Life Stress, Genes, and Depression: Multiple Pathways Lead to Increased Risk and New Opportunities for Intervention

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Introduction

Depression is among the most disabling of all medical disorders. It frequently appears early in life, can run a chronic course, and adversely affects the prognosis of other medical illnesses, such as coronary vascular disease, diabetes, and osteoporosis. A World Health Organisation study has reported that depression is the leading global cause of years of life lived with disability and the fourth leading cause of disability-adjusted life-years. Disability-adjusted life-years refers to the reduction in an individual's productive life and is a measure that takes into account premature mortality (*1*, *2*).

Suicide is not an uncommon event in patients with severe depression. In 1999, there were approximately 30,000 suicides in the United States, nearly twice the number of homicides (*3*, *4*). Tragically, suicide has become the third leading cause of death in individuals 15 to 24 years old (*5*). It is estimated that 85 to 90% of individuals who die from suicide have a diagnosable psychiatric disorder, with the largest number suffering from severe depression.

In the context of the high morbidity and mortality associated with depression, it is unfortunate that the psychological and neurobiological determinants of depression have not been precisely defined. This is due, in part, to the fact that depression, as currently diagnosed by means of a diagnostic system (DSM-IV) based on phenomenology, likely represents a heterogeneous set of disorders with multiple causes. A primary goal of current and future research in this area is to develop a diagnostic system based on etiology (*6*). This goal is becoming increasingly closer to reality because of advances in identifying the neural circuits, neurochemicals, and signal transduction mechanisms involved in the pathophysiology and treatment of depressive illness (*7*, *8*). Progress toward specifying the contribution of genetic factors (*9*), psychosocial stressors (*10*, *11*), and perhaps most important, gene-environment interactions to vulnerability to depression is also taking place (*12*–*14*).

Stress and Vulnerability to Depression

Stressful life events, such as divorce, financial problems, and being a crime victim, have a substantial causal association with depression (*10*). However, despite a strong correlation between stressful life events and depression, part of this apparent association is noncausal, because genetic risk factors for some stressful life events are correlated with a genetic predisposition to major depression (*13*). Stressful life events are not experienced at random; some individuals have a persistent tendency to place themselves in situations that have a high probability of producing stressful life events. Further, genetic risk factors for stressful life events are positively correlated with genetic risk factors for major depression (*11*). The type of stressful life event also affects vulnerability to a subsequent depressive episode. Stressful life events, such as serious marital problems, that occur in relation to a person's behavior are more likely to produce a depressive episode than are random events (*10*). Men and women are, in general, equally sensitive to the depressogenic effects of stressful life events, but their responses vary depending on the nature of the event itself. Men are more likely to have depressive episodes after divorce, separations, or work difficulties, whereas women are more sensitive to events in their social network, such as difficulty getting along with an individual, serious illness, or death (*11*). There is compelling evidence that early life stress, such as childhood neglect, physical or sexual abuse, or early parental loss, constitutes a major risk factor for the subsequent onset of depression (*15*). A widely held view is that the combination of genetics, early life stress, and ongoing stress may ultimately determine individual responsiveness to stress and the vulnerability to psychiatric disorders, such as depression.

It is clear that a comprehensive model of vulnerability to depressive illness is needed to facilitate advances in diagnostic accuracy, prevention, and treatments. Such a model must assume that both genetics and psychosocial stress play a causal role in depression and that multiple brain neural systems contribute to specific functional and structural abnormalities in discrete neuronal networks that mediate the complex symptom pattern in depressed patients. The model must be capable of linking the neurobiological effects of stress to depression vulnerability genes and the neurobiological abnormalities repeatedly observed in depressed patients. Progress is being made toward the creation of such a model, which provides for pivotal roles for 5-HT (5-hydroxytryptamine, serotonin), norepinephrine, extrahypothalamic CRH (corticotropin-releasing hormone), the HPA (hypothalamic-pituitary-adrenal) axis, and various associated signal transduction pathways and suggests potential vulnerability genes that ought to be studied in the context of the impact of life stress.

