Postmortem Studies in Mood Disorders Indicate Altered Numbers of Neurons and Glial Cells

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The influence of stress and glucocorticoids on neuronal pathology has been demonstrated in animal and clinical studies. It has been proposed that stress-induced changes in the hippocampus may be central to the development of depression in genetically vulnerable individuals. New evidence implicates the prefrontal cortex (PFC) in addition to the hippocampus as a site of neuropathology in depression. The PFC may be involved in stress-mediated neurotoxicity because stress alters PFC functions and glucocorticoid receptors, the PFC is directly interconnected with the hippocampus, and metabolic alterations are present in the PFC in depressed patients. Postmortem studies in major depression and bipolar disorder provide the first evidence for specific neuronal and glial histopathology in mood disorders. Three patterns of morphometric cellular changes are noted: cell loss (subgenual PFC), cell atrophy (dorsolateral PFC and orbitofrontal cortex), and increased numbers of cells (hypothalamus, dorsal raphe nucleus). The relevance of cellular changes in mood disorders to stress and prolonged PFC development and a role of neurotrophic/neuroprotective factors are suggested, and a link between cellular changes and the action of therapeutic drugs is discussed. The precise anatomic localization of dysfunctional neurons and glia in mood disorders may reveal cortical targets for novel antidepressants and mood stabilizers. Biol Psychiatry 2000;48:766–777 © *2000 Society of Biological Psychiatry*

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Stress and Morphopathology of Depression

Cortical regions such as the hippocampus (limbic archicortex) and prefrontal cortex (PFC; association neocortex) have been implicated in the neuropathology of depression and the response to stress. Reductions in the volume of the hippocampus are reported in subjects with a history of depression (Bremner et al 2000; Krishnan et al 1991; Shah et al 1998; Sheline et al 1996; 1999). Interestingly, the loss of hippocampal volume is correlated with the total lifetime duration of depression but not with the age of the patients (Sheline et al 1999). It has been proposed that repeated stress during recurrent depressive episodes may inflict cumulative hippocampal injury as reflected in the loss of structural volume (Duman 1999).

The volume reduction in the hippocampus in depression may be a result of a loss in the number of neurons due to a neurotoxic effect of glucocorticoids (McEwen 1997; Sapolsky et al 1990, 1991). Glucocorticoids are adrenal steroids that are secreted in increased amounts during stress and are essential for maintaining homeostasis through the hypothalamic–pituitary–adrenal (HPA) axis. The biological actions of glucocorticoids are mediated via mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) located in the hippocampus, frontal cortex, and other regions (Lopez et al 1998; McEwen 1991). In a recent study, significant reductions in MR messenger RNA (mRNA) in the hippocampus and GR mRNA in the PFC were detected in suicide victims with a history of mood disorders (Lopez et al 1998; 42). Thus, stress-induced alterations in glucocorticoids and subsequent decreases in MR or GR populations in the hippocampus and PFC may be a mechanism whereby stress triggers or exacerbates depression.

Chronic stress or the repeated administration of glucocorticoids to animals results in the degeneration of specific types of hippocampal neurons (Sapolsky et al 1990, 1991; Watanabe et al 1992). Stress- or glucocorticoid-related cellular changes observed in the primate and rodent hippocampus include dendritic atrophy, shrinkage of the neuronal cell body, and nuclear pyknosis (Sapolsky et al 1990; Watanabe et al 1992). Stressful experience also suppresses the normal production of granule cells in the hippocampal dentate gyrus during postnatal development and in adulthood (Gould and Tanapat 1999). Such postnatal reductions in the production of new cells are likely to alter the structure and function of the adult hippocampal formation and related structures. Thus, it has been proposed that changes in the hippocampus, secondary to stress, may be central to the development of depression in genetically vulnerable individuals (Duman 1999).

The influence of stress and glucocorticoids on neuronal

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pathology in depression is likely to involve several other brain regions and circuits in addition to those in the hippocampus. The PFC may be another site in which stress-mediated neurotoxicity contributes to depression. The PFC has strong reciprocal connections with the hippocampus and other stress-related structures. Moreover, animal and clinical studies indicate that stress alters prefrontal cognitive functions, and depressed suicide victims have a decrease in GR mRNA in the PFC. Finally, glucocorticoids may play a role in metabolic disturbances detected by imaging techniques in the PFC in living patients with mood disorders and produce the changes in cell morphometry that are reported in postmortem studies of mood disorders.

