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Development of the Brain Stem in the Rat. V. Thymidine-Radiographic Study of the Time of Origin of Neurons in the Midbrain Tegmentum

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ABSTRACT Groups of pregnant rats were injected with two successive daily doses of ^{3}H-thymidine from gestational day E12 and 13 (E12 + 13) until the day before parturition (E21 + 22) in order to label in their embryos the proliferating precursors of neurons. At 60 days of age the proportion of neurons generated (no longer labeled) on specific embryonic days was determined quantitatively in 18 regions of the midbrain tegmentum. The neurons of the oculomotor and trochlear nuclei are generated concurrently on days E12 and E13. There was a mirror image cytogenetic gradient in these nuclei and this was interpreted as the dispersal of neurons derived from a common neuroepithelial source to the medial longitudinal fasciculus. Neurons in three other components of the tegmental visual system are produced in rapid succession after the motor nuclei. In the nucleus of Darkschewitsch peak production time was on days E12 and E13, extending to day E15; in the Edinger-Westphal nucleus the time span was the same but with a pronounced peak on day E13; finally, the neurons of the parabigeminal nucleus were produced between days E13 and E15 with a peak on day E14. The neurons of the periaqueductal gray were generated between days E13 and 17 with a pronounced ventral-to-lateral and lateral-to-dorsal gradient.

In the red nucleus the neurons were produced on days E13 and E14 with a caudal-to-rostral gradient: the cells of the magnocellular division preceding slightly but significantly the cells of the parvocellular division. The neurons of the interpeduncular nucleus originated between days E13 and E15; the peak in its ventral portion was on day E13, in its dorsal portion on days E14 and E15. A ventral-to-dorsal gradient was seen also in both the dorsal and the median raphe nuclei in which neuron production occurred between days E13 and E15. The neurons of the pars compacta and pars reticulata of the substantia nigra were both produced between days E13 and E15 with a modified lateral-to-medial gradient. This gradient extended to the ventral tegmental area where neurons of the pars medialis were produced between days E14 and E16. With the exception of the central gray, neuron production was rapid and relatively early in the structures situated ventral to the midbrain tectum. A comparison of the cytogenetic gradients in the raphe nuclei of the lower and upper medulla, the pontine region, and the midbrain suggests that they originate from at least three separate neuroepithelial sources.

The preceding papers of this series (Altman and Bayer, '80a–d) dealt with the time of origin of neurons in the caudal parts of the brain stem: the medulla and the pons. The present paper is concerned with neurogenesis in the midbrain tegmentum. We shall deal in separate publications with the time of origin of neurons in the inferior and superior colliculi (Altman and Bayer, '80e,f). The tegmental structures examined in this paper include nuclei concerned with visual functions: the trochlear and oculomotor nuclei, the nucleus of Darkschewitsch, the Edinger-Westphal nucleus, and the parabigeminal nucleus. The other tegmental structures that we quantified are the dorsal and median raphe nuclei, the red nucleus, the interpeduncular nucleus, the ventral tegmental area, and the substantia nigra, and we also included the central gray.

To our knowledge there is presently no comprehensive quantitative study available of the time of origin of neurons in the rat tegmentum.

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Hanaway et al. ('70) have provided data on the time of origin of neurons in the substantia nigra, ventral tegmental area, and interpeduncular nucleus of the rat, and Lauder and Bloom ('74) studied the time of origin of neurons in the raphe nuclei and substantia nigra in the same species. Taber Pierce ('73) summarized her semiquantitative results of ^{3}H-thymidine labeling in the mouse brain stem. Datings of the birth dates of neurons in the nuclei of the extraocular muscles of the Peking Duck were presented by Sohal and Holt ('77).

As in the previous papers of the series, we make an attempt here to identify groups of structures which in terms of similarities in their dates and patterns of cell production, and shared cytogenetic gradients, may constitute discrete cytogenetic zones or systems. The assumption is that all structures belonging to the same cytogenetic zone derive from a single and a unique germinal site in the embryo, possibly from the same cell line. The datings obtained in these radiographic studies in adults specify the ages when the postulated germinal site should be maximally active in the embryo, and the cytogenetic gradients (lateral-to-medial, ventral-to-dorsal, etc.) offer the first clue as to where in the neuroepithelium the germinal site in question may be located and what routes the migrating cells take to reach their settling site.

MATERIALS AND METHODS

The thymidine-radiographic material used in this study is identical with that used in the preceding investigations of the caudal parts of the brain stem (Altman and Bayer, '80a–d). The proportion of labeled cells was quantified in samples of 100 or more cells in 18 regions in six animals each of all relevant injection groups from days E12+13 to days E21+22. The method and rationale of the progressively delayed comprehensive labeling procedure used in these studies to estimate the proportion of neurons produced (ceasing to divide) on a particular day were presented in the first paper of the series (Altman and Bayer, '80a) together with a description and justification of the statistical test used (Conover's sign test) to evaluate the results.

RESULTS

Summary quantitative data

The locations of the components of the mesencephalic tegmentum that were evaluated quantitatively are indicated schematically in two coronal planes (Fig. 1A,B). The estimated time of origin of neurons in 18 structures is presented in Figures 1C–G and 2A,B. Details of the results and relevant statistics are provided below together with qualitative observations.

The nuclei of the ocular motor system

The oculomotor and trochlear nuclei. The trochlear nucleus innervates the superior oblique muscle of the eye; the oculomotor nucleus innervates the superior rectus, the levator palpebrae, the inferior rectus, the inferior oblique, and the medial rectus. On the basis of experimental work in the monkey, Warwick ('53) concluded that in the oculomotor nucleus the different eye muscles are represented by separate groups of motor neurons. The pattern of localization in the oculomotor nucleus of different species appears to vary (Tarlov and Tarlov, '71; Gacek, '74; Akagi, '78). Moreover, there is disagreement about the exact localization of different muscles in the cat. Tarlov and Tarlov ('71), using the retrograde degeneration technique, have described three dorsoventrally arranged longitudinal groups of motor neurons for the medial rectus, inferior oblique, and inferior rectus, respectively. In contrast, Gacek ('74) and Akagi ('78), using retrograde labeling with horseradish peroxidase, reported an arrangement of longitudinal slabs of motor neurons from lateral to medial. The pattern found in the rat (Glicksman, '80) resembles that of the rabbit (Akagi, '78).

Afferents reach the oculomotor and/or trochlear nucleus from the vestibular nuclei (Szentágothai, '50; Highstein, '73; Yamamoto, et al., '78; Gacek, '79); from each other and the abducens nucleus (Strominger and Strominger, '65); and from the accessory oculomotor nuclei (Carpenter et al., '70). Other inputs come from the prepositus nucleus (Graybiel and Hartwieg, '74; Baker and Berthoz, '75; Gacek, '79) and the reticular formation (Keller, '74; Büttner-Ennever and Henn, '76). The major fiber tract by which afferents reach the eye muscle nuclei is the medial longitudinal fasciculus, but some fibers from superior vestibular nucleus have been traced by way of the brachium conjunctivum (Yamamoto et al., '78).

The oculomotor and trochlear nuclei approximate each other very closely in the rat brain. In order to assure that the two were not confused in the quantification, scanning of the cells of the trochlear nucleus was started in the most posterior sections in which they could be identified, and the scanning of the cells of the oculomotor nucleus was started in the most anterior sections. In most animals a small gap between the two nuclei could be identified, and, quite consistently, the neurons of the oculomotor nucleus were situated closer to the midline than the neurons of the trochlear nucleus (Fig. 3). The lateral displacement of the trochlear neurons could be related to the

presence medially of the rostral portion of the dorsal raphe nucleus. In both ocular muscle nuclei cell counts were restricted to the large multipolar (motor) neurons.

In the trochlear nucleus close to 60% of the cells originated on day E12 and the rest on day E 13 (Fig. 2A). A similar pattern of cytogenesis was obtained in the oculomotor nucleus, with a slightly higher percentage of cells arising on day E13 (Fig. 2A). Occasional labeled neurons were seen in the oculomotor nucleus of E14 + 15 rats. Observations in coronal sections indicated mirror-image intranuclear gradients in the two nuclei: in the oculomotor nucleus from rostral to caudal (anteroventral to posterodorsal) and in the trochlear nucleus from caudal to rostral. In sagittal sections of the oculomotor nucleus (Figs. 4, 5) the cytogenetic gradient was identified as being proximal-to-distal in relation to the medial longitudinal fasciculus. As shown in Figure 6A, the medial longitudinal fasciculus curves between the oculomotor and trochlear nuclei. Accordingly, the mirror-image gradients in the two nuclei may reflect the dispersal of neurons derived from a common neuroepithelial source (the embryonic aqueduct) in two directions (Fig. 6B) and the settling of the earliest generated neurons closest to the fibers of the medial longitudinal fasciculus. The difference between the generation time of the trochlear and oculomotor nuclei was not significant ($P < 0.125$).

The Edinger-Westphal nucleus and the nucleus of Darkschewitsch. The Edinger-Westphal nucleus is an unpaired, midline structure made up mostly of small, densely packed cells. It begins caudally as a narrow band between the oculomotor nuclei, and it widens and dips ventrally as it extends in the rostral direction (Berman, '68). It has been assumed for some time that the Edinger-Westphal nucleus is the source of the parasympathetic outflow to the ciliary ganglion, which supplies fibers to the muscles of pupillary constriction and lens accomodation. This conclusion was supported by the retrograde degeneration study of Warwick ('54). However, recent studies utilizing anterograde and retrograde axoplasmic tracer techniques (Loewy and Saper, '78; Loewy et al., '78) suggest that only some of the cells of the Edinger-Westphal nucleus supply fibers to the ciliary ganglion. Rather, there is a widespread descending projection to the inferior olive, the parabrachial nucleus, components of the trigeminal complex, and to the spinal cord. Horseradish peroxidase applied to the root of the oculomotor nerve (Sugimoto et al., '78) backfilled only a few cells in the Edinger-Westphal nucleus; others were found in the adjacent central gray and tegmentum.

Abbreviations

A	aqueduct
c	caudal
CGd	central gray, pars dorsalis
CGl	central gray, pars lateralis
CGp	periventricular central gray
CGv	central gray, pars ventralis
cp	cerebral peduncle
cs	commissure of the superior colliculus
dl	dorsolateral
DR	dorsal raphe nucleus
DRd	dorsal raphe nucleus, pars dorsalis
DRv	dorsal raphe nucleus, pars ventralis
EW	Edinger-Westphal nucleus
HI	hippocampus
hp	habenulopeduncular tract
IC	inferior colliculus
IP	interpeduncular nucleus
IPd	interpeduncular nucleus, pars dorsalis
IPl	interpeduncular nucleus, pars lateralis
IPm	interpeduncular nucleus, pars medialis
l	lateral
LC	locus coeruleus
m	medial
MB	mammillary body
MG	medial geniculate body
MLF	medial longitudinal fasciculus
MRd	median raphe nucleus, pars dorsalis
MRv	median raphe nucleus, pars ventralis
MRF	mesencephalic reticular formation
NC	nucleus cuneiformis
ND	nucleus of Darkschewitsch
NO	nucleus of the optic tract
pc	posterior commissure
PG	parabigeminal nucleus
PGd	parabigeminal nucleus, pars dorsalis
PGm	parabigeminal nucleus, pars medialis
PGv	parabigeminal nucleus, pars ventralis
PN	pontine gray nuclei
PO	posterior thalamic nucleus
r	rostral
RE	retrofacial nucleus
RM	raphe magnus
RN	red nucleus
RNm	red nucleus, pars magnocellularis
RNp	red nucleus, pars parvocellularis
ROd	raphe obscurus, pars dorsalis
RPM	raphe pontis magnocellularis
RPP	raphe pontis parvocellularis
RPv	raphe pallidus, pars ventralis
SC	superior colliculus
SNc	substantia nigra, pars compacta
SNr	substantia nigra, pars reticulata
so	subcommissural organ
tdd	dorsal tegmental decussation
tdv	ventral tegmental decussation
vm	ventromedial
VTl	ventral tegmental area, pars lateralis
VTm	ventral tegmental area, pars medialis
III	oculomotor nucleus
IV	trochlear nucleus
VI	abducens nucleus
IX	glossopharyngeal nucleus
XII	hypoglossal nucleus

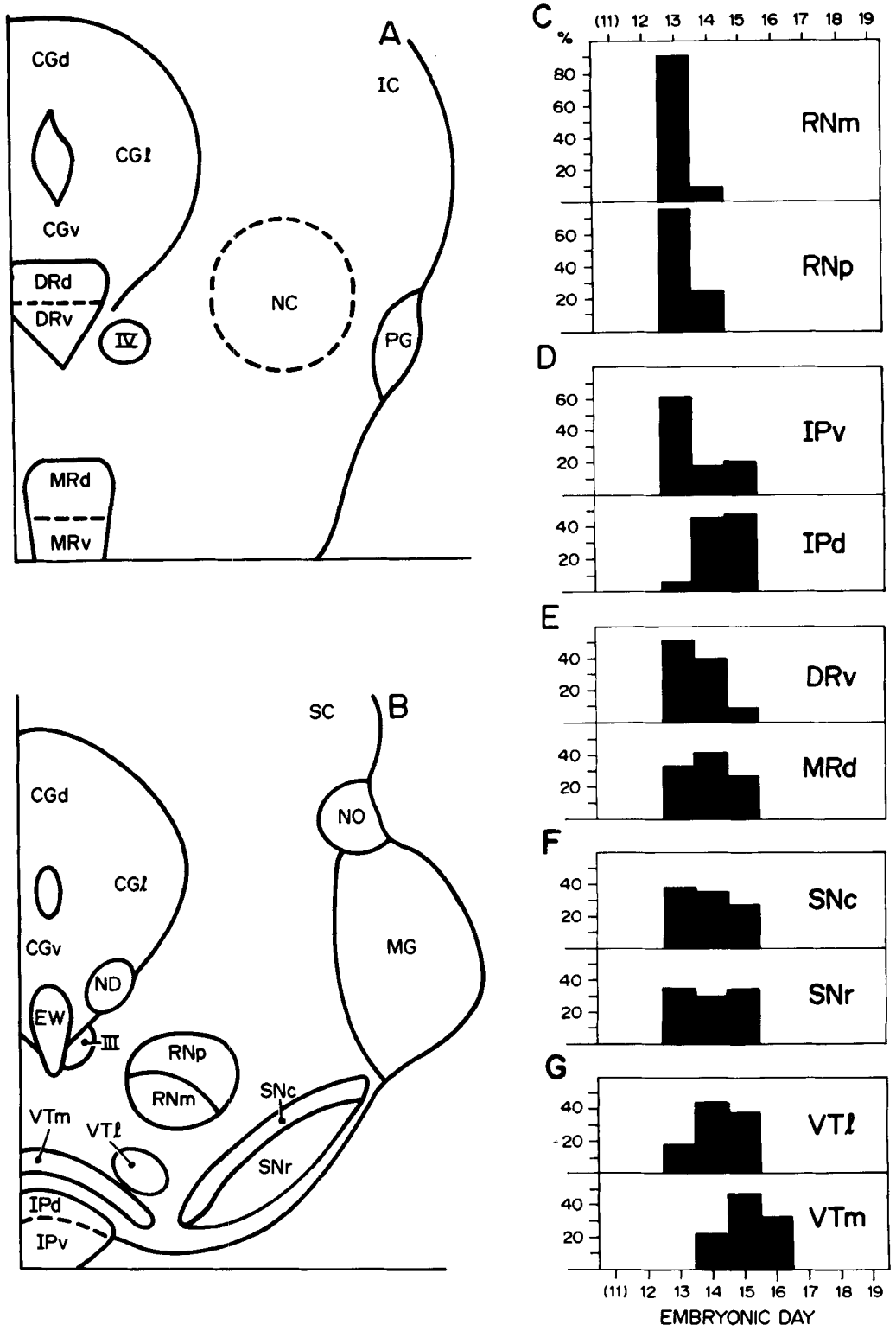

Fig. 1. Location of the structures quantified in the caudal tegmentum (A) and in the rostral tegmentum (B). C-G. Time of origin of neurons in ten tegmental structures.

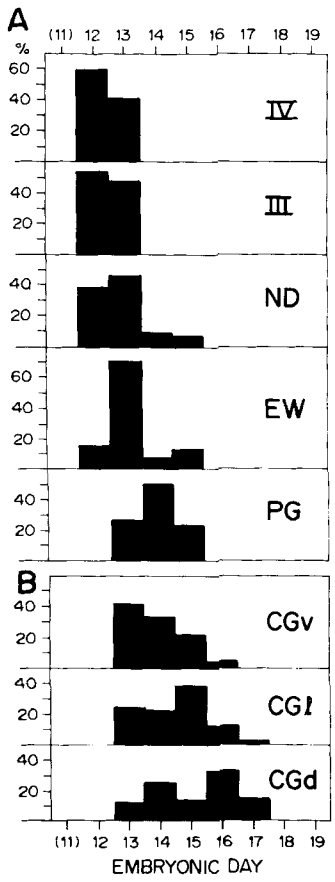

Fig. 2. A and B. Time of origin of neurons in eight additional tegmental regions.

Relatively little attention has been paid to the nucleus of Darkschewitsch (Carpenter et al., '70). In rodents it is situated rostral to the oculomotor nucleus and laterodorsal to the Edinger-Westphal nucleus (Slotnick and Leonard, '75). Its cells tend to be of intermediate size between those of the third nerve nucleus and the Edinger-Westphal nucleus. Afferents to the nucleus of Darkschewitsch have been described from the superior colliculus (Altman and Carpenter, '61; Graybiel and Hartwieg, '74) and the medial longitudinal fasciculus (Carpenter and Hanna, '62). Among its efferents are fibers to the facial nucleus (Panneton and Martin, '79) and possibly to the ocular muscle nuclei (Carpenter et al., '70).

Figure 7 illustrates the location of the nucleus of Darkschewitsch and the Edinger-Westphal nucleus in a coronal autoradiogram anterior to the level of the oculomotor nucleus. In this autoradiogram from a rat injected on days E13+14 many of the intermediate-sized cells of the nucleus of Darkschewitsch are no longer labeled but the majority of the smaller cells of the Edinger-Westphal nucleus are labeled. The quantitative results show (Fig. 2A) that the time span of cell generation time overlaps in these two nuclei between days E12 and E15 but that the peak occurs later in the Edinger-Westphal nucleus, with 70% of its neurons being generated on day E13. The difference was statistically significant ($P < 0.001$). The differences between the generation times of neurons of the nucleus of Darkschewitsch were also significant with respect to the trochlear nucleus ($P < 0.0001$) and the oculomotor nucleus ($P < 0.001$).

The parabigeminal nucleus. The parabigeminal nucleus is formed of a tight cluster of cells in the lateral aspect of the midbrain beneath the brachium of the inferior colliculus. In the rat the nucleus has been subdivided into a dorsal, a middle, and a ventral part (Tokunaga and Otani, '78). Afferents have been traced to the nucleus from the superior colliculus (van Noort, '69), either from cells located in the superficial layers (Abplanalp, '71; Harting et al., '73; Graham, '77) or the deep layers (Benevento and Fallon, '75; Baleydier and Magnin, '79). According to the last authors, afferents also come from the ventral nucleus of the lateral geniculate body, the cuneiform nucleus, the periaqueductal gray, and a few other structures. The efferents of the parabigeminal nucleus were traced to the superior colliculus (Kawamura et al., '77; Baleydier and Magnin, '79), where they terminate in the superficial layers (Graybiel, '78b). Other efferents reach the pretectal area, the suprachiasmatic nucleus, and the lateral geniculate body (Kawamura et al., '77). Tokunaga and Otani ('78) suggested that the neurons of the middle region of the parabigeminal nucleus are the efferent elements and that the dorsal and ventral regions are composed of intrinsic neurons. Most investigators denied the existence of connections with the inferior colliculus. The assumption that the parabigeminal nucleus is part of the brain stem visual system is supported by physiological evidence (Sherk, '78).

The medium-sized roundish neurons of the parabigeminal nucleus (Fig. 8) are generated between days E13 and 15 (Fig. 2A). Scanning the parabigeminal nucleus from anterior to posterior in coronal sections the following gradient was observed. In anterior sections there is a single cluster of cells and these were no longer labeled in the E15+16 injection group (Fig. 8B). Proceeding posteriorly three clusters could be identified, corresponding to the dorsal, medial, and ventral parts described by Tokunaga and Otani ('78). Labeled cells were still present in the E15+16 group dorsally and

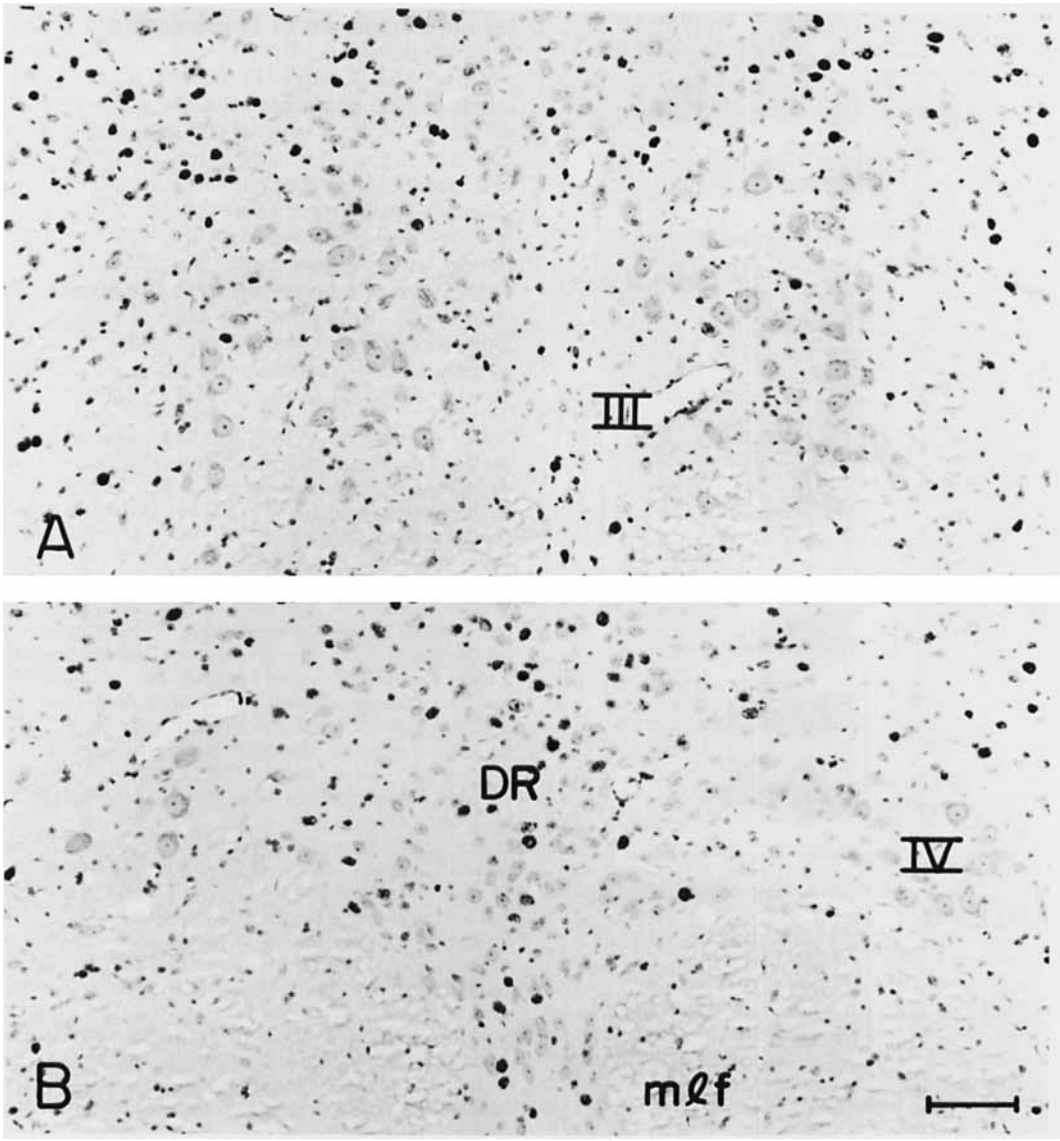

Fig. 3. A. The region of the oculomotor nucleus and B, of the trochlear nucleus, from an animal injected on days E14 + 15. Scale: 100 μm.

