CHAPTER III

Development of dopamine-containing systems in the CNS

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1. INTRODUCTION

In the late 1940s Raab et al. extracted different brain regions of several mammalian species, including man, and identified a catechol compound with a sympathomimetic biological activity (Raab 1948; Raab and Gigee 1951). Although they were unable to chemically characterize the substance which they called 'encephalin', it was clear that it differed from adrenaline and noradrenaline in several aspects. Its concentration was highest in the caudate nucleus and increased with intraperitoneal injections of L-dihydroxyphenylalanine (L-DOPA). It later became obvious that the researchers were unknowingly dealing with brain extracts containing dopamine.

In 1957, Montagu found rather high amounts of an unknown substance 'X' in whole brains of several species. Although he tentatively identified 'X' as dopamine, she furnished no proof for her suggestion. However, within a year Montagu's claim was substantiated when it was established that dopamine was normally present in the brain in approximately the same concentration as noradrenaline (Weil-Malherbe and Bone 1957; Carlsson et al. 1958). Shortly thereafter it was demonstrated that dopamine is concentrated in specific regional brain areas, with the neostriatum containing 70-80% of the brain's dopamine (Bertler and Rosengren 1959; Sano et al. 1959) and additional high concentrations being found in the substantia nigra (Bertler 1961).

Dopamine belongs to the family of biogenic monoamines which can be defined as a group of compounds formed in one or more steps from the aromatic amino acids tyrosine, phenylalanine, and tryptophan. From these, tyrosine gives rise to the catecholamines dopamine, noradrenaline, and adrenaline. Because dopamine has only small sympathomimetic properties in the periphery, it was long neglected in comparison to the closely related biogenic catecholamines adrenaline and noradrenaline. Originally its role was limited to being an intermediate (precursor) in the biosynthesis of noradrenaline and adrenaline from L-tyrosine. The high concentration and the remarkable distribution of dopamine in the brain led Bertler and Rosengren (1959) to suggest that dopamine might not serve only as a precursor of noradrenaline, but could also function as a distinct neurotransmitter.
2. VISUALIZATION OF DOPAMINE

Although this Chapter deals with the development of dopaminergic systems, we will first describe the methods used to demonstrate dopamine in adult neural tissue. The study of the distribution of dopamine and other catecholamines (CA) in the central nervous system (CNS) was aided tremendously by the discovery that these monoamines could be visualized in tissue sections under a fluorescent microscope (Falck et al. 1962). Using this method the intraneuronal monoamines are converted to strongly fluorescent molecules by condensation with formaldehyde. A major problem of the fluorescence method, however, was the inability to distinguish between the fluorescence derived from noradrenaline and that derived from dopamine in standard histochemical fluorescence preparations. Therefore, it was necessary to classify fluorescent structures as either catecholaminergic (green fluorescence) or serotonergic (yellow fluorescence). Although several fluorescence histochemical methods have been introduced for further differentiation between dopaminergic and noradrenergic systems, it remained unclear whether the two amines in a given catecholaminergic terminal field were differentially distributed. The history of the fluorescence methodology and the various procedures currently in use have been reviewed extensively by Björklund (Vol. 1, This Series, pp. 50–112).

In parallel with the fluorescence technique, another method for identifying monoaminergic neuron systems in the mature brain was developed based upon the selective reuptake mechanisms of nerve cells. High-affinity uptake of tritiated transmitters enables the selective visualization of monoaminergic fibers in autoradiographs. Specificity for a given monoamine, however, is seriously hampered since the high-affinity uptake processes are not selective. The only developmental radiographic study on monoamines also suffered from these specificity problems (Dupin et al. 1976).

Knowledge of the anatomical distribution of dopaminergic neurons and their projection fields was improved further by immunocytochemical methods using antibodies directed against transmitter-synthesizing enzymes. Tyrosine hydroxylase (TH) acts on tyrosine via ring hydroxylation to produce the immediate precursor of dopamine, L-DOPA, and as such is a reliable marker for dopaminergic neurons. Recently, it has become possible with in-situ hybridization histochemistry to visualize the expression of genes coding for enzymes that regulate the synthesis of secretory products. Thus far, this new technique has been used primarily to map the distribution of TH mRNA-containing cells in the adult brain. Developmental studies using in-situ techniques have only recently appeared (Kedzierski and Porter 1989; Burgunder and Young 1990). However, since TH is not only involved in the synthesis of dopamine, but is also used as an intermediate in the synthesis route of noradrenaline and adrenaline, marking this enzyme may visualize adrenergic, noradrenergic as well as dopaminergic systems. Thus, for a further differentiation, comparative studies using antibodies against the other catecholamine-synthesizing enzymes are necessary. Dopamine neurons should contain only TH and aromatic amino acid decarboxylase (AAAD, which converts L-DOPA to dopamine), and lack both dopamine-beta-hydroxylase (DBH, that converts dopamine to noradrenaline) and phenylethanol-amine N-methyltransferase (PNMT, that converts noradrenaline to adrenaline).

It is clear that a major limitation of all the methods mentioned above is the indirect labeling of the dopaminergic structures. Inevitably, these methods should be combined with pharmacological tools, lesion techniques or double-staining techniques to separate the dopaminergic system from other intermingling monoaminergic systems. The fact that these techniques must be performed in developing animals, makes these methods
even more difficult and precarious. The definitive answer concerning the precise anatomical localization of dopamine-containing neurons and fibers, therefore, requires antibodies raised directly against the neurotransmitter itself. In 1984 Geffard and colleagues succeeded in developing such an antibody by coupling dopamine to a protein carrier with glutaraldehyde. After perfusion with glutaraldehyde the dopamine complex could be specifically and directly visualized by both light and electron microscopy (Voorn and Buijs 1987). A number of studies have appeared describing only selective parts of the dopamine system in the adult animal with the use of such specific antibodies, viz. dopamine innervation of the lateral septum (Onteniente et al. 1984), the hypothalamus (Buijs et al. 1984), the ventral striatum (Voorn et al. 1986), the prefrontal cortex (Van Eden et al. 1987), the mesencephalic trigeminal nucleus (Copray et al. 1990), and the distribution of dopamine-containing neurons in the midbrain of the squirrel monkey (Arsenault et al. 1988).

The present Chapter summarizes the current knowledge on the development of the dopaminergic systems. The majority of the data on this subject have been obtained using mice and rats as experimental animals. Although scarce, existing data on primates (including human) will also be reviewed. Furthermore, the appearance of the dopaminergic cell groups and the pre- and postnatal development of 3 of its major projection systems (i.e. the mesostriatal, the mesocortical, and the hypothalamic systems) will be described in detail using an ontogenetic series from our laboratory. This series comprises embryonic, fetal, and postnatal ages from embryonic day (E) 11 until postnatal day (P) 90. Since dopamine antibodies were raised against glutaraldehyde-conjugated dopamine, the application of these antibodies avoided difficulties previously encountered in processing immature brains. It was now possible to combine an optimum fixation with the specific demonstration of endogenous dopamine without having to perform various pharmacological manipulations. Only 2 earlier developmental studies have appeared using antibodies to dopamine in order to describe the development of the dopamine innervation in the striatum (Voorn et al. 1988) and the prefrontal cortex (Kalsbeek et al. 1988). The following account is based and extends upon the results of these studies.

3. DEVELOPMENT OF THE MESOTELSENFALIC DOPAMINERGIC SYSTEM

As with other neurotransmitter systems, the understanding of the ontogeny of the dopaminergic system was preceded by extensive work in the adult. For a detailed and current description of the present knowledge on the organization of the central dopamine systems in the adult rat, the reader is referred to Björklund and Lindval’s chapter (Vol. 2, This Series, pp. 55–122). The major populations of dopamine-containing cell bodies are confined to the mesencephalon (A8–A10) and the hypothalamus (A11–A15). In addition, dopamine-containing neurons can be found in the olfactory bulb (A16) and the retina.