Evidence for Involvement of the Prefrontal Cortex in the Neuropathology of Depression

Several lines of evidence suggest that the PFC is involved in the neuropathology of major depressive disorder (MDD) and bipolar disorder (BPD, manic–depressive). In both MDD and BPD abnormal symptoms such as disturbances of social behavior, depressed moods, and deficits in working memory suggest pathophysiologic involvement of the PFC. Direct evidence obtained from neuroimaging studies further suggests that the PFC may be a common site of neuropathology in mood disorders. In BPD and MDD the PFC displays reductions in gray and/or white matter volume and sulcal widening as well as alterations in glucose metabolism and blood flow (Cohen et al 1989; Drevets et al 1997; Elkis et al 1996; Swayze et al 1990). Volumetric changes in the PFC in depression, similar to those described in the human hippocampus, are also reported in familial mood disorders (Drevets et al 1997). Recent examinations of the PFC in MDD and BPD in postmortem tissue provide the first morphometric evidence that specific changes in cell number packing density and size are associated with mood disorders (Ongur et al 1998; Rajkowska et al 1999; G. Rajkowska et al, unpublished data). These cellular changes in MDD and BPD may be the basis for morphometric alterations detected by the neuroimaging studies in gray and white matter in these disorders.

Stress is implicated in the volumetric and cellular alterations in the PFC because of the reciprocal cortical connections with the hippocampus and with other key structures involved in the stress response (e.g., paraventricular nucleus of the hypothalamus, amygdala, monoaminergic nuclei of the brainstem; for a review, see Lopez et al 1999). Moreover, stress alters the level of GRs in the PFC. The exposure to chronic unpredictable stress results in downregulation of GR mRNA in the rat PFC (Herman et al 1995; J.F. Lopez, unpublished observations, 2000). Decreases in GR mRNA are also detected in the PFCs of depressed suicidal individuals (Lopez et al 2000). It is suggested that downregulation of GRs in the PFC may be a compensatory response to elevated levels of circulating glucocorticoids. Interestingly, GR mRNA downregulation is not observed in the hippocampus from these same individuals (Lopez et al 2000). Instead, marked decreases in MR mRNA are found in the hippocampus of depressed suicide victims.

The involvement of different types of GRs in the PFC and hippocampus pathology suggests different sensitivity to circulating glucocorticoids of cells in these two regions. Another possibility is that other mechanisms may also contribute to PFC dysfunction during stress. For example, increases in catecholamine release during stress are reported in the PFC by behavioral and biochemical animal studies. Exposure to mild to moderate uncontrollable stress impairs working memory performance as well as other prefrontal functions in monkeys and rats (Arnsten and Goldman-Rakic 1998; Arnsten et al 1999; Mizoguchi et al 2000; Seamans et al 1998). Neurocognitive prefrontal deficits are observed in unmedicated patients with major depression, and these deficits are correlated with the severity of depression (Merriam et al 1999). It has been proposed that stress may impair cognitive functions through altering catecholamine actions on the dendritic stem of PFC neurons and that this mechanism involves G protein–linked intracellular pathways (for a review, see Arnsten and Goldman-Rakic 1998). It has been further suggested that increases in catecholamine release in the PFC during stress may occur due to blockage of catecholamine transporters by circulating steroids. This mechanism may have a survival value. The PFC may be taken offline during stress exposure, allowing faster, more habitual mechanisms regulated by subcortical and posterior cortical structures to adjust behavioral responses (Arnsten and Goldman-Rakic 1998). Further neuroanatomic, biochemical, and genetic studies will elucidate the unique role of the PFC in the stress response and the neurobiology of depression.

Affect is regulated not only by circulating levels of glucocorticoids, but also by gonadal hormones (Kathol 1985; Lopez et al 1998; Murphy 1991; Rubinow and Schmidt 1995). Prefrontal cortex functions are also regulated by circulating gonadal hormones, as recent animal studies reveal that changes in the ovarian hormone level alter the density of catecholamine, serotonin, and cholinergic axons in the dorsolateral PFC (dlPFC) of adult female rhesus monkeys (Kritzer and Kohama 1999; Kritzer et al 1999). Moreover, the responsiveness of cortical catecholamine innervation to gonadal steroid stimulation changes with life stage and maturity of the PFC (Kritzer et al 1999). These responses are different before

and after puberty. Thus, the development of the catecholamine and other prefrontal systems is influenced by gonadal hormones as well as glucocorticoids, providing further support for the relationship between stress, hormones, mood, and the PFC dysfunctions.

Cell Pathology in Depression

Recent postmortem studies demonstrate that mood disorders are characterized by specific histopathologic changes in both neurons and glial cells. These alterations at the microscopic level may give rise to the volume reductions and metabolic abnormalities reported in mood disorders in neuroimaging studies and contribute to identifying dysfunctional neuronal circuits and their cellular components in these disorders.

Two independent postmortem studies morphometrically estimated cell number and density in the subgenual prefrontal region (Ongur et al 1998), dlPFC, and orbitofrontal region (ORB; Rajkowska et al 1999). Significant reductions in glial cells were noted in these brain regions in subjects with mood disorders as compared with psychiatrically normal control subjects. In addition, neuronal cell packing density and the size of neuronal cell bodies are decreased in the lateral orbitofrontal cortex (areas 10 and 47) and in the dlPFC (Brodmann's area 9) and in MDD and BPD (Rajkowska et al 1999; G. Rajkowska et al, unpublished data). The reduction in cell number and density is accompanied by a 38–40% decrease in the volume of gray matter in the subgenual region (Drevets et al 1997; Ongur et al 1998) and a 12–15% reduction of cortical thickness in the lateral orbitofrontal cortex (Rajkowska et al 1999).

