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Dynamic Ordering of Early Generated Striatal Cells Destined to Form the Striosomal Compartment of the Striatum

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Abstract

The mature striatum is divided into a labyrinthine system of striosomes embedded in a surrounding matrix compartment. We pulse-labeled striosomal cells (S cells) and matrix cells (M cells) in cats with ³H-thymidine and followed their distributions during fetal and post-natal development. We identified three maturational phases in S-cell distributions. The early phase (sampled at E27–E35 following E24–E28 ³H-thymidine) was characterized by a transient medial accumulation of synchronously generated S cells within the caudate nucleus adjoining the ganglionic eminence, potentially a waiting compartment. Band-like arrangements of synchronously generated S cells then formed beyond this medial band. During the second phase (sampled at E38–E45), the loosely banded S-cell distributions were transformed into clustered arrangements typical of developing striosomes. In the third phase (sampled from E52 into the postnatal period), these developed into the typical mature striosomal architecture. At adulthood, gentle mediolateral birthdate-gradients in S cells were still evident, but M cells, produced over mid to late prenatal ages, became broadly distributed, without apparent gradients or banding arrangements. These findings suggest that the maturational histories of the striosomal and matrix neurons are influenced by their generation times and local environments, and that future S cells have transient, non-striosomal distributions prior to their aggregation into striosomal clusters, including a putative waiting compartment. Further, the eventual patterning of the striosomal compartment reflects outside-in, band-like gradient patterns of settling of synchronously generated S cells, patterns that could be related both to neural processing in the mature striatum and to patterns of vulnerability of striatal neurons.

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CONFLICT OF INTEREST ATATEMENT

The authors have no conflicts of interest.

ROLE OF AUTHORS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: AMG. Acquisition of data: AMG, HN. Analysis and interpretation of data: AMG, HN, F-CL. Drafting of the manuscript: AMG, HN. Critical revision of the manuscript for important intellectual content: AMG, HN, F-CL. Obtained funding: AMG. Study supervision: AMG.

Keywords

basal ganglia; striosomes; ganglionic eminence; migration; pattern formation

INTRODUCTION

Modular arrangement of functional organization within a single neural structure represents an elegant design in information processing by the nervous system. The striatum contains two neurochemically distinct compartments, the striosomes (“striatal bodies”) and the large extrastriosomal matrix around them (Graybiel, 1990; Gerfen, 1992; Crittenden and Graybiel, 2011), distinguished by many of the neurotransmitter and neuromodulator-related substances known (Graybiel and Ragsdale, 1978; van der Kooy, 1984; Gerfen et al., 1985; Graybiel et al., 1986; Graybiel, 1990; Gerfen, 1992; Kawasaki et al., 1998). During development, a repertoire of neurotransmission-related genes and signaling molecules are expressed at differentially high levels in striosomes, including dopamine, tyrosine hydroxylase (TH), dopamine D1 receptor, DARPP-32, multiple glutamate receptor subtypes (particularly GluR1), calcium calmodulin-dependent kinase II and Nr4a1/NGFI-B/Nur77 (Olson et al., 1972; Moon Edley and Herkenham, 1984; Ouimet et al., 1984; van der Kooy, 1984; Graybiel, 1984a; Newman-Gage and Graybiel, 1988; Voorn et al., 1988; Caille et al., 1995; Snyder-Keller and Costantini, 1996; Davis and Puhl, III, 2011). In human prenatal striatum, striosomes also are clearly distinguished neurochemically (Graybiel and Ragsdale, 1980).

The distinct neurochemical profiles in striosomal and matrix compartments are rooted in differential developmental programs underlying these two populations of striatal neurons. Neurons in striosomes and matrix have different profiles of neurogenesis, establish different connectivity with other brain regions, and differentially degenerate in neurodegenerative diseases (Graybiel and Hickey, 1982; van der Kooy and Fishell, 1987; Graybiel, 1990; Gerfen, 1992; Crittenden and Graybiel, 2011). Unlike the ordered, domain-specific units of the columnar organization present in the cerebral cortex and the rhombomeres of the hindbrain, striosomes, which comprise some 15–20% of the striatum, are embedded in a surrounding matrix to form a complementary labyrinthine structure (Graybiel and Ragsdale, Jr., 1978; Graybiel and Hickey, 1982; Groves et al., 1988; Desban et al., 1989; Johnston et al., 1990). This complex labyrinthine design suggests that novel cellular mechanisms may underlie compartment formation in the developing striatum.

Pioneering developmental studies of the striatum have been performed in rodents, whose gestational time is short. In the rat, the majority of striosomal neurons (S cells) are born at embryonic day (E) 13–15, whereas matrix neurons (M cells) are born at E18–20 (van der Kooy and Fishell, 1987; Song and Harlan, 1994). In the mouse, likewise, there is a compressed temporal development of neurons in the two compartments (S cells are born at as early as ~E10 and are plentifully born at least through ~E11.5 to ~E13.5, whereas M cells are born at later time windows, most after ~E16.5 (Liao et al., 2008). Neurogenesis is substantially protracted with increased gestational times in larger mammals. For example, in the cat, the subject of this study, gestation lasts for ca. 63 days. The S cells of the cat’s

striatum are born over an extended time window from E22 to E30 (Graybiel and Hickey, 1982), and matrix cells mainly thereafter (Graybiel, 1984b). The prolonged neurogenesis of compartment progenitor cells allows appraisal of the dynamics of neuronal settling, and an opportunity to approach the important issue of how neurons destined to have the same compartmental phenotype, but having different birthdays, are organized during compartment formation. This question is a critical one, as neurogenesis, cell migration and pattern formation are inextricably interwoven during neural development.

To approach this issue, we traced the migration patterns of striatal neurons at early developmental stages by pulse-labeling embryos *in vivo* with ^3H -thymidine during S-cell and M-cell generation windows, and then culling the embryos at varying times after this initial labeling. We found that, in contrast to the labyrinthine structure of striosomes in the adult striatum, band-like arrangements of synchronously generated S cells are transiently present as the S cells migrate into the striatal primordium, prior to the emergence of striosome-matrix compartments identifiable by tight clusters of S cells. The presence of a persistent medial aggregation of S cells labeled on successive days further indicated that a transient waiting compartment could exist in the developing striatum. This pattern suggests that S cells with the same birthdays may be organized in this medial band prior to migrating through the entire striatum, and that successive groups of synchronously born S cells are arranged to set up the labyrinthine form of the striosomal system by band-like outside-in migration patterns.

MATERIALS AND METHODS

Animals and Surgery

All experimental procedures were approved by the Committee on Animal Care of the Massachusetts Institute of Technology and were in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. To obtain fetal cats of specific ages, mating pairs from the laboratory colony were housed together overnight, and the following day was designated as E0. After a gestational period of 63–65 days, kittens were usually born at night, and the following first postnatal day (P) was counted as P1.