Fig. 4. Sagittal sections through the oculomotor nucleus in rats injected on days E12 + 13 (A), E13 + 14 (B), and E14 + 15 (C). Scale: 100 μm.

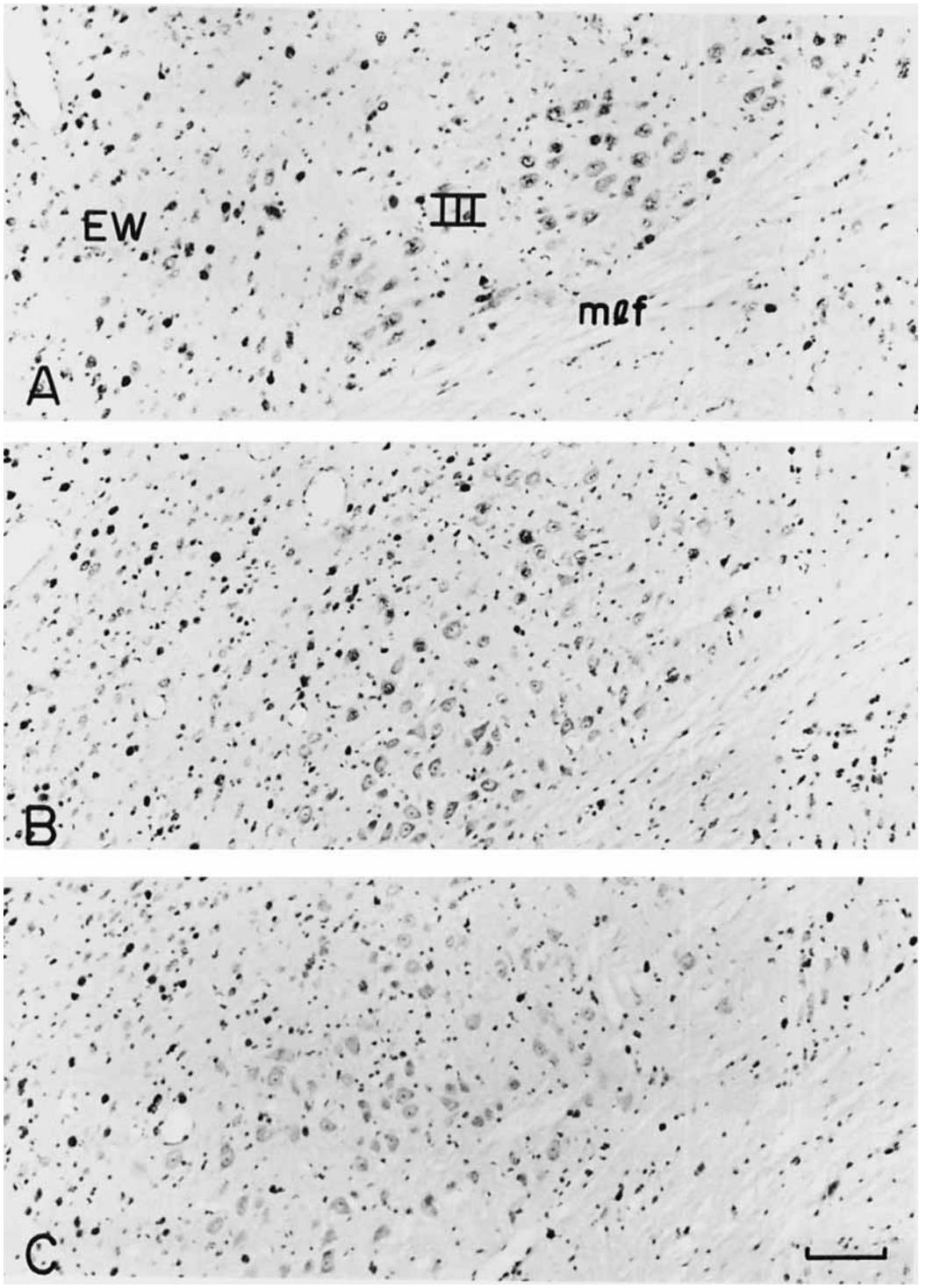

Figure 4

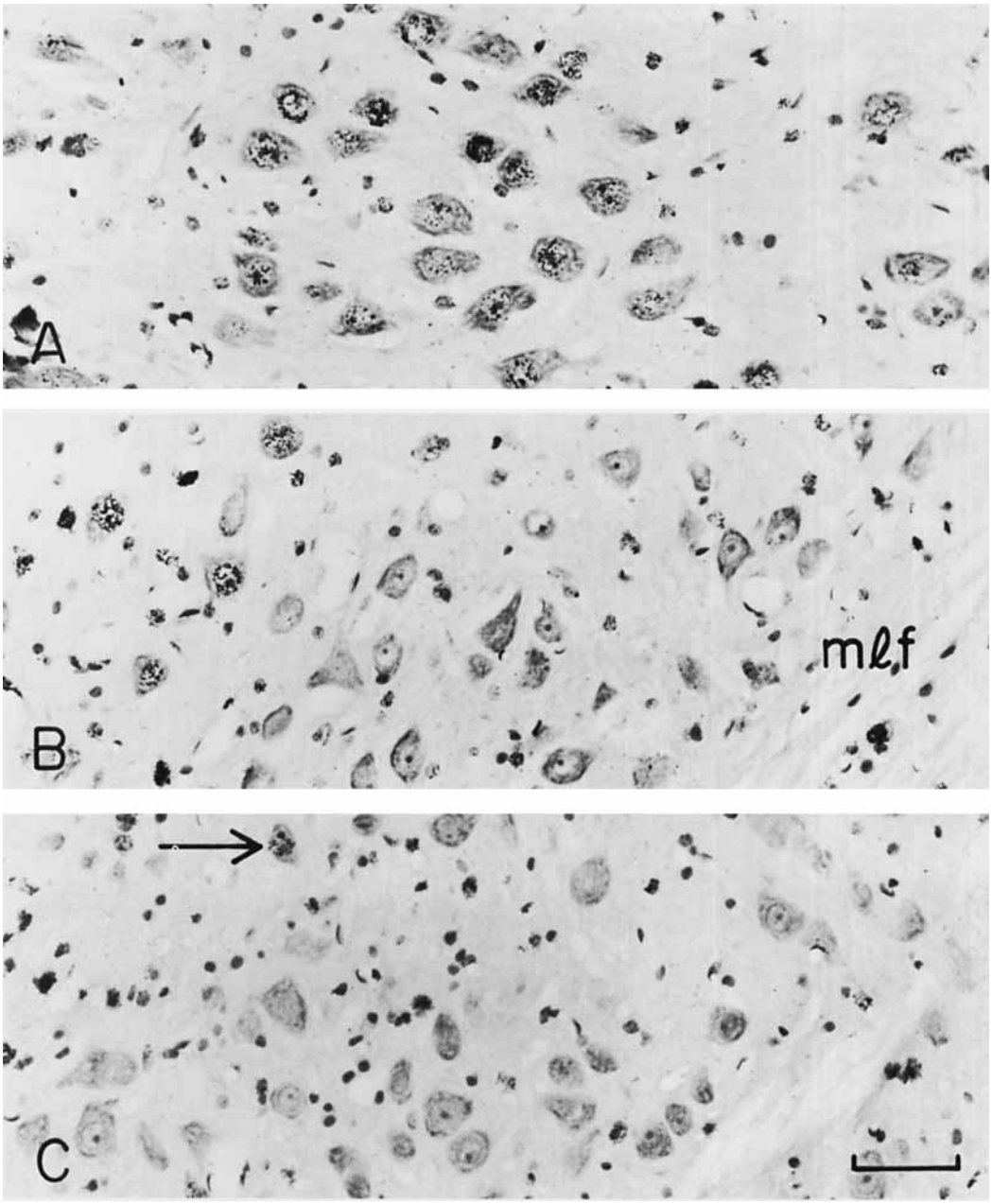

Fig. 5. Higher magnification of oculomotor neurons in sagittal sections from rats injected on days E12 + 13 (A), E13 + 14 (B), and E14 + 15 (C). Scale 50 μm.

ventrally. The reconstruction of the labeling pattern in a sagittal view (Fig. 9) suggests that the neurons of the larger cluster of cells forming the pars medialis, the efferent cells of Tokunaga and Otani ('78), are produced earlier than the other two components of the nucleus. The statistical analysis showed that the neurons of the parabigeminal nucleus, as a whole, arise significantly later ($P < 0.0001$) than the neurons of the trochlear, oculomotor, and Edinger-Westphal nuclei, and the nucleus of Darkschewitsch. They thus represent the latest-arising components of the midbrain tegmentum related to the optic system.

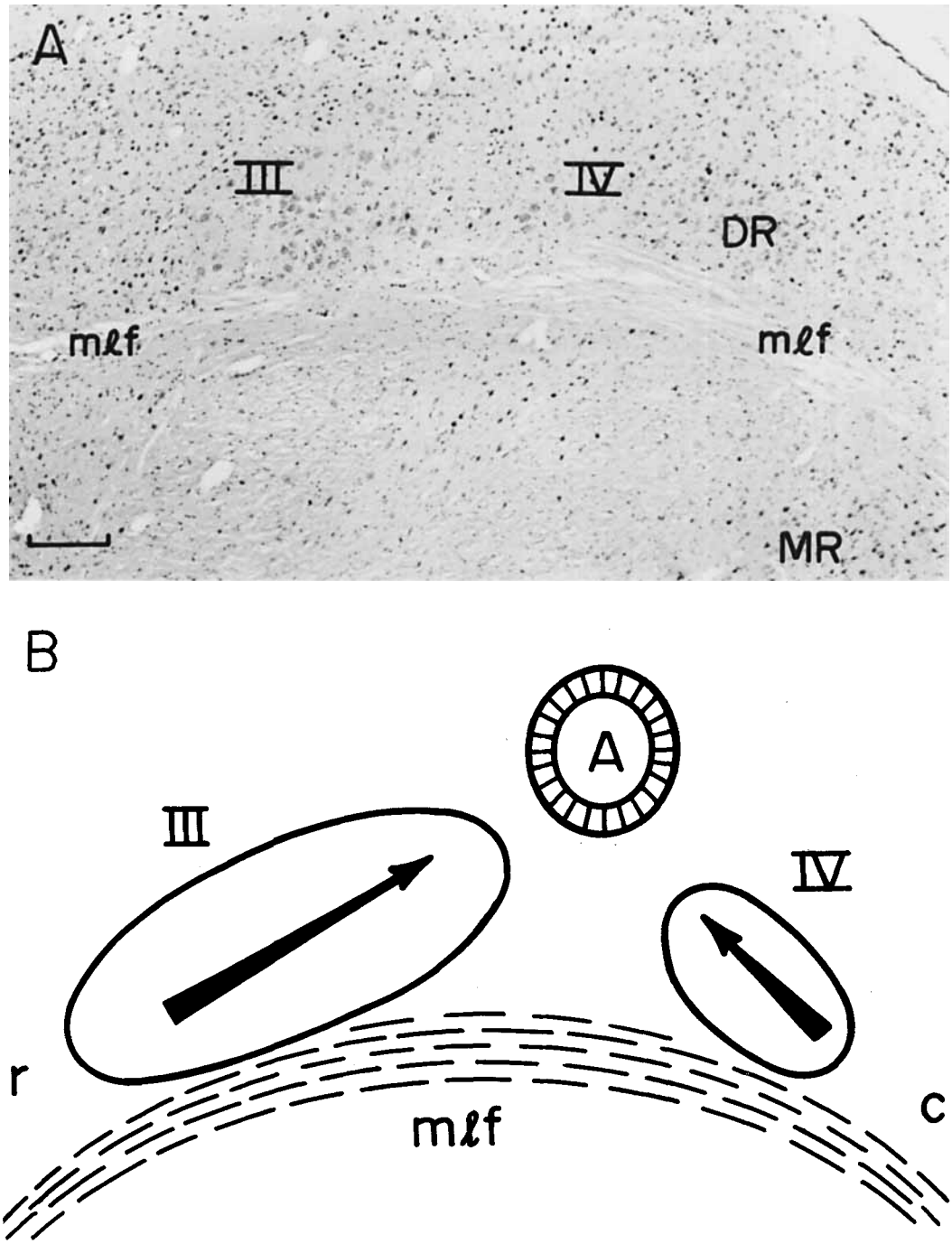

Fig. 6. A. Low-power autoradiogram showing the relation of the oculomotor and trochlear nuclei to the medial longitudinal fasciculus in sagittal section. Scale: 200 μm. B. Schematic illustration of cytogenetic gradients in the two nuclei with a hypothesis of their origin in a single germinal source in the embryonic aqueduct.

The periaqueductal gray

Traditionally the central gray of the mesencephalon is divided into a dorsal portion surrounded by the tectum and a ventral portion surrounded by the tegmentum (Castaldi, '23; Hamilton and Skultety, '70). Another classification (Olszewski and Baxter, '54; Hamilton, '73a) is into a nucleus dorsalis overlying the aqueduct, the nucleus medialis adjacent to the aqueduct laterally and extending

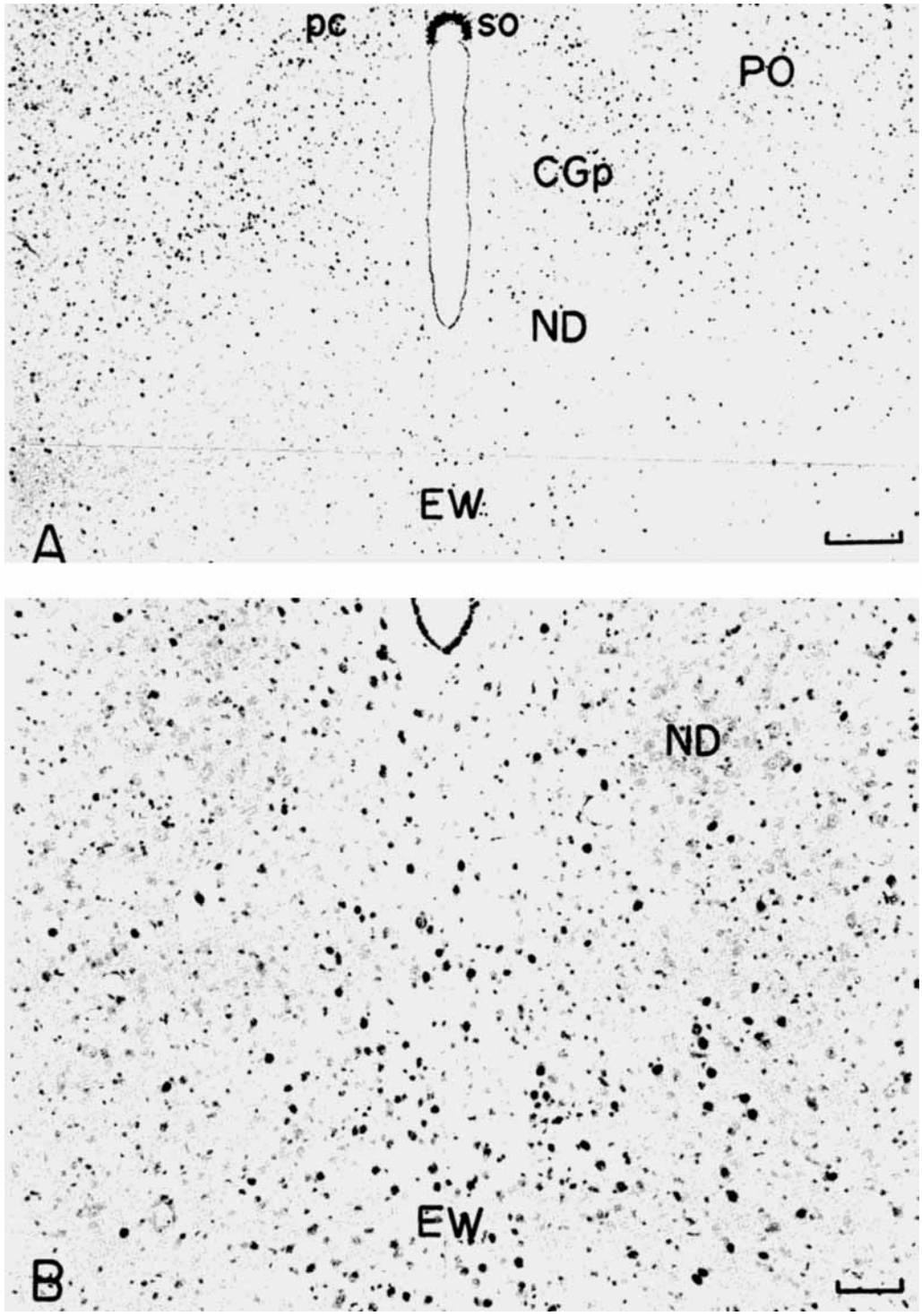

Fig. 7. A. Coronal section, anterior to the level of the oculomotor nucleus. Scale: 200 μm. B. Enlargement of the area of the nucleus of Darkschewitsch and the Edinger-Westphal nucleus. Rat injected on days E13 + 14. Scale 100 μm.

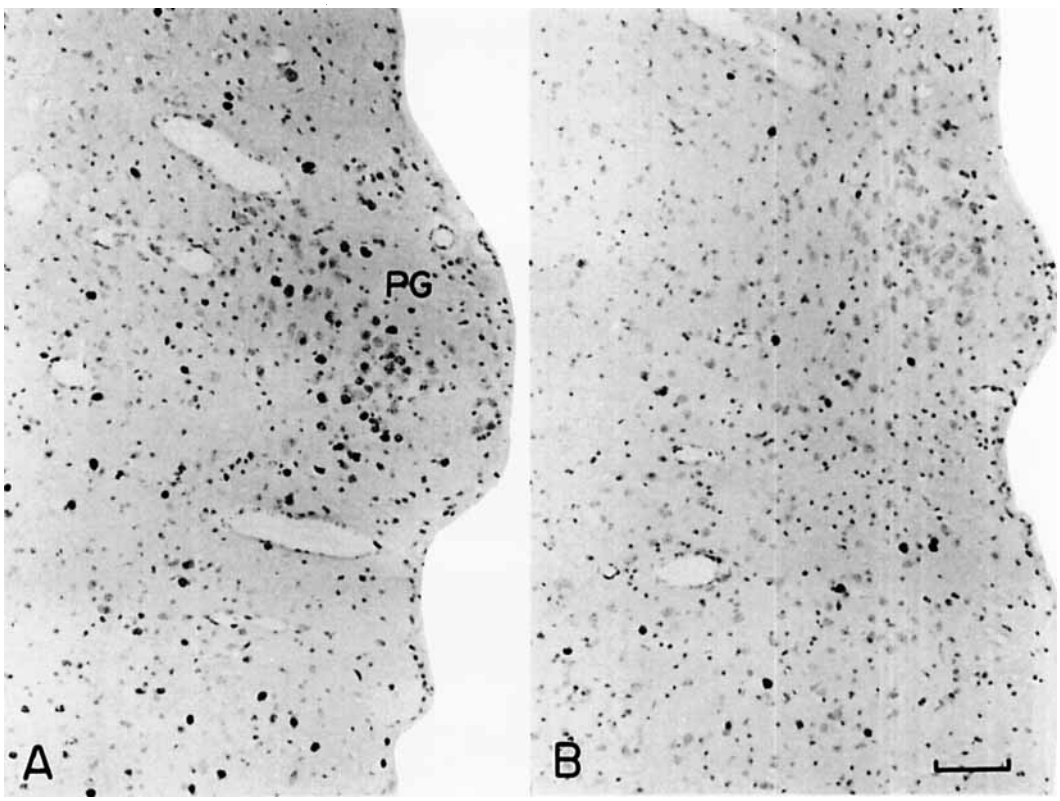

Fig. 8. Neurons of the parabigeminal nucleus in anterior coronal sections from rats injected on days E14 + 15 (A) and E15 + 16 (B). Scale: 100 μm.

