Published in final edited form as:

Brain Struct Funct. 2008 September ; 213(0): 119–127. doi:10.1007/s00429-008-0185-1.

Cell proliferation in the striatum during postnatal development: preferential distribution in subregions of the ventral striatum

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Abstract

The ventral striatum receives topographic input from the orbital frontal cortex (OFC), anterior cingulate cortex (ACC), and ventral medial prefrontal cortex (vmPFC), areas associated with incentive-based learning. In addition, cortico-striatal projections from the dorsal prefrontal cortex (dorsal PFC) and the motor/premotor areas are also organized topographically. Cortico-ventral basal ganglia circuitry is associated with a variety of mental health disorders including obsessive-compulsive disorder, and drug addiction, disorders that emerge during childhood through young adulthood. Growing evidence indicates that cortical and striatal development continues through this period. Moreover, cell proliferation, which is associated with development and plasticity, also continues in the cortex and striatum through adulthood. Given the implication of cortico-basal ganglia circuitry in diseases emerging during postnatal development, we studied cell proliferation in striatal regions associated with specific frontal cortical areas at different ages. The results show cell proliferation throughout the striatum at all postnatal ages. The majority of the new cells were immunoreactive for the chondroitin sulfate NG2, a marker for glia, but not NeuN, a neuronal marker. Although neurogenesis was not observed, approximately 30% of the new cells were associated with neurons. There was a significantly higher degree of cell proliferation during the first year postnatal compared to other striatal regions. Finally, the ventral striatal areas receiving input from the vmPFC and OFC have significantly more new cells throughout the juvenile years compared to other striatal regions. Taken together, these results indicate that new cells in the ventral striatum may be particularly important for the refinement of the cortico-striatal network, and in the formation of neural ensembles fundamental to learning during behavioral development.

Keywords

Reward; glia; plasticity; orbital cortex; cingulate cortex; dorso-lateral prefrontal cortex; ventral; medial prefrontal cortex

Introduction

The concept of the ventral striatum was first introduced by Lennart Heimer to describe the ventral extension of the striatum that included the nucleus accumbens, the medial and ventral portions of the caudate and putamen, and the striatal cells of the olfactory tubercle and anterior perforated substance (L. Heimer, 1978). Since that seminal discovery, the ventral striatum and its connections have been at the center of the reward circuit, incentive-based learning, and the development of habit formation and addictions (W. C. Drevets et al., 2001; A. E. Kelley et al., 2002; G. Pagnoni et al., 2002; R. Elliott et al., 2003; P. W. Kalivas and N. D. Volkow, 2005; A. H. Evans et al., 2006; A. R. Hariri et al., 2006). The prefrontal cortical (PFC)-areas most associated with reward are the ventral medial prefrontal cortex (vmPFC) and the orbital prefrontal cortex (OFC) (E. T. Rolls, 2000; R. Elliott et al., 2003; M. R. Roesch and C. R. Olson, 2004; C. Padoa-Schioppa and J. A. Assad, 2006). These frontal regions project to different parts of the ventral striatum; in particular, to the ventral medial striatum, (the shell and along the medial wall of the caudate n.) and to the ventral putamen and caudate n. respectively (H. W. Berendse et al., 1992; S. N. Haber et al., 2006). In contrast, the dorsolateral prefrontal cortex (dorsal PFC) that is involved in cognitive processing, projects to the dorsal caudate n. and central putamen, and motor control areas project to the lateral striatum (R. Calzavara et al., 2007).