Laparotomies were performed under strict sterile conditions on time-mated pregnant cats deeply anesthetized with 10–40 mg sodium pentobarbital i.v. following tranquilization with 50 mg ketamine hydrochloride i.m. The exact dose of pentobarbital was determined by monitoring standard reflex responses. Each horn of the uterus was successively exposed, and 0.25 ml of fresh ^3H -thymidine (specific activity, 82.3 Ci/mmol, 0.003 mg/ml, New England Nuclear, Boston, MA) was injected directly into the amniotic fluid of each fetal sac with a tuberculin syringe and 25G, 0.5 in. needle. In order to minimize possible overlap with M-cell generation times, we used a narrower window of thymidine exposure than the window characteristic of the total S-cell generation time. Fourteen animals were exposed once between E24 and E28, and were then sacrificed at relatively short survival times (see Table 1). To determine how populations of S cells with varying exposure dates during the S-cell window were distributed at adulthood, we analyzed ^3H -thymidine labeling patterns in 2 young adult cats that had been exposed to ^3H -thymidine embryonically at E24 and E30.

Preparation of Brain Tissue

At specific post-injection intervals (Tables 1 and 2), sterile laparotomies were performed to cull the fetuses with prenatal survival times. The uterus was incised at the cephalic pole of the fetal sac, and the fetus with its membranes and placenta was delivered and immediately perfused transcardially. The uterine incision was sutured shut, and the body wall was closed in 3 layers. The remaining kittens were usually delivered within the normal gestational period. Postnatal animals were anesthetized and also transcardially perfused. The perfusate contained 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4, room temperature). Fixed brain tissue was removed immediately and postfixed in fresh fixative at 4°C for 24–48 hours. Larger brains were cut into blocks containing the striatum. After post-fixation, blocks were soaked in 20% and then 30% sucrose and were then frozen. Serial frozen transverse sections were cut at 30 µm on a sliding microtome.

Immunohistochemistry

Immunostaining was performed by preincubation of free-floating sections in successive 0.1 M Tris saline solutions containing 10% methanol and 3% hydrogen peroxide, 0.2% Triton X-100, and 3% normal goat serum, with several Tris saline washes in between steps. Sections were then incubated in rabbit polyclonal antiserum against TH (1:250–1:1000, a gift of Dr. T.H. Joh or purchased from Eugene Tech International, Inc., Allendale, NJ) or in antiserum against substance P (SP) (gift from Dr. R. Ho, G6, diluted 1:250) with 1% normal goat serum and 1% normal cat serum for 24–72 hours at 4°C and were subsequently processed according to the peroxidase-antiperoxidase method as described previously (Graybiel and Chesselet, 1984). Control sections were identically processed except that primary antiserum was omitted.

Acetylcholinesterase Histochemistry

Histochemistry for detection of acetylcholinesterase (AChE) activity was a modified Geneser-Jensen and Blackstad method as previously described (Graybiel, 1984a).

Autoradiography

³H-thymidine autoradiography (Angevine and Sidman, 1961) was performed as previously described (Graybiel and Hickey, 1982; Graybiel, 1984a). Briefly, individual sections were mounted on slides, defatted and dipped in molten emulsion (Kodak NTB2, Rochester, NY). After 5–9 week exposure times, the emulsion-coated slides were developed, carefully washed, stained for Nissl substance and coverslipped. Cells with more than 15 overlying grains were considered as labeled.

Antibody Characterization

Table 3 lists all antibodies used in this study. Both of the two TH antibodies were tested at the laboratory of origin (Dr. Joh) or company (Eugene Tech), and in our laboratory at the Massachusetts Institute of Technology by omission of primary antiserum (Graybiel et al., 1987). These antibodies were extensively used in our laboratory and published in other articles (e.g., Graybiel et al., 1981a, 1987; Graybiel 1984a). The antibody against substance

P was tested in the donor laboratory (Dr. Ho) and in our laboratory by primary omission and was used in our previous report (Graybiel et al., 1981b).

Data Analysis

Comparative analyses of serial sections were made with the aid of photography, charting, and direct visual inspection at magnifications of 100×, with adjacent microscopic fields aligned by means of fiducial blood vessels. Photographic projection images of autoradiographically labeled slides were made, and macroprojector tracings of adjacent AChE or TH stained sections, outlining striosomes or dopamine islands, were drawn to the same scale and overlain on the photographs. Local blood vessels and borders were again used as fiducial markers. Selected sections were also drawn with a camera lucida attachment focused on a computer graphics screen coupled to a digitizing pad and computer program (Cajal Microscope Charting System, Infinite Automata, Cambridge, MA). The program was calibrated to the microscope objectives and thus allowed recording of data (e.g., the location, type of cell, type of space—blood vessel, outlines, etc.) at any magnification on the microscope for an individual section. Hard copies of recorded data were generated and compared with projections or overlay tracings of adjacent sections.

RESULTS

In the domestic cat, neurons that become postmitotic between E23–E30 primarily reside in striosomes at adulthood, whereas neurons of the future matrix are born later during striatal neurogenesis (Graybiel and Hickey, 1982; Graybiel, 1984b). Such neurons are herein designated, respectively, as S cells and M cells. We labeled cells born between E24 and E45 to determine early patterns of migration and distribution of these cells within the feline caudate nucleus and to determine whether these were reflected in the eventual distribution of neurons with S-cell and M-cell birthdates. We followed the distribution of cells labeled with ³H-thymidine over increasingly long survival intervals through the perinatal developmental phase and into adulthood. We compared these distributions to those of arriving and maturing extrinsic dopaminergic afferents marked by TH immunoreactivity and the appearance and distribution of an intrinsic early striosomal marker, AChE.

Striosomal Window

Chronology of striosomal cell distribution during development—We identified three phases of development in the distributions of S cells within the developing striatum: (1) an early phase, in which the S cells migrated into and settled within the striatal anlage (observed in embryos culled between E27 and E35); (2) a transitional phase, in which clustering of the S cells occurred (observed in embryos culled between E38 and E45); and (3) a late phase, in which the forerunner of the future striosomal architecture matured (examined in cases culled between E52 and adulthood).

Formation of a medial band of striosomal cells: a putative waiting compartment for cell migration in the striatum. Early distribution (culled between E27 and E35)—We injected ³H-thymidine during the first half of the S-cell birthday window and culled the embryos at short (3 and 6 day) survival times to examine the

early distribution of S cells (case KP45, E24–E27; and case KP101, E26–E32). At the earlier cull date (E27), the most abundant labeling was of the germinal epithelia in the rostral forebrain and the meninges; because of the extreme immaturity of the fetal feline brain, it was not possible to delimit definitively the ventricular edge of the nascent striatum at this earliest (E27) cull date (images not shown). Labeled cells were present in the ventricular and subventricular zones of the ganglionic eminence (GE). With a slightly later cull date (E32), most labeled cells had left the GE and had begun to settle in the striatum, especially in the putamen (Fig. 1). This shift in position suggests that many neurons born during the S-cell window remained within the GE for periods of at least 3 days, but that such neurons had migrated into the striatum by 6 days. By E32, TH-immunoreactive fibers had also invaded the putamen and were beginning to invade the most ventral part of the caudate nucleus.

