CHAPTER 7

Dopamine Systems in the Cingulate Gyrus: Organization, Development, and Neurotoxic Vulnerability

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Chapter contents

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A principal feature differentiating one cortical area from another is the connectivity of the area, particularly, its afferents. Areas in the anterior and midcingulate regions, for example, have rich dopaminergic (DAergic) afferents and those to the anterior cingulate gyrus are the strongest such innervations of all cortical regions (Gaspar *et al.*, 1989; Crino *et al.*, 1993). These projections to cingulate and prefrontal cortices have been implicated in a variety of functions including cognition, reward-seeking behaviors, motor activity (Ashby *et al.*, 1999; Dehaene and Changeux, 2000; Tzschentke and Schmidt, 2000; Mathon *et al.*, 2003; McCoy and Platt, 2005), and a pivotal contribution of anterior cingulate cortex (ACC) to reward is considered by Rolls in Chapter 8.

Among the specific hypotheses of the function of DAergic projections to the cerebral cortex is that of incentive motivation. This refers to the drive-like effects of an encounter with an otherwise neutral stimulus that acquires motivational importance through prior association with a primary reward (Wise, 2004). The early role of ACC in reward and motivated behavior was identified with electrical self-stimulation and drug self administration (Routtenberg and Sloan, 1972; Routtenberg, 1979). Glucose metabolism during electrical self-stimulation in the ventral tegmental area (VTA) was also very high in frontal cortex including ACC (Porrino, 1993 - Table 15.1) and the ACC is one of the few cortical regions that support cocaine self administration in the rat (Goeders *et al.*, 1986). Finally, a direct link has been made between dopamine (DA) catabolism via two genotypes for the enzyme catecholamine-O-methyltransferase (COMT) and cingulate cortex activation during working memory tasks (Egan *et al.*, 2001; Meyer-Lindenberg *et al.*, 2005).

DAergic dysfunction is associated with a variety of disorders such as drug addiction and schizophrenia (Kalivas and Volkow, 2005; Chapter 31). Since each of these disorders has a developmental component (Brady and Sinha, 2005; Rehn and Rees, 2005; Miller and Spear, 2006), the present consideration of the DAergic innervation is made in terms of developmental stages and periods of vulnerability. It is important to note that the developing DAergic system is a key player in the overall development of the central nervous system; for example, it is involved in the proliferation of neural precursors and the survival of post-mitotic neurons. This development is profoundly affected by prenatal exposure to drugs of abuse such as cocaine and ethanol. Thus, understanding of the structure and function of the DAergic system in the adult cingulate cortex can be obtained by appreciating the role of DA during development as well as the effects of substances upon that development.

In addition to the developmental issues and the general associations between DA functions and cingulate gyrus innervation, many findings over the past decade require a reconsideration of the organization and functions of this system. The location and features of the two cingulate motor areas are now understood in great detail and provide a specific motor substrate whereby DA might influence behavior (Dum and Strick, 1993; Chapter 5). Also there are now more detailed cytoarchitectural analyses available in human and monkey that provide a context for re-evaluating this system (Vogt *et al.*, 2005; Chapter 3). Most importantly, one of the primary sites of action for drugs of abuse, including cocaine and ethanol, is the ACC and these actions can now be analyzed in terms of the details of DAergic innervation in the cingulate gyrus.

Goals of This Chapter

This chapter provides an integrated, neurobiological perspective on dopamine innervation of the cingulate gyrus. It describes the structure and functions of the DAergic projection in the adult, develops a circuit model to guide future studies of this system, and it explores normal and abnormal development of this system. Although the focus is on primate brain, there is a paucity of information on developing primates and this information is garnered from rodent models. The specific goals of this chapter include the following:

1. Review DA system organization in terms of structure-function relations within the cingulate cortex.
2. Define the area and laminar patterns of DA innervation in the context of recent cytoarchitecture.
3. Review the Seaman-Yang model and consider how it explains the role of DAergic projections in mediating intracingulate and cingulate/prefrontal information processing during a "D1, gate-closed state."
4. Evaluate links between allelic variations in the expression of the DA-degrading enzyme catechol-O-methyltransferase and specific functions mediated by cingulate cortex.
5. Review how the DA system modulates cingulate gyrus development including the general principles of development of neurons, synaptogenesis, and axonal and dendritic differentiation.
6. Consider the neural developmental consequences of early exposure of DA system toxins such as cocaine and ethanol on cingulate gyral anatomy.
7. Present the first observations in a monkey model of changes in DAergic innervation of the cingulate gyrus following prenatal ethanol exposure and the implications of this primate model for studies of ethanol toxicity.

Dopamine System Organization

The first evidence of a cortical DAergic system in the mammalian brain comes from Thierry and colleagues (1973). They described stores of DA in the cerebral cortex and surmised that it was distributed in nerve terminals. This has been documented with autofluorescence, immunohistochemical detection for the expression of DA associated substances, and electron microscopy. Using these approaches, investigators characterized the organization of the DAergic system over the subsequent two decades and clarified the innervation patterns of particular regions, areas, and layers including the cingulate gyrus.

Sites of origin

Dopaminergic somata are distributed in various sites within the midbrain and telencephalon. These include the retrorubral nucleus (the A8 cell group), the substantia nigra (A9), and the ventral tegmental area (A10). In general, each of these cell groups projects to a specific brain region(s). The former two groups generate mesostriatal projections that terminate in the caudoputamen and globus pallidus. Some DAergic somata are local circuit neurons (LCNs), such as those in the caudate nucleus, and do not project to distant structures such as the cingulate gyrus.

Dopaminargic afferents to cingulate cortex arise primarily from neuronal somata in the VTA as shown in Figure 7.1 (Kalivas, 1993; Haber and Fudge, 1997; Korotkova *et al.*, 2005). The VTA is a region of the midbrain ventrolateral to the interpeduncular nucleus and medial to the substantia nigra. TH positive (+) VTA neurons have been identified in the brainstem of monkeys by many investigators (Mishra *et al.*, 1975; Brown and Goldman, 1977; Björklund *et al.*, 1978; Francois *et al.*, 1999). Most neurons in the VTA express DA and many, if not all, give rise to cortical projections. This conclusion is supported by a retrograde labeling study in which horseradish peroxidase (HRP) was injected into sites in prefrontal cortex (Porrino and Goldman-Rakic, 1982) and HRP was traced back to the VTA. Indeed, this projection is topographically organized. Prefrontal cortex (medial and lateral orbital cortex) receives input from the medial VTA, whereas more dorsal cortex, including cingulate cortex, is innervated by neurons in the lateral VTA and the medial segment of the substantia nigra.

Observations from the rat confirm and extend the monkey findings. Multiple neurons in the VTA project to prefrontal and cingulate cortices (Carter and Fibiger, 1977; Beckstead *et al.*, 1979). Moreover, the VTA is rich in DA histoflurescence (Fuxe *et al.*, 1974; Emson and Koob, 1978; Fink and Smith, 1980). Neurons retrogradely labeled with a fluorescent marker applied to prefrontal cortex were co-labeled with DA histofluorescence (Albanese and Bentovoglio, 1982; Harris and Nestler, 1996). It is noteworthy that retrograde labeling is also evident in non-DAergic VTA neurons.

The cell bodies of VTA neurons are large multipolar neurons: ~20m long and ~10m wide (e.g., Kalivas, 1993; Haber and Fudge, 1997; Francois *et al.*, 1999). These neurons are nestled among the rootlets of the oculomotor nerve. Thus, the appearance of these neurons is similar to the somata of other brainstem catecholaminergic projection neurons, for example, those in the locus coeruleus and dorsal raphe, however, they do not have melanin inclusions.

VTA neurons fire spontaneously and their activity is driven by an endogenous mechanism. Based on studies of knock-out mice, these autoreceptors appear to be D2 receptors (Keefe *et al.*, 1992; Mercuri *et al.*, 1997). In part, this is regulated by DA autoreceptors on VTA neurons that apparently tonically down-regulate DA activity.

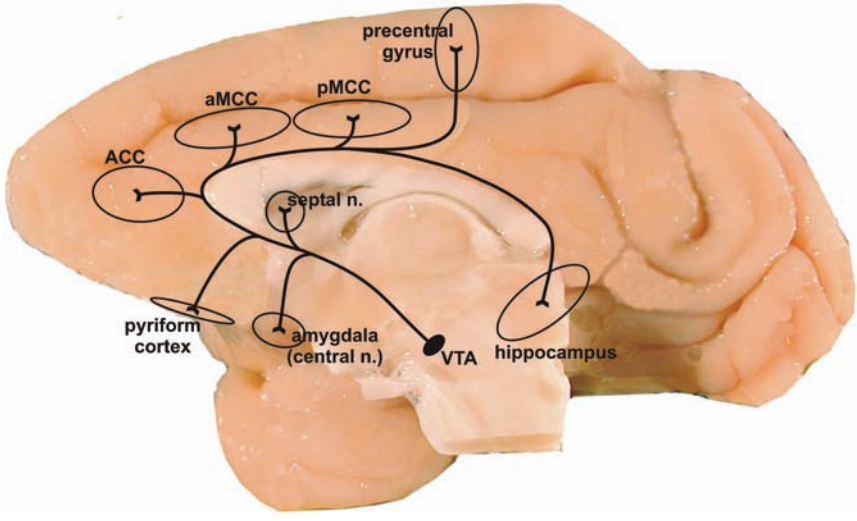

Fig. 7.1 Monkey dopamine connections. The DAergic pathways to cingulate cortex arise in the ventral tegmental area (VTA) and terminate in three regions including the anterior cingulate cortex (ACC) and the anterior and posterior midcingulate cortices (aMCC and pMCC, respectively). In addition, the VTA projects DAergic fibers to the central nucleus of the amygdala, the septal nuclei, the hippocampus, and various other cortical regions.

VTA activity is also shaped by the activity of gamma aminobutyric acid (GABA) and glutamatergic afferents from, for example, the midbrain, striatum, and cortex (Grace and Bunney, 1984, 1985; Tong *et al.*, 1996; Paladini *et al.*, 1999). As might be expected, the GABAergic afferents inhibit the activity of VTA neurons and activation of glutamatergic afferents stimulates VTA DAergic activity. Application of glutamate to DAergic neurons or stimulation of glutamatergic afferents elicits burst firing by VTA neurons (Grace and Bunney, 1984; Chergui *et al.*, 1996, 1997).

General organization

Explorations of brain catecholamines were catapulted by the work of Falck and Hillarp (Falck *et al.*, 1963; Hillarp, 1966). They linked autofluorescence in formaldehyde-fixed tissue with catecholaminergic systems. These early histofluorescence studies showed that primate cortex contained a network of axons that was densest rostrally and declined caudally. Two different types of axons were discriminable (Lindvall and Bjorklund, 1974). One was fine and had few small varicosities. The other was a larger caliber and expressed large, regularly spaced varicosities.