Neurobiological and Neuroanatomical Substrates of Depression

Positron emission tomography (PET) imaging studies have revealed multiple abnormalities of regional cerebral blood flow (CBF) and glucose metabolism in limbic structures and the prefrontal cortex (PFC) in mood disorders. Although disagreement exists regarding the specific locations and the direction of some of these abnormalities, in unmedicated subjects with familial major depression, regional CBF and metabolism are consistently increased in the amygdala,

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orbital cortex, and medial thalamus, and decreased in the dorsomedial/dorsal anterolateral PFC and anterior cingulate cortex ventral to the genu of the corpus callosum (in other words, subgenual PFC) relative to healthy controls (*16*, *17*). These abnormalities implicate limbic-thalamic-cortical and limbic-cortical-striatal-pallidal-thalamic circuits, involving the amygdala, orbital and medial PFC, and anatomically related parts of the striatum and thalamus in the pathophysiology of major depression. These circuits have also been implicated more generally in emotional behavior by the results of electrophysiological, lesion analysis, and brain mapping studies of humans and experimental animals (*16*).

During symptom remission some of these abnormalities reverse, which implicates areas where neurophysiological activity may increase or decrease to mediate or respond to the emotional and cognitive manifestations of depression. Nevertheless, in many of these areas, CBF and metabolism do not entirely normalize during effective antidepressant treatment. In the latter regions recent morphometric magnetic resonance imaging (MRI) and postmortem investigations have also demonstrated abnormalities of brain structure that persist independently of mood state and may contribute to the corresponding abnormalities of metabolic activity (*18*, *19*).

Structural imaging studies have demonstrated reduced gray-matter volumes in areas of the orbital and medial PFC, ventral striatum, and hippocampus, and enlargement of third ventricles in mood-disordered individuals relative to healthy control samples (*16*). Complementary postmortem neuropathological studies have shown abnormal reductions in cortex volume, and either glial cell counts, neuron size, or both, in the subgenual PFC, orbital cortex, dorsal anterolateral PFC, and amygdala (*18*–*20*). It is not known whether these deficits constitute developmental abnormalities that may confer vulnerability to abnormal mood episodes, compensatory changes to other pathogenic processes, or the sequelae of recurrent affective episodes per se. Understanding these issues will partly depend on experiments that delineate the onset of such abnormalities within the illness course and determine whether they antedate depressive episodes in individuals at high familial risk for mood disorders. Nevertheless, the marked reduction in glial cells in these regions has been particularly intriguing, in view of the growing appreciation that glia play critical roles in regulating synaptic glutamate concentrations and central nervous system (CNS) energy homeostasis and in releasing trophic factors that participate in the development and maintenance of synaptic networks formed by neuronal and glial processes (*21*, *22*). Abnormalities of glial function could thus prove integral to the impairment of structural plasticity and overall pathophysiology of major depression.

Taken together with other clinical and preclinical data regarding these structures' specific roles in emotional processing, the neuroimaging and neuropathological abnormalities in major depression suggest that major depression is associated with activation of regions that putatively mediate emotional and stress responses (for example, amygdala), whereas areas that appear to inhibit emotional expression (such as posterior orbital cortex) contain histological abnormalities that may interfere with the modulation of emotional or stress responses (*23*). For example, in major depression, the elevation of CBF and metabolism in the amygdala is positively correlated with depression severity, consistent with this structure's role in organizing the autonomic, neuroendocrine, and behavioral manifestations of some types of emotional responses.

In contrast, some of the medial and orbital PFC areas where metabolism is abnormal in major depression appear to play roles in reducing autonomic and endocrine responses to stressors or threats and in extinguishing behavioral responses to fear-conditioned stimuli that are no longer reinforced (*23*). Activation of the orbital cortex during depressive episodes may thus reflect endogenous attempts to interrupt unreinforced, aversive thought and emotion. However, the histopathological abnormalities identified in these areas in major depression post-mortem suggest that the ability to mediate these functions may be impaired. The hypothesis that dysfunction of these regions may contribute to the development of depression is consistent with evidence that lesions (such as strokes or tumors) involving either the PFC or the striatum (a major target of efferent projections from the PFC), as well as degenerative diseases affecting the striatum (for instance, Parkinson's and Huntington's diseases), are associated with increased risk for developing the depressive syndrome (*24*, *25*).

