Pattern Formation in the Striatum: Developmental Changes in the Distribution of Striatonigral Neurons

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The striatum of the mammalian forebrain can be divided into 2 compartments, the patches and the matrix. We have investigated embryonic events involved in the formation of these compartments in rats. Early in development, dopamine fibers from the substantia nigra selectively innervate the patches. In the perinatal striatum, we observed a close match between the distributions of striatal cell bodies with axonal projections to the substantia nigra and patches of afferent dopamine fibers. Striatal cells projecting to the nigra are first seen in the ventrolateral striatum at embryonic day (E) 17. Striatonigral cell bodies are distributed homogeneously through the striatum from E18 to 19. At E20 and until postnatal day 4, these cell bodies are organized into discrete patches. After this time, striatonigral cell bodies assume the dense and homogeneous distribution characteristic of the adult striatum. A retrograde tracer injection in the nigra at E18 (during the early period of homogeneous striatonigral distribution) produces a patchy striatonigral distribution if the embryo is not sacrificed until E21. The number of retrogradely labeled striatonigral cell bodies in a midstriatal section, at times immediately before and after the early homogeneous to patchy changeover did not differ significantly. We suggest that the neurons of the patch compartment of the striatum are born first and project to the substantia nigra first. The patch neurons only become restricted to "patchy" areas as the later-born matrix neurons migrate out into the striatum.

Pattern formation, how groups of cells aggregate to form functional units, is one of the major questions of developmental biology. We have investigated this phenomenon in the striatum of the mammalian forebrain. The striatum can be divided into 2 compartments, the patches and the matrix, which are defined on the basis of their connections, neurotransmitters, enzymes, and receptor types. Patch areas can be delineated in the adult by high levels of opiate receptors (Pert et al., 1976; Kent et al., 1982), substance P (Gerfen, 1984; Haber and Watson, 1985), neurotensin (Goedart et al., 1984), and afferents from the prefrontal cortex (Gerfen, 1984). In complementary fashion, the matrix compartment can be identified in the adult by high levels of somatostatin (Gerfen, 1984), neurotensin receptors (Goedart et al., 1984), AChE (Graybiel, 1984), and thalamic terminals

from the centromedian parafascicular complex (Herkenham and Pert, 1981). Furthermore, recent work by Gerfen (1984, 1985) suggests that, patch and matrix neurons in the adult rat have selective projections to the compacta and reticulata portions respectively, of the substantia nigra (SN). In light of the large reciprocal projection between the SN and the striatum in the adult animal, the development of these connections must be integral to any theory of striatal compartmentalization. The developmental mechanisms underlying the formation of the patch and matrix compartments in the striatum are currently unknown. Clues as to the origins of striatal compartments come largely from the identification of early patch markers and the time course of their development. In particular the development of striatal opiate receptors and the dopaminergic nigrostriatal pathway have been of interest (Graybiel et al., 1981; Moon Edley and Herkenham, 1984; van der Kooy, 1984; Lanca et al., 1985). Both of these markers show a similar early pattern of development. As early as embryonic day (E) 14 both of these markers show a homogeneous distribution (Specht et al., 1981; Moon Edley and Herkenham, 1984). Over the later portion of the rat's 21 d gestation period, first dopamine and then opiate receptors take on an identical patchy distribution (Moon Edley and Herkenham, 1984; Murrin and Ferrier, 1984; van der Kooy, 1984). While striatal opiate receptors maintain this patch distribution in the adult, a second wave of dopamine innervation during the first postnatal week changes the early distribution nigrostriatal terminals from patchy to homogeneous (Moon Edley and Herkenham, 1984; Saji et al., 1984; van der Kooy, 1984). Until recently, the perinatal dopamine distribution was the earliest known marker of striatal patch areas. While it is tempting to suggest that these early dopaminergic projections have an inductive role in the formation of the striatal patch compartment, neuroleptic blockade of prenatal dopamine using haloperidol (Moon, 1984; van der Kooy, 1984) has proven unsuccessful in blocking or delaying compartment formation (using opiate receptor density as a marker of the compartments).