Since the cerebral cortex contains two types of catecholamines; DA and norepinephrine, the challenge was to differentiate these systems from one another. Though it was surmised from histofluorescence studies that the fine axons were DAergic and that the larger axons were noradrenergic (Lindvall and Bjorklund, 1974), it was only with the application of immunohistochemical methods that this discrimination became certain (e.g., Levitt *et al.*, 1984).

The key to finishing the puzzle of catecholamine identity was the use of antibodies directed against tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH). These enzymes catalyze the rate-limiting steps in the synthesis of DA and norepinephrine, respectively. The dilemma is that though DAergic neurons have TH and do not have DBH, noradrengeric neurons contain both enzymes. This complication has been addressed by a series of studies showing that TH and DBH are specific markers.

1 TH and DBH have non-overlapping distributions within cortex. DBH is expressed in areas that are deficient in TH and vice versa (Campbell *et al.*, 1987; Lewis *et al.*, 1987; Gaspar *et al.*, 1989; Noack and Lewis, 1989; Akil and Lewis, 1993; Berger *et al.*, 1993).

2 TH and DBH are expressed in axons with different features. TH is in fine axons with few small varicosities and DBH is in larger axons with many large varicosities.

3 TH co-localizes with DA (Akil and Lewis, 1993) and the DA transporter.

4 Lesions of the locus coeruleus with 6-hydroxydopamine (6-OHDA) eliminates cortical DBH-immunolabeling, but has no effect on cortical TH expression (Lewis *et al.*, 1987, 1988). In contrast, a lesion confined to the VTA causes a loss of TH labeling in cingulate and prefrontal cortices.

5 [^{3}H]DA applied to cortex is taken up by axons throughout cortex (Berger *et al.*, 1986, 1988).

Before reviewing the distribution of TH+ axons and neurons in the cingulate gyrus with an antibody to TH, the specificity of the antibody is emphasized with Figure 7.2 to validate the above argument in this tissue from an adult cynomolgus monkey. This figure shows the location of the VTA and locus coeruleus (LC) with an antibody to intermediate neurofilament proteins (SMI32) that was counterstained with thionin. Although the VTA neurons are only Nissl stained because they express low levels of these proteins, those in LC are heavily labeled with SMI32. The DBH enzyme is also heavily expressed in the LC, whereas the VTA neurons are not immunoreactive for DBH and TH. In contrast, the VTA, SN, and parabrachial pigmented nucleus are all heavily reactive for TH.

In general, cortical DAergic innervation is strongest in rostral cortex and progressively diminishes at caudal levels. For example, TH is strongly expressed in dorsomedial prefrontal area 9 and weakly expressed in occipital area 17. This does not mean, however, that the rostral-to-caudal gradient is a simple pattern (Campbell *et al.*, 1987; Lewis *et al.*, 1987; Akil and Lewis, 1993). For example, among the most richly innervated segment of the lateral cortical mantle is the precentral gyrus (motor area 4). Indeed, the plexus in motor cortex is denser than it is in area 9. In contrast, postcentral, somatosensory areas 1, 2, and 3a have few TH+ fibers. The lumpiness of this pattern is further emphasized by the TH network in inferior paretial area 7 and along the cingulate gyrus as discussed below.

In most areas of primate cortex, DAergic afferents are in two bands. One is comprised of thin fibers and located in superficial layers I-III and the other in deep layers V and VI is comprised of larger caliber fibers. This pattern has been demonstrated with histofluorescence and by the immunohistochemical distribution of TH, DA, and DA transporter.

Cingulum bundle

The ACC and MCC have a higher amount of DAergic innervation than does posterior cingulate cortex (PCC) or retrosplenial cortex (RSC; Crino *et al.*, 1993) and this impression is verified by looking at the cingulum bundle white matter underlying the cingulate gyrus. Figure 7.3 shows one level of the anterior MCC (areas a24a′b′c′)

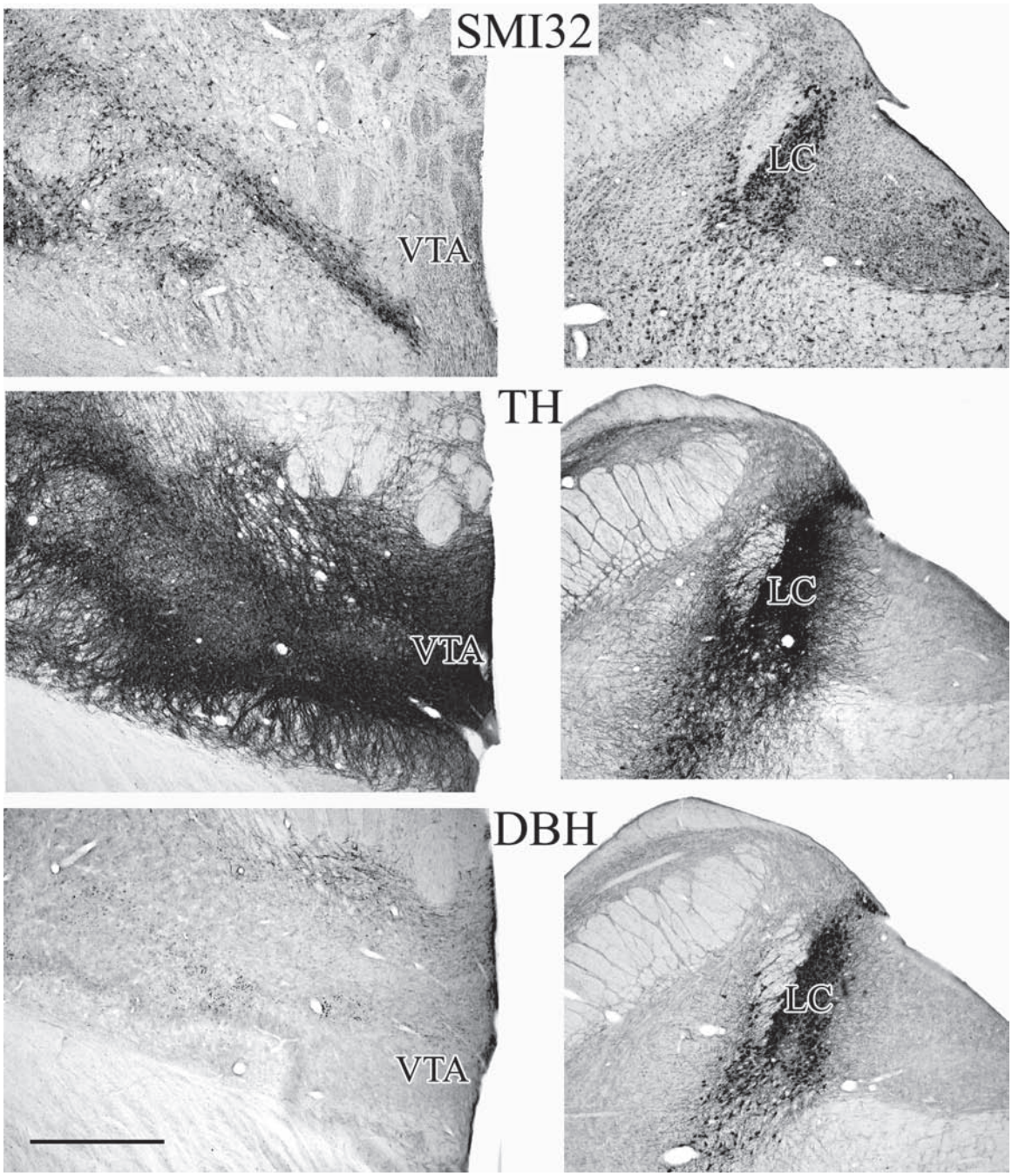

Fig. 7.2 Demonstration of TH and DBH immunoreactivity in the VTA/SN and LC. In this SMI32-immunostained section, the parabrachial nucleus is adjacent to the VTA and it has a high level of expression of intermediate neurofilament proteins. Although the LC is highly reactive for both antibodies, the VTA is not immunoreactive for DBH. Scale bar, 200 μm.

and another through dorsal PCC/RSC. In the rostral section, there is a dense plexus in the dorsal part of the cingulum bundle (#3) that penetrates to cortex along the depths of the cingulate sulcus. Medial to this plexus (#2), there is a much thinner plexus and fewer fibers penetrate the cortex. Posterior, although there is substantial TH innervation of the indusium griseum (IG), innervation of RSC area 29 is moderate and much less to area 30. The border of the cingulum bundle is shown with asterisks in the figure and this emphasizes that the greatest density of TH+ white matter axons is displaced from the cortex and does not penetrate into