Infancy through young adulthood is that critical time in which incentive-based learning forms the basis for behavioral guiding rules and the development of habits. It is also a vulnerable time for developing addictions and compulsive behaviors. Consistent with rapid behavioral development, the cortex and striatum undergo maturation during this time (M. DiFiglia et al., 1980; N. Gogtay et al., 2004; R. K. Lenroot and J. N. Giedd, 2006). Cell proliferation, which is associated with development and plasticity, continues in the cortex and striatum through adulthood. The subventricular zone (SVZ), which retains a population of neuronal progenitor cells that migrate adjacent to the ventral striatum, to reach the olfactory bulb (D. R. Kornack and P. Rakic, 2001; V. Pencea et al., 2001), is thought to give rise to newly generated striatal glial or neuronal cells (J. M. Van Kampen et al., 2004; A. G. Dayer et al., 2005; A. Bedard et al., 2006; F. Luzzati et al., 2006; D. Tande et al., 2006). However, little is known about cell proliferation in the striatum during post-natal development. Whether glia or neurons, these newly formed cells during this critical period likely play a key role in elaboration and refinement of neural connections associated with early learning and habit formation (W. P. Ge et al., 2006; M. Paukert and D. E. Bergles, 2006).

Given the implication of cortico-basal ganglia circuitry in diseases emerging during childhood through young adulthood, we studied postnatal cell proliferation in striatal regions receiving inputs from cortical areas associated different aspects of incentive-based learning compared to areas that receive inputs from motor control areas. The results show that there is a significantly higher degree of cell proliferation throughout the striatum during the first year postnatal compared to other age groups. While there was little evidence for neurogenesis, approximately 30% of the new cells were associated with neurons. The ventral striatal areas receiving input from the vmPFC and OFC have significantly more new cells throughout the juvenile years compared to that observed in other regions. Since the

development of goal-directed behaviors depends on the integrity of the ventral striatum (B. J. Everitt and T. W. Robbins, 2005), proliferation of new cells may play a critical role in refining of neural ensembles during postnatal development, a time of rapid learning.

Methods

Twenty-three male macaque monkeys (*Macaca Fascicularis*) were used in this study. Animals were on a 12-hour light-dark cycle, with food and water present *ad libitum*. All of the experimental procedures and care of laboratory animals conformed to and were approved by the University Committee on Animal Resources (UCAR) and the ILAR Guide for the Care and Use of Laboratory Animals (C. o. L. S. Institute of Laboratory Animal Resources, National Research Council, 1996). To evaluate the number and distribution proliferating cells in the striatum during post-natal development, animals received 2 intravenous injections of 5-bromo-2-deoxyuridine (BrdU) (see below). There were four animals in each of the following age groups: 5 months, 1 year, 2 years and 3 years. To determine whether cell fate or final distribution depended on the length of time between injection and sacrifice, two animals in each group were sacrificed 5 weeks following BrdU treatment and two animals were sacrificed 10 following treatment. As there was no difference between these two groups in the number and distribution of BrdU⁺ cells, animals within each age group were combined for further analysis. In addition, for comparison, there were two, 12 years old animals and two, 18 years old animals that were sacrificed 5 weeks following BrdU-treatment. Finally, to determine whether new cells were derived from resident progenitors or the SVZ, one animal was sacrificed 24 hours following BrdU-treatment and compared to two age-matched controls sacrificed 5 weeks following BrdU treatment.

Bromodeoxyuridine Treatment and Tissue Preparation

The injections of BrdU (50 mg/kg; Sigma) were given intravenously at a rate of 5 ml/minute, twice, two days apart. A 10 mg BrdU/ml solution in sterile saline was prepared immediately before use by vortexing until completely dissolved, titrating with 10 N sodium hydroxide to a pH 7.4, and filter sterilizing. Animals were sacrificed by intracardiac perfusion 5 or 10 weeks, or one day following the last BrdU injection. Animals were initially anesthetized with Ketamine (10mg/kg, intramuscularly) and then deeply anesthetized with pentobarbital (25mg/kg, intravenously) and perfused with saline, followed by a 4% paraformaldehyde/1.5% sucrose solution in 0.1 M phosphate buffer, pH 7.4. Brains were postfixed overnight and cryoprotected in increasing gradients of sucrose (10%, 20%, and 30%). 50 µm serial sections were cut on a freezing microtome. Tissue processed for BrdU immunohistochemistry was transferred into 50% formamide/sodium chloride and sodium citrate (2x SSC) at 65°C for 2 hours, to denature the DNA and expose incorporated BrdU. Following a 5 minute rinse in 2x SSC at room temperature, tissue was transferred into 2 N HCl at 37°C for 30 minutes to degrade the nucleoprotein and further expose the DNA, then rinsed in 0.1 M boric acid, pH 8.5, at room temperature for 10 minutes to neutralize the 2 N HCl. Tissue was rinsed once in 0.1 M phosphate buffer with 0.3% Triton X-100 (TX, Sigma, St. Louis, MO) for 5 minutes, preincubated in 10% normal goat serum (NGS, from Invitrogen, Grand Island, NY) and 0.3% TX in 0.1M phosphate buffer for 30 minutes, then