Biochemical data have frequently been used to describe the development of the dopaminergic systems. Whole brain data show a steep increase in dopamine content from E15 onwards until approximately the third postnatal week. After a plateau between P20 and P30, dopamine concentrations increase further to reach adult levels at about P60 (Agrawal et al. 1966; Breese and Traylor 1972; Loizou 1972; Coyle and Henry 1973; Keller et al. 1973; Coyle 1977; Stancheva et al. 1985). This increase is also reflected in the dopamine levels found in the cerebrospinal fluid (Shaywitz et al. 1985). However,
whole brain data may be very misleading. There is a significant variation in the developmental timetable of the different dopaminergic cell groups, and there are considerable differences between the appearance of the dopaminergic cell bodies and the completion of their projection fields. The steep increase from E15 to about P20 is primarily due to the expansion of the cell groups in the mesencephalon (Noi5in and Thomas 1988; Parés-Herbuté et al. 1989). After P30 the increasing density of the dopaminergic innervation in the striatum is responsible for a further increase in whole brain levels of dopamine (Loizou and Salt 1970; Keller et al. 1973; Nomura et al. 1976; Hedner and Lundborg 1981; Daszuta et al. 1982; Crawford et al. 1984; Giorgi et al. 1987; Broadus and Bennett 1990). Dopamine levels in the hypothalamus rise slowly and do not show rapid increases. Adult levels are reached between P80 and P100 (Leret and Freile 1985; Orosco et al. 1986; Stanley and Watts 1985).

3.1. CELL GROUPS IN THE VENTRAL MESENCEPHALON

The first indication of neurons with a dopaminergic phenotype within the CNS can be detected in the rat after 12.5 d of gestation (Specht et al. 1981a). At this age TH-positive structures can be seen in the mesencephalic flexure and the ventral prosencephalon. These structures eventually give rise to the mesencephalic and hypothalamic dopaminergic cell groups. Although they lack detailed descriptions, 2 other studies mention a similar distribution pattern of TH-positive perikarya existing by E12 (Rothman et al. 1980) or even as early as E11.5 (Foster et al. 1983b). Similar differences have been reported for the first appearance of TH-containing cell bodies in the hypothalamus (see below). Reasons for these differences remain unclear. Although the timing of developmental stage, crown-rump length and strains used are all similar, it is unclear why these 3 studies report different data on TH cells. It seems acceptable, however, that the visualization of these first putative dopaminergic neurons, with only low amounts of TH, depends critically on both the sensitivity of the antibody and the fixation procedure. Similarly, the detection of dopamine also depends upon either the sensitivity of the fluorescence technique or the employed antibody to dopamine. Using a monoamine oxidase inhibitor to accumulate dopamine in the cell body, Olson and Seiger (1972) were able to visualize dopamine fluorescence for the first time at E13. Twelve hours later (E13.5) the first cells exhibiting dopamine immunoreactivity appeared in the ventral mesencephalon (Voorn et al. 1988). Conventional histofluorescent studies usually show the first fluorescent cells in the mesencephalon at E14-E15 (Maeda and Dresse 1968; Cardilhac and Pons 1976). In other rodent species the mesencephalic dopamine-containing cell bodies show a similar developmental pattern. At E13 and E14 the first faint green fluorescence can be observed in the rostral mesencephalon of the mouse and the rabbit, respectively (Golden 1972, 1973; Tennyson et al. 1973). Using TH immunocytochemistry, Foster et al. (1988) were able to visualize the mesencephalic dopamine cell group for the first time at E11 in the mouse. Only recently, DiPorzio et al. (1990) reported the appearance of TH-positive cells in the mouse mesencephalon by day 8.5–9 of gestation.

These first dopaminergic cells are localized exclusively in the intermediate zone of the mesencephalon, a region which contains only post-mitotic neurons. Autoradiographic studies on the general neuro- and histogenesis of the ventral mesencephalon show that evolving dopaminergic cells incorporate [3H]thymidine in their nuclear DNA during E11–E15, with a peak on E13 (Hanaway et al. 1971; Lauder and Bloom 1974; Altman and Bayer 1981; Marchand and Poirier 1983). These data demonstrate that cell division
of the dopaminergic neurons in the ventral mesencephalon ceases by day 16 of gestation. This suggested the possibility that phenotypic characteristics might be acquired prior to withdrawing from the cell cycle. The absence, however, of any TH-positive structures in the ventricular zone implied that the rate-limiting enzyme of the dopamine synthesis appears only after the precursor cells have ceased dividing. Rothman et al. (1980) confirmed this suggestion by showing a lack of DNA synthesis in tyrosine-positive neurons, as well as the reverse situation, i.e. an absence of the enzyme in cells still dividing (at least in the central nervous system). The second enzyme in the synthetic pathway of catecholamines (AADC), however, is already detectable in the ventricular layer when the presumptive CA neurons are still dividing (Teitelman et al. 1983). Alternatively, migrating neurons do contain TH (Specht et al. 1981a; DiPorzio et al. 1990), as well as the transmitter (Olson and Seiger 1972; Seiger and Olson 1973; Tennyson et al. 1973; Voorn et al. 1988) (see also Fig. 1). However, this is not necessarily an intrinsic property of all dopaminergic neurons, but may be different for the various groups of dopaminergic neurons and may also depend on target-afferent interactions (Cochard et al. 1978; Friedman et al. 1988; Denis-Donini 1989). Dopamine-containing neurons of the olfactory bulb, for instance, lack TH activity until they have reached their destination in the glomerular layer (McLean and Shipley 1988).

The pioneering dopaminergic elements in the mesencephalon are still rather undifferentiated and exhibit a rounded appearance with 1 or at most 2 processes (Olson and Seiger 1972; Lauder and Bloom 1974; Specht et al. 1981a,c; Voorn et al. 1988). Although labeled processes have been shown to extend from the dopaminergic cells in the mesencephalic flexure, they are confined to the marginal zone close to their origin. Although they are formed primarily in the developing cortex and striatum, TH-positive processes can be observed temporally in the ventricular zone of virtually all regions of the brain (E12–E16). These fibers emanate, however, from structures intrinsic to the ventricular zone, rather than from neurons located in the mesencephalic flexure (Specht et al. 1978).

Initially (i.e. E12.5), the dopaminergic cell group in the mesencephalon comprises 2 separated clusters of neurons located on either side of the midline along the rostral portion of the mesencephalic flexure (Specht et al. 1981a). One day later, both TH and dopamine immunocytochemistry show 2 rows of labeled perikarya in the ventral mesencephalon (Fig. 1) (Specht et al. 1981a; Voorn et al. 1988). Olson and Seiger (1972), using the formaldehyde-induced fluorescence technique, showed 2 similar separate clusters of green fluorescent cell bodies in these early stages of development. Rostrally, the cell groups lie in a ventrolateral position, whereas caudally, at the level of the future interpeduncular nucleus, the 2 groups merge in the midline. At E14 and E15 a large compact group of dopaminergic neurons is present in the midline of the mesencephalon over the interpeduncular nucleus, corresponding to the A10 cell group. From this midline A10 group, neurons extend rostrolaterally to form the neurons of the substantia nigra or A9 group. Caudally, the compact medial cell group concentrates towards the midline, forming the A8 cell group (Olson and Seiger 1972; Tennyson et al. 1973; Specht et al. 1981a; Voorn et al. 1988). At E16 and E17 the rather loosely arranged lateral cells tend to aggregate into what probably represents the primordia of the pars compacta and the pars reticulata of the substantia nigra (Olson and Seiger 1972; Specht et al. 1981a; Voorn et al. 1988). In the last days before birth, differentiation proceeds and the outlines of the substantia nigra and ventral tegmental area (VTA) gradually become more distinct (Fig. 2).

During the first postnatal week the dopaminergic cells in the ventral mesencephalon become organized into a pattern similar to that seen in adult animals. The dissociation
between the pars compacta and pars reticulata neurons in the substantia nigra is completed around P7 (Fig. 3). It takes until P14, however, before all adult characteristics, for instance the orientation and organization of the dendrites of the pars compacta neurons, have become evident.

