CHAPTER 31

Course and Pattern of Cingulate Pathology in Schizophrenia

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Chapter contents

Schizophrenia is a psychiatric disorder that has attracted the greatest interest by neuroscience. As early as 1919, Emil Kraepelin provided the first systematic descriptions of its symptoms and, in so doing, concluded that schizophrenia probably involves an organic process in the brain. A serious impediment to neuropathological studies of schizophrenic and bipolar disorder that historically has discouraged many investigators from studying this disorder has been the lack of hallmark histopathological features that could serve as a focus of investigation. Patients with schizophrenia characteristically demonstrate a slower deterioration during the first 10 years of illness, but then go on to live a full life with stable deficits. The mechanisms that give rise to schizophrenia likely involve alterations of normal brain circuitry that progress initially and later persist as a stable entity for an indefinite period of time, whereas those for bipolar disorder may show progression later. Taken together with the lack of any obvious brain changes, these clinical observations imply that these disorders may involve rather subtle abnormalities in neural circuitry. As discussed below, the diverse symptoms of schizophrenia and bipolar disorder make it unlikely that only one brain region is involved in the pathophysiology of this disorder. This issue is explored in detail in the discussion that follows by examining various functions that are abnormal in this disorder. The ones focused on represent the 'core' of schizophrenia that usually persists after the more apparent, though less specific, hallucinations and delusions have remitted following treatment with anti-psychotic medication. Similar categories of behavior are affected in almost all forms of psychopathology, such as bipolar disorder. In schizophrenia, these core symptoms are said to constitute a 'defect state' that many believe is the essence of this disorder and the source of the marked functional impairments.

Goals of this Chapter

This chapter addresses the question of why anterior cingulate cortex (ACC) is an important brain region to investigate in relation to schizophrenia and other psychiatric disorders. It begins with a description of some core features of this disorder and discusses how this region and other limbic and neocortical areas with which it is connected can be implicated in its pathophysiology as shown in Figure 31.1. Finally, recent postmortem findings in this region are described and a model is proposed for how cingulate circuitry may be altered in both schizophrenia and bipolar disorder. As schizophrenia and bipolar disorder both present during adolescence and early adulthood, some believe that late postnatal maturational changes may 'trigger' the onset of symptoms. To explore this possibility, the postnatal development and plasticity of both intrinsic and extrinsic fiber systems with the anterior cingulate region are discussed.

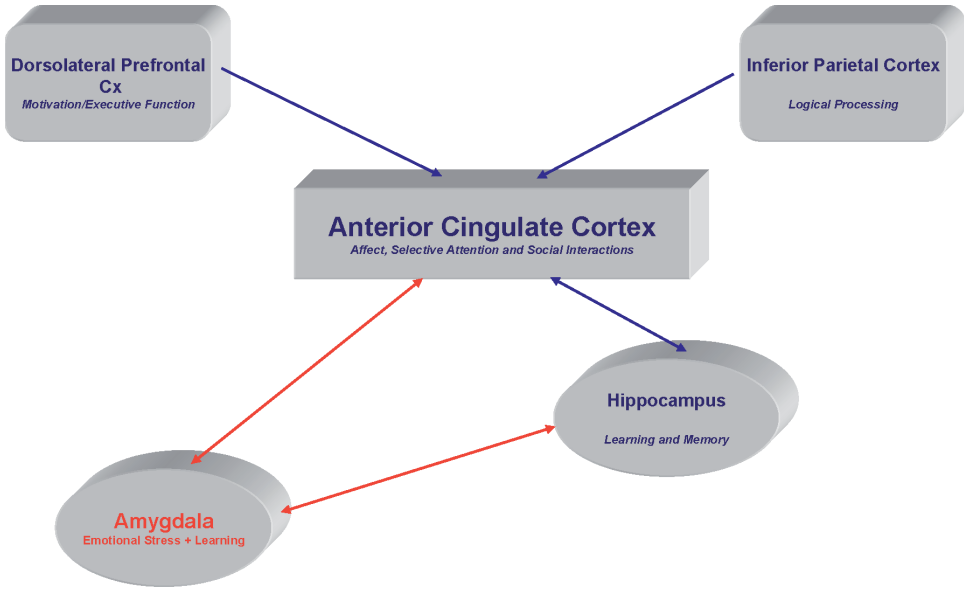

Fig. 31.1 The corticolimbic system in mammalian brain. The anterior cingulate cortex (ACC) plays a central role in processing affective and selective attentional responses that involve motivation, learning and logical processing. These functions are mediated through the dorsolateral prefrontal cortex, hippocampus and inferior parietal area, respectively. The amygdala, particularly its basolateral division, integrates stress responses in this system and sends major projections to the ACC and hippocampal formation.

The Corticolimbic System and Symptoms of Schizophrenia

The symptoms of schizophrenia typically involve changes of emotion, motivation, attentional responses, sociability, and reasoning (Fig. 31.1). In a broad sense, the behaviors disturbed in schizophrenia are also central features of personality, an entity that can be defined as the intrinsic constitution or endophenotype of the individual, together with the total set of experiences of that individual to the surrounding environment. It has been suggested that alterations of temperament can be induced in both monkeys (Ward, 1948a,b) and humans (Tow & Whitty, 1953) following surgical ablations of certain corticolimbic regions, such as the ACC. In the discussion that follows, several features of personality, known to be abnormal in psychotic disorders, will be considered in relation to the ACC and the other corticolimbic areas with which it is extensively connected.

Affectivity

A hallmark feature of schizophrenia, particularly when it takes on a chronic course, is a progressive disappearance of affect (Bleuler, 1952). Schizophrenics characteristically show a facial expression that lacks emotion, a reflection of their inability to generate appropriate affective responses to surrounding events. In some cases, an affective response is noted but is inappropriate; in others, it may be 'shallow'. In general, schizophrenics are unable to tolerate a high degree of emotion expressed by others around them; such an occurrence seems to predispose them to relapse (Barrelet *et al.*, 1990).

As depicted in Figure 31.2, a variety of studies have demonstrated that the anterior cingulate region is a true 'visceral' cortex, that is, it has direct descending and ascending connections with structures at brainstem and spinal cord that modulate the activity of the parasympathetic (dorsal vagal nucleus) and parasympathetic (intermediolateral cell column) nervous systems (for a review, Neafsey, 1993). In addition, the periaqueductal gray **(PAG)** mediates rage responses at midbrain levels and the activity of the larynx and pharynx by the nucleus ambiguus at pontine levels. In turn, visceral afferents from the peripheral autonomic end-organs, including the heart, blood vessels, gastrointestinal tract and bladder, carry sensory information back to the nucleus solitarius at medullary levels and these eventually influence the processing of information in the anterior cingulate region. Accordingly, changes in the conscious experience of affect probably involve this important visceral cortical region and disturbances in its circuitry could certainly result in abnormal affective behaviors.

The participation of the amygdala in emotional responses was first highlighted by the work of (Kluver & Bucy, 1937) in which temporal lobe lesions in monkeys were associated with 'psychic blindness' in which events seem to lose their emotional implications.

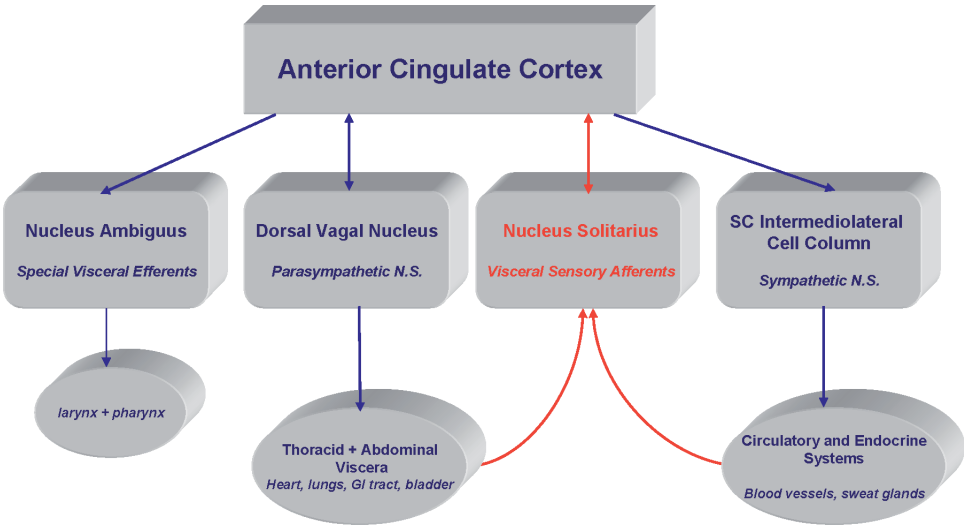

Fig. 31.2 A schematic diagram depicting the corticobulbar and corticospinal connections of the anterior cingulate cortex (ACC) with various nuclei at brain stem and spinal cord levels, including the nucleus ambiguus, dorsal vagal nucleus, and intermediolateral cell column. All three of these nuclei, in turn, send visceromotor projections to peripheral autonomic structures that include the larynx and pharynx, abdominal viscera and cardiovascular system, respectively. The nucleus solitarius relays viscerosensory information back to the ACC.

Although the brain mechanisms associated with emotion continued to elude study by the neuroscience community, the past 10 years has brought a renaissance of interest in this area. For example, it is now well recognized that the amygdala plays a central role in fear conditioning (Ledoux, 2000). Although, many aspects of these responses involve the subcortical connectivity of the amygdala, the participation of the ACC is probably equally critical. This is not surprising given the extensive connectivity that exists between these latter two regions (Van Hoesen *et al.*, 1993; Chapter 6). It is becoming increasingly clear that the affective responses and selective attention normally associated with anterior cingulate function may be driven, at least in part, by discharges of activity from the basolateral nuclear complex. Together, they comprise a critical arms of the great limbic lobe (Broca, 1878).

Selective Attention

Bleuler (1952) was perhaps the first to emphasize that schizophrenics have great difficulty in selective focusing and this is reflected in their low scores on the continuous performance task (CPT; Kornetsky & Orzack, 1978). This loss of selective attention in schizophrenia may be due to a defective central filtering mechanism (Detre & Jarecki, 1971) that gives rise to 'over-inclusive' thinking (Cameron, 1938; Payne & Friedlander, 1962). Typically, patients with schizophrenia cannot distinguish relevant objects in their perceptual field from irrelevant objects, a defect that seems to arise early in the course of the illness and one that might involve an impairment of inhibitory mechanisms (McGhie & Chapman, 1961; see below). Some believe that the cingulate and parietal cortices may cooperate in the performance of directed attention (Mesulam, 1983). In monkeys, lesions of the ACC bilaterally have been associated with neglect of surrounding objects and even cage mates (Glees *et al.*, 1950). A similar syndrome has been observed in cats with cingulate ablations (Kennard, 1955). In humans with bilateral infarction of the cingulate gyrus, a lack of attentiveness to the surrounding environment has been observed (Laplane *et al.*, 1981). Moreover, a recent cerebral blood flow study reported that human subjects show a marked increase of activity in the anterior cingulate region during performance of a Stroop attentional conflict paradigm (Pardo *et al.*, 1990). In schizophrenic subjects, a slower response to targets in the right, but not in the left visual fields, and attentional deficits similar to those following left hemispheric lesions were also noted (Posner *et al.*, 1988). It is noteworthy that abnormalities of smooth pursuit eye movements also have been found in schizophrenics and in their first-degree relatives, and were thought to reflect a 'latent trait' for this disorder (Holzman *et al.*, 1988). The neglect occurring with lesions of the cingulate cortex is thought to involve alterations in the relationship of the cingulate region with frontal eye field in area 8 (Belaydier & Maugierre, 1980), but this may be an indirect effect mediated through connections of this region with the prefrontal and inferior parietal areas. Nevertheless, the smooth pursuit abnormalities seen in schizophrenic subjects may reflect a role of frontal eye field 8 in the attentional deficits also seen in patients with this disorder. Patients with unilateral neglect syndromes arising from lesions of the frontal or parietal regions also show some emotional disturbances and these defects are consistent with a 'parallelism in the integrity of attention and emotion' (Mesulam & Geschwind, 1978, pp. 252), a concept that Bleuler first suggested to be pertinent to our understanding of schizophrenia.