incubated with rat antiserum to BrdU (1:1000; Accurate Chemical) in 0.1 M phosphate buffer with 0.3% TX and 10% NGS for four nights at 4°C. Following extensive rinsing, the tissue was preincubated in 0.1M phosphate buffer with 0.3% TX and 10% NGS and then incubated in biotinylated secondary antibody followed by incubation with the avidin-biotin complex solution (Elite Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Immunoreactivity was visualized using standard 3,3'-diaminobenzidine tetra-hydrochloride (DAB) procedures (S. N. Haber et al., 2006).

The greatest changes in cell number occurred between the 5mo./1yr. age and the 2 yr. age groups (see results), therefore, detailed analysis and co-localization studies were done with tissue from these groups. We performed double-label immunohistofluorescence experiments to determine whether cells differentiated into neurons or glia. These included: BrdU + NeuN (1:4000, Chemicon), S-100 (1:4000; Novocastra), GFAP (1:4000; Sigma), NG2 (1:100; Chemicon or gift from Dr. Joel Levine). We also doubled stained sections to determine the striatal neural type that BrdU+ cells were associated with. This included calbindin (CaBP-1:1000; Chemicon) for medium spiny projection neurons, and a marker for each of the four interneurons, calretinin (CR-1:15,000; Swant), parvalbumin (PV-1:15,000; Swant), nNOS 1:30,000; Sigma), choline acetyl transferase (ChAT-1:100; Chemicon). Sections were denatured as described above, incubated in antiserum cocktails for one night at 4°C, rinsed, and incubated in fluorescent secondary antibody cocktail (Alexa 488 goat anti-rat; 1:200 and either Alexa 555 goat anti-mouse; 1:200, Alexa 555 goat anti-rabbit).

Analysis

We divided the striatum into four regions based on cortical inputs from 3 prefrontal areas associated with different aspects of decision-making and incentive learning, and one motor control region. These divisions were based on projections from frontal cortex, which involve primarily the rostral striatum (S. N. Haber et al., 2006). Since cortico-striatal inputs terminate in both separate and converging regions, regional borders were drawn to exclude overlapping focal projections fields. The divisions were as follows: ventral medial striatum- input from the vmPFC, areas 25, 32 (subgenual cingulate), and 14; ventral, central striatum- input from OFC, areas 11, 12, and 13; dorsal striatum- input from the dorsal PFC; and the dorsolateral striatum- input from rostral motor control regions (see figure 1a and 3b for striatal divisions) (S. N. Haber et al., 2006; R. Calzavara et al., 2007). The rostral migratory stream (RMS) lies adjacent to the ventral striatum, and the border of the ventral striatum was drawn to exclude RMS cells. For each animal, in all age groups, BrdU⁺ cells were charted and cell counts were determined using unbiased stereology (Stereoinvestigator, Micro-Brightfield) for each of the four striatal regions. Sections, (1 in 6), were analyzed throughout the rostral striatum where inputs from the prefrontal cortex are primarily found. Co-localization studies using double-stained sections were analyzed with a Leica (6500) fluorescent microscope (60X oil emersion objective), equipped with deconvolution algorithms. In addition, selective sections were also analyzed using a Zeiss microscope (100X oil emersion objective) equipped with confocal microscopy. Preliminary cell counts did not show a significant difference in BrdU⁺ cells associated with NeuN between the 5 mo. and 2 yr. age groups. Since the 5 mo. age group showed the greatest number of new cells, we used this group to evaluate BrdU⁺ cell association with NeuN, CaBP, and CR in

