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NEUROGENESIS IN THE RAT NEOSTRIATUM

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Abstract—Neurogenesis in the rat neostriatum was examined with [^{3}H]thymidine autoradiography. For the animals in the prenatal groups, the initial [^{3}H]thymidine exposures were separated by 24 h; they were the offspring of pregnant females given two injections on consecutive embryonic (E) days (E13–E14, E14–E15, . . . E21–E22). For the animals in the postnatal (P) groups, the initial [^{3}H]thymidine exposures were separated by 48 h, each group receiving four consecutive injections (P0–P3, P2–P5, P4–P7). On P60, the percentage of labeled cells and the proportion of cells originating during either 24 or 48 h periods were quantified at several anatomical levels for both the large and medium-sized neurons. Neurogenesis of the large neurons occurs mainly between E13 and E16 in a strong caudal-to-rostral gradient. The medium-sized neurons throughout the neostriatum are generated in a prominent ventrolateral-to-dorsomedial gradient so that ventrolateral cells originate mainly between E14 and E18, dorsomedial cells between E18 and E21–22 (fewer than 10% originate between P0 and P4). Medium-sized neurons also show two other gradients. First, there is a superficial-to-deep gradient in the anterior part of the caudoputamen, while more posterior levels have a deep-to-superficial gradient. Second, anterior parts have a caudal-to-rostral gradient while posterior parts have a gradient in the opposite direction. This shift in neurogenetic gradients along both superficial–deep and rostrocaudal directions is developmental evidence that an anterior 'caudate' can be separated from a posterior 'putamen' in the rat. Finally, neurogenetic gradients in the medium-sized caudoputamen neurons can be linked to the patterns of their anatomical interconnections with the substantia nigra.

Key words: Caudoputamen, Neostriatum, Rat, Neurogenesis, [^{3}H]thymidine autoradiography.

The neostriatum is the focus of intense neurochemical research due to increasing evidence that damage to dopamine terminals from the nigro-striatal projection may be the basis for degenerative neurological disorders such as Parkinson's disease and Huntington's chorea.[50] The working hypothesis of this study is that rich anatomical interconnections between specific brain structures are the outcome of precisely timed developmental events, beginning with the timetables of neurogenesis. This hypothesis has been confirmed in studies of the limbic[4] and olfactory systems.[6] The present results will show that neurogenetic gradients in both the neostriatum (data to be presented) and the substantia nigra[1] can be correlated with axonal termination patterns.

Pulse labeling with single injections of [^{3}H]thymidine has been used to establish approximate times for neuronal birth dates and the direction of obvious neurogenetic gradients in the rodent neostriatum mainly on a qualitative basis.[2,19,47,54] The present study utilizes comprehensive labeling with multiple injections of [^{3}H]thymidine.[7] This method allows an accurate delineation of both the onset and cessation of neurogenesis as well as the determination of the proportion of neurons that originate during single days of embryonic life. This extensive quantitative data permits the correlation of chronologies of neurogenesis with anatomical patterns.

METHODS

Since neurogenesis in the rat caudoputamen complex extends beyond the day of birth, both prenatal and postnatal developmental series were used. All series contained groups of Purdue–Wistar rats given successive daily (between 9 and 11 a.m.) s.c. injections of [^{3}H]thymidine (Schwarz–Mann; specific activity 6.0 Ci/mM: 5 μCi/g body weight), to insure comprehensive cell labeling. The prenatal developmental series contained 10 groups, the offspring of pregnant females given two successive daily injections progressively delayed by 1 day between groups (E12–E13, E13–E14, E21–E22). Two or more pregnant females were injected for each group; the day of sperm positivity was embryonic day 1 (E1). Normally, births occur on E23, which is also designated as postnatal day 0 (P0). The postnatal developmental series had three groups of rat pups, each group containing males from at least two litters. The pups were given four (P0–P3, P2–P5, P4–P7) consecutive daily injections.

All animals were perfused through the heart with 10% neutral formalin on P60. The brains were kept for 24 h in Bouin's fixative, then were transferred to 10% neutral formalin until they were embedded in paraffin. The brains of at least six animals from each group were blocked cornally according to the stereotaxic angle of the Pellegrino *et al.*[42] atlas. Every 15th section (6 μm) through the caudoputamen complex was saved. Slides were dipped in Kodak NTB-3 emulsion, exposed for 12 weeks, developed in Kodak D19, and post-stained with hematoxylin and eosin.

Anatomically matched sections were selected at six levels throughout the anteroposterior extent of the caudoputamen complex;[42] (Fig. 7). Medium-sized cells were counted in a total of 30 unit areas (0.085 mm^2) throughout these levels. The few scattered large cells were counted at levels A6.0 and A8.6. The proportion of labeled cells was determined microscopically at ×312.5 with the aid of an ocular grid. All cells with reduced silver grains overlying the nucleus in densities above background levels were considered labeled; obvious endothelial and glial cells were excluded. The determination of the proportion of cells arising (ceasing to divide) on a particular day utilized a modification of the progressively delayed comprehensive labeling procedure,[7] and is described in detail elsewhere.[4] Briefly, a progressive drop in the proportion of labeled neurons from a maximal level (>95%) in a specific population indicates that the precursor cells are actively producing neurons; when all neurons are unlabeled, all population members have originated. By analyzing the rate of decline in labeled neurons, one can determine the proportion of neurons originating over blocks of days (or single days) during development. Table 1 shows the data and calculations for the dorsomedial part of the caudoputamen at level A8.6. Trends in cell labeling were analyzed in individual animals with the sign test;[14] the rationale for the use of this statistic is provided elsewhere.[4]