Using a combination of embryonic surgery and retrograde axonal tracing techniques, we have studied the development of the major efferent projections from striatal cell bodies (i.e., the striatonigral pathway) to investigate early intrinsic indications of compartmentalization in the developing striatum. Striatal neurons with perinatal axon projections to the SN were found to occur in patches, which match the distribution of the dopamine afferent innervation at late prenatal and early postnatal times (Fishell and van der Kooy, 1984). More importantly, at earlier embryonic ages (E18), when only a minority of the adult striatal population is postmitotic (Bayer, 1984), many of the striatal neurons already have projections to the SN. Furthermore, these early embryonic projection neurons are homoge-

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neously distributed throughout the striatum. At later embryonic stages (E20), the major portion of the striatal perikarya are postmitotic. The later-born neuronal population migrates out into the striatum, separating the previously present striatonigral neurons into patches. These "matrix" neurons make their connections with the substantia nigra postnatally. Indeed, ^3H -thymidine studies on striatal development revealed that patch neurons become postmitotic at earlier embryonic times than matrix neurons (van der Kooy and Fishell, 1987). Similar to our findings, there is a temporal correlation between the growth of callosal projections from cortical neurons and the birthdates (and spatial distributions) of these neurons (Floeter and Jones, 1985a).

In support of this general scenario, recent work suggests that neurons may be committed to a specific phenotype around the time that they cease mitosis (Floeter and Jones, 1985b). Deep cortical neurons, which become postmitotic at discrete early embryonic times (Angevine and Sidman, 1961; Rakic, 1975), develop their expected axonal projections, even when transplanted at later embryonic times to neonatal host tissue (Floeter and Jones, 1985b). These studies on the cerebral cortex support the idea emerging from our experiments on the striatum that a neuron's birthdate is a good indicator of its presumptive phenotype. Our results suggest that either genetic programming of lineage or transient environmental inducers have determined compartmental fate before, or soon after, cells cease mitosis and leave the ventricular zone.

Materials and Methods

Comparison of the distributions of striatonigral cell bodies and dopamine terminals. Early postnatal rat pups ($n = 5$) were processed for the simultaneous visualization of striatonigral cell bodies and tyrosine hydroxylase (TH), a synthetic enzyme present in dopamine neurons. On postnatal day 2 (PND2) rats received unilateral SN injections (0.2–0.4 μl) of a 5% solution of the fluorescent retrograde axonal tracer True blue (TB). After a 24 hr survival period, these animals were processed for immunocytochemistry (van der Kooy and Sawchenko, 1982). Animals were deeply anesthetized and transcardially perfused with 2–4 ml of saline, followed by 10 ml of 4% paraformaldehyde (both at 4°C). The brains were removed and stored in 10% sucrose (in 0.1 M PBS, pH 7.4) at 4°C overnight. Brains were frozen and cut into 32 μm sections and collected in ice-cold PBS. Sections were incubated in a 1:100 rabbit anti-TH solution (Eugene Tech) containing 0.3% Triton X-100 and 1% normal goat serum (NGS) for 48 hr at 4°C. After washing in PBS (with 1% NGS and 0.3% Triton X-100) for 10 min, sections were incubated for 1 hr in 1:10 fluorescein isothiocyanate (FITC) goat anti-rabbit solution at room temperature. Following a final wash in PBS, sections were mounted and coverslipped with glycerol and water (pH 7.8) and viewed under excitation light of 470 nm wavelength to see the FITC-labeled TH and under a 360 nm wavelength excitation to visualize TB.

This experiment was also performed on E20 embryos ($n = 3$). A TB injection was made in the ventral midbrain at E19 by the modified injection procedure for *in utero* surgery described below. Subsequent fixation and processing of tissue adhered to the protocol used for the visualization of TH and retrogradely labeled cells in the early postnatal animals.