each of the four regions. Data were analyzed with a one-way ANOVA with Bonferroni Multiple Comparison tests when comparing one variable, and a two-way ANOVA with Bonferroni Multiple Comparison tests was used for comparing two variables (functional regions across age groups). For analysis of BrdU⁺ cells densities, densities from different regions of the same animal were matched and a two-way ANOVA with Bonferroni Multiple Comparison tests was performed to compare both region and age variables. We judged significance to be at $p < .05$.

Results

General Distribution of BrdU⁺ cells and cell fate

BrdU⁺ cells were found throughout the rostral striatum in animals of all age groups (Figs. 1a; Fig. 3a). There were no differences in the number or distribution of cells between animals sacrificed 5 weeks or 10 weeks post injection. This data therefore was combined for each age group. The labeled cells in the animal sacrificed 24 hours following BrdU treatment were not preferentially distributed adjacent to the SVZ compared to animals sacrificed with longer survival times (Fig. 1b). However, the mean percentage of BrdU⁺ cells dividing or found closely associated with other BrdU⁺ cells, (referred to as doublets) (Fig. 1c), was more than two standard deviations higher in the animal with a 1-day survival time ($59\% \pm 5.7$), compared to control animals ($32\% \pm 1.8$ and $39\% \pm 5.1$). We found no co-localization between BrdU⁺ cells and NeuN or the astrocyte markers, S-100 or GFAP. However, both the confocal and deconvolution methods demonstrated cells that the majority of cells were double-stained for BrdU and NG2 (Fig. 1d). Specifically, the results showed that 83% of the BrdU⁺ cells were double labeled for NG2 in the 5-month-old group, and 70% of the BrdU⁺ positive cells were double labeled for NG2 in the two year old animals.

Relationship between BrdU⁺ cells and neurons

Despite the lack of evidence for neurogenesis, sections double-stained for BrdU and NeuN showed that there was a particularly close association between a subpopulation of BrdU⁺ cells and neurons (Fig. 2a-c). Preliminary counts indicated that approximately one-third of BrdU⁺ cells in the 5 month and 2-year-old animals, were associated with at least one neuron. Since there were no significant differences between these age groups and there are relatively small numbers of BrdU⁺ cells found in the 2 year old animals, complete cell counts were carried out for the 5 month old age group. We found that 29% of BrdU⁺ cells were associated with at least one neuron (Fig. 2a-c). We double stained sections for BrdU and a marker for each of the four striatal interneuron types, and for calbindin to label the medium spiny projection neurons. Approximately 11% of the BrdU⁺ cells were closely associated with CaBP⁺ medium spiny neurons (Fig. 2d) and approximately 1.3% of BrdU⁺ cells were associated with the calretinin interneuron (Fig. 2e). In contrast, no BrdU⁺ cells were associated with the other three interneurons, parvalbumin, NOS, or acetylcholine.

Distribution of BrdU⁺ cells across age

The greatest total number of cells was found in the 5-month and 1 year old animals (Fig. 3a) with no significant difference between these age groups. However, the individual variability

was quite high which is consistent with a rapid increase of brain volume during the first year of life (Malkova et al. '06). We therefore combined the data from these two groups. There was a significant difference in the total mean number of BrdU⁺ cells across the different age groups ($p < 0.006$; Fig. 3a). Animals in the 5-month/1year age group had significantly more BrdU⁺ cells than animals in the 2 years or 3 years age groups ($p < 0.01$; $p < 0.05$; Fig. 3a-c). The largest decrease in total number of BrdU⁺ cells (86%) was observed from the 1 year to 2 years age group (Fig. 3a,b). There was no difference in cell number between the 2 and 3 years old groups. We also compared the number of BrdU⁺ cells just between the older animal groups since brain growth is more stable in these animals compared to the first year of life. There was a significant difference in mean total BrdU⁺ cells among the older age groups ($p < 0.01$; Fig. 3d), with the 12 year old animals showing significantly more BrdU⁺ cells than animals in the 2 year, 3 year, and 18 year age groups ($p < 0.01$; $p < 0.01$; $p < 0.01$; Fig. 3d).