3.2. THE MESOSTRIATAL INNERVATION

The mesostriatal fiber tract is the most massive projection issued by the mesencephalic dopaminergic cell groups (see Björklund and Lindvall, Vol. 2, This Series, pp. 55–122, for review). Biochemical data show that in the adult rat brain the striatum has the highest specific activity for TH and the highest total content of dopamine (Coyle 1972; Versteeg et al. 1976). TH activity can already be detected in the rat telencephalon at E17 (Coyle and Axelrod 1972). At birth the enzyme activity in the rat striatum is 10–20% of its maximum value and the activity levels increase progressively during the following weeks (Coyle and Axelrod 1972; Kalaria and Prince 1988). The pattern of increase correlates well with the changes in the concentration of dopamine in the striatum of the rat and other species (Connor and Neff 1970; Loizou and Salt 1970; McGee et al. 1971; Tennyson et al. 1972; Coyle and Henry 1973; Keller et al. 1973; Nomura et al. 1976; Hedner and Lundborg 1981; Pickel et al. 1981; Daszuta et al. 1982; Crawford et al. 1984; Giorgi et al. 1987; Broaddus and Bennett 1990). The total content of dopamine in the rat striatum reaches adult levels around P60 (Keller et al. 1973).

Several histochemical studies have provided the morphological basis for the biochemical developmental characteristics. The year 1972 saw a series of publications in which the pre- and postnatal development of the catecholaminergic innervation of the striatum, as revealed by histofluorescence, was described for the first time in different species (Golden 1972; Maeda and Astic 1972; Olson and Seiger 1972; Olson et al. 1972; Tennyson et al. 1972). The findings of these first reports were confirmed and considerably ex-
Fig. 2. Transverse sections through the mesencephalic dopaminergic cell groups on E17. (a) Rostrally the 2 dopaminergic cell groups have lateral positions. The arrow indicates the projection fibers to the prosencephalon that leave the cell groups medially. CM, corpus mammillare. (b) More caudally, the lateral portion of each dopaminergic cell group consists of scattered cells (arrow). This is shown in more detail in (c). Note that the dendritic processes are oriented from dorsomedial to ventromedial (single arrow) or from ventromedial to dorsolateral (double arrow). (d) At the most caudal level, 1 dopaminergic cell group is located in the midline extending dorsally to the cerebral aqueduct (AQ). (a,b,d) × 31; (c) × 124.
Fig. 3. Perinatal development of the dopaminergic cell groups in the mesencephalon. (a) Detail of the lateral region of the substantia nigra on E20. (b,c) Transverse sections on E21 at a rostral (b) and a more caudal level (c). (d,e) Postnatal development of the ventral mesencephalon at P7 in a rostral (d) and a more caudal section (e). The single arrows and double arrows in a and d point to dendrites that are oriented from dorsomedial to ventrolateral and from ventromedial to dorsolateral, respectively. CM, corpus mammillare. (a) × 102; (b,c,d) × 31.
Fig. 4. Sagittal sections through the brain of a 14-d-old embryo near the midsagittal plane. A thick bundle of dopaminergic fibers (arrow in a) runs through the diencephalon and enters the ganglionic eminence (GE) (b). (a) × 15.5; (b) × 124.

Abbreviations: LV, lateral ventricle; AQ, cerebral aqueduct; IV, fourth ventricle.

Expanded upon in later years. The application of immunocytochemistry for TH was crucial for the continuation of this line of research (Specht et al. 1981a,b; Graybiel 1984a; Moon-Edley and Herkenham 1984; Murrin and Ferrer 1984; Van Der Kooy 1984; Martin et al. 1989). It is only recently that antibodies against the transmitter itself have been employed to study in detail the ontogeny of the dopaminergic innervation of the rat striatum (Voorn et al. 1988).
The first dopaminergic fibers in the striatal anlage are seen on E14 in the form of the forefront of a massive bundle of dopaminergic fibers traversing the diencephalon and then entering the telencephalon from a ventrolateral position (Fig. 4b). With catecholamine-induced histofluorescence the first striatal fibers were recognized on E14 (Olson and Seiger 1972), whereas the first TH-immunopositive fibers were seen half a day earlier, on E13.5 (Specht et al. 1981a). In the mouse the first fluorescent catecholaminergic fibers were detected on E14 (Golden 1972), in the rabbit on E19 (Tennyson et al. 1972), and in the guinea pig on E38–E39 (Maeda and Astic 1972). On E14 varicosities cannot be distinguished in the dopaminergic fibers. Over the next 2 d, E15–E16, dopaminergic fibers become more numerous and cover a larger area (Fig. 5). They also distribute to the ventralmost region of the striatum where the nucleus accumbens is developing. The majority of the fibers remains confined to the zone of cellular differentiation, but some fibers do penetrate the subventricular zone of neurogenesis. At the point of entry into the striatum, the fibers are aggregated in thick bundles. On E17, thick bundles of fibers are present in the ganglionic eminence and from there a number of fibers proceed toward the cortex (Fig. 6). The dopaminergic fibers can be seen to invade the dorsolateral stria-
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Fig. 6. Sagittal section through the prosencephalon on E17. Bundles of dopaminergic fibers spread to the dorsal striatum and the ventral striatum (arrow). × 31.

Abbreviations: GE, ganglionic eminence; LV, lateral ventricle; TH, thalamus.

tum from a ventrolateral position. Ventromedially, they grow around the tip of the lateral ventricle into the nucleus accumbens and make their way to the septum and the medial prefrontal cortex. On E18, thick twisted fibers run along the ventricular zone and enter the ventral striatum. More caudally, an intricate fiber network is beginning to develop, exhibiting a clear pattern of fiber distribution that consists of dense networks along the lateral margin of the striatum and in a more medially located region, with a relatively fiber-free zone in between (Fig. 7). No varicosities can be recognized in the fibers (Fig. 8). On E19, it is clear that the dopaminergic fibers remain within the region where fiber bundles of the internal capsule are present. There is a clear anatomical relationship between the 2 types of fibers in that the dopaminergic fibers appear to climb into the striatum along the internal capsule fibers (Fig. 10). In rostral areas of the striatum, the dopaminergic fibers are located in a lateral striatal region (Fig. 9a), whereas more caudally the fibers occupy both a lateral and a ventromedial zone (Fig. 9b). At this stage dopaminergic fibers are also detected in the olfactory tubercle and in the nucleus accumbens (Fig. 9a). In all areas the fibers retain a smooth appearance. Caudally in the caudate putamen, several loci can be distinguished with extensive fiber ramifications (Fig. 11). Figure 12 presents a clear view of the fiber ramifications along the caudo-rostrally oriented dopaminergic fibers on E21. These loci represent the first indications of the patches of dopaminergic fibers that can be found at later stages. During the next 2 d, E20–E21, the highest density of fibers is found dorsolaterally in the striatum. In the nucleus accumbens the outgrowth of dopaminergic fibers follows closely that of the developing nucleus (Fig. 13) (Bayer 1981). Gradually, fibers approach the lateral ventricle where bundles of the internal capsule appear. Loci with extensive dopaminergic fiber ramifications, that were first recognized caudally and dorsally in the striatum on E19, have further differentiated and on E21 they are also visible in rostral regions. These loci
now appear as patches with high densities of dopaminergic fibers. On E20 these patches are primarily visible along the concave contour of the external capsule, medial to the relatively fiber-free zone that became apparent on E18 (Fig. 13). Especially caudally in the striatum, it is clear that the patches first develop in the aforementioned area. No patches can be found in the relatively fiber-free zone. On E21, a patch-like distribution of dopaminergic fibers is also present in ventral-striatal areas such as the olfactory tubercle and medially and ventrally to the anterior commissure.

To summarize the prenatal pattern of dopaminergic fiber ingrowth, these fibers enter the caudal portion of the striatum from the ventromedially located mesencephalo-prosencephalic dopaminergic fiber bundle. Then outgrowth takes place in dorsal, lateral, and medial directions. Rostrally, high fiber densities develop along the ventrolateral border of the striatum. Comparison of these spatiotemporal patterns with those of the neurogenesis and cytodifferentiation in rodent striatum shows remarkable similarities (Ten Donkelaar and Dederen 1979; Bayer 1981, 1984; Marchand and Lajoie 1986). Caudally, a clear ventromedial to dorsolateral cytogenetic gradient is seen, whereas in rostral regions the earliest generated cells settle along the ventrolateral striatal border and younger cells take up positions closer to the lateral ventricle. The development of the dopaminergic innervation in the dorsal striatum evidently follows these cytogenetic
gradients closely, both rostrally as well as caudally. The same holds true for the nucleus accumbens, in which neurogenesis follows a ventrolateral to dorsomedial course that proceeds underneath the ventral tip of the lateral ventricle (Bayer 1981). In both the dorsal and the ventral striatum, the dopaminergic fibers arrive at a time point when the first cells are generated in the respective regions. Neurogenesis occurs from E12 to P2 in the caudate putamen and from E13 to P6 in the nucleus accumbens, with peaks on E15 and E17, respectively (Bayer 1981, 1984; Marchand and Lajoie 1986). It may be concluded that the dopaminergic fibers arrive in the striatal subdivisions before the peaks in the neurogenesis occur and that the fiber ingrowth evolves along the same spatiotemporal gradients as those for neurogenesis and cytodifferentiation.