Striatonigral time course. In order to study the time course of the development of the striatonigral projection, injections of TB were made from E16 to PND7. Injections into prenatal rats were done using 2 or more timed pregnant rats for each day between E16 and E20, inclusive. The pregnant rats were anesthetized and given a laparotomy. Every second rat fetus was oriented inside the uterus, and the dorsal calvarian suture lines were used to estimate the location of the ventral midbrain. After puncturing the uterine wall with a 25 gauge needle, a 1 μl Hamilton syringe was used to make a 0.2–0.4 μl injection of 5% TB into the SN. The hole in the uterine wall was sealed by cauterization. The pregnant rat was sutured and allowed to recover. After 16 hr survival, the mother was again anesthetized and a cesarean section was performed. Previously injected fetal rat pups were removed and immediately perfused transcar-

dially with 5 ml of 20% formalin. The brains were removed, postfixed in the same solution for 4 hr, and then stored in 10% sucrose. Frozen sections, 32 μm , were cut and mounted on gelatin-coated slides. In order to investigate the possible migration and/or death of the early striatonigral neurons, fetuses from 3 additional pregnant rats were injected with 5% TB on E18 and allowed to survive until E21. Except for the length of the survival period, processing was exactly as described above.

For postnatal injections, a minimum of 5 animals was used on each day between PND1 and PND7, inclusive. The pups were either cold anesthetized (PND1–5) or given a 0.2 ml injection of 0.3% sodium pentobarbital (PND6–7). Animals received unilateral stereotaxic injections of 0.2–0.4 μl of 5% TB into the SN. After 16 hr, the rat pups were deeply anesthetized and perfused transcardially with 2–4 ml saline, followed by 10 ml of 20% formalin. Subsequent tissue processing and visualization of striatonigral neurons followed the procedures described above.

Finally, to help determine the length of time the retrograde tracer is available for uptake, a long-term survival experiment was undertaken. Using techniques outlined above, striatal injections of 5% TB were made at E19. The mother was allowed to give birth, and the pups were sacrificed at PND10. The appearance at PND10 of either a late prenatal or a 1 week postnatal striatal labeling pattern should allow a rough estimation of the time the tracer is available for uptake at the injection site.

Quantitative analysis. Midstriatal sections were quantitated in order to estimate the changes in the number of striatonigral neurons over the perinatal period. In all cases analyzed, regardless of age, a 1:1 match of TH and early projecting striatonigral neurons was observed. (Although TH immunofluorescent patches were weak in some cases, they were always present in areas containing numerous retrogradely labeled striatonigral neurons.) This allowed us to use the clusters of retrogradely labeled neurons themselves to delineate patch areas. Counts were made from complete collages of coronal sections through the middle of the head of the striatum (at the level of the septal nuclei), at E19, E21, and PND3 (1 midstriatal section from each of 5 animals at each age). The use of a midstriatal section for quantification is based on the observation that the ratio between areas comprising the patch and the matrix compartments seems relatively invariant from rostral to caudal striatal sections (Lanca et al., 1985). Thus, it is reasonable to assume that analysis of a specifically identifiable part of the striatum is representative of overall trends.

Two correction factors were applied to the absolute cell counts to give total counts. All cell counts were adjusted by these factors prior to any comparative analyses. These 2 corrections adjusted for changes in neuronal cell size and total striatal volume during development; allowing unbiased comparisons between ages despite the growth of neurons and expansion of the striatum itself. First, the average cell size at each age was assessed using a diameter calculated from cross-sectional area measurements for 50 randomly chosen cells, measured from calibrated photographs of Nissl sections. All cell counts were corrected by the method of Ambercrombie (1946), as modified by Königsmark (1970). Second, to allow comparison between age groups, a correction for the increasing size of the striatum during development was made. Striatal volume corrections were done by treating the striatum as a sphere and calculating the radius on the basis of area measurements of the 5 striatal cross sections used for counts at each age. The ratios of the radius of the striatum at E19 to the radii at E21 and PND3 were used to correct for striatal growth at the 2 later ages. Estimations of coronal midstriatal counts were also made with one E20 injection–E21 sacrifice animal and one PND6 injection–PND7 sacrifice animal. These were done by counting a smaller area of a coronal cross section and then adjusting the counts according to the area of the whole section. Corrections for cell size and striatal area were done as described above.