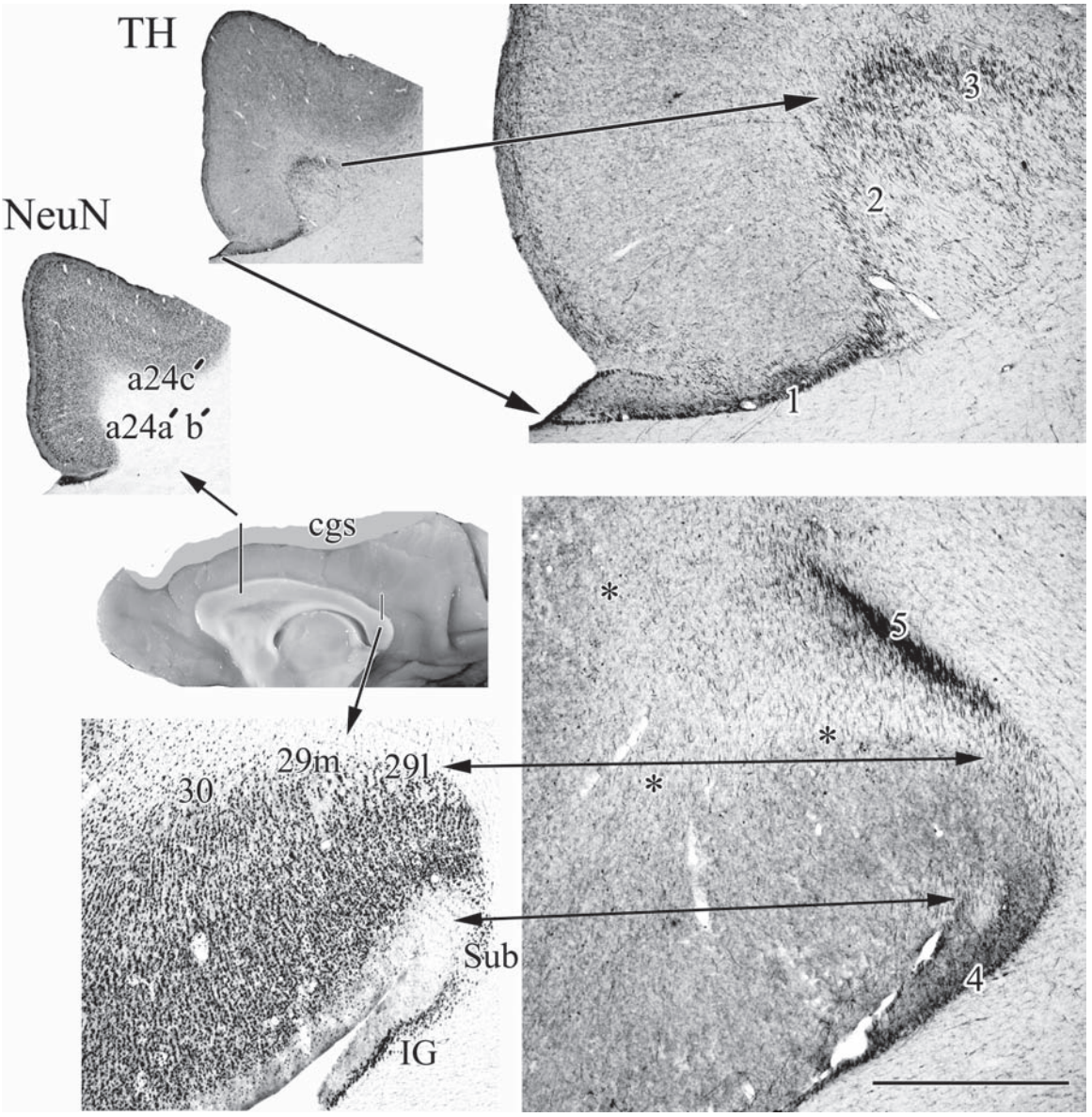

Fig. 7.3 Distribution of TH-labelled axons at two levels of the cingulate gyrus. Neurons at these two levels are shown with an antibody to neuron-specific nuclear binding protein (NeuN) and the cingulate areas are labeled. The double arrows provide points of orientation in the matched sections such that the immunoreactive axons can be seen in terms of particular areas and layers. Three TH+ fiber tracts are labeled in the rostral section (#1-#3) and two caudally (#4, #5); #1 and #4 are associated with the indusium griseum (IG) at both levels. In the anterior level the greatest density of axons is in the dorsal part of the cingulum bundle (#3), whereas fewer are ventral to this underlying the gyral part of area a24′. At the caudal level the boundary of the cingulum bundle with cingulate cortex is marked with three asterisks to emphasize that the very dense axonal plexus (#5) lies deep into PCC/RSC and does not penetrate cingulate cortex. The cingulate sulcus (cgs) on the medial surface was unfolded by an amount equal to the distance between the dorsal apex of the cingulate gyrus and the fundus and marked with a light gray tone. Scale bar, 200 µm.

the posterior cingulate gyrus. These fibers appear to pass caudally to the hippocampal formation. Thus, TH+ fibers in the white matter demonstrate that most such terminations are associated with the ACC and MCC and more in cortex of the anterior cingulate sulcus than on the gyral surface.

Area and laminar terminations

Differences in the density of TH+ axons throughout the cingulate gyrus are shown in greater detail in Figure 7.4 and for samples along the full gyrus. Although many studies report the general pattern of TH+ axons, few considered it in the context of detailed cytoarchitecture

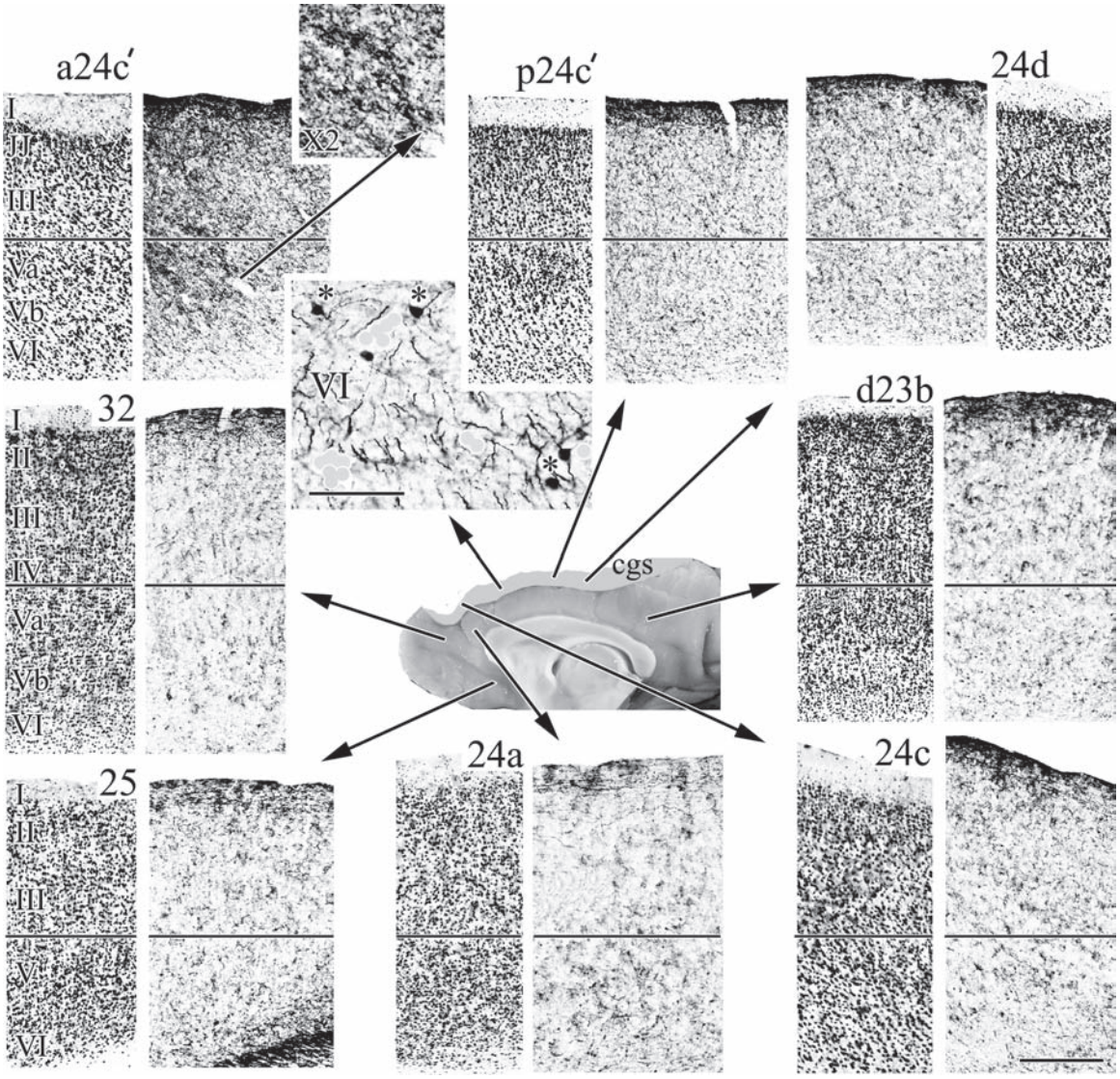

Fig. *7.4 TH-immunoreactivity in cingulate cortex. TH-immunoreative fibers and neurons throughout the monkey cingulate gyrus are shown with arrows from the medial surface. The ventral bank of the cgs is shown in gray. Each pair of sections is a NeuN and TH reacted section and they are aligned with a black line at the top of layer Va (calibration bar, 200 μm). The TH section from area a24c′ has a 2X pullout and another photograph of layer VI (scale, 100 μm) to show TH+ somata*. The laminar patterns of DA innervation are shown and the overall density is lowest in area d23b and 24c, with moderate amounts in areas 25, 32, and 24a, whereas greatest numbers of TH+ fibers are in a24c′.

and none have linked these projections to functionally unique cingulate subregions. We previously observed the greatest density of TH+ axons in the rostral part of the cingulate sulcus including areas 24c and 24c' (Vogt *et al*., 1997) and this was also observed in the present cases but with some refinement to area a24c´ based on a recent cytoarchitectural map of this region (Vogt *et al*., 2005; Chapter 3). Areas with a moderate density of axons include areas 25, 32, 24, p24´, whereas area 23 has a light density of axons as do the RSC areas. Thus, the most dense, DAergic innervation is to cortex that contains the trunk and limb representations of the rostral cingulate motor area (rCMA) in the cingulate sulcus as reviewed in Chapter 5. In contrast, there is moderate termination in areas a24a´b´ on the gyral surface.

A characteristic of all areas, regardless of overall density of axons, is a limited number of TH+ neurons in layer VI and a dense plexus in layer I. Areas with a limited density of TH+ axons had most in superficial layers as in PCC and RSC. Areas with an overall moderate density of TH+ axons had most in layers I-III and some

usually in layer V as is characteristic for the areas in ACC.

The most dense terminations are in area a24c' and, since this area contains part of the rCMA, it is emphasized with a second level of magnification in Figure 7.4 (X2). This photograph is of a varicose axonal plexus that spans between layers III and Va. This plexus is very dense and no equivalent structures are present in other cingulate areas. The TH+ neurons in layer VI are also shown at higher magnification, however, this density is the same for all cingulate areas. Finally, notice in the full-depth area a24c' strip that layers I-V have a higher density of TH+ axons than any other cingulate area. Photographs were taken and processed in the same manner to assure they are comparable.

Synaptology

Varicosities on the TH+ axons form synapses. These varicosities contain two types of vesicles: mostly large, clear-core and a small number of dense-core vesicles (Smiley and Goldman-Rakic, 1993). Elements with this morphology are distributed through all layers of cortex. Serial section analyses show that less than half of the DA+ varicosities form synapses (Smiley and Goldman-Rakic, 1993). This is particularly intriguing because the tacit assumption is that a varicosity is synonymous with at least one synapse.

The most common targets of DA and TH+ axons in cingulate cortex are dendritic spines (Goldman-Rakic *et al.*, 1989, 1992; Krimer *et al.*, 1997; Yang *et al.*, 1999). These synapses are often on the necks of thin spines and are symmetric (i.e., inhibitory) synapses as shown in Figure 7.5. As all spines form at least a single symmetric synapse (Peters and Kaiserman-Abramof, 1969; Miller and Peters, 1981), the additional DAergic symmetric synapse constitutes a triad. The positioning of the DA terminal on the neck of the spine strategically permits DA to modify the excitatory input to the spine head. A small number of DAergic terminals are associated with asymmetric (i.e., excitatory) synapses (Smiley and Goldman-Rakic, 1993). It is important to note that DA+ axons also form synapses with dendritic shafts and somata (Bergson *et al.*, 1995).

The targets of DAergic axons have been identified in electron microscopy studies. As DA+ axospinous synapses are common (Smiley *et al.*, 1992; Yang *et al.*, 1999) and pyramidal neurons are the most common source of spiny dendrites (Feldman, 1984; Whitford *et al.*, 2002; Lund *et al.*, 2003; Bannister, 2005), it is presumed that the spines are those of pyramidal neurons.

Dopamine-expressing axons also form synapses with LCNs (Smiley and Goldman-Rakic, 1993; Sesack *et al.*, 1995; Vincent *et al.*, 1995; Mrzljak, *et al.*, 1996; Krimer *et al.*, 1997; Chapter 31). These synapses are primarily on the shafts of dendrites and the somata of these LCNs.