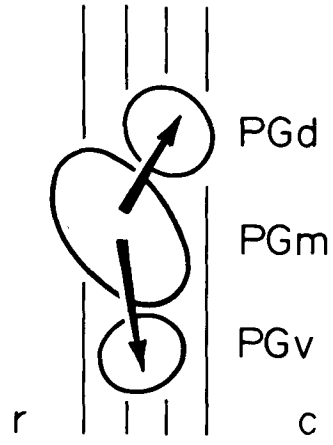

Fig. 9. Schematic illustration of cytogenetic gradients in the dorsal, medial, and ventral parts of the parabigeminal nucleus in the sagittal plane.

ventrally, and a nucleus lateralis. These three divisions were described to be composed of different cell types (Hamilton, '73a). The dorsal longitudinal fasciculus of Schütz is the major tract associated with the periaqueductal gray. Efferents from the dorsal nucleus were traced (Hamilton, '73b) to the pretectal area and the lateral habenular nucleus. From the nucleus medialis radiating fibers reach the adjacent tegmentum, and other fibers were traced to the fields of Forel, the ventral tegmental area, and the dorsal tegmental nucleus. Finally, from the nucleus lateralis fibers reach the tectum and midbrain tegmentum, the periventricular gray of the diencephalon, various thalamic nuclei, and the posterior hypothalamus. From the ventral portion of the central gray a descending projection was described to the facial nucleus (Panneton and Martin, '79).

In the cat, afferents to the central gray were described from the inferior colliculus (Powell and Hatton, '69) and the superior colliculus (Edwards

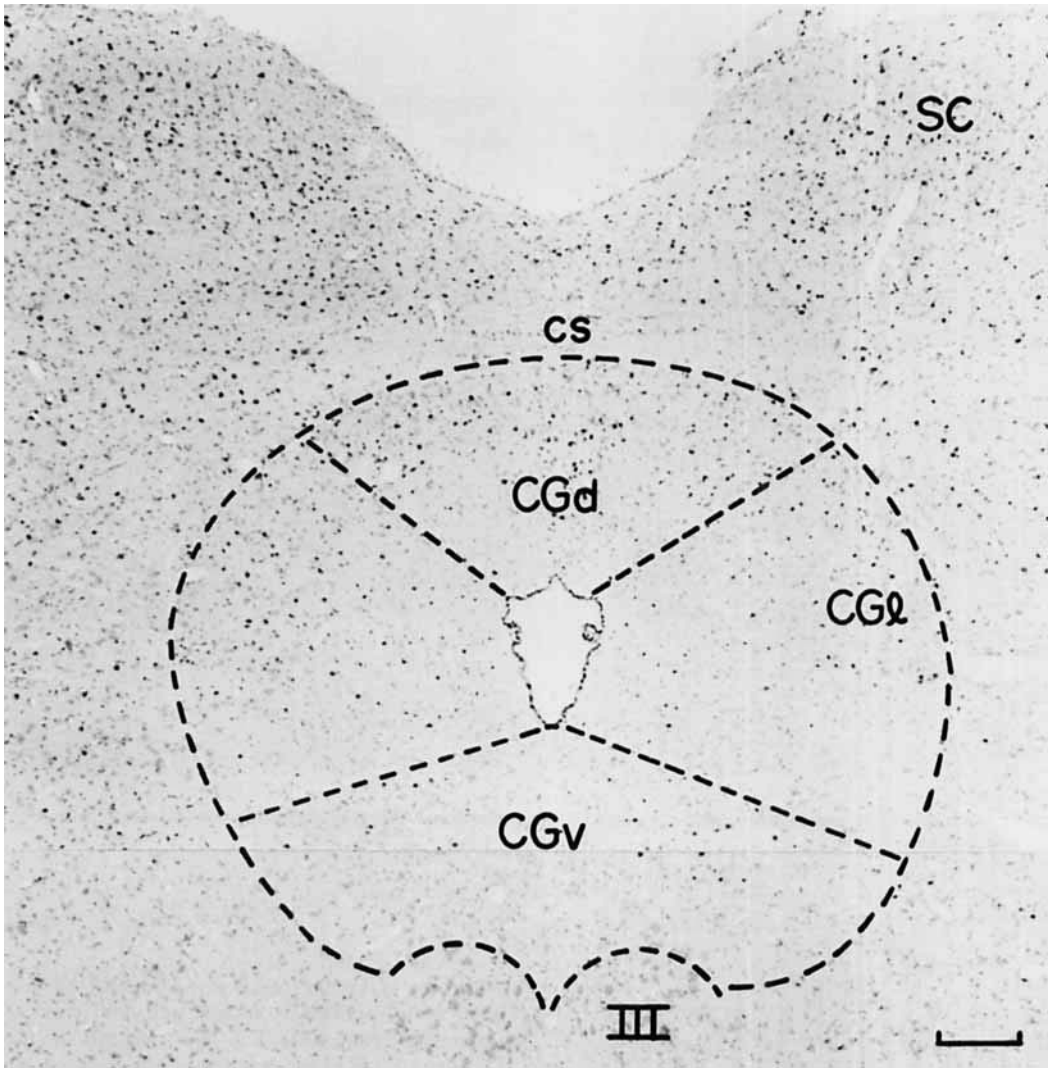

Fig. 10. Labeling pattern in the periaqueductal gray in a rat injected on days E15+16. Note dorsal-to-ventral gradient within each subdivision of the central gray. Scale: 200 μm.

and Henkel, '78). In the squirrel monkey, a widespread projection is said to exist from a large number of telencephalic, diencephalic, and mesencephalic structures implicated in vocalization (Jürgens and Pratt, '79). Physiological and pharmacological studies (eg, Skultety, '58; Mayer and Liebeskind, '74; Jacquet and Lajtha, '76) suggest the involvement of this brain region in nociceptive functions.

The gradient in the labeling pattern (Fig. 10) suggests a subdivision of the periaqueductal gray into a ventral, lateral, and dorsal portion. Neuron production begins in all three components on day E13 (Fig. 2B). However, peak production time is on day E13 in the pars ventralis, on day E15 in the pars lateralis, and on day E16 (possibly with a biphasic pattern) in the pars dorsalis. The differences between these components were significant ($P < 0.0001$). In addition to this overall ventral-to-dorsal gradient, a ventral-to-dorsal gradient was also noted within each subdivision (Fig. 10). However, the gradient in the ventral nucleus was complicated by the proximity of

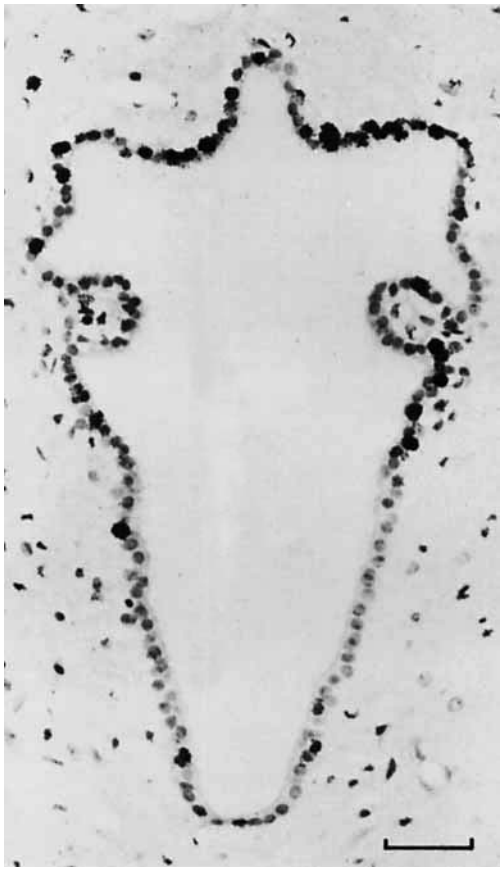

Fig. 11. Ventral-to-dorsal gradient in the labeling pattern of the cells of the ependymal lining of the aqueduct in a rat injected on days E16+17. Scale: 50 μm.

structures (the dorsal raphe nucleus caudally and the Edinger-Westphal nucleus rostrally) that become partially embedded in the central gray. The earlier cessation of neuron production in the ventral nucleus of the periaqueductal gray, and the ventral-to-dorsal gradient, is reflected in the labeling pattern of the ependymal lining of the aqueduct (Fig. 11).

The dorsal raphe and median raphe nuclei

The serotonin-containing cell bodies (Dahlström and Fuxe, '64) of the dorsal raphe nucleus (group B7) and the median raphe nucleus (group B8; nucleus centralis superior) are the principal source of the "serotonergic" fibers of the nervous system (Andén et al., '66; Kostowski et al., '68; Sheard and Aghajanian, '68; Ungerstedt, '71; Olson and Seiger, '72). From these nuclei ascending fibers have been traced (Conrad et al., '74; Bobillier et al., '76; Taber Pierce et al., '76; Azmitia and Segal, '78) to a large number of telencephalic and diencephalic structures by way of the medial forebrain bundle and several other fiber tracts. Descending projections have been described to the locus coeruleus, the pontine reticular formation, the periaqueductal gray, and a few other regions (Conrad et al., '74; Taber Pierce et al., '76). Bobillier et al. ('75, '76), however, stated that these descending projections are poor or nonexistent from the dorsal raphe nucleus but extensive from the median raphe nucleus. From the latter region efferents reach many structures, including the cerebellum, the auditory nuclei of the medulla, and the inferior olive.

Afferents to the dorsal raphe nucleus have been described from the habenular nuclei (Nauta, '58; Akagi and Powell, '68; Wang and Aghajanian, '77), particularly the lateral habenular nucleus (Herkenham and Nauta, '79). The transmitter in this pathway may be substance P (Neckers et al., '79). Projections have also been described from the retina (Foote et al., '78) and the lateral geniculate nucleus (Leger et al., '75). But, surprisingly, one horseradish peroxidase study indicated (Mosko et al., '77) that the dorsal raphe nucleus receives mostly intrinsic afferents.

The cells of the dorsal raphe nucleus are distinguishable from the cells of the periaqueductal gray by their larger size (Figs. 12, 13). Because the two cell types are intermingled in the dorsal portion of the nucleus, cell counts were made in its ventral part in coronal sections at a level immediately caudal to the trochlear nucleus. The bulk of the neurons of the ventral part of the dorsal raphe nucleus are generated on days E13 and E14 (Fig. 1E). There is a pronounced ventral-to-dorsal gradient and many cells are still labeled dorsally in animals in which injection was begun on day E15 (Figs. 12, 13); at least some of these labeled cells were typical, medium-sized dorsal raphe nucleus neurons (Fig. 13).

The median raphe nucleus appears to be composed of at least two parts: a small-celled dorsal component (Figs. 14, 15) and a large-celled ventral part (Fig. 15). The dorsal part could be traced as far rostrally as the ventral tegmental area of Tsai. The ventral part, which is quite limited in extent in the rostrocaudal plane, is situated more caudally. It consists of spindle-shaped cells that are horizontally oriented in coronal sections. Most of these cells were no longer labeled in animals injected on days E14+15 (Fig. 15B). The cell counts were made in the small-celled part of the nucleus at a coronal level containing the rostral boundary of the pontine gray and the caudal tip of the interpeduncular nucleus (Fig. 14). The results indicate (Fig. 1E) a generation time between days E13 and E15 with a slight peak on day E14. There is apparently a ventral-to-dorsal gradient in both the dorsal raphe and median

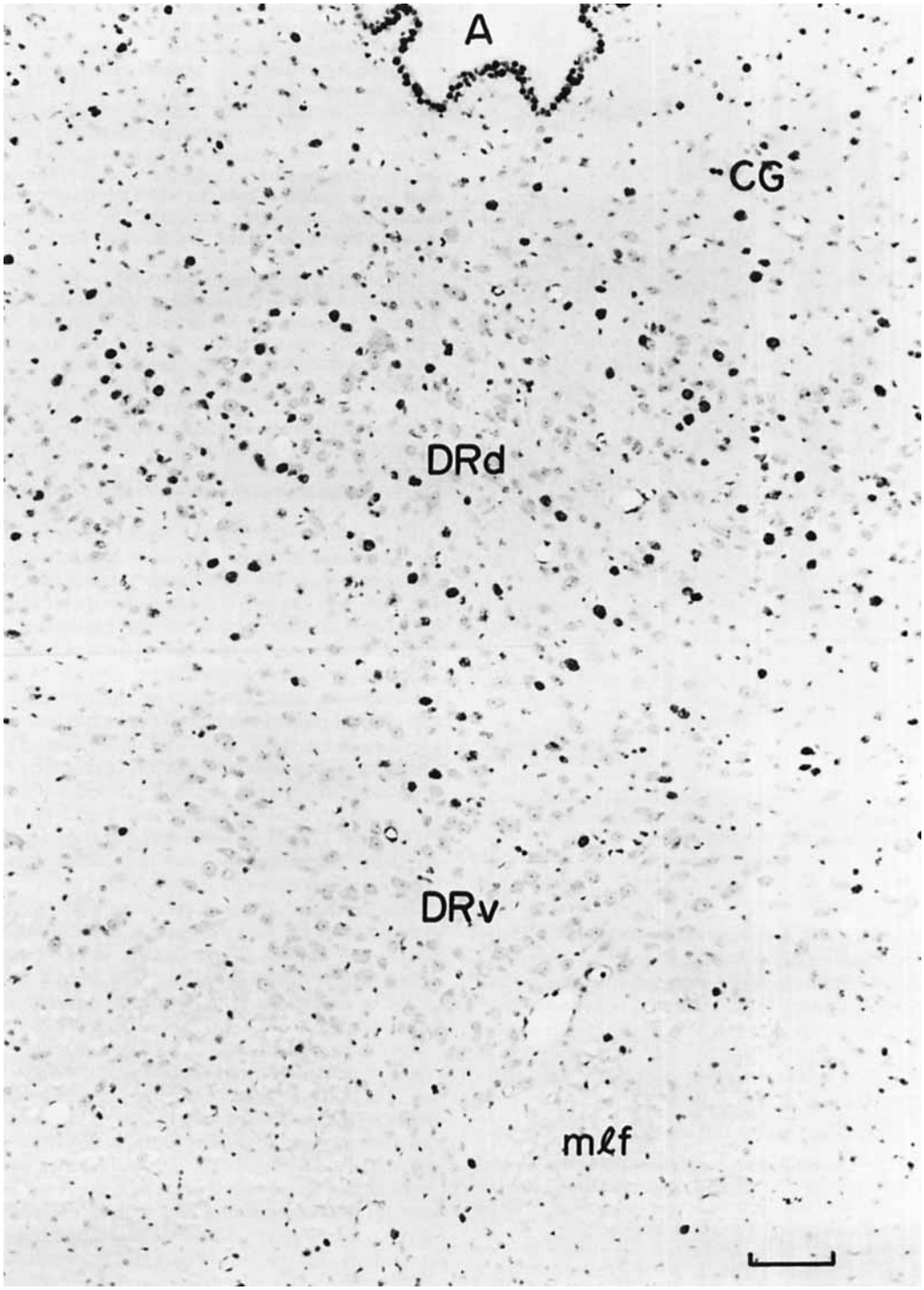

Fig. 12. The dorsal and ventral portions of the dorsal raphe nucleus in a rat injected on days E15+16. Scale: 100 μm.

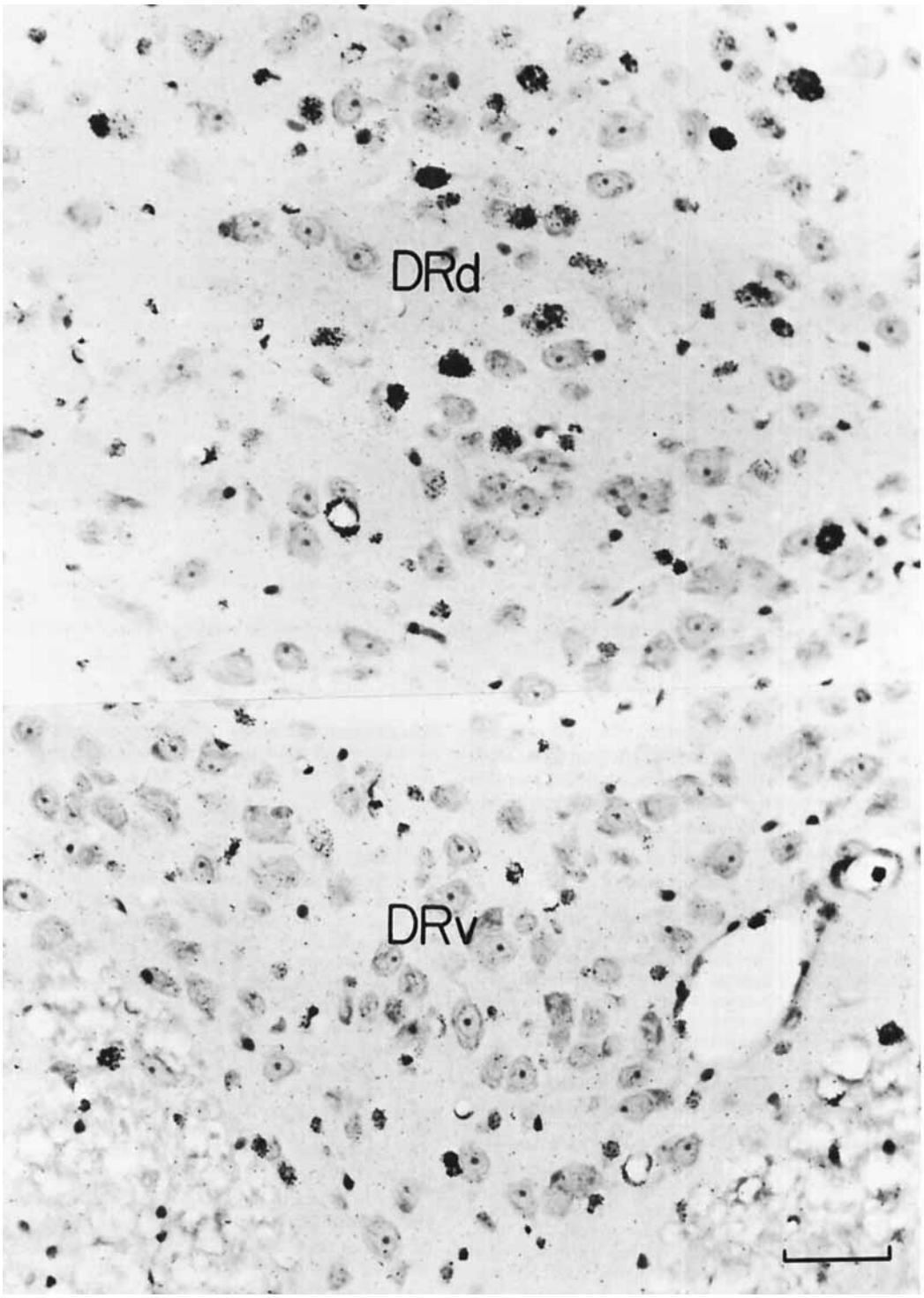

Fig. 13. Higher magnification of the dorsal and ventral portions of the dorsal raphe nucleus to show the ventral-to-dorsal intranuclear gradient. Scale: 50 μm.

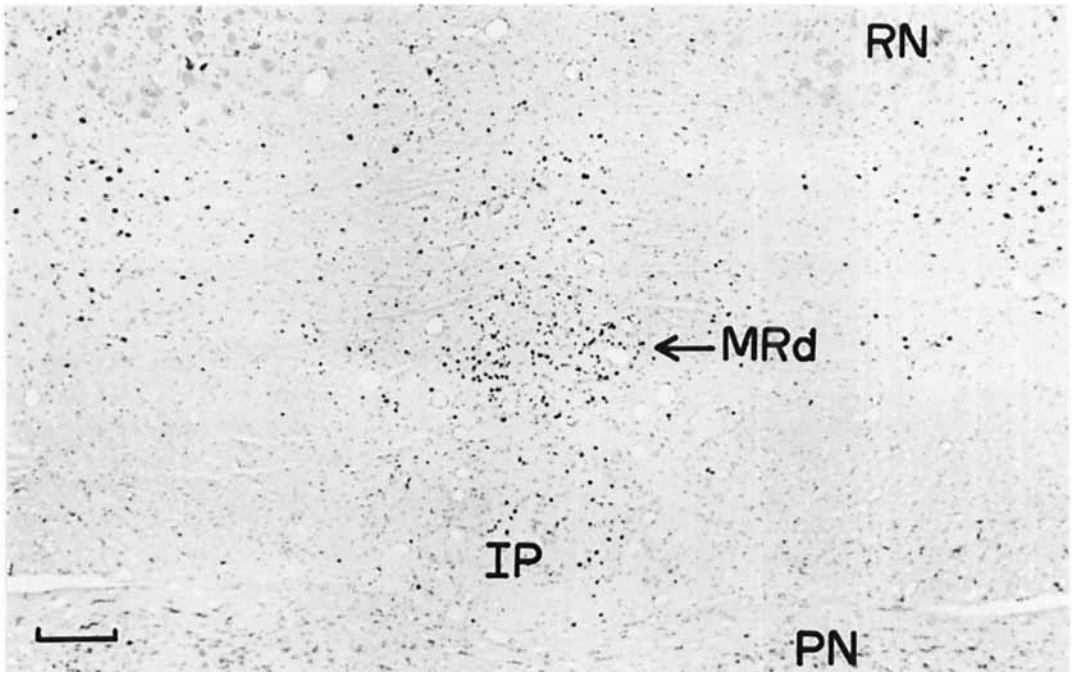

Fig. 14. The median raphe nucleus (pars dorsalis) at the level of the rostral border of the pontine gray and the caudal tip of the red nucleus. Rat injected on days E14+15. Scale: 200 μm.

raphe nuclei. The later production of neurons in the median raphe nucleus (dorsal part) with respect to the dorsal raphe nucleus (ventral part) indicated in Figure 1E may reflect merely the intranuclear gradient existing within both structures.

The red nucleus

The red nucleus is composed of a caudal magnocellular and a rostral parvocellular division. Comparative studies have indicated (Hatschek, '07; Monakow, '10; Massion, '67; Reid et al., '75) that the magnocellular division is well developed in lower mammals and regresses in higher forms, and that the converse holds for the parvocellular division.