A second analysis was performed to assess cell migration during development. At E19, E21, and PND3 the percentage of striatal neurons retrogradely labeled in each of the 2 striatal compartments after a SN injection was calculated by comparing labeled neurons in a 4 mm² midstriatal area to Nissl-stained neurons counted in the same area. The 4 mm² areas selected for counts were from the centers of individual patch and matrix regions. Again, the 2 corrections discussed above were applied to both the labeled and Nissl counts prior to calculating the percentage of labeled cells. At E19, we counted 6 such areas for each of the 5 animals evaluated. Five similar measurements were made in the subcallosal area of a single E19 animal, in order to measure the apparent higher density in this area. At E21 and PND3, 12 areas (6 patch and 6

Figure 1. Matching of the distributions of striatonigral cells and dopamine fibers in perinatal rats. *A*, Outlined area represents the region shown in *B* and *C*. *B*, Visualization of fluorescent retrogradely labeled (5.0% True blue, TB) striatonigral cells at PND3. *C*, Dopamine-rich areas in the same section as *B* demonstrated by immunocytochemical (FITC) visualization of TH. *D*, Higher-power photomicrograph of the distribution of TB retrogradely labeled striatonigral neurons at E20. Bright profiles adjacent to retrogradely labeled neurons indicate labeled neurons slightly out of the plane of focus. *E*, Dopamine fibers in the same field as *D*, visualized as described in *C*. *F*, Nissl-stained cells in the same field as *D* and *E*. Arrows indicate the same fiber bundle landmarks for photomicrograph comparison. Abbreviations: *C*, cortex; *CC*, corpus callosum; *LV*, lateral ventricle; *ST*, striatum.

matrix) were sampled from 5 animals at each age. Labeled TB cells were counted in a 4 mm² area under a Leitz epifluorescence microscope. The microscopic coordinates and tissue landmarks were recorded for each area to aid in identification after Nissl staining. After the sections were stained and coverslipped, the same 4 mm² areas were recounted to yield total neuron number.

Results

Overlap of dopamine and striatonigral cell patches

The distribution of striatonigral cell bodies exactly matched the distribution of TH-stained fibers in the striatum, at both E20 and PND3 (Fig. 1). This relationship is less easily seen as one moves medially in the striatum because of the weaker TH staining here (e.g., the retrogradely labeled patch in the lower-left portion of Fig. 1*B* is matched by only a weakly stained area in Fig. 1*C*). Similar matching in the striatum was observed (Fishell and van der Kooy, 1984) using glyoxylic acid for direct visualization of endogenous dopamine (van der Kooy, 1984) and another fluorescent retrograde axonal tracer (propidium iodide) in early postnatal animals. While striatonigral cell bodies are seen clustered through the entire early postnatal striatum, islands of TH staining (although present throughout the striatum) are best visualized in the dorsolateral quarter (Graybiel, 1984; Moon Edley and Herkenham, 1984). Previous investigators have shown that the perinatal distribution of dopamine matches areas of high opiate receptor density, the latter being a marker of the adult patch compartment of the striatum (Moon Edley and Herkenham, 1984; Murrin and Ferrier, 1984; van der Kooy, 1984). This suggests that the distribution of striatal cells with early nigral projections provides an effective perinatal marker for developing patch areas of the striatum. The following time course investigates the earliest point at which striatonigral cells exist in a patchy distribution, in an attempt to elucidate the events underlying patch formation.

Striatonigral time course

Through numerous inaccurate injections it became clear that only those that included the SN resulted in retrograde labeling of striatonigral cell bodies. For example, injections that infringed only on the dorsal midbrain produced no retrograde labeling in the striatum. All results presented below are drawn from injections filling the ipsilateral ventral midbrain (Fig. 2). A further criterion for injections considered acceptable for analysis was restriction of the injection site to the level of the most caudal thalamus and the midbrain. Furthermore, within any given age group all injections that filled the ipsilateral ventral midbrain yielded a similar distribution of retrogradely labeled striatal neurons. What follows is a description of the developmental changes in labeled striatonigral neurons in a midstriatal section. All cell counts reported are corrected for changes in neuronal cell size and changes in total striatal volume during development, as described in Materials and Methods.

E16 injection (E16i)/E17 sacrifice (E17s). A small number of retrogradely labeled neurons were seen restricted to the ventrolateral edge of the striatum at this age ($n = 5$) (Fig. 3*A*). As at all other embryonic ages, mitotically active cells in the ventricular zone were not labeled. However, there was also a large medial striatal area containing postmitotic neurons with no nigral projections. All the labeled neurons were clumped against the edge of the developing external capsule. The labeling of so few neurons in a small portion of the striatum suggests that E17 represents a time soon after the first axons of striatal cells reach the nigra.

