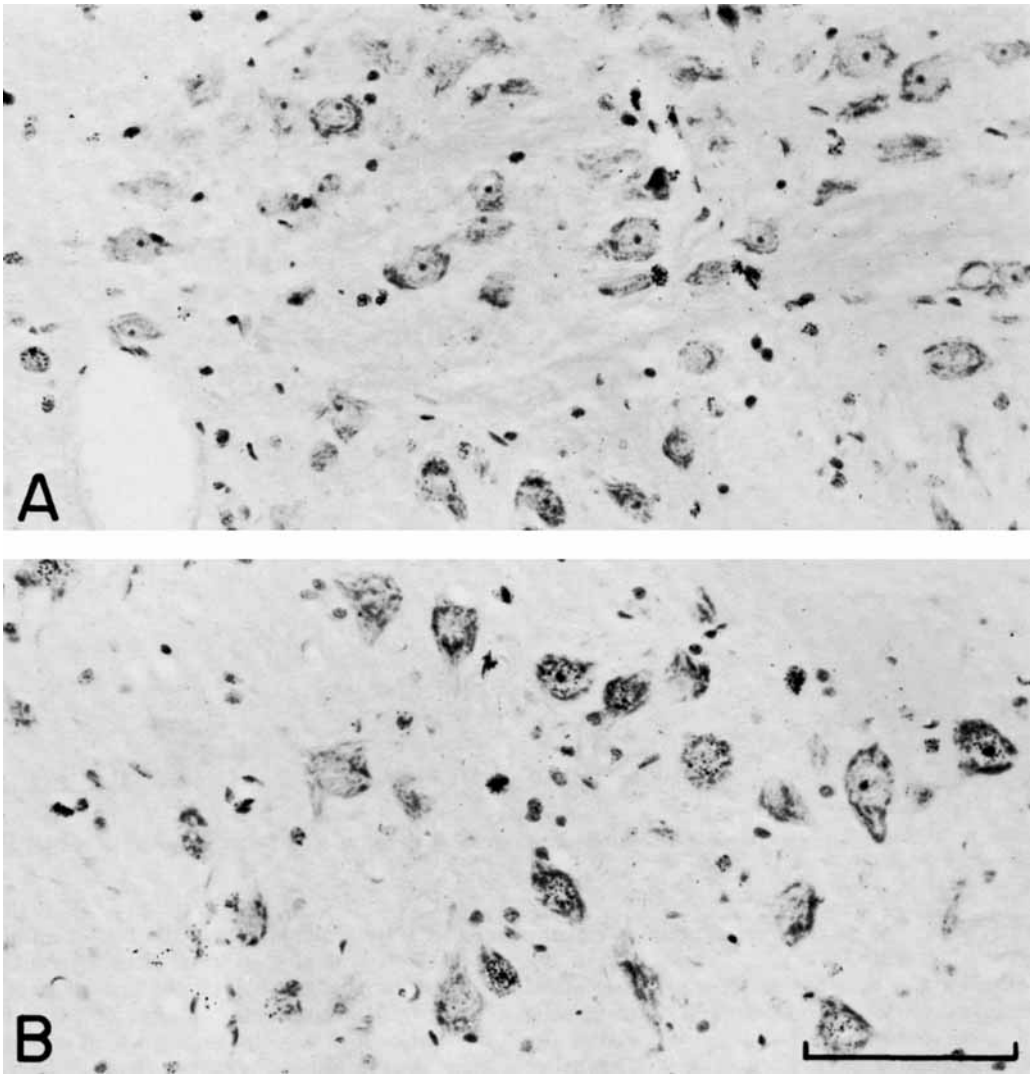


Fig. 4. Neurons of the retrofacial (A) and the facial (B) nuclei, from a rat injected on days E13 + 14. For the location of the regions see Figure 1. Scale, 50 μm.

the exact day of their production was not determined. The neurons of the retrofacial nucleus, as a group, are the earliest forming cells of the upper medulla and all comparisons with the other motor nuclei of the upper medulla were significant ($p < 0.0127 - 0.0001$).

The abducens nucleus. The sixth cranial nerve nucleus is composed of motor neurons that innervate the lateral rectus muscle that abducts the eye. It has relatively few cells in the rat (Fig. 5). In different mammalian species, projections have been described to the abducens nucleus from all (McMasters et al., '66) or some (Tarlov, '70; Gacek, '79) of the vestibular nuclei, the pontine reticular nuclei (Highstein et al., '76), the gigantocellular tegmental field, and the nucleus prepositus (Maciewicz et al., '77). Central efferents from the abducens nucleus reach the contralateral medial rectus subdivision of the oculomotor nucleus (Bienfang, '78).

Close to 90% of the large neurons of the abducens nucleus are generated on day E12;

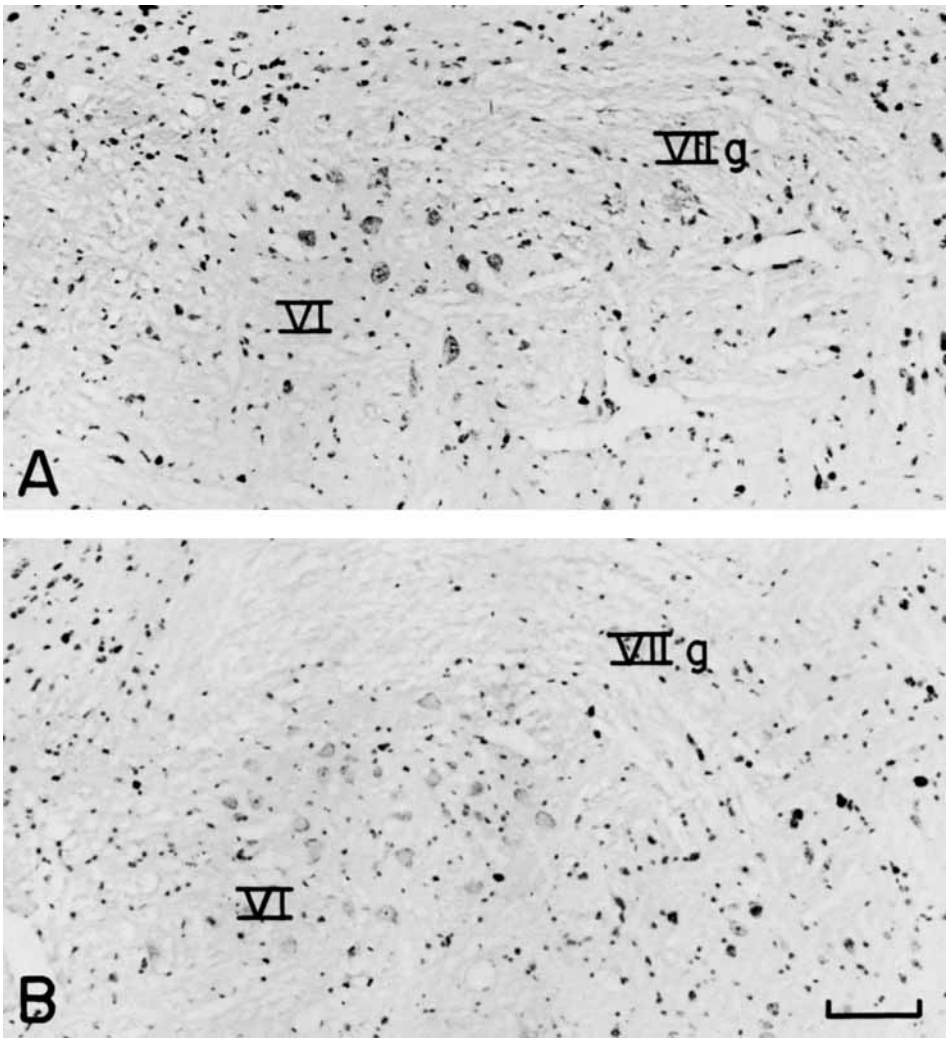


Fig. 5. Abducens nucleus in sagittal sections from rats injected on days E12 + 13 (A) and E13 + 14 (B). Only small cells are labelled in the latter. Scale, 100 μm.