Cases examined at E34 and E35 demonstrated that, regardless of exposure date within the S-cell window, there are transient, non-striosomal arrangements of S neurons well before the aggregation of these neurons into striosomal clusters. Figure 2 illustrates case KP46, exposed to ³H-thymidine at E24. The GE was divided into a thin ventricular zone and an extensive subventricular zone in which cells were very densely packed but appeared unoriented. Large numbers of thymidine-labeled neurons that had been born during the first half of the S-cell window had already reached the lateral edge of the caudate nucleus and the putamen, and there were labeled neurons in the cell bridges traversing the internal capsule (Fig. 2A,B).

In the caudate nucleus, the labeled S cells formed a medially situated band of cells (arrows in Fig. 2B) that filled most of the width of the nucleus but did not reach the lateral part of the nucleus. Lateral to this main population of labeled cells was a small elliptical, less densely populated region, next to the internal capsule (x in Fig. 2B; compare to same region the Nissl-stained section shown in Fig. 2A), in which there were only a few sparsely distributed labeled neurons. Only at the lateral edge of the caudate nucleus, next to the internal capsule and putamen, were there a few more dense collections of labeled cells. Medially, the main population of labeled S cells was also separated from the GE by a thin zone in which very few labeled neurons were present. Patterns in the nearly adjoining Nissl-stained section suggest that the density of cells in this region is also sparse. Within the medial band of labeled S cells, the cells were closely packed and homogeneously distributed at all anteroposterior levels. The medial gap between the GE and the labeled band was present at all rostrocaudal levels, although its width differed at different levels (data not shown). It was notably larger anteriorly than posteriorly, suggesting the possibility that more unlabeled cells were accumulated in the rostral region due to subsequent cell divisions than in the caudal caudate nucleus. Additionally, the sparsely labeled zone lateral to the labeled cell mass and immediately adjacent to the internal capsule was not present at levels rostral to the putamen. These patterns suggest that, from medial to lateral, there was a series of somewhat banded zones: the thin zone next to the GE, containing few labeled cells; the heavily labeled medial band of S cells (arrows in Fig. 2B), separated from the GE by the thin band; then a weakly labeled zone (x in Fig. 2B); and finally the few more punctate but strongly labeled cells in the lateral caudate nucleus, as well as the heavily labeled conduits to the putamen, and the putamen itself (Fig. 2B). In the rostrocaudal dimension, this labeled band could be described as roughly tracing an arc from the full width of the rostral pole of the caudate

nucleus, then bulging medially toward the GE and then extending more laterally again as the sections progressed caudalwards.

The band-like arrangement of neurons in the caudate nucleus was accentuated in a slightly older embryo (case KP38, E35), with a later age of birth-dating (E28). The strip of thymidine-labeled S cell formed a relatively thin layer, and was flanked by a relatively large, less densely labeled zone at mid-anteroposterior levels (data not shown but cf. Fig. 2B). Presumably, many of the unlabeled cells in the lateral caudate nucleus had been born earlier in the S-cell generation window. The medial band of dense labeling was visible as far caudal as the caudal extreme of the caudate nucleus, but at rostral levels, the band of thymidine labeling appeared to curve laterally around the rostral pole so that it came to fill nearly the full cross-section of the caudate nucleus. The medial edge of the cell labeling in the caudate nucleus either abutted or nearly abutted the medial edge of the GE except rostrally, where a progressively wider label-poor gap appeared just lateral to the GE (cf. Fig. 2). A similar thinning of the medial aggregation of thymidine-labeled cells could be seen in KP38 (Fig. 3), which illustrates the ^3H -thymidine labeling and accompanying Nissl-stained section together with a nearby section stained for TH. Again, the medial accumulation of labeled cells is not as pronounced, and more labeled neurons are visible across the width of the growing striatum.

Spatial Relationship of the Medial Band and the Early Striosomal Markers of AChE and TH

AChE and TH are early markers for immature striosomes (Graybiel, 1984a). As shown in Figure 2C (TH) and 2D (AChE) for the E24-34 case, the shape of the lateral edge of the labeled S-cell band corresponded closely to the curved shape of the medial edge of the densely AChE-stained part of the caudate nucleus (Fig. 2D); the general match in shape of the medial edge of the AChE-stained zone and the medial edge of the band of labeled neurons was present at all anteroposterior levels. However, the AChE stain did not appear to push completely to the full medial extent of the medial band (compare arrows in Fig. 2B showing medial edge of medial band with arrows in Fig. 2C,D showing medial edge of AChE). The main meshwork of TH-immunostained fibers (Fig. 2C) was restricted to the lateral part of the caudate nucleus and putamen (most concentrated ventrally, compare fiducial arrows in Fig. 2C,D), but some of the individual TH-positive fibrils extended far medially into the GE.

Notably, the advancing medial edge of the TH-rich zone did not match either the medial edge of the AChE staining or the lateral edge of the thymidine-labeled band of S cells (Fig. 2B–D). Comparison of these labeled S-cell distributions for earlier born S cells with those born at later stage (e.g., E28–E35 cases, not illustrated) suggested that many of the later-generated E28 labeled cells were located medial to the advancing TH front rather than within it. Thus, during the first half of gestation, it is clear that later born cells (E28) are located relatively more medially in the caudate nucleus than their earlier born predecessors (E24).

Emergence of striosomes. Transitional maturation of S-cell distributions (culled between E38 and E45)—The S-cell labeling seen in the caudate nucleus at E38

was even more obviously divided into an intensely labeled medial band and a more lateral zone of weak labeling at mid-anteroposterior levels of the caudate nucleus (case KP102, E26–E38, Figs. 4,5). As shown in Figure 4A, the medial band (arrow in Fig. 4A) formed an arc parallel to the lateral edge of the GE. The band was displaced from the GE only slightly at mid-anteroposterior levels of the caudate nucleus (Fig. 4B), but the gap between the labeled cells and the GE was wider rostrally. Again, lateral to the medial band was a zone of weak labeling and, more laterally, the densely labeled lateral rim of the caudate nucleus and the putamen (Fig. 4A,B). Thus, as in the cases labeled at earlier S-cell generation times (see Fig. 2B), multiple zones appeared in a loosely banded arrangement from medial to lateral. But the relatively larger size of the middle sparsely labeled zone had increased.