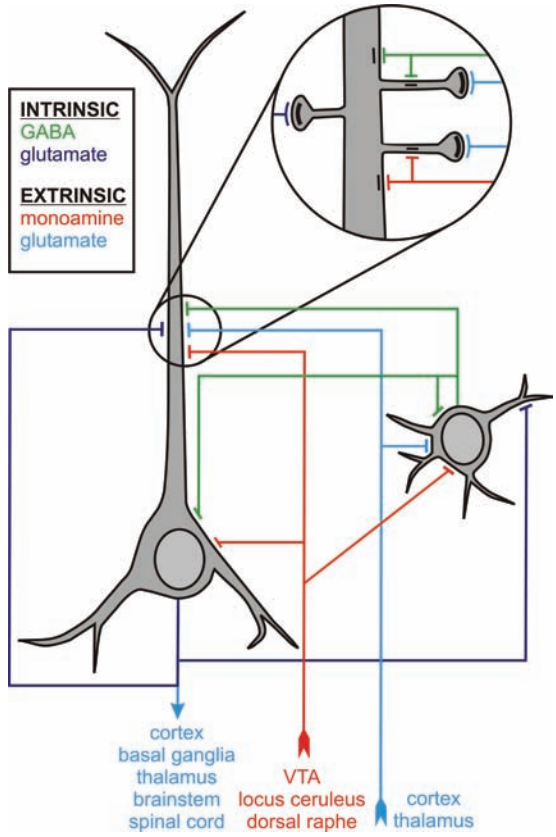

Fig. 7.5 Synaptology of dopaminergic afferentation of cingulate cortex. DA+ fibers (red) arise from the VTA and form synapses with projection neurons (left) and local circuit neurons (right). The DAergic axons terminate on the shafts of dendrites, spine necks, somata of projection neurons, and on the somata and smooth dendrites of local circuit neurons. GABA, γ-aminobutyric acid.

This conclusion is largely based on statistical analyses and ultrastructurally by identifying dendrites as being from a pyramidal neuron or LCN if it has spines or was smooth, respectively. Existence of DAergic axon-LCN synapses is further demonstrated by DA+ axons forming synapses with γ-aminobutyric acid-containing dendrites (Sesack *et al.*, 1995). DA-receptive LCNs express parvalbumin, but do not appear to be calretinin-immunoreactive (Sesack *et al.*, 1998a, b). The implication from these data is that DAergic axons innervate chandelier neurons, but not double-bouquet neurons (Condé *et al.*, 1994). Chandelier neurons have distinctive radial arrays of axonal varicosities that form synapses with axon hillocks and initial segments of pyramidal neurons (DeFelipe *et al.*, 1985, 1999; Akil and Lewis, 1992). Thus, this synaptology would mean that DA would inhibit the activity of chandelier neurons, powerful inhibitors of minicolumns of pyramidal neurons, through a feedforward mechanism and activation of DAergic projections leads to the disinhibition of excitatory pyramidal

neurons. Though chandelier neurons are considered to regulate the activity of pyramidal neurons in a minicolumn, it is unknown whether passage of DA activity through chandelier neurons imbues the DAergic system with focal effects.

The synaptology of DA afferents underlies physiological changes induced by DA. Application of DA to prefrontal cortex can increase (Yang and Seamans, 1996; Shi *et al*., 1997) or decrease (Gulledge and Jaffe, 1998; Gorelova and Yang, 2000) neuronal excitability. Two complementary explanations for this bimodal response reflect (a) DA receptor features and membrane conductance and (b) the balance of the activities of projection and local circuit neurons.

Stimulation of D1 receptors can (1) increase the sodium plateau potential, (2) lower sodium current reversal potentials (Gorelova and Yang, 2000), (3) decrease the slow potassium conductance (Yang and Seamans, 1996), and (4) activate an L-type, calcium conductance (Yang *et al*., 1999). Together these changes generate more negative resting potentials and increase excitability. Thus, prefrontal neurons that express D1 receptors and are in a depolarized state are excited by D1 agonists, whereas hyperpolarized neurons do not respond to DA (Lavin and Grace, 2001). Most of these changes are particularly apparent for pyramidal neurons, especially the effect on calcium channels on their proximal dendrites. The implication of these findings is that DA is a potent regulator of glutamatergic excitation.

The two types of cortical neurons have different overall response characteristics. Pyramidal neurons respond to a stimulus with a phasic burst, whereas LCNs are tonically active and have a graded response (Connors and Gutnick, 1990). DA activation of LCNs largely mediates a feed-forward inhibition of pyramidal neurons. In the most common functional circuit, DA stimulates a pyramidal neuron and a LCN simultaneously, and the LCN-mediated inhibition reaches the pyramidal neuron with a slight delay. Thus, the mutliple changes caused by DA on prefrontal neurons reflects the early phasic increase in pyramidal neuron excitability followed by a delayed decrease mediated by the LCN. Thus, the temporal aspects of cortical circuitry output are critical for interpreting DA-mediated responses.

Some DA may function through an extrasynaptic mechamism. This conclusion is supported by evidence that (1) DA terminals distributed in the deep layers of prefrontal cortex do not have DA transporters (Sesack *et al*., 1998a and b) and (2) much of the released DA is deaminated in noradrenergic terminals (Gresch *et al*., 1995). This allows for broad diffusion of DA-mediated signals and stimulation of extrasynaptic D1 receptors on pyramidal neurons (Smiley *et al*., 1994).

Dopamine Receptor Model of Cingulate-Prefrontal Information Processing

Seamans and Yang (2004) present a compelling theoretical model based on single neuron electrophysiology whereby the two DA receptor systems could engage in different aspects of prefrontal cortical processing and this model can be amplified for the organization of DA-regulated cingulate cortex. They propose that strong D2-receptor activation reduces N-methyl-D-Aspartic acid (NMDA) and $GABA_A$ currents and could disrupt working-memory performance in the presence of distractors with less stimulus-dependent tuning while many items are represented simultaneously. In a second state with increased D1 activation, there is an increase in NMDA currents and inhibition that contribute to a very active and stable state. This D1-dependent "gate closing" provides for a more restricted information processing mode with higher resolution and more detailed processing. In this latter D1 state, working-memory representations are more robust (Durstewitz *et al*., 2000). This model may be relevant to processing in the cingulate gyrus and it may provide insight into DA-enhanced prefrontal-cingulate interactions. We suggest that DA specifically enhances information interchanges between prefrontal and cingulate cortices in the "D1 state."

Although little is known about the distribution of D2 receptors in the cingulate gyrus, D1 binding in the human brain in conjunction with the above noted cingulate distribution of TH-expressing axons leads to a number of interesting observations and hypotheses. In Chapter 2, Palomero-Gallagher and Zilles show that highest D1 binding is in the aMCC. The pattern of moderate to low levels in the ACC and much of dorsal PCC matches the TH immunohistochemistry findings reported above and, where binding is most dense, it is mainly in layers I-III. Although there is one site of elevated binding in PCC area 31 that does not match the TH-immunoreactivity and will not be considered further here, there also are potentially important differences in aMCC. Surprisingly, highest D1 binding is on the gyral surface rather than in the cingulate sulcus. This may suggest that D1 activation does not contribute to a single functional pattern of activation as suggested by the Seamans-Yang model or there may be critical differences in monkey and human DA-mediated regulation of the cingulate gyrus.

The model predicts a single D1-receptor-mediated state in prefrontal cortex, however, evaluation of the cingulate gyrus and its circuitry raises new issues. To the extent that the two state model (D2/gate open and D1/gate closed) may be relevant, there are differences

in gyral and sulcal binding that suggests a differential, D1 gate closing that focuses mainly on areas a24a′b′. A weaker or delayed closing could occur in area a24c′. This observation has important and related outcomes in terms of cingulate interactions with prefrontal cortex and intracingulate connectivity.

Prefrontal-cingulate engagement during the D1-dominant, closed-gate state could become restricted. As discussed in Chapter 5 and shown in Figures 5.6 and 5.14, frontal projections to area a24a′b′ arise from dorsolateral areas 6, 9, 8, and 46; cingulate areas 25, 32, 24, and 23; as well as orbitofrontal and supplementary motor areas. Although most of these areas are also connected with the rCMA in area a24c′, primary motor cortex projects to the rCMA and projections from the supplementary motor areas are also greater to the rCMA. Thus, although connection diagrams show wide similarities in the inputs to gyral and sulcal areas, differences in density of projections suggest a greater motor and pre-motor regulation of the rCMA than for gyral cortex. In this context, differential regulation by D1 receptors would enhance information flow from the cingulate areas into the gyral cortex and later the flow of motor areas into the rCMA.

Interestingly, D1 gate closing could be associated with elevated processing on the cingulate gyral surface with moderate rCMA engagement. This would represent a truly pre-motor processing condition before corticospinal outputs are generated. Heavy and reciprocal connections between the surface and gyral areas assure that such activity is coordinated (Van Hoesen *et al.*, 1993). Although the Seaman-Yang model does not provide for different D1 receptor-processing modes, this hypothesis could be evaluated in the cingulate gyrus. Thus, timing of DA/D1 receptor activation could lead to gate closing first on the gyrus and later in the rCMA when and where specific behavioral outputs are defined.

Dopamine and COMT-Genotype Regulation of Cingulate-mediated Functions

The role of ACC in reward and motivated behavior was first identified with electrical stimulation and drug self administration. The ACC was a key part of neural circuits that established reinforcement behavior during electrical self stimulation (Routtenberg and Sloan, 1972; Routtenberg, 1979) and these sites in limbic cortex were unique in that stimulation of neocortical areas did not support this behavior. These medially located sites were plotted by Porrino (1993) and included areas 25, 32, and both divisions of area 24 in the rat. An important aspect of self stimulation in the rat is that dopamine depletion does not interfere with this behavior because other transmitters mediate the cortical part of the response (Duvauchelle and Ettenberg, 1991). Also, there is no cingulate sulcal cortex in the rat that contains the cingulate motor areas and the main DA innervation in primates as discussed above.

Since the rat medial cortex is limited in terms of the number and extent of areas, strict homologies among cingulate cortical functions are difficult as are extrapolations among species for the actions of DA. Walton *et al.* (2005) reported that DA depletion of ACC in the rat plays no role in guiding effort-related decisions and this appears to conflict with the many reports in primates suggesting a role in incentive motivation. It is likely that providing "effort" is not the major contribution of primate cingulate cortex to incentive motivation and behavior. Also, as discussed in detail in Chapter 3, the part of cingulate cortex that was ablated with the DA-neurotoxin by Walton *et al.* (2005) is not equivalent to the active regions in human studies that include the rCMA. In fact, the component of the corticospinal tract in the rat that originates from the medial surface is in area 32 and the most rostral part of area 24 (Miller, 1987a). In contrast, the corticospinal system in monkey originates from the CMAs in the cingulate sulcus which does not have a counterpart in the rat. Finally, in terms of DA functions, the highest level of DAergic innervation is to the rCMA. Thus, effortful behaviors in rodent may not be equivalent to incentive motivation in human because the rodent VTA and other subcortical systems may be more critical in this species than in primates.