the rest is added on days E13 and E14 (Fig. 2C). Because of the small size of the nucleus (Fig. 5), it was not possible to determine the presence of a cytogenetic gradient. Some small cells of unknown identity were labelled after day E14. The neurons of this nucleus are generated significantly later than the retrofacial nucleus neurons ($p < 0.0129$); but they form significantly earlier than the neurons in the caudal portion of the facial nucleus ($p < 0.0225$). However, the difference between the abducens nucleus and the rostral portion of the facial nucleus was not significant ($p < 0.7744$).

The superior salivatory nucleus. Descriptions of the salivatory nucleus usually refer to two parts: (a) a superior nucleus which is the source of preganglionic fibers that leave by way of the nervus intermedius and influence the lacrimal, submandibular, and sublingual glands; and (b) an inferior nucleus which contributes fibers to the glossopharyngeal nerve that influences the parotid gland. The site of these nuclei has been debated for a long time (Shute and Lewis, '60). Using a modification of Koelle's technique for staining fibers and cell bodies for the presence of cholinesterase, Shute and Lewis showed that the strongly

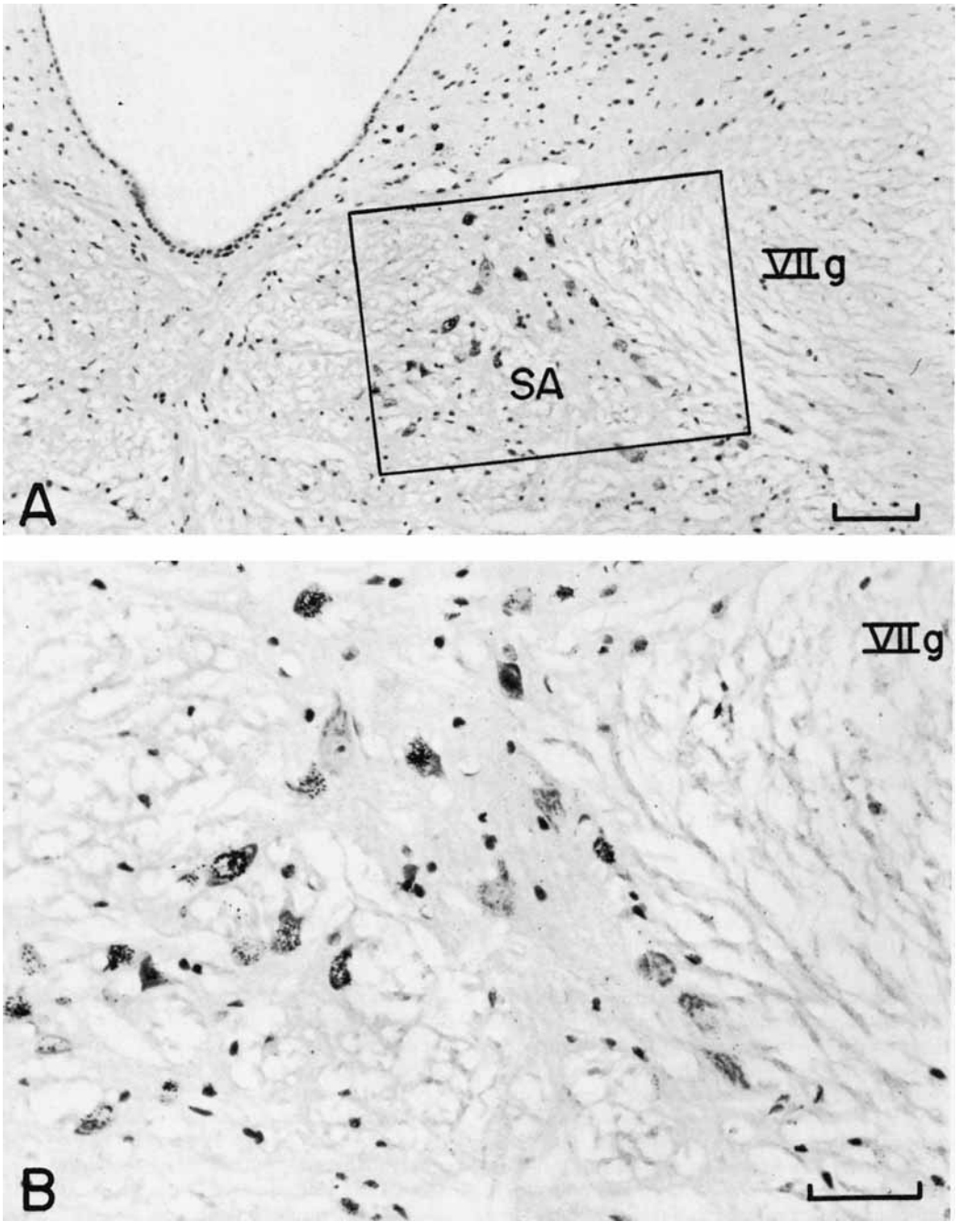


Fig. 6. Superior salivatory nucleus in coronal section from a rat injected on days E15 + 16. Scales: A, 100 μm; B, 50 μm.

staining fibers of the nervus intermedius in the rat originate in two cell groups flanking the genu of the facial nerve, and named them the lateral and medial salivatory nuclei. They could not confirm the existence of an inferior salivatory nucleus. A slightly different interpretation of the lateral and medial nucleus was offered by Brown and Howlett ('68).

Cells of variable size and shape, but smaller than the neurons of the abducens nucleus, were found labelled in the E15 + 16 animals (Fig. 6), and in small numbers in the E16 + 17 group. These late-forming cells flanked medially and laterally the genu of the facial nerve or were embedded in the white matter. In terms of appearance and location, they seem to be identical to the superior salivatory nucleus of Shute and Lewis ('60) and Brown and Howlett ('72). No attempt was made to quantify the date of neuron production in this irregular structure, but apparently the bulk of its neurons arises on day E15.

In summary, the earliest forming motor neurons of the upper medulla are those of the retrofacial nucleus, followed in succession by the abducens nucleus and then the facial nucleus (Fig. 2C); the preganglionic neurons of the salivatory nucleus are generated much later than the others.