Motivation

A defect of motivation is another core feature of schizophrenia. A model for understanding a loss of motivation and interest comes from the description of massive frontal lobe lesions resulting in the apathico-akinetico-abulic syndrome (Luria, 1973). As in schizophrenia, individuals with such lesions are passive, lack desires, and have poor hygiene (Luria, 1973). In some studies, schizophrenics who failed to activate cerebral blood flow in the dorsolateral prefrontal cortex also performed poorly on the Wisconsin Card Sort, a functional marker for this area (Weinberger *et al.*, 1986), and the differences did not appear to be related to either poor attention or global cortical dysfunction (Berman *et al.*, 1986). Using near infra-red imaging, studies of executive function using the Stroop interference paradigm have demonstrated significant differences between children and adults in the prefrontal area (Schroeter *et al.*, 2004). Presumably, significant changes are probably occurring during adolescence when schizophrenia typically begins. Accordingly, the timing of such developmental changes could play a critical role in determining the nature of the clinical deficits noted in patients with schizophrenia.

Bleuler may have been the first to suspect a link between affect and motivation. He wrote, 'The will, a resultant of all the various affective and associative processes, is of course disturbed in a number of ways, but above all by the breakdown of the emotions' (Bleuler, 1952, pp. 70). The interaction of motivation and affect in humans is illustrated by a distinct syndrome called 'akinetic mutism,' seen in patients with bilateral destructive lesions of the ACC (Barris & Schumann, 1953). Acute infarctions of this type are associated with an inability to move or speak, as well as considerable negativism. This is quite similar to the catatonic state in which muteness, lack of movement, and negativism are also observed. Patients who had akinetic mutism arising from bilateral occlusion of the anterior

cerebral arteries and who later recovered, have described a sudden loss of the experience of affect and a concomitant absence of the will to move (Damasio & Van Hoesen, 1983). A similar concurrence of defects in motivation and emotional experience, as seen in schizophrenia, could arise from a disturbance in communication between the dorsolateral prefrontal area and the ACC. A recent imaging study has demonstrated that there is a reduced correlation of structural parameters between the cingulate gyrus and prefrontal area (Mitelman *et al*., 2005), a change that implies that there is probably an imbalance in the functional interactions between these two regions in schizophrenia.

Sociability

Schizophrenics have great difficulty engaging in relationships with other people, and this results in a marked degree of isolation. It is no surprise then that these patients rarely marry and raise families. Female schizophrenics, in general, are unable to nurture their children. The inability of these patients to 'relate' to others and share empathetically in their feeling states is probably central to their impaired social skills, both within family units and outside them.

The cingulate gyrus has been implicated in the mediation of interpersonal relations because ablations of this region are associated with a loss of maternal activities, such as nursing, nest building, and retrieval of the young (Slotnick, 1967; Stamm, 1955). It has been suggested that separation calls and play activities, key features associated with the appearance of social interaction, may have emerged in parallel with the development of the cingulate gyrus during the phylogenetic progression of reptiles into mammals (MacLean, 1985). MacLean has suggested that separation calls that also first appeared in mammals may be mediated by the cingulate gyrus. In support of this, vocalizations can be elicited by stimulation of the anterior cingulate region in monkeys (Smith, 1945). More extensive ablations that also include the medial prefrontal cortex (mPFCx) and the pregennal cingulate cortex result in a complete loss of spontaneous isolation calls (MacLean, 1985; Chapter 15).

If the cingulate gyrus plays a role in social interactions, it is possible that the unrelatedness and poor social skills typically observed in schizophrenia may also be related, at least in part, to associate defects in motivation and attentiveness. It seems likely, however, that lack of interest in and neglect of one's surroundings might also contribute to diminished interactiveness.

Logical Processing

One of the most significant core defects in schizophrenia is a formal thought disorder, in which there are illogical sequences of unrelated concepts. Attempts have been made to characterize the nature of the defective thinking found in schizophrenic patients. Toward this end, methodologies for evaluating the central processing of incoming information have been developed to study the thought disorder in this disorder. One such study characterized the abnormalities of central integration in schizophrenics as involving serial processing of information, but with a limited channel capacity (Callaway & Naghdi, 1982). When an informational target stimulus is followed at varying intervals by a non-informational masking stimulus, a temporal delay in a two-choice forced discrimination task is observed in schizophrenics (Saccuzzo & Braff, 1986). This information processing defect appears to be trait-dependent, rather than an epiphenomenon of the psychotic state, because individuals with the schizotypal personality profile also show it (Saccuzzo & Braff, 1986). Patients with focal left-inferior parietal lesions demonstrate a marked impairment in the ability to formulate thoughts that involve the communication of relationships among ideas (Luria, 1973). Neurons in posterior parietal cortex of monkey are activated by hand–eye coordinated movements, particularly when 'desirable' objects that can satisfy thirst or hunger are the focal points (Mountcastle *et al*., 1975). This motivationally driven response is believed to require not only limbic connections with the parietal region, but also an attentional component (Mesulam & Geschwind, 1978). Consistent with this proposal, the inferior parietal region has extensive connections with the ACC and the presubiculum (Pandya & Kuypers, 1969; Jones & Powell, 1970; Petras, 1971; Seltzer & Pandya, 1978; Seltzer & Van Hoesen, 1979). There is a unilateral neglect syndrome in which patients with right-sided posterior parietal lesions do not attend to left extracorporeal space, but show a peculiar inability to perceive their defects (Luria, 1973). The failure to perceive one's defects is commonly seen in schizophrenics and many patients do not notice that a listener is unable to comprehend what they are saying. The simultaneous occurrence of illogical thinking and neglect in schizophrenia is consistent with the idea that both the left and right inferior parietal areas might be dysfunctional in this disorder. As noted above, neglect of the surrounding environment occurs with cingulate lesions, and the coincidence of attentional problems and disturbances of thinking could reflect the extensive connections between the anterior cingulate and inferior parietal cortices.

Does Anterior Cingulate Cortex Play a General Role in Psychopathology?

Based on the above discussion of the symptoms of schizophrenia and the functions of several corticolimbic regions, it has been suggested that the ACC and other regions with which it connects, may be of central importance to the symptomatology, and possibly even the

etiology of schizophrenia. The functions collectively subsumed by these regions cover a broad range of behaviors, including affect, attention, motivation, social behavior, cognition and the influence of stress on the modulation of these behaviors. It is relevant to this discussion, to consider whether other psychiatric disorders may possibly involve alterations in similar cortical regions.

In bipolar mood disorder, as mania alternates with depression, patients may show increases or decreases of emotional expression, motivation, attention, interpersonal relations, and thinking. Manic individuals typically show euphoric affect alternating with dysphoria and irritability. When manic, there is a 'flight of ideas' and a general inability to maintain attention on any single focus, whereas depression typically drives their thinking toward pessimistic ideas. In considering the brain areas that might play a role in these mood disturbances, it is noteworthy that chronically depressed patients show improvement of their mood following bilateral anterior cingulotomy (Ballantine *et al.*, 1967; Tow & Whitty, 1953), a procedure that does not produce deficits in, and may even improve, overall cognitive function (Long *et al.*, 1978). Other psychiatric syndromes, such as panic disorder and phobias, frequently occur comorbidly with depression. Adult individuals with phobias have been found to be prone to separation anxiety as children and many were branded as school phobics during childhood (Klein *et al.*, 1978). Paul MacLean suggested that the cingulate area that might mediate separation behaviors (see above) could provide a theoretical basis for understanding this syndrome (MacLean, 1985), which also responds well to treatment with antidepressant medication. Another syndrome occurring comorbidly with depression is panic disorder, characterized by episodes of acute anxiety in which an individual experiences shortness of breath, tachycardia, profuse sweating, and pallor in response to an irrational perception of impending doom. Because the amygdala (Davis, 2000) and ACC (Anand & Dua, 1956; Kaada *et al.*, 1949) are involved in the regulation of autonomic visceromotor responses, it is possible that these regions, and/or areas with which they connect, could participate in the generation of panic attacks as well.

Postmortem Studies of Cingulate Cortex in Schizophrenia and Bipolar Disorder

Gamma-aminobutyric acid (GABA) dysfunction is believed to play a role in various neuropsychiatric disorders. For example, as early as 1972, Eugene Roberts postulated that this compound might be abnormally regulated in schizophrenia (Roberts, 1972). Schizophrenia typically involves impaired attentional responses (McGhie & Chapman, 1961) and disruptions of normal information processing (Saccuzzo & Braff, 1986; Braff *et al.*, 1991) that has been described as being 'overinclusive' in nature, that is, there is an inability to filter out extraneous information (Cameron, 1938; Payne *et al.*, 1961; Payne & Friedlander, 1962). This has lead to the speculation that an impaired central filtering mechanism may be present in this disorder (Detre & Jarecki, 1971), as schizophrenics are unable to distinguish relevant objects in the perceptual field (Matussek, 1951). Using physiological recordings from schizophrenics, decreased auditory-evoked P50 response to repeated stimuli (Adler, 1982) and defective sensorimotor gating (Braff *et al.*, 1978; Geyer *et al.*, 1990; Swerdlow *et al.*, 1994) have been noted in schizophrenics. The most consistent electrophysiological abnormality observed in schizophrenics, however, is a reduced amplitude and increased latency of the P300-evoked potential (Blackwood *et al.*, 1991). These changes are related to a diminished ability to habituate selective attentional responses to a stimulus and could reflect defective GABAergic inhibitory modulation in schizophrenia (Benes, 1999, 2000).

Microscopic Evidence for a GABA Defect in ACC in Schizophrenia

In 1991, a postmortem study in which pyramidal and non-pyramidal neurons were differentially counted using a two-dimensional (2D) method, revealed a decreased density of non-pyramidal cells in layers II–VI of the anterior cingulate area of schizophrenic and schizoaffective subjects (Benes *et al.*, 1991a). These changes are primarily significant in layer II of the schizoaffective group, suggesting the possibility that they might show a stronger covariation with affective disorder than with schizophrenia. This finding is, however, controversial as other groups using three-dimensional (3D) methods have not demonstrated a similar change in the ACC of subjects with schizophrenia (Ongür *et al.*, 1998; Cotter *et al.*, 2001).

In a subsequent replication study using a 2D method, a pattern similar to that previously reported was observed (Benes *et al.*, 2001c). The manic depressives showed approximately a 30% decrease, while the schizophrenics showed a 16% decrease. It is noteworthy that, in a *post hoc* analysis of another study in which tyrosine hydroxylase-immunoreactive (TH-IR) fibers were analyzed Benes *et al.* (1997a) also showed an 18% reduction in the density of non-pyramidal neurons in layer II of ACC (Benes, 1998). More recently, the findings from these three studies have been combined and a meta-analysis performed (Todtenkopf *et al.*, 2005). The data indicate that the reduction in the density of non-pyramidal neurons in layer II of ACC was 16% in the schizophrenics

($n = 25$), 25% in schizoaffectives ($n = 18$) and 30% in manic depressives (Benes & Todtenkopf, 1998). More recently, a meta-analysis of 3 cell counting studies in the ACC using 2D methodologies and one using 3D optical disector counting has been reported. It indicated that the decrease of non-pyramidal cells in layer II of this region was also found when a 3D optical disector method was employed (Todtenkopf *et al*., 2005). There was a remarkable correspondence in the magnitude of the effect sizes obtained, that is, approximately 12–15% in schizophrenics and 25–35% in bipolars. Why have there been discrepancies in the cell counting findings in the ACC in schizophrenia?

There are significant methodological factors that probably contributed to these failures to replicate the reduced density of non-pyramidal cells. Not least among these are the difficulties in comparing data obtained from three dimensional (optical disector) with those obtained from two dimensional cell counting methods with those obtained with 3D techniques (Benes & Lange, 2000b). It is essential to consider the object counting methodology employed in evaluating the differences in findings reported in schizophrenia. Other differences in methodology probably contributed to these discrepancies, as a comparison of 2D and 3D methods in tissue from the same cases showed a similar reduction in the numerical density of non-pyramidal neurons (NPs) in the ACC of subjects with schizophrenia and bipolar disorder, although the latter showed the most larger effect size (Todtenkopf *et al*., 2005). Taken together, these data suggest that a loss of interneurons does occur, but is more striking in affective disorder, than in schizophrenia.

More compelling evidence for defective GABA function in schizophrenia has come from studies in which a variety of cytochemical and molecular approaches have been employed. For example, when a high resolution technique was employed to study the distribution of the $GABA_A$ receptor, a highly selective increase of binding was observed on pyramidal, but not non-pyramidal neurons in layers II and III of subjects with schizophrenia (Benes *et al*., 1992b). Tissue from bipolars was not available at that time this latter study was undertaken, but subsequent preliminary evidence pointed to a similar change in bipolars as well. Taking together the cell counting and receptor binding studies, the results were consistent with the hypothesis that a decrease of GABAergic activity is present within the cingulate cortex of schizophrenics and probably results in a compensatory upregulation of the $GABA_A$ receptor on postsynaptic pyramidal neurons.