Table 1. Neurogenesis of the dorsomedial medium-sized neurons in the caudoputamen complex (level A8.6)

Injection group	*N*	% Labeled cells (Mean ± S.D.)	Day of origin	% Cells* originating
E16–E17	12	(A) 98.17 ± 1.27	E16	2.39 (A–B)
E17–E18	9	(B) 95.78 ± 2.22	E17	5.11 (B–C)
E18–E19	6	(C) 90.67 ± 3.88	E18	15.0 (C–D)
E19–E20	6	(D) 75.67 ± 5.85	E19	24.96 (D–E)
E20–E21	7	(E) 50.71 ± 6.16	E20	21.88 (E–F)
E21–E22	6	(F) 28.83 ± 5.27	E21–E22	23.66 (F–G)
P0–P3	6	(G) 5.17 ± 1.72	P0–P1	3.6 (G–H)
P2–P5	7	(H) 1.57 ± 1.62	P2–P3	1.57 (H–I)

* These data are graphically represented in Fig. 2.

RESULTS

Medium-sized neurons

Golgi studies have repeatedly shown that the medium-sized neurons have spiny dendrites and are by far the most numerous (96%) cell type in the striatum.[13,20,32,43] Earlier investigators[20,32] thought these cells were interneurons. More recent Golgi studies[43] and the intracellular injection of horseradish peroxidase[33] showed that besides extensive axon collaterals within the striatum, one of the axonal branches is long, approaches the internal capsule, and leaves the striatum. Somogyi & Smith[48] showed that the medium spiny neurons are retrogradely labeled after horseradish peroxidase injections into the substantia nigra. Today, the medium spiny cells are considered the major output neurons of the striatum.

A statistical analysis of the data for all 30 unit areas in which medium-sized cells were counted showed significant differences in the time of neuron origin along superficial-deep planes, ventrolateral–dorsomedial planes, and between homologous areas in different sections along the rostrocaudal plane. As a result of this chronological 'mosaic', none of the raw data were combined. The medium-sized neurons had several prominent gradients of neurogenesis throughout the caudoputamen complex; in the following sections, data from selected unit areas will be presented to illustrate these gradients.