In the first week after birth, the area bordering the lateral ventricle becomes more and more occupied by dopaminergic fibers. Concomitantly, the patches in this area become more conspicuous. On P2, patches can also be discerned in the most rostral striatal regions, both ventrally and dorsally, so that as from this stage on the compartmental distribution of dopaminergic fibers in both dorsal and ventral striatum can be clearly appreciated (Fig. 14). The patches generally coincide with cell-sparse regions (Fig. 15), as observed previously by Van der Kooy (1984). However, in the dorsomedial part of the nucleus accumbens, the high-fiber densities match cell-dense regions rather than cell-sparse regions (Fig. 15).

The morphology of the dopaminergic fibers changes over the first postnatal week. Gradually, thin fibers start to make up the majority of the fiber contingent and varicosities start to develop. On P4, they can be clearly distinguished (Fig. 16a). Their number increases rapidly over the following weeks (Fig. 16b); however, at 3 weeks of age the
Fig. 9. Transverse sections at a rostral (a) and a caudal (b) level through the striatum on E19. The highest densities of dopaminergic fibers (white arrows) are present at different locations in the 2 levels. Note fiber ingrowth into the ventral striatum (arrowhead in a). H, hippocampus. × 31.

The highly varicose appearance of the dopaminergic fibers in the adult animal has not yet been attained (compare Figs 16b and 16c).

It is unlikely that the actual dopaminergic neurotransmission depends on the presence of varicosities. Release and uptake of dopamine are already detected on E17–E18 and there is a marked increase in both processes in the postnatal stage, with the sharpest increase occurring between E18 and P1 (Nomura et al. 1981; Yotsumoto and Nomura 1981). This occurs prior to the appearance of varicosities. In addition, the development of varicosities does not appear to be paralleled by an increase in the number of dopamine uptake sites (Broaddus and Bennett 1990).

In the second postnatal week a major developmental change occurs in the distributional pattern of the dopaminergic fibers. The patches of dopaminergic fibers disappear from medial striatal regions and the fiber distribution becomes rather diffuse (Fig. 17). In the third week the distribution obtains near-adult characteristics. Dopaminergic fibers are now detected throughout the entire extent of the striatum, including the most rostral regions bordering the lateral ventricle. At this stage of development, the most conspicuous patches lie along the lateral perimeter of the striatum (Fig. 18a). In the adult animal only these particular patches remain detectable (Fig. 18b). Contrary to the caudate putamen, where the patches become blurred, in the ventral striatum the patches become clearly outlined and remain this way until the adult stage (Voorn et al. 1986, 1988, 1989).

The development of the patchy distribution of dopaminergic fibers is generally in
Fig. 10. Transverse section through the striatum on E19. Dopaminergic fibers are associated with non-dopaminergic fiber fascicles of the internal capsule. IC, fiber bundle of internal capsule. × 497.

Fig. 11. Transverse (a) and sagittal (b and c) sections through the striatum. On E19 loci with strongly ramifying dopaminergic fibers are situated caudolaterally in the striatum (arrows in a). In (b) and (c) such loci are shown in more detail. (a) × 155; (b,c) × 497.
agreement with the descriptions given in previous studies employing catecholamine-induced fluorescence or TH immunocytochemistry (Olson and Seiger 1972; Olson et al. 1972; Tennyson et al. 1972; Golden 1973; Graybiel 1984a; Murrin and Ferrer 1984; Van der Kooy 1984) (in the opossum heterogeneities in the striatal dopaminergic fiber distribution were less extensive than observed in other species, Martin et al. 1989). The patches in the dopaminergic fiber distribution that can be distinguished in the developing animal, and to some extent also in the adult, have been shown to correspond to distributional heterogeneities of other markers (Graybiel 1984a; Moon-Edley and Herkenham 1984; Murrin and Ferrer 1984; Van der Kooy 1984; Newman-Gage and Graybiel 1988). In the adult animal these heterogeneities outline 2 separate striatal compartments: the 'striosome or patch' compartment and the 'matrix' compartment (see Graybiel 1984b; Gerfen 1987; Gerfen et al. 1987). These 2 compartments receive efferents from specific subdivisions of the mesencephalic dopaminergic cell groups. The striatal compartmentation appears to have a cytoarchitectonic basis. According to Fishell and Van der Kooy (1986) and Marchand and Lajoie (1986), cells belonging to the 'patch' compartment are generated until E18–E19 and subsequent isochronously generated 'patch' cells are arranged in clusters through isolation by late-generated 'matrix' neurons. Thus, before E19 the striatal anlage consists primarily of 'patch' cells. The study of Voorn et al. (1988) shows that from E19 on the dopaminergic fibers establish extensive ramifications in the patch compartment while at the same time invading the developing matrix compartment. This means that both compartments receive dopaminergic fibers from the moment they are generated. It is not clear which processes are involved in the 'disappearance' of the dopaminergic fiber patches in the postnatal stages. There is some evidence indicative of a 'second wave' of dopaminergic fiber ingrowth, predominantly to the matrix compartment (Tennyson et al. 1972; Gerfen et al. 1987).

A peculiar finding in the second and third postnatal weeks is the presence of numerous

Fig. 12. On E21 high densities of fibers in the striatum appear in the sagittal plane as extensive ramifications in the course of the fiber bundle (rostral is left side of figure). × 78.
Fig. 13. Transverse section through the telencephalon on E21. Dorsal as well as ventral (single arrow) striatal regions are occupied by dopaminergic fibers. High densities of fibers are present along the external capsule (arrowhead) and in patches in the caudate putamen (CP) and nucleus accumbens. Patches are absent from a zone just medial to the external capsule (double arrow). × 31.

dopamine-immunopositive cell bodies lying along the rostral margins of both the nucleus accumbens and the caudate putamen (Fig. 19). A small number of cells can also be seen in the adult rat (Voorn et al. 1986). In the primate a population of TH-immunopositive cell bodies has been demonstrated, located in the white matter adjacent to the nucleus accumbens and the putamen and in the outer rim of the neostriatum throughout its anterioposterior extent (Dubach et al. 1987). The cell populations in rat and primate may be homologous. However, since in adult rats the number of cells was rather low compared to that in the developing animals, this area may be similar to other brain areas in that some of the cells transiently express TH (see Section 6).

There are some indications for the presence of sex differences in the dopaminergic innervation of the striatum. Fewer striatal D2-receptor binding sites are found in females compared to males, and different sex-related responses to neuroleptics have been observed (Miller 1983). Furthermore, Becker (1990) claims that steroid hormones modulate the striatal dopamine release in females but not in males. Crowley and coworkers (1978) have demonstrated a higher concentration of dopamine in the striatum of male...
rats than in that of females. It is not clear whether this difference can be attributed to a higher content of dopamine per fiber or whether there is a higher density of dopaminergic fibers. Thus far, such a sexual dimorphism has not been reported in histochemical studies. Furthermore, this difference does not appear to come about by a developmental influence of sex steroids, since neither neonatal castration nor ovariectomy disrupts the differences between the sexes. A possible developmental influence has been suggested in studies in which a clear effect of steroid hormones was demonstrated on the outgrowth of cultured E14 mesencephalic TH-immunopositive neurons (Reisert et al. 1987; Engele et al. 1989).