The Serotonergic System and Depression

Evidence for abnormal serotonergic function in depression includes the ability of tryptophan (the precursor for 5-HT biosynthesis) depletion—and the consequent lowering of brain 5-HT—to trigger a transient return of depressive symptoms in remitted depressed patients (*26*, *27*) and reduced density of both the 5-HT_{1A} receptor and the 5-HT transporter in depressed patients and in the postmortem brain tissue of suicide victims (*28*, *29*). Furthermore, a recent report suggests that a polymorphism associated with 5-HT_{1A} receptor transcription is more common in depressive illness (*30*).

Transgenic mice with knockout of the 5-HT_{1A} receptor early in life exhibit a behavioral phenotype of increased sensitivity to stress that persists even when the 5-HT_{1A} receptor is restored (*31*). Serotonin transporter knockout mice demonstrate a similar behavioral phenotype, which may also be related to 5-HT_{1A} receptor function (*32*). Chronic psychosocial stress decreases 5-HT_{1A} receptor density (*33*, *34*).

Recently, a seminal report by Caspi and colleagues found that one or two copies of the short allele of a 5-HT transporter promoter polymorphism, in conjunction with life stress, increased the risk for depression (*12*). Given the data reviewed above, it is possible that a similar relation exists for genes related to the 5-HT_{1A} receptor.

The Noradrenergic System and Depression

Key observations supporting noradrenergic system dysfunction include both increased (*35*) and decreased norepinephrine metabolism (*36*) in depressed patients; increased density of alpha-2 adrenergic receptors in the locus coeruleus (LC, the major brain norepinephrine-containing nucleus) in suicide victims with a history of major depression (*37*); increased activity of tyrosine hydroxylase (the rate-limiting enzyme of catecholamine biosynthesis) in the LC (*38*); and decreased density of the norepinephrine transporter in the LC (*39*). These effects are reproduced by depletion of brain norepinephrine (*40*, *41*) and reversed by antidepressant administration (*42*, *43*). Moreover, remitted drug-free depressed patients experience a return of depressive symptoms after administration of the tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine (AMPT), which lowers norepinephrine and dopamine levels (*44*).

The α_2-adrenergic receptor subtypes may be particularly relevant to the relation between life stress, noradrenergic system function, and vulnerability to depression. Several investigations using α_{2a}- and α_{2c}-

adrenergic receptor knockout mice suggest that the α_{2a}-adrenergic receptor is stress-protective, whereas the α_{2c}-adrenergic receptor mediates stress susceptibility (*45*, *46*). Prolonged exposure to stress decreases α_{2a}-receptor density in the amygdala and hippocampus (*33*). Thus, as with the 5-HT system, the interaction among stress effects on noradrenergic system function, noradrenergic system–related genes such as those encoding the α_2-adrenergic receptor subtypes, and vulnerability to depressive illness need further study.

Neuroplasticity and Depression

More recently, research into the pathophysiology and treatment of major depression has focused on intracellular signaling pathways regulating long-term neuroplastic events. Multicomponent cellular signaling pathways interact at various levels, thereby forming complex signaling networks that allow neurons to receive, process, and respond to information and to modulate the signal generated by multiple different neurotransmitter and neuropeptide systems. These signaling pathways are undoubtedly involved in neuroplastic events that regulate complex psychological and cognitive processes, as well as diverse functions such as appetite and wakefulness. Consequently, recent evidence demonstrating that impairments of neuroplasticity and cellular resilience may underlie the pathophysiology of major depression, and that antidepressants exert major effects on signaling pathways that regulate neuroplasticity and cell survival, have generated considerable excitement among the clinical neuroscience community and are reshaping views about the neurobiological underpinnings of these disorders. We now review these data and discuss their implications not only for changing existing views regarding the pathophysiology of major depression, but also for the strategic development of improved therapeutics.