E18i/E19s. The retrogradely labeled striatonigral cell population at this age is striking in its widespread distribution throughout the striatum ($n = 5$) (Fig. 3*B*). The homogeneously distributed striatonigral cells filled the entire striatum and accounted for 32% of the neurons present (average of 23 ± 1.4 labeled neurons (LN)/ 74 ± 1.8 total neurons (TN) per 4.0 mm²). The lateral edge of the striatum (the future subcallosal streak) showed a higher density of labeled neurons, 44% (35.4 LN/ 80.6 TN). There is also a suggestion of clustering of the neurons at the extreme lateral edge of the striatum. The average number of retrogradely labeled striatonigral neurons in a midstriatal coronal section was 4095 ± 145 .

E19i/E20s. The first clear evidence of patches in the distribution of striatonigral neurons is seen at this age ($n = 5$) (Fig. 3*C*). Furthermore, dense retrograde labeling of the lateral edge of the striatum demarcates a clear subcallosal streak. In areas outside of the patches and the subcallosal streak, low levels of retrogradely labeled cells (approximately 10% of that seen in patchy areas) were seen. It is important to note that the homogeneous to patchy crossover seems to follow both caudal to rostral and ventrolateral to dorsomedial gradients, and thus the patches are first seen in the caudal ventrolateral striatum.

E18i/E21s. Striatonigral labeling is almost exclusively restricted to patches at E21 ($n = 5$) (Fig. 3*D*). In the patches the percentage of neurons that were retrogradely labeled was 49% (31 ± 0.8 LN/ 62 ± 3.5 TN), whereas retrograde labeling in the matrix accounted for only 3% of the total neurons present in this compartment (2.2 ± 0.1 LN/ 63 ± 1.3 TN). As mentioned above, because of the ventrolateral to dorsomedial gradient in the crossover from homogeneous to patchy striatonigral neurons, the dorsomedial striatum is the last area to take on a patchy distribution. This effect is reflected in the small, but homogeneous region of retrogradely labeled cells visualized in the dorsomedial striatum at E21 (Fig. 1*D*). The general clustering of the striatonigral neurons was reflected in a significant increase in the percentage of labeled neurons in the patches at E21 (49%) compared with the labeling in the striatum at E19 (32%) (significant interaction of age and of cell counts, $F_{1,8} = 27$, $p < 0.05$) (Fig. 4*B*). The average number of retrogradely labeled striatonigral neurons in a midstriatal coronal section was 4172 ± 144 . Although the distribution of cells has changed from homogeneous at E19 to patchy at E21, remarkably the number of retrogradely labeled striatonigral neurons in animals sacrificed at these 2 ages remained constant. There is no significant difference in the average number of retrogradely labeled neurons in striatal coronal sections between these 2 ages ($t = 0.4$, $df = 8$, $p > 0.05$) (Fig. 4*A*). An estimate of retrogradely labeled neurons (~ 4927) in a coronal midstriatal section from an animal injected on E20 and sacrificed on E21 appears to be higher than the mean count from E18i/E21s animals. The E20i/E21s count was generated using a single animal, but it does fall, however, within the range of counts observed in the long-term survival (E18i/E21s) group ($n = 5$). Furthermore, the patchy visual appearance of striatal labeling in a number of E20i/E21s animals ($n = 3$) indicates that the results obtained are similar to those from the E18i/E21s animals.

PND2i/PND3s. Although the percentage of neurons that can be retrogradely labeled in patchy areas at PND3 (55%; 32 ± 1.5 LN/ 59 ± 1.8 TN; $n = 5$) has not changed significantly ($F_{1,8} = 1.3$, $p > 0.05$), labeling in the matrix areas (32%; 18 ± 0.9 LN/ 58 ± 1.5 TN) has increased significantly ($F_{1,8} = 86.7$, $p < 0.05$) from the 3% labeling at E21 (Fig. 4*B*). Despite this drastic in-

Figure 2. *A–D*, Diagrams showing the maximum and minimum extent of tracer spread in coronal midbrain sections from cases considered adequate for the quantitative analysis shown in Figure 4. *A*, E16/E17s; *B*, E18/E19s; *C*, E18/E21s; and *D*, PND2/PND3s. *E*, Typical injection site (from an E18/E21s animal) showing the distribution of the retrograde tracer TB in the ventral midbrain. The pattern of retrogradely labeled neurons in the striatum resulting from this injection is the same as shown in Figure 3*D*.