Functional Distribution of BrdU⁺ cells

There was a significant effect of striatal region on the percentages of total BrdU⁺ cells ($p < 0.0001$; Fig. 4a). Moreover, there was a significant interaction between age and region ($p < 0.01$; Fig. 4a). The region receiving input from the OFC had more cells compared to the other functional areas. In the 5 months/1 year age group, the striatal region receiving input from the OFC had a significantly larger percentage of BrdU⁺ cells than regions receiving projections from the vmPFC ($p < 0.001$), dorsal PFC ($p < 0.001$), and combined premotor and motor cortices ($p < 0.001$; Fig. 4a).

Interestingly, despite the overall decrease in new cell number, the 2 years age group showed an increase in the percent of BrdU⁺ cells in the region receiving input from the vmPFC. In this group the area receiving input from both the OFC and the vmPFC had a significantly higher percentage of BrdU⁺ cells than regions receiving projections from the dorsal PFC ($p < 0.01$; $p < 0.001$) and the motor control cortices ($p < 0.01$; $p < 0.01$; Fig. 4a). In the 3 years age group, the percentage of BrdU⁺ cells across regions is more evenly distributed. The relative number of BrdU⁺ cells in the region that receives vmPFC projections was greater than in the OFC projection field, but did not reach significance. However, it did reach significance compared to the striatal region receiving input from the motor control cortices ($p < 0.05$; Fig. 4a). In addition, there was a trend towards a relative increase of new cells in the dorsal caudate n. (input from the dorsal PFC) in the 3 and 12 years age groups. There was no difference between the percentage of BrdU⁺ cells associated with neurons across regions or across age groups.

Regional trends in BrdU⁺ cell density were consistent with the percent distribution of total BrdU⁺ cells across areas. There was a significant effect of age ($p = 0.05$), region ($p < 0.0001$), and interaction ($p < 0.0001$) on BrdU⁺ cell density (Fig. 4d). Moreover, in the youngest animals, the striatal regions receiving input from the vmPFC/OFC had significantly higher BrdU⁺ cell densities than regions receiving input from the dorsal PFC ($p < 0.001$; $p < 0.001$) or motor/premotor regions ($p < 0.001$; $p < 0.001$; Fig. 4d). In the 2-year age group, only the region receiving input from the vmPFC had a significantly higher BrdU⁺ cell density than in the regions receiving input from the dorsal PFC ($p < 0.05$) or motor/premotor ($p < 0.01$; Fig.

4d). In the 12years age group, there is an increase in BrdU⁺ cells across all striatal regions. Overall, the region receiving input from the vmPFC, OFC, and dorsal PFC all had a significantly higher BrdU⁺ cell density than the region receiving input from the motor/premotor ($p<0.001$; $p<0.001$; $p<0.05$; Fig. 4d). Of particular interest is that the density of the region receiving input from the dorsal PFC in the 12 yr. age group is similar to that in the 5months/1year age groups.

Summary

BrdU⁺ were found throughout the striatum at all ages under normal conditions. These cells appear to be primarily derived from resident progenitor cells rather than from the subventricular zone (SVZ). We did not detect BrdU⁺ cells that double-labeled for NeuN, suggesting that few, if any were newly generated neurons. However, a subpopulation of BrdU⁺ cells was closely associated with neurons, in particular, with calbindin-medium spiny cells and the interneuron, calretinin. The 5months/1year age group had significantly more BrdU⁺ cells than other age groups. Finally, we demonstrated a differential distribution of BrdU⁺ cells that related to the input from specific functional regions of cortex. In particular, the vmPFC and OFC had overall a greater proportion of new cells compared to other functional regions.