This rearranging invasion of medial-band cells into the previously mostly unlabeled lateral core of the caudate nucleus signified the beginning of the process of transition to a striosomal architecture as detectable with the birth-dating technique. This rearrangement, beginning at mid and ventral levels of the caudate nucleus, did not appear to follow TH, AChE, or even early cell-distribution gradients and patterns. It appeared to begin at a mid-anteroposterior and ventral and lateral zone of the caudate nucleus. For example, at the levels shown in Figures 4 and 5, the medial band was present throughout the caudate nucleus, but at mid-caudal levels, labeled neurons that, in brains from younger embryos, occupied the ventral part of the medial band had begun to be dispersed into the lateral width of the nucleus. Thus, by E38 the medial band appeared only dorsally at mid-caudate levels. As was the pattern already in the E35 case, the cell-sparse zone disappeared rostrally, and labeled cells filled the rostral pole of the caudate nucleus. At caudal levels, the majority of the E26 ³H-thymidine-labeled cells were medial to the leading edge of the AChE staining in the caudate nucleus, whereas rostrally, the majority of labeled cells had come to lie within the AChE-stained region. As at the earlier culling ages, the leading edge of the dense TH-immunoreactive fiber meshwork (Fig. 5C) did not match either the leading edge of the AChE staining or the edge of the medial band.

Expression of intrinsic and extrinsic markers of proto-striosomes in the medial band—The second developmental landmark that we observed at the age of E38 was the appearance of distinct TH-positive dopamine islands, the forerunners of striosomes (Fig. 5C). These were forming along the lateral edge of the caudate nucleus at mid-anteroposterior levels. At this developmental stage (exemplified by case KP102, Fig. 5), crisp SP-positive patches were present in the caudate nucleus. These SP-positive cell clusters extended medial to the zone in which TH-positive dopamine islands were present (Fig. 5B,C). Remarkably, at this level, the E26-labeled neurons still appeared largely homogeneously distributed (Fig. 5A). The fact that E26-labeled cell clusters were not apparent does not preclude a clustered arrangement of other, possibly earlier generated, S cells within these SP-positive patches, but the observation of patches of a presumably intrinsically generated substance (SP) suggests that the early discrete clustering, and possibly the maturation of striosomal neurons, can occur in a more extensive field than is suggested by the distribution of nigrostriatal afferents in dopamine islands.

This evidence suggests that S cells that had migrated into the caudate nucleus could have been following intrinsic cues, and that they did not obligatorily cluster when in the vicinity

of clustered nigrostriatal afferents, (i.e., a dopamine island) or an already established cell cluster (i.e., an SP-positive patch)—both known to be forerunners of mature striosomes. Interestingly, there was still only a slight suggestion of inhomogeneity in the AChE staining in this case (data not shown).

Transformation of the medial band into cell clusters of representing proto-striosomes—By E39-45, the medial band that had been so prominent at earlier cull stages was disappearing ventrally (Fig. 6). It was still identifiable only in the dorsal caudate nucleus at caudal levels (Fig. 6C). It was as if cells that formerly occupied the ventral part of the medial band had dispersed into the lateral region of the nucleus, where they now appeared in clusters (Fig. 6C), but that at caudal levels, medial band cells lagged in their disbursement (Fig. 6C). The relative position of the medial band in progressively older cases suggests that this maturation and movement of cells into the caudate nucleus takes place along a ventral-to-dorsal gradient, and from mid and caudal striatal levels rostralward and caudalward. As a consequence, the medial band appeared to be receding from the ventral part of the mid and caudal caudate nucleus with increasing age.

At this time, the AChE front was extending farther medially toward the GE in the ventral parts of mid and caudal sections. The fact that the curve of the medial AChE front departed from a strictly parallel relationship to that of the GE was important in suggesting that different maturational processes coexisted within different parts of the caudate nucleus. The principal features evident in the material were (1) the early, relatively concentric, hemispheric birthdate-specific cell distribution, (2) the wave of maturation as represented by cell disbursement from the medial band, and (3) the different distributions of AChE staining and TH immunostaining from mid to caudal locations in the dorsal and medial dimensions. These combined to result in the asymmetric disappearance of the medial band with increasing age, leading in late stages to the appearance of a dorsolateral "cap" of labeled cells at mid and caudal striatal levels as the remnant of the medial band. At the rostral pole, the labeled S cells continued to maintain a widely distributed appearance.

By E42, in case KP109, cells exposed to ^3H -thymidine at E27 were present throughout the full width of the caudate nucleus. By E45 (Fig. 6), the variegated S-cell distribution had been transformed into one of sharply defined clusters of labeled cells (Fig. 6B). This pattern was apparent in all four cases culled at E45 (cases KP32, 39 and 40, exposed at E28; and case KP27, exposed at E25). TH immunostaining and AChE staining in the caudate nucleus were now nearly coextensive. For illustrative purposes, we show in Figure 6D a magnified view of the mid-transverse level (Fig. 6B), together with a similarly magnified view of the adjoining AChE-stained section (Fig. 6E).

The distributions of S-cell clusters in these cases indicated a gentle lateral-to-medial gradient in the settling of progressively later generated cells. With earlier ^3H -thymidine exposure, the labeled S cells were slightly more concentrated in cell clusters coincident with dopamine islands at the lateral edge of the caudate nucleus (data not shown), but the medial dopamine islands contained few labeled cells (Fig. 6). The most heavily labeled clusters of cells generated at E28, on the other hand, tended to be located in both middle and lateral regions of the caudate nucleus. Many of the S-cell clusters were clearly in register with

dopamine islands, but other S-cell clusters were medial to the still-expanding front of dopamine islands. The medial caudate nucleus contained fewer labeled cells, and these were diffusely distributed within the more medial of the dopamine islands.

Striosomal distributions of S cells (culling window between E52 and adulthood)—

From E52 through P8 and into adulthood, cells that were thymidine-labeled during the S-cell window continued to be clustered in dopamine islands and in striosomes. The dopamine islands were prominent features throughout the extent of the caudate nucleus until the first postnatal week, but then TH immunoreactivity and AChE staining began to exhibit the characteristic changes that result in appearance of mature striosomal patterns.

The distribution of S cells radiolabeled at E24 exposure exhibited no medial band by E52 (Fig. 7A) except in the most caudal sections, in which the caudate nucleus was still very narrow. Labeled cell clusters in register with dopamine islands were present throughout the remaining rostrocaudal extent of the nucleus. The most intensely labeled cell clusters were found in the lateral half of the nucleus (Fig. 7A). Later ^3H -thymidine injections (e.g., at E27, observed at P1 in case KP56, Fig. 7B) produced a similar pattern with the exceptions that the arc of densely labeled cell clusters was slightly more medial, and no medial band was present at any level. S-cell distributions studied at adulthood (KPZ24-56 and KPB30-5, Fig. 7C,D) corroborated these patterns.