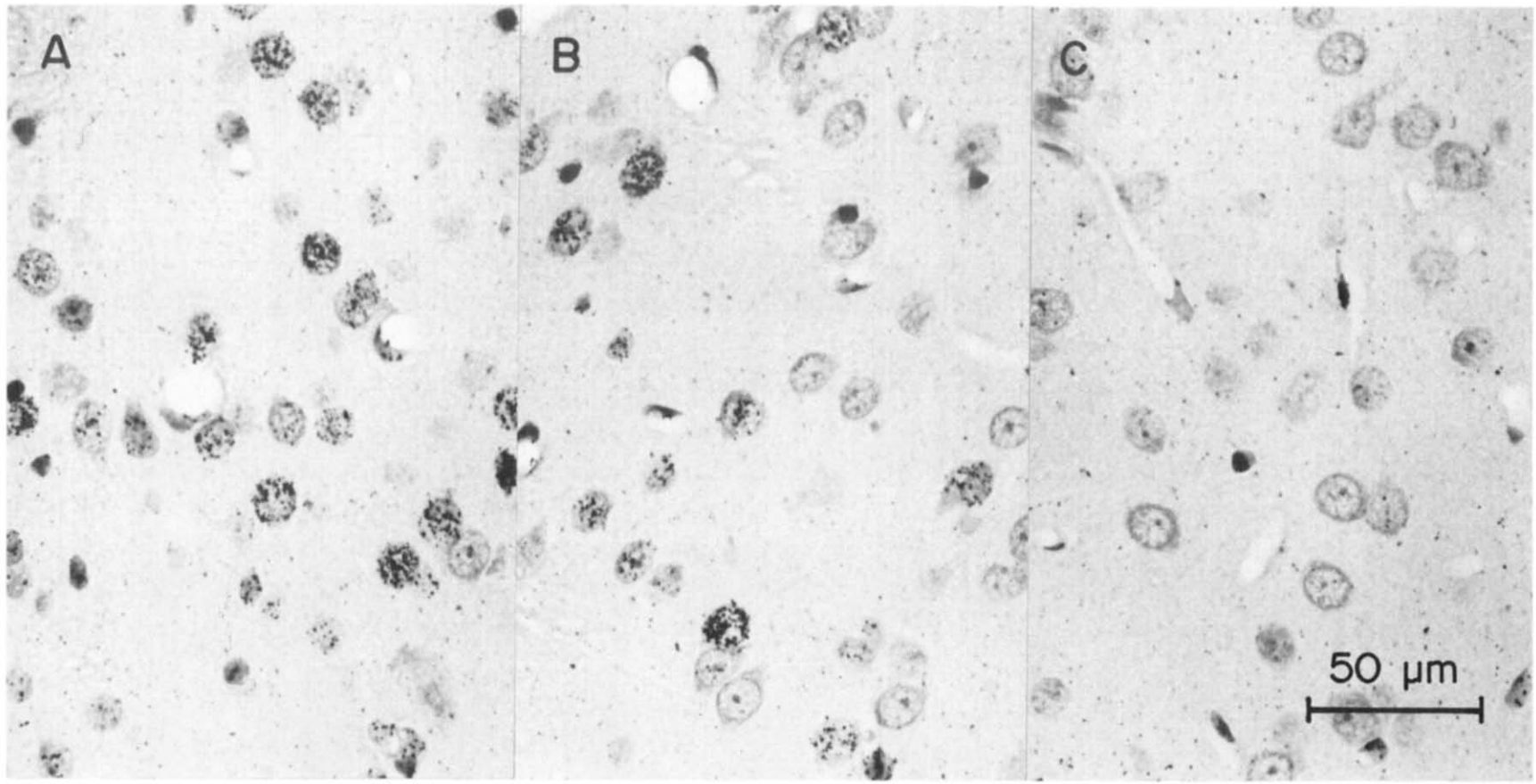

Fig. 1. Autoradiograms of superficial parts of the caudoputamen complex at level A7.8 from an animal exposed to [^{3}H]thymidine on E19–E20 and killed on P60 (6 μm, paraffin, hematoxylin and eosin). (A) Dorsomedial, (B) dorsolateral, (C) ventrolateral areas. Note that the proportion of labeled cells (those with reduced silver grains in the emulsion layer) decreases from A to C.

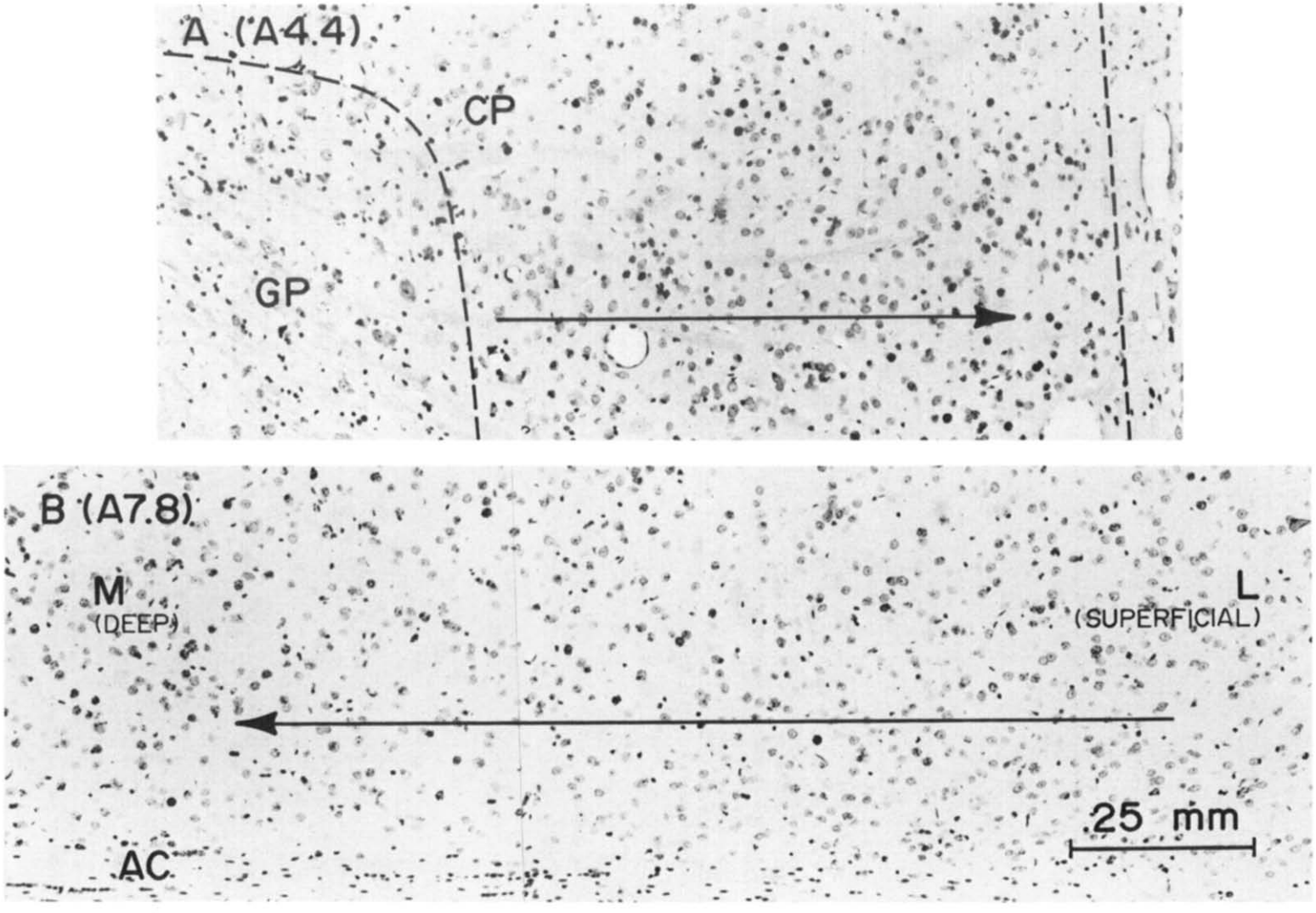

Fig. 4. Autoradiograms of the ventral caudoputamen complex (paraffin, 6 μm, hematoxylin and eosin). (A) Level A4.4 from an animal exposed to [^{3}H]thymidine on E17–E18 and killed on P60. The narrow caudoputamen (CP) is separated from the globus pallidus (GP) by the left dashed line. The right dashed line delineates the superficial border of the CP. Many superficial cells are labeled while most deep cells are unlabeled indicating a deep-to-superficial gradient (arrow). (B) Level A7.8 from an animal exposed to [^{3}H]thymidine on E18–E19 and killed on P60. The temporal limb of the anterior commissure (AC) lies along the base. More deep cells (on left) than superficial cells (on right) are labeled indicating a superficial-to-deep gradient (arrow).