"Neuroplasticity" subsumes diverse processes of vital importance by which the brain perceives, adapts to, and responds to various internal and external stimuli. The manifestations of neuroplasticity in the adult CNS include alterations of dendritic function, synaptic remodeling, long-term potentiation (LTP), axonal sprouting, neurite extension, synaptogenesis, and even neurogenesis (*47*).

Stress Modulates Neural Plasticity

Most studies of adaptive plasticity of neurons in response to stress, as well as to hormones of the HPA axis, have focused on the hippocampus. This is due, in part, to the well-defined and easily studied neuronal populations of hippocampal regions, including the dentate gyrus granule cell layer, and the CA1 and CA3 pyramidal cell layers. These cell layers and their connections (mossy fiber pathway and Schaffer collaterals) have also been used as cellular models of learning and memory (such as LTP). Another major reason that the hippocampus has been the focus of stress research is that the highest levels of glucocorticoid receptors (GRs) are expressed in this brain region (*48*). However, it is clear that stress and glucocorticoids also influence the survival and plasticity of neurons in other brain regions (such as prefrontal cortex, vide infra) that have not yet been studied in the same detail as the hippocampus.

One of the most consistent effects of stress on cellular morphology is the dendritic remodeling of hippocampal neurons (*49*, *50*). The remodeling of dendrites is observed in the CA3 pyramidal neurons as profound atrophy-decreased number and length of the apical dendritic branches. The stress-induced atrophy of CA3 neurons occurs after 2 to 3 weeks of exposure to restraint stress or more long-term social stress and has been observed in rodents and tree shrews (*49*, *50*). Although the effects of chronic stress tend to be the greatest in the CA3, subtle structural changes are also found in the CA1 and the dentate gyrus after a 1-month multiple-stress paradigm (*51*). In addition, profound changes in the morphology of the mossy fiber terminals and significant loss of synapses have also been observed. The hippocampus has a very high concentration of glutamate and expresses both type I and type II corticosteroid receptors, although type II receptors may be relatively scarce in the hippocampus of primates (*52*, *53*), and more abundant in cortical regions. Mineralocorticoid (type I corticosteroid receptor, MR) activation in CA1 is associated with reduced calcium currents, whereas activation of glucocorticoid or type II receptors causes increased calcium currents and enhanced responses to excitatory amino acids. Very high levels of type II receptor activation markedly increase calcium currents and lead to increased *N*-methyl-D-aspartate (NMDA) receptor throughput that could predispose to neurotoxicity. Indeed, as we discuss in greater detail below, a growing body of data has implicated glutamatergic neurotransmission in stress-induced hippocampal atrophy and death (*50*).

Although not as extensively studied as the hippocampus, recent research has also demonstrated histopathological changes in rat prefrontal cortex after corticosterone administration (*54*). Thus, using a Golgi-Cox procedure, Wellman (*54*) investigated pyramidal neurons in layer II-III of medial prefrontal cortex and quantified dendritic morphology in three dimensions. This study demonstrated a significant redistribution of apical dendrites in corticosterone-treated animals; the amount of dendritic material proximal to the soma was increased, and distal dendritic material was decreased. These findings suggest that stress may produce a significant reorganization of the apical dendritic arbor from medial prefrontal cortex in rats.

The observations that glucocorticoids may exert deleterious effects on neural plasticity and morphology are noteworthy with respect to the pathophysiology of mood disorders, because many patients with mood disorders display some form of HPA axis activation, and the subtypes of depression most frequently associated with HPA activation are those most likely to be associated with hippocampal volume reductions (*49*). A high percentage of patients with Cushing's disease, in which pituitary gland adenomas result in cortisol hypersecretion, are also known to manifest prominent depressive symptoms, as well as hippocampal atrophy. In addition, some patients with Cushing's disease show a reduction in hippocampal volume that correlates inversely with plasma cortisol concentrations; after corrective surgical treatment, enlargement of hippocampal volume in proportion to the treatment-associated decrement in urinary free cortisol concentrations is observed (*55*, *56*).