Glial Pathology

The unexpected prominent reductions in glial cell number and packing density are reported in independent laboratories in postmortem brains from subjects with mood disorders in different regions of the PFC and in the anterior cingulate cortex (Cotter et al 2000; Ongur et al 1998; Rajkowska et al 1999). Such a striking cellular deficit in mood disorders suggests that glia may be unique targets for novel strategies in the treatment of major depression.

Stereological estimates of the number of glial cells in MDD and BPD patients reveal significant reductions in glial number in the subgenual medial prefrontal region in both mood disorders (Ongur et al 1998). The most prominent glial reductions are found in subgroups of subjects with familial MDD (24%) or BPD (41%).

Parallel morphometric studies of glial cells in the dlPFC and ORB cortex also reveal marked decreases in overall (11–15%) and laminar (20–30%; layers III–VI) cell packing densities in subjects with MDD, as compared with control subjects (Rajkowska et al 1999). Comparable reductions in glial densities are detected in the dlPFC from subjects with BPD (G. Rajkowska et al, unpublished data). These reductions in glial density in MDD and BPD are accompanied by a significant enlargement in the size of glial nuclei in specific cortical layers.

Increases in glial nuclear sizes are also reported in the same prefrontal region in subjects with a neurodegenerative disorder—Huntington's disease (Rajkowska et al 1998). In Huntington's disease, however, the enlargement of glial cells is accompanied by marked gliosis manifested by *increases* in glial cell densities (Selemon et al 1995). Therefore, the cortex from subjects with mood disorders does not exhibit the classic morphometric signature of gliosis (i.e., glial hypertrophy in conjunction with glial proliferation). Rather, fewer but larger glial cells are present, and these cells may have more elaborate cytoplasmic processes, although these studies are yet to be completed. The lack of glial proliferation in mood disorders suggests that the glial pathology in mood disorders is not a response to ongoing neurodegenerative changes in the cortex; however, if fewer neurons are present in the cortices of depressed patients, the reductions may have occurred before the disease onset or may represent a more prolonged or moderate process of degeneration such that full-scale gliosis has not been triggered.

Taken together, the data suggest that there is disease-specific glial pathology in mood disorders. A link between reductions in glial cells revealed by postmortem analyses and altered glucose metabolism reported in neuroimaging studies can be suggested, since glial cells are involved in the active processing of glucose metabolism and are reported as the primary sites of glucose uptake and phosphorylation during neuronal activity (Tsacopoulos and Magistretti 1996).

It is also interesting to speculate that glial pathology might be related to the dysfunction of serotoninergic and/or noradrenergic systems reported extensively in depression (Heninger and Charney 1987; Hollister and Claghorn 1993), since glia may play a role in serotonin and norepinephrine neurotransmission via postsynaptic receptors distributed on their cell bodies and processes (Griffith and Sutin 1996; Milner et al 1998; Shimizu et al 1996; Whitaker-Azmitia et al 1993; Wilkin et al 1991). Recognition and precise anatomic localization of dysfunctional glial types and their receptors may offer a new cortical model for development of antidepressant and mood-stabilizing medications.

Neuronal Pathology

Morphometric analysis of the density and size of prefrontal neurons in the dlPFC and ORB regions in MDD and

BPD reveals significant reductions, in comparison to control subjects (Rajkowska et al 1999; G. Rajkowska et al, unpublished data). These neuronal reductions are more subtle than the corresponding glial alterations, and they are detected only when specific morphological size-types of neurons are analyzed in individual cortical layers. For example, marked reductions in the density of large neurons (corresponding to pyramidal glutamatergic excitatory neurons) are found in layers III and V of the dlPFC in BPD and MDD. In other prefrontal regions such as the rostral ORB, the most prominent neuronal reductions in MDD are confined to layer II cells (mostly corresponding to nonpyramidal inhibitory local circuit neurons; Rajkowska et al 1999). Interestingly, reductions in the density of specific populations of layer II nonpyramidal neurons containing the calcium-binding protein calretinin are reported in the anterior cingulate cortex in subjects with a history of mood disorders (S. Diekmann et al, unpublished observations, 1998). Thus, the layer-specific cellular changes in the PFC reported in mood disorders imply the involvement of several neural circuits and neurotransmitter systems in the neuropathology of depression (for more details, see Rajkowska 2000).

The reductions in neuronal densities are paralleled by smaller sizes of neuronal somatas and significant 12–15% decreases in cortical thickness observed in the rostral and middle parts of the ORB region in MDD (Rajkowska et al 1999, submitted). Neuronal loss (in contrast to glial loss) is not reported in the subgenual region in familial mood disorders (Ongur et al 1998); however, in this study specific types of neurons were not analyzed in individual cortical layers.

Cell Loss versus Cell Atrophy

Recent postmortem studies reveal several patterns of morphometric cellular changes in mood disorders: cell loss (subgenual prefrontal cortex), cell atrophy (and possibly cell loss, dlPFC and ORB), or increased numbers of cells (hypothalamus, dorsal raphe nucleus) are reported.