The nucleus oralis of the trigeminal complex

The nucleus caudalis and nucleus interpolaris of the trigeminal complex were considered in the preceding paper (Altman and Bayer, '80a). The nucleus oralis is the rostral continuation of this system (Olszewski, '50; Torvik, '56). It occupies the lateral aspect of the rostral medulla and is composed of an admixture of cell types, including a much higher proportion of large neurons than other portions of this complex. There are few specific descriptions of the anatomical connections of this nucleus. Physiological studies (Sessle and Greenwood, '76) suggest that the nucleus oralis shares with the principal nucleus of the trigeminal complex nociceptive afferents from the region of the face, mouth, jaw, pharynx, and larynx. Efferents from the large and smaller neurons have been traced to the cerebellum (Karamanlidis, '68; Ikeda, '79). It has been claimed that, unlike the nucleus interpolaris, the nucleus oralis is devoid of those small cells that are the source of trigeminothalamic efferents (Fukushima and Kerr, '79). The prinicipal nucleus will be dealt with in a succeeding paper of this series (Altman and Bayer, '80c).

The cell composition of the nucleus oralis (Fig. 7) is reminiscent of the subnucleus magnocellularis of the nucleus caudalis of the trigeminal complex. The majority of its neurons arise, with a relatively even distribution, between days E13 and E15 (Fig. 2E). The earliest arising cells are a type of small, round neurons (Fig. 7); the majority of these are generated on day E14. In contrast to what is seen in most brain regions, the latest arising cells are the largest ones and some of those were still labelled in a few animals injected as late as days E16 + 17 (Fig. 8). There was no statistically significant difference between the generation times of neurons of the nucleus oralis and either the nucleus interpolaris ($p < 0.1435$) or the subnucleus magnocellularis of the nucleus caudalis ($p < 0.1435$) of the trigeminal complex (Altman and Bayer, '80a).

The raphe and reticular nuclei

The raphe magnus. The raphe neurons of the rostral medulla correspond to the nucleus raphe magnus of several authors (e.g., Taber, '61; Valverde, '62) and the inferior central nucleus of others (e.g., Berman, '68), which may be a separate nucleus (Fig. 2A). The cells range in size from the largest found in the medulla to very small (Figs. 9,10). The serotonin-containing cells of the region were designated as group B2 (Dahlström and Fuxe, '64). Evidence has been presented that, in the rat, the afferents of the medullary raphe nuclei originate rostral to the pons, especially in the central gray and the tegmentum of the mesencephalon (Gallager and Pert, '78). Projections from the neocortex have also been described (Valverde, '62; Bentivoglio et al., '78). Descending efferents of the raphe magnus have been traced to the ventral horn of the spinal cord of the cat (Bobillier et al., '76) by way of the dorsolateral funiculus (Martin et al., '78). Ascending projections have been found to the central gray, the superior colliculus, the pretectal area, interpeduncular nucleus, parts of the thalamus and hypothalamus, and as far rostrally as the diagonal band of Broca (Bobillier et al., '76).

For purposes of quantification, cells of the raphe magnus were scanned ventral to the nucleus prepositus. The results (Fig. 2D) indicate peak formation time on days E12 and E13 in the dorsal raphe magnus. In some regions, particularly ventrally, there was no difference in the labelling pattern of large and small neurons (Fig. 9). In other regions (Fig. 10), the larger cells were no longer labelled in

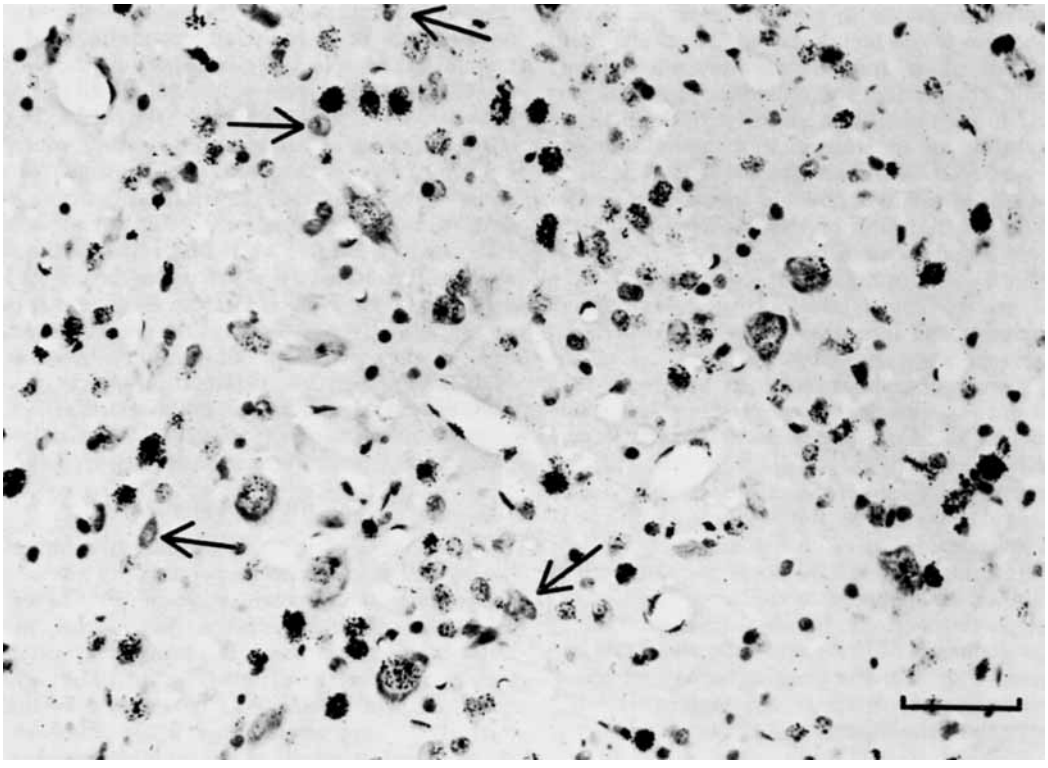


Fig. 7. Nucleus oralis of the trigeminal complex, from a rat injected on days E14 + 15. All large neurons are labelled but some smaller neurons (arrows) are not. Scale, 50 μm.

the E13 + 14 group while the smaller cells were still labelled. These differences may be related to the differential time of origin of the neighboring motor nuclei. The raphe neurons situated between the relatively late-forming facial nucleus neurons (Fig. 9) were the ones that were labelled in higher proportion than the raphe neurons situated in the vicinity of the earlier generated abducens nucleus neurons (Fig. 10) Neuron production in the dorsal aspect of the raphe magnus antedated significantly the generation of neurons in the raphe pallidus ($p < 0.0001$) and the raphe obscurus ($p < 0.0001$) of the lower medulla (Altman and Bayer, '80a).

The rostral medullary reticular formation. The reticular formation of the rostral medulla is bounded medially by the nucleus raphe magnus, dorsally by the vestibular nuclei, the nucleus prepositus, and the rostral extension of the solitory nucleus, and laterally by the nucleus oralis of the spinal trigeminal complex. Its neurons range in size from the largest to the smallest cells (Figs. 11–12). Afferents to it have been described from the sensory and motor cortices (Valverde, '62), the tectum (Peterson et al., '74), and from the level of the pons as far caudally as the spinal cord (Gallager and Pert, '78). An electrophysiological study in the rat (Groves, et al., '73) suggested that there is a differential distribution of sense modalities in the reticular formation from the level of the midbrain caudally, and that neurons at the rostral medullary level tend to respond to tactile stimulation of the anterior parts of the body. The relation of the medullary reticular formation to the trigeminal system has been discussed previously (Altman and Bayer, '80a), and the relation of the pontine and midbrain reticular formation to the vestibular and ocular systems will be described in a later paper (Altman and Bayer, '80c). Efferents to the spinal cord are well established as far caudally as the lumbar level (Torvik and Brodal, '57).