The magnocellular red nucleus, which also contains medium and small neurons, is the source of the crossed, descending rubrobulbar and rubrospinal tracts (Pompeiano and Brodal, '57; Kuypers and Lawrence, '67; Reid et al., '75). The bulbar projection has been traced to a large number of structures, such as the lateral reticular, inferior vestibular, and inferior olivary nuclei (Edwards, '72). In the rat, a projection to the cranial motor nuclei has been denied (Waldron and Gwyn, '69) but a projection has been found in the cat to the facial nucleus (Edwards, '72). The spinal projection reaches the thoracic level (Prendergast and Stelzner, '76); the termination pattern overlaps with that of the corticospinal tract (Nyberg-Hansen and Brodal, '64).

The afferents of the magnocellular division derive mainly from the contralateral cerebellum (Massion, '67; Gwyn and Flumerfelt, '74), particularly the interpositus nucleus (Massion, '67), but it does not appear to receive afferents from the cerebral cortex (Gwyn and Flumerfelt, '74; Brown, '74a). The cortical afferents terminate in the rostrally located parvocellular nucleus. The cerebellar projection to this region is mainly from the dentate nucleus (Courville, '66; Massion, '67). The projections from the cerebral cortex and cerebellum may converge on the same parvocellular units (Oka and Jinnai, '78) and the latter show a topographical organization with respect to the fore and the hindlimbs (Padel et al., '73). The efferent outflow of parvocellular neurons is ipsilateral, mainly to the inferior olive (Walberg, '56; Courville and Otabe, '74) and the spinal cord (Nyberg-Hansen and Brodal, '64; Waldron and Gwyn, '69; Brown, '74b; Martin et al., '74).

The neurons of the magnocellular division of the red nucleus, with a rare exception, were labeled in rats injected on days E13+14 (Figs. 16, 17). The majority of cells were no longer labeled in the magnocellular division of the

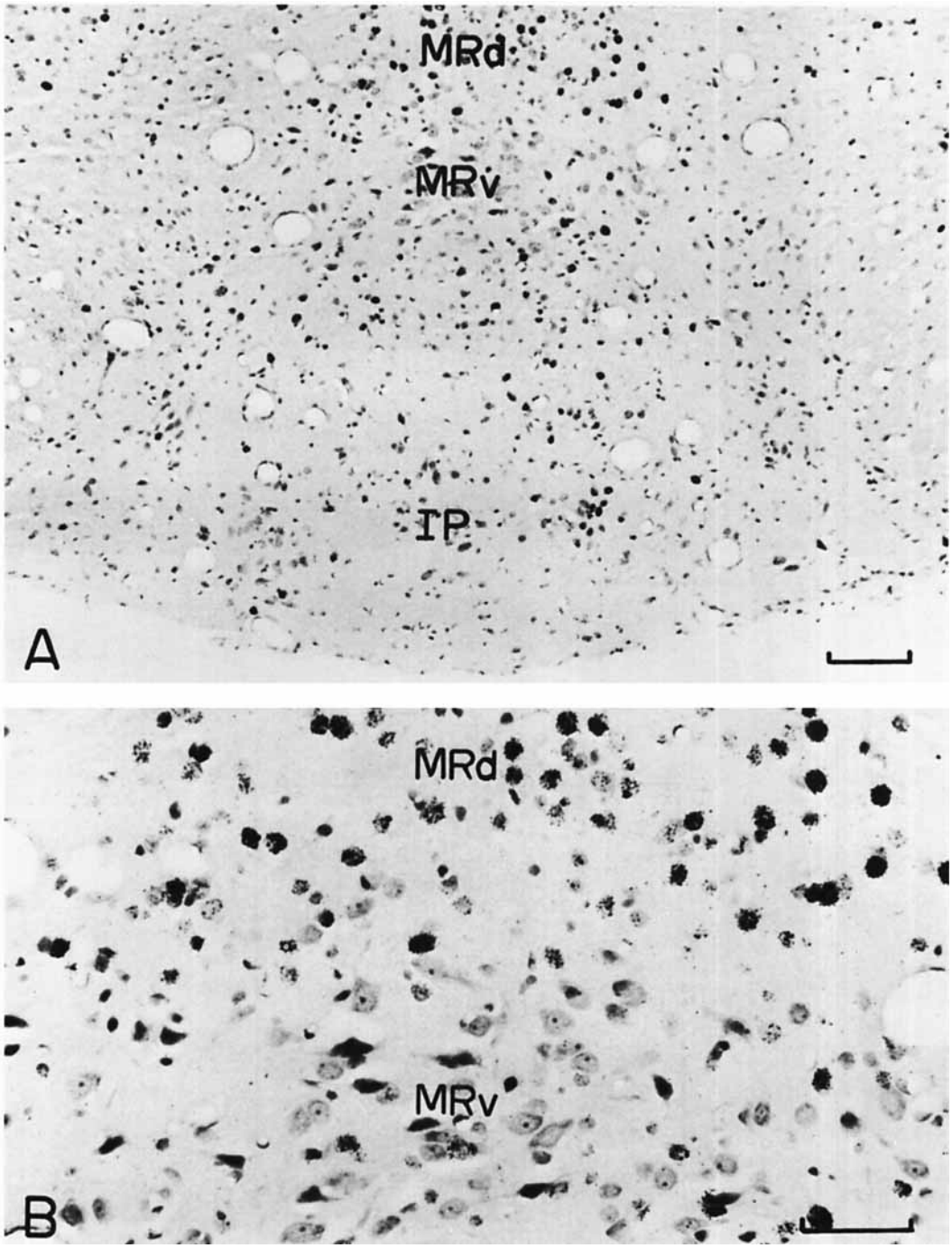

Fig. 15. A. The small-celled dorsal portion, and the large-celled ventral portion of the median raphe nucleus at the level of the posterior half of the interpeduncular nucleus. Scale: 100 μm. B. Higher magnification of the median raphe nucleus. Scale: 50 μm.

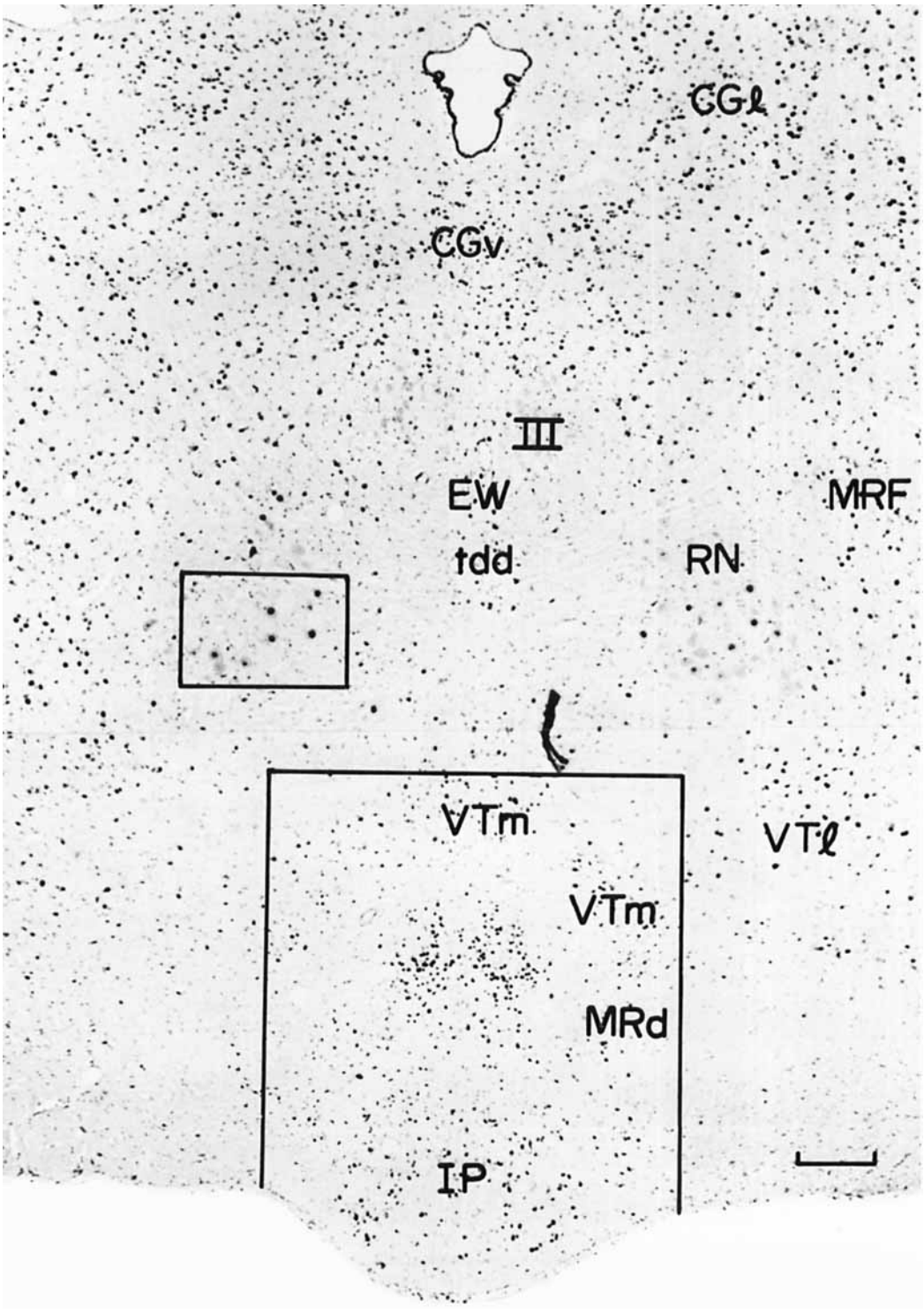

Fig. 16. Low-power radiogram of the middle portion of the tegmentum from a rat injected on days E13 + 14. Area in small rectangle shown enlarged in Figure 17; area in larger rectangle enlarged in Figure 20. Scale: 200 μm.

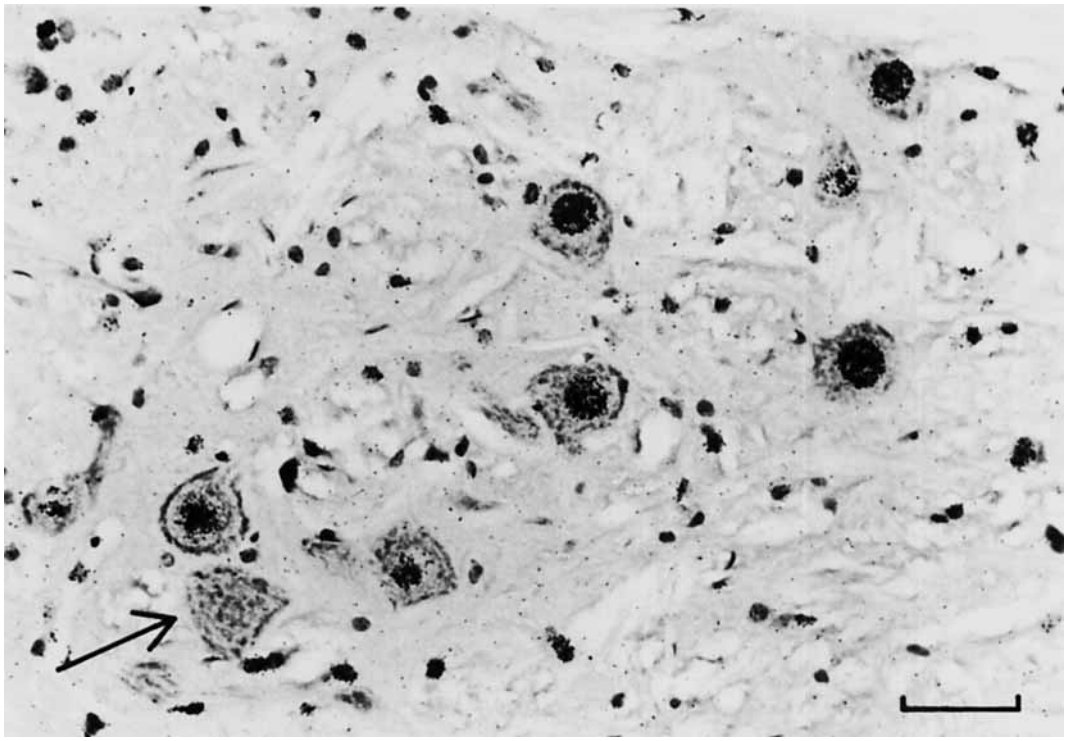

Fig. 17. Labeled neurons of the magnocellular division of the red nucleus in a rat injected on days E13+14. The arrow points to a cell which would not be included in the cell counts because its nucleus is not in the plane of sectioning. Scale: 50 μm.

nucleus in animals injected on days E14+15 (Fig. 18) and the few cells that were labeled tended to be located rostrally. Cell counts were carried out in coronal sections beginning at the caudal level where the nucleus could first be identified. The boundary between the magnocellular and parvocellular divisions is not clear and the cell counts included some smaller neurons. The results indicate (Fig. 1C) that over 90% of the neurons are generated here on day E13 and the rest on the next day.

Scanning of the parvocellular nucleus was begun rostrally (Fig. 19). The majority of cells were of intermediate size but the large neurons present were also included in the counts. The results showed (Fig. 1C) that 25% of the cells were still labeled in the group injected on days E14+15. Typically, the labeled cells were intermediate-size neurons (Fig. 19B). There was no indication of a gradient within the parvocellular nucleus. The temporal difference in the generation of neurons in the two divisions of the red nucleus was statistically significant ($P < 0.002$).

The interpeduncular nucleus

The unpaired interpeduncular nucleus has been divided in rodents into a pars medialis, pars lateralis, and a pars dorsalis (Ives, '71). The bulk of its afferents come, in the cat, from the habenular nuclei (Akagi and Powell, '68; Smaha and Kaelber, '73), and exclusively from the medial habenular nucleus in the rat (Herkenham and Nauta, '79). According to Herkenham and Nauta the projection from the medial habenular nucleus is topographic: its medial portion projects to the ventral aspect of the interpeduncular nucleus, whereas its lateral portion projects to the dorsal part of the habenular nucleus. According to Ramón y Cajal ('11) the fibers of the habenulopeduncular tract reach the interpeduncular nucleus from its lateral aspect and then enter the nucleus and cross and recross the midline. The efferents of the interpeduncular nucleus reach rostrally the lateral habenular nucleus, the septum, and some nuclei of the medial thalamus (Massopust and Thompson, '62; Mitchell, '63; Smaha and Kaelber, '73); the caudal projection is to the dorsal and deep tegmental nucleus (Smaha and Kaelber, '73). Both the afferent and efferent connections are bilateral.

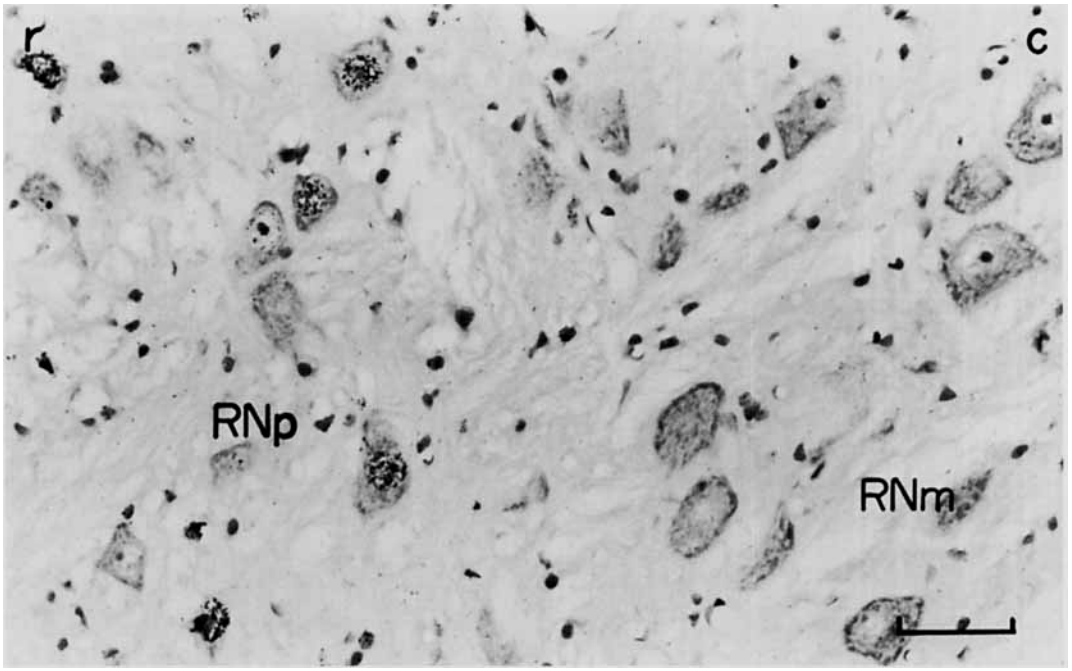

Fig. 18. Unlabeled neurons of the magnocellular division and the labeled neurons of the parvocellular division from a sagittal section in a rat injected on days E14+15. Scale: 50 μm.

The pars medialis extends throughout the entire ventral aspect of the interpeduncular nucleus from caudal to rostral (Figs. 20, 23), and is characterized by a low cell concentration in contrast to the pars dorsalis, with its high packing density. The pars dorsalis is relatively small caudally at the level of the rostral tip of the median raphe nucleus (Fig. 20) but becomes larger rostrally (Figs. 21, 23). The pars lateralis is small and is clearly seen only caudally (Fig. 20); its cell packing density is similar to the medial nucleus. As a convenience, the latter two structures were treated as a single entity for cell counting purposes, called the pars ventralis; and it was distinguished from the overlying, more tightly packed pars dorsalis.

There was a pronounced ventral-to dorsal intranuclear gradient in the generation of neurons of the interpeduncular nucleus (Fig. 22). Peak generation time in the pars medialis ventrally was on day E13, but only a fraction of the cell population was generated on this day in the pars dorsalis (Fig. 1D). The bulk of the neurons of the pars dorsalis was produced on days E14 and E15. The difference between the two regions was highly significant ($P < 0.0001$). A semilunar band of cells was still labeled in the group injected on days E16+17 (Figs. 22C, 23). These cells are situated above the dorsal interpeduncular nucleus in rostral sections in a fibrous band situated beneath the ventral tegmental nucleus. This cell group may be identical with the interfascicular nucleus of the ventral tegmental area described by Phillipson ('79a; see below).

The ventral tegmental area

The ventral tegmental area of Tsai ('25) contains the dopaminergic neurons of group A10 (Dahlström and Fuxe, '64; Fallon and Moore, '78). There are different descriptions of the subdivisions of this area in the cat (Taber, '61; Simon et al., '79) and the rat (Fallon and Moore, '78; Phillipson, '79a). Phillipson described and illustrated the position of four nuclei. The two nuclei dorsal to the interpeduncular nucleus are the interfascicular nucleus, rostrally, and the nucleus linearis raphe caudalis, caudally. The nucleus paranigralis is situated lateral to the interpeduncular nucleus and the nucleus parabrachialis pigmentosus dorsolaterally.

The afferents to the ventral tegmental area of the rat have been traced from several rostral and caudal brain structures (Phillipson, '79b). Among the

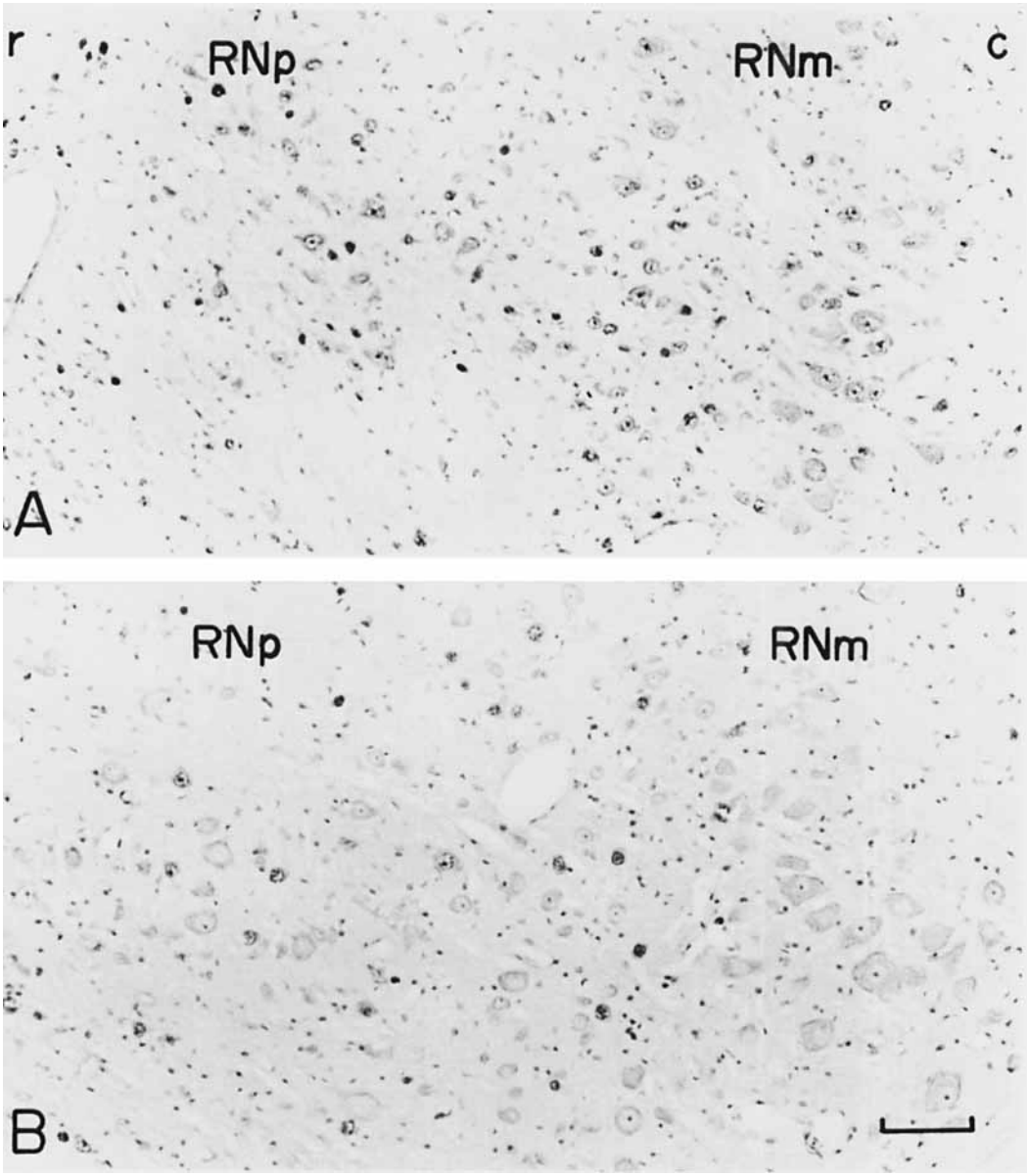

Fig. 19. Sagittal sections through the red nucleus in a rat injected on days E12 + 13 (A), and E13 + 14 (B). Scale: 100 μm.