Catechol-O-methyltransferase (COMT) is the enzyme that metabolizes DA, thus, modulating COMT activity determines the rate of DA clearance. Genomic variations in COMT levels have been identified with polymorphisms in the COMT gene with a $Val^{108/158}Met$ substitution enhancing DA clearance by as much as four-fold; methionine homozygotes have greatly reduced COMT activity and greater blood flow and working memory capacity than do the valine-allele carriers (Meyer-Lindenberg *et al.*, 2005). These allelic variations alter functions that probe prefrontal function and they have been demonstrated in post-mortem tissues (Chen *et al.*, 2004). Importantly to the present consideration, allelic variations in COMT genotype lead directly to validation of the role DA in cingulate functions.

Two types of functional studies of COMT genotypes have generated similar findings, though not exactly the same in terms of cingulate responses. Meyer-Lindenberg *et al.* (2005) correlated cerebral blood flow with midbrain DA and showed significant correlations with ACC during 0-back testing PET analysis of regional cerebral blood flow with radioactive water or presynaptic function with 6[^{18}F]3,4dihydroxyphenylalanine (F-DOPA).

Higher uptake of F-DOPA was detected in valine-allele carriers than methionine-allele homozygotes. Methionine allelic reduction in COMT activity was associated with a positive correlation of midbrain DA during 0-back and 2-back working memory tasks, whereas the same tasks had negative correlations for the valine allelic carriers.

Egan *et al.* (2001) evaluated fMRI activity in 175 schizophrenics, 219 unaffected siblings, and 55 controls in relation to allele dosage during the Wisconsin Card Sorting Test. They found that the methionine allele enhanced cognitive performance for all subjects. In a separate N-back task in the three separate groups, they found that in family trios of subjects, there were significant increases in transmission of the Val allele to the schizophrenic offspring. The fMRI analysis showed that pACC and aMCC were most active in both hemispheres during the two-back working memory task by genotype. This is rather compelling evidence that regulation of COMT activity, and hence DA levels, substantially impacts the function of the region in primate brain that has the highest density of DAergic inputs and the highest level of D1 receptors. It also continues to point to the role of impaired cingulate functions in schizophrenia as pivotal to risks for this disease as discussed in Chapters 30 and 31.

Development of Dopaminergic Systems

The early development of cingulate neurons depends on DAergic innervation and cingulate cortex experiences two waves of DAergic projections as shown in Figure 7.6. The DAergic neurons in the VTA are born between Gestational (G) days 9.5-G13 in mice, G11-G15 in rats, and between weeks 6-8 in humans. These neurons are born in the ventricular zone (VZ) of the fourth ventricle (Lauder and Bloom, 1974; Reisert, 1990). Shortly after being generated, the young neurons begin their migration to the VTA and during this migration they become immunoreactive for TH (Reisert *et al.*, 1990).

First wave of innervation

A first wave of innervation is marked by the initial appearance of TH-expressing afferents issuing from the VTA. These fibers ultimately arborize within infragranular laminae of the adult (Verney *et al.*, 1982). During their development, however, these fibers reach the anlage of cingulate cortex on G16 in mouse (Berger *et al.*, 1991; the equivalent of G17 or G18 in the rat per Clancy *et al.*, 2001) or at six weeks of age in human (Zecevic and Verney, 1995). These fibers extend caudorostrally from the VTA giving rise to the medial forebrain bundle and they then extend past the basal ganglia and to the telencephalic wall. The fibers then penetrate the frontolateral cortical anlage through the intermediate zone (IZ) and sparsely through the marginal zone (MZ). This is referred to as Stream 1. A second stream of fibers enters the telencephalic anlage frontomedially soon thereafter and these fibers invade the subplate and penetrate the cortical plate from below.

Neurons in cingulate cortex are generated concurrent with those in the VTA. In the rat, neurons that occupy presumptive cingulate cortex pass through their last mitotic divisions between G11 and G20 (Bayer, 1990; Miller, 1992a). The early-generated neurons take about one day to move from their proliferative zones into the developing cortex and the late-generated neurons take as much as eight days (Berry and Rogers, 1965). Hence, by G17, the cortical plate is comprised of layer VIa neurons that have completed their migrations, and by the day of birth, neurons in layers IV and V have completed their migrations. Synapses in cortex initially appear during the first postnatal week (Miller, 1981; Miller and Peters, 1981; Jung and Bennet, 1996; Levitt *et al.*, 1997b; Diaz *et al.*, 1997). Thus, DAergic fibers are present in the developing cortical plate prior to the onset of synaptogenesis. If DA is released from these neurons in a functionally significant manner, it must act through a non-synaptic mechanism, possibly via an autocrine/paracrine pathway.

Berger and colleagues (1995) suggest an alternative, non-neural source of cortical DA (Berger *et al.*, 1995). Accordingly, both DA and norepinephrine (NE) can be derived from blood cells. This conclusion is supported by evidence that catecholamine concentrations throughout the monkey fetal cerebral wall (on G70, G90, and G120) are 70-80 times higher prior to formation of the blood brain barrier than those measured neoantally, that is, after the establishment the blood brain barrier. This concept is supported by evidence that monoamines can easily cross the placenta.

The arrival of DA afferents is not temporally aligned with the appearance of cortical synapses. The temporal expression of the DA receptor, like that of its native agonist, precedes that of presynaptic terminals. The D1 receptor is first detected on G16 in the rat and D1 mRNA is distributed in both proliferative and differentiation compartments of the cerebral wall (Reinoso *et al*, 1996). During migration, cortical neurons express both the D1 and D5 receptors (Lidow, 1995) and DA receptors are also expressed by radial glia and astrocytes (Lidow, 1998).

Second wave of innervation

The second wave of DAergic innervation of cingulate cortex occurs during the first and second postnatal weeks in rats (Verney *et al.*, 1982) and week 11 in humans (Zecevic and Verney, 1995). These fibers issue

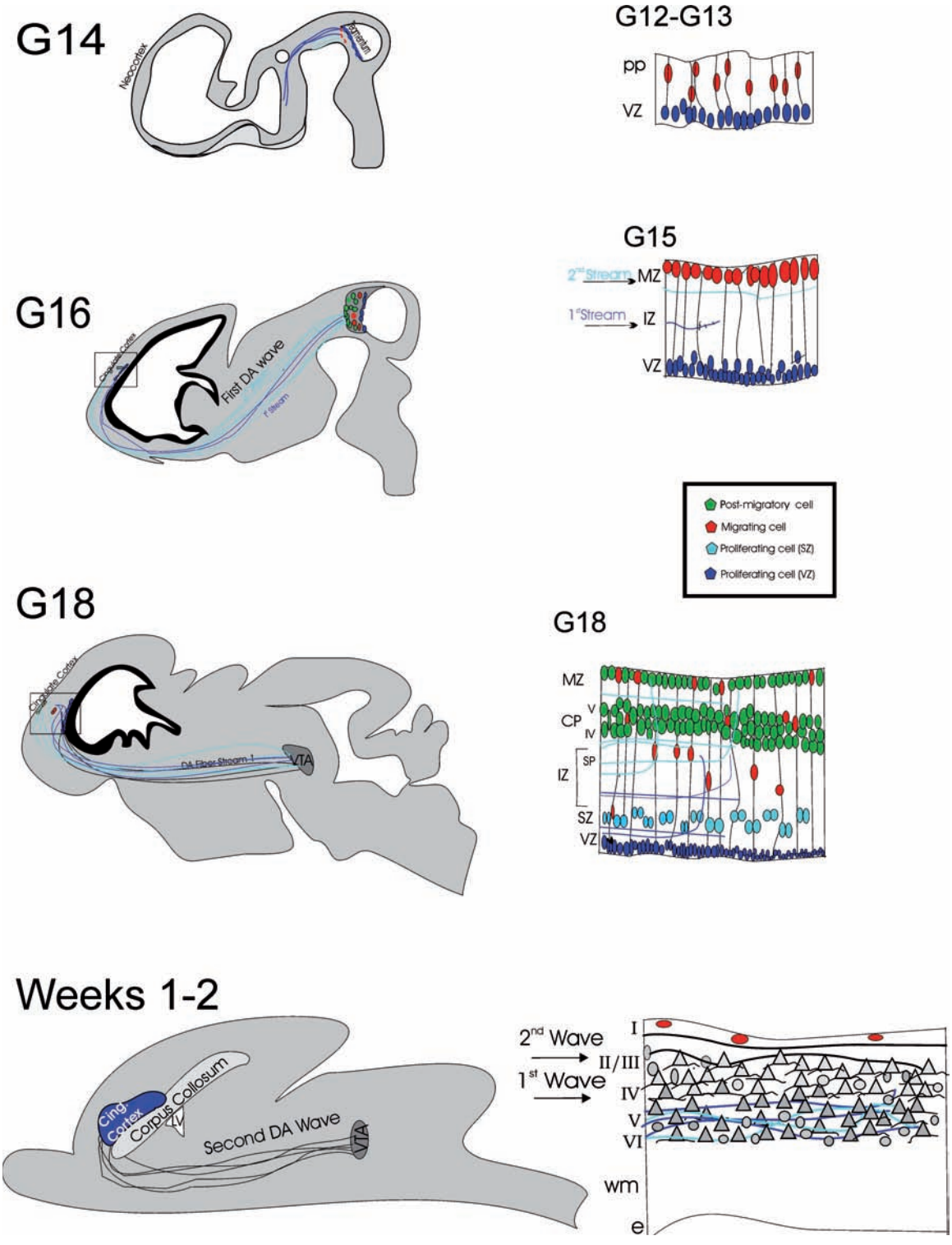

Fig. 7.6 Development of DAergic fibers in cingulate cortex. Neurons in the VTA are born on G12 and G13. The DA-positive fibers move to the developing cingulate cortex in a wave that passes through the basal telencephalon on G14 and arrives in cingulate cortex on G16. These fibers follow one of two streams, one passes through the marginal zone (MZ; blue) and the other that passes through the intermediate zone (IZ; purple). Fibers ramify in the cortex by G18. A second wave of DAergic afferents arrives in cortex during the first two postnatal weeks.

from the medial VTA and distribute rostrocaudally to superficial layers. This contrasts with the first wave which arborizes mainly in layers V and VI.

By the day of birth in rodents or the start of the third trimester in humans, TH+ fibers are robust in both the prefrontal and ACC. These DAergic fibers are distributed through all layers invading the subplate layer and finally the cortical plate. The progression of this wave coincides with the appearance of cortical synapses (Miller, 1981; Miller and Peters, 1981; Lauder, 1988; Jung and Bennet, 1996; Levitt *et al*., 1997b; Diaz *et al*., 1997). The density of all DA receptors increases steadily during this second wave, that is, the first two postnatal months in the human. These receptors concentrate preferentially in the superficial cortical layers. At postnatal month four of the monkey, receptor density begins a decrease that subsides by puberty (Lidow and Rakic, 1992).