The rostral medullary reticular formation is a heterogeneous region. In an effort to

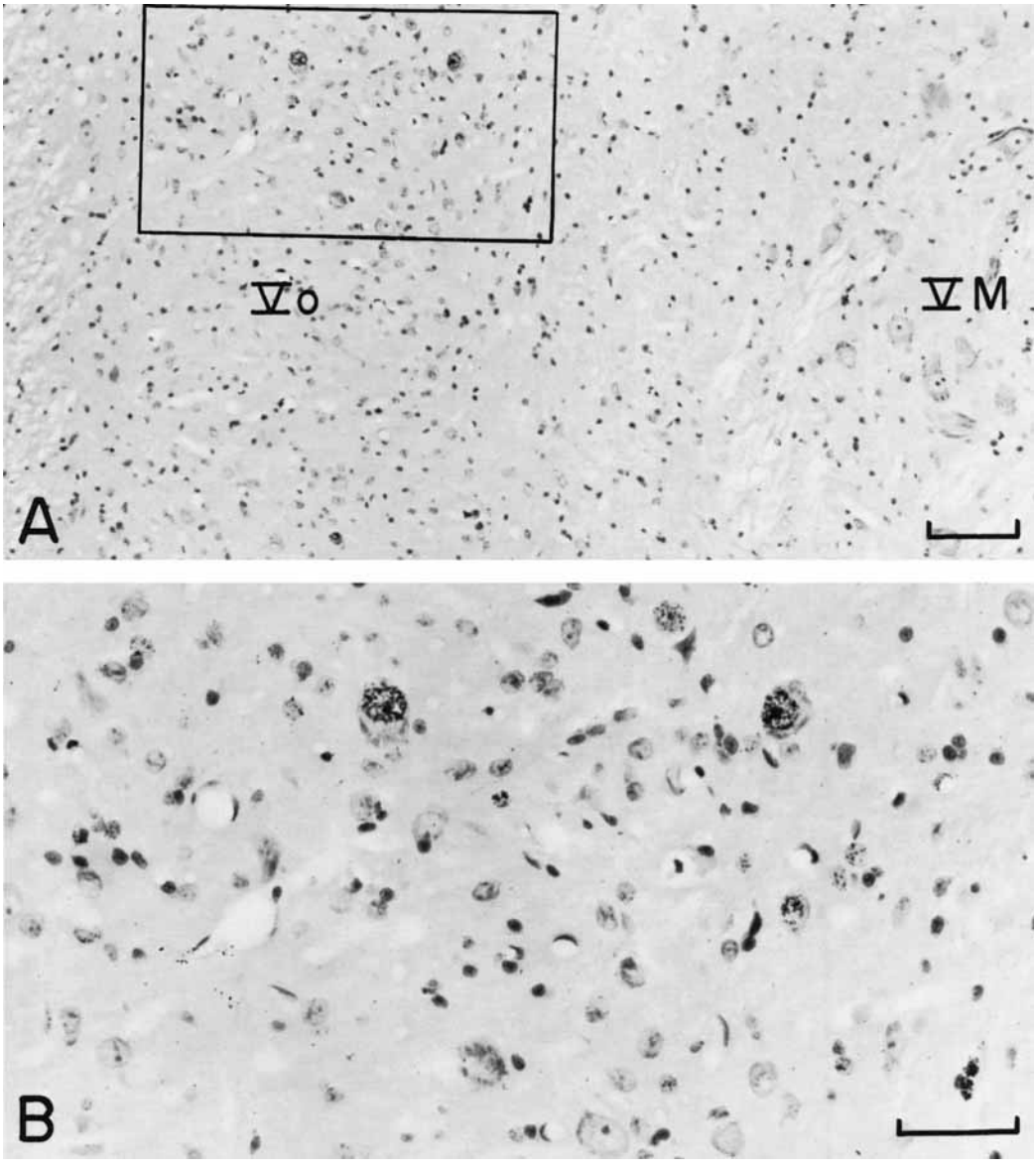


Fig. 8. A. Rostral part of the nucleus oralis of the trigeminal (near caudal portion of the motor nucleus of the trigeminal); coronal section from a rat injected on days E16 + 17. The few labelled cells include the largest. Area in rectangle is shown at higher magnification in B. Scales: A, 100 μm; B, 50 μm.

sample comparable regions, cell counting was restricted to a small-celled region ventrolateral to the prepositus nucleus. The results indicated peak cell acquisition time on days E13 and E14, with a few cells arising on day E15 (Fig. 2D). In adjacent regions with a higher proportion of large cells there was a tendency for some of the largest cells to be labelled when some of the smaller cells were no longer labelled (Fig. 12), resembling in this respect the adjacent nucleus oralis of the trigeminal tract. More caudally and ventrally, the large cells of the gigantocellular region (Fig. 13) typically originated on days E12 and E13.