rostral regions are the prefrontal cortex, the bed nucleus of the stria terminalis, the diagonal band of Broca, the amygdala, the preoptic area, widespread regions of the hypothalamus, and some nuclei of the medial thalamus. Among the caudal structures sending afferents to the ventral tegmental area are the superior colliculus, the nucleus raphe dorsalis, the pontine and the medullary raphe nuclei, the parabrachial nucleus, the locus coeruleus, and the deep cerebellar nuclei. The efferents of the area form the mesolimbic pathway (Ungerstedt, '71). These fibers ascend with the nigrostriatal fibers and terminate in the nucleus accumbens, the bed nucleus of stria terminalis, and the olfactory tubercle. More recent studies (Simon et al., '79; Beckstead et al., '79) describe additional rostral projections and also ef-

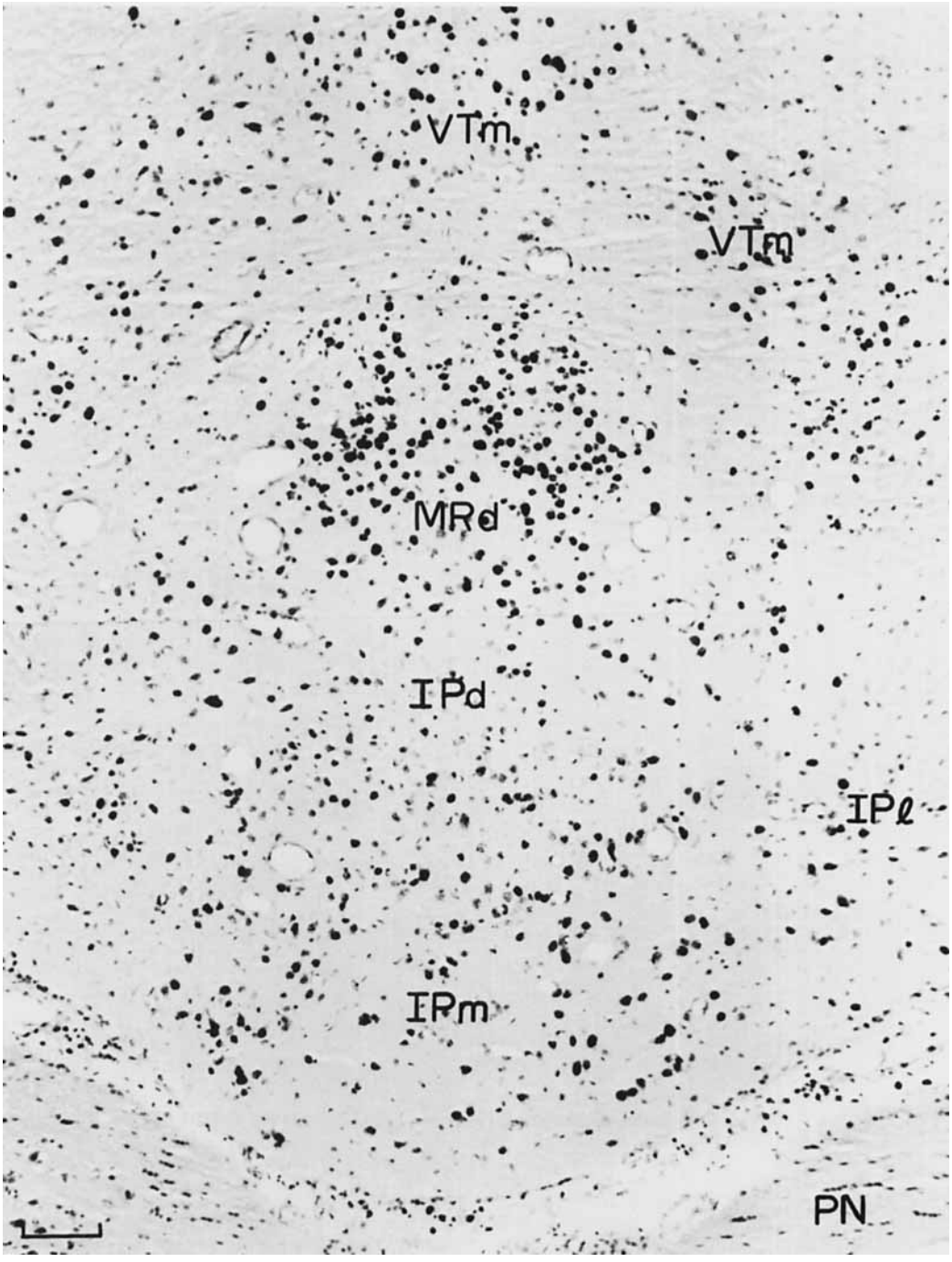

Fig. 20. The interpeduncular nucleus in a caudal coronal section. From a rat injected on days E13+14. (See Fig. 16 for survey photograph). Scale: 100 μm.

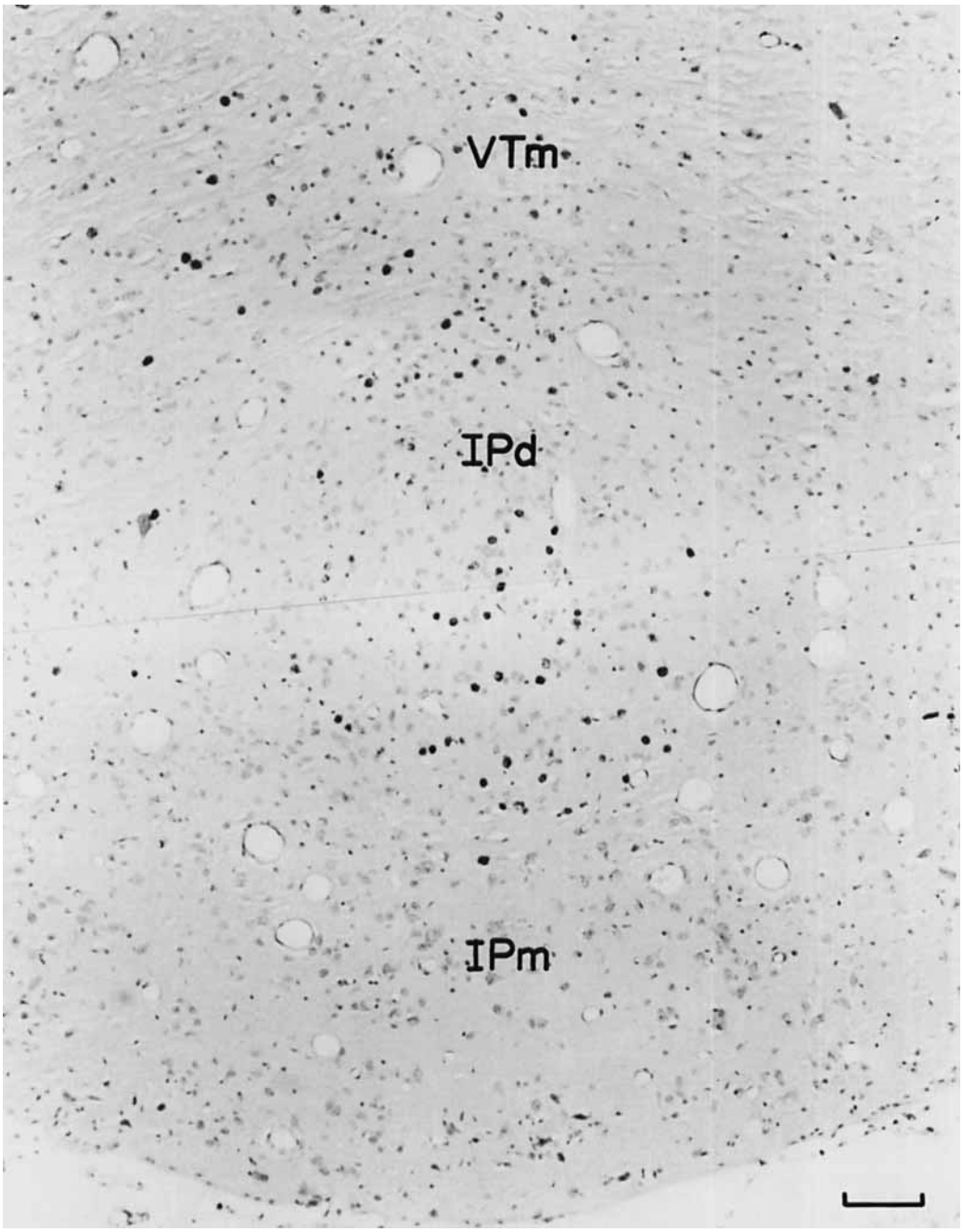

Fig. 21. The interpeduncular nucleus in a midcoronal section. From a rat injected on days E15 + 16. Scale: 100 μm.

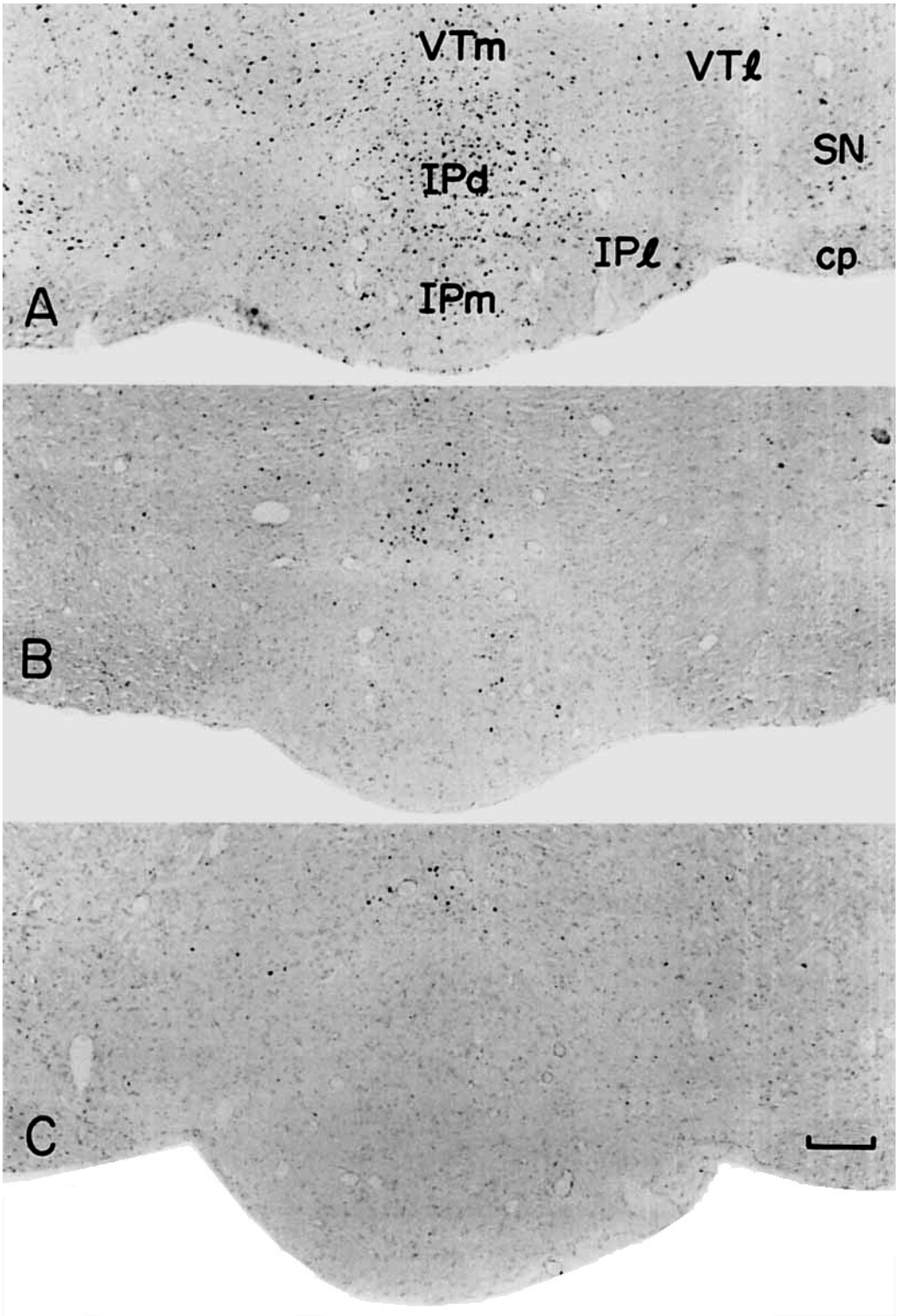

Figure 22

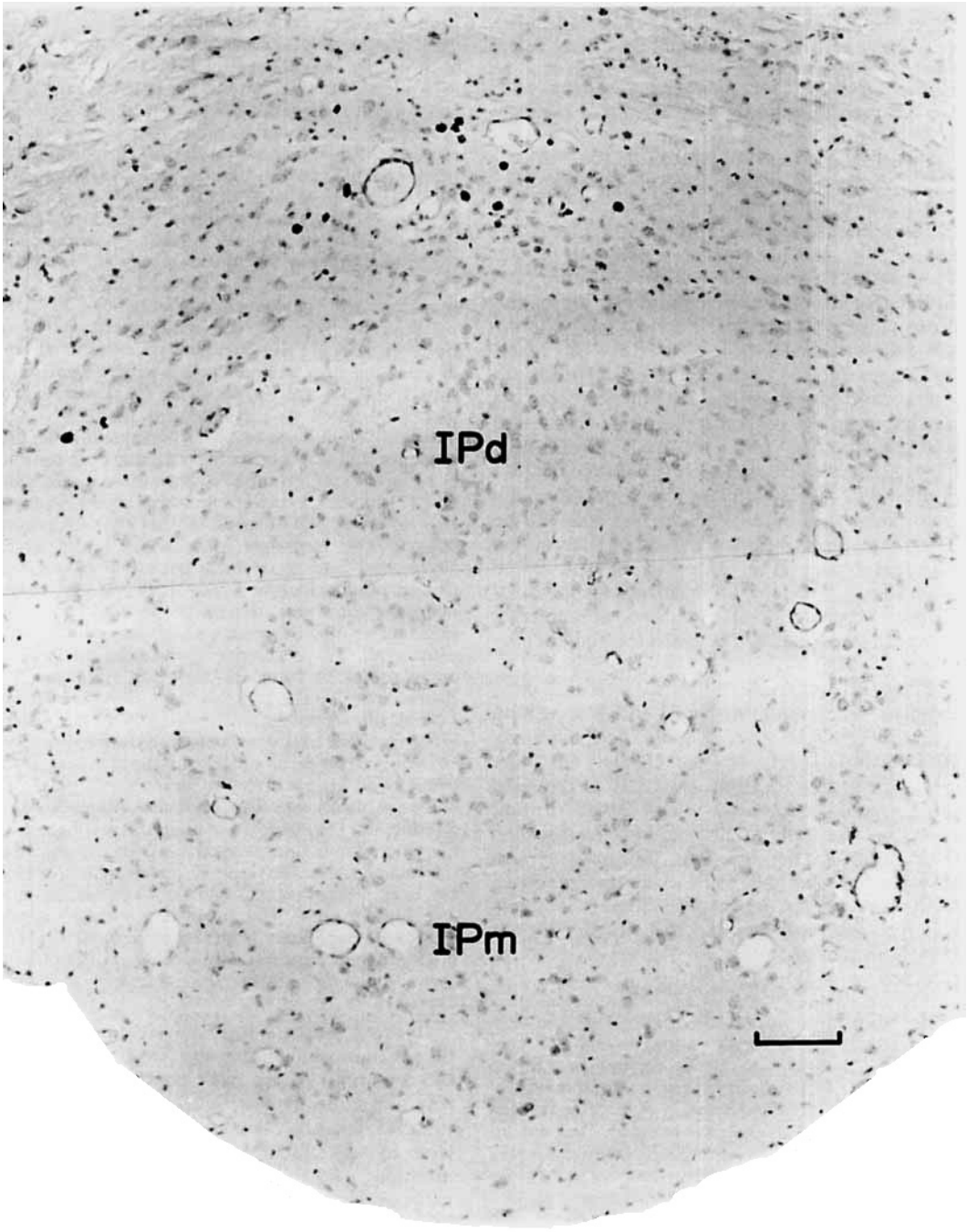

Fig. 23. Higher magnification of Figure 22C to show that the band of cells labeled in the E16 + 17 injection group is not part of the pars dorsalis of the interpeduncular nucleus. It is probably identical with the interfascicular nucleus of the ventral tegmental area. Scale: 100 μm.

Fig. 22. Labeling pattern in the interpeduncular nucleus in rats injected on days E14 + 15 (A), E15 + 16 (B), and E16 + 17 (C). Scale: 200 μm.

ferents to such caudal regions as the central gray and tegmentum of the midbrain, the dorsal raphe nucleus, and the locus coeruleus. According to Fallon and Moore ('78) the efferent projection from the ventral tegmental area is topographically organized.

The different subdivisions of the ventral tegmental area have uncertain boundaries. On the basis of radiographic labeling pattern the nucleus linearis raphe caudalis was identified tentatively as the rostral extension of the small-celled, nucleus raphe dorsalis, pars dorsalis. It is seen over the caudal portion of the interpeduncular nucleus (Figs. 14–16, 20) but not more rostrally (Figs. 21–23). The interfascicular nucleus appeared to be of semilunar shape caudally (Figs. 16, 20) but narrows to a medial structure rostrally (Fig. 22). The lateral portion may be identical with Phillipson's ('79a) nucleus paranigralis but because of their continuity, and similar labeling pattern, the two nuclei were quantified as one and will be referred to as the pars medialis of the ventral tegmental area (Fig. 1B). We distinguished from this, as the pars lateralis, an aggregate of loosely scattered cells situated dorsolateral from the pars medialis beneath the red nucleus (Figs. 16, 22).

The scattered cells of the pars lateralis are generated between days E13 and E15 with a peak on day E14 (Fig. 1G). The more densely packed and mostly smaller cells of the pars medialis are produced on days E14 to day E16 with a peak on day E15. The latest-forming cells of the pars medialis tended to be situated near the midline (Figs. 22C, 23), indicating a lateral-to-medial gradient. The difference in the birth dates of neurons of the pars lateralis and pars medialis was statistically significant ($P < 0.0001$).

The substantia nigra

The substantia nigra is composed of the dorsomedially situated pars compacta and the ventrolaterally situated pars reticulata. Some anatomists also distinguish a dorsolaterally situated pars lateralis (Hanaway et al., '70). The cell bodies of neurons of the pars compacta exhibit intense dopamine fluorescence (Dahlström and Fuxe, '64) and the axons of these neurons form the nigrostriatal pathway that terminates in the caudate nucleus and putamen (Andén et al., '65; Bédard et al., '69; Moore et al., '71). In the monkey (Carpenter and Peter, '72), the lateral part of the substantia nigra projects to the dorsal region of the putamen, and its medial part to the ventral region of the putamen. In the rat, Fallon and Moore ('78) found that medial and lateral sectors of the substantia nigra project to medial and lateral parts of the striatum. A medial-to-lateral arrangement of nigral fibers throughout the length of the neostriatum was also reported in the rat by Beckstead et al. ('79).

In addition to the nigrostriatal pathway, there is a large nigrothalamic outflow (Cole et al., '64; Afifi and Kaelber, '65; Faull and Carman, '689; Carpenter and Peter, '72) The cell bodies of this outflow are located predominantly in the pars reticulata (Rinvik, '75; Kultas-Ilinsky et al., '78; Beckstead et al., '79). Within the thalamus the nigral fibers terminate in the rat primarily in the nucleus ventromedialis (Faull and Carman, '68; Beckstead et al., '79) in the target region of cerebellar fibers, with a few fibers reaching the parafascicular nucleus and the paralamellar zone of the nucleus mediodorsalis (Beckstead et al., '79). The pars reticulata neurons are also a source of fibers to the superior colliculus (Graybiel, '75, '78a; Rinvik et al., '76; Jayaraman et al., '77). There is physiological (Anderson and Yoshida, '77) and anatomical (Bentivoglio et al., '79) evidence that some individual reticulata neurons are sources of axon terminals to both the thalamus and the tectum. But a retrograde tracer study in the rat showed (Faull and Mehler, '78) that the cells projecting to the thalamus and tectum tend to be segregated into separate longitudinal columns in the rat pars reticulata. Most of the pars reticulata neurons show no dopamine fluorescence; the transmitter may be gamma-aminobutyric acid (Vincent et al., '78). Afferents of the substantia nigra come primarily from the striatum (Voneida, '60; Szabo, '62; Nauta and Mehler, '66; Cowan and Powell, '66; Grofová and Rinvik, '70). There is some evidence that striatal projection to the pars compacta is derived predominantly from the globus pallidus (Nauta and Mehler, '66; Hattori et al., '75) but this has been questioned (Bunney and Aghajanian, '76). In contrast, the afferents of the pars reticulata may derive predominantly from the caudate nucleus and putamen (Szabo, '62; Grofová and Rinvik, '70; Hattori et al., '75) rather than the globus pallidus (Nauta and Mehler, '66). The projection appears to be topographical: Fibers from the caudate nucleus end in the rostral pars reticulata, whereas fibers from the putamen terminate in the caudal pars reticulata (Szabo, '62).