Gradients of striosomal cell distribution at adulthood—To determine whether cell generation time influenced final S-cell distributions at maturity, we compared the locations of ^3H -thymidine-labeled cell clusters in autoradiographic sections from young adult cats exposed to ^3H -thymidine at varying prenatal dates. Exposure dates ranged from E24 to E30 (see Table 1). Comparisons of S-cell distributions from cases with increasingly later thymidine exposure dates demonstrated that S cells are distributed in a quasi-banded, hemispheric pattern that is skewed in the dorsoventral dimension. With the earliest exposure dates, S-cell clusters (striosomes) were primarily found laterally (Fig. 7C). With increasingly later exposure dates, labeled S-cell clusters appeared farther medially, although they were still within the lateral half of the caudate nucleus, and no labeled S-cell clusters were found at the lateral edge of the caudate nucleus. Approaching the end of the S-cell generation window (Fig. 7D), the most densely labeled striosomal clusters were found in the medial half to one-third of the nucleus. Thus, there was a general lateral-to-medial distribution gradient of S-cell clusters dependent upon birthdate. Because this banded pattern of S-cell cohorts was also skewed in the caudal to rostral plane, sections through the middle third and most of the caudal third of the nucleus cut across several of these loose layers as well as the early generated core, whereas sections through the rostral part of the nucleus cut through fewer layers and missed the early generated core. This geometry resulted in an absence of labeled cells in sections from the rostral third of the caudate nucleus in brains exposed to ^3H -thymidine during the earliest phase of S-cell generation, but the presence of later generated S cells (data not shown).

Matrix Window

Chronology of matrix cell distribution—To follow M cells during striatal maturation, ^3H -thymidine injections were made at various times after E30 (E33–E35, E37–E38, E41–E45). Some of these animals were culled at short survival times, but the prenatal culling times were more restricted than those for the S cells. The remainders were taken for study at young adulthood (Table 2).

Early matrix neurogenesis (E33–E35 thymidine exposure)—The eventual distributions of cells labeled between E33 and E35 were dramatically different from those of cells labeled before E30. The post-E30 born cells were distributed throughout the caudate nucleus, lacked the clustered arrangements characteristic of cells exposed to thymidine at earlier dates (case KP169, Fig. 8A; and case KP146, Fig. 8B), and lacked overt rostrocaudal and dorsoventral gradients in labeled cell density. Comparisons with serial sections stained to demonstrate striosomes showed that labeled M cells tended to avoid striosomes throughout the rostrocaudal extent of the striatum, and especially clearly in the dorsolateral sector (Fig. 8). This tendency was least clear in the most rostral sections.

Interestingly, the labeled M cells were frequently at or near the edges of striosomes. The few labeled M cells within striosomal boundaries preferentially occupied medial striosomes at middle and ventral levels of the caudate nucleus. This intermixing could represent some overlap of the lateral-to-medial S-cell generation schedule with the emerging M-cell generation schedule. Labeled cells also preferentially avoided the densely stained cell clusters visible in Nissl stains.

Mid matrix neurogenesis (E37–E39 thymidine exposure)—Tritiated thymidine exposures at E37–E39 (e.g., case KM2, Fig. 9C,D) labeled cells that clearly avoided dopamine islands when viewed 10–20 days later, at E46 or E57. However, many cells were again aligned along the edges of dopamine islands, and occasional cells could be found within dopamine island/striosomes in every section. Thus there was not complete separation of these cells from the striosomes. There were few labeled cells in the putamen, but these also avoided the AChE-rich zones. No general gradients in labeled cell density were visible along dorsoventral or rostrocaudal axes.

The striatum of young adult cats (4–6.5 months old) exposed to ^3H -thymidine at E38 showed obvious pockets lacking labeled neurons, and these corresponded to striosomes throughout the entire caudate nucleus (Fig. 8C,D). The occasional labeled cells that were found within striosomes were at the most medial edge of the ventral half of the caudate nucleus.

Late matrix neurogenesis (E41–E45 thymidine exposure)—Even as early as 3 days after E45 exposure to ^3H -thymidine (Fig. 9A,B), most of the labeled cells tended to avoid dopamine islands, but there was not yet exclusive separation of M cells and the future striosomes. Many of the labeled cells were located at the edges of and within the boundaries of these future striosomes. This observation raises the question of continued cell migration and possibly cell death as sculpting factors in pattern formation in the caudate nucleus. One additional interesting observation of cells labeled at this time and culled shortly thereafter is

the finding of a significant amount of diffusely distributed labeling over the GE, as though cells had remained there and continued to divide, rather than migrating into the maturing striatum, with these cell divisions progressively diluting the concentration of radioactivity within individual cells (Fig. 9A).

Fewer labeled cells were found in adults with ^3H -thymidine exposure at these late embryonic ages, especially in the putamen and at very rostral and caudal poles of the caudate nucleus. The cells that were labeled were medium-sized cells scattered through the matrix without any apparent mediolateral or dorsoventral distribution gradient. A few small glial cells had overlying grains.

DISCUSSION

The most striking finding of this study is that neurons of the striosomal compartment of the striatum undergo transient, patterned arrangements during the period of pattern formation in the developing striatum. By using the strategy of combining a range of birthdate labeling with a range of post-labeling survival times, we were able to track the fates of synchronously generated groups of these neurons from shortly after their births into adulthood. We found that during the early phase of striatal development, striosomal cells (S cells) move into a waiting compartment in the caudate nucleus next to the ganglionic eminence (GE). S cells then leave this medial band to enter the more lateral, expanding bulge of the caudate nucleus. Only at the latest stages of S-cell generation does the medial band begin to disappear. The S cells then undergo a radical shift in their distributions. Synchronously generated S cells that had appeared in the medial band and then in loosely banded distributions within the caudate nucleus begin to form striosomal clusters, so that by E45, crisp S-cell clusters are a dominant feature of the striatum. These developmental patterns may leave their mark at adulthood in the tendency for longitudinal organizations within the striosomal labyrinths (Graybiel and Ragsdale, 1978; Graybiel et al., 1981b; Graybiel, 1984a; Groves et al., 1988; Desban et al., 1989). In sharp contrast to this S-cell developmental pattern, synchronously generated matrix cells (M cells) lack the eventual clustering that is typical of S cells, and also the tendency toward earlier banding patterns, at least for the middle and late stages of M-cell development that we studied. These findings suggest that the neurons of striosomes and matrix, despite settling nearby one another in the striatum, have markedly different maturational histories influenced by their birthdates as well as their local environments (Fig. 10).