Loss of glial but not neuronal cells is observed in mood disorders in the subgenual prefrontal region, whereas lamina-specific reductions in the density of both neurons and glia are reported in the dlPFC and ORB regions in MDD and BPD (Figure 1a). Whether these prominent reductions in cell density represent cell loss or only atrophy of cell bodies and/or their processes has not been established. It is not entirely clear whether cell loss accounts for the reductions in cell packing density because density measurements are dependent not only on the total number of cells present but also on the total volume in which cells are counted. For the estimation of total number of neurons or glia in a particular brain region, it is essential that the borders of this region be established so that sampling is confined to the region within these borders (Gundersen et al 1988; West 1993). Since the entire extent and borders of the previously studied cortical areas were not available for examination, estimates of total cell number were not possible. On the other hand, indirect evidence from morphometric analyses of cell sizes and cortical and laminar thickness suggests that some cell loss in addition to cell atrophy takes place in the PFC in mood disorders.

In MDD, the densities of the largest neurons are significantly reduced by 22–37% in the rostral part of the lateral ORB and in the dlPFC region (Rajkowska et al 1999). In contrast, the densities of small neurons are *increased* by 6–27% in those regions. The latter observation suggests that either neuronal shrinkage or a developmental deficiency rather than neuronal loss accounts for the overall smaller sizes of neuronal soma in those cortical layers (Figure 1b). If neuronal loss had occurred, it is likely that the density of large neurons would have been decreased without associated increases in the density of small neurons, as was demonstrated in Huntington's disease (Rajkowska et al 1998).

In contrast to MDD, in BPD the decreased densities of large- and medium-sized neurons are not accompanied by increases in small neurons' density (G. Rajkowska et al, unpublished data). Therefore, in BPD these decreases in the densities of large and medium types of neurons suggest neuronal loss rather than a diminution in neuronal size in this disorder (Figure 1c). Another indication of cell loss in BPD comes from the observation that the width of layer IIIc in the dlPFC is increased as compared with control subjects. In this layer, marked increases in the size of glial nuclei are also observed in BPD. In light of the increased width of layer IIIc, an increase in interneuronal neuropil, perhaps including the processes of hypertrophied glial cells and/or enlarged dendritic trees, might account for the reduction in neuronal and glial densities or cell loss in this layer; however, definitive answers regarding cell loss in the PFC in mood disorders await stereological studies in which the total numbers of specific types of neurons and glia will be estimated.

Cell pathology in depression is not universally marked by a decreased density or number of neurons. *Increased* numbers of specific types of hypothalamic neurons (arginine vasopressin [AVP]–immunoreactive neurons, oxytocin [OXT]–expressing neurons, and corticotropin-releasing hormone [CRH] neurons) are detected in the paraventricular nucleus (PVN) of the hypothalamus in MDD and BPD (Purba et al 1996; Raadsheer et al 1995). Moreover, increases in CRH mRNA and increased number of CRH neurons colocalizing AVP are also found in depressed patients (Raadsheer et al 1994; Swaab et al 1993), as are increased corticotropin-releasing factor con-