Discussion

Cell proliferation and Cell fate

There were BrdU⁺ cells throughout the rostral striatum in all age groups. BrdU⁺ cells in the short survival animal were more often found in 'doublets' indicating that they had recently divided. However, the overall number and distribution of BrdU⁺ cells in this animal did not differ from the animals with longer survival times. While several studies suggest that new striatal cells can migrate from the SVZ (V. Pencea et al., 2001; J. M. Van Kampen et al., 2004; A. B. Tonchev et al., 2005; A. Bedard et al., 2006), taken together, our results support the idea (F. Luzzati et al., 2006) that newly formed striatal cells are derived from resident progenitor cells rather than from multipotent stem cells migrating from the SVZ.

Since neurogenesis occurs in selected areas (olfactory bulb and hippocampus), and the fact that the striatum is adjacent to the RMS, has lead to a particular interest in potential neurogenesis in the striatum and its potential for therapeutics. Most studies reporting neurogenesis in the striatum in different species including primates used lesions or infusion of growth factors to induce neurogenesis (V. Pencea et al., 2001; J. M. Van Kampen et al., 2004; A. B. Tonchev et al., 2005; A. Bedard et al., 2006; F. Luzzati et al., 2006). Our findings indicate that, under normal conditions, neurogenesis is limited in the post-natal, Old World primate striatum. However, limited postnatal neurogenesis in the striatum does not diminish the potential importance of striatal cell proliferation during critical periods of learning. In particular, glia play a critical role in the elaboration and refinement of neural connections during development.

The majority of BrdU⁺ cells was NG2⁺. NG2⁺ cells express voltage-dependent ion channels and ionotropic neurotransmitter receptors and are now defined as a separate glial population,

which are morphologically and functionally distinct from oligodendrocytes, microglia, and astrocytes (A. Nishiyama, 2001). In the hippocampus, NG2⁺ cells receive direct synaptic inputs from both glutamatergic and GABAergic neurons (M. Paukert and D. E. Bergles, 2006) (D. E. Bergles et al., 2000) (S. C. Lin and D. E. Bergles, 2004). These synapses show activity dependent potentiation consistent with long-term potentiation (LTP). NG2⁺ cells contain Ca²⁺-permeable AMPA receptors (CaPARs) which mediate excitatory polysynaptic currents that are larger in young animals suggesting an important role during development (W. P. Ge et al., 2006). While little is known whether these types of synapses occur in the striatum, since close to 30% of the BrdU⁺ cells were closely associated with at least one neuron suggests that such a relationship may exist. Eleven percent of BrdU⁺ cells counted were CaBP⁺ medium spiny neurons and 1.3 % were calretinin⁺ interneurons. Given that we did not find any BrdU⁺ cells associated with the other three interneuron types (NOS, PV, or Ach), the remainder of identified neurons are likely to be associated with medium spiny neurons that are not CaBP⁺.

Developmental changes

The greatest number of new cells was found in the 5months/1year group. During the first years of life the volume of both the frontal cortex and striatum continue to increase at a relatively rapid rate (E. R. Sowell et al., 2004; L. Malkova et al., 2006) and myelination, transmitter systems, and connections become more mature during this period (M. DiFiglia et al., 1980; P. S. Goldman-Rakic and R. M. Brown, 1982; J.-P. Bourgeois et al., 1994; D. R. Rosenberg and D. A. Lewis, 1995; C. J. Machado and J. Bachevalier, 2003). Consistent with developmental changes and cortical and striatal synapse refinement during the first year of life, there is also a rapid development of social behaviors in Macaque monkeys. Animals are weaned, begin peer interactions, and develop appropriate social responses (C. J. Machado and J. Bachevalier, 2003). Our results show that, during this time period, there is profuse striatal, NG2⁺ cell proliferation. Approximately a third of these NG2⁺ cells are closely associated with neurons.