The ventrolateral-to-dorsomedial gradient. Figure 1 illustrates this gradient qualitatively in photomicrographs of areas in the superficial part of the caudoputamen complex at level A7.8 from an animal exposed to [^{3}H]thymidine on E19–E20. The ventrolateral (C) area contains fewer labeled cells than the dorsolateral (B) area, which in turn contains fewer labeled cells than the dorsomedial (A) area.

Figure 2 shows the proportion of labeled cells in ventrolateral, dorsolateral and dorsomedial unit areas in the superficial caudoputamen complex at levels A8.6, A7.8 and A6.0. In all sections, neurons in the ventrolateral areas begin to originate earliest, those in the dorsolateral areas are next, while neurons in the dorsomedial areas are last (all levels and comparisons, $P<0.0001$, sign

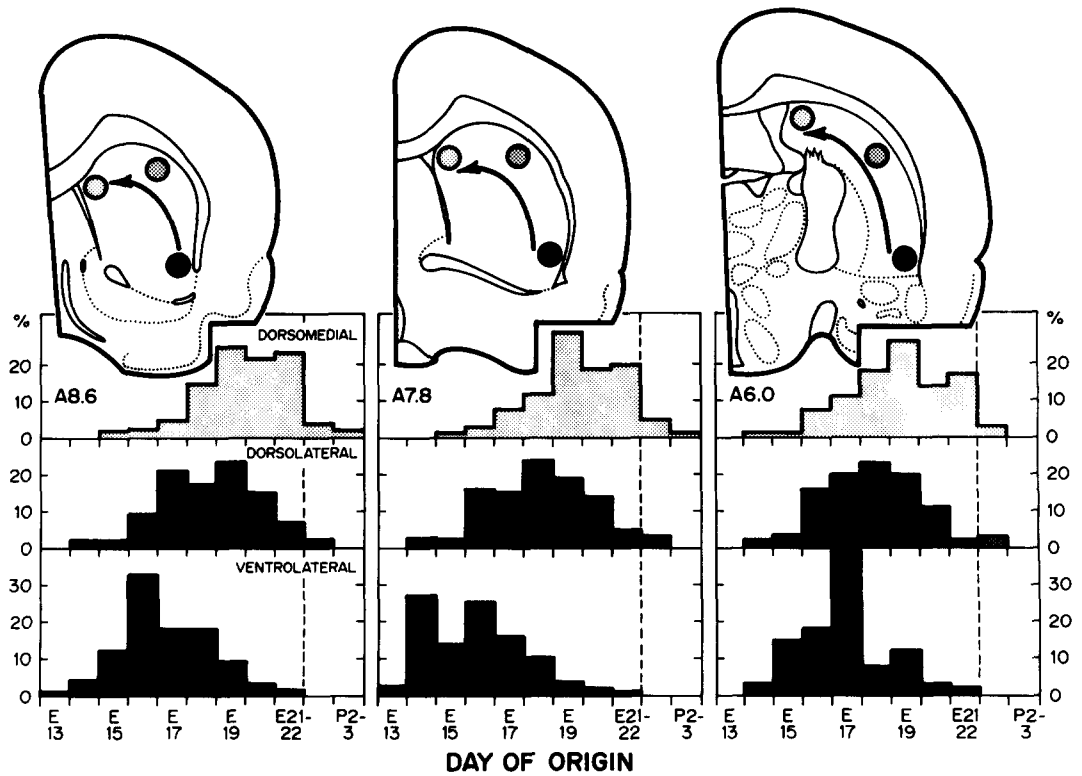

Fig. 2. The proportion of labeled medium-sized cells in *superficial* ventrolateral (solid), dorsolateral (dark stipple), and dorsomedial (light stipple) areas of the caudoputamen at levels A8.6 (left column of graphs), A7.8 (center column), and A6.0 (right column). Bar graphs indicate the proportion of cells originating within 24 h periods during embryonic life; Table 1 indicates how the data were calculated. Note the strong ventrolateral to dorsomedial gradient (arrows).

test). This same gradient is shown in levels A9.6 and A4.4 (data are not illustrated). Figure 3 shows the proportion of labeled cells in ventral, intermediate, and medial areas of the deep caudoputamen complex. Neurons in ventral (and slightly more lateral) areas originate significantly earlier than those in medial (and slightly more dorsal) areas (all levels and comparisons, $P<0.0001$, sign test). Deep areas in level A4.4 show the same trend (data are not illustrated).

Superficial–deep gradients. Figure 4 shows photomicrographs of the ventral caudoputamen complex at levels A4.4 (A) and A7.8 (B). There is a superficial-to-deep gradient at A7.8. At A4.4, the caudoputamen complex has considerably narrowed, but one can see a prominent gradient in the opposite direction.