Figure 3. Coronal striatal sections of fluorescent retrogradely labeled (5.0% TB) striatonigral cells taken at 5 perinatal times. All successful ventral midbrain injections within any single age group yielded similar results. *A*, At E17, labeled striatonigral neurons are restricted to the ventrolateral portion of the striatum. *B*, At E19, striatonigral cells are homogeneously distributed; however, early evidence of a subcallosal streak and adjacent patchiness is seen on the lateral border of the striatum. *C*, At E20, the emergence of a patchy pattern in the distribution of striatonigral neurons is clearly evident. *D*, At E21, striatonigral cells exist almost exclusively in patches, except for a small area in the dorsolateral quadrant (discussed in text). *E*, By PND3, while patches of striatonigral cells are still visible, a specific increase in matrix labeling is readily apparent.

Figure 4. *A*, Change in the total corrected number of striatonigral cells in a midstriatal coronal section during the perinatal period. After an E18 injection, no change was observed in the number of striatonigral neurons labeled after survival until E19 or E21. However, a significant increase in labeled neurons is seen between the E18i/E21s and PND2i/PND3s animals. Data represent means $\pm$ SEM. *B*, Percentage of striatal neurons in patch and matrix areas that are retrogradely labeled from the nigra, during the same perinatal time frame as Figure 3*A*. Patchy areas show a significant increase in the percentage of labeling from E19 to E21, but not from E21 to PND3. Matrix areas, which do not exist until E20, show a significant increase in the percentage labeling from E21 to PND3. Data represent means $\pm$ SEM.

crease in matrix labeling, dense patches of retrogradely labeled striatonigral neurons are still discernible (Fig. 3*E*). The total corrected number of striatonigral cells in a midstriatal section has gone up significantly from 4172 ± 144 at E21 to $11,750 \pm 1229$ at PND3 ($t = 10.4$, $df = 8$, $p < 0.05$) (Fig. 4*A*). The changes in the percentage of labeling mentioned above strongly suggest that this increase in total striatonigral labeling is primarily a reflection of the increased striatonigral projections from matrix regions. Similar to the prenatal homogeneous to patchy crossover, a caudal to rostral gradient was also observed in the changes from patchy to homogeneous postnatal labeling. However, a ventrolateral to dorsomedial gradient was not readily apparent.

Kent et al. (1982) reported an overall decrease in the density of Nissl-stained cells in the patch compartment over the perinatal period. We found small decreases in the total number of Nissl-stained cells per unit area of the patch compartment (particularly between E19 and E21), but our Nissl counts were from small sample areas at the center of patches, rather than from entire patches as in Kent et al. (1982).

PND6i/PND7s. By this age, many neurons situated throughout the striatum have projections to the SN. An estimate of the total number of labeled striatonigral neurons in a midstriatal section ($\sim 16,345$) suggests that there has been at least a 50% increase in the number of striatonigral projections (primarily from the matrix compartment) between PND3 and PND7.

E19i/PND10s. Clearly defined patches of labeled neurons are visible in the striatum (Fig. 5). These patches exist throughout the striatum; homogeneous striatal labeling is not observed. Thus, areas in the striatal matrix appear to be completely unlabeled. This stands in clear contrast to the results from the PND6i/PND7s animals described above, where the striatal labeling is homogeneous and dense. These results suggest that TB is available for uptake in the SN for not more than a few days after the E19 injection, during which time only the striatal patches are labeled retrogradely.

Discussion

The perinatal matching of striatal cells projecting to the nigra, with dopamine fibers, indicates that during early development (E20–PND5) these striatonigral cell bodies provide an excellent marker for the patch compartment of the striatum. The time course of development suggests that 2 distinct waves of striatonigral connections occur. An early population, which is initially distributed homogeneously in the striatum, makes connections with the SN from E17 to E19. A second population (matrix neurons) generated during the late gestation period (E18–E21) migrates out into the striatum, separating out the previously established striatonigral neurons into patches. The second, matrix population of striatal neurons does not send its axonal projections to the SN until the early postnatal period.