When an *in situ* hybridization (ISH) study in which mRNA for the NR_{2A} subunit of the NMDA receptor was co-localized with mRNA for GAD67 (Woo *et al*., 2004), a pronounced reduction of double labeled neurons was observed in layer II of both schizophrenics (52%) and bipolars (35%). These decreases are of particular interest because the reduction in affective disorder is similar to that observed in previous cell counting studies (12–15%), whereas, in schizophrenics, the reductions detected with ISH were much greater than those previously reported (12–15%). Taken together, these findings suggest that a loss of GABAergic activity in bipolar disorder may be related to overt cell death, whereas in schizophrenics it may be related to cellular dysfunction in the absence of cell death. Interestingly, when *in situ* end-labeling of single-stranded DNA breaks was used as a marker for apoptosis, schizophrenics showed a pronounced 72% reduction, whereas bipolars showed no change (Benes *et al*., 2003; Fig. 31.3). This study

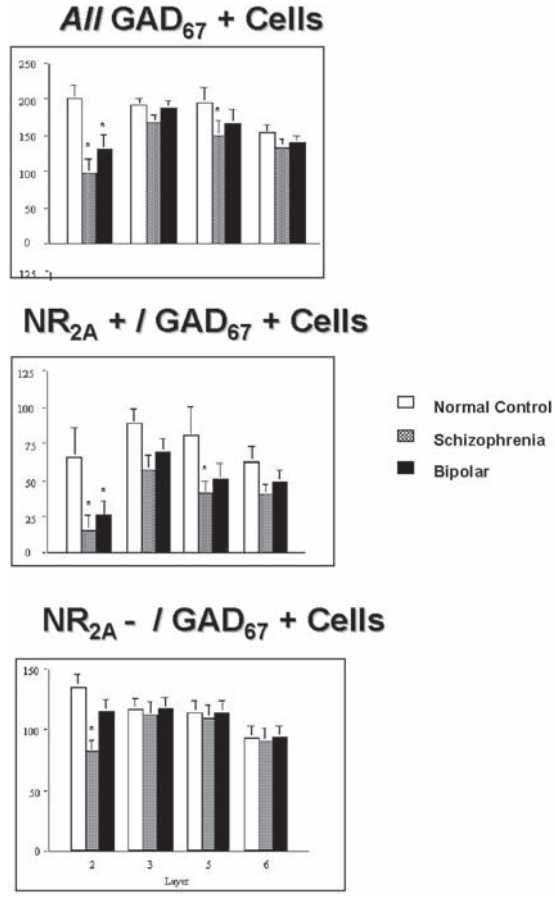

Fig. 31.3 Graphs showing the results of a double *in situ* hybridization study in which mRNA representing GAD_{67} and the NR_{2A} subunit of the NMDA receptor were co-localized in a cohort of normal controls, schizophrenics and bipolars. There is a highly selective reduction in the number of GAD67-positive cells in layer II of both schizophrenic and bipolar subjects, whether or not they are expressing mRNA for the NR_{2A} subunit. However, the double-labeled cells show the largest reductions, suggesting that GABA cells that receive NMDA-mediated excitatory inputs may be more vulnerable to either reduced expression of the respective messages or possibly even to apoptotic cell death (see text for details).

suggested the possibility that apoptosis is probably not occurring in schizophrenia. Using gene expression profiling, a recent study has demonstrated a marked increase in expression of apoptosis genes in the hippocampus of subjects with bipolar disorder, whereas in schizophrenics, the expression of similar genes was down-regulated (Benes *et al.*, 2004).

Dopamine Inputs to GABA Cells in Schizophrenia

The dopamine (DA) system has long been suspected of playing a role in the pathophysiology of schizophrenia (Kety & Matthysse, 1972), even though convincing empiric evidence for a primary defect in this system has been lacking (Carlsson, 1978). Seymour Kety was the first to suggest, however, that subtle changes in connectivity could occur in the absence of any discernible biochemical alterations (Kety, 1959). Using antibodies against TH to localize DA fibers (Lewis *et al.*, 1987; Gaspar *et al.*, 1989; Noack & Lewis, 1989; Samson *et al.*, 1990; Williams & Goldman-Rakic, 1993), a study of the distribution of TH-IR varicosities in the anterior cingulate of schizophrenic brain has suggested that there may be a decrease of these fibers on pyramidal neurons, but an increase on interneurons in layer II of the ACC; this change was not observed in the prefrontal cortex (Benes *et al.*, 1997a). In layers V and VI, there was a significant reduction in the density of TH-IR varicosities and this compares favorably with an analysis of fiber length in the prefrontal cortex of schizophrenics where a significant reduction was found in layer VI (Akil *et al.*, 1999). In the ACC, however, this change was only found in patients treated with neuroleptic drugs, whereas the apparent shift of TH-IR varicosities from pyramidal to non-pyramidal cells of layer II was found in all schizophrenic subjects, whether or not they were treated with these drugs (Benes *et al.*, 1997a). As shown in Figure 31.4, a subsequent *post hoc* analysis of the data yielded different working models that lead to the conclusion that these data were best explained by a trophic shift of TH-IR fibers from pyramidal to non-pyramidal neurons. The model strongly suggested that a loss of GABAergic interneurons was not required for this pattern to occur, although such a change could co-exist with a trophic shift. If these findings were correct, they would suggest that dopaminergic afferents might be providing a non-adaptive hyperinnervation of a subpopulation of GABAergic interneurons, perhaps ones that are intrinsically impaired in schizophrenia. As DA appears to exert an inhibitory effect on cortical GABA cells (Retaux *et al.*, 1991), these findings would predict that an excessive release of DA under conditions of stress (Thierry *et al.*, 1976; Roth *et al.*, 1988) could lead to an impairment of GABAergic function and ultimately to a decompensation of the intrinsic circuitry in layer II of ACC (Benes, 1997).

Evidence for Glutamatergic Dysfunction

It is well known that phencyclidine (PCP) exposure in normals can results in a schizophrenia-like psychotic state, whereas in schizophrenics, it results in an immediate exacerbation of pre-existing psychotic symptoms (Javitt & Zukin, 1991). Based on data obtained from a rodent model using PCP-induced neurotoxicity, Olney and Farber (1995) suggested that GABA cell dysfunction in the cingulate region might be related to a hypofunctioning of NMDA-mediated glutamatergic activity. In an attempt to integrate the PCP model with the growing evidence that the GABA system is dysfunctional in schizophrenia, it was suggested that NMDA hypofunction was specifically occurring on GABAergic interneurons and causing a reduction in the amount of inhibitory modulation provided to excitatory projection neurons.

In contrast to the Olney–Farber model derived from rodent studies and clinical observations, postmortem studies of the ACC have demonstrated changes suggestive of an increase of glutamatergic activity. Specifically, axons visualized with antibodies against phosphorylated epitopes of the 200K neurofilament protein were found to be increased by 25% in layer II and upper portions of IIIa in the ACC of schizophrenics (Benes *et al.*, 1987a); this change occurred selectively for vertical, but not horizontal axons in this region. A subsequent study using antibodies against glutamate demonstrated a much more robust increase of 74% for vertical axons in the same laminae of the anterior cingulate region (Benes *et al.*, 1992a). As discussed below, this change could be potentially related to other abnormalities in the GABA system in layer II of this region.

It is noteworthy that cells coexpressing both GAD_{67} and the NR_{2A} subunit of the NMDA receptor show a much more robust reduction than cells expressing only GAD_{67} in schizophrenics (Woo *et al.*, 2004). One interpretation of this finding is that GABA cells receiving NMDA-mediated glutamatergic activity might receive excessive incoming glutamatergic activity and thereby be more prone to oxidative stress and apoptosis. Such an interpretation is compatible with the NMDA hypofunction model, except that the reduced amount of NMDA receptor activity may be viewed as being a secondary or compensatory change, rather than a primary one.

Model for Altered Neural Circuitry in ACC of Schizophrenics

As depicted in Figure 31.5, postmortem studies of layer II in the ACC have reported a reduced density of NPs (Benes *et al.*, 1991b, 2001d; Todtenkopf *et al.*, 2005), increased $GABA_A$ receptor binding activity on pyramidal neurons (Benes *et al.*, 1992b) and an increase of TH-IR varicosities on non-pyramidal neurons, but a

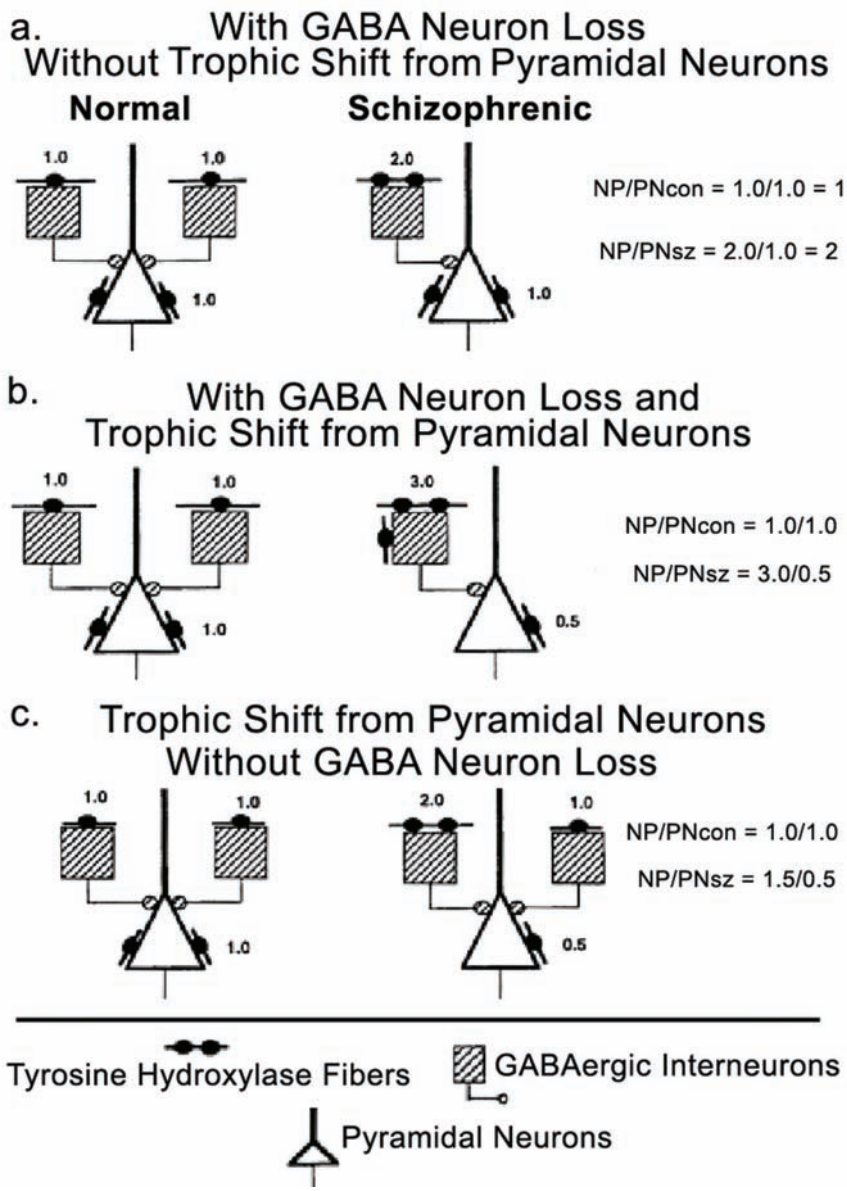

Fig. 31.4 Models generated from studies of the interaction of tryosine-hydroxylase-immunoreactive fibers with pyramidal (PNs) and non-pyramidal (NPs) neurons in ACC of normal controls and schizophrenics (Benes, 1998). The size of PNs and NPs was normalized according to the empirically derived cell areas. The number of TH-IR varicosities per cell was adjusted in proportion to the values determined. The three mechanisms considered in the diagram are (a) GABA neuron loss alone, (b) GABA neuron loss, together with a trophic shift of TH varicosities from PNs to NPs, and (c) a trophic shift of TH varicosities from PNs to NPs with no loss of GABA cells. A ratio of the density of TH-IR varicosities on NPs versus PNs (NP/PN_{Con} and NP/PN_{SZ}) was computed for the control and schizophrenia groups, respectively, using each model. These numbers were compared with those empirically obtained from the microscopic analysis (Benes *et al.*, 1997a). Only the last model yields a theoretical ratio (3.0) virtually identical to that obtained for the TH-IR fibers staining in layer II of the ACC of schizophrenia subjects. This suggests that GABA cell loss is not necessary to explain an increase of dopaminergic inputs to remaining interneurons in layer II of schizophrenics.

decrease on pyramidal cells (Benes *et al.*, 1997b). Taken together, these findings have suggested a model in which pyramidal neurons in layer II of the cingulate cortex receive are conceptualized as receiving inadequate amounts of inhibitory modulation from GABAergic interneurons. Because, dopaminergic afferents are believed to exert an inhibitory influence on cortical neurons, a sprouting of these fibers could worsen this situation by causing the GABA cells to fire even less. If such fibers are providing excessive excitatory drive to the same pyramidal neurons and possibly also the GABAergic cells, the overall amount of excitatory activity in the circuit shown in Figure 31.3 could push the GABA cells toward oxidative stress, and as discussed above, apoptosis and perhaps even cell death. Such a mechanism seems plausible in the setting of data suggesting that NMDA receptor expression is preferentially down-regulated in relation to GABAergic interneurons in layer II of the cingulate region (Woo *et al.*, 2004).