Figure 5 graphs the proportion of labeled cells in superficial and deep parts of the ventral caudoputamen at various rostrocaudal levels. At the crossing of the temporal limb of the anterior commissure (A7.8) and anterior to this (A8.6), there is a significant superficial-to-deep gradient (both levels, $P<0.0001$, sign test). There is a significant deep-to-superficial gradient at levels A6.0 ($P<0.45$) and A4.4 ($P<0.0001$). This relationship also holds for paired comparisons between intermediate (deep) and dorsolateral (superficial) locations and for medial (deep) and dorsomedial

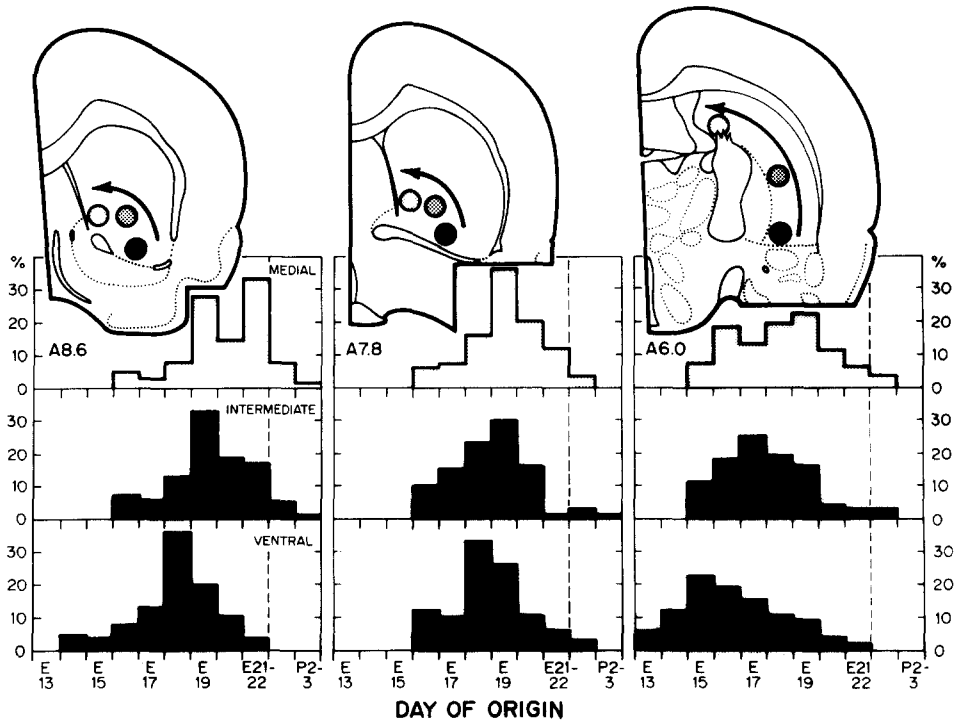

Fig. 3. The same as Fig. 2 for the deep ventral (solid), intermediate (dark stipple), and medial (light stipple) areas of the caudoputamen complex.

(superficial) locations (data are not illustrated). In all cases, the precommissural caudoputamen has significant superficial-to-deep gradients, while deep-to-superficial gradients are present at postcommissural levels.

Caudal–rostral gradients. Directionality in neurogenetic gradients along the rostrocaudal plane are variable depending on which area of the caudoputamen complex is under consideration. Since the superficial ventrolateral area has the longest rostrocaudal extent throughout the complex, only these data are illustrated in Fig. 6. The oldest medium-sized neurons in the entire caudoputamen complex are located in the superficial ventrolateral area at level A7.8 ($P<0.0001$, sign test). Both rostrally and caudally there are significantly younger neurons in homologous areas. The youngest medium-sized neurons in the superficial ventral caudoputamen are, surprisingly, the most caudal ones at level A3.6. They are slightly but significantly later in time of origin than the superficial ventral neurons at level A9.6 ($P<0.018$, sign test). Could this be the 'tail' of the caudate nucleus?

The dorsomedial area within the caudoputamen does not show this type of gradient along the rostrocaudal plane; rather, anterior levels are significantly younger than more posterior levels ($P<0.001$, sign test; quantitative data are not illustrated). Caudal to level A7.8, the caudoputamen complex is gradually pushed more laterally by the dorsal hippocampus, which eliminates the dorsomedial part in the levels quantified at A6.0 and beyond. Consequently, the dorsomedial part is only present in the precommissural caudoputamen, and it follows the same gradient as the ventrolateral caudoputamen in the precommissural part of the complex; there is a caudal-to-rostral gradient between levels A7.8 and A9.6 (Fig. 6).

Large neurons

In agreement with the observations of Mensah[36] in the mouse, the relatively rare (1–2%) large neurons in the caudoputamen complex of the rat are more likely to be located in a centrally placed core. Several Golgi studies have shown these neurons to have long, aspiny dendrites.[13,21,32,43] Kemp & Powell[32] and Fox *et al.*[21] thought these cells had long axons. The Golgi studies of Pasik *et al.*[43] found that these neurons have extensively arborizing short axons that remain within the striatum. Recent studies by Vincent *et al.*[59] showed that the large aspiny cells contain acetyl-