3.3. THE MESOCORTICAL INNERVATION

Initiated by the earlier work of Olson and Seiger (Olson and Seiger 1972; Seiger and Olson 1973), the ontogeny of the catecholaminergic innervation in the rat cerebral cor-
Dopamine-containing systems

Fig. 15. Dopamine-immunostained (a) and Nissl-stained (b) transverse sections through the striatum on P4. High densities of dopaminergic fibers in both the caudate putamen (arrowheads in a) and the nucleus accumbens (arrows in a) coincide with areas characterized by a low staining intensity in the Nissl-stained section (corresponding arrowheads and arrows in b), indicating low cell densities. The dopaminergic fiber patch dorsomedially in the nucleus accumbens corresponds to an area of high cell density (double arrows in a and b). × 24.

text has been described extensively (see reviews by Coyle 1977; Berger and Verney 1984; Foote and Morrison 1987). Specific reports on the development of the dopaminergic cortical innervation are scarce (Verney et al. 1982; Kalsbeek et al. 1988), however, or only start postnatally (Lorén et al. 1976; Schmidt et al. 1982; Berger et al. 1985a,b; Verney et al. 1985, 1987). While the lack of specificity of certain methods used in describing dopamine cell body development can be overcome by the knowledge of neural localization in the adult brain and by lesion techniques, specificity is of the utmost importance for the description of outgrowing fiber systems. This is especially true for areas where dopamine-containing fibers are heavily intermingled with noradrenergic projections, such as the cerebral cortex and the hypothalamus. In fact, the low levels of dopamine present in many noradrenaline-rich regions was often taken to indicate that the dopamine found was serving solely as a precursor for noradrenaline. Using TH-like immunoactivity as a 'selective' marker (viz. anti-TH immunocytochemistry was claimed to reveal preferentially dopaminergic terminals), Verney et al. (1982) provided the first detailed description of dopamine cortical innervation from E15 onwards. Later, other studies using TH confirmed these results without focusing specifically on cortical dopamine innervation (Foster et al. 1987). Only recently has the development of dopamine innervation in the cortex been described using the glutaraldehyde-conjugated dopamine antibody (Kalsbeek et al. 1988).
Fig. 16. Transverse sections through the ventrolateral part of the caudate putamen illustrating the development of varicosities in the dopaminergic fibers. (a) P4, (b) P20, (c) adult. Arrows point to varicosities. × 497.

Figures 20–24 show the entrance of the dopaminergic fibers in the developing cortex from E14 to E21, as observed with our specific antibodies to dopamine. On E14 the first dopaminergic-containing fibers appear in the telencephalon, but do not reach beyond the ganglionic eminence (Fig. 4). One day later (E15) the number of fibers entering the developing striatum has clearly increased, and by now a number of fibers can be followed into the intermediate zone of the cortical anlage (Fig. 20). The dopaminergic-containing fibers seem to fan out in front of the striatal anlage to the lateral and frontal cortices, but no further than its most ventral part. On E16 the first fibers can be detected in the subplate region beneath the thin rim of cortical plate cells in the lateral wall of the hemisphere (Fig. 5), entering it in a ventral-to-dorsal direction. In the medial wall of the cerebral hemisphere, no dopaminergic-containing fibers can yet be detected. In the following days (E17–E18) the number of dopamine-positive fibers passing the striatal cluster increases considerably and a number of fibers can be followed into the olfactory bulb. The majority of these labeled fibers, however, can be observed to curve upwards and invade the subplate of the frontal cortex at its most ventral part, and then turn back-
Fig. 17. Transverse section through the striatum on P13. Dopaminergic fiber patches are still present along the medial, dorsal and dorsolateral margins of the striatum, but can no longer be discerned in its central part. Note that magnification is lower than in previous overviews. × 15.5.

Fig. 18. Transverse sections through the striatum on P20 (a) and in adult age (b). Well-developed dopaminergic fiber patches are present in the nucleus accumbens (double arrows), whereas in the caudate putamen patches can be identified only dorsally and dorsolaterally (single arrows). × 15.5.

Fig. 19. Dopaminergic neurons along the ventrolateral border of the nucleus accumbens (ACC) on P11 (a) and in the caudate putamen on P20 (b). × 310.
Fig. 20. Sagittal section through the brain of a 15-d-old embryo taken near the midsagittal plane (a). (b,c) Detail of the massive bundle of dopaminergic fibers traversing the diencephalon (c) and entering the ganglionic eminence (GE) (b). (d) A single fiber can be observed reaching the frontal pole and entering the subplate beneath the cortical plate (asterisk in a). (a) × 15; (b,c) × 35; (d) × 100.

Abbreviations: LV, lateral ventricle; AQ, cerebral aqueduct; MF, mesencephallic flexure; TH, thalamus; SN/VTA, substantia nigra/ventral tegmental area.

Towards to enter more dorsal and posterior cortical regions (Fig. 21). Occasionally a positive fiber can be detected even in the most caudal cortical regions. Although most of the immunoreactive fibers remain confined to the subplate region beneath the cortical plate, sometimes a fiber traverses the cell-dense cortical plate to enter the marginal zone (see inset Fig. 21). In coronal sections, a more medial fiber bundle can be observed containing dopaminergic fibers heading for the septal pole and the subplate in the medial wall of the hemisphere (Fig. 22). The medial wall is thus innervated via 2 routes: a medially situated bundle innervating its ventral part and a more lateral ‘striatal’ bundle arching around the forceps minor to innervate the dorsal part. At this stage (i.e. E17–E18), however, dopamine-containing fibers have only reached dorsal portions of the cortex in the most rostral sections. In more caudal sections the dorsal half of the cerebral hemispheres is still devoid of dopamine-positive fibers. Along the ventrolateral border of the striatum, an occasional fiber can be observed in the perirhinal and entorhinal cortices. These fibers are derived from a lateral branch of the medial forebrain bundle which
Fig. 21. Sagittal section at E18 showing dopaminergic fibers passing the striatum (STR) and entering the subplate of the frontal cortex. At a higher magnification, the inset shows a positive fiber leaving the subplate (SP), transversing the cortical plate (CP) and entering the marginal zone (MZ). × 100; detail × 360.

Abbreviations: LV, lateral ventricle; VZ, ventricular zone; IZ, intermediate zone; GE, ganglionic eminence.
Fig. 22. Transverse section at E18 showing the dopaminergic fiber bundle situated ventromedial to the striatal bundle (a). (b) Twisted fibers head for the medial part of the frontal cortex and the septum (arrow). (a) × 12; (b) × 78.

Abbreviations: LV, lateral ventricle; VZ, ventricular zone of cell division.
Fig. 23. Sagittal sections from E16, E18 and E20 showing dopaminergic fibers growing through the developing striatum (STR) heading for the frontal cortex. At E20 the external capsule forms a clear frontal border. CP, cortical plate. × 100.
Fig. 24: Transverse sections at E20 (a) and E21 (b) showing the progressive invasion of more caudal cortical regions by the dopaminergic fibers (arrows). Furthermore, it can be seen that at E21 dopaminergic fibers are no longer confined to the subplate but enter the basal layers of the cortex. $\times$ 95.

**Abbreviations:** CP, cortical plate; CPu, upper part of the cortical plate; GE, ganglionic eminence; IZ, intermediate zone; LV, lateral ventricle; STR, striatum; V/VI, cortical layers.
Fig. 25. Development of dopaminergic innervation in the medial part of the prefrontal cortex at E18 (a,b), E20 (c,d), P4 (e,f), and P10 (g,h). Dopaminergic fibers can be seen entering the first formed cortical layers (i.e. Layers V and VI) just before birth and continuing through to the last formed layers (i.e. Layers II and III) around P10. Arrowheads indicate borderlines of the different cortical layers. (a,b) × 280; (c,d) × 200; (e–h) × 100.

Abbreviations: CP, cortical plate; CPu, upper part of the cortical plate; IZ, intermediate zone; LV, lateral ventricle; MZ, marginal zone; SP, subplate layer; STR, striatum; VZ, ventricular zone; I, V, VI, cortical layers.
splits off at the beginning of the striatum. On E18 the dopamine innervation of the amygdaloid complex also starts to appear. Fibers are first recognizable in what appears to be the presumptive central nucleus of the amygdala. As shown by \(^{3}H\)thymidine experiments, this nucleus is also the first part of the amygdala to be generated (Bayer 1980).