^3H -thymidine Labeling Technique as an Advantageous Method for Studying Neuronal Development during Prolonged Neurogenesis

The ^3H -thymidine labeling technique was the first method that was applied to study of cell migration in the developing rodent brain (Angevine and Sidman, 1961), and for years was the main method for birth-dating developing neurons. We used the ^3H -thymidine birth-dating method to mark and trace striosomal and matrix neurons in the developing striatum during the prolonged period of neurogenesis and cell settling of the striatum in the cat, for which there is much evidence indicating that many early-born cells come to reside in striosomes. The prolonged neurogenesis provided greater temporal resolution in analyzing

developmental events, particularly the pattern of cell migration in detail, than possible in most studies of striatal neurogenesis. Most developmental studies have used the DNA analogue of 5-bromo-2'-deoxyuridine (BrdU) rather than ^3H -thymidine for tracing cell migration, because of safety concerns and the short processing time afforded by this method, as well as its advantages in combining BrdU labeling with other types of labeling. We used the more conservative approach of using the ^3H -thymidine method nonetheless, given its long history of accurate birthdate labeling, the absence of transgenic or other markers, and in the light of the suggestion that problems might occur with BrdU with the prolonged survival times used here. By simultaneously comparing developing cortical cells labeled with ^3H -thymidine or BrdU, Rakic's group has reported BrdU toxicity in cell survival and evidence that BrdU-labeled cells can be prone to erroneous migration in prolonged development of the cerebral cortex of monkey (Duque and Rakic, 2011). It has been reported that BrdU labeling per se can alter cell fate specification to form false-positive isochronic clusters in the chick dorsal telencephalon (Rowell and Ragsdale, 2012).

Thus our study with ^3H -thymidine has the disadvantage particularly of lacking forms of double or higher order joint labeling, and the disadvantage of not being performed in genetically engineered animals, but does have the advantage of being performed with a highly reliable method suitable for a detailed mapping of the migration patterns of S and M cells in an extended framework of feline neurogenesis.

The Medial Band of S Cells: A Putative Waiting Compartment in the Developing Striatum

A key feature of the early pattern formation that we found in the striatum is the occurrence of the medial band, consisting of S cells born, at minimum, between E24–E28 within the S-cell window and forming an arc roughly concentric with the curve of the GE. Our evidence suggests that other mediolaterally displaced arcs of synchronously generated S cells are also present. The early-generated S cells migrating into the caudate nucleus come to reside in loosely band-like arrangements accumulating with a lateral to medial gradient over time. Even as S cells later leave the medial band to settle in deeper parts of the caudate nucleus, they thus maintain a general lateral early-born to medial later-born banding pattern. The mechanisms underlying the formation of the medial band/waiting compartment of S cells is not yet known. An interesting possibility is that dynamic interaction between selective adhesion within S cells themselves and repulsion by other cells could set up and maintain this transient compartment before S cells migrate into the mantle zone of caudate nucleus (see below).

These patterns probably limit the mingling of cells with adjoining birthdays prior to their assuming final positions within the caudate nucleus. Our observations from successively later ^3H -thymidine exposure dates indicate that, in the more lateral regions as well as in the medial band, there may be some mixing of S cells born at different times. Such intermixing is not complete or random, however, because S cells maintain a quasi-banded distribution upon settling and on into adulthood. The incomplete separation into layer-like arrangements suggests that striosomes emerge from environments with some heterogeneity.

The medial band evident with early ^3H -thymidine exposures was flanked medially by a relatively large gap of unlabeled cells between the band and the GE at mid-anteroposterior

levels. With later ^3H -thymidine exposures, however, the medial band was relatively close to the GE at these levels. This evidence suggests that the medial band may represent a type of waiting compartment. Studies of neurogenesis in the rat striatum have shown that S cells maintain a homogeneous distribution late into prenatal development, at ca. E20. They are, in fact, intermingled with M cells at this developmental stage, prior to the formation of compartments (Krushel et al., 1995). We did not perform double or multiple labeling experiments to trace S and M cells simultaneously with different markers, and the possibility remains open that the medial band compartment contains M cells. The formation of the striosomal compartment (sometimes called patch compartment) in the rat striatum has been suggested to result from the reaggregation of S cells in response to the invasion of M cells (Marchand and Lajoie, 1986; Fishell and van der Kooy, 1987; Krushel et al., 1995). The apparent mediolateral banding of synchronously formed S cells that we observed here could provide a key constraint on the mixing of S and M cells, leading to the branched labyrinthine form of the mature striosomal system.

Our findings in material with prolonged survival times suggest that there may be some intermingling of future S and M cells, including some M cells bordering closely developing S-cell clusters. Such alignments could reflect repulsive forces or attractive forces, depending on the on-going dynamics, or other developmental constraints. The main pattern of development that we observed in the case material available was one in which S cells form early patterned aggregations near the ganglionic eminence that then evolve into striosomal clusters as M cells migrate into the striatum. Thus our findings suggest an alternate model (Fig. 10) to the model of S and M cells being fully intermingled early on before separating. Such lack of intermingling would be more easily aligned than full intermixing with the later labyrinthine structure of the striosomal system as a whole.

Different Developmental Processes Underlying Generation of S and M Cells

We were unable to detect mediolateral, caudorostral, or dorsoventral patterns of M-cell distribution during middle and late stages of striatal development. We did not follow early-generated M cells at short survival times, but the contrast with S cells at adulthood was striking. The lack of distinct gradients and local patterning of M cells might reflect the cell adhesion properties of this large population of striatal neurons, at least in the middle and late stages of striatal development. We would, however, not have detected adequately evidence that cell death and/or reverse migration might occur, which could in turn lead to a compartmental remodeling of M cells resulting in a loss of M-cell gradient.

Our suggestion of privileged S-cell patterning and aggregation through striatal development is in accord with much evidence from cell culture work and work with adhesion molecule markers. Previous studies on striatal cell migration in the rodent striatum have shown that cultured rat striosomal cells are selectively adherent to one another, forming cell clusters *in vitro*, whereas cultured matrix cells remain dispersed through the cell cultures (Krushel et al., 1989; Krushel et al., 1995). The postulated S cell-specific cell adhesion molecules remain yet to be identified, but cadherin-8 is enriched in the matrix of postnatal rat striatum (Korematsu et al., 1998). As opposed to cell adhesion as a mechanism for aggregation of S cells, cell repulsion could be required for sorting out of S cells from M cells. Preferential

expression of repulsive signaling molecules in S and M cells has been documented. EphA4 and its ligands ephrin-A5 and ephrin-B2 are preferentially expressed in developing striosomal and matrix compartments (Janis et al., 1999; Passante et al., 2008). Moreover, *in vitro* cell culture study and *in vivo* EphA4 and ephrin-A5 knockout mice study suggest that bi-directional repulsive signaling mediated by ephrin-A5/B2 binding to EphA4 is required for sorting S cells from M cells during development (Passante et al., 2008).

Null mutations of the homeobox genes *Dlx-1* and *Dlx-2* in mice have been found to impair selectively the migration of M cells without affecting the migration of S cells (Anderson et al., 1997). This differential effect suggests that the early phase of S-cell migration is independent of the late phase of M-cell migration, despite the fact that S and M cells in rodent are reported to be intermixed prior to compartment formation (Krushel et al., 1995). Rubenstein and his coworkers have proposed that the early cell migrations from the ventricular zone generate early-born S cells, whereas the late and dominant cellular output of the subventricular zone gives rise to the late-born M cells (Anderson et al., 1997). Consistent with this hypothesis, it has been proposed that netrin-1, which is expressed in the striatal ventricular zone, repulses cells expressing its receptor, DCC, from the subventricular zone into the mantle zone during neurogenesis of matrix cells at E18 of rats (Hamasaki et al., 2001).