HPA-axis hyperactivity in patients with mood disorder is identified by increased cortisol levels in plasma (especially at the circadian nadir), urine, and CSF; increased cortisol response to adrenocorticotropin hormone (ACTH); blunted ACTH response to CRH challenge; enlarged pituitary and adrenal glands; and down-regulation at postmortem examination of frontal cortical CRH. Reduced corticosteroid receptor feedback is implicated in this process by challenge studies with dexamethasone alone or dexamethasone plus CRH in subjects with bipolar and unipolar disorders (*57*, *58*).

A cautionary note concerning the interpretation of the clinical studies that suggest a simple causal relation between stress and hippocampal atrophy is sounded by the results of recent longitudinal studies undertaken to investigate the effects of early life stress and inherited variation on monkey hippocampal volumes (*59*). In these studies, paternal half-siblings raised apart from one another by different mothers in the absence of

fathers were randomized to one of three postnatal conditions that disrupted different aspects of early maternal care. These researchers found that paternal half-siblings with small adult hippocampal volumes responded to the removal of all mothers after weaning with initially larger relative increases in cortisol levels (*59*). Plasma cortisol levels 3 and 7 days later and measures of cortisol-negative feedback in adulthood were not, however, correlated with hippocampal size. Thus, these studies suggest that small hippocampi also reflect an inherited characteristic of the brain and highlight the need for caution in attribution of causality in the cross-sectional human morphometric studies of the hippocampus.

A recent study by Gilbertson *et al*. also supports the hypothesis that smaller hippocampal volume is associated with susceptibility to stress (*60*). The brains of monozygotic twin pairs, in which one twin experienced combat in Vietnam and the other did not, were imaged by MRI. As reported in prior studies, veterans who had developed PTSD (post-traumatic stress disorder) displayed reduced hippocampal volume compared with those who did not. However, it was also observed that the combat-naïve twins of the PTSD-sufferers also had reduced hippocampal volume, in comparison to twins of veterans who never developed PTSD. Likewise, the hippocampal volume of the combat-naïve twins was inversely correlated with the severity of PTSD symptoms in their veteran counterparts. These data suggest that a genetic contributor to PTSD susceptibility is associated with reduced hippocampal volume.

Cellular mechanisms underlying the deleterious effects of stress on neural plasticity. Microdialysis studies have shown that stress increases extracellular levels of glutamate in hippocampus and that NMDA glutamate receptor antagonists attenuate stress-induced atrophy of CA3 pyramidal neurons (*50*, *61*). Although various methodological issues remain to be fully resolved, the preponderance of the evidence to date suggests that the atrophy, and possibly death, of CA3 pyramidal neurons arises, at least in part, from increased glutamatergic neurotransmission (*50*, *61*). It should be noted, however, that although NMDA antagonists block stress-induced hippocampal atrophy, there have not been any studies demonstrating that they are able to block the cell death induced by severe stress. This suggests that the mechanisms underlying atrophy and death may lie on a continuum, with severe (or prolonged) stresses "recruiting" additional pathogenic pathways in addition to enhanced NMDA-mediated neurotransmission. As discussed, stress increases extracellular levels of glutamate and sustained activation of NMDA, as well as non-NMDA, ionotropic receptors could result in high intracellular levels of calcium. Overactivation of glutamate ionotropic receptors is known to contribute to the neurotoxic effects of various insults, including repeated seizures and ischemia Neurotoxicity follows as a response to overactivation of calcium-dependent enzymes and the generation of oxygen free radicals (*50*, *61*). Stress or glucocorticoid exposure also compromises the metabolic capacity of neurons, thereby increasing the vulnerability to other types of neuronal insults. Activation of the HPA axis appears to play a critical role in mediating these effects, because stress-induced neuronal atrophy is prevented by adrenalectomy and duplicated by exposure to high concentrations of glucocorticoids (*49*, *50*, *62*).