In most animals injected on days E13+14 all the neurons of the substantia nigra were labeled, both in its pars compacta and pars reticulata. In a few animals an occasional unlabeled neuron was seen in the rostral portion of the substantia nigra (Fig. 24). In animals injected on days E14+15 many neurons were no longer labeled rostrally (Fig. 25A,B) but the majority of them were labeled caudally (Fig. 25A,C). In animals injected on days E15+16 there were few labeled neurons rostrally, and virtually none at this level dorsolaterally (Fig. 26C). Caudally, the labeled cells were mainly concentrated ventromedially (Fig. 26A,B). The rarity of labeled cells in this

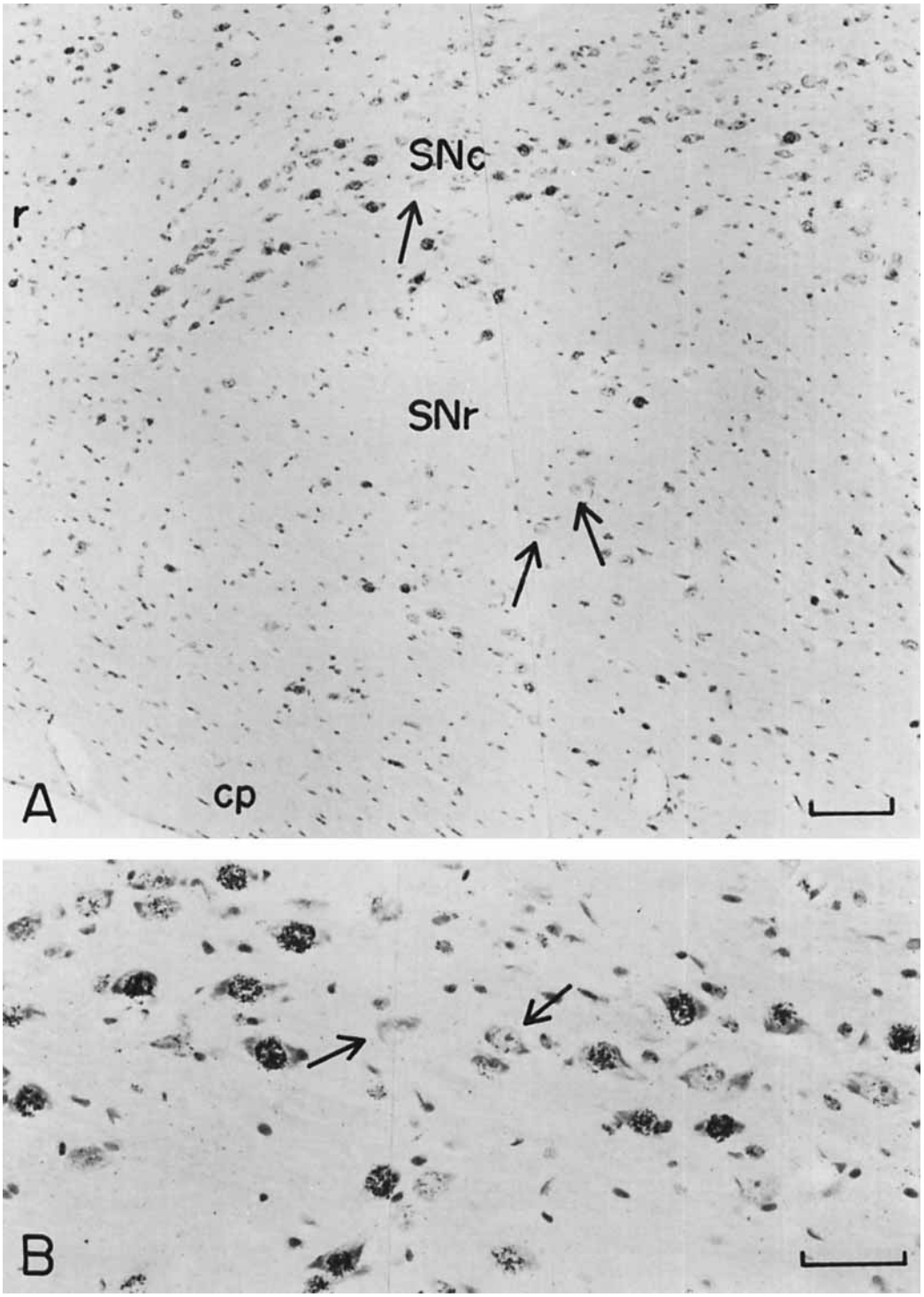

Fig. 24. A. Pattern of cell labeling in the pars compacta and pars reticulata of the rostral part of a substantia nigra. Sagittal section from a rat injected on days E13 + 14. Scale: 100 μm. B. The pars compacta at higher magnification. Arrows point to unlabeled cells. Scale: 50 μm.

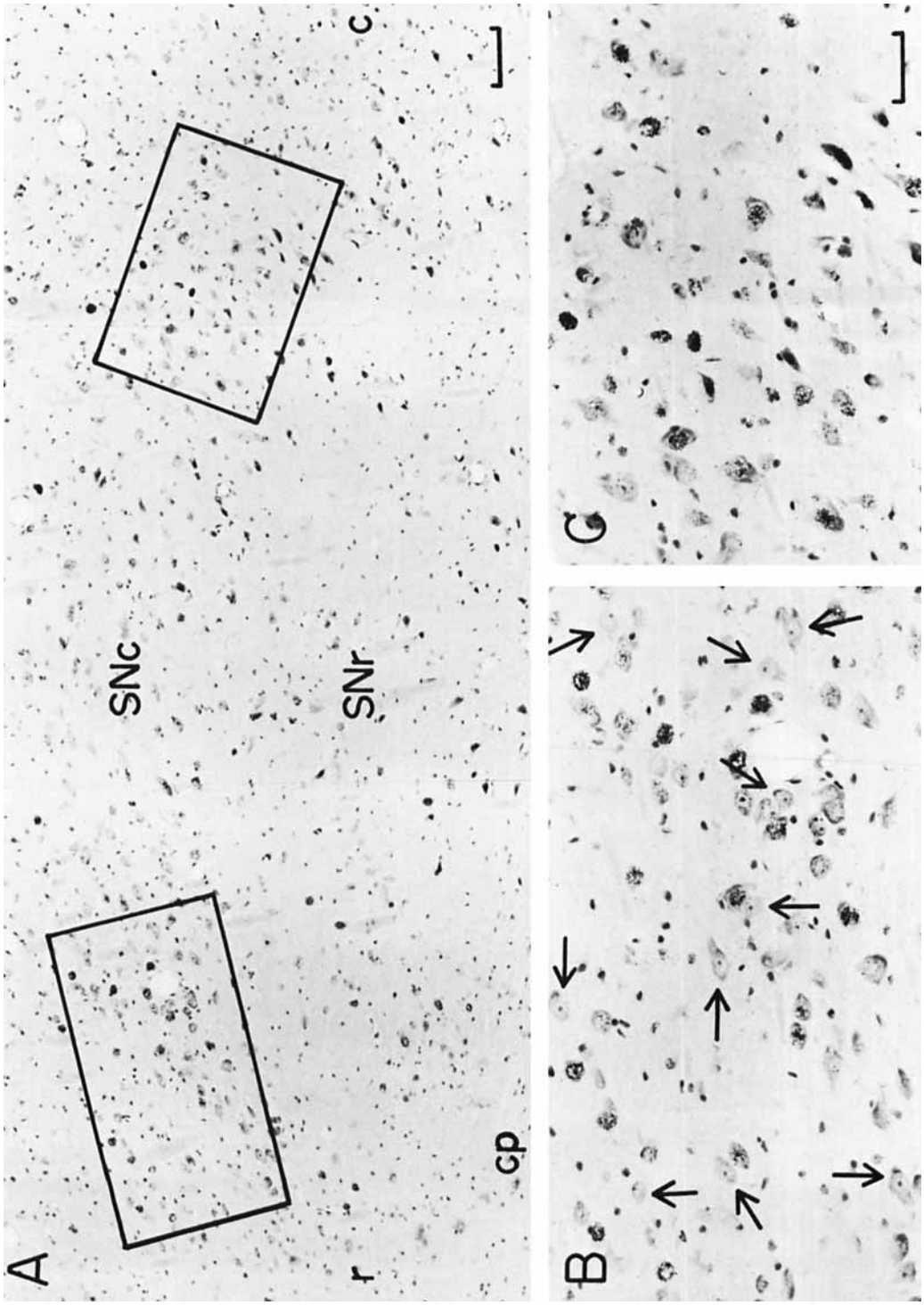

Fig. 25. A. Pattern of cell labeling in a sagittal section of the substantia nigra from a rat injected on days E14 + 15. Scale: 100 μm. Higher magnification of the pars compacta rostrally is shown in B, and caudally in C. Arrows point to unlabeled cells rostrally. Scale for B and C: 50 μm.

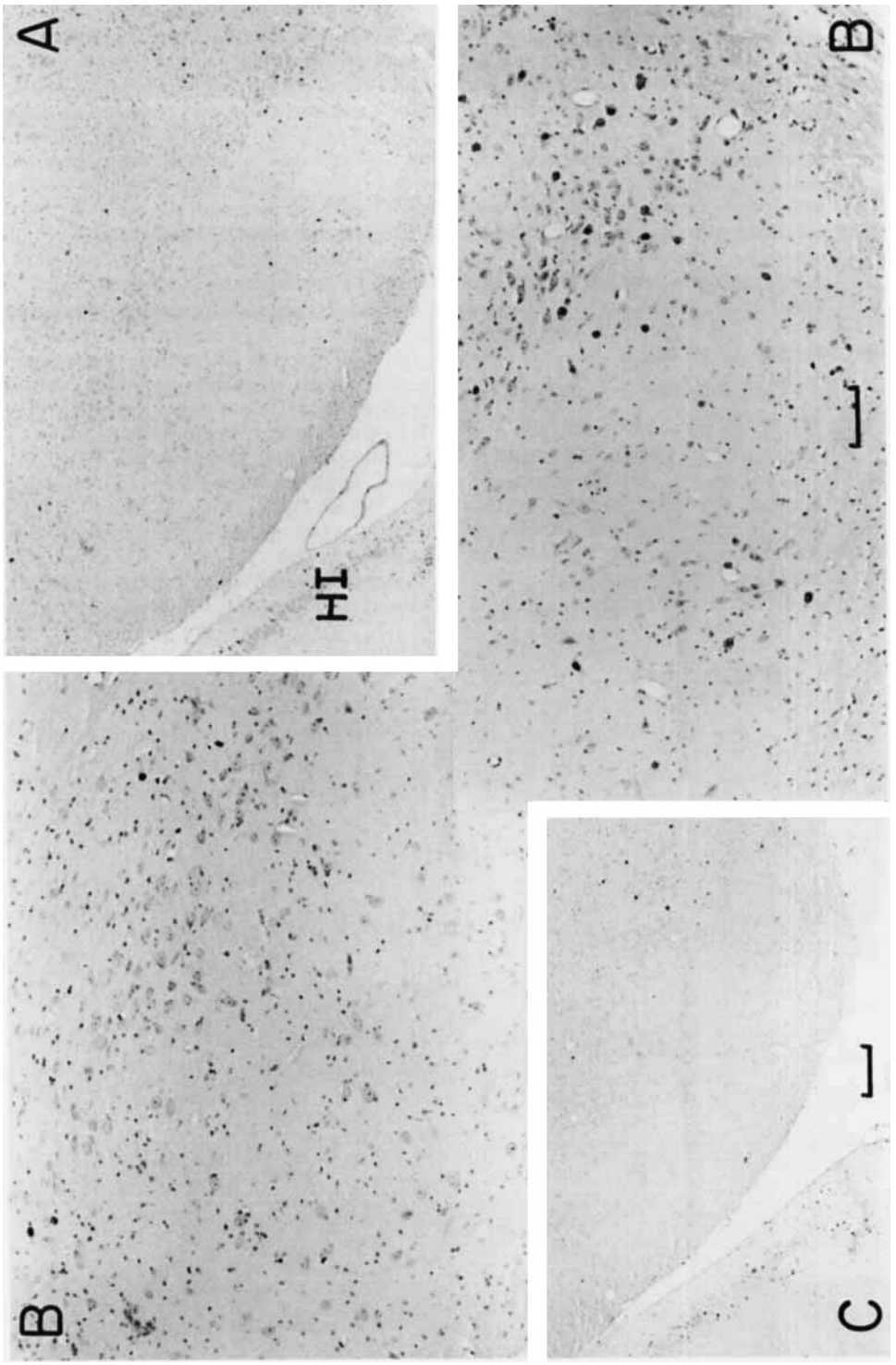

Fig. 26. Pattern of cell labeling in the substantia nigra in coronal sections from a rat injected on days E15 + 16. A and B. Caudal sections at low and high magnification, respectively. C. A rostral section. A and C, Scale: 200 μm. B, Scale: 100 μm.

injection group rostrally and particularly dorsally, and their relative abundance caudally and particularly ventrally, are illustrated in Figure 27 in horizontal sections. At the different levels there was no obvious difference in labeling pattern between the pars compacta and pars reticulata. These observations suggest an anterodorsal-to-posteroventral gradient in the substantia nigra.

For quantitative purposes the attempt was made to scan matched midcoronal sections of the substantia nigra. The results indicate (Fig. 1F) that the neurons are generated on days E13, E14, and E15 at a relatively even rate with no difference between the pars compacta and pars reticulata ($P < 0.146$).

DISCUSSION

The nuclei of the visuomotor system

In the midbrain tegmentum the earliest generated neurons, except for the scattered cells of the mesencephalic nucleus of the trigeminal (Altman and Bayer, '80d), are those of the trochlear and oculomotor nuclei. They precede the neurons of other tegmental nuclei implicated in visual functions, the nucleus of Darkschewitsch, the Edinger-Westphal nucleus, and the parabigeminal nucleus. The neurons of the trochlear and oculomotor nuclei also antedate: (1) all cellular components of the superior colliculus, including the large neurons that are believed to be the source of the tectospinal tract (Altman and Bayer, '80f); (2) the cells of the mesencephalic raphe and central gray nuclei; and (3) all the suprasegmental nuclei of the area (nuclei that do not have analogs in the more caudal regions of the brain stem), namely, the red nucleus, the interpeduncular nucleus, and the substantia nigra.

The early production of the trochlear and oculomotor neurons may be related to the early generation time of all somatic motor neurons. Figure 28 presents a summary of birth dates of components of this system in the brain stem. We added to the oculomotor and trochlear nucleus the abducens nucleus (Altman and Bayer, '80b), the hypoglossal nucleus (Altman and Bayer, '80a), and also the retrofacial nucleus, which we have described as a possible source of the somatic efferents of the glossopharyngeal nerve (Altman and Bayer, '80b). Within the group of ocular muscle nuclei,

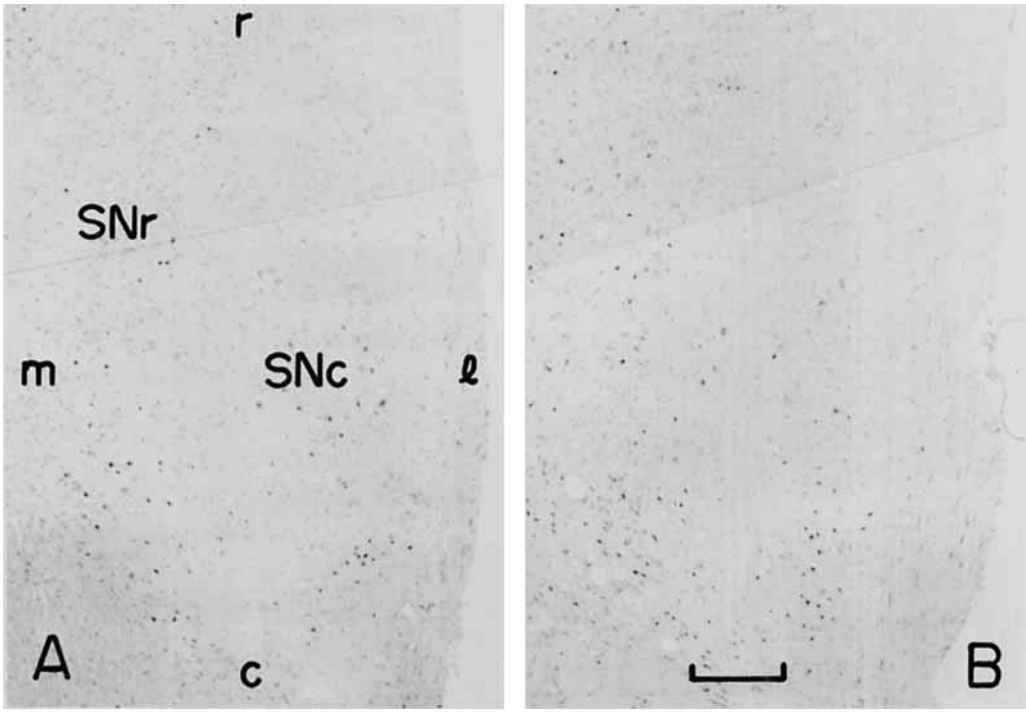

Fig. 27. Horizontal sections from a rat injected on days E15 + 16; A, dorsal; B, ventral. Scale: 300 μm.

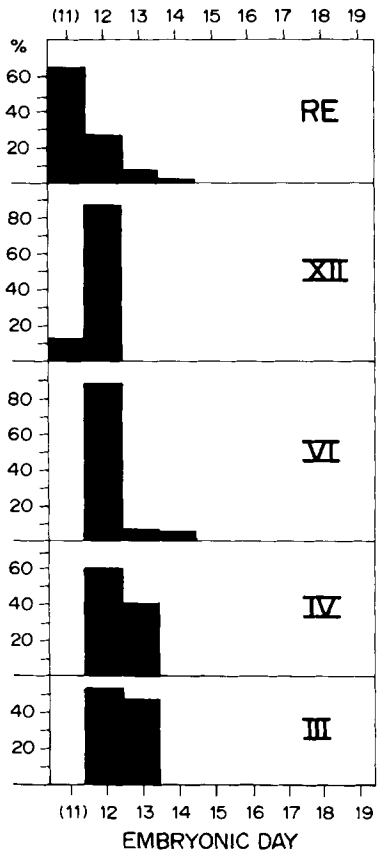

Fig. 28. Summary of the time of origin of neurons of the somatic motor nuclei of the brain stem. (Data for the retrofacial nucleus and for the abducens nucleus from Altman and Bayer, '80b, and for the hypoglossal nucleus from Altman and Bayer, '80a.)

89% of the neurons of the abducens neurons are produced on day E12 but only 50% of the trochlear neurons and 53% of the oculomotor neurons. This indicates a caudal-to-rostral internuclear gradient with respect to the latter two nuclei. The same gradient is also indicated if the hypoglossal nucleus is included in this system, which we have designated earlier as part of the MS cytogenetic zone (Altman and Bayer, '80b). It is not clear whether the earliest-produced neurons of the retrofacial nucleus belong into this system (Fig. 29). The retrofacial nucleus differs from the others by a relatively long time span of neuron production (it is likely that some of its neurons are produced before day E11) and by their distal settling site from the ventricular lumen.

Because of its small size, we could not detect an intranuclear gradient in the abducens nucleus but we noted gradients within the trochlear nucleus and, more clearly, in the oculomotor nucleus. We interpreted the mirror-image intranuclear gradients in the latter two structures (Fig. 6B) as proximal-to-distal in relation to the medial longitudinal fasciculus. The implication was that the earliest-produced (and presumably earliest-arriving) neurons settle closest to the fiber tract with which they establish intimate contact and the later-arriving neurons pile up behind them. We have described such a proximal-to-distal gradient in the case of neurons of the pontine gray in relation to the pyramidal tract (Altman and Bayer, '78b). But unlike in the latter case, the migratory path of the neurons of the ocular muscle nuclei is very short. More parsimoniously the gradient can be described as an outside-in pattern in relation to the aqueduct (Fig. 6B). The cytogenetic gradient could not be related to the subdivisions of the different eye muscles in the oculomotor nucleus of the rat (Glicksman, '80).

The somatic motor neurons of the eye muscles are generated before the neurons of the nucleus of Darkschewitsch and the Edinger-Westphal nucleus. However, the relative precocity of the Darkschewitsch neurons in relation to the rest of the mesencephalon is noteworthy; it is unfortunate that so little is known about its connections and functions. The classical view that the Edinger-Westphal nucleus is a preganglionic structure in relation to the ciliary ganglion (Warwick, '54) has failed to gain support in recent studies with axoplasmic tracer techniques (Loewy and Saper, '78; Loewy et al., '78; Sugimoto et al., '78). Only a few of its neurons seem to supply fibers to the ciliary ganglion; others were traced to various medullary sites and to the spinal cord. We proposed previously (Altman and Bayer '80b: Fig. 15) that the preganglionic neurons of the dorsal nucleus of the vagus and the superior salivatory nucleus may constitute a preganglionic cytogenetic zone (PG?) with a caudal-to-rostral gradient. We added later (Altman and Bayer, '80d: Fig. 18B) the infra- and supratrigeminal nuclei as possible rostral components of the same system. Since a high proportion of the neurons of the infratrigeminal nucleus are produced on day E16 (Altman and Bayer, '80d: Fig. 1C) the earlier-generated (Fig. 2A), more rostrally situated Edinger-Westphal nucleus cannot be part of the gradient of the hypothesized

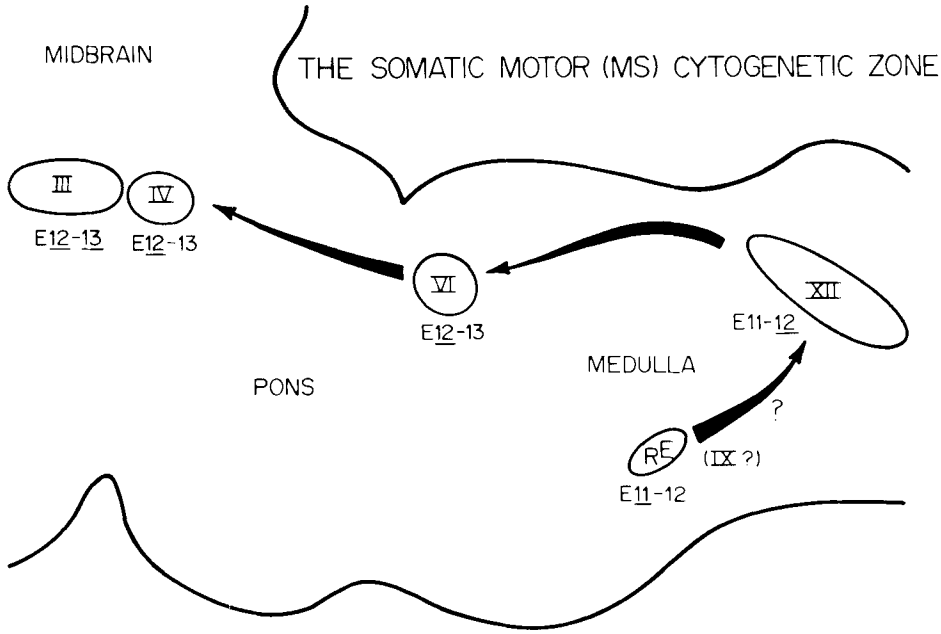

Fig. 29. Caudal-to-rostral internuclear gradient in the somatic motor (MS) cytogenetic zone of the brain stem. It is questionable whether the retrofacial nucleus is part of the system.

preganglionic cytogenetic system. The cells constituting the second peak on day E15 might be the preganglionic elements of the Edinger-Westphal nucleus. The temporal order of neuron production in the ocular muscle nuclei, the nucleus of Darkschewitsch, and the Edinger-Westphal nucleus (Fig. 2A) suggests a possible hierarchical organization within the system in which there is a descending order in terms of cell size. The latest-generated parabigeminal nucleus may be part of another system more closely related to the late-forming superior colliculus (Altman and Bayer, '80f).