These results fit with our demonstration that S and M cells are different both in their neurogenetic schedules and in their migratory and settling patterns, suggesting that distinct genetic programs underlie the neurogenesis of S and M cells. In accord with this possibility, genetic knockout of *Notch1* results in mouse in specific disruption of neurogenesis of early-born S cells, but double knockouts of *Notch1* and *Notch3* significantly reduce neurogenesis of both S cells and M cells in the mouse striatum (Mason et al., 2005). Genetic inactivations of transcriptional regulators, including *RAR β* , *Ctip2* and *Isl1*, also impair neurogenesis of S cells in the mouse striatum (Arlotta et al., 2008; Liao et al., 2008; Ehrman et al., 2013; Lu et al., 2014). Genetic deletions of M cell-enriched transcriptional regulators, including *Ebf1* and *Ikaros-1*, result in defective development of M cells in the mouse striatum (Garel et al., 1999; Lobo et al., 2006; Martin-Ibanez et al., 2010).

The ganglionic eminences of the rodent basal telencephalon have been identified as major sources not only of future striatal neurons, but also of interneuron precursors that migrate tangentially to the dorsal telencephalon, including to the cerebral cortex and hippocampus (Marin and Rubenstein, 2001; Gelman et al., 2012). It is likely that some of the cells labeled with ³H-thymidine in our experiments were among such populations of tangentially migrating cells en route to the overlying cerebral cortex, but it seems probable that the majority of labeled cells that we observed in the medial band and the GE were striatal precursors.

Developmental Anatomy of Caudate Nucleus and Putamen

In embryos in which S cells labeled at E24 were studied with 3 day survival times, a majority of the labeled cells still resided in the GE. By 6 days post-exposure, most cells had begun to migrate and were within the putamen as well as the lateral rim of the caudate nucleus and the cell bridges connecting these two nuclei. If, as seems likely, cells destined

for the putamen migrate directly through the caudate nucleus, then synchronously generated S cells can have very different destinations within the striatum. Some migrate laterally for long distances and ultimately reside in the striosomes of the putamen, whereas others travel relatively short distances and accumulate, at least temporarily, in the medial band. These findings suggest that the caudate nucleus and putamen, although being part of the same structure (the neostriatum), and although being combined in one “caudoputamen” in the brains of species such as rats and mice, go through different developmental progressions.

The cues that lead to such differential migrations are, as yet, unknown. They apparently are not dependent upon incoming TH-containing afferents or early AChE-positive elements, given that both the early-born cells of the putamen and those of the caudal medial band are synchronously generated and are at least initially in or near the GE and so are exposed to the same environment. Lesions of mesostriatal afferents at late gestational stages in rat embryos and depletion of dopamine synthesis by null mutation of TH have no apparent effect on the neurochemical compartmentation of the striatum (Snyder-Keller, 1991; Zhou and Palmiter, 1995). Nevertheless, the possibility of dopaminergic influence within the GE should not be discounted, given that TH-positive fibrils have been found in the GE of the rat at early gestational stages (Specht et al., 1981).

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Figure 1.

Generation of early-born striosomal cells (S cells) in the ganglionic eminence (GE). The embryo (case KP101) was pulse-labeled with ^3H -thymidine at E26 and culled at E32. Photomicrographs show adjacent sections illustrating the distribution of ^3H -thymidine-labeled cells in the GE. **A:** Nissl-stained section showing anatomical structure. **B:** Dark-field autoradiogram showing ^3H -thymidine labeled cells. Most labeled cells have moved out of the ventricular and subventricular zones of the GE and are located in the ventrolateral part of GE. (arrow in **B**). Ctx, cerebral cortex; LV, lateral ventricle. Scale bar = 0.5 mm.

Figure 2.

Early appearance of ^3H -thymidine-labeled cells in the developing caudate nucleus (CN) and putamen (P). ^3H -thymidine was injected at E24, and the embryo (case KP46) was culled at E34. **A,B:** Same section processed by Nissl staining (**A**) and by autoradiography (**B**). **C,D:** Adjacent sections, nearby the section shown in **A** and **B**, processed for tyrosine hydroxylase (TH) immunostaining (**C**) and acetylcholinesterase (AChE) staining (**D**). Many ^3H -thymidine-labeled S cells are present in both the caudate nucleus and the putamen, and there are labeled cells in the cell bridges traversing the internal capsule (IC). At this

developmental stage, the labeled cells in the caudate nucleus (**B**) fill the lateral part of the caudate nucleus (arrows delineate medial edge of dense band). This labeled zone reaches farther medially than either the TH-positive (arrows in **C**) or AChE-positive (arrows in **D**) part of the caudate nucleus. The arrows in **C** and **D** are fiducially placed with respect to each other and delineate the medial-most extent of the AChE staining. Note the zone of sparse cell labeling (example at $\times$ in **B**) in the caudate nucleus between the medial band of dense ^3H -thymidine labeling (arrows) and the lateral edge of the nucleus; See Figure 3 for comparable images from in KP-38, labeled at E28 and culled at E35. LV, lateral ventricle; GE, ganglionic eminence; Sp, septum. Scale bars = 0.5 mm in **A** (applies to **A,B**) and in **C** (applies to **C,D**).

Figure 3.

Appearance of ³H-thymidine labeling in KP-38, labeled at E28 and culled at E35, illustrating the thinning of the medial band of ³H-thymidine-labeled cells in the medial region of the medial band, and the spreading of labeled cells through the striatum into the lateral, putamen region. **A,B:** Same section processed by Nissl staining (**A**) and by autoradiography (**B**). **C:** Nearby section stained for TH, illustrating early arriving TH-immunoreactive fibers, especially prominent laterally. Scale bar = 0.5 mm.

Figure 4.

Illustration of the medial band (putative waiting compartment) in the developing caudate nucleus. **A:** The embryo (case KP102) was pulse-labeled with ^3H -thymidine at E26 and was culled at E38. **B:** Case KP109, pulse-labeled with ^3H -thymidine at E27 and culled at E42. A prominent ^3H -thymidine-labeled band (arrow) appears in the medial part of the caudate nucleus adjacent to the ganglionic eminence (GE). Note zone of sparse labeling in lateral caudate nucleus (cf. Fig. 2). LV, lateral ventricle; Sp, septum. Scale bar = 0.5 mm.

Figure 5.