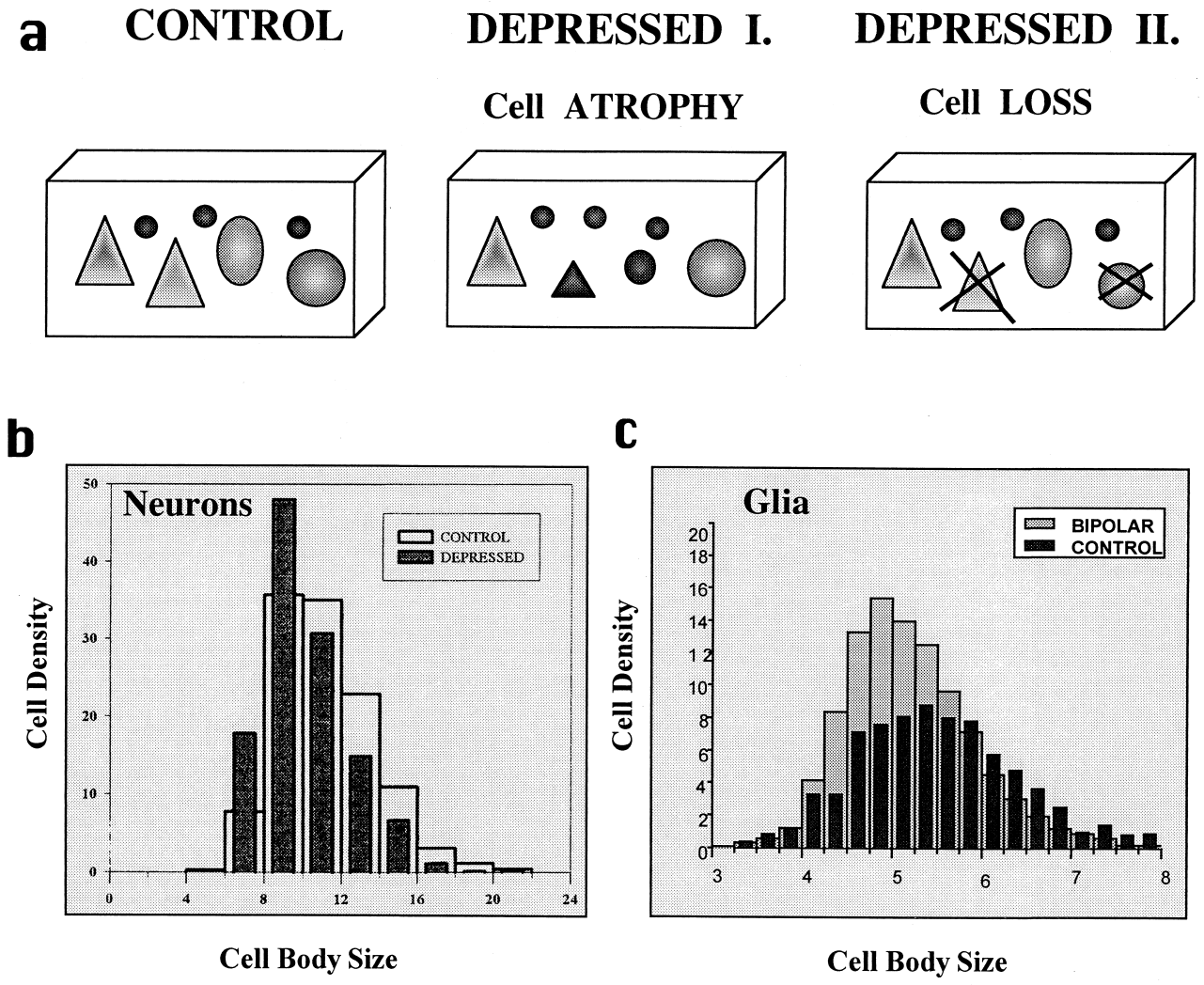

Figure 1. Hypothetical model of cellular alterations in mood disorders reflecting two patterns of morphometric changes reported in various cortical regions. The "Depressed I" panel in **a** and the histogram in **b** illustrate cell atrophy reported in the dorsolateral prefrontal cortex and orbitofrontal region in major depression. In these regions there is a decrease in the density of large neurons that is accompanied by parallel increases in the density of small neurons (compare size and number of "cells" in the Depressed I model with a "Control"). The latter observation suggests that neuronal shrinkage (atrophy) or a developmental deficiency rather than neuronal loss accounts for the overall smaller neuronal sizes in those cortical layers. If neuronal loss had occurred, the density of large neurons would have been decreased without associated increases in the density of small neurons as it is presented in the panel "Depressed II." The Depressed II panel and the histogram in **c** represent glial loss reported in the subgenual prefrontal cortex and in the dorsolateral prefrontal cortex in mood disorders.

centrations in cerebrospinal fluid (Banki et al 1987). These findings of increases in neurons are consistent with the evidence of activation of the HPA axis in some subsets of depressed patients (Holsboer et al 1992). Increased numbers of CRH, AVP, or OXT cells suggest an increase in related cell function, which may in turn have a modulatory effect on cortical or brain stem neurons. The PFC may be influenced by hypothalamic overactivation because this region is directly connected with the PVN and indirectly via monoaminergic brain stem nuclei. Moreover, the PFC can modulate the activity of the HPA axis (Herman and Cullinan 1997; Herman et al 1996), and messenger RNA for CRH receptors has been detected in the PFC (Steckler and Holsboer 1999).

Increased AVP and OXT cell numbers are related to the increased production of these neuropeptides in the HPA axis (Frolkis et al 1982; Hoogendijk et al 1985; Lucassen et al 1994) and in turn may be associated with the increased activity of signaling systems reported in mood disorders (Hughes and Dragunow 1995; Hyman and Nestler 1996; Manji and Lenox 1999; Pandey et al 1999).

Neuron–Glia Interactions

The cellular changes described here indicate that both types of brain cells, neurons and glia, are abnormal in mood disorders. The question remains whether depressed patients are genetically predisposed for the cellular changes detected postmortem and had smaller neurons and/or less glia from birth, or whether the cellular changes are a consequence of MDD. Alternatively, those genetically predisposed to the greatest histopathologic alterations may exhibit a greater vulnerability to depression. Cell loss or atrophy may also be related to developmental factors such as diminished amounts of neurotrophic factors and/or malfunctions in programmed cell death (apoptosis).