Figure 31.6 shows the repeated observation that there are preferential changes in layer II of the cingulate

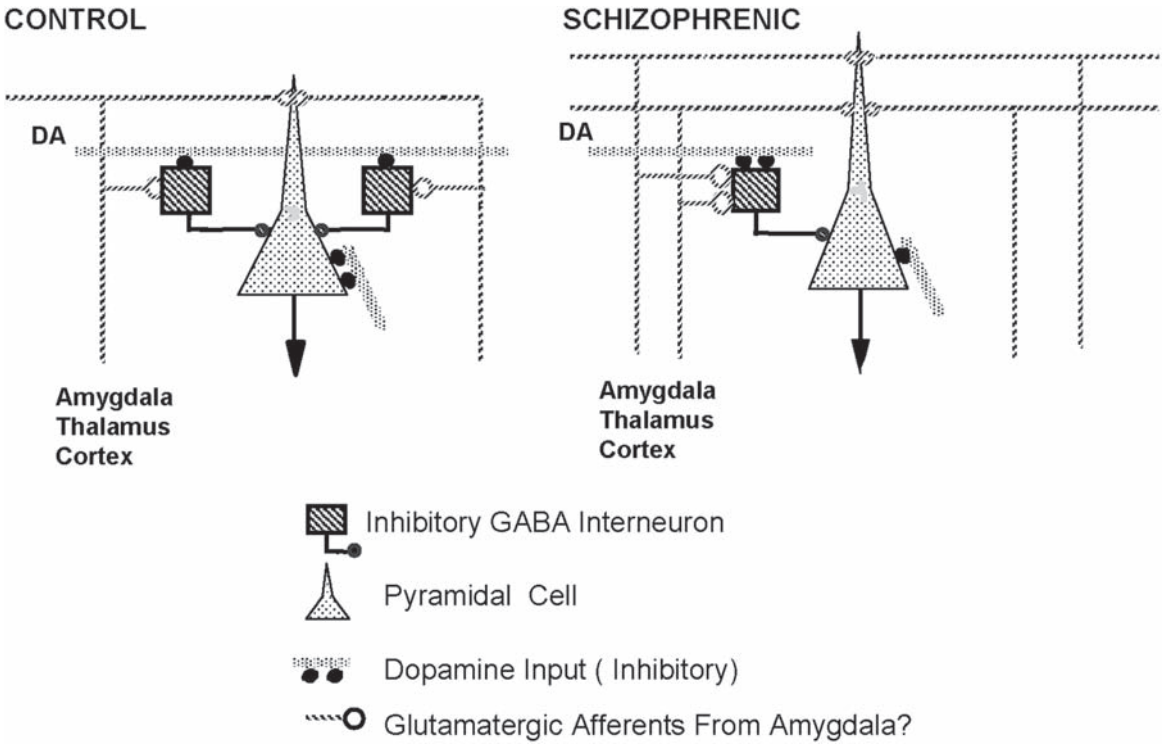

Fig. 31.5 A model depicting changes in the intrinsic circuitry of layer II in ACC of subjects with schizophrenia. In normal controls (left), pyramidal neurons receive excitatory afferents from a variety of cortical and subcortical regions that include the dorsolateral prefrontal cortex, amygdala and thalamus. At the same time, these projection neurons receive an inhibitory input from GABAergic interneurons. These latter cells, in turn, receive an inhibitory modulatory influence from dopaminergic projections. In the schizophrenic circuit (right), evidence from postmortem studies suggest that there are excessive numbers of excitatory afferents, possibly originating in the thalamus, basolateral amygdala or other cortical regions, and projecting toward layers I and II. Under conditions of emotional stress, these fibers could induce excitotoxicity. As these inhibitory interneurons begin to fail metabolically, they may possibly succumb to apoptotic cell death (Benes, 2000).

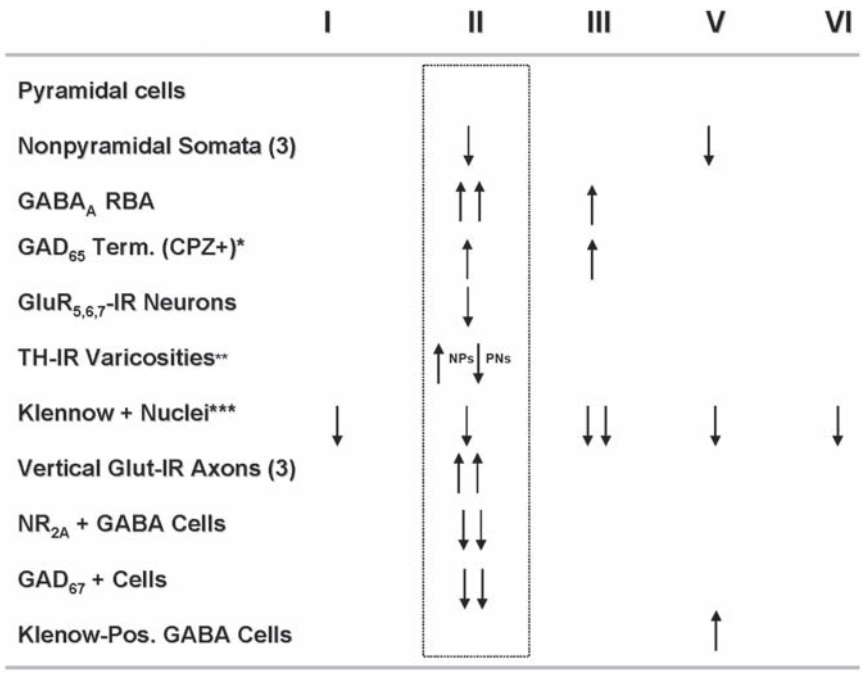

Fig. 31.6 Tabulation of postmortem studies in ACC using a variety of approaches and markers, a preponderance of alterations have been found in layer II of ACC. The studies in this figure include: (Benes & Bird, 1987; Benes *et al.*, 1987b, 1991b, 1992b,c, 1997b, 2000b, 2001b 2003; Woo *et al.*, 2004).

cortex suggesting the possibility that the changes noted in schizophrenia might be related in some way to projections from the basolateral amygdala which sends a 'massive' input to this specific site (Van Hoesen *et al.*, 1993). Also noteworthy is the finding that the same subdivision of the basolateral amygdala that projects to layer II of the ACC also projects to sectors CA2/3 of the hippocampus (for a review, see Benes & Berretta, 2001). Several postmortem studies have also shown abnormalities in these sectors in schizophrenia including increased $GABA_A$ binding (Benes *et al.*, 1996b, 2001a), an uncoupling between $GABA_A$ and benzodiazepine binding (Benes *et al.*, 1997), a decrease of non-pyramidal cells, neuroleptic-related increases in GAD_{65}-containing terminals (Todtenkopf & Benes, 1998), reductions in the distribution of TH-IR varicosities (Benes & Todtenkopf, 1999), and a reduction of immunoreactivity for the $GluR_{5,6,7}$ subunits of the kainate receptor on pyramidal cell dendrites (Benes *et al.*, 2001a).

As shown in Figure 31.7, these observations suggested a 'partial' rodent model for schizophrenia in which increased activation of the basolateral amygdala might be responsible for the induction of abnormalities in layer II of the ACC and sectors CA3/2 of the hippocampus (Benes & Berretta, 2001). Although, most reports to date have focused on the acute (Berretta *et al.*, 2001) and long-term (Berretta *et al.*, 2004) effects of picrotoxin infusion in the BLa on GABA cells in the hippocampus, more recent work have been conducted in the ACC. Preliminary studies in the anterior cingulate region of rats receiving chronic infusion of picrotoxin in the BLa, have demonstrated a significant reduction of calretinin-containing interneurons in layer II (Fig. 31.8 postnatal development; see below). Consistent with this finding is the observation that rats receiving similar infusions of picrotoxin in the basolateral amygdala between P30 and P35 show a decreased amplitude of GABA currents recorded from pyramidal neurons in layer II of this region (Fig. 31.9). Overall, these studies support the viewpoint that projections from the BLa may play a role in the induction of GABA cell abnormalities in layer II of the ACC.

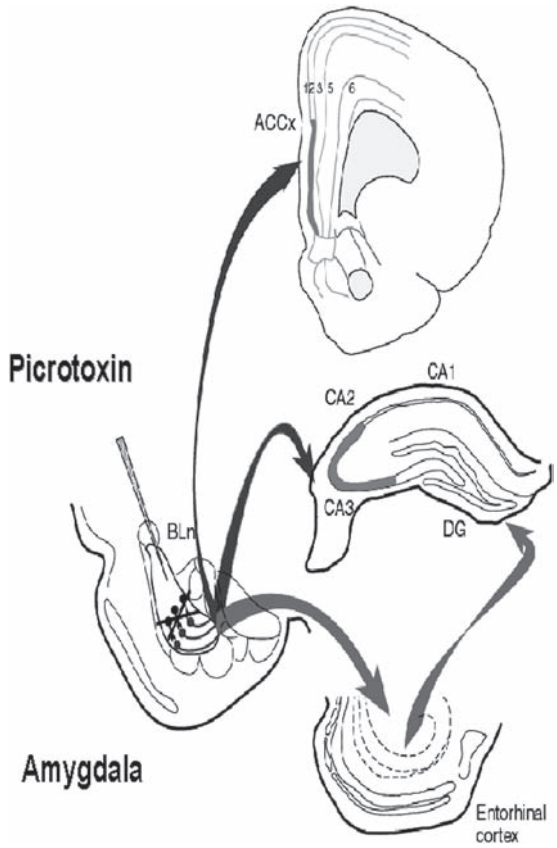

Fig. 31.7 'Partial' modeling for dysfunctional interactions between basolateral amygdala (BLa) and the anterior cingulate cortex (ACC) and hippocampus (HIPP). The BLa sends a 'massive' direct projection to layer II of ACC and sectors CA3/2 of the HIPP (left). Using a stereotaxic placement of an indwelling cannula and infusion of picrotoxin into the BLa, the influence of amygdalar excitation on GABA cells of ACCx and HIPP has been studied in awake, freely moving adult rats. Adapted from Benes and Berretta (2000).

Potential Influence of Pre- and Postnatal Stress on Cingulate Circuitry in Schizophrenia

The role of stress in the induction of changes in the cortical GABA system in schizophrenia and bipolar disorder is an interesting issue to explore. For example, glucocorticoid hormones have the ability to bind to the $GABA_A$ receptor (Sutanto *et al.*, 1989) and have been found to directly increase its activity (Majewska *et al.*, 1985; Lambert *et al.*, 1987). It is important to point out, however, that stress is believed to *increase* rather than decrease the activity of the GABA system (Woodbury, 1952; Feldman & Robinson, 1968; Pfaff *et al.*, 1971; Miller *et al.*, 1978), although it is possible that chronic stress, particularly when preceded by stress in utero, might result in an eventual decrease in the activity of this transmitter system. This possibility is particularly intriguing when the marked sensitivity of GABAergic neurons to excitotoxic injury (Schwarcz & Coyle, 1977) is taken into account. It is believed that cell death in this setting probably requires both an increase of excitatory activity and an increased release of glucocorticoid hormone (Sapolsky, 1992).