Both waves contribute fibers that are distributed in the deep laminae, however, it is appealing to speculate that even in the adult, the fibers derived from the two waves function in different manners. Accordingly, the DAergic fibers produced during the 1st wave use DA in an autocrine/paracrine mechanism (Tiu *et al*., 2003), whereas DAergic fibers arriving during the second wave participate in synaptic-regulated activities. Even in the adult, there is evidence of non-synaptic DA release, and this is confined exclusively to the deep laminae. The deep layers are the major termination site of the first wave of innervation interneurons (Goldman-Rakic *et al*., 1999; Zoli *et al*., 1999; Pickel, 2000). Moreover, there is consensus that cortical DA exerts diffuse or non-synaptic signaling via DA receptors on projection neurons and LCNs.

Role of dopamine in cell proliferation

The cells in the VZ that generate cingulate neurons are responsive to DA and they exhibit DA-induced changes in cell proliferation *in vivo*. Furthermore, cells in cortical proliferative zones of fetal monkeys and rodents express high concentrations of the D1 receptor (Schamabra, 1994; Lidow, 1995; Lidow and Rakic, 1995; Reinoso *et al*., 1996; Wang *et al*., 1997; Lidow *et al*., 1999; Popolo *et al*, 2004). Treatment of primary cultures of fetal cortical progenitor cells taken from the ganglionic eminence (GE; a source of cortical LCNs) and from the cortical neuroepithelium (the site of origin of projection neurons) respond to a D1 agonist, SKF 81297 (Popolo *et al*, 2004). Treatment with a high dose of the agonist results in reduced bromodeoxyuridine (BrdU) labeling in both the VZ and SZ of the GE (n.b. BrdU is a DNA precursor taken up by cells passing through S-phase of the cell cycle; Miller and Nowakowski, 1988; Nowakowski *et al*., 1989). Interestingly, the agonist only reduces BrdU incorporation in the cortical VZ and the concentration of the agonist needs to be greater for the cortical cells than for the GE cells (Popolo *et al*, 2004). Similar results for the cortical cells can be obtained using another D1-specific agonist, SKF82958 (Zhang and Lidow, 2002).

DA affects the expression and activity of proteins that regulate the cell cycle. These regulators include: cyclin D (a promoter of the transition from G1 to S) and p27 (an inhibitor of cyclin-dependent kinase; Zhang *et al*., 2005). Application of a D1 agonist (SKF38393) decreases the expression of cyclin D. In contrast, application of this agonist has paradoxical effects on p27 expression; at low doses it increases p27 expression, yet at high doses it decreases expression. As p27 induces a lengthening of the cell cycle or encourages neurons to exit the cell cycle (Coqueret, 2003), we can imply from the results with the D1 agonist that the reduction in BrdU incorporation results from an increase in the proportion of cells in the G0 or G1 phases of the cell cycle.

Whereas the VZ progenitors appear to respond primarily to D1 activation, cells of the SZ appear to be more responsive to D2 activation. D2 mediates an increase in the SZ of the GE and neuroepithelium of prefrontal cortex (Popolo *et al*, 2004). Blocking D1 with a specific antagonist (Schering 23390) establishes a situation wherein endogenous DA can only activate D2 receptors. Application of this antagonist to a rat fetus on G15 increases BrdU incorporation in the SZ of the PFC, but not the VZ. A role of D2 receptors in the proliferation of SZ cells is further substantiated by DA depletion studies focusing on the neocortical SZ and in the SZ-like region in the hippocampal formation, the intrahilar zone (aka the subgranular zone). Depletion of DA decreases cell proliferation in these two zones. This proliferation can be restored, both *in vivo* and *in vitro*, by administration of a D2 agonist (ropinirole; Höglinger *et al*., 2004). Thus, stimulation of D2 receptors is pro-proliferative and the opposite of the anti-proliferative effects mediated by D1 receptors.

DA can affect cell-cycle progression via the dopamine transporter (DAT). DA inhibits the growth of SK-N-MC neuroblastoma and HEK293 cells transfected with DAT by blocking cell cycle progression (Woldman *et al*., 2005). It does so by increasing the number of cells in the G1-phase of the cell cycle, decreasing BrdU incorporation, and increasing the expression of cyclin A (a promoter of transit through the S-phase). Interestingly, the expression of other cell cycle regulators (e.g., cyclins D2, D3, E, cdk4, and p21) is unchanged. Thus, it appears that the DAT is critical for progression through the S-phase of the cell cycle, whereas the action of DA on D1 receptors is key for the transition from the G1-phase to the S-phase.

Neurite outgrowth

DA stimulates outgrowth of neurites from post-migratory neurons, this growth is concentration-dependent; low concentrations promote the expression of cytoskeletal components in maturing axons and high doses halt neurite outgrowth (Reinoso *et al.*, 1996). Lack of D1 receptor expression in D1 receptor knock out mice results in decreased bundling and increased irregularity in the pattern of radially oriented apical dendrites of pyramidal neurons in cingulate cortex (Stanwood *et al.*, 2005). These dendrites weave an irregular course toward the pial surface. This pattern contrasts with the straight route taken by apical dendrites in the cortices of wild-type animals. Interestingly, regions that are weakly innervated by DA, such as somatosensory and visual cortices, develop and appear normal.

When hyperactivity of the D1 system is induced by exogenous application of a DA agonist (SKF82958) to mouse cortical neurons, changes opposite those of the knockout are evident. For example, treatment of cortical cultures with a D1 receptor agonist reduces the area occupied by neuronal processes (Song *et al.*, 2002). In D1 receptor over-expressing mice, layer V pyramidal neurons have shorter apical dendrites and stunted branches in the dorsal part of the anterior cingulate cortex. These changes occur in the absence of a change in cortical thickness or lamination. Though the apical dendrites appear to enter layer I, they do not extend through the entire layer as in the wild-type animals. These mice also exhibit increased phosphorylation of microtubule-associated protein 2 (MAP2) in cingulate cortex. Furthermore, both the decreases in neurite extension and the increases in MAP2 phosphorylation induced by D1 agonists are inhibited by the addition of a D1 antagonist. Therefore, data obtained both *in vivo* and *in vitro* with either knockout or over-expressing animals, suggests that the D1 receptor plays an important role in the development of dendrites, and that this effect is mediated through MAP2 signaling.

Like D1, the D2 receptor is involved in DA-induced neurite outgrowth. Unlike D1, however, stimulation of the D2 receptor results in changes that are opposite of those caused by activating the D1 receptor. Treatment of cultured cortical neurons with a D2 agonist (quinpirole) results in a 3- to 10-fold increase in the length of neurites and in the number of branch points per neurite (Todd, 1992).

Studies of Abnormal Development

Insight into the role of DA in normal development can be garnered from studies of abnormal development. In this regard, two sets of data are instructive. First, studies examining the effects of toxins that induce rearrangement of the monoaminergic systems (e.g., 6-hydroxydopamine, 6-OHDA; 5,7-hydroxytryptamine, 5,7-DHT) and second, studies of the effects of drugs of abuse such as cocaine and ethanol.

Toxin-induced monoaminergic reorganization

Information about the role of DA in development of the central nervous system is available from the use of selective DA system toxins. The most commonly used catecholinergic neurotoxin is 6-OHDA. It reduces DA and depletes NAergic stores in nerve endings and intrastriatal injection of 6-OHDA reduces the density of monoaminergic projections by as much as 40% (DeBeir *et al.*, 2005; Towle *et al.*, 1989).

A competitive interaction exists between the 5-HT and DA systems and this has been shown using ablation of 5-HT neurons with 5, 7-DHT. A significant increase in the number of TH+ fibers occurs after 5-HT fibers are ablated with 5, 7-DHT. Therefore, the 5-HT system presumably exerts a suppressive effect on the sprouting of DAergic fibers. Further, the 5-HT and DA systems may compete for targets. Lesions of VTA neurons with 6-OHDA decrease the density of 5-HT fibers in layers II and III of young rats. This implies that DA has a tropic effect on 5-HT development wherein DA facilitates the in-growth of 5-HT fibers (Cunningham *et al.*, 2005). Likewise, it appears that 5-HT suppresses the ingrowth of TH-expressing fibers.

Exposure to drugs of abuse: Cocaine

Two of the most widely abused drugs are cocaine and ethanol, and both affect DA neurotransmission and the use and/or abuse of either substance by pregnant women can lead to dysregulation of DAergic signaling in the offspring. Cocaine binds to monoamine transporters; it blocks the re-uptake of monoamines by pre-synaptic elements. Thus, cocaine leads to a pre-synaptic increase in DA, NE, and 5-HT. That said, in mice, only DA turnover and metabolism is affected by prenatal exposure to cocaine; NE and 5-HT are unaffected (Miller *et al.*, 1995). It should be noted that the effect of prenatal exposure to cocaine varies among species (Harvey *et al.*, 2004). Rats show little structural damage, however, mice, rabbits, and primates have pronounced structural abnormalities, particularly in the growth and laminar organization of the cerebral cortex. Further, prenatal exposure to cocaine in mice and primates leads to a loss of neurons of cerebral cortex and hippocampus, but only if the exposure occurs during corticogenesis. Similar effects are also evident in rabbits, but only following exposure to high doses of cocaine (Harvey *et al.*, 2004).

Most research into the effects of prenatal primate cocaine exposure on development has been performed

in the laboratory of Michael Lidow. In these studies, monkeys were exposed to cocaine (25 mg/kg/day) daily throughout the period of cortical neuronogenesis (G40-G102). Prenatal cocaine exposure leads to decreased body weights during early postnatal development (Lidow, 1998). By two months of age this weight difference is gone and the animals do not present with abnormal physical characteristics. Such transient changes are also evident in the brain.

The brains of monkeys exposed to cocaine during gestation were examined as two-month olds or as three-year olds. The changes detected at two months of age often persist into adolescence (Lidow and Song, 2001) and anatomical defects occur primarily in the cingulate cortex. For example, at both ages, the cortex is marked by disrupted gray and white matter. The volume and density of neocortex is reduced; these changes correspond to a decrease in the total number of neurons.

The white matter in monkeys prenatally exposed to cocaine has an unusually high number of cells and these cells appear to be neurons (Lidow, 1995). Since this may represent defects in neuronal migration, potential migration defects have been addressed in a [^{3}H]thymidine ([^{3}H]dT) birth-dating study. In this study, [^{3}H]dT was administered on G64 and G65, a time when supragranular neurons are normally generated as shown in Figure 7.7 (Lidow, 1995, 1998). In animals exposed to cocaine prenatally, radiolabeled cells are often widely distributed in ectopic sites. Few [^{3}H]dT labeled cells, most of which are neurons, are in the supragranular layers; the majority is dispersed in layer VI and the subcortical white matter. Note that the persistence of these neurons in the ACC of the adolescent monkey shows that the ectopic neurons survive and it implies that they are functional. Nevertheless, the increase in the number of neurons in the white matter does not account for the reduction in neuronal number in the cortical parenchyma; the aberrantly migrated neurons only account for half of the total number of "missing" neurons.