It is unknown at this stage whether cellular changes in the brains of depressed adults can also be found in depressed adolescents. There are no reports on morphometry in postmortem tissues from adolescent patients. Reductions in the density and size of neurons and glia found in adults could be a contributing factor to the pathophysiology of depression or could worsen as the illness progresses. It is interesting to speculate that the PFC or hippocampus from adolescents with depression will exhibit primarily glial pathology due to genetic and/or early adverse environmental events that over time (and with recurrence of depressive episodes) leads to neuronal pathology. Lack of sufficient glial support (e.g., less structural support due to a reduced number of glial cells or a shortage in glucose, the energy substrate provided to neurons by glia) may lead to neuronal pathology. Thus, it can be hypothesized that glial pathology in adolescents precedes neuronal changes (Figure 2). Alternatively, if the pathology in depressed adolescents is found in both glia and neurons, it may suggest that depression-induced reductions in activity of neurons with altered morphology will require less glial support, which may be reflected in a reduced number or density of glial cells. Pathology of both neurons and glia may worsen with increasing duration of illness and recurrent depressive episodes, and that pathology may eventually lead to cell loss.

The hypothesis that glial changes precede neuronal changes is supported by our recent observations on the distribution of glial fibrillary acid protein (GFAP), a marker for immunoreactive astroglial cells, in the dlPFC in MDD (Miguel-Hidalgo et al, in press). In MDD, there is a significant positive correlation between age and GFAP immunoreactivity (i.e., the GFAP areal fraction and packing density of GFAP-immunoreactive glia). The correlation between age and GFAP immunoreactivity suggested that glial pathology in younger subjects might be different from that in older subjects. In younger adults (23–45 years) with MDD, the GFAP areal fraction is smaller than the smallest value of the control subjects, whereas there is a tendency for a larger GFAP areal fraction in older (46–86) MDD subjects, as compared with age-matched control subjects. Thus, GFAP-immunoreactive astroglia may be differentially involved in the pathology of MDD at the early and late stages of this disorder.

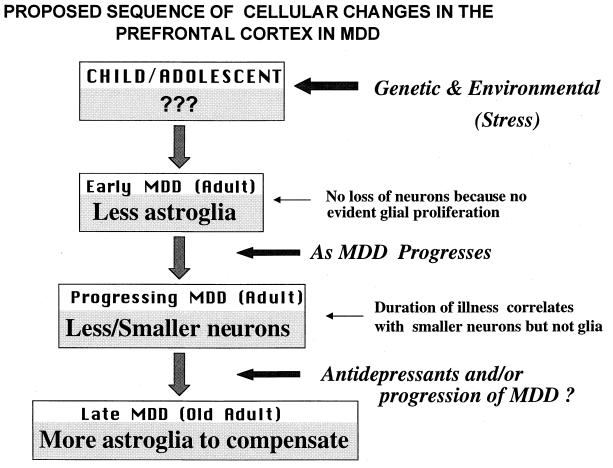

Figure 2. Scheme of hypothetical sequence of cellular changes in mood disorders. We propose that the prefrontal cortex and hippocampus from young adults with depression will exhibit primarily glial pathology (reductions in the number or density of cells) due to genetic and/or early adverse environmental events such as stress. Decreased level of glial fibrillary acid protein (GFAP)–immunoreactive astroglial cells is reported in young adults with major depression (Miguel-Hidalgo et al, in press). Lack of sufficient glial support may lead over time and with recurrence of depressive episodes to neuronal pathology. Reduced density of neurons and smaller sizes of their cell bodies are found to be correlated with a duration of depression. Progressing neuronal pathology (in combination with medication affect) may in turn stimulate glial activity and with time lead to increased levels of immunoreactive astroglial cells. Increased levels of GFAP-immunoreactive astroglia are observed in older individuals with major depression. MDD, major depressive disorder (Miguel-Hidalgo et al, in press).

Cellular Changes and Prolonged PFC Development

The most pronounced reductions in neuronal density in MDD and BPD are observed in superficial prefrontal layers II and III in both mood disorders. Neurons of these layers show greater plasticity than neurons of deep layers V and VI due to their late neurogenesis and extremely prolonged postnatal development. The prolonged postnatal development may render these neurons more susceptible to environmental factors related to the appearance of depression. The maturation and stabilization of neural elements and synapses on cells in layers II and III continue until adulthood (Koenderink et al 1994; Kostovic et al

1988; Mrzljak et al 1990, 1992). For example, during the developmental progression to adolescence there is an increase in the number of myelinated axons and an outgrowth of dendritic trees on layer III pyramidal neurons. These changes in neuronal and synaptic density may play critical roles in the remodeling of the basic cyto- and chemoarchitecture of the PFC.

The late structural and chemical maturation of prefrontal neurons and associated glia, especially those located in upper cortical layers, makes them more vulnerable to postnatal environmental and experience-dependent insults. The final formation of the prefrontal framework is stimulated during postnatal development by environmental factors such as personal experience and neuroendocrine factors. Exposure to chronic stress during the maturation of cellular elements in the PFC may lead to overactivation of the HPA axis and to hypercortisolemia. Stress may alter functioning of the developing PFC in a manner similar to that in the hippocampus. In the developing hippocampus, stress inhibits the proliferation of granule cell precursors (Tanapat et al 1998). Adult neurogenesis in the dentate gyrus is also regulated by adrenal steroids (Cameron and Gould 1994). Most recently, postnatal generation of new prefrontal neurons in the monkey PFC (Gould et al 1999) suggests prolonged plasticity in this region. Thus, circulating glucocorticoids or adrenal steroids may alter prefrontal cell morphology or even suppress adult cell production, as revealed in animal studies (Kritzer et al 1999; Kritzer and Kohama 1999; Lopez et al 1999).