Another important component to the stress response is the increased release of DA that occurs in the mPFCx (see above). Relevant to this discussion is the fact that,

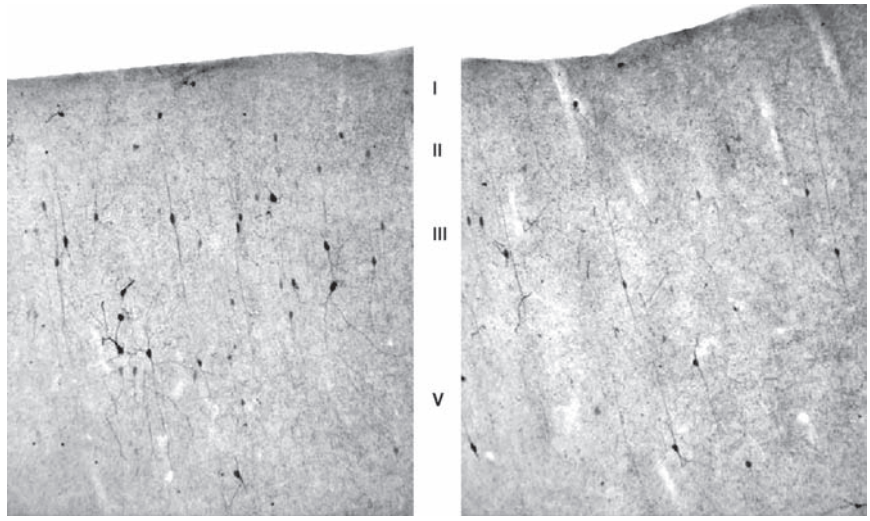

Fig. 31.8 Photomicrographs of calretinin-containing interneurons in layer II of the anterior cingulate cortex from rats treated chronically with either vehicle (left) or picrotoxin (right) infusion in the basolateral amygdala. The picrotoxin-treated rat shows a marked reduction in the number of calretinin-containing neurons when compared with the vehicle treated.

an increase of DA varicosities forming appositions with GABAergic interneurons has been induced by exposing rats both pre- and postnatally to stress-related doses of corticosterone (Benes, 1997). Thus, it is possible that the postnatal maturation of GABA cells in the cingulate cortex may be normally influenced by the in growth of DA fibers, but abnormally affected when this occurs in individuals for whom pre- and postnatal stress are comorbid factors. In this latter case, it would have to be assumed that gene(s) involving the DA system and perhaps also cortical GABA cells would be affected by prenatal exposure to stress and would be permanently sensitized in such individuals. Based on studies using the 'partial' rodent model, the effects of stress on the cingulate cortex of subjects with psychotic disorders, such as schizophrenia and bipolar disorder, it seems reasonable to postulate that projections from the basolateral nucleus to the ACC, particularly layer II, may be a preferential site for the induction of such stress-related changes.

Postnatal Development of ACC and the Onset of Schizophrenia

Given the characteristic onset of schizophrenia between 16 and 25 years of age, it is relevant to consider whether normal developmental changes during this period may contribute to the appearance of prodromal changes. To explore this question, it is relevant to explore how the principle intrinsic and extrinsic neurotransmitter systems are changing during the equivalent of adolescence and early adulthood, when schizophrenia is beginning to present clinically. It has long been suspected that the development of corticolimbic regions of human brain may continue well beyond birth (Flechsig,

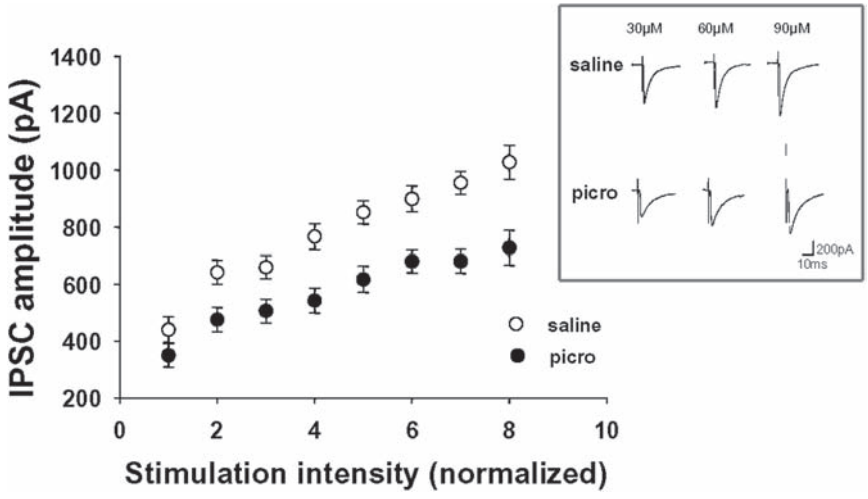

Fig. 31.9 Single-cell recordings of GABA currents from pyramidal neurons in layer II of anterior cingulate cortex from rat treated chronically with either vehicle (left) or picrotoxin (right) infusion in the basolateral amygdala. There is a significant reduction in GABA currents in adult rats receiving picrotoxin infusion in the basolateral amygdala (BLa) between P30 and P35.

1920; Yakovlev & Lecours, 1967; Benes, 1989, 1994b). This idea has received increased attention with the growing realization that normal maturational changes probably play an important role in the appearance of various neuropsychiatric diseases at specific stages of postnatal life (Benes, 1988; Weinberger, 1987). In other words, a normal ontogenetic change at a critical stage of development could potentially act as a 'trigger' for the onset of a given disorder at that stage. Consistent with this concept, many studies in rodent brain have demonstrated that there are significant changes in several key neurotransmitter systems that occur at key stages of the postnatal period (for comprehensive reviews of developmental neurochemical findings, see Parnavelas *et al.*, 1988; Johnston, 1988).

GABA System

GABA has long been considered the most important inhibitory neurotransmitter in the mammalian brain and extensive neurochemical studies of its development in rodent brain suggest that its maturation continues well into the postnatal period (Johnston, 1988). For example, GABA-accumulating cells show a progressive increase in numerical density until P11 (Chronwall & Wolff, 1980). In contrast, the concentration of GABA and the specific activity of GAD (Coyle & Enna, 1976), as well as GABA receptor binding activity (Coyle & Enna, 1976; Palacios *et al.*, 1979), and the mRNA for this receptor (Gambarana *et al.*, 1990), all increase until the third postnatal week.

At birth, the numerical density of GAD-IR somata in the ACC reaches a peak at approximately postnatal day 5 (PN5), then diminishes until PN20 when the thickness of the cortical mantle is maximal (Vincent *et al.*, 1995). During this same period, the relative amount of neuropil surrounding all cell bodies in this region is expanding as dendritic and axonal fibers are increasing. As shown in Figure 31.10, GABAergic terminals show a gradual increase in their number, and by P20–40, they attain a maximum numerical density distribution. Accordingly, the neuropil in rat mPFCx shows a gradual expansion, probably involving an increase of both

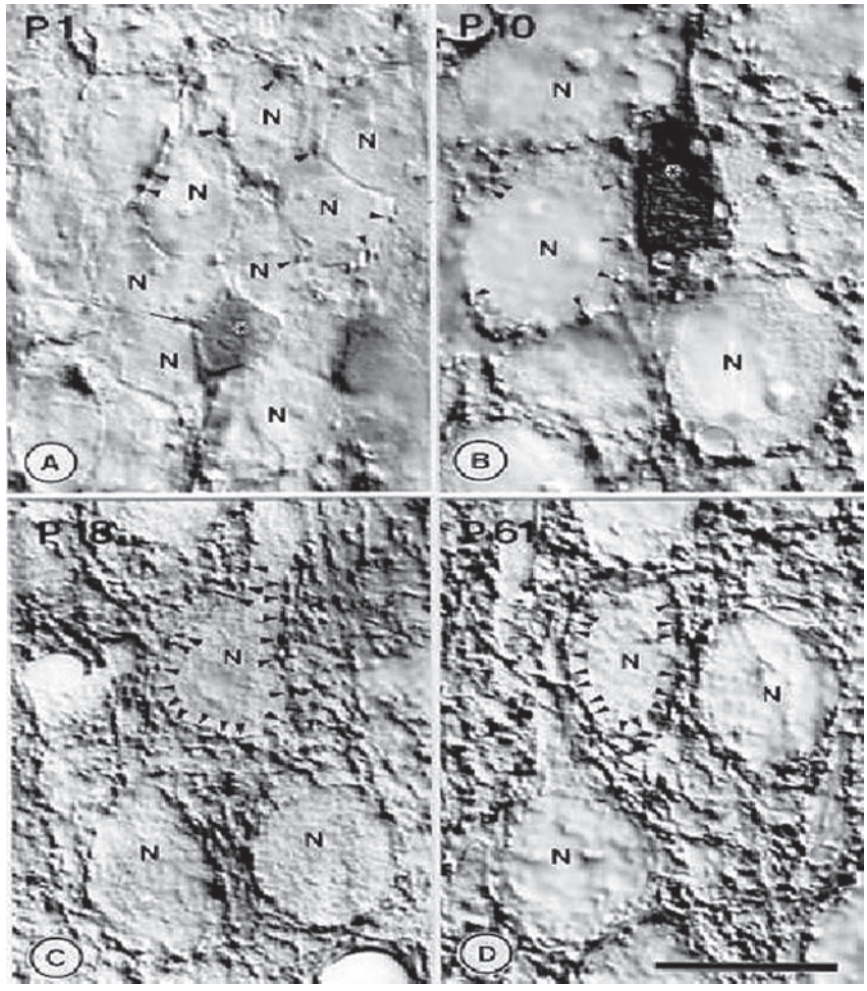

Fig. 31.10 Postnatal development of GABA-immunoreactive terminals in rat ACC. At postnatal day 1 (P1), there is a paucity of GABA terminals, but the numbers show progressive increase at P10 and P18 and are maximal at approximately P25 (not shown). At P61, the GABA terminals in ACC show a typical adult pattern of distribution. N, neuronal somata; arrowheads, GABA terminals. Adapted from Vincent *et al.* (1995).

dendritic branches and terminals of GABAergic neurons, a process that continues in the superficial layers until P25 (Vincent *et al*., 1995) when the efficacy of GABAergic synaptic transmission also becomes optimal (Luhmann & Prince, 1991). In general terms, the maturation of the GABA system continues for approximately 2–3 weeks postnatally. As such, its full maturation within mPFCx is probably complete *before* the DA system attains its full postnatal profile. Presumably, then, the dendritic branches of GABAergic interneurons lay in waiting for ingrowing DA fibers to target them for the formation of functional interactions.

Important questions regarding the role of a neurodevelopmental disturbance in the induction of altered phenotypes of GABA cells are when and how such changes manifest during the life cycle in individuals who carry the susceptibility genes for schizophrenia and bipolar disorder. One possibility is that the GABA cells are abnormal from birth; however, the clinical observation that most subjects with schizophrenia are relatively normal during childhood and early adolescence argues against this possibility. As discussed above, the cortical GABA system continues to develop until the equivalent of early adolescence. Taking together these observations, a second possibility is that the GABA cells may be relatively normal during childhood when they are also relatively immature, but become abnormal as their maturation process is completed as putative gene(s) associated with schizophrenia or bipolar disorder begin their expression. In this latter case, it would be assumed that both disorders would share common genes and these would be capable of altering the normal functioning of GABA cells. A third possibility is that the GABA cells are either relatively normal or abnormal during childhood, but their activity is quiescent, while they await the ingrowth of extrinsic fiber systems that form functional connections with intrinsic neurons of the anterior cingulate region. As discussed below, these systems include projections from the amygdala and the ventral tegmental area (VTA).

Ingrowth of AmygdaloCingulate Projections

Verwer *et al*. (1996), using the retrograde tracer fast blue injected into the mPFC, and the anterograde tracer, PHA-L, first suggested that various subregions of the basolateral amygdaloid nucleus may continue to grow into the mPFC until the early adult period (Verwer *et al*., 1996). During the post-weanling period (P6–P40), these authors demonstrated that the number of fast blue-labeled cells increased within the amygdala, and this change was paralleled by an associated increase in the density of amygdalofugal fibers within mPFCx. In a more recent study (Fig. 31.11), the development of the amygdalo-mPFCx innervation at distinct stages of postnatal development was quantified and it was demonstrated that there is a dynamic and robust sprouting of amygdalocortical projections during the post-weanling and early adult periods (Cunningham *et al*., 2002).