On E19 and E20 the main changes are the clear fronto border of the striatum formed by the external capsule (Fig. 23), an increasing number of fibers in the subplate region of the fronto cortex, and the start of dopamine innervation in the medial field of the lateral septum (Fig. 27a). Dopamine-containing fibers from both the medial and 'striatal' bundle have reached the dorsal 'shoulder' region of the fronto cortex where they seem to intermingle. After arching around the forceps minor, dopaminergic fibers can be followed all along the supragenual cortex up to the retrosplenial cortex (Fig. 24). At the same time fibers can frequently be observed entering the perirhinal and entorhinal cortices as well as the subplate beneath the visual and auditory cortex. A few positive fibers can also be detected consistently in the subicular region. As indicated by the direction of the growth cones, these fibers reach the hippocampus via the septal pole. Similar observations concerning dopamine innervation of the hippocampus were previously re-
Fig. 27. Perinatal development of dopaminergic innervation in the septum at E20 (a), P5 (b), P10 (c) and P20 (d). At E20 dopamine-immunoreactive fibers invade the medial field. At P5 dopaminergic innervation of the medial field has extended and pericellular baskets (arrows) can be observed; the lateral field is hardly innervated at this time. At P10 dopaminergic innervation has also reached the lateral part of the lateral septum, and at P20 the full-grown innervation pattern has been reached. × 60.

Abbreviations: CC, corpus callosum; LV, lateral ventricle; MS, medial septal nucleus.
Fig. 28. Postnatal development of dopaminergic innervation in the amygdaloid complex. At P5 (a) the central nucleus (C) already contains a clear plexus of dopamine fibers, whereas the basolateral complex (BL, demarcated by arrowheads) is virtually empty. (b) Four days later (P9) the basal nucleus (B) contains some dopamine fibers, and in the neighboring piriform cortex (PC) dopamine-immunoreactive fibers can also be discerned. At P14 (c) the adult-like pattern has been reached with a moderate dopamine innervation in the basal nucleus and almost no fibers in the lateral nucleus (L). The central nucleus and the intercalated nuclei (arrows), however, show a dense pattern of dopamine fibers. PX, caudoventral portion of the putamen. × 52.
ported by Verney et al. (1985). The most striking change upon the last day before birth (E21) is that dopaminergic fibers no longer stay confined to the subplate, but start to innervate the lower regions of the cortical plate (Fig. 24b), presumed to represent the developing Layers VI and V (Uylings et al. 1990). The projection area, on the other hand, seems to have narrowed down to the medial regions of the frontal cortex. At the same time the morphology of the immunopositive fibers changes drastically; they become thinner and varicosities start to appear (Kalsbeek et al. 1988). Just before birth, therefore, all the ‘appropriate’ cortical areas (as derived from the adult innervation pattern) have been reached by dopamine fibers, and it seems that by now the first functional contacts have also been formed.

The postnatal development of dopamine innervation in the different cortical areas is displayed in Figures 25–28. In all areas innervation follows the developmental sequence of the various cortical layers (Uylings et al. 1990); basal layers are innervated first (E21) and the more superficial last (about P10–P12) (Fig. 25). Dopamine innervation of the prefrontal cortex is already well developed at P10, especially its medial part. The only difference with the adult situation is the lower density of the innervation. Dopamine innervation in the olfactory and entorhinal cortex lags a few days behind the frontal innervation scheme. The development of dopamine innervation in the supragenual cortex, especially in its superficial layers, however, is delayed by many days and only reaches its adult appearance after P20 (Fig. 26). After birth dopamine fibers also start to innervate the lateral part of the lateral septum. The characteristic pericellular baskets are not observed before P4 (Fig. 27), confirming the data of Verney et al. (1987). The development of dopamine innervation in the amygdaloid complex is shown in Figure 28. At P5 the central nucleus already contains a dense complex of dopamine fibers, whereas the basolateral complex is clearly outlined by its complete lack of dopamine-containing fibers. At P9 the first dopamine-positive fibers appear in the posterior division of the basolateral complex. At P19 adult characteristics are reached, with dopamine fibers concentrated in the central nucleus, the basolateral complex and intercalate masses. The cortical and lateral nuclei are almost devoid of dopamine fibers at this time.

4. DEVELOPMENT OF HYPOTHALAMIC-DOPAMINERGIC SYSTEMS

Relatively many studies devoted to dopaminergic development have concentrated on hypothalamic cell groups (Björklund et al. 1968; Hyypä 1969; Smith and Simpson 1970; Loizou 1971; Specht et al. 1981a,b; Daikoku et al. 1986; Ugrumov et al. 1989a,b; Reisert et al. 1990). Immunocytochemical studies in adult animals have shown that the majority of CA cell bodies in the hypothalamus, as recognized with TH immunocytochemistry or by histofluorescent methods, are in fact dopaminergic (Chan-Palay et al. 1984; Hökfelt et al., Vol. 2, This Series, pp. 277–379). Using TH immunocytochemistry, the first dopaminergic neurons in the lateral wall of the hypothalamic anlage have been reported to appear at either E12.5 (Specht et al. 1981a), E13 (Ugrumov et al. 1989a), or E13.5 (Daikoku et al. 1986). These scattered cells extend from just beyond the optic chiasm to a position ventral to the caudal striatum. In the following days (E13–E15) the caudally located neurons form 2 populations, one in the basal region of the hypothalamus lateral to the median eminence and the other more dorsally at the junction of the hypothalamus and the thalamus just lateral to the third ventricle. In the hypothalamus migration and aggregation continues after E14, so that at E18 the major dopaminergic cell groups as described by Hökfelt et al. (Vol. 2, This Series, pp. 277–379) can be
recognized. Using fluorescent-histochemical preparations the first dopamine-containing cell bodies in the hypothalamus are observed only at E18 (Seiger and Olson 1973), E20 (Hyvönen 1969), or even later (Loizou 1971). In the mouse, dopamine-containing cell bodies in the hypothalamus also appear only in the last few days before birth (Björklund et al. 1968).

The development of the hypothalamic dopamine systems as observed in our material closely resembles the developmental pattern described by Ugrumov et al. (1989a,b) on the basis of TH immunocytochemistry: the only difference being a time lag of 3–4 d between the first appearance of TH- and dopamine-positive cell bodies. A few very faintly stained neurons can be observed at E15 in the caudal telencephalon/rostral hypothalamus region, adjacent to the third ventricle. On E17–E18 the number of hypothalamic dopamine-positive cells has increased considerably, although they are still only weakly stained. The most significant accumulations are observed in the periventricular region running into a ventral zone lateral to the median eminence (corresponding to the A14 and A12 group, respectively) (Fig. 29). A more darkly stained cluster of cells can be
observed somewhat more caudally in the dorsal hypothalamic regions of the zona incerta and dorsomedial and posterior nuclei (from rostral to caudal these cells correspond to the A13 and A11 groups). The main change observed on E19–E20 is a further separation between the periventricular cells and those belonging to the arcuate A12 group (Fig. 30). Compared to previous stages, no evident changes in the number or distribution of dopamine cells are observed on E21.

The hypothalamic population of dopamine-producing neurons thus shows a remarkable time lag between the appearance of TH and the transmitter itself. This is in marked contrast with the situation in the mesencephalon, where dopamine can be detected shortly after the first appearance of its rate-limiting enzyme. This could mean that the presence of the enzyme in detectable quantities does not necessarily indicate that it is actually active in the synthesis of dopamine. However, biochemical data show that TH-catalytic activity can already be detected at E13, although at low levels (Friedman et al. 1988, 1989). At E15 the specific activity of the enzyme in whole brain is still only 5–10% that of the adult brain, and from there on it shows a linear increase during the last week of development (Breese and Traylor 1972; Coyle and Axelrod 1972). On the
other hand, it may be that the synthesized amount of dopamine in the hypothalamic neurons is too low to reach the biochemical or immunocytochemical detection limit. Biochemical experiments have indeed demonstrated that there are considerable metabolic differences between hypothalamic and mesencephalic neurons in adulthood, with lower values for TH activity in the hypothalamus (Costa et al. 1974; Bacopoulos and Bhatnagar 1977; Demarest and Moore 1979; Moore and Wuerthele 1979). It was recently shown that the CA cells of the hypothalamus show remarkably low levels of TH mRNA compared with the substantia nigra and superior cervical ganglion cells (Kedzierski and Porter 1989). Dopaminergic cells have also been shown to be sensitive to gonadal steroids (Crowley et al. 1978; Wilson and Agrawal 1979; Leret and Fraile 1985; Stanley and Watts 1985; Reisert et al. 1987). Because TH is the rate-limiting enzyme for dopamine biosynthesis, it is likely that gonadal steroids can regulate intracellular levels of dopamine by altering TH expression (Simerly 1989).