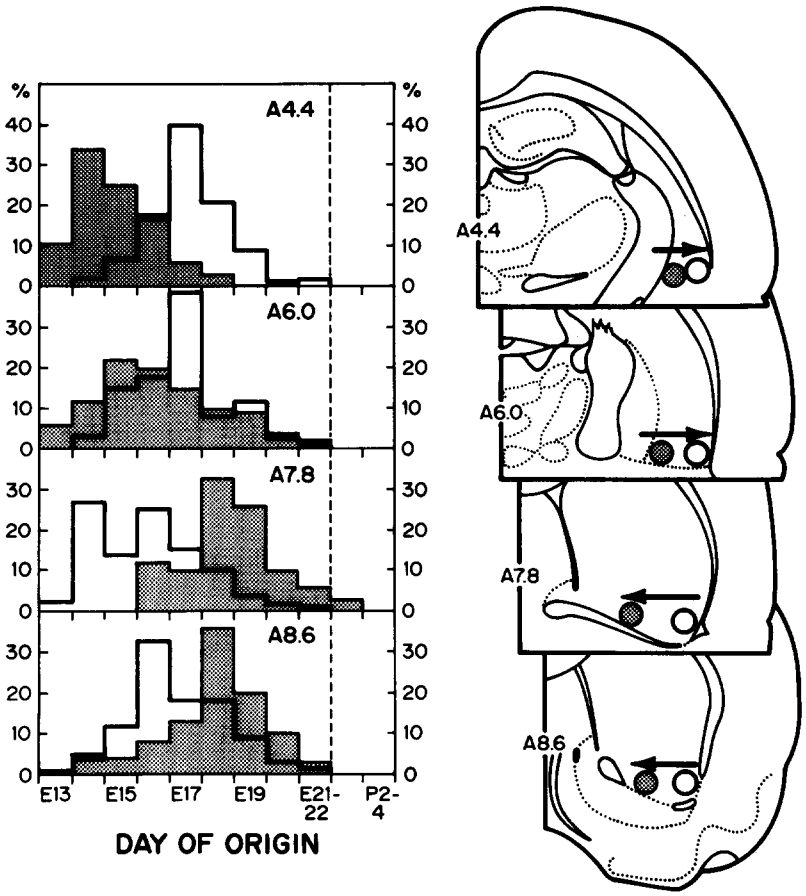

Fig. 5. Neurogenesis of medium-sized cells in superficial (clear) and deep (stipple) parts of the ventral caudoputamen complex from levels A4.4 (top graph and drawing) to A8.6 (bottom graph and drawing). Bar graphs are the proportion of cells originating on the embryonic day indicated. Rostral levels (A7.8 and A8.6) have a superficial-to-deep gradient (arrows in two bottom drawings) while caudal levels (A6.0 and A4.4) have the opposite gradient (arrows in two top drawings).

cholinesterase (ACHE) and their axonal ramifications are presumably responsible for the dense ACHE staining seen in the striatum.

To quantify the time of origin of large neurons, the proportion of labeled cells was determined by scanning an entire section at both a rostral level (A8.6) and at a caudal level (A6.0) of the caudoputamen complex. Due to the small number of large neurons in each 6 μm section, no attempt was made to determine time of origin in medial vs lateral or in dorsal vs ventral locations (see Fig. 7 for results). The time of neuron origin is very early, with a prominent caudal (peak on E14) to rostral (peak on E15) gradient. The time of origin of those neurons in level A6.0 is similar to the generation time of the large neurons in the globus pallidus at that location (S. A. Bayer, in preparation). At both levels, the large neurons are nearly completely generated before the medium-sized cells begin to originate (compare Fig. 7 with Fig. 2).

Small cells

Fox and coworkers[22,23] considered the small cells in the striatum to be predominantly oligodendroglia. Since these cells could not be distinguished from glial cells in paraffin sections, the time of origin of this cell population was not quantified. Small cells are either very lightly labeled or unlabeled in many of the prenatal injection groups, but are heavily labeled in the postnatal injection groups that were examined during the quantification of the medium-sized cells. These observations indicate a predominantly postnatal origin for the small cells.

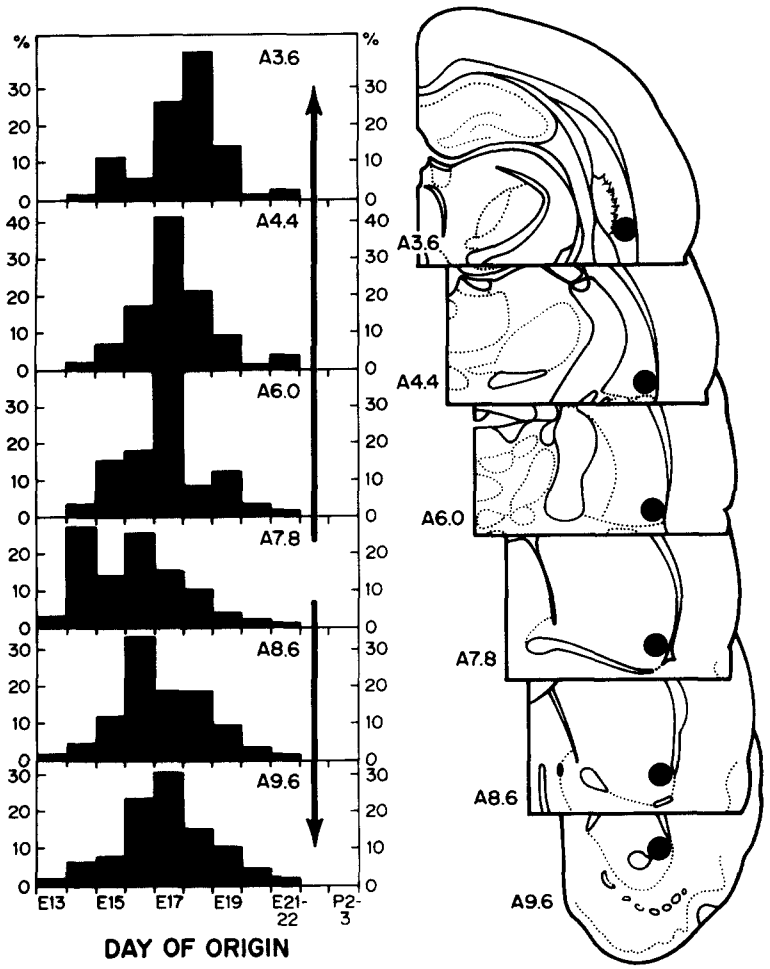

Fig. 6. Neurogenesis of medium-sized cells in the superficial ventrolateral area of the caudoputamen from caudal level A3.6 (top graph and drawing) to rostral level A9.6 (bottom graph and drawing). Bar graphs indicate the proportion of cells originating on the days indicated. Oldest neurons are found at level A7.8 with progressively younger neurons located caudally (top arrow in graphs) and rostrally (bottom arrow in graphs).