Electrophysiological studies have demonstrated that stimulation of the BLA results in a preponderance of

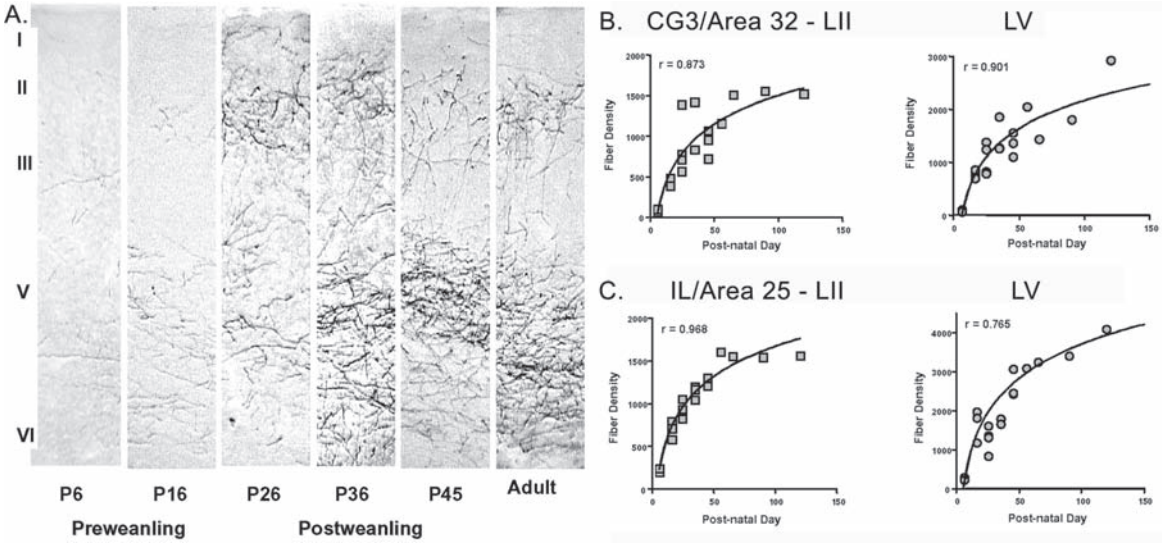

Fig. 31.11 A. Anterogradely traced fibers originating in the basolateral amygdala and projecting to rat ACC. Between the preweanling and early adult periods, there is a gradual increase in the density of BLa projections to ACC. B. Using quantitative microscopic analyses, a curvilinear increase in the BLa occurs between P0 and P60 and this occurs in both layers II and V of areas 32 and 25; L, layer. Adapted from Cunningham *et al*. (2002).

inhibitory postsynaptic potentials within the mPFCx (Perez-Jaranay & Vives, 1991). This suggests that BLa fibers may form preferential connections with GABAergic neurons. As shown in Figure 31.13, an ultrastructural analysis of anterogradely-traced BLa fibers in the ACC of adult rats demonstrated that the majority of terminations form asymmetric synapses with dendritic spines (Cunningham *et al.*, 2002), presumably associated with pyramidal neurons, although the dendrites of calretinin-containing GABA cells also have spines.

Axospinous synapses formed by BLa projections show a linear increase in density between birth and the early adult period at P60 (Fig. 31.12). Many BLa terminations are also found on aspiny dendritic shafts that are probably associated with GABAergic interneurons, but these do not show classic synaptic profiles with a postsynaptic membrane specialization. These axodendritic appositions also show a linear increase through to P60 or the early adult period. Light and confocal studies have demonstrated that BLa fibers make frequent contacts with GABAergic cell bodies and processes in rat ACC, although electron microscopic studies suggest that these are not classical synaptic profiles (Cunningham *et al.*, 2002). If oxidative stress does occur in GABA cells during the prodrome for schizophrenia, it could theoretically occur in response to direct stimulation coming from the BLa; however, this would likely be modulatory in nature. As shown in Figure 31.9, chronic stimulation of BLa neurons between P30 and P35, the equivalent of adolescence in rats, results in a significant reduction of GABA currents recorded from pyramidal cells during the early adult period. As amygdalocortical projection neurons are glutamatergic (McDonald *et al.*, 1989), and are therefore likely to be excitatory in nature, it is possible that direct amygdalofugal projections to GABAergic interneurons could play a central role in the pathophysiology of schizophrenia during late adolescence and early adulthood. It is equally plausible; however, that the effect of BLa projections may be an indirect one that involves stimulation of pyramidal neurons and collateral excitation of GABA cells.

Dopamine System

Dopaminergic projections to rat mPFCx have been found to increase progressively beyond the weanling stage until the early adult period (Verney *et al.*, 1982; Kalsbeek *et al.*, 1988), and a coincident increase in DA transporters has also been described (Coulter *et al.*, 1996). As shown in Figure 31.13, the relative distribution of DA-IR varicose fibers in rat mPFCx is quite low during the first few postnatal weeks, but attains the highest density during the early adult period. As described by others (Lindvall & Bjorklund, 1978), the density of DA-containing fibers in rat mPFCx is greatest in layer VI and shows a progressive decrease toward layer I. Unlike noradrenergic fibers in mPFCx (Lindvall & Bjorklund, 1984), the DA system does not show long, vertical fibers traveling either vertically toward layer I or horizontally

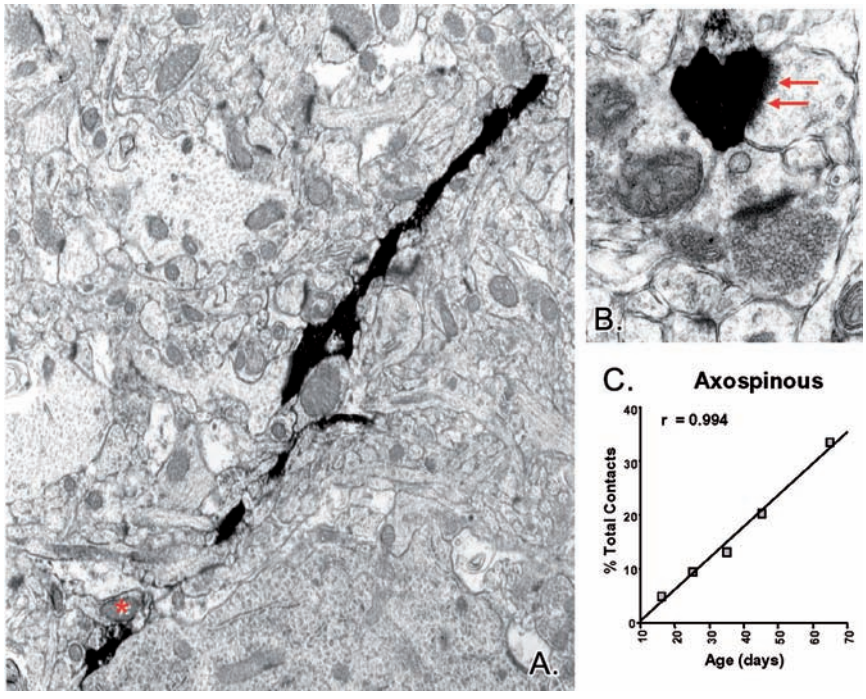

Fig. 31.12 Ultrastructure of anterogradely labeled axons from the basolateral amygdala as they course through the neuropil of ACC (left) and form asymmetric synapses with dendritic spines (upper right). These synapses show a linear increase between birth and the early adult period (lower right; Cunningham *et al.*, 2002).

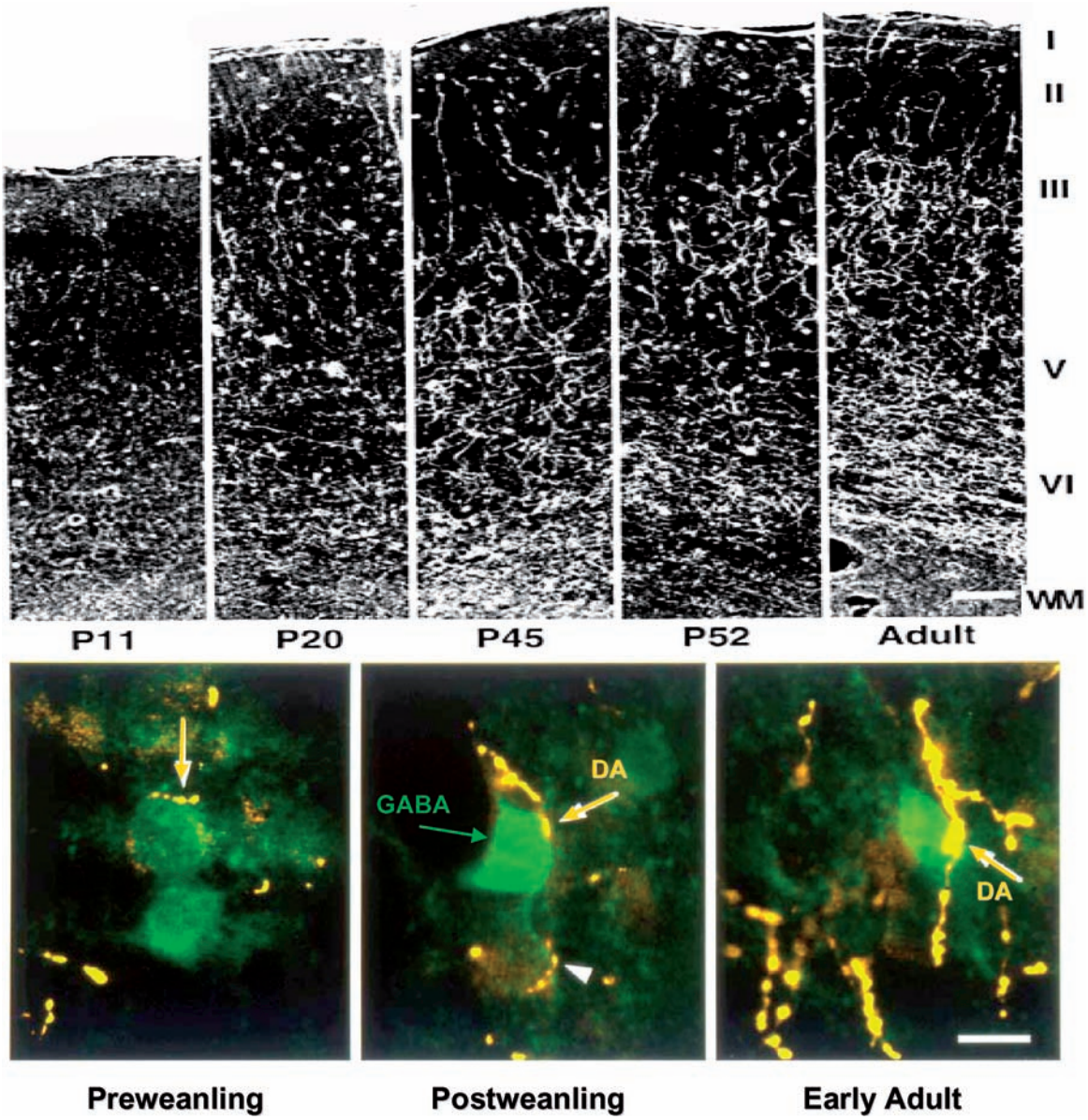

Fig. 31.13 Postnatal increases in the interaction of dopamine (DA) fibers and GABAergic neurons in rat ACC. Upper: A set of darkfield photomicro-graphs showing DA-IR fibers in the rat ACC at various postnatal ages. There is a progressive increase in the density of these fibers that continues until the early adult period. Lower: The cell somata of GABA neurons (green) are densely distributed during the early pre-weanling period, but very few DA fibers (yellow) are in evidence. During the post-weanling period, the numerical density of GABA cells decreases as surrounding neuropil expands. Increased numbers of DA fibers with varicosities are present in the neuropil and form non-random appositions with the GABA cells. By early adulthood (P60), there is a marked increase (approximately 150%) in both the DA fiber density and the number of appositions formed with any given GABA cell. Adapted from Benes *et al.* (1996a).

within this lamina. This contrasts with the distribution of DA fibers seen in primate prefrontal cortex where abundant fibers are also found in layer II and superficial portions of layer III (Krimer *et al.*, 1997). Together with the fact that the densest distribution of fibers observed is in deeper layers, it seems unlikely that noradrenergic axons have been included in these analyses, particularly since the antibody preparation employed in one of these studies (Benes *et al.*, 1993) is 50 times less selective for norepinephrine-glutaraldehyde-protein conjugates than to those with made DA (Geffard *et al.*, 1984). Thus, it seems unlikely that a significant number of noradrenergic fibers were included in the count of DA-IR varicosities.