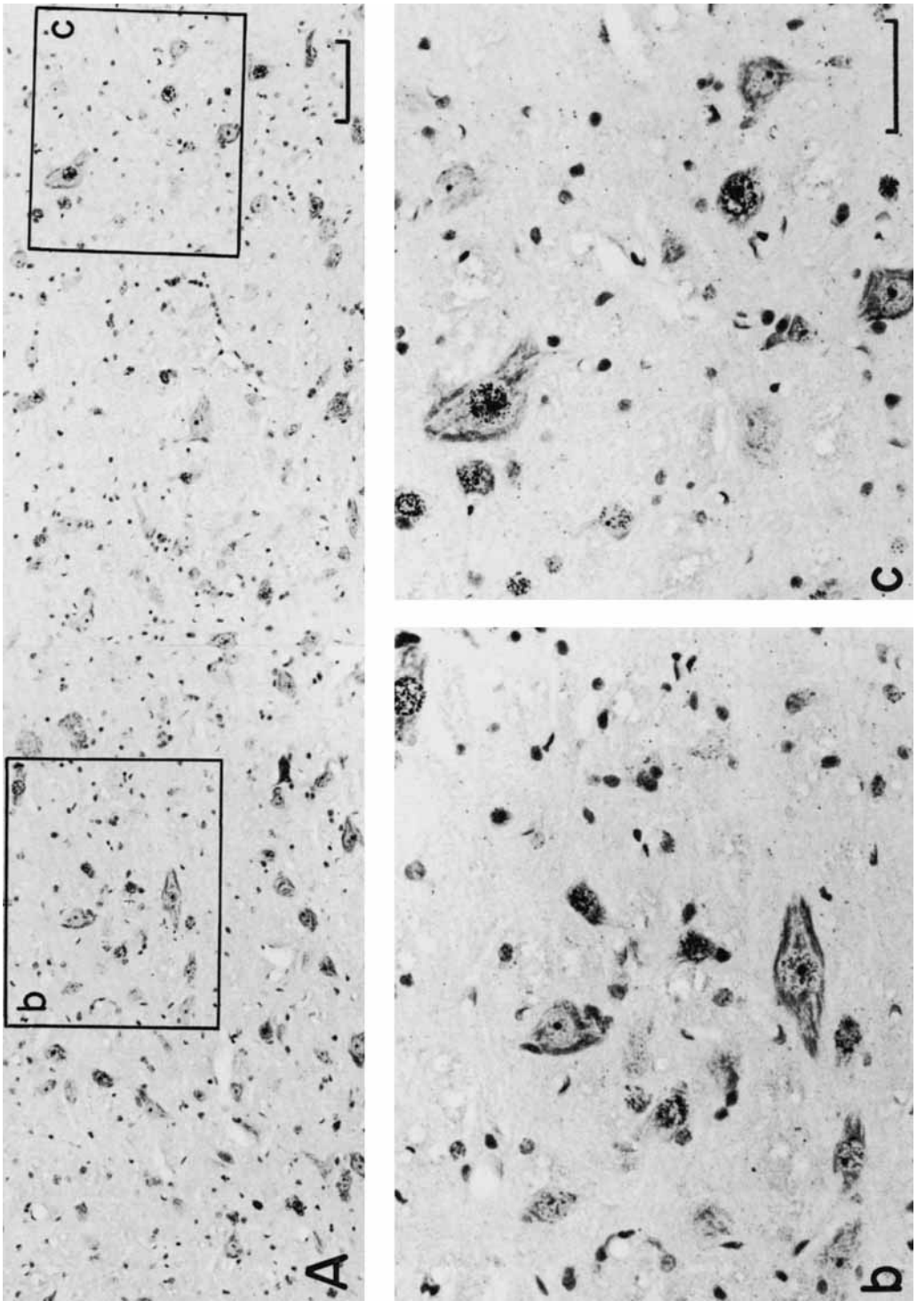


Fig. 9. A. Coronal section of neurons of raphe magnus (or inferior central nucleus between the facial nuclei), from a rat injected on days E13 + 14. Areas shown in rectangles b and c are magnified in 9b and 9c. Note the labelling of some cells of variable sizes. Scales: A, 100 μ; b and c, 50 μm.

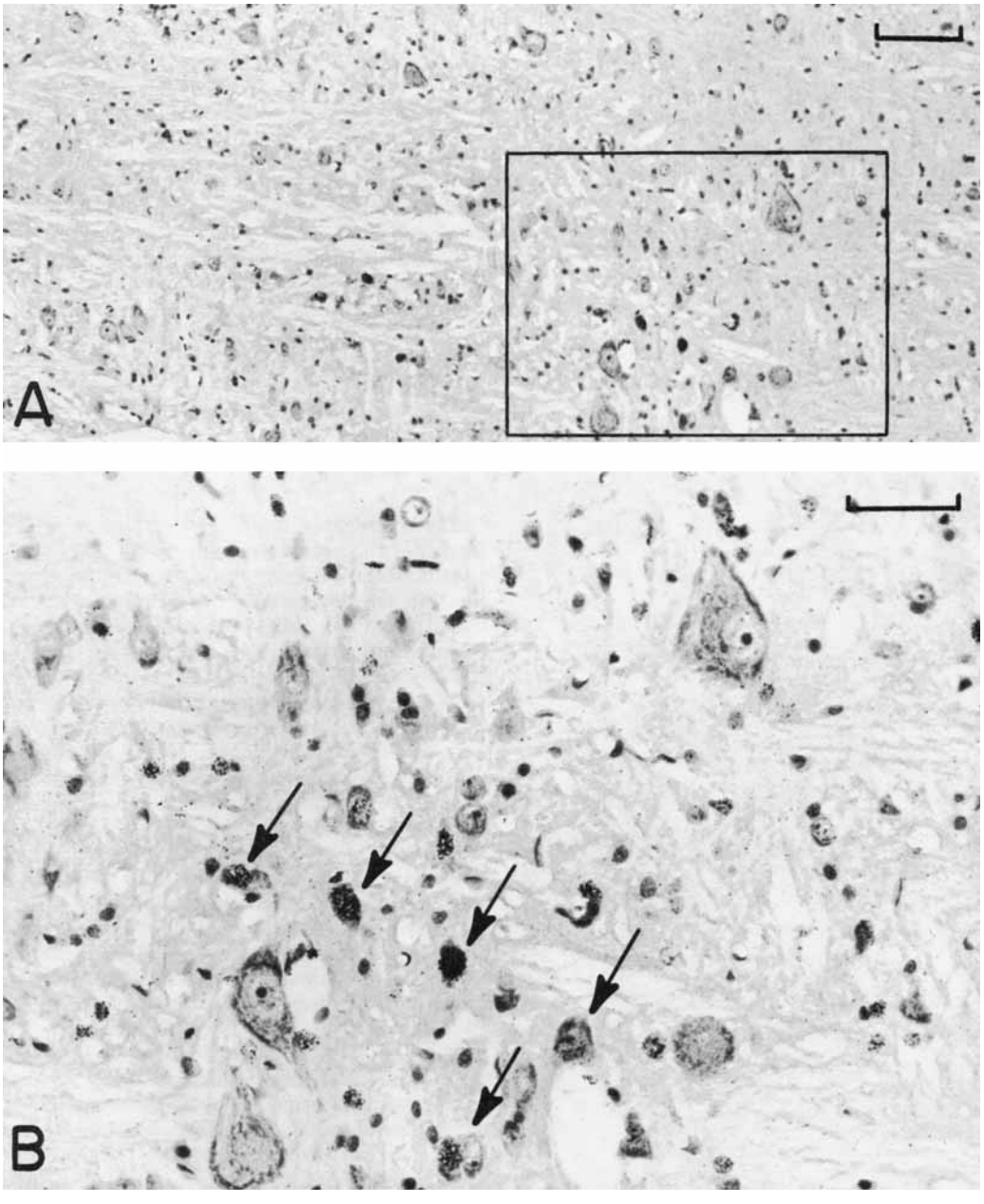
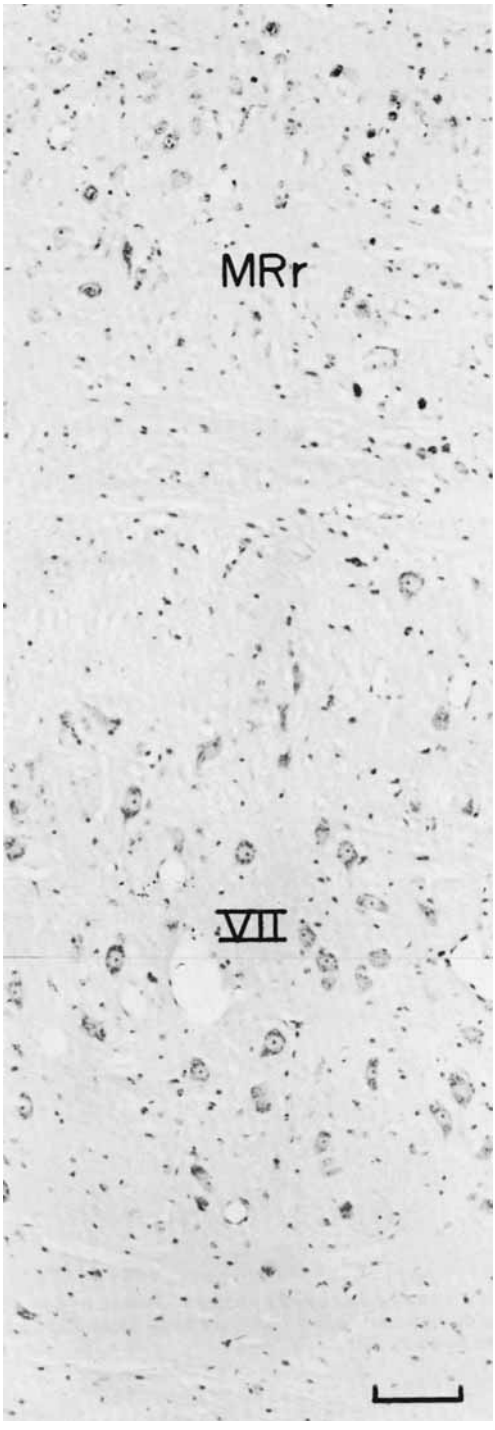