DISCUSSION

Relationships between neurogenetic gradients and anatomical subdivisions of the caudoputamen complex

Figure 8 diagrams the two most prominent gradients in the striatum. There is a strong ventrolateral-to-dorsomedial gradient shown throughout the entire rostrocaudal extent of the complex, confirming earlier studies.[2,19,47,54] The superficial-to-deep gradient at and anterior to the plane of the crossing of the temporal limb of the anterior commissure (A7.8) switches to a deep-to-superficial gradient posteriorly. This shift in neurogenetic gradients in precommissural vs postcommissural levels has not been noted in previous [^{3}H]thymidine autoradiographic studies of the striatum. Classical anatomical descriptions of the caudate and putamen remark that these two nuclei are histologically identical.[10,32] In the rat, the caudate and putamen are not separated as in cats and primates, and the two nuclei are together referred to as the caudoputamen complex. This study shows that there is neurogenetic evidence for separation of the caudate and putamen. The precommissural part of the complex which shows the prominent superficial-to-deep gradient could be considered the 'caudate' (Fig. 8). Gurdjian[27] noted that the precommissural neostriatum was similar in all respects to the caudate nucleus of other species. On the other hand, the postcommissural part of the complex which shows the deep-to-superficial gradient could be considered the 'putamen'

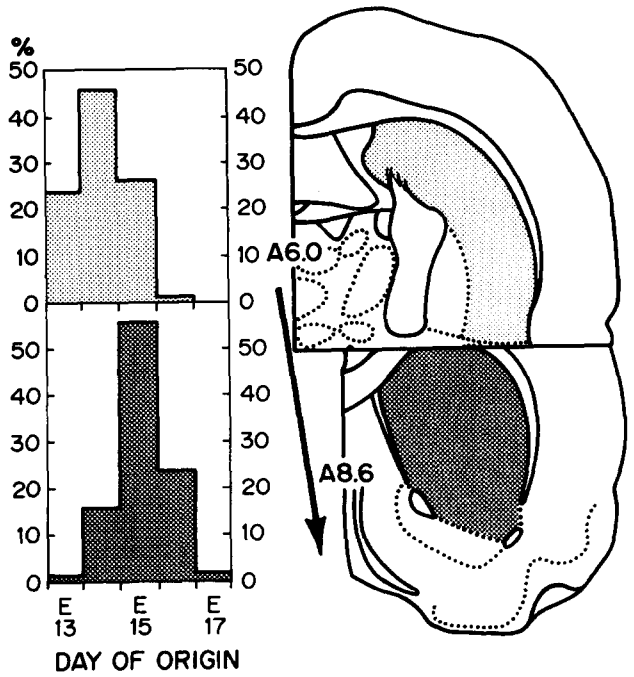

Fig. 7. Neurogenesis of large neurons at levels A6.0 (top graph and drawing) and A8.6 (bottom graph and drawing). Bar graphs indicate the proportion of cells originating on the days indicated. There is a prominent caudal-to-rostral gradient (arrow).

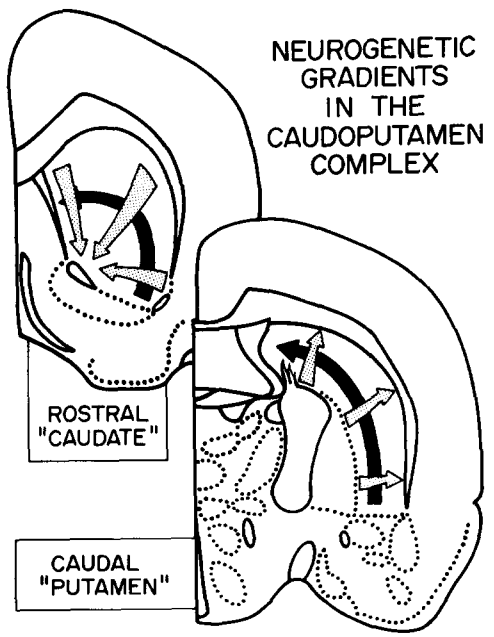

Fig. 8. The entire caudoputamen complex shows a prominent ventrolateral-to-dorsomedial gradient (solid arrows); stippled arrows indicate gradients along the superficial–deep plane. Rostral levels show a superficial-to-deep gradient while caudal levels show a gradient in the opposite direction. This neurogenetic shift is evidence that a 'caudate' can be delineated from a 'putamen' in the rat.

(Fig. 8). Gurdjian[27] also noted that the appearance of the neostriatum at these levels had all the characteristics of the putamen in other species. It is also important to note that the caudate and putamen can be distinguished by neurogenetic gradients in the rostral–caudal plane. The caudate has a caudal-to-rostral gradient, while the putamen has a gradient in the opposite direction (Fig. 6). Earlier [^{3}H]thymidine autoradiographic studies of the caudoputamen complex observed a caudal-to-rostral gradient[19,47,54] but failed to notice the rostral-to-caudal gradient at posterior levels.