The periaqueductal gray

The neurons of the periaqueductal gray were produced later and over a more protracted period than the neurons of the ocular muscle nuclei and associated structures embedded in it. The pattern, in terms of duration of neuron production, resembled the superior colliculus (Altman and Bayer, '80f). But in several layers of the superior colliculus neuron, production started somewhat later and in most of them continued longer than in the central gray.

The cytogenetic gradient observed in the periaqueductal gray was essentially a ventral-to-dorsal one, with possibly a similar gradient within each of its three subdivisions (Fig. 10). One interpretation of this is that the earlier-produced pars ventralis is related to the ontogenetically older tegmental neurons and the later-produced pars dorsalis to the younger neurons of the tectum. An implication of this view is that the pars ventralis is derived from the embryonic basal plate and the pars dorsalis from the alar plate. However, the type of cells in the periaqueductal gray, and their late and prolonged production, suggest that the entire periaqueductal gray is of alar plate derivative. The extant ependymal lining of the aqueduct, which shows a labeling gradient (Fig. 11) similar to that of the central gray, may be derived from the alar plate. If so, the basal plate, or tegmental portion, of the large embryonic aqueduct (mesencoele) must be obliterated during development. The possibility may be entertained that the dorsal parts of the central gray are related to the optic layers of the superior colliculus and its lateral and ventral parts to the nonoptic layers of the superior colliculus and to the satellite structures of the eye muscle nuclei (Altman and Bayer, '80f).

As an extension of this argument, we suggest a hierarchical organization in the development of the midbrain visual system. The motor neurons of the extraocular muscles and some associated nuclei are generated first, the intercalated neurons of the central gray next, while many of the neurons of the superior colliculus, particularly those located in layers receiving optic and other afferents, are produced last. Such a view is reconcilable with the intimate afferent and efferent connections of the periaqueductal gray with the superior colliculus, as reviewed earlier, and the widely held view that the connection between the superior colliculus and the eye muscle nuclei is an indirect one. We shall return to this topic in a future paper of this series (Altman and Bayer, '80f). However, in such considerations we must not forget that the central gray has been implicated in functions other than vision, particularly nociception.

The red nucleus

Neurogenesis in the red nucleus was singularly rapid. The bulk of the cell population was produced on day E13 and the process was completed on day E14. Since the temporal pattern of cell production was very similar in both the magnocellular and parvocellular divisions, we may tentatively assume that the two derive from a single neuroepithelial locus; we shall designate the system as cytogenetic zone RN. The boundaries of the two components are not sharp and our cell counts of the rostral portion undoubtedly included magnocellular cells and vice versa. But in spite of this procedural difficulty we obtained a significant difference between the generation time of the magnocellular and parvocellular divisions.

The earlier production of the caudal magnocellular neurons with respect to the rostral parvocellular neurons may be related to phylogenetic differences in the origin of these two parts of the red nucleus (Hatschek, '07; Monakow, '10; Massion, '67; Reid et al., '75). Probably related to these phylogenetic considerations is the evidence that the parvocellular neurons are involved in the control of the limbs not of the axial musculature (Padel et al., '73). This later-generated region receives afferents from the neocerebellum (the dentate nucleus; Courville, '66) and the neocortex (Gwyn and Flumerfelt, '74; Brown, '74a; Oka and Jinnai, '78). The earlier-generated magnocellular division does not receive a cortical projection, only a cerebellar one (Gwyn and Flumerfelt, '74; Brown, '74a), mainly from the interpositus nucleus (Massion, '67). The neurons of the deep cerebellar nuclei are generated on days E13 to E15 with a peak on day E14 (Altman and Bayer, '78a), whereas the neurons of the cortex that project to the cerebellum, assuming that they are layer V pyramidal cells, are generated later, about day E16 (Berry and Rogers, '65; Hicks and D'Amato, '68; Bisconte and Marty, '75). In this context it is noteworthy that the cells both in the magnocellular and parvocellular divisions of the red nucleus are generated before the cells of their major afferents.

The interpeduncular nucleus

The neurons of the interpeduncular nucleus originate between days E13 and E15 with a pronounced ventral-to-dorsal gradient: Peak production time of the neurons is day E13 ventrally, and days E14 and E15 dorsally (Fig. 1D). If the settling pattern of the cells in this nucleus follows the outside-in principle indicated for most ganglionic structures, then the germinal source of these neurons must be situated dorsally in the embryonic aqueduct. We shall designate this system as cytogenetic zone IP.

We noted in our study of the projections of the medial and lateral habenular nuclei (Altman and Bayer, '79: Fig. 10) that the fibers of the earlier-generated lateral habenular nucleus project to the earlier-generated dorsal tegmental nucleus, whereas the fibers of the later-generated medial habenular nucleus make connections with the later-generated deep tegmental nucleus. A similar relationship was seen in the efferent projections of the dorsal and deep tegmental nuclei to the lateral and medial mammillary nuclei, respectively. The implied "first come-first served" principle does not seem to hold for the projection of the medial habenular nucleus to the interpeduncular nucleus. Neurogenesis in the lateral portion of the medial habenular nucleus antedates cell production in its medial part (Altman and Bayer, '79). However, according to Herkenham and Nauta ('79) the lateral portion of the medial habenular nucleus (the early component) projects to the dorsal part of the interpeduncular nucleus (its late component), and the medial part of the medial habenular nucleus projects to the ventral interpeduncular nucleus. We cannot resolve this inconsistency at the present. It is possible that the principle applies only to different structures (lateral versus medial habenular nucleus, for instance) but not to components of a given structure (in this case

the medial and lateral parts of the medial habenular nucleus). It is also possible that because of the very late arrival of the axons from the medial habenular nucleus (the cells themselves are generated between days E15 and E19) an organization has already been established in the interpeduncular nucleus which precludes specification of its components by medial-habenular fibers. Indeed, the habenulopeduncular tract is clearly recognizable in day E15 embryos (Altman and Bayer, '79: Fig. 2), indicating the presence of connections between these two structures at this early age. The operation of complex forces in the establishment of habenulopeduncular connections is suggested by the crossing or recrossing of these fibers in the habenula (Ramón y Cajal, '11; Herkenham and Nauta, '79). Finally, the possibility must be entertained that the "first come-first served" principle is not a valid one and that the relationship observed in our previous study was coincidental.

The substantia nigra

Hanaway et al. ('70) concluded on the basis of single injections of ^{3}H-thymidine in the rat that the neurons of the substantia nigra are produced between days E11 and E15 with maximal production on days E14 and E15. The same temporal range was reported with the flash-labeling procedure by Lauder and Bloom ('74) but with a peak production time on day E13. Our results with the comprehensive labeling procedure showed that with injections beginning on day E13 practically all the neurons of the substantia nigra could be labeled. The quantitative analysis indicated that the neurons are produced between days E13 and E15 with a rather even distribution throughout these 3 days (Fig. 1F).

According to the evidence reviewed earlier the pars compacta neurons project preferentially to the striatum and are rich in dopamine, whereas the neurons of the pars reticulata project preferentially to the thalamus and tectum and do not contain dopamine. In spite of these differences in anatomical connections and transmitter chemistry we could not detect any systematic difference in the cytogenesis of the two components of the substantia nigra. Rather, at any given coronal level the labeling pattern was similar between the two divisions. The gradient that we noted could be best described as one from rostral and dorsolateral to caudal and ventromedial (Fig. 30). This gradient suggests that the two divisions of the substantia nigra originate in a shared, medial neuroepithelial locus situated posteroventral from the settling site and that the cells are distributed in an outside-in pattern. We shall designate this system as cytogenetic zone SN with the proviso that the germinal locus may be composed of two separate cell lines that proliferate and migrate out at the same time. Olson and Seiger ('72) reported that dopamine fluorescence first appears in the tegmentum of rat embryos of 9 mm crown-rump length. This corresponds to day E13, the first day when neuron production begins in the substantia nigra. Whether chemical differentiation begins before or after cell division has stopped is uncertain. The report of Olson and Seiger seems to indicate that the dopamine-fluorescing cells have left the vicinity of the ventricular lumen and already have ascending processes.

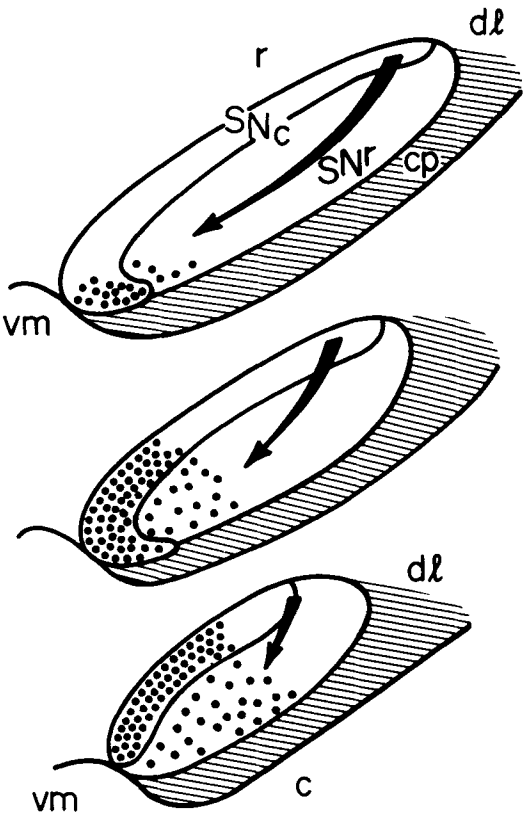

Fig. 30. Pattern of cell labeling in the substantia nigra of rats injected on days E15 + 16. Arrows indicate the inferred intranuclear gradients from dorsolateral to ventromedial.

The reconstructed cytogenetic gradient may be considered a topologically distorted lateral-to-medial pattern. This would be similar to the lateral-to-medial gradient in the basal ganglia (unpublished observations). However, it may be unjustified to attach too much significance to the corresponding topographic distribution of nigral fibers in the striatum (Fallon and Moore, '78; Beckstead et al., '79) because it is difficult to see any further relationship between nigral cytogenetic and fiber projection patterns. The neurons of the striatum and of the superior colliculus, as a whole, are produced later than the neurons of the thalamus. However, the distribution pattern of nigrothalamic, nigrotectal, and nigrostriatal cells, as described by Faull and Mehler ('78), is not easy to match with the cytogenetic gradient obtained in this study.

In recent descriptions, the ventral tegmental area of Tsai is usually classified with the adjacent substantia nigra because an unspecified proportion of its cells contribute fibers to the ascending dopaminergic pathway (Dahlström and Fuxe, '64; Ungerstedt, '71; Fallon and Moore, '78; Simon et al., '79; Beckstead et al., '79). We found that the neurons of both the pars lateralis and pars medialis of this diffuse area are produced later than the neurons of the substantia nigra ($P < 0.003-0.0001$) and that there was a pronounced lateral-to-medial cytogenetic gradient in the ventral tegmental area. The region may therefore constitute part of cytogenetic zone SN and its gradient be part of the lateral-to-medial distribution of cells inferred for the substantia nigra.

The raphe nuclei of the brain stem

The present study showed that neurons of both the dorsal and the median raphe nuclei are generated between days E13 and E15. This is reconcilable with the results of Lauder and Bloom ('74: Fig. 4), who found that some of the cells in these nuclei could be labeled on day E15 but no longer on day E16. We sampled for procedural reasons different components of the two nuclei – the ventral region in the dorsal nucleus and the dorsal region in the median nucleus. Because in both nuclei there was a ventral-to-dorsal gradient it seemed unjustified to compare statistically the dates from the two nuclei. It is likely that the two nuclei are cytogenetically contemporaneous and that the neurons originate from a shared source situated dorsal to the settling sites.

The study of cytogenesis in the dorsal and median raphe nuclei completes our survey of the raphe nuclei of the brain stem (Fig. 31). The earliest component of this serotonergic system (Andén et al., '66; Ungerstedt, '71) is the raphe magnus in the upper medulla; its neurons are produced significantly before the more caudally situated neurons of the nuclei raphe pallidus and obscurus (Altman and Bayer, '80b). There was no difference in cytogenesis between the latter two nuclei (Altman and Bayer, '80a). The raphe neurons of the upper and lower medulla may constitute a single cytogenetic zone with a rostral-to-caudal gradient; it is designated tentatively as RR1 (Fig. 32). A rostral-to-

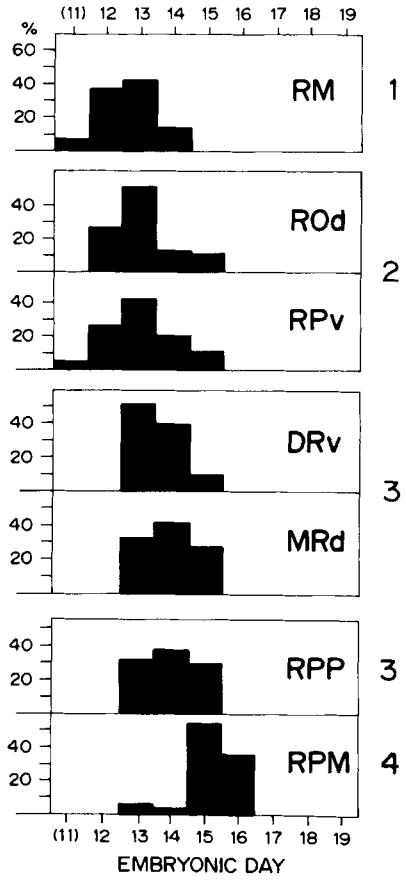

Fig. 31. Comparison of the time of origin of neurons in the brain stem raphe nuclei. (Data for the nuclei of raphe obscurus and raphe pallidus from Altman and Bayer. '80a; for raphe magnus from Altman and Bayer, '80b; and for raphe pontis parvicellularis and magnocellularis from Altman and Bayer, '80d.) Numerals refer to chronological order in cytogenesis among those nuclei that differed from the others significantly.

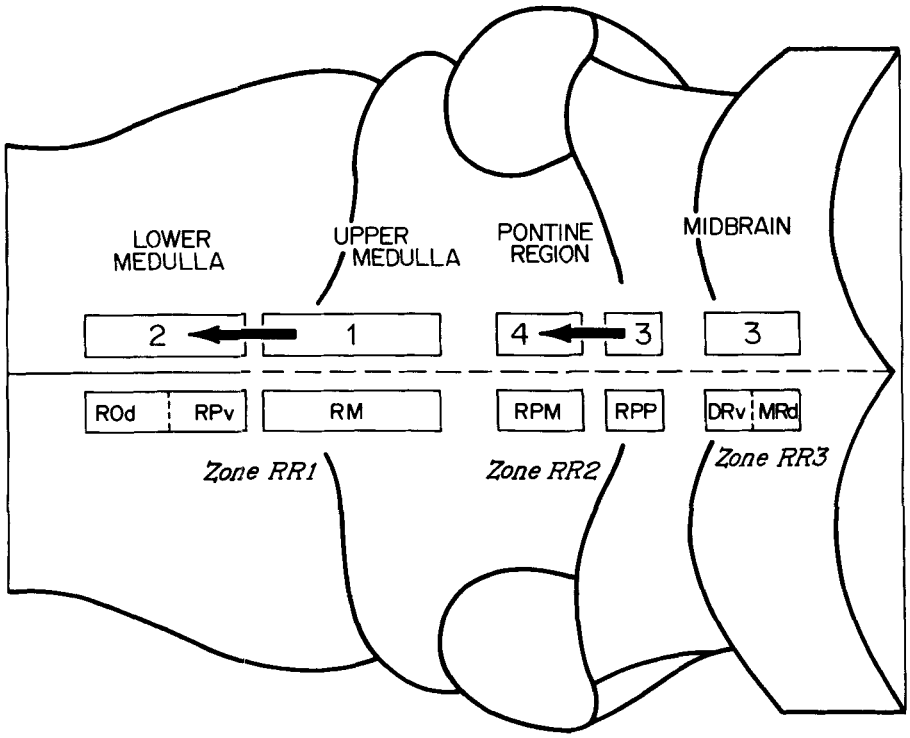

Fig. 32. Summary diagram of the sequence of neuron origin in the raphe nuclei of the brain stem. The raphe nuclei in the caudal medulla and in the rostral medulla show separate rostral-to-caudal gradients. It is postulated that the raphe nuclei constitute a minimum of three distinct cytogenetic zones.

caudal gradient was also obtained between the nucleus parvocellularis and nucleus magnocellularis of the pontine raphe (Altman and Bayer, '80d). The large neurons of the latter nucleus are the latest-produced elements of the raphe nuclei. These two raphe nuclei may constitute another cytogenetic zone, designated as RR2. The time of origin of neurons in the dorsal and median raphe nuclei appears to overlap with the nucleus parvocellularis raphe pontis (Fig. 31). It is unclear at present whether or not the two are cytogenetically related. Tentatively we shall designate the midbrain raphe nuclei as parts of cytogenetic zone RR3. In general, a clear internuclear gradient is not indicated between the raphe nuclei of the midbrain, pons, and the medulla. It is possible that the components of this complex, although they are similar in terms of a midline location and transmitter chemistry, are of heterogeneous embryonic derivation.

The monoamine neurons of the brain stem

There has been considerable interest in recent years in the monoamine-containing neurons of the brain. The major components of this system in the region of the pons and the midbrain are the locus coeruleus (noradrenaline fibers), the dorsal and median raphe nuclei (serotonin fibers), and the substantia nigra and its vicinity (dopamine fibers). A shared property of these rostral monoamine-containing structures is that their ascending axons are distributed diffusely and widely over the forebrain. In addition to implicating this neuronal system in a great variety of visceral and behavioral functions, it has also been ascribed a regulatory role in neurogenesis. The original hypothesis was based on biochemical and pharmacological investigations dealing with early embryogenesis (Baker and Quary, '69; Buznikov et al., '68, '70; Gustafson and Toneby, '70) but our studies do not bear on this. But our cytogenetic findings are relevant to an extension of the hypothesis to neurogenesis in mammals. Olson and Seiger ('72) reported that in the rat serotonin-containing neurons begin to appear in 8-mm embryos (about day E12.5), dopamine-containing neurons in 9-mm embryos (about day E13), and noradrenaline-containing neurons in 11-mm embryos (day E14). The early origin of these neurons was confirmed in a subsequent autoradiographic study by Lauder and Bloom ('74). Olson and Seiger suggested

that these early-differentiating neurons may exert a regulatory role on later-developing structures to which they project, and this proposition was examined by Lauder and Bloom in relation to neurogenesis in the cerebellum and the hippocampus.

Our studies of cytogenesis in these monoamine-rich nuclei confirm the early origin of one of its components, namely the locus coeruleus, with 75% of its cells forming on day E12 (Altman and Bayer, '80d). Curiously, the noradrenaline-containing locus coeruleus neurons were described by Olson and Seiger ('72) as the latest chemically differentiating elements of the group. The cells of the other monoamine-containing nuclei are not distinguished by early cytogenesis. As we noted earlier (Fig. 31), the neurons of the raphe nuclei arise over an extended period, and the rostral nuclei, which are the major source of ascending fibers, are produced later than the caudal nuclei. The neurons of the nuclei raphe pontis (Fig. 31), which are sources of cerebellar fibers (Bobillier et al., '76), are produced with a peak either on day E14 (parvocellular nucleus) or day E15 (magnocellular nucleus). These cells arise later than the cells of origin of the climbing fibers in the inferior olive, where over 75% of the neurons are produced on day E12 or earlier and cytogenesis is completed by day E13 (Altman and Bayer, '78b: Fig. 3). The onset of cytogenesis in the median and dorsal raphe nuclei (day E13) does antedate cell production in most of the relay nuclei of the thalamus (E14; Altman and Bayer, '79), but the distances that the fibers of the thalamus have to grow to their target structures are considerably shorter. While these considerations do not rule out a neurotrophic role for the monoamine neurons of the brain stem they appear to provide little support for such a concept.

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LITERATURE CITED

Abplanalp, P. (1971) The neuroanatomical organization of the visual system in the tree shrew. Folia Primatol. *16*:1–34.

Afifi, A., and W.W. Kaelber (1965) Efferent connections of the substantia nigra in the cat. Exp Neurol. *11*:474–482.

Akagi, Y. (1978) The localization of the motor neurons innervating the extraocular muscles in the oculomotor nuclei of the cat and rabbit, using horseradish peroxidase. J. Comp. Neurol. *181*:745–762.

Akagi, K., and E.W. Powell (1968) Differential projections of habenular nuclei. J. Comp. Neurol. *132*:263–274.

Altman, J., and S.A. Bayer (1978a) Prenatal development of the cerebellar system in the rat. I. Cytogenesis of the deep nuclei and the cortex of the cerebellum. J. Comp. Neurol. *179*:23–48.

Altman, J., and S.A. Bayer (1978b) Prenatal development of the cerebellar system in the rat: II. Cytogenesis and histogenesis of the inferior olive, pontine gray, and the precerebellar reticular nuclei. J. Comp. Neurol. *179*:49–76.

Altman, J., and S.A. Bayer (1979) Development of the diencephalon in the rat. IV. Quantitative study of the time of origin of neurons and the internuclear chronological gradients in the thalamus. J. Comp. Neurol. *188*:455–472.