The changes in cell number are at least partially due to a reduction in the proliferation of neural progenitors. These changes may be caused by the temporary reductions in the binding of α-adrenergic receptors in the proliferative zones, IZ, and cortical plate (CP).

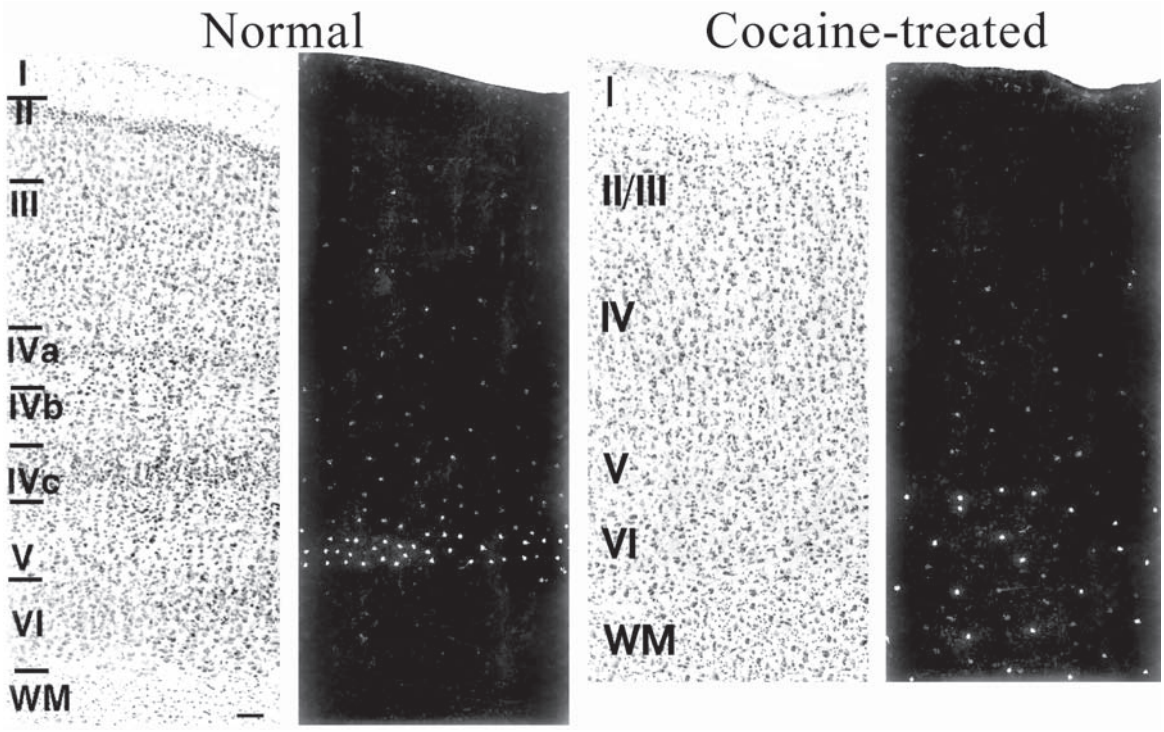

Fig. 7.7 Effect of prenatal cocaine on DAergic systems in cingulate cortex. Neurons generated on gestational day (G) 64 and G65 (i.e., labeled with [^{3}H]dT) with darkfield photomicrography show labeled neurons (white) in the adult monkey. In the normal monkey, these cells were mainly in layer V. In monkeys exposed to cocaine prenatally, the distribution of these cells was broader, extending from layer V to the white matter (WM). Also notice that the six layers distinguishable in the Nissl-stained section of the normal monkey are dispersed following prenatal exposure to cocaine.

Prenatal cocaine exposure in monkeys also increases the binding and/or number of D1 receptors on first wave DAergic projections to the VZ, IZ, and CP. Activation of D1 receptors during development inhibits cell proliferation (see Role of Dopamine in Cell Proliferation) and dendritic extension (see Neurite Outgrowth). Therefore, the cocaine-induced increase in the number of D1 receptors may underlie cocaine-induced decreases in neuron number.

The effect of prenatal cocaine exposure on cortical DA concentration was determined with high pressure liquid chromatography with electrochemical detection (Lidow, 1998). On G70, DA content is increased (~133%) in the VZ, IZ, and CP. Twenty days later (on G90), however, DA concentration is ~32% below that detected in normal monkeys. DA concentration normalizes by G120. Monkeys exposed to cocaine prenatally also have structural abnormalities. The most striking is a cortex with laminae that are difficult to distinguish (Lidow, 1998). Part of this is due to migration defects. A pulse injection of [^{3}H]dT normally leads to a tight tangential clustering of cohort cells. After prenatal exposure to cocaine, however, the radiolabeled cells are more broadly distributed. Indeed, the late-generated cells which normally find residence in superficial cortex, are distributed broadly and most notably in ectopic sites in deep cortex (Fig.7.7). This is reminiscent of changes caused by prenatal exposures to other substances such as ethanol (see below).

Prenatal cocaine exposure affects the ACC of rabbits. One of the most conspicuous changes is anomalous dendritic growth. The dendrites of pyramidal neurons in layers III and V are 30-50% longer than in controls and the apical dendrites traverse an irregular path, as opposed to the straight one in controls. Likewise, ACC neurons harvested from cocaine-exposed rabbit fetuses exhibit greater outgrowth *in vitro* (Jones *et al.*, 2000). The *in vivo* changes are accompanied by an increase in GABAergic LCNs. The effects on pyramidal neurons and LCNs are only evident in the DA-rich ACC and they are absent in DA-poor regions such as somatosensory cortex. As in monkeys, prenatal cocaine exposure in rabbits does not induce gross-structural abnormalities and the distribution of 5-HTergic and TH-positive fibers in cortex. In contrast to the monkey, the total number of neurons is not affected by prenatal exposure to cocaine in the rabbit.

Exposure to drugs of abuse: Ethanol

There are similarities in cortical development between monkeys exposed prenatally to cocaine and rats exposed prenatally to ethanol. The cortices of ethanol-treated rats have significantly fewer neurons and glia and much of this decrease results from a reduction in cell proliferation (Miller, 2006a). Furthermore, ethanol induces migration defects whereby many neurons migrate only as far as layer VI (Miller, 1986; Siegenthaler and Miller, 2006) and show dendritic hypertrophy (Miller *et al.*, 1990). Specific ethanol-induced effects on the ACC have been described. Neuronal generation is delayed and reduced in both the ACC and RSC (Miller, 1987b; Miller and Robertson, 1993). These effects include decreases in the total number of paravalbumin-immunoreactive, GABAergic LCNs with no corresponding changes in structural volume or neuronal size (Moore *et al.*, 1998). These changes appear to be limited to prenatal exposures because no changes are evident in cingulate cortex when the exposure occurs between postnatal day (P) 4 through P10 (Mitchell *et al.*, 2000).

Though ethanol affects a wide variety of systems, the parallel nature of the defects caused by ethanol and cocaine suggest that at least some of the teratogenic mechanisms are similar. This notion is supported by evidence that prenatal exposure affects cortical DAergic systems. Following prenatal exposure there is an increase in the two metabolites of DA, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) that persists into early adulthood. In the developing cortices of normal rats, there is a transient increase in DOPAC concentration and a decrease in HVA concentration. Following treatment with ethanol prenatally, there is a persistent reduction in DA uptake, a decrease in the number of receptor binding sites, and a decrease in DA metabolites in cell body and terminal areas of DA neurons (Cooper and Rudeen, 1988; Druse *et al.*, 1990). Note that DA neurons in the VTA are also affected by prenatal ethanol exposure. They have smaller cell bodies, retarded dendritic growth, and exhibit an age-dependent decrease in the number of spontaneously active DA fibers in the VTA (Shetty *et al.*, 1993).

Prenatal Ethanol Exposure: A Monkey Model

The organization of rat and monkey cingulate cortex are substantially different from that of the rat as shown in Chapter 3. To the extent that monkey cingulate cortex is more representative of the human, it is important that the developmental consequences of ethanol exposure be explored in a monkey model. For example, the greatest density of DAergic fibers appears to terminate in area a24c′ in the rostral cingulate sulcus of aMCC. Since the rat does not have a cingulate sulcus or any areas therein, it cannot be used to analyze DAergic alterations in area a24c′. Only observations in the monkey will have value to this end and such work has important consequences for understanding the role of ethanol toxicity during human pregnancy.

The model involves exposure of monkeys to ethanol (Et) one day per week for the first six or 24 weeks

of gestation; Et6 or Et24, respectively. The first six weeks include the period of neurulation and a time of active neural stem cell proliferation. Cortical neurogenesis and the migration and differentiation of the young cortical neurons occurs during the subsequent 18 weeks of gestation. Thus, only the Et24 monkeys were exposed to ethanol during the period of cortical development.

Some aspects of the macroscopic organization of cortex appear normal in Et6 and Et24 monkeys. That is, the thickness of cortex is not significantly affected by prenatal treatment (Miller, 2006b) and cytoarchitectonic distinctions can be made. The distribution of cortical projection and local circuit neurons may not be affected by ethanol treatment, although more detailed analyses will be needed to prove this. On the other hand, the corpora callosa of the Et24 monkeys are altered; they are larger than in control monkeys (Miller *et al.*, 1999). The most conspicuous difference is the increased size of the rostral segment of the corpus callosum. This is the portion carrying information between the homotopic sites in the ACC, MCC, and the prefrontal cortex of the two hemispheres.

To assess the condition of the DAergic system, the Et6 and Et24 monkeys were prepared for TH immunohistochemistry and compared to control cases. A number of interesting and important post-mortem pathologies were observed in the cingulate gyrus including the organization of the cingulum bundle and the density of TH-positive varicosities. Figure 7.8 shows the variation in the structure of the cingulum bundle. In the control and Et6 animals, the TH+ axons established a primarily dorsal position in MCC (#3 in Fig. 7.3; asterisk in Fig. 7.8-Anterior) and the plexus underlying PCC was vertically placed and did not approach the cortex/white matter border (asterisk in Fig. 7.8-Posterior). In contrast, the Et24 monkeys have variations in both midcingulum and posterior cingulum bundles. In the midcingulum bundle (Fig. 7.8B), the fibers are dispersed in an almost equal density throughout the bundle. At the posterior level, the displaced plexus arches over the cortex instead of maintaining a vertical orientation. These differences are not due to planes of section as the differences appear in all sections at the level of the genu and splenium of the corpus callosum. Thus, the pathways for DAergic projections to the cingulate gyrus are present but altered in size and orientation by the ethanol exposure as was also true for the contralateral innervations through the corpus callosum. These changes predict alterations particularly in MCC where DAergic innervation is greatest.