A similar pattern of fiber staining can be distinguished at all postnatal stages examined. The increase of fiber density occurs to a proportionate degree across layers VI–II and does not progress in a distinct 'inside-out' manner. The size of the DA-IR varicosities also increases from approximately 1.2 µm at P20 to approximately 2.4 µm by P60; however, it is not likely that this change in size accounts for the increase in the density of fibers during the postnatal period, because the dimensions of the largest varicosities are still quite small relative to the thickness of the sections (40 µm). For sections in which single immunoperoxidase-processing is combined with cresyl violet staining, DA-containing varicose fibers can be observed throughout the neuropil, but very commonly, such fibers course toward neuronal cell bodies (Benes *et al.,* 1993). Many cell bodies have one or more varicosities in close apposition, and such contacts are observed in both superficial and deep laminae at all stages of postnatal life examined, although the density of varicosities in layers II and III is characteristically quite low. A Poisson analysis indicated that the appositions with cell bodies occur in a highly non-random manner, suggesting that they are probably functional in nature.

Postnatal increases in the density of dopaminergic projections to rat mPFCx are paralleled by an increase of D_2 receptor binding activity that begins prenatally (Bruinink *et al*., 1983) and continues until the fourth postnatal week (Deskin *et al*., 1981). Interestingly, administration of 6-OH-dopamine prevents this latter increase of D_2 receptor binding (Deskin *et al*., 1981), an effect that is associated with dystrophic changes in the basal dendrites of pyramidal neurons (Kalsbeek *et al*., 1988). Lesions induced in the prefrontal cortex of adult monkeys using 6-OH-dopamine result in an impaired performance of the spatial delayed alternation task (Brozoski *et al*., 1979) and it seems likely that this functional deficit would be associated with changes in the D2 receptor on pyramidal neurons.

Development of Dopamine–GABA Interactions in Cingulate Cortex

As previously reported (Benes *et al*., 1993), specimens of rat mPFCx processed with a double-immunostaining technique that localizes both DA varicosities and GABA cell bodies show a progressive increase in the interaction of these two neuronal elements between the pre-weanling period (Fig. 31.11) and the early stages of the post-weanling period. An increasing number of such varicosities form contacts with GABA neurons as the post-weanling period progresses, and this becomes most apparent at the beginning of the adult period. When the latter double-IF preparations are subjected to blind, semiquantitative analysis, a progressive linear increase in the percentage of GABA-IF cell bodies with apposed DA-IR varicosities occurs between P0 and P60, and these data best fit a first order polynomial equation ($r = 0.75$, $p = 0.0005$). During the pre-weanling period, any given GABA-IR cell body, on average, can show approximately one apposed varicosity. During the post-weanling period, however, the number of DA-containing varicosities in contact with GABA-containing cell bodies shows a rise through P60 ($r = 0.81$, $p = 0.0005$). Some neurons had no varicosities forming appositions with their somata, while others had more than one, making the average number per cell greater than 1.0. For these latter data, a second order polynomial equation provides the best fit. When an index of interaction is computed by multiplying the percentage of GABA cell somata having apposed DA varicosities and the number of such varicosities in contact with any given GABA-cell body, post-weanling rats have an index that is 1.5 times higher than that seen in pre-weanling animals. By adulthood, this index increases 1.8 times with respect to post-weanling rats and 2.5 times when compared with pre-weanling animals (Benes *et al*., 1996a).

Dopaminergic fibers are known to interact extensively with dendritic processes throughout the neuropil (Seguela *et al*., 1988; Goldman-Rakic *et al*., 1989; Verney *et al*., 1990; Smiley & Goldman-Rakic, 1993), although the somata of both pyramidal and non-pyramidal neurons probably also serve as *non-random* targets for these fibers in rat mPFCx (Taylor & Benes, 1996; Benes *et al*., 1993; Verney *et al*., 1990; Vincent *et al*., 1993, 1995; Huntley *et al*., 1992; Davidoff & Benes, 1998; Davidoff *et al*., 2000). In primate PFCx, TH-IR varicosities have not been found on neuron somata (Krimer *et al*., 1997); however, in PFCx of human brain, TH-IR varicosities are present on the somata of both pyramidal and non-pyramidal neurons (Benes *et al*., 1997). Thus, it appears that the association of DA fibers with neuronal cell bodies may vary in degree from one species to another. Based on rodent studies, serotonergic fibers appear to have a distribution that is similar to that of TH-IR fibers, although the latter also include some noradrenergic elements, particularly in the superficial layers where DA fibers are quite sparse. Serotonergic fibers probably interact with both projection cells and interneurons (Sheldon and Aghajanian, 1991; Morilak *et al*., 1993; Taylor & Benes, 1996; Smiley & Goldman-Rakic, 1996).

In primates, dopaminergic inputs to neuron somata appear to be minimal (Goldman-Rakic *et al*., 1989), while in human cortex, TH-IR varicosities have been shown to form non-random appositions with the cell bodies of both pyramidal and non-pyramidal neurons with a remarkable degree of consistency across many cases (Todtenkopf & Benes, 1998). Using a Poisson analysis

(Benes *et al.*, 1993) of a large number of varicosities (>10,000) counted in 15 normal human cases, neurons in layers II, III, V and VI of the ACC were found to have non-random contacts with TH-IR varicosities. The data obtained in a parallel analysis of a schizophrenic cohort ($n = 10$) were remarkably similar. Overall, the 'observed' percentage of varicosities in contact with cell bodies ranges from approximately 3–7% and is much lower than that seen for DA-IR (Benes *et al.*, 1993) and TH-IR (Taylor & Benes, 1996) in rat cingulate cortex. Although the majority are clearly associated with the neuropil, the proportion on cell somata is nevertheless quite significant in a Poisson sense. It is not clear, however, why the primate and human brain show such a low density, although even a small number of varicosities could potentially exert a significant modulatory influence at the level of the cell body.

Taken together, the somata of GABAergic neurons probably act as sites with which sprouting dopaminergic fibers may form appositions. In this process, GABA cells may be 'passive' targets for the formation of interactions. Alternatively, they might exert an 'active' neurotrophic influence on fiber sprouting and/or contact formation (Spoerri, 1988). Either way, it seems likely that dopaminergic fibers are capable of exerting an increasing modulatory influence on the activity of inhibitory interneurons during the postnatal period, particularly as DA receptors are localized on non-pyramidal cell bodies in rat mPFCx (Vincent *et al.*, 1993, 1995). Moreover, both agonists and antagonists of DA can alter the postsynaptic potentials recorded in GABAergic interneurons in pyriform (Gellman & Aghajanian, 1993) and frontal (Zhou & Hablitz, 1999) cortices. Agonists of the D_2 receptor (i.e., RU24926 and LY171555) have been found to inhibit the release of [^{3}H]GABA (Retaux *et al.*, 1991a, 1991b; Tam & Roth, 1990) and DA itself can influence the firing of GABAergic neurons (Penit-Soria *et al.*, 1987). Taken together with postmortem results and the model shown in Figure 31.5, it is plausible that the ingrowth of DA fibers during adolescence could influence the activity of GABAergic interneurons.

Convergence of Serotonin and Dopamine Fibers on Cortical Neurons

Based on electrophysiological studies conducted in rat pyriform cortex, a convergence of DA and serotonin fibers was postulated to occur on GABAergic interneurons (Gellman & Aghajanian, 1993). To test this hypothesis (Taylor & Benes, 1996; Benes *et al.*, 2000a), a triple co-localization of TH, serotonin (5HT) and glutamate decarboxylase (GAD) revealed that both DA and serotonin fibers do form appositions with GABAergic cell bodies (Fig. 31.14). Interestingly, this convergence occurred on approximately 25% of the GAD-immunoreactive cells, a number that is remarkably similar to that obtained in the co-localization of DA GABA described above (Benes *et al.*, 1996a). In addition to GABA cells, 'ghosts' of pyramidal neurons (i.e., a characteristic somal profile marked by the absence of staining) that also showed a similar convergence, although, in these cases, GABAergic terminals were also observed on the same cell bodies, leading to the suggestion that there is a 'trivergence' of dopamine, serotonin and GABA inputs on the principle projection neurons of the cortex (Fig. 31.14).

Influence of Serotonergic Fibers on the Cortical Dopamine Innervation

To assess the nature of the potential interaction between dopaminergic and serotonergic fibers in the developing anterior cingulate region, a series of experiments were conducted in which the 5HT projections from the nucleus raphe dorsalis (NRD) were lesioned during the neonatal period using the selective toxin, 5,7-dihydroxytryptamine [5,7-DHT] (Taylor *et al.*, 1998). As depicted in the model shown in Figure 31.15, the sham-lesioned rats in which only vehicle was injected ($n = 4$) showed a dense distribution of 5HT-IR neuronal cell bodies in ventral portions of the PAG at the level of the decussation of the superior cerebellar peduncle. The NRD is particularly prominent at this brainstem level and lesioned rats show a marked reduction in the number of immunoreactive cells and fibers. At more rostral levels of the midbrain, both sham and lesioned rats show abundant TH-IR fibers in the substantia nigra (SN) and VTA. It seems unlikely that 5,7-DHT adversely affects DA neurons, since the rats have been treated with nomifensine, which blocks its uptake into these latter cells. However, it is hypothetically possible that this treatment could have had an adverse effect on other neuronal populations, particularly those that receive an abundant serotonergic innervation; however, there was no obvious indication of this. Rather than being decreased, TH-IR fibers appeared to be increased in both dopaminergic nuclei of the 5,7-DHT-lesioned rats suggesting that the NRD may exert a suppressive effect on the projection neurons of the SN and VTA.

Overall, these findings are not consistent with the idea that 5HT may act trophically to facilitate the ingrowth of DA fibers during the late post-weanling and early adult periods. Rather, it seems more likely that the opposite is the case, that is, the 5HT system seems to be exerting an inhibitory effect on the normal postnatal ingrowth of TH-IR fibers. One interpretation of the findings described above is that the 5HT and DA systems may be competing with one another for functional territory on

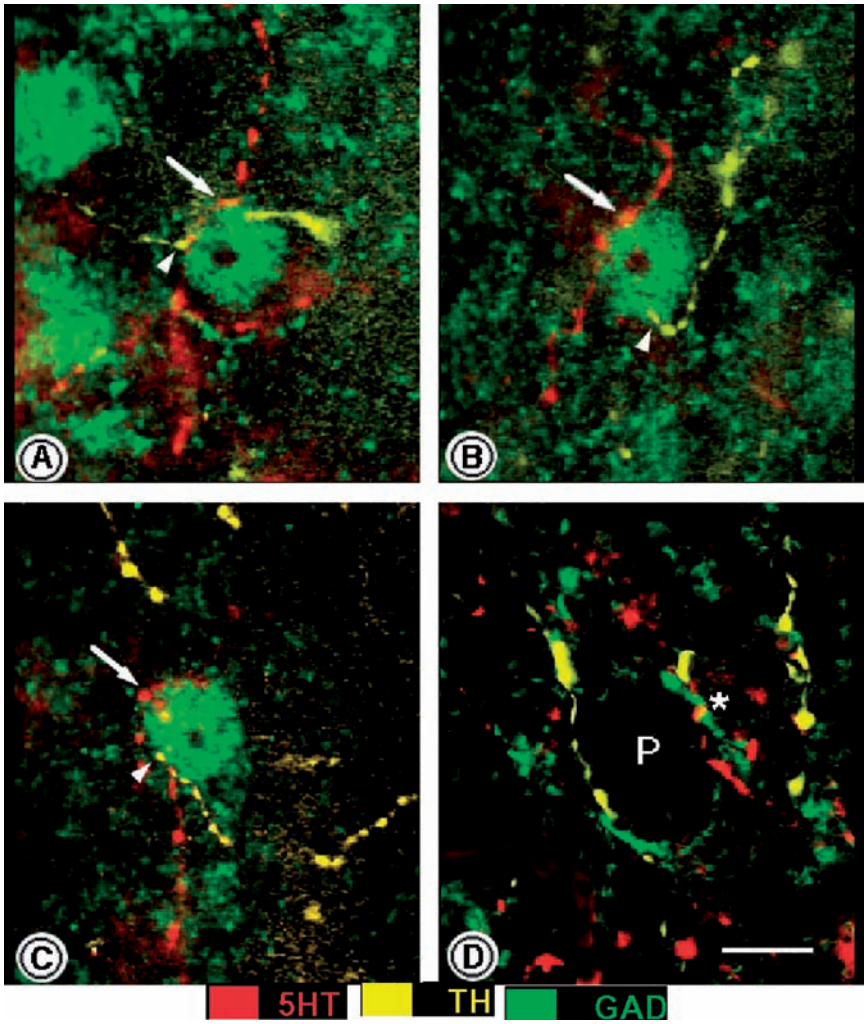

Fig. 31.14 A co-localization of tyrosine hydorxylase (TH), serotonin (5HT) and glutamic acid decarboxylase (GAD) immunofluorescent (IF) elements in rat ACC. Both TH- and 5HT-IF fibers converge on the same GAD-IF neuron somata. This occurs for about 25% of all GAD-IF cells visualized with the triple localization method. In the lower right corner, the ghost of a pyramidal neuron shows a 'trivergence' of TH-, 5HT-, and GAD-IF fibers. Adapted from Benes *et al.* (2000a).