Altman, J., and S.A. Bayer (1980a) Development of the brain stem in the rat. I. Thymidine-radiographic study of the time of origin of neurons of the lower medulla. J. Comp. Neurol., *194*:1–35.

Altman, J., and S.A. Bayer (1980b) 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 J. Comp. Neurol., *194*:37–56.

Altman, J., and S.A. Bayer (1980c) Development of the brain stem in the rat. III. Thymidine-radiographic study of the time of origin of neurons of the vestibular and auditory nuclei of the upper medulla. J. Comp. Neurol., *194*:877–904.

Altman, J., and S.A. Bayer (1980d) Development of the brain stem in the rat. IV. Thymidine-radiographic study of the time of origin of neurons in the pontine region. J. Comp. Neurol., *194*:905–929.

Altman, J., and S.A. Bayer (1980e) Time of origin of neurons of the rat inferior colliculus and the relations between cytogenesis and tonotopic order in the auditory pathway. Exp. Brain Res. (In press).

Altman, J., and S.A. Bayer (1980f) Time of origin of neurons of the rat superior colliculus in relation to other components of the visual and visuomotor pathways. Exp. Brain Res. (In press).

Altman, J., and M.B. Carpenter (1961) Fiber projections of the superior colliculus in the cat. J. Comp. Neurol. *116*:157–177.

Andén, N.-E., A. Dahlström, K. Fuxe, and K. Larsson (1965) Further evidence for the presence of nigro-neostriatal dopamine neurons in the rat. Am. J. Anat. *116*:329–334.

Andén, N.-E., A. Dahlström, K. Fuxe, K. Larsson, L. Olson, and U. Ungerstedt (1966) Ascending monoamine neurons to the telencephalon and diencephalon. Acta Physiol. Scand. *67*:313–326.

Anderson, M., and M. Yoshida (1977) Electrophysiological evidence for branching nigral projections to the thalamus and the superior colliculus. Brain Res. *137*:361–364.

Azmitia, E.C., and M. Segal (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J. Comp. Neurol. *179*:641–668.

Baker, P.C., and W.B. Quary (1969) 5-hydroxytryptamine metabolism in early embryogenesis and the development of brain and retinal tissues. A review. Brain Res. *12*:273–295.

Baker, R., and A. Berthoz (1975) Is the prepositus hypoglossi nucleus the source of another vestibulo-ocular pathway? Brain Res *86*:121–127.

Baleydier, C., and M. Magnin (1979) Afferent and efferent connections of the parabigeminal nucleus in cat revealed by retrograde axonal transport of horseradish peroxidase. Brain Res. *161*:187–198.

Beckstead, R.M., V.B. Domesick, and W.J.H. Nauta (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res *175*:191–217.

Bédard, P., L. Larochelle, A. Parent, and L.J. Poirier (1969)

The nigrostriatal pathway: A correlative study based on neuroanatomical and neurochemical criteria in the cat and the monkey. Exp. Neurol. *25*:365–377.

Benevento, L.A., and J.H. Fallon (1975) The ascending projections of the superior colliculus in the rhesus monkey (*Macaca mulatta*). J. Comp. Neurol. *160*:339–362.

Bentivoglio, M., D. Vander Kooy, and H.G.J.M. Kuypers (1979) The organization of the efferent projections of the substantia nigra in the rat. A retrograde fluorescent double labeling study. Brain Res. *174*:1–79.

Berman, A.L. (1968) The Brain Stem of the Cat. Madison: University of Wisconsin Press.

Berry, M., and A.W. Rogers (1965) The migration of neuroblasts in the developing cerebral cortex. J. Anat. (Lond.) *99*:691–709.

Bisconte, J.-C., and R. Marty (1975) Etude quantitative du marquage radioautographique dans le système nerveux du rat. II. Charactéristiques finales dans le cerveau de l'animal adulte. Exp. Brain Res. *22*:37–56.

Bobillier, P., F. Petitjean, D. Salvert, M. Ligier, and S. Seguin (1975) Differential projections of the nucleus raphe dorsalis and nucleus raphe centralis as revealed by autoradiography. Brain Res. *85*:205–210.

Bobillier, P., S. Seguin, F. Petitjean, D. Salvert, M. Touret, and M. Jouvet (1976) The raphe nuclei of the cat brain stem: A topographical atlas of their efferent projections as revealed by autoradiography. Brain Res. *113*:449–486.

Brown, L.T. (1974a) Corticorubral projections in the rat. J. Comp. Neurol. *154*:149–168.

Brown, L.T. (1974b) Rubrospinal projections in the rat. J. Comp. Neurol. *154*:169–188.

Bunney, B.S., and G.K. Aghajanian (1976) The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. Brain Res. *117*:423–435.

Büttner-Ennever, J.A., and V. Henn (1976) An autoradiographic study of the pathways from the pontine reticular formation involved in horizontal eye movements. Brain Res. *108*:155–164.

Buznikov, G.A., I.V. Chudakova, L.V. Berdysheva, and N.M. Vyazmina (1968) The role of neurohumours in early embryogenesis. II. Acetylcholine and catecholamine content in developing embryos of sea urchin. J. Embryol. Exp. Morphol. *20*:119–128.

Buznikov, G.A., A.N. Kost, N.F. Kucherova, and L.V. Berkysheva (1970) The role of neurohumours in early embryogenesis. III. Pharmacological analysis of the role of neurohumours in cleavage divisions. J. Embryol. Exp. Morphol. *23*:549–569.

Carpenter, M.B., and G.R. Hanna (1962) Effects of thalamic lesions upon cerebellar dyskinesia in the rhesus monkey. J. Comp. Neurol. *119*:127–147.

Carpenter, M.B., J.W. Harbison, and P. Peter (1970) Accessory oculomotor nuclei in the monkey: Projections and effects of discrete lesions. J. Comp. Neurol. *140*:131–154.

Carpenter, M.B., and P. Peter (1972) Nigrostriatal and nigrothalamic fibers in the rhesus monkey. J. Comp. Neurol. *144*:93–116

Castaldi, L., (1923) Quoted from Hamilton and Skultety (1970).

Cole, M., W.J.H. Nauta, and W.R. Mehler (1964) The ascending efferent projections of the substantia nigra. Trans. Am Neurol. Assoc. *89*:74–78.

Conrad, L.C.A., C.M. Leonard, and D.W. Pfaff (1974) Connections of the median and dorsal raphe nuclei in the rat: An autoradiographic and degeneration study. J. Comp. Neurol. *156*:179–206.

Courville, J. (1966) Somatotopical organization of the projection from the nucleus interpositus anterior of the cerebellum to the red nucleus. An experimental study in the cat with silver impregnation methods. Exp. Brain Res. *2*:191–215.

Courville, J., and S. Otabe (1974) The rubro-olivary projection in the macaque: An experimental study with silver impregnation methods. J. Comp. Neurol. *158*:479–494.

Cowan, M.W., and T.P.S. Powell (1966) Strio-pallidal projection in the monkey. J. Neurol. Neurosurg. Psychiat. *29*:426–439.

Dahlström, A., and K. Fuxe (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand. Suppl. 232, *62*:1–55.

Edwards, S.B. (1972) The ascending and descending projections of the red nucleus in the cat: An experimental study using an autoradiographic tracing method. Brain Res. *48*:45–63.

Edwards, S.B., and C.K. Henkel (1978) Superior colliculus connections with the extraocular motor nuclei in the cat. J. Comp. Neurol. *179*:451–468.

Fallon, J.H., and R.Y. Moore (1978) Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J. Comp. Neurol. *180*:545–580.

Faull, R.L.M., and J.B. Carman (1968) Ascending projections of the substantia nigra in the rat. J. Comp. Neurol. *132*:73–92.

Faull, R.L.M., and W.R. Mehler (1978) The cells of origin of nigrotectal, nigrothalamic and nigrostriatal projections in the rat. Neuroscience *3*:989–1002.

Foote, W.E., E. Taber-Pierce, and L. Edwards (1978) Evidence for a retinal projection to the midbrain raphe of the cat. Brain Res. *156*:135–140.

Gacek, R.R. (1974) Localization of neurons supplying the extraocular muscles in the kitten using horseradish peroxidase. Exp. Neurol. *44*:381–403.

Gacek, R.R. (1979) Location of trochlear vestibuloocular neurons in the cat. Exp. Neurol. *66*:692–706.

Glicksman, M.A. (1980) Localization of motoneurons controlling the extraocular muscles of the rat. Brain Res. *188*:53–62.

Graham, J. (1977) Autoradiographic study of the efferent connections of the superior colliculus in the cat. J. Comp. Neurol. *173*:629–654.

Graybiel, A.M (1975) Anatomical organization of retinotectal afferents in the cat: An autoradiographic study. Brain Res. *96*:1–23.

Graybiel, A.M. (1978a) The organization of nigrotectal connections: An experimental tracer study in the cat. Brain Res. *143*:339–348.

Graybiel, A.M. (1978b) A satellite system of the superior colliculus: The parabigeminal nucleus and its projections to the superficial collicular layers. Brain Res. *145*:365–374.

Graybiel, A.M., and E.A. Hartwieg (1974) Some afferent connections of the oculomotor complex in the cat: An experimental study with tracer techniques. Brain Res. *81*:543–551.

Grofová, I., and E. Rinvik (1970) An experimental electron microscopic study on the striatonigral projection in the cat. Exp. Brain Res. *11*:249–262.

Gustafson, T., and M. Toneby (1970) On the role of serotonin and acetylcholine in sea urchin morphogenesis. Exp. Cell Res. *62*:102–117.

Gwyn, D.G., and B.A. Flumerfelt (1974) A comparison of the distribution of cortical and cerebellar afferents in the red nucleus of the rat. Brain Res. *69*:130–135.

Hamilton, B.L. (1973a) Cytoarchitectural subdivisions of the periaqueductal gray matter in the cat. J. Comp. Neurol. *149*:1–28.

Hamilton, B.L. (1973b) Projections of the nuclei of the periaqueductal gray matter in the cat. J. Comp. Neurol. *152*:45–58.

Hamilton, B.L., and F.M. Skultety (1970) Efferent connections of the periaqueductal gray matter in the cat. J. Comp. Neurol. *139*:105–114.

Hanaway, J., J.A. McConnell, and M.G. Netsky (1970) Cytoarchitecture of the substantia nigra in the rat. Am. J. Anat. *129*:417–438.

Harting, J.K., W.C. Hall, I.T. Diamond, and G.F. Martin (1973) Anterograde degeneration study of the superior colliculus in *Tupaia glis*: Evidence for a subdivision between superficial and deep layers. J. Comp. Neurol. *148*:361–386.

Hatschek, R. (1907) Zur vergleichenden Anatomie der Nucleus Ruber tegmenti. Arb. Neurol. Inst. Univ. Wien *15*:89–135.

Hattori, T., H.C. Fibiger, and P.L. McGeer (1975) Demonstration of pallido-nigral projection innervating dopaminergic neurons. J. Comp Neurol. *162*:487–504.

Herkenham, M., and W.J. H. Nauta (1979) Efferent connections of the habenular nuclei in the rat. J. Comp. Neurol. *187*:19–48.

Hicks, S.P., and C.J. D'Amato (1968) Cell migrations to the isocortex in the rat. Anat. Rec. *162*:619–634.

Highstein, S.M. (1973) The organization of the vestibulo-oculomotor and trochlear reflex pathways in the rabbit. Exp. Brain Res. *17*:285–300.

Ives, W.R. (1971) The interpeduncular nuclear complex of selected rodents. J. Comp. Neurol. *141*:77–94.

Jacquet, Y.F., and A. Lajtha (1976) The periaqueductal gray: Site of morphine analgesia and tolerance as shown by 2-way cross tolerance between systemic and intracerebral injections. Brain Res. *103*:501–513.

Jayaraman, A., R.R. Batton, and M.B. Carpenter (1977) Nigrotectal projections in the monkey: An autoradiographic study. Brain Res. *135*:147–152.

Jürgens, U., and R. Pratt (1979) Role of the periaqueductal grey in vocal expression of emotion. Brain Res. *167*:367–378.

Kawamura, S., S. Ozawa, N. Fukushima, S. Hattori, and T. Tashiro (1977) The efferent projections of the parabigeminal nucleus in the cat. Acta Anat. Nippon *52*:42–43. Quoted from A. Tokunaga and K. Otani (1978).

Keller, E.L. (1974) Participation of medial pontine reticular formation in eye movement generation in monkey. J. Neurophysiol. *37*:316–332.

Kostowski, W., E. Giacalone, S. Garattini, and L. Valzelli (1968) Studies on behavioral and biochemical changes in rats after lesions in midbrain raphé. Europ. J. Pharmacol. *4*:371–376.

Kultas-Ilinsky, K., I.A. Ilinsky, L.C. Massopust, P.A. Young, and K.R. Smith (1978) Nigrothalamic pathway in the cat demonstrated by autoradiography and electron microscopy. Exp. Brain Res. *33*:481–492.

Kuypers, H.G.J.M., and D.G. Lawrence (1967) Cortical projections to the red nucleus and the brain stem in the rhesus monkey. Brain Res. *4*:151–188.

Lauder, J.M., and F.E. Bloom (1974) Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. I. Cell differentiation. J. Comp. Neurol. *155*:469–482.

Leger, L., K. Sakai, D. Salvert, M. Touret, and M. Jouvet (1975) Delineation of dorsal lateral geniculate afferents from the cat brain stem as visualized by the horseradish peroxidase technique. Brain Res. *93*:490–496.

Loewy, A.D., and C.B. Saper (1978) Edinger-Westphal nucleus: Projections to the brain stem and spinal cord in the cat. Brain Res. *150*:1–27.

Loewy, A.D., C.B. Saper, and N.D. Yamodis (1978) Reevaluation of the efferent projections of the Edinger-Westphal nucleus in the cat. Brain Res. *141*:153–179.

Martin, G.F., R. Dom, S. Katz, and J.S. King (1974) The organization of projection neurons in the opossum red nucleus. Brain Res. *78*:17–34.

Massion, J. (1967) The mammalian red nucleus. Physiol. Rev. *47*:383–436.

Massopust, L.C., and R. Thompson (1962) A new interpedunculo-diencephalic pathway in rats and cats. J. Comp. Neurol. *118*:97–106.

Mayer, D.J., and J.C. Liebeskind (1974) Pain reduction by focal electrical stimulation of the brain: An anatomical and behavioral analysis. Brain Res. *68*:73–93.

Mitchell, R. (1963) Connections of the habenula and of the interpeduncular nucleus in the cat. J. Comp. Neurol. *121*:441–457.

Monakow, C. von (1910) Der rote Kern, die Haube und die Regio subthalamica bei einigen Säugetieren und beim Menschen. Arb. Hirnanat. Inst. Zürich *4*:103–226.

Moore, R.Y., R.B. Bhatnagar, and A. Heller (1971) Anatomical and chemical studies of a nigro-neostriatal projection in the cat. Brain Res. *30*:119–135.

Mosko, S.S., D. Haubrich, and B.L. Jacobs (1977) Serotonergic afferents to the dorsal raphe nucleus: Evidence from HRP and synaptosomal uptake studies. Brain Res. *119*:269–290.

Nauta, W.J.H. (1958) Hippocampal projections and related neural pathways to the mid-brain in the cat. Brain *81*:319–341.

Nauta, W.J.H., and W.R. Mehler (1966) Projections of the lentiform nucleus in the monkey. Brain Res. *1*:3–42.

Neckers, L.M., J.P. Schwartz, R.J. Wyatt, and S.G. Speciale (1979) Substance P afferents from the habenula innervate the dorsal raphe nucleus. Exp. Brain Res. *37*:619–623.

Nyberg-Hansen, R., and A. Brodal (1964) Site and mode of termination of rubro-spinal fibers in cat. An experimental study with silver impregnation methods. J. Anat. (Lond.) *98*:235–253.

Oka, H., and K. Jinnai (1978) Electrophysiological study of parvocellular red nucleus neurons. Brain Res. *149*:239–246.

Olson, L., and Å. Seiger (1972) Early prenatal ontogeny of central monoamine neurons in the rat: Fluorescence histochemical observations. Z. Anat. Entw. Gesch. *137*:301–316.

Olszewski, J., and D. Baxter (1954) Cytoarchitecture of the Human Brain. Philadelphia: Lippincott.

Padel, Y., A.M. Smith, and J. Armand (1973) Topography of projections from the motor cortex to rubrospinal units in the cat. Exp. Brain Res. *17*:315–332.

Panneton, W.M., and G.F. Martin (1979) Midbrain projections to the trigeminal, facial and hypoglossal nuclei in the opossum. A study using axonal transport techniques. Brain Res. *168*:493–511.

Phillipson, O.T. (1979a) The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of Tsai in the rat. J. Comp. Neurol. *187*:85–98.

Phillipson, O.T. (1979b) Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: A horseradish peroxidase study in the rat. J. Comp. Neurol. *187*:117–144.

Pompeiano, O., and A. Brodal (1957) Experimental demonstrations of a somatotopical origin of rubrospinal fibers in the cat. J. Comp. Neurol. *108*:225–252.

Powell, E.W., and J.B. Hatton (1969) Projections of the inferior colliculus in cat. J. Comp. Neurol. *136*:183–192.

Prendergast, J., and D.J. Stelzner (1976) Changes in the magnocellular portion of the red nucleus following thoracic hemisection in the neonatal and adult rat. J. Comp. Neurol. *166*:163–172.

Ramón y Cajal, S. (1911) Histologie du Système Nerveux de l'Homme et des Vertébrés. 2 vols, Paris: Maloine.

Reid, J.M., D.G. Gwyn, and B.A. Flumerfelt (1975) A cytoarchitectonic and Golgi study of the red nucleus in the rat. J. Comp. Neurol. *162*:337–362.

Rinvik, E. (1975) Demonstration of nigrothalamic connections in the cat by retrograde axonal transport of horseradish peroxidase. Brain Res. *90*:313–318.

Rinvik, E., I. Grofová, and O.P. Otterson (1976) Demontration of nigrotectal and nigroreticular projections in the cat by axonal transport of proteins. Brain Res. *112*:388–394.

Sheard, M.H. and G.K. Aghajanian (1968) Stimulation of the midbrain raphe: Effect on serotonin metabolism. J. Pharmacol. Exp. Ther. *163*:425–430.

Sherk, H. (1978) Visual response properties and visual field topography in the cat's parabigeminal nucleus. Brain Res. *145*:375–379.

Simon, H., M. Le Moal, and A. Calas (1979) Efferents and afferents of the ventral tegmental-A10 region studied after local injection of [^{3}H] leucine and horseradish peroxidase. Brain Res. *178*:17–40.

Skultety, F.M. (1958) The behavioral effects of destructive lesions of the periaqueductal gray matter in adult cats. J. Comp. Neurol. *110*:337–362.

Slotnick, B.M., and C.M. Leonard (1975) A Stereotaxic Atlas of the Albino Mouse Forebrain. Rockville, Maryland: U.S. Public Health Service.

Smaha, L.A., and W.W. Kaelber (1973) Efferent fiber projections of the habenula and the interpeduncular nucleus. An experimental study in the opossum and cat. Exp. Brain Res. *16*:291–308.

Sohal, G.S., and R.K. Holt (1977) Autoradiographic studies on the time of origin of neurons of the eye-muscle nuclei. Exp. Neurol. *56*:227–236.

Strominger, N.L., and A.I. Strominger (1971) Ascending brain stem projections of the anteroventral cochlear nucleus in the rhesus monkey. J. Comp. Neurol., *143*:217–242.

Sugimoto, T., K. Itoh, and N. Mizuno (1978) Localization of neurons giving rise to the oculomotor parasympathetic outflow: A HRP study in the cat. Neurosci Lett. *7*:301–305.

Szabo, J. (1962) Topical distribution of the striatal efferents in the monkey. Exp. Neurol. *5*:21–36.

Szentágothai, J. (1950) The elementary vestibulo-ocular reflex arc. J. Neurophysiol. *13*:395–407.

Taber, E. (1961) The cytoarchitecture of the brain stem of the cat. I. Brain stem nuclei of cat. J. Comp. Neurol. *116*:27–70.

Taber Pierce, E. (1973) Time of origin of neurons in the brain stem of the mouse. Progr. Brain Res. *40*:53–65.

Taber Pierce, E., W.E. Foote, and J.A. Hobson (1976) The efferent connection of the nucleus raphe dorsalis. Brain Res. *107*:137–144.

Tarlov, E., and S.R. Tarlov (1971) The representation of extraocular muscles in the oculomotor nuclei: Experimental studies in the cat. Brain Res. *34*:37–52.

Tokunaga, A., and K. Otani (1978) Neuronal organization of the corpus parabigeminum in the rat. Exp. Neurol. *58*:361–375.

Tsai, C. (1925) The optic tracts and centers of the opossum *Didelphis virginiana*. J. Comp. Neurol. *39*:173–216.

Ungerstedt, U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Suppl. *367*:1–48.

van Noort, J. (1969) The Structure and Connections of the Inferior Colliculus. Assen: Van Gorcum.

Vincent, S.R., T. Hattori, and E.G. McGeer (1978) The nigrotectal projection: A biochemical and ultrastructural characterization. Brain Res. *151*:159–164.

Voneida, T.J. (1960) An experimental study of the course and destination of fibers arising in the head of the caudate nucleus in the cat and monkey. J. Comp. Neurol. *115*:75–88.

Walberg, F. (1956) Descending connections to the inferior olive. J. Comp. Neurol. *104*:77–173.

Waldron, H.A., and D.G. Gwyn (1969) Descending nerve tracts in the spinal cord of the rat. J. Comp. Neurol. *137*:143–153.

Wang, R.Y., and G.K. Aghajanian (1977) Physiological evidence for habenula as major link between forebrain and midbrain raphe. Science *197*:89–91.

Warwick R. (1953) Representation of the extra-ocular muscles in the oculomotor nuclei of the monkey. J. Comp. Neurol. *98*:449–503.

Warwick R. (1954) The ocular parasympathetic nerve supply and its mesencephalic sources. J. Anat. (Lond.) *88*:71–93.

Yamamoto, M., I. Shimoyama, and S.M. Highstein (1978) Vestibular nucleus neurons relaying excitation from the anterior canal to the oculomotor nucleus. Brain Res. *148*:31–42.