The 2 remarkable changes in the distribution of cells forming the striatonigral projection occur at E19–E20 and at PND2–PND6. These 2 time windows see changeovers in the striatonigral projection from homogeneous to patchy and from patchy to homogeneous, respectively. Most interesting in terms of patch/matrix formation is the first of these phenomenon. This early homogeneous to patchy changeover may occur as the result of striatonigral neuron cell death, withdrawal of striatal nerve terminals from the nigra, or migration of neurons within the striatum.

A cell death explanation demands an overproduction of patch neurons to allow for massive cell death. The constancy of the number of striatonigral neurons in a midstriatal section, before and after the homogeneous to patchy changeover, makes this possibility appear unlikely. If continued uptake of tracer results in significant retrograde labeling in long-term survival experiments, then an alternative to our interpretation would be that new connections made in patch zones are accompanied by cell death in matrix areas. However, the long-term survival experiment (E19i/PND10s), designed as a general test for continued

Figure 5. Retrograde labeling in a coronal section of the lateral striatum at PND10, after an E19 injection of TB into the SN. Retrogradely labeled neurons are seen only in the patches and in the subcallosal streak. Although a large matrix striatonigral projection exists by this point in development, the striatal matrix perikarya are not labeled by a late embryonic injection. This suggests effective uptake of the retrograde tracer TB is limited to a very few days.

tracer uptake at the injection site, revealed no striatal matrix labeling at PND10, although axons from the striatal matrix should have started to reach the SN injection site in the first few postnatal days. This suggests that the retrograde tracer TB is available for uptake for only a few days after injection and that continued tracer uptake does not obscure any putative cell death of other striatal neurons. Furthermore, a requirement for the exact balancing of labeled cell death and new striatonigral projections over the entire late embryonic period makes cell death an unlikely explanation for the homogeneous to patchy change in the distribution of striatonigral neurons.

If withdrawal of nigral nerve terminals by some striatal cells accounted for patch formation, then we should expect that striatal cells retrogradely labeled during the early homogeneous distribution period (E18–E19) would still be labeled in the same homogeneous distribution if the animals were allowed to survive until well into the patchy distribution period (E20–PND2). Our E18i/E21s experiment showed exactly the opposite result, a patchy distribution at sacrifice. Thus, nerve terminal withdrawal does not explain patch formation.

The most parsimonious mechanism for the development of the patches is migration. This presumably takes 2 forms. First, the massive migration of matrix cells from the proliferative

ventricular zone, out into the striatum, probably accounts for the majority of patch formation. The homogeneously distributed striatonigral neurons at E18 may be physically segregated into patches by the large numbers of new matrix neurons migrating into the striatum after this time. Second, the gradual focusing of patches around E20 suggests that patch cells themselves may have some mutual attraction for each other. The evidence for mutual attraction is the increase in the percentage of neurons retrogradely labeled in the developing patch compartment, from 32% at E19 to 49% at E21. Furthermore, this patching of striatonigral neurons occurs despite the fact that the total number of labeled neurons does not change over this period. Recent *in vitro* results have also provided evidence that patch cells selectively reaggregate with other patch cells in dissociated embryonic striatal cultures (Krushel et al., 1986). These results raise the possibility that striatonigral cell bodies are able to migrate *in vivo*, at least short distances, even after they have sent long axonal projections to the midbrain.

While the role of the early dopaminergic nigrostriatal projection in striatal compartmentalization (Moon, 1984; Moon Edley and Herkenham, 1984; van der Kooy, 1984; Lanca et al., 1985) should not be discounted, its importance must be reassessed in light of the finding that the striatonigral perikarya themselves also show early compartmentalization. A striking parallelism is seen in the development of the reciprocal connections between the striatum and the SN. Nigrostriatal projections reach the striatum as early as E14 (Moon Edley and Herkenham, 1984), and we first observed striatonigral projections at E16. However, before either projection shows its characteristic patchy distribution in the perinatal striatum, both are seen homogeneously throughout this structure. This suggests that the putative patch cells may all receive dopaminergic input at this point in early development, and that all striatal cells receiving such input project to the SN. The prenatal proximity of dopamine terminals to retrogradely labeled striatonigral cells (see Fig. 1, *D, E*) strongly supports this hypothesis. Furthermore, the fact that both these markers assume a patchy distribution at about the same time (E19–E20) suggests that early reciprocal connections between the striatum and the SN may be important in establishing early environmental cues necessary for patch/matrix compartmentalization. However, there is no direct experimental evidence bearing on the question of whether either projection provides inductive factors essential to the formation of striatal compartments.