Fig. 10. A. Sagittal section of the nucleus raphe magnus from a region adjacent to the abducens nucleus, from a rat injected on days E13 + 14. Area shown in rectangle magnified in B. Note that in this region the large neurons are not labelled but many of the intermediate and small cells (arrows) are. Scale: A, 100 μm; B, 50 μm.



Granular layer of the raphe. The granular layer of the raphe is a term applied by Berman ('68, p. 17) to an unpaired narrow band of cells situated basally in the midline of the medulla; it is illustrated in horizontal sections of the cat brain stem by Berman ('68, e.g., pp. 44, 45). It can be identified in other atlases of the brain stem but is usually not named, unless it is identical with the arcuate nucleus of Wünscher et al. ('65: Figs. 28a,29a). This layer or midline nucleus is prominent in the rat and contains many small and some intermediate-sized cells.

In autoradiograms this raphe nucleus stands out by the relatively late generation of its neurons: Many of the cells are labelled in the E16 + 17 group (Fig. 14) and a few of them were labelled in animals injected as late as E17 + 18. This region is the latest forming component of the rostral medulla.

DISCUSSION

The motor nuclei

There are four motor nuclei in the upper medulla: the retrofacial, the facial, the abducens, and the superior salivatory. Of these, the neurons of the retrofacial nucleus are produced first. The latter, as we shall see in the succeeding papers, are the earliest-arising motor neurons of the entire brain stem. The only cells that are generated before the retrofacial neurons in the brain stem are those of the mesencephalic nucleus of the trigeminal (Altman and Bayer, '80c), which are considered to be primary afferent neurons, analogous to the neurons of the spinal and cranial ganglia. It is interesting that the retrofacial neurons are contiguous with the neurons of the ambiguus nucleus which, in sharp contrast, are among the last produced motor neurons of the brain stem (Altman and Bayer, '80a). Indeed, as we described earlier, what we have identified here as the retrofacial nucleus (Wünscher et al., '65) is often labelled as a portion of the ambiguus nucleus. But our thymidine-radiographic datings show clearly that they are cytogenetically different: Peak generation time of retrofacial neurons is day E11 or earlier (we lack an E11 + 12 injection group and therefore cannot date specifically

Fig. 11. The rostral medullary reticular formation in sagittal section from a rat injected on days E14 + 15. A high proportion of the cells of the reticular formation are labelled but very few are in the facial nucleus. Scale, 100μm.

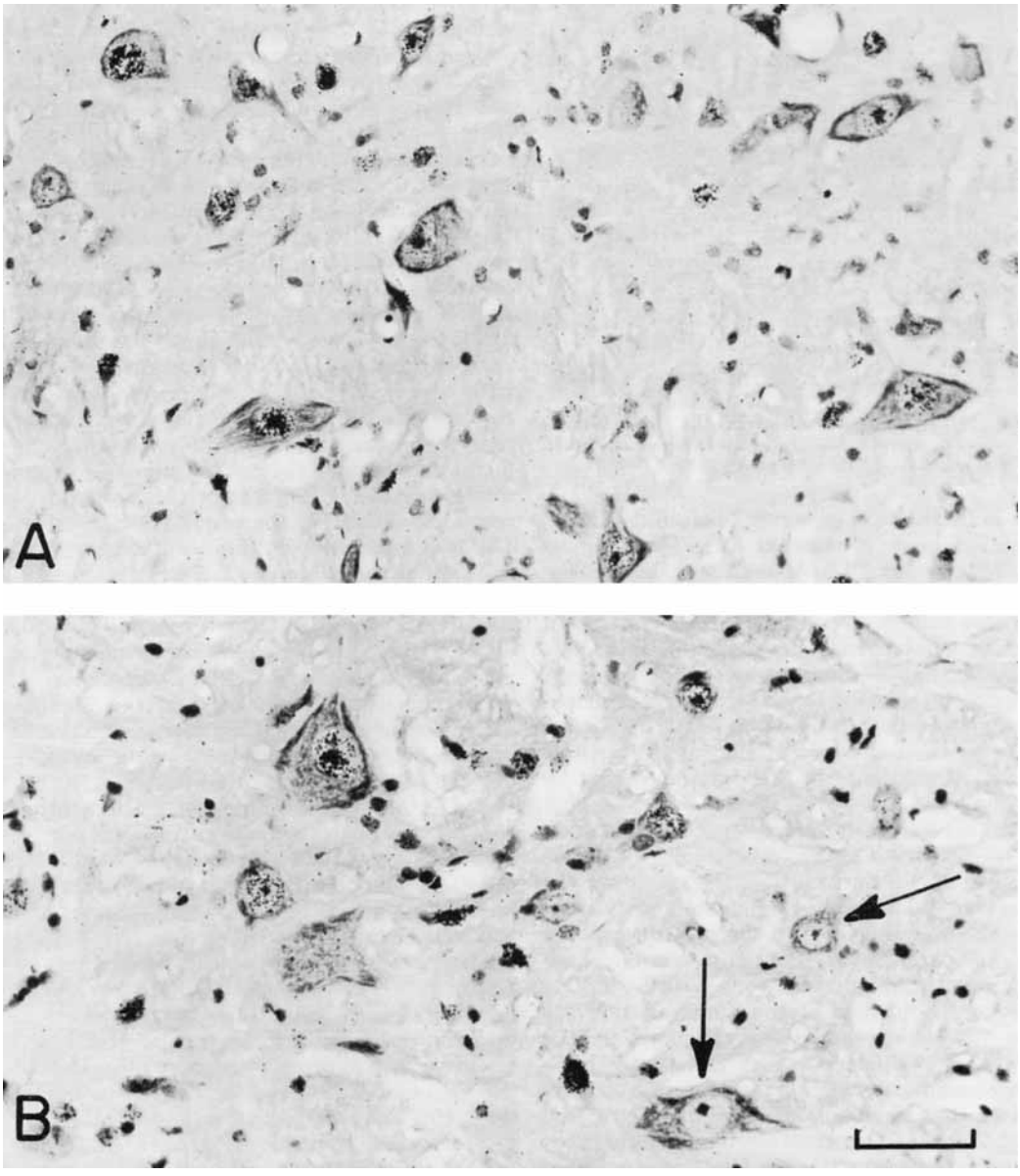


Fig. 12. Neurons of the rostral medullary reticular formation from rats injected on days E12 + 13 (A) and E13 + 14 (B). In A all the cells are labelled whereas in B a very large cell is labelled but two smaller cells (arrows) are not. Scale: 50 μm.