Recent data also suggest a critical role for CRH in long-term effects of early life stress on hippocampal integrity and function. Thus, the administration of CRH to the brains of immature rats reduces memory function throughout life; these deficits are associated with progressive loss of hippocampal CA3 neurons and chronic up-regulation of hippocampal CRH expression, effects that do not require the presence of stress levels of glucocorticoids (*63*). The CRF_1 receptor, which binds CRH with higher affinity than does the CRF_2 receptor, plays a major role in regulating ACTH release and has been implicated in animal models of anxiety. Indeed, the central administration of CRF_1 antisense oligodeoxynucleotides has anxiolytic effects against both CRH and psychological stressors. Although CRF_2 receptors appear to act in an antagonistic manner (in other words, CRF_1 activates and CRF_2 attenuates the stress response), the precise role of the CRF_2 receptor is still being characterized (*58*). Note that pretreatment with a CRH antagonist also attenuates the stress-induced increases in MR levels in hippocampus, neocortex, frontal cortex, and amygdala. Rats that underwent a stressor also showed increased ACTH and cortisol levels after the administration of an MR antagonist, which suggests that up-regulation of MR in the stressed group is associated with increased inhibitory tone of the HPA axis (*64*).

In addition to directly causing neuronal atrophy, stress and glucocorticoids also appear to reduce cellular resilience, thereby making certain neurons more vulnerable to other insults, such as ischemia, hypoglycemia, and excitatory amino acid toxicity (*49*). The precise mechanisms by which glucocorticoids reduce cellular resilience remain to be fully elucidated, but appear to involve the inhibition of glucose transport (thereby diminishing capability of energy production and augmenting susceptibility to hypoglycemic conditions) and the aberrant, excessive facilitation of glutamatergic signaling (*49*).

Stress regulates hippocampal neurogenesis. The demonstration that neurogenesis occurs in the adult human brain has reinvigorated research into the cellular mechanisms by which the birth of new neurons is regulated (*65*). Pluripotent progenitor cells and thus neurogenesis occur in restricted brain regions. The greatest density of new cell birth is observed in the subventricular zone and the subgranular layer of the hippocampus. Cells born in the subventricular zone migrate largely to the olfactory bulb, and those in the subgranular zone migrate largely into the granule cell layer. The newly generated neurons send out axons and appear to make connections with surrounding neurons, which indicates that they are capable of integrating into the appropriate neuronal circuitry in hippocampus and cerebral cortex. Neurogenesis in the hippocampus is increased by enriched environment, exercise, and hippocampal-dependent learning (*66*–*68*). Up-regulation of neurogenesis in response to these behavioral stimuli and the localization of this process to hippocampus has led to the proposal that new cell birth is involved in learning and memory (*66*).

Recent studies have shown that decreased neurogenesis occurs in response to both acute and chronic stress (*66*). Removal of adrenal steroids (through adrenalectomy) increases neurogenesis, and treatment with high levels of glucocorticoids reproduces the down-regulation of neurogenesis that occurs in response to stress. Aging also influences the rate of neurogenesis. Although neurogenesis continues into late life, the rate is significantly reduced (*69*). The decreased rate of cell birth could result from the up-regulation of the HPA axis and higher levels of adrenal steroids that occur in later life. Lowering glucocorticoid levels in aged animals restores neurogenesis to levels observed in younger animals, which indicates that the population of progenitor cells remains stable but that it is inhibited by glucocorticoids (*69*). As an interesting parallel, studies in glucocorticoid receptor knockout mice showed significant alterations in hippocampal neurogenesis (*70*). A reduction of granule cell neurogenesis (up to 65% of control levels) was found in $MR^{-/-}$

mice, whereas $GR^{-/-}$ mice did not show neurogenic disruption, implicating the MR receptor in the pathogenesis of hippocampal changes observed in chronic stress and affective disorders (*70*). These preclinical observations raise the interesting possibility that CRH and GR antagonists, currently being developed for the treatment of mood and anxiety disorders (vide infra), may have particular utility in the treatment of elderly depressed patients. Also of potential relevance (noting the effect of hormonal fluctuations on mood disorders) for our understanding of the neurobiology and treatment of mood disorders, ovariectomy decreases the proliferation of new cells in the hippocampus, an effect that is reversed by estrogen replacement. The rate of neurogenesis fluctuates over the course of the estrus cycle in rodents, and the total rate of cell birth is higher in female rodents relative to males. In addition to potentially playing a role in the beneficial cognitive effects of estrogen, the regulation of neurogenesis by this gonadal steroid may also provide important clues about certain sexually dimorphic characteristics of mood disorders.