Appearance of SP-positive cell clusters and TH-positive dopamine islands in fields of homogeneously distributed S cells in the developing caudate nucleus (CN). **A–C:** adjacent sections processed for autoradiography (**A**), SP immunostaining (**B**) and TH immunostaining (**C**) showing case KP102, which was ^3H -thymidine-labeled at E26 and perfused at E38. The E26-labeled cells appear relatively evenly distributed in the caudate nucleus. However, there are distinct SP-positive patches in the caudate nucleus that are aligned with TH-positive dopamine islands (examples shown at the asterisks in **B** and **C**). LV, lateral ventricle; GE, ganglionic eminence; P, putamen. Scale bar = 0.5 mm.

Figure 6.

Transformation of the medial band into cells that cluster in proto-striosomes. **A–C:** Autoradiograms of, respectively, rostral to caudal sections from case KP27, which was ^3H -thymidine-labeled at E25 and then culled at E45. The ^3H -thymidine-labeled medial band has disappeared at rostral and mid-anteroposterior levels (**A,B**). The medial band is still present in the dorsal part of the caudate nucleus (arrows) at caudal levels (**C**), but disappears ventrally (arrowheads). Accompanying the disintegration of the medial band, ^3H -thymidine-labeled cell clusters corresponding to proto-striosomes begin to appear (examples at asterisks) in **A–C**. Note finger-like extensions from remaining medial band penetrating the

caudate nucleus (f's in **C**), which might represent S cells caught in the midst of migrating inward. **D,E**: Close correspondence between the ³H-thymidine-labeled cell clusters (**D**, magnified view of section shown in **B**) and AChE-labeled striosomes visible in the adjoining section (**E**). CN, caudate nucleus; LV, lateral ventricle; GE, ganglionic eminence; P, putamen. Scale bar = 0.5 mm in **B** (applies to **A–C**) and in **E** (applies to **D,E**).

Figure 7.

Maturation and covert band-like patterning of striosomal cell clusters. **A:** Autoradiogram of case KP74, ^3H -thymidine-labeled at E24 and culled at E52. **B:** Autoradiogram of case KP56, ^3H -thymidine-labeled at E27 and culled at P1. In both cases, the medial band no longer exists, whereas many crisp S-cell clusters (examples at asterisks) appear in the caudate nucleus. **C:** Autoradiograms of case KPZ24-56, ^3H -thymidine-labeled at E24 and culled at adulthood. **D:** Autoradiogram of case KPB30-5, ^3H -thymidine-labeled at E30 and culled at adulthood. Early-born E24-labeled S cells are located in the lateral part of the

caudate nucleus (example at asterisk in **C**), whereas late-born E30-labeled S cells tend to be located in the medial part of the caudate nucleus (example at asterisk in **D**). LV, lateral ventricle; Sp, septum. Scale bars= 0.5 mm.

Figure 8.

Maturation of the M-cell compartment. **A,B:** Case KP169, ³H-thymidine-labeled at E33 and perfused at adulthood. **C,D:** Case KP146, ³H-thymidine-labeled at E38 and perfused at 4 months of age. Adjacent sections were processed for autoradiography (**A,C**) and AChE staining (**B,D**). Neither E33-labeled M cells (**A**) nor E38-labeled M cells (**C**) are located within AChE-positive striosomes in the caudate nucleus (examples at asterisks). The distribution of the M cells labeled at earlier and late stages of the M-cell birthday window does not appear to follow specific gradients in the caudate nucleus, as do S cells (see Fig. 7).

AC, anterior commissure; NAc, nucleus accumbens; CN, caudate nucleus; P, putamen; LV, lateral ventricle. Scale bar = 0.5 mm.

Figure 9.

Development of the M-cell compartment. Adjacent sections were processed for autoradiography (A,C) and acetylcholinesterase (AChE) staining (B,D). A,B: Case KP34, ^3H -thymidine-labeled at E45 and perfused at E48. The E45-labeled cells in the caudate nucleus (CN) are not in AChE-positive striosomes (example at asterisks). Many labeled cells are located in the ventricular zone (arrows). C,D: Case KM2, ^3H -thymidine-labeled at E37 and perfused at E57. The E37-labeled M cells also avoid AChE-positive

striosomes (example at asterisks). LV, lateral ventricle; Sp, septum. Scale bars = 0.5 mm in **B** (applies to **A,B**) and in **D** (applies to **C,D**).

Figure 10.

Schematic drawings illustrating the proposed progression pattern of S-cell development. **A** shows the medial band (MB), a putative waiting compartment for newly generated S cells, just medial to the ganglionic eminence (GE). Color code for dots in successive drawings illustrates distributions of successively generated cohorts of S cells (order: red, blue, green). The later-born population of M cells is shown in solid gray. A quasi-banded distribution pattern of S cells, shown here highly schematically (**B,C**), precedes the emergence of striosomal clusters (**D,E**), which appear as neurons of the matrix migrate into the striatum. Horizontal arrow denotes progression of time.

Summary of Times of ³H-thymidine Exposure and Culling Times for Embryos Injected with ³H-thymidine during the E20–E30 Time Window of S-Cell Generation

Table 1

Age at Injection	Age at Culling (E ¹)				
	E20–E29	E30–E39	E40–E49	E50–E59	Postnatal
E24	KP45 ² (E27) ³	KP46 (E34)		KP74, KP75 (E52)	KPZ24-56 (adult)
E25			KP27, KP28 (E45)		KP31 (P8)
E26	KP101 (E32), KP102 (E38)				
E27	KP108 (E39)		KP109 (E42)		KP56 (P1)
E28	KP38 (E35)		KP39 (E45)		
E30					KPB30-5 (adult)

¹ E, Embryonic day

² Case identification number

³ Cull date in parentheses

Table 2

Summary of Times of ^3H -thymidine Exposure and Culling Times for Embryos Injected with ^3H -thymidine during the E30–E45 Time Window of M-Cell Generation

Age at Injection (E ¹)	Age at Sacrifice			
	E46–48	E54	E57	Postnatal-Adult ²
E33–E35				KP163, KP166, KP167, KP168, KP169, KP178, KP179
E37–E38	KP157 ³	KP180, KP181, KP182	KM2	KP129, KP130, KP146, KP159
E41–E45	KP34			KP44, KP184, KP185

¹ E, Embryonic day

² Older than 2.5 months

³ Case identification number

Table 3

Primary Antibodies Used in the Study

Antigen	Description of Immunogen ¹	Source, Host Species, Lot #, RRID ²	Concentration Used
Tyrosine Hydroxylase	Joh laboratory	Dr. T.H. Joh, Rabbit, N/A, N/A	1:250–1:1000 Dilutions
Tyrosine Hydroxylase	Eugene Tech Lab	Eugene Tech International, Rabbit, Lot #: 1012, N/A	1:250–1:1000 Dilutions
Substance P	Ho Lab	Dr. R. Ho, Rabbit, Lot #: G6 (1983–1984), N/A	1:250 Dilutions

¹We relied on these highly reputable laboratories.

²RRIDs (Research Resource Identifiers) were not in use when the case material was originally collected.