The second change in the distribution of the striatonigral cells (the postnatal change from patchy to homogeneous) can be attributed to new connections made from the matrix specifically. This suggestion is supported by the massive increase in the percentage of retrogradely labeled neurons in matrix (but not patch areas) observed at PND3.

We hypothesize that striatal cell birthdate underlies the compartmentalization of the mammalian striatum. In other words, the time at which a striatal cell undergoes its last mitotic division is key in determining whether it becomes part of the patch or the matrix. While numerous investigators have carried out studies of the time course of striatal development in rats and other animals (Brand and Rakic, 1979; Donkelaar and Dederen, 1979; Smart and Sturrock, 1979; Fentress et al., 1981; Graybiel and Hickey, 1982; Bayer, 1984), their primary emphasis has been on identifying gradients of cell division within the striatum. While both Brand and Rakic (1979) and Graybiel and Hickey (1982) observed the presence of patches of radiolabeled neurons

after prenatal ^3H -thymidine injections, a clear scenario of the time course of patch versus matrix development has not been outlined. Our results suggest that patch and matrix cells develop during separate and specific time windows. The earliest striatal cells will form the patch component, followed by a time window of matrix cell production. ^3H -thymidine pulse label experiments strongly support this suggestion (van der Kooy and Fishell, 1987). This parallels work of Floeter and Jones (1985a), who showed that 2 successive waves of both cortical neurogenesis and callosal projections exist for the deep and superficial layers of the cortex. Our studies indicate that neurons becoming postmitotic at E13 contribute almost solely to the adult patch compartment. In contrast, neurons born at later times (E18–E21) have a significantly higher probability of joining the adult matrix compartment (van der Kooy and Fishell, 1987). We suggest that the compartments are physically formed around E20, with the migration of the later-born matrix cells out among the earlier-born striatal patch cells. Further support for this hypothesis comes from comparing the caudal to rostral and ventrolateral to dorsomedial gradients seen in the homogeneous to patchy embryonic changeover of striatonigral perikaryal distribution (the present results) with the very similar gradients in striatal cell birthdates from the detailed studies of Bayer (1984).

It has long been known that neuronal birthdate predicts lamination of the cerebral cortex, with the first neurons to become postmitotic joining the deep layers and later-born neurons forming the superficial layers (Angevine and Sidman, 1961; Rakic, 1975). Of particular note is the transplantation work by Floeter and Jones (1985b), which suggests that the commitment to phenotypic markers such as neurotransmitter and projection target is made in cortical neurons at times soon after mitosis ceases. Similarly, sheets of E15 rat cerebral cortex transplanted to newborn hosts in an inverted orientation develop a reverse pattern of lamination (Chang et al., 1986), suggesting that at least the lamination of these neurons is intrinsically regulated. Further evidence of early determination of cortical neurons comes from studies in which the migration of cortical neurons to their normal layer is prevented by exposing developing rat cortex to ionizing radiation at E17 (Jensen and Killackey, 1984). Although inappropriately located, these neurons make connections with their correct subcortical targets.

In relation to cell birthdate, the important question with regard to both the striatum and cerebral cortex is whether neurons are programmed to a given fate on the basis of their cell lineage or whether their specific time window of postmitotic migration allows interaction with transient, inductive environmental cues. The primary question to be addressed in future striatal experiments is whether cells acquire their role as part of the patch or matrix compartments through a mechanism intrinsic or extrinsic to the striatum. Are the compartments already determined in the proliferative ventricular zone of the striatum, or are they induced postmitotically in the striatum by some environmental or extrinsic factor? Perhaps events controlling cell birth date help determine commitment to a compartment, but extrinsic factors induce the differentiation and expression of the numerous intracompartamental phenotypes.

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