the surface of intrinsic cortical neurons within rat mPFCx. An interaction of this type would tend to produce a reciprocal relationship between the two systems. An alternative possibility, however, is that the 5HT and DA systems mainly influence one another at the level of their respective brainstem nuclei. Accordingly, lesioning of the NRD may result in a stimulation or release of dopaminergic neurons within the VTA to sprout the distal portion of their fiber projections in various termination sites, such as the mPFCx. Consistent with this idea, an increase of TH-IR staining was observed in the SN, VTA and PAG of 5,7-DHT-lesioned rats. Physiological studies have yielded contradictory results regarding the manner in which the DA and 5HT systems may be influencing one another. On the one hand, some believe that 5HT can increase the release of DA in nucleus accumbens (Van Bockstaele *et al.*, 1994; Broderick & Phelix, 1997), corpus striatum (Broderick & Phelix, 1997; West & Galloway, 1996; Gudelsky & Nash, 1996) and prefrontal cortex (Gudelsky & Nash, 1996; Iyer & Bradberry, 1996). Contrariwise, some studies suggest that 5HT may actually decrease the release of DA, as exposure to selective 5HT receptor antagonists has been associated with an increase of extracellular DA concentrations (Pehek, 1996; Howell *et al.*, 1997). The latter pattern is consistent with the idea that there may be a competitive interaction between these two monoaminergic systems. This idea is a particularly appealing one because the VTA receives a direct input from serotoninergic fibers (Van Bockstaele *et al.*, 1994).

Influence of Dopamine Fibers on the Cortical Serotonergic Innervation

When the converse experiment was conducted and 6-hydroxy-dopamine (6-OH-DA) was used to lesion the

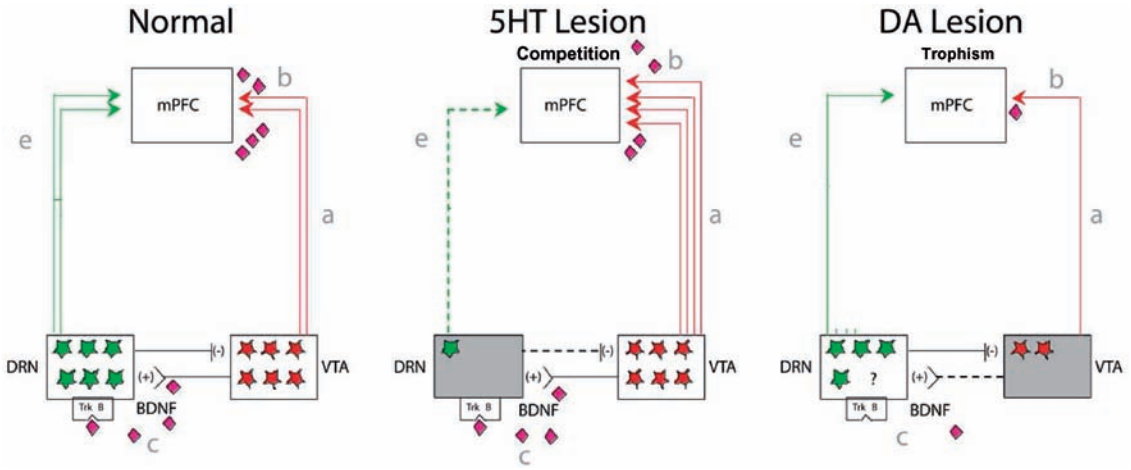

Fig. 31.15 A model for the convergent 5HT and DA fibers in rat ACC. (A) Normal interaction of 5HT fibers originating in the dorsal raphe nucleus (DRN) and DA fibers originating in the ventral tegmental area (VTA). The DA input to ACC is predominantly inhibitory, while the VTA input into DRN is predominantly excitatory. VTA DA neurons (red stars) produce brain-derived neurotrophic factor (BDNF) (diamonds) that is released from DA terminals in an activity-dependent manner. 5HT neurons (green stars) express the TrkB receptor, and 5HT neurite extension is promoted by BDNF. (B) When 5HT cells in DRN are ablated with 5,7-dihycroxytryptamine (5,7-DHT), an increase of DA fibers is observed in ACC and the brainstem. This suggests that the 5HT and DA fibers may be competing for the same space on the terminal fields of neurons and opportunistically occupying more space when one or the other system is ablated. (C) When DA cells are lesioned with 6-hydroxydopamine (6-OH-DA), the DA projections to ACC are predictably diminished, the 5HT fibers are also decreased, possibly reflecting decreased levels of BDNF in this terminal field. Accordingly, the DA and 5HT projections to ACC are probably interacting in complex ways that include both competitions and trophism. Adapted from Benes *et al.* (2000a).

VTA, it was expected that the opposite pattern would be observed (Cunningham *et al.*, 2005). Specifically, it was postulated that the lesion would result in a reduction of DA fibers in rat mPFCx, but an increase of 5HT fibers, reflecting the competition that seems to exist between the two fiber systems. As shown in Figure 31.15, both DA and serotonin fibers were found to be reduced in number. It is noteworthy that thermal ablation of the VTA in neonatal rats has been associated with a 30% decrease of basal dendritic branches of pyramidal neurons (Kalsbeek *et al.*, 1989b). This latter treatment resulted in a depletion of not only of DA levels, but also those of serotonin (Kalsbeek *et al.*, 1989a).

The data from the 6-OH-DA lesions are not consistent with the competition hypothesis suggested by the 5,7-DHR lesions of the DRN. Rather than the competitive interaction previously reported with ablation of the 5HT system, when the serotonin system is lesioned, there appears to be a non-reciprocal, or 'inverse' trophic relationship between the two developing monoaminergic systems. In other words, the DA system may be seen as promoting the survival of 5HT fibers in the ACC, while the 5HT system may inhibit or compete with the DA projections to this region.

A model of neurotrophic interactions provides a compelling explanation for the non-reciprocal relationship seen during the development of the DA and 5HT systems. Brain-derived neurotrophic factor (BDNF) is produced by DA neurons in the VTA (Seroogy *et al.*, 1994) and is involved in the maintenance of both the 5HT and DA systems in the developing and mature brain. BDNF has been shown to have numerous and diverse effects in its modulation of the 5HT system. For example, BDNF has been shown to promote sprouting in both uninjured and damaged 5HT neurons (Mamounas *et al.*, 1995, 2000), prevent neurotoxin-induced 5HT denervation in rat striatum (Frechilla *et al.*, 2000), increase 5HT neuronal firing rate in DRN (Celada *et al.*, 1996), elevate 5-HIAA and 5HT turnover in rat hippocampus, cortex, striatum, nucleus accumbens, SN, and hypothalamus (Siuciak *et al.*, 1996), and increase both tryptophan hydroxylase and 5HT levels (Siuciak *et al.*, 1998). Additionally, 5HT cells in the DRN express tyrosine kinase-B (TrkB) receptor mRNA that encodes the high affinity-receptor for BDNF (Madhav *et al.*, 2001). As illustrated in the model shown in Figure 31.5, the DA system may exert a supportive, or trophic, influence on the 5HT system by serving as a source of BDNF, supplied by activity-dependent anterograde transport (Fawcett *et al.*, 1998; Kohara *et al.*, 2001) along DA projections to the DRN (Conner *et al.*, 1997) and/or the DRN terminal field in ACC (Fig 31.4A). Thus, lesioning the DA system may have resulted in decreased trophic support of 5HT neurons, thereby reducing sprouting

within ACC, whereas lesioning of the 5HT system results in a competitive response by DA fibers. With regard to the schizophrenia model presented in Figure 31.5, a convergence of monoaminergic fibers on GABAergic cells implies the presence of complex interactions between the DA and serotonin systems. Accordingly, their functional interaction with GABAergic interneurons is probably much more complex than may have heretofore been appreciated.

Mis-Wiring of Cingulate Circuits as a Pathological Substrate of Schizophrenia

The ACC is a pivotal brain region with a central role in the integration of motivation, emotion, social interaction and logical processing during the postnatal period. An essential component to the normal functioning of this region is its extensive connectivity with the basolateral amygdala and hippocampal formation. Together, these phylogenetically older regions complete the cognitive–behavioral continuum that swirls through the ACC by mediating emotionally related learning, particularly that which occurs under stressful conditions. An intriguing aspect of the ACC is the fact that it undergoes late postnatal maturational changes in the integration of key extrinsic projection systems to this region. These include glutamatergic projections from the BL amygdala and dopaminergic projections principally originating in the VTA.

The postnatal increase in the density of amygdalocingulate fibers and their synapses parallels the emotional maturation that is associated with early and late adolescence. Concurrent with these behavioral changes are major shifts in gonadal steroids and adrenal androgens during the teenage period (Spear, 2000). Although the evidence for immediate effects of such neuroactive steroids on behavior is tenuous, such hormones may exert subtle, but long-term effects on both the structure and function of developing amygdalocortical connections. Increased glucocorticoid levels seen with stress may also have a potentially important influence on the integration of amygdaloid function during adolescence. Developmental differences in the influence of gonadal steroids on amygdalar projections to the anterior cingulate region are also relevant to understanding the pathophysiology of schizophrenia because the age of onset in males occurs much earlier than in females (Larsson *et al.*, 2005).

It seems evident that cognitive and emotional development is related to amygdalocingulate connectivity. As projections from the amygdala to ACC have been implicated in social functioning (MacLean, 1985), the continued development of this system during adolescence may enable an individual to modulate anxiety and fear, and become more socially adept. Thus, normal emotional maturation during the teenage period may involve ontogenetic changes in amygdalocingulate connectivity like those demonstrated in the present study. Contrariwise, abnormal emotional maturation could hypothetically be related to aberrant sprouting of this connectivity within the cingulate cortex during the adolescent period. As psychosis, depression, substance abuse, violence, and suicidality all typically present during adolescence, a comprehensive understanding of postnatal changes in amygdalocingulate connections will provide a unique opportunity for increasing our understanding of both normal and abnormal behaviors that manifests during this critical stage of emotional development.

Based on its functional integration and early lesion studies, the ACC presents itself as a logical region in which abnormalities might be found in subjects with various forms of psychopathology. Indeed, postmortem studies over the past 20 years have demonstrated rather compellingly that a variety of alterations in the glutamate, GABA and DA systems are probably present within its intrinsic circuitry. More specifically, it appears that extrinsic fiber systems from the amygdala (glutamatergic) and VTA (dopaminergic) are probably involved in 'mis-wired' circuits that have been identified in schizophrenia, particularly with respect to GABAergic interneurons.

It seems plausible that the onset of symptoms in schizophrenia during adolescence may be triggered by normal developmental changes in the intrinsic circuitry of ACC. Based on the current state of our knowledge, such changes likely involve environmental influences on the glutamatergic and dopaminergic projections from the basolateral amygdala and VTA, respectively. The manner in which these fiber systems are integrated with intrinsic neurons of the cingulate cortex probably plays an important role in the regulation of gene expression, particularly as it relates to environmental stress during the pre- and postnatal periods. The most challenging issues, however, are those related to the identification of susceptibility genes for schizophrenia and how they define the endophenotype for this disorder. Are these genes expressed in the ACC or the basolateral amygdala, or possibly both? How are they influenced by the expression of other genes that are regulated by amygdalar and/or ventral tegmental neurons and their projections to the ACC? And finally, how do changes in the expression of susceptibility genes and genes regulated by environmental stress translate into specific alterations in the intrinsic circuitry within layer II of the ACC during adolescence and early adulthood? The answers to these questions will point to the development of novel pharmacologic agents that can be applied to the treatment of

schizophrenia in its earliest stages. A strategy of this type may eventually help to diminish or perhaps even eliminate the deterioration in functioning that typically occurs in patients with schizophrenia.

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