An assessment of TH-positive axons in the same cases was undertaken as part of a qualitative survey of the entire cingulate gyrus. The terminal arbors of TH fibers in the Et24-treated animals are substantially affected by the prenatal exposure. Figure 7.9 shows that the normal terminal varicosities in all layers of area a24c′ are greatly reduced leaving the larger caliber primary axons. This morphology suggests that, although primary axons are guided to their cortical targets, they fail to generate synaptic contacts in the ethanol-exposed animals. The drastic effect this might have on functions of the rCMA can be predicted from the circuit diagram in Figure 7.5. This organization suggests a severe loss of DA modulation of both pyramidal and LCNs in the rCMA and throughout much of the cingulate gyrus. Thus, exposure of the fetus at critical periods of DA generation can lead to a massive DA deafferentation that could disrupt many functions including those associated with cingulate-mediated reward processes.

Dopamine-mediated Cingulate Functions, Development, and Vulnerability to Toxic Insults

Dopaminergic systems are pivotal to the executive functions of cingulate cortex, regulation of its motor outputs, and possibly intermediating between cingulofrontal interactions. The selective vulnerability of this system to drug exposure and developmental anomalies, such as in schizophrenia, play a key role in the pathophysiology of a number of psychiatric diseases. Although the cingulate gyrus has not played a prominent role in understanding the function and dysfunction of this system, the present analysis suggests there are many more DA stones to be turned before the DA system is understood.

The myriad of complex single neuron discharge properties in the DAergic inputs and cortical neurons still does not provide a single and coherent view of one or more functional states by which DA regulates prefrontal functions. The Seamans/Yang model has great potential, if it can be modified to accommodate the unique specializations of the cingulate gyrus. Hypotheses are provided as to the likely scenarios by which intracingulate and cingulofrontal interactions are modified by DA. These specific hypotheses can be tested as we devise a more precise circuit model by which this system operates. Just as important, it will be necessary to reconcile responses evoked during incentive motivation and pain processing as discussed in the other chapters in this volume. As noted in Chapter 12, cognitive processes must be viewed in the larger context of cortical processing in aMCC and this means consideration of all functions, not just those hypothesized and probed in a single study design. Thus, dynamic circuit models have much to consider beyond the two state model of DA function in prefrontal cortex.

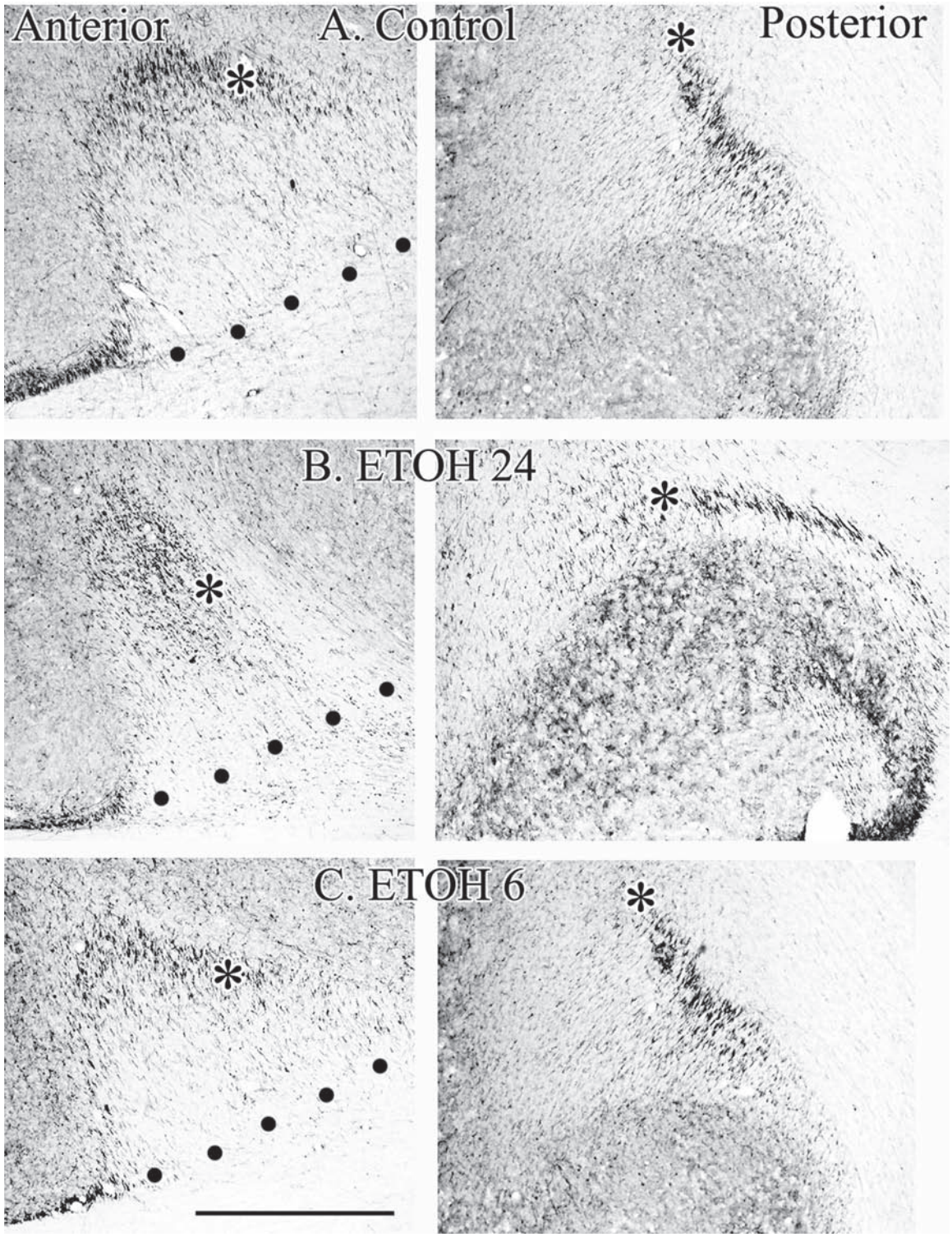

Fig. 7.8 Distribution of TH immunoreactive axons (asterisks) in the cingulum bundle in control (A), Et24 (B) and Et6 (C) monkeys. The midcingulate cingulum bundle is delineated with dots to emphasize differences in axonal distributions. The primary effect of ethanol exposure was an MCC reduction of DAergic axons in the Et6 animals and a reduction and reorientation of axons in Et24 suggesting a significant MCC involvement in these cases. Scale bar, 1 mm.

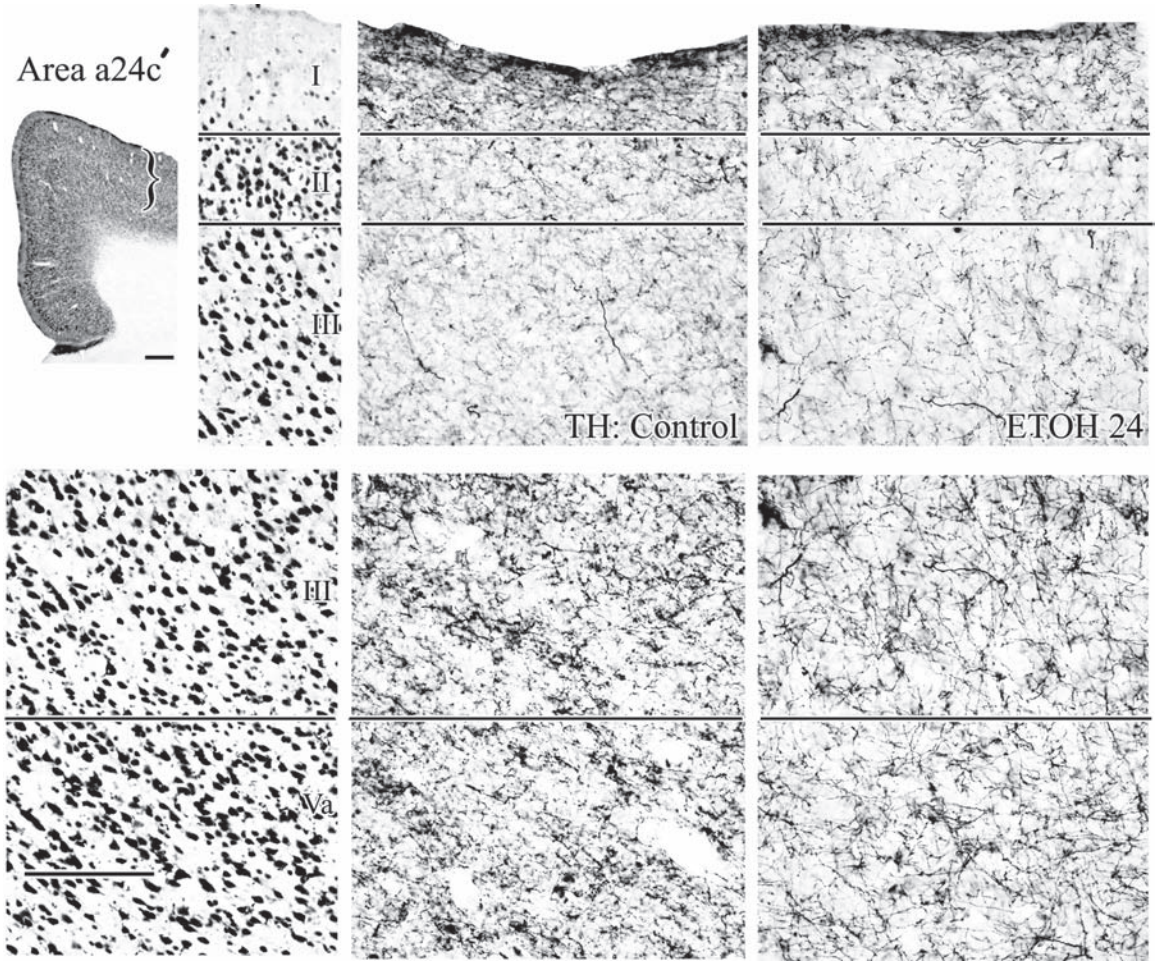

Fig. 7.9 TH immunohistochemistry showing axonal plexuses in an Et24 animal compared to a control. The neuron-specific nuclear binding protein reacted (NeuN) coronal section shows the position of magnified cortical strips for layers I-superficial III and deep layers III-Va. The loss of heavily varicose plexi is particularly evident in deep layer III and layer Va when compared to similar sections in the control case. Calibration bars; coronal section, 500 μm; cortical strips, 200 μm.

DA is a critical player in the developing nervous system and specifically the cingulate cortex. It is involved in establishing the cortical anlage wherein DA is used as an autocrine/paracrine messenger. The subsequent development of a complex array of DAergic synapses apparently overlays, but does not replace this early developing system. Proper development of these two systems, the focused (synapse-mediated) and diffuse (autocrine/paracrine) DA-mediated systems are susceptible to toxins such as cocaine and ethanol. Parallels between the effects of prenatal exposure to cocaine and ethanol on the developing cortex, regardless of the species, are remarkable. These include changes in cell proliferation and defects in neuronal migration. They show that central events of early development are under DA regulation. Moreover, understanding the action of these toxins provides an appreciation of mental dysfunction associated with diseases such as schizophrenia and mental retardation as well as providing a unique insight into normal development.

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