Shifts in neurogenetic gradients often indicate differential germinal sources during embryonic development. The basal telencephalon contains two ridges during early morphogenesis which later fuse.[34] These ridges are believed to be the sources of the striatum, globus pallidus, and possibly the amygdala. It is tempting to speculate that the putamen is derived from the more posterior of the two ridges while the caudate comes from a more anterior primordium. An extensive embryonic study of the basal telencephalon (S. A. Bayer & J. Altman, in preparation) will be completed after neurogenetic timetables have been prepared for the entire basal telencephalon.

The striatum has been postulated by Heimer & Wilson[30] to continue ventrally to include both the nucleus accumbens and the olfactory tubercle. There is neurogenetic evidence to support this hypothesis since the striatal cell bridges and many of the small cells in the olfactory tubercle closely fit in with the neurogenetic gradients of the caudate nucleus (S. A. Bayer, in preparation). Neurogenetic gradients in the nucleus accumbens show a ventrolateral-to-dorsomedial gradient[3,5] so that the youngest neurons tend to be located in the vicinity of the anterior horn of the lateral ventricle, just as is found in the caudate nucleus.

Relationships between neurogenetic gradients and anatomical connections of the medium-sized neostriatal neurons

One of the best documented anatomical connections of the neostriatum is the projection from the substantia nigra. Rosegay[44] found chromatolytic changes in the substantia nigra after lesions of the caudate nucleus. Later lesion studies with the Nauta technique were unable to trace fine fiber degeneration into the caudate–putamen after nigral damage.[17] Ungerstedt[56] traced dopamine fibers from the substantia nigra into the striatum, and both lesion studies with the Fink–Heimer stain[11,29,35,53] and retrograde transport studies[18,39,45,46,49] have confirmed this projection. Some of the recent studies have shown the presence of a topographic relationship such that the ventromedial substantia nigra and adjacent ventral tegmental area project to a dorsomedial strip of the caudoputamen complex, while the dorsolateral substantia nigra projects to the more lateral and ventral areas.[8,12,57,58] The neurogenetic gradients in both the substantia nigra[1] and caudoputamen complex (present study) can be correlated with this topographic projection. Figure 9 diagrams both the anatomical projections and the neurogenetic gradients. Note that the oldest substantia nigra neurons are located in the dorsolateral part (generated before E15), while the younger neurons (generated after E15) are located in the ventromedial part[1] (large outlined arrows, Fig. 9). The axonal projections of the nigra neurons are arranged so that the oldest ones project to the regions of the neostriatum containing the oldest neurons, while the youngest nigra neurons project to the youngest neostriatal neurons.

There is equally strong anatomical evidence, demonstrated by a variety of methods, that the striatum projects to the substantia nigra.[15,24–26,28,31,37,38,40,41,51,52,60] Some of the more recent studies have demonstrated a topographic relationship in the rat so that the ventrolateral striatal cells project to the dorsolateral substantia nigra, while the dorsomedial striatal cells project to the ventromedial nigra.[9,16] This projection also correlates with the neurogenetic gradients (Fig. 9) so that older striatal neurons project to older nigral targets while younger neurons project to younger targets.

The ingrowing dopamine axons into the striatum may arrive at a particular target at a time when striatal neurons are just ready to receive their axons. Tennyson *et al.*[55] found that one of the first areas to develop dopamine fluorescence in the fetal rabbit was a region in the ventral putamen in the neighborhood of the temporal limb of the anterior commissure. In the rat, this area contains the earliest generated medium-sized cells. Tennyson also reported two stages of ingrowing axons. The first preferentially arborized in the morphogenetically more mature putamen, while the second group of axons arborized in the caudate, then extended later into the putamen. Perhaps the shifts in the superficial-to-deep gradients may be related to this two-stage growth of monoamine axons.

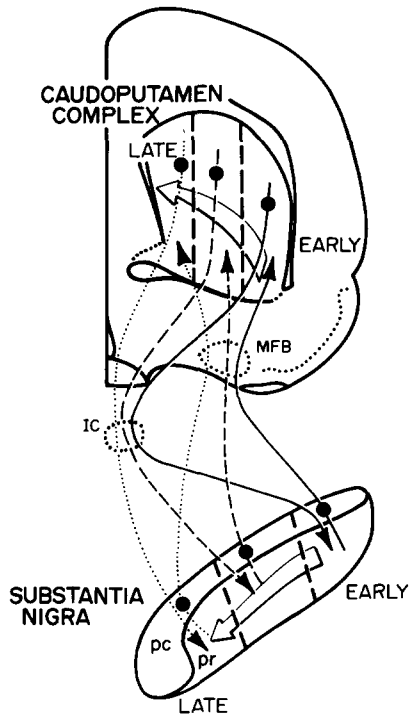

Fig. 9. Correlations between neurogenetic gradients and anatomical connections in the nigro-striatal system. Axons of early originating nigral neurons (solid lines) project via the medial forebrain bundle (MFB) to early-originating cells in the ventrolateral part of the caudoputamen complex. To complete the circuit, axons from superficial neurons in the lateral part of the complex (solid lines) project via the internal capsule (IC) to the dorsolateral substantia nigra. Thus early originating cells in both structures are interconnected. The same relationship holds for those neurons intermediate in age (axons are represented as dashed lines), and late in age (axons are represented as dotted lines). It should be noted that the ventromedial nigra projects throughout the medial tier of the caudoputamen, not just to the ventral part as shown in the diagram.

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