Neurotrophic/Neuroprotective Factors and Cell Pathology

Experimental data with in situ hybridization histochemistry indicate that the development of cortical neuronal circuits may be related to the expression of specific target-derived neurotrophic factors such as brain-derived neurotrophic factor (BDNF; Huntley et al 1992). Expression of BDNF mRNA increases during later stages of prefrontal cortical development and continues into adulthood (Friedman et al 1991; Maisonpierre et al 1990), and the deprivation of neurotrophic factors activates cell death in neurons. Thus, any reduction in the supply of the neurotrophic factor could lead to a greater degree of neuronal death. Neurotrophic factors act by suppressing the latent biochemical pathway (a suicide program) present in all cells (for a review, see Jessel and Sanes 2000). Once the program is activated, cells die by apoptosis, and one of the early features of the apoptotic process is cell shrinkage. Therefore, it can be speculated that the neuronal shrinkage observed in the PFC in mood disorders represents an early stage of apoptosis. Currently, however, there is no postmortem evidence for apoptotic markers in depression.

There is a temporal correlation between the ingrowth of afferent axons into the PFC and detectable expression of BDNF mRNA (Murer et al 1999; Siuciak et al 1998). Therefore, it is likely that BDNF production by prefrontal neurons is required for a normal growth of afferent systems targeting those or neighboring neurons. The PFC is a target of overlapping cortical afferents coming from multimodal sensory areas, motor and limbic cortices and subcortical afferents such as thalamocortical axons, cholinergic projections from the basal ganglia, dopamine axons of the ventral tegmental area, serotonin axons of the dorsal raphe, and norepinephrine axons of the locus coeruleus (for a review, see Fuster 1997, 6–42). The last three mentioned systems constitute monoaminergic projection systems implicated in the pathophysiology of depression (Heninger and Charney 1987; Hollister and Claghorn 1993). Parallel studies of the locus coeruleus (Klimek et al 1997) or the dorsal raphe nucleus (Stockmeier et al 1999) in MDD, however, do not reveal severe morphological changes or the loss of norephinephrine or serotonin cell bodies. Rather, the number of serotonin neurons in the dorsal raphe nucleus may actually be increased in MDD (Underwood et al 1999). Thus, altered input from brain stem monoaminergic systems as well as other cortical regions may contribute to the pathology of prefrontal neurons in depression.

The survival of appropriate populations of synaptically connected neurons and supporting glial cells depends on neurotrophic factors such as BDNF (Ghosh et al 1994; Ohgoh et al 1998). For example, separation-induced cell death can be suppressed by BDNF. Separation of astroglial cells from cortical neurons in culture was shown to lead to neuronal death (Ohgoh et al 1998). Inasmuch as separation-induced cell death is suppressed by neurotrophic factors such as BDNF, glial cell production of neurotrophic factors such as glial-derived neurotrophic factor appears crucial to cortical neuron survival (Ohgoh et al 1998).

Localization of BDNF in the neocortex has been thoroughly established by recent studies on primates. Brain-derived neurotrophic factor–like immunoreactivity was observed in somata, processes, and axons of discrete neuronal populations as well as glial subpopulations in the monkey and human brain (Kawamoto et al 1999; Murer et al 1999). In our novel observations, BDNF-like immunoreactivity is observed in the human PFC primarily within the cell bodies of large pyramidal neurons of deep layer III and in processes of cells with astrocytic morphology (Miguel-Hidalgo and Rajkowska 1999; J.J. Miguel-Hidalgo and G. Rajkowska, unpublished data). We are currently testing whether prefrontal cells from postmortem

brains of depressed subjects will exhibit reduced levels of BDNF, as compared with matched control subjects.

Other neurotrophic factors such as astroglial neuroprotective protein S-100-β or fibroblast growth factor (FGF) may also be involved in neuron–glial interactions associated with the pathophysiology of depression. For example, loss of astroglial protein S-100-β was evoked in the hippocampi of rats that underwent serotonin depletion during early postnatal development (Mazer et al 1997). The glial FGF factor was shown in other animal studies to regulate the size of the cerebral cortex during embriogenesis (Vaccarino et al 1999). The cortical localization of glia–derived trophic factors has not yet been examined in the human brain.

A Link between Cellular Changes and the Action of Therapeutic Drugs

The neurotrophins and monoamine neurotransmitters appear to play related roles in stress, depression, and therapies for treating depression. From animal studies reporting that stress and antidepressant treatments regulate specific neurotrophin-related target genes within the central nervous system, it has been proposed that, in individuals genetically predisposed to clinical depression, cellular changes may be related to stress-induced changes in neurotrophin-related intracellular mechanisms (Duman 1999). These researchers further proposed that in depression precipitated by stress, hypoxia–ischemia, neurotoxins, or viral infections vulnerable neurons and glia may undergo atrophy or damage caused by increased levels of glucocorticoids and decreased levels of BDNF (Duman 1999). It is further hypothesized that upregulation of the BDNF gene could reverse the atrophy or damage of vulnerable neurons or protect these neurons from further damage. A role for BDNF in treatments for depression is also revealed in studies where the repeated treatment of rats with chronic electroconvulsive seizure or antidepressant drugs is shown to block the stress-induced decrease in BDNF in the hippocampus (Nibuya et al 1995).