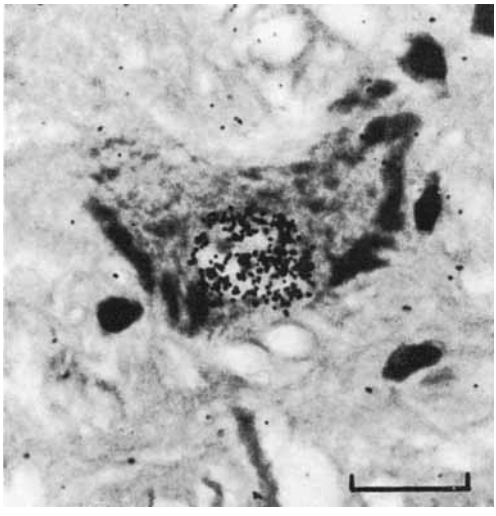


Fig. 13. A labelled neuron from the gigantocellular region of the rostral medullary reticular formation, from a rat injected on days E13 + 14. Scale, 20 μm.

the cells that are generated before day E12), whereas peak generation time of ambiguus neurons is day E15 (Altman and Bayer, 80a: Fig. 4A).

Lawn's ('66) investigations in the rabbit with the retrograde degeneration technique showed that a compact cell mass situated ventrolateral to the rostral tip of the principal column of the ambiguus nucleus is distinguished from the rest of the nucleus (which sends its efferents by way of the vagus) by sending its efferents by way of the glossophyaryngeal nerve. If this compact cell mass is identical with what we describe in the rat as the retrofacial nucleus then the latter may be considered a nucleus of glossopharyngeal efferents. We suggested in the preceding paper (Altman and Bayer, '80a) that the late generation of ambiguus neurons with respect to the dorsal nucleus of the vagus (which are believed to be preganglionic efferents) and the hypoglossal nucleus (which innervates muscles of somitic derivation) is due to the circumstance that the ambiguus neurons are related to muscles of branchial derivation (pharynx, larynx, and part of the esophagus). If so, then the early generated retrofacial neurons might innervate muscles of somatic derivation. The best conjecture is that these muscles might be involved in voluntary swallowing (Doty, '68; Hockman et al., '79).

In addition to the nucleus ambiguus, two other nuclei are considered to constitute parts of the branchial efferent system (Patten and Carlson, '74): the facial nerve nucleus and the motor nucleus of the trigeminal complex. The facial nucleus is distinguished from the other motor nuclei so far considered (excepting the ambiguus) by the relatively late production of its neurons. Peak generation time (over 80% of cells) of the hypoglossal neurons (Altman and Bayer, '80a) and of the abducens neurons (this paper) is day E12; that of the facial nucleus neurons is day E13. This might suggest that the motor neurons supplying somatic muscles (the tongue and the lateral rectus, respectively) are produced before motor neurons supplying branchial muscles (the facial and the abducens). But, as we shall document in a succeeding paper (Altman and Bayer, '80c), the peak production time of motor neurons of the trigeminal nerve is also day E12, with nearly 80% of the cells forming on that day. As an alternative possibility we suggest that the motor nucleus of the trigeminal, the facial nucleus, and the nucleus ambiguus form a single cytogenetic system with a pronounced rostral-to-caudal internuclear gradient (Fig. 15). It is interesting in this respect that there is a similar rostral-to-caudal intranuclear gradient within the facial nerve nucleus. We shall tentatively designate this branchial longitudinal chain of motor neurons as cytogenetic zone MB (Altman and Bayer, '80a).

For the present we shall defer a general discussion of the motor neurons of muscles of somatic derivation until we have quantitative datings of the trochlear and oculomotor neurons. The data at hand suggests a caudal-to-rostral internuclear gradient from the hypoglossal nucleus to the nuclei of the extraocular muscles, the opposite of that of the branchial motor neurons (Fig. 15). Interestingly, the intranuclear gradient within the hypoglossal nucleus is also caudal-to-rostral (Altman and Bayer, '80a), the opposite of that seen in the facial nucleus. A caudal-to-rostral gradient is also indicated for the two nuclei of preganglionic motor neurons—the dorsal nucleus of the vagus and the superior salivatory nuclei (Fig. 15). They may constitute another cytogenetic system, tentatively designated as zone PG. Another noteworthy feature is the different settling patterns of motor neurons supplying muscles of somatic and branchial derivation: the former being located dorsally and medially (remaining near the ventricle), the latter more laterally and ventrally. The exception to this is the retrofacial nucleus. The source and migration routes of these two nu-