With regard to the development of dopamine innervation in the hypothalamus, it is difficult to compare our data with studies using TH immunocytochemistry (Ugrumov et al. 1989b) or histofluorescence studies (Hyypä 1969; Khachaturian and Sladek 1980), since there is a dense noradrenergic, and to a lesser degree adrenergic, innervation in the hypothalamus (see references in Chan-Palay et al. 1984) and these 3 types of innervation are extremely difficult to separate using those techniques. Biochemical studies, however, show that dopamine can be detected in all hypothalamic areas (Palkovits 1981). Due to the lack of specific antibodies to noradrenaline and dopamine, only a few anatomical studies have been able to make a distinction between the dopamine and noradrenaline compartments of catecholamine innervation within the hypothalamus. With regard to the supraoptic and paraventricular nuclei, it has been shown that, besides a considerable noradrenaline innervation, these nuclei also receive a dopamine input (Buijs et al. 1984; Lindvall et al. 1984; Decavel et al. 1987). In contrast, the suprachiasmatic nucleus appeared to receive little or no dopamine innervation.

Dopamine innervation of the paraventricular and dorsomedial nuclei and the periventricular area develops particularly in the first postnatal days. Only recently, were Reisert et al. (1990) able to detect TH-immunoreactive fiber bundles entering the hypothalamus as early as E14 in whole-mount preparations. However, all 3 mesencephalo-hypothalamic projections had largely disappeared by E20 and were no longer detectable as distinct fiber bundles thereafter. In our preparations the first dopaminergic fibers in the hypothalamus were detected just before birth (E21), while even as late as P4 they are still scarce without specific preference for any of the hypothalamic nuclei. Only in the mammillothalamic tract is there a 'striking' accumulation of fibers, probably belonging to the incertohypothalamic system. On the other hand, the medial part of the lateral habenula and the paraventricular nucleus of the thalamus already show an adult-like innervation pattern at this age. Only at P9 can an accumulation of fibers be observed in the medial part of the hypothalamus, especially in the paraventricular nucleus (Fig. 31c). The dorsomedial nucleus, however, still shows hardly any fibers (Fig. 31a). At P14 both the paraventricular and dorsomedial nucleus are clearly outlined by the presence of dopaminergic fibers in these nuclei (Fig. 31b). Although the characteristics of the adult innervation pattern can be recognized 2 weeks after gestation, adult densities of this innervation still have not been reached at 3 weeks of age. This delayed development of the dopaminergic innervation in the hypothalamus is in line with the biochemical data (see introduction to this paragraph) as well as with the scarce data from histofluorescence- or TH immunocytochemistry-employing studies (Khachaturian and Sladek 1980; Foster et al. 1985b, 1987).
Fig. 31. Transverse sections showing the dopaminergic innervation of the hypothalamic nuclei at P9 (a,c) and P14 (b). Above (dorsal) the dorsomedial nucleus (a,b; DMH), the caudal extension of the A13 cell group can be observed along the ventromedial aspects of the fasciculus mammillothalamicus (fm). Large multipolar cells of the A11 group can be observed dorsomedially of the small A13 cells. A number of dopamine-immunoreactive perikarya belonging to the A14 group are visible ventral to the paraventricular nucleus (c; PVN). 3V, third ventricle. (a,b) × 52; (c) × 140.
5. DEVELOPMENT OF DOPAMINE-CONTAINING STRUCTURES IN THE BRAINSTEM

Dopamine-containing neurons have also been detected caudal to the mesencephalon, where CA cell groups are usually considered to synthesize only noradrenaline or adrenaline. As shown by Buijs et al. (1984), however, this dopamine staining is not due to cross-reactivity with the other CAs, but must represent dopamine as a precursor or as a distinct transmitter. Although histofluorescent studies have never found any evidence for the existence of dopamine-containing cell bodies in the brainstem, biochemical data show concentrations of dopamine larger than expected if it were only a precursor for noradrenaline (Van der Gugten et al. 1976; Versteeg et al. 1976). Immunocytochemical studies have strengthened the idea of dopamine-synthesizing neurons within the brainstem, especially in the dorsomedial medulla (Armstrong et al. 1982; Kalia et al. 1985). In the hamster, too, there is a large discrepancy between the number and localization of TH- and DBH-immunoreactive cells in the A2 area, suggesting the existence of dopamine-containing neurons in this structure (Davis and Jang 1988; Vincent 1988). However, since at least a part of these TH-positive and DBH-negative perikarya also seem to lack AADC, the enzyme that converts L-DOPA to dopamine (Jaeger et al. 1984; Vincent and Hope 1990), the existence of dopamine-synthesizing neurons was still not proven. Only recently have Manier et al. (1990) provided evidence for the existence of dopamine-producing cell bodies in the caudal part of the dorsal motor nucleus of the vagus nerve in the rat.

The first dopamine-positive cell bodies were detected at E16 in the ventral part of the pontine flexure near the IV ventricle. This corresponds with the position of the noradrenaline-containing neurons of the locus ceruleus. At E18 dopamine-containing cell bodies are observed in the presumptive region of the A1–A3 cell groups. The staining intensity of these brainstem groups, however, is considerably lower than in the mesencephalic dopamine groups (Fig. 32), as was also noticed with TH immunocytochemistry (Reisert et al. 1990). The appearance of the A1–A7 cell groups with dopamine immunocytochemistry is also considerably later than observed with other methods; using TH immunocytochemistry, these cells can be observed from E12.5 onwards. In histofluorescent studies, brainstem CA-containing neurons are usually recognized 1–2 d later (Maeda and Dresse 1968; Olson and Seiger 1972). Also, the other enzymes involved in the synthesis of noradrenaline and adrenaline, i.e., DBH and PNMT, are already present at E13 and E14 (Foster et al. 1985b; Bohn et al. 1986). Thus, there seems to be a slow accumulation of dopamine in these neurons from the start of noradrenaline or adrenaline synthesis. The dopamine-containing perikarya of the A1 and A2 cell groups show a clear difference in their staining intensity (Fig. 33). Whereas the lighter stained cell bodies may represent noradrenaline-synthesizing neurons containing dopamine as an intermediate product, the darkly stained perikarya may represent dopamine-producing neurons. It would therefore seem that, besides noradrenaline and adrenaline, the medulla oblongata also contains dopamine-synthesizing neurons at least at these younger ages.

At the same time, dopamine-containing fibers can be detected heading for brainstem and spinal cord regions (Fig. 32b), as indicated by their leading growth cone. The descending dopamine system has received little attention and is not yet well characterized. Descending projections of the mesencephalic dopamine cell groups have been described in the locus ceruleus, lateral parabrachial nucleus, dorsal raphe nucleus, cerebellum, and spinal cord (Björklund and Lindvall, *Vol. 2*, This Series, pp. 55–122; Copray et al. 99
Fig. 32. Noradrenergic brainstem nuclei showing dopamine-immunoactivity at E19. (a) In sagittal sections the locus ceruleus or A6 and some scattered cell bodies of the A5 group can be observed. Notice the difference in staining intensity between the dopamine-synthesizing cells in the substantia nigra/ventral tegmental area (SN/VTA) complex (arrow points to the dorsocaudal extension of this complex in the midsagittal plane) and the noradrenaline-synthesizing cells. (b) Somewhat more caudally in the pontine flexure (big arrows), the A5 group can be observed in the ventrolateral pons. The processes of the generally bipolar cells are perpendicular to the axis of the brainstem. The small arrow points at a descending dopamine-immunoreactive fiber. MF, mesencephalic flexure. × 52.