Upregulation of BDNF occurs via increased serotonin and norephinephrine neurotransmission and upregulation of the cyclic adenosine monophosphate (cAMP)–cAMP response element binding protein cascade. A large body of evidence supports a role for BDNF in regulating the physiology and morphology of the serotonin system (Celada et al 1996; Mamounas et al 1995; Siuciak et al 1998; Vaidya et al 1997). For example, the infusion of BDNF into the neocortex substantially increases the surrounding density of serotonin axons (i.e., induces the sprouting of mature, uninjured serotonin axons; Mamounas et al 1995). The administration of 2,5-dimethoxy-4-iodoamphetamine (DOI), a serotonin$_{2A}$ receptor agonist, dramatically increases the expression of BDNF mRNA in the rat frontal and parietal cortices in layers II/III and V/VI (Vaidya et al 1997). These are precisely the layers in which prominent reductions in neuronal and glial cell density and enlargements of glial nuclei are observed in postmortem brains of depressed subjects (Rajkowska et al 1999). Thus, serotonin receptor–mediated regulation of BDNF suggests that serotonin may play a role in the neuronal and glial changes reported in the neocortex in depressed patients.

Therapeutic medications are likely to exert their therapeutic actions via networks of interconnected neurotransmitter pathways and their signal transduction systems (in particular, guanine nucleotide–binding proteins, adenylyl cyclases, and protein kinase C isozymes). Recent molecular studies in human cell culture and the animal brain suggest that antidepressant and mood-stabilizing medications alter the genomic level of various neurotrophins, receptors, and enzymes involved in neurotransmitter biosynthesis (Hughes and Dragunow 1995; Hyman and Nestler 1996; Manji and Lenox 1999; Manji et al, in press). For example, genes of several endogenous proteins, including neurotrophin receptors, are known to be regulated by the activator protein 1 family of transcription factors. Activator protein 1 DNA binding activity was markedly increased in the frontal cortex and hippocampus of rats treated chronically with mood stabilizers (Manji and Lenox 1999). Moreover, recent experiments with mice chronically treated with lithium, the major therapeutic mood stabilizer, reveal enhanced production of new cells in the dentate gyrus (Chen et al, in press). Thus, lithium has significant effects on the regulation of gene expression in the central nervous system.

Evidence has emerged suggesting that lithium may also have neuroprotective or neurotrophic actions in BPD (Manji et al 1999). Robust increases are noted in the level of bcl2, a major neuroprotective protein, in layers II/III of the rat PFC after chronic lithium treatment (Chen et al 1999). These increases in bcl2, suggesting a neuroprotective effect for lithium, are found in the same cortical layer III in which pathologic changes are detected postmortem in BPD.

Recent in vivo magnetic resonance spectroscopy studies conducted by the same group demonstrate that the administration of lithium at therapeutic doses increases volume of the cortical grey matter (Moore et al, in press) and concentration of brain *N*-acetyl-aspartate (NAA) in bipolar patients (Moore et al 2000). Those increases in NAA are found in a number of brain regions including the frontal cortex. *N*-Acetyl-aspartate is a marker of neuronal viability or function. A relative increase in this compound in BPD may reflect a neuroprotective effect of lithium in response to malfunctioning frontal neurons and may be

related to increases in the width of specific cortical layer(s) observed postmortem in BPD subjects. Most BPD subjects used for this study took lithium months or years before their deaths (Rajkowska et al, submitted). Of relevance to this hypothesis is the observation that the thickness of sublayer IIIc is greater and pyramidal cell density tends to be lower in subjects with a long exposure to lithium. Thus, a compensatory increase in dendritic (and/or glial) neuropil and consequent decrease in neuronal density may be a response to medication. Other therapeutic drugs may have a similar neuroprotective effect on cortical cells. Treatment with deprenyl, a neuroprotectant and antidepressant drug, enhances performance in cognitive tasks and is linked to increased dendritic tree aborization in the primate PFC (Shankaranarayana Rao et al 1999).

In summary, alterations in cell number or density revealed postmortem in mood disorders may be relevant to stress-induced changes in gene expression related to signal transduction pathways and cell survival (neurotrophic/neuroprotective) factors. These changes may be reversed by antidepressant and mood-stabilizing medications by altering genomic level of various neurotrophins, receptors, and enzymes involved in neurotransmitter biosynthesis. The precise anatomic localization of dysfunctional neurons and glia, in conjunction with molecular genetic approaches, may facilitate our understanding of the neurobiology of mood disorders and may reveal new cortical targets for action of novel antidepressant and mood-stabilizing medications.

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