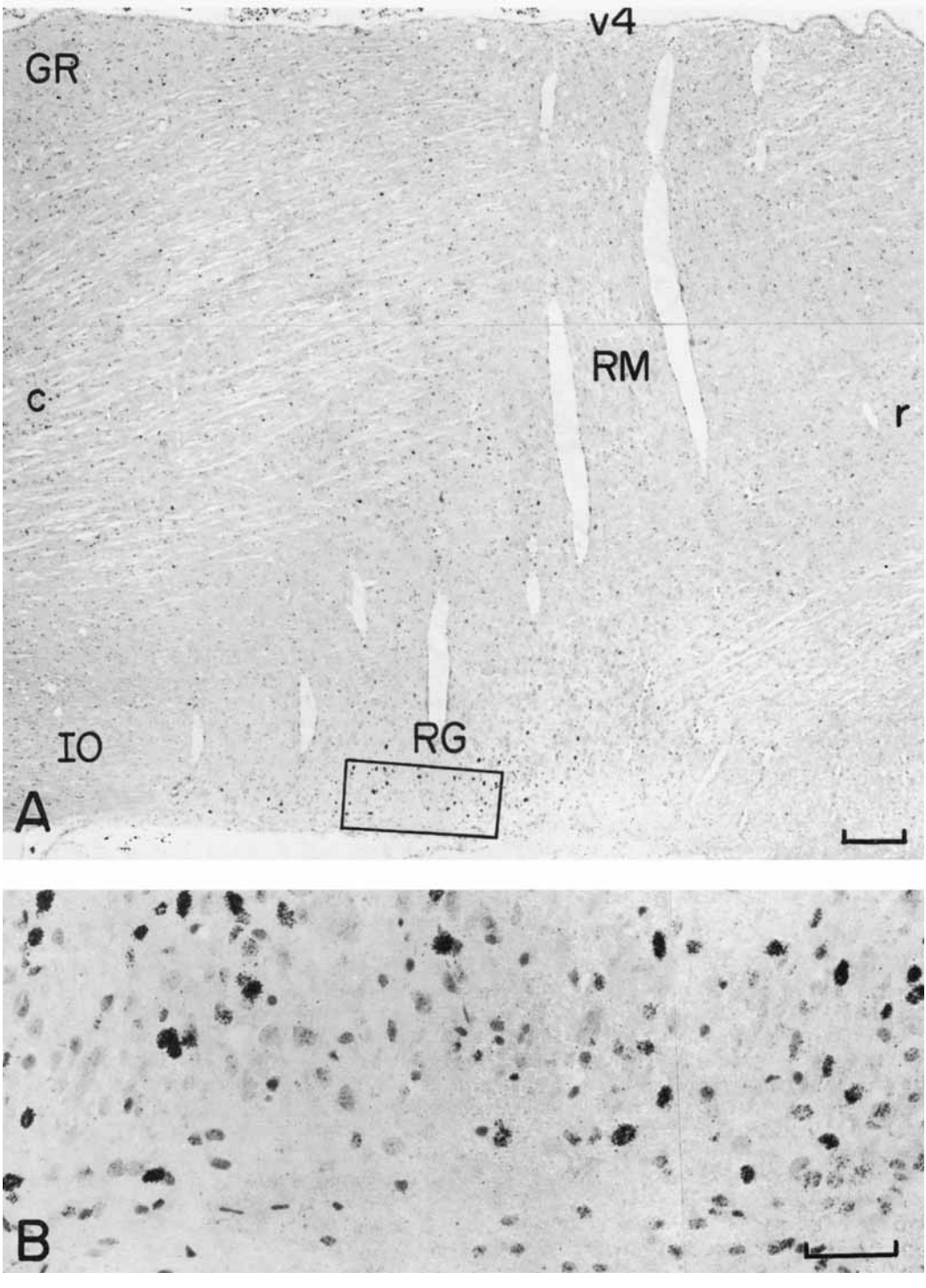


Fig. 14. A. Midsagittal section of the medulla showing the labelled cells of the granular layer of the raphe. The area in the rectangle is shown at higher magnification in B. Scales: A, 200 μm; B. 50 μm.

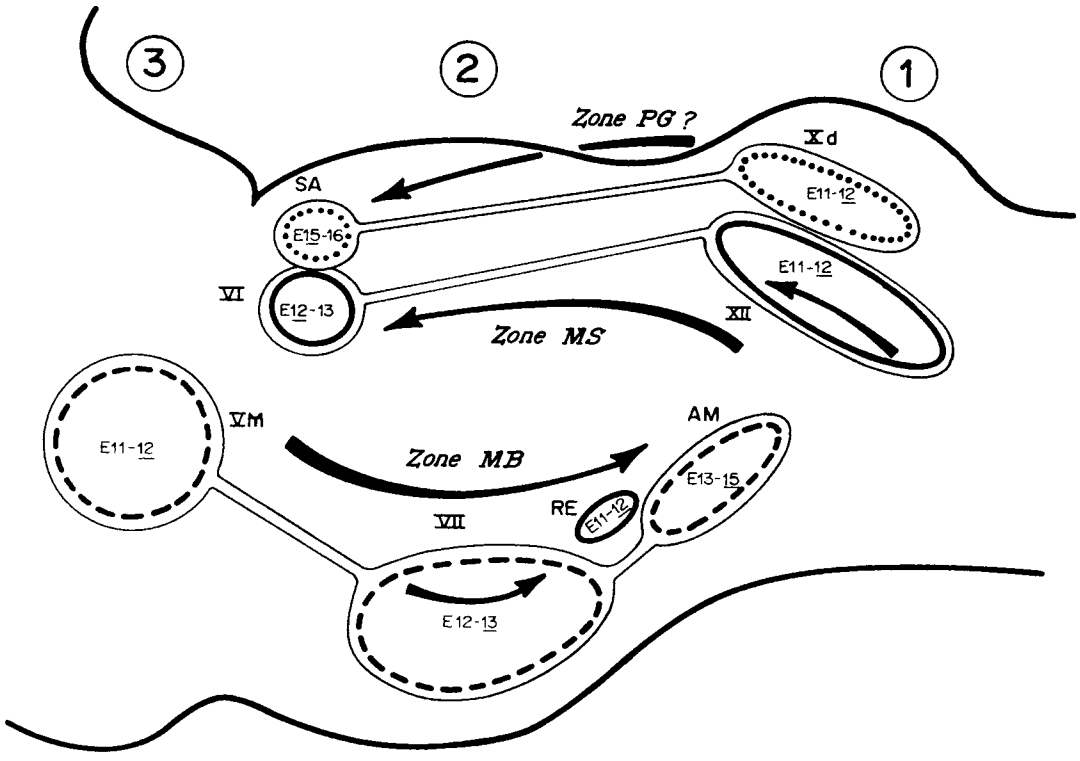


Fig. 15. Summary diagram of the three postulated cytogenetic zones (MB, MS, and PG), extending from the lower medulla (1), through the upper medulla (2) to the pontine region (3). The numerals within the nuclei are approximate dates of neuron generation; the day underlined represents peak production time. Arrow within a nucleus designates direction of intranuclear cytogenetic gradient; large outside arrows are the internuclear gradients of the cytogenetic zones.

clear systems will have to be examined in embryonic material. Tentatively we shall designate the abducens nucleus as part of the MS cytogenetic zone (Altman and Bayer, '80a).

Other regions of the upper medulla

The nuclei related to the two special sensory systems of the upper medulla—the vestibular and auditory systems—will be described in the next paper (Altman and Bayer, '80b). The single sensory structure that we dealt with here is the nucleus oralis of the trigeminal nerve. The bulk of the neurons of the nucleus oralis are generated between days E13-15. Excepting some nuclei of the auditory system (cochlear and superior olivary), some of the neurons of the nucleus oralis form later than those so far considered. It was an unusual finding that the last produced cells of the nucleus oralis tend to be its largest neurons (some of which arise as late as day E16), which is the converse of what is seen in most brain regions. As we shall see, the large neurons are also generated last more rostrally in the principal nucleus of the trigeminal (Altman and Bayer, '80c). In contrast, in the nucleus caudalis, where very small cells are found in high concentration in particular in the subnucleus marginalis, the latter are the youngest elements. Taking the trigeminal complex as a whole, the smallest and largest cells are its youngest elements and an intermediate cell type its oldest. Although there are studies about the projections of different cell types of the trigeminal (Karamanlidis, '68; Ikeda, '79; Fukushima and Kerr, '79) the significance of the cytogenetic difference cannot as yet be assessed.

There are some difficulties in trying a comparison of the time or origin of neurons in the different nuclei of the trigeminal complex because the caudal nucleus is laminated and the nucleus oralis and nucleus interpolaris are not. If the comparison is made between the

latter two and the subnucleus magnocellularis of the nucleus caudalis, which is composed of large as well as smaller cells, there is no difference in their generation time. We conclude tentatively that in the trigeminal nuclei (what we designated as cytogenetic zone TR; Altman and Bayer, '80a: Fig. 26) there is no longitudinal gradient present. The late generation of the cells of the subnucleus zonalis of the caudal nucleus might represent a medial-to-lateral (inside out) intranuclear gradient. An internuclear medial-to-lateral gradient is also present at the level of the nucleus oralis with respect to the nucleus raphe magnus and the rostral medullary reticular formation (Fig. 2C,E). At present it is not clear whether or not all three components of the upper medulla are functionally related; the physiological evidence reviewed earlier suggested a relationship between the trigeminal nuclei and the medullary reticular formation. In general, the following cytogenetic order is indicated for the structures of the upper medulla so far considered: The motor nuclei of somatic muscles are first and the sensorsy nucleus of the trigeminal is last. The branchial motor nucleus of the facial, the raphe magnus and the reticular formation occupy an intermediate position.

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