1990). The dopamine projection to the spinal cord, however, originates primarily in the diencephalic A11 group (Skagerberg et al. 1982). The first descending dopamine-positive fibers can already be detected at E15, just caudal from the mesencephalic dopamine cell groups. In the following prenatal days dopamine fibers gradually reach the spinal cord. Postnatally, these fibers can be observed in the vicinity of dopamine-stained neurons in the A1–A3 cell groups (Fig. 33), however, the origin of these fibers is not known. The innervation of CA brainstem cell groups by dopamine fibers was already suggested by biochemical studies (Rea et al. 1982).
6. DEVELOPMENT OF OTHER DOPAMINERGIC SYSTEMS

The large majority of the dopamine fibers projecting to more rostral regions leaves the mesencephalon via the nigrostriatal tract or the medial forebrain bundle. However, some of the dopamine fibers found in thalamic regions have been shown to leave the
Fig. 34. Dopaminergic fibers (arrow) leaving the ventral tegmental area (VTA) via the fasciculus retroflexus as observed in a sagittal section at E16 (a) and E19 (b). (a) × 41; (b) × 124.

Abbreviations: MF, mesencephalic flexure; SN, substantia nigra.
mesencephalic dopamine cell groups via the fasciculus retroflexus. These fibers can be observed leaving the VTA via the fasciculus retroflexus from E16 onwards (Fig. 34). At E17–E18 dopamine fibers in the fasciculus retroflexus can be followed all along their course through the dorsal thalamus to the anterior hypothalamus. Dopamine fibers from the fasciculus retroflexus start entering the lateral habenula between E19 and E20. Figure 35 shows the adult innervation pattern in the medial part of the lateral habenula and the paraventricular nucleus of the thalamus at P9.

Besides the well-known populations of dopamine neurons in the mesencephalon and the hypothalamus, dopamine-containing neurons have been described in 2 other locations, namely the olfactory bulb and the retina. Dopamine-containing elements in the olfactory bulb and retina appear around birth or even a few days later. TH-positive neurons in the glomerular layer of the olfactory bulb appear only on the last day before birth (Specht et al. 1981b; Matsutani et al. 1988; McLean and Shipley 1988). In our material, a few faintly stained neurons were already detected at E21, while the number of dopamine-containing neurons in the glomerular layer increased considerably in the fol-
following 2 postnatal weeks (Fig. 36). Dopamine cell bodies appear only after birth in the rat retina. As shown with TH immunocytochemistry these cells appear between P3 and P7 (Nguyen-Legros et al. 1983; Foster et al. 1985a). The dopamine-containing amacrine cells in the mouse retina can be seen with TH immunocytochemistry for the first time at P6, concurrent with an increase in the endogenous dopamine content of the retina (Wulle and Schnitzer 1989). The rabbit retina shows a similar delayed development of TH activity and dopamine content (Fung et al. 1982; Parkinson and Rando 1984). Uptake mechanisms for dopamine, however, can already be demonstrated prenatally (Fung et al. 1982). In the guinea pig a significant degree of development of the dopaminergic system in the retina occurs before birth (Parkinson et al. 1985). Although the time course of appearance of retinal dopamine cells varies greatly between species, the relative timing (i.e. compared to the period of ‘blindness’) is strikingly similar (Mitrofanis and Finlay 1990). In the golden hamster the tentative dopamine-containing neuron-like cells in the pineal gland first show TH activity at P6 (Jin et al. 1989). In our material, however, no dopamine-positive structures could be detected in the pineal gland at any age studied.

In the late prenatal and early postnatal period, transient populations of TH-positive perikarya have been observed in various brain regions. TH-positive neurons can be observed in the amygdala and the bed nucleus of the stria terminalis from E17 to 7 weeks postnatally (Verney et al. 1988). In the mouse anterior olfactory nucleus, a transient population of TH-positive cells was described from E16 to P28 (Nagatsu et al. 1990). During early postnatal life transient populations of TH-positive perikarya have been described in the inferior colliculi from P3 to P21 (Hökfelt et al. 1976; Jaeger and Joh 1983). In the hamster, however, TH-immunoreactive cells in the tectum are still present in adulthood (Vincent 1988). Berger et al. (1985c) described a ‘transient’ (see Kosaka et al. 1987) population of TH-ir cells in the rat cerebral cortex from P7 to P24. Cortical TH-ir cells have also been described in the adult human and monkey (Köhler et al. 1983; Gaspar et al. 1987; Hornung et al. 1989; Kuljis et al. 1989; Trottier et al. 1989). Furthermore, TH-ir cells have been reported in transplanted or in-vitro-grown embryonic neocortex (Park et al. 1986; Iacovitti et al. 1987). In all cases TH was the only detectable aminergic trait in these neuronal populations, and no other catecholaminergic synthetic enzymes or endogenous catecholamines could be detected. Moreover, this phenomenon is not only restricted to mammalian species, but has also been reported in reptiles (Smeets and Steinbusch 1990). These observations once more stress, therefore, that care
has to be taken with the extrapolation of data from TH immunocytochemistry to the dopamine system (see also the discussion in Hökfelt et al., Vol. 2, This Series, pp. 157–276 and in Vincent and Hope 1990), especially in developmental studies.

7. DOPAMINERGIC SYSTEMS IN THE HUMAN FETUS

Although most studies on the ontogeny of the dopaminergic system have been performed in rodents, there are quite a few studies dealing with the prenatal development of dopaminergic systems in humans. Remarkably few studies have been published on the development of dopamine systems in nonhuman primates, presumably as a result of the excessive costs of the animals and long gestation periods. Levitt and Rakic (1982) described the genesis of the different CA nuclei in the monkey using [3H]thymidine. In addition, 2 published biochemical studies describe the postnatal development of CAs in the cortex (MacBrown and Goldman 1977; Goldman-Rakic and Brown 1982). Ehinger and Hornykiewicz (1960) reported small amounts of dopamine in the hypothalamus and striatum of a fetus that had a crown-rump length (CRL) of 400 mm. Bertler (1961) noted dopamine (0.04–0.07 μg/g) in some parts of the brain of a 5-month-old fetus, with a higher amount being present in an 8-month-old fetus. High amounts of dopamine can be found in the hypothalamus after 11 weeks of gestation (Hyypä 1972; Masudi and Gilmore 1983). At the same age, green fluorescent fibers could be detected in the supraoptic and arcuate nucleus of the hypothalamus (Hyypä 1972). The presence of CA-containing structures in the human fetal brain was demonstrated for the first time by Olson and Ungerstedt in 1970; using the Falck-Hillarp fluorescence technique they showed CA-containing cell bodies and fibers in a 9-week-old fetus (CRL 400 mm). Detailed anatomical descriptions were provided by Nobin and Björklund (1973) and by Olson et al. (1973). Green fluorescent neuroblasts could already be observed in the 7-week-old fetus, indicating that the initial appearance of catecholamine-containing neuroblasts must be even earlier. At 10 weeks of age a dense aggregation of small weakly green-fluorescent, rounded neuroblasts were found at the level of the mesencephalic flexure, occupying the middle third of the brain width. Green-fluorescent axon bundles were found to ascend from the lateral aspects of the cell complex to the developing striatal area. A sparse group of small green-fluorescent cell bodies were found medially in the most rostral part of the hypothalamus. Between 16 and 20 weeks after gestation a further subdivision of the large mesencephalic cell group takes place. In the hypothalamus the number of fluorescent cells has increased considerably not only in the ventromedial but also the dorsolateral regions.

Using TH immunocytochemistry, no TH-immunoreactivity could be detected in the 5.5–6-week-old fetal brain, although TH-ir was present in the developing sympathetic chains of the peripheral nervous system. Clusters of TH-ir neurons were detected in the 9–10-week-old fetus in the substantia nigra and ventral tegmental area (Pearson et al. 1980). The basal ganglia can be distinguished from the surrounding tissue at this time through a dense network of TH-ir-positive axons, but primarily in the ventrolateral region (Pickel et al. 1980). In 17–21-week-old fetuses the TH-positive neurons have a more mature appearance and a clustering occurs that forms more distinct nuclear groups. TH-containing perikarya first appear in the human hypothalamus between 14 and 17 weeks of gestation.
8. ACKNOWLEDGMENTS

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9. REFERENCES


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