During the second and third year of life, there was a dramatic decrease in overall cell proliferation. This is a time that cortical development slows down considerably (L. Malkova et al., 2006). Despite this decrease, the percentage of BrdU⁺ cells associated with neurons did not significantly change, suggesting that some plasticity is maintained through adulthood. Interestingly, the fully mature adult, 12 years old animals showed a significant increase in BrdU⁺ cells compared to the juveniles, particularly, in the dorsal PFC receptive striatal region. Unfortunately, our sample did not include brains from adolescent monkeys (between ages 3.5-5). It would be particularly informative to compare post-natal development through this critical developmental period.

Functional distribution of BrdU+ cells

The greatest overall number and percentage distribution of new cells was located in the ventral striatum, the area that receives input from the OFC and the vmPFC. This region maintains a high percentage of the BrdU⁺ cells in the striatum at each age point. The ventral striatum is a key component for the development of responses to rewarding cues and the association between actions and outcomes. Over time, as these cues transfer to a stimulus-

response or habit learning, the dorsal striatum is activated (B. J. Everitt and T. W. Robbins, 2005). During the first year of life, as monkeys first explore the world, they learn the basic behavioral guiding rules that govern their interactions with the environment. The large proportion of new cells in the striatal region that receives input from the ventral prefrontal cortex is consistent with its role in reward based learning and plasticity necessary during that critical time (M. J. Thomas and R. C. Malenka, 1999; P. Calabresi et al., 2000; G. Pagnoni et al., 2002; W. Schultz et al., 2003). The relatively high distribution of NG2+ cells, particularly those associated with neurons may be a key factor in the strengthening of synapses during reward learning during behavioral development. Thus NG2+ cells may be particularly important for the refinement of neural ensembles fundamental to learning and the development of habits.

Acknowledgments

We thank April Whitbeck, for the excellent technical support and Dr. Joel Levine for providing NG2 antisera. This work was supported by NIH grant MH45573.

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Figure 1.

Schematic chartings and microphotographs of BrdU⁺ cells found to be in doublets and co-localized with NG2 in the striatum. **a, b** Distribution of BrdU⁺ cells found to be in doublets in an animal sacrificed 5 weeks (**a**) and 1 day (**b**) after BrdU injection in each of the four functionally defined striatal regions. Red=inputs from the vmPFC; orange=inputs from the OFC; yellow=inputs from the dorsal PFC; green=inputs from rostral motor control areas. Black dots indicate doublet pairs of BrdU⁺ cells. **c.** A series of microphotographs showing BrdU⁺ cells in double pairs. Scale bar, 25µm. **d.** Cell double labeled with BrdU and NG2 shown at successive z levels. Scale bar, 5.35 µm.

Figure 2.

Photomicrographs of double-labeled immunofluorescence. **a-c.** BrdU⁺ cell associated with a neuron marked with NeuN. Scale bar, 10.064 μ m. **d.** BrdU⁺ cell associated with a CaBP⁺ neuron. **e.** BrdU⁺ cell associated with a CR⁺ interneuron..

Figure 3.

Schematic chartings and graphs demonstrating the change in total number of BrdU⁺ cells across development. **a** Mean total number of BrdU⁺ cells in animals for all age groups. Error bars indicate the SEM, $n=4$ for 5 months, 1 year, 2 years, and 3 years age groups, $n=2$ for the 12 years and 18 years age groups. **b,c** Chartings of BrdU⁺ cells in the striatum of a 5 months old animal (**b**) and a 2-year-old animal (**c**). **d**. Mean total number of BrdU⁺ cells in animals of the older age groups. Error bars indicate the SEM.

Figure 4. Functional distribution of total BrdU⁺ cells and BrdU⁺ cell density for animals in each age group. **a.** Mean percentage of total BrdU⁺ cells found in each of the four functionally defined striatal regions. Red=inputs from the vmPFC; orange=inputs from the OFC; yellow=inputs from the dorsal PFC; green=inputs from rostral motor control areas. Error bars indicate the SEM. **b,c.** 3-D models of BrdU⁺ cell density for a 5 month old (**b**) and a 2 years old animal (**c**). **d.** Mean BrdU⁺ cell density in each functional region of the striatum. Error bars indicate the SEM.