Stress regulates growth-factor cascades. In addition to the cellular mechanisms described above, it is now clear that stressors may exert major effects on cellular plasticity and resilience by regulating the expression and function of growth factor cascades (*71*, *72*). Neurotrophic factors [such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), and glial derived neurotrophic factor (GDNF)], as well as cytokines and insulin-like growth factor–1 (IGF-1), increase cell survival (*73*, *74*). These factors are now known to promote cell survival by suppressing intrinsic, cellular apoptotic machinery, not by stimulating cell survival pathways (*74*, *75*). This occurs through binding of these factors to membrane receptors and regulation of intracellular signal transduction pathways that can control apoptosis, including regulation of Bcl-2 family members. Signal transduction cascades that are currently believed to mediate many of the effects of neurotrophic factors include the mitogen-activated protein kinase (MAPK) cascade, the phosphotidylinositol-3 kinase (PI3K)-Akt pathway, and the phospholipase C-γ cascade (*76*) (Fig. 1). Neurotrophic factor signaling is mediated by a family of receptors known as Trks, which contain an intrinsic tyrosine kinase domain. Nerve growth factor binds to the TrkA receptor, and BDNF binds to TrkB. Receptor activation results in the activation of effectors, including PI3K, as well as leading, through a series of proteins, to activation of the extracellular signal–regulated kinase (ERK) 1 and 2 (ERK1/2) MAPK cascade (*76*). Recent studies have demonstrated that the activation of the MAPK pathway can inhibit apoptosis by inducing the phosphorylation of Bad (a major proapoptotic protein) and increasing the expression of Bcl-2 (a major antiapoptotic protein), the latter effect likely involves the cAMP response element–binding protein (CREB) (*77*, *78*). Phosphorylation of Bad occurs through activation of a downstream target of the MAPK cascade, ribosomal S-6 kinase (Rsk). Rsk phosphorylates Bad and thereby promotes its inactivation. Activation of Rsk also mediates the actions of the MAPK cascade and neurotrophic factors on the expression of Bcl-2. Rsk can phosphorylate CREB, and this leads to induction of Bcl-2 gene expression. Accumulating data suggest that not only is Bcl-2 neuroprotective, but it also exerts neurotrophic effects and promotes neurite sprouting, neurite outgrowth, and axonal regeneration (*79*–*81*).

A growing body of evidence suggests that the cellular effects of various antiapoptotic signals is mediated by several signaling cascades (including the PI3K-Akt, the ERK1/2-MAPK, the Ca^{2+}-CaMK, and the cAMP-PKA pathways) that converge on the CREB family of leucine-zipper transcription factors (*82*, *83*). This family of transcription factors, which bind as homo- or heterodimers to the CRE (cAMP-response element), a cis-acting enhancer element in the regulatory region of various genes, is activated by phosphorylation. Not only is CREB activated by BDNF (as discussed above), the BDNF gene promoter also contains CREs (*84*, *85*). Thus, activating the cAMP-PKA cascade leads to BDNF gene expression in a CREB-dependent manner (*86*). Although the activation of diverse receptors by direct agonists can activate activate adenylyl cyclase and thereby increase cAMP levels (*87*), the receptors rapidly undergo tachyphylaxis—making this a less useful long-term pharmacologic strategy. One alternate pharmacologic strategy to increase cAMP levels that is being increasingly explored is the use of inhibitors of cAMP-specific phosphodiesterases (PDEs), the enzymes responsible for the breakdown of cAMP (*88*, *89*). Recent studies have shown that CNS-penetrant PDE inhibitors are indeed capable of increasing BDNF expression. Other pharmacologic strategies to increase BDNF expression are also being investigated, most notably the AMPA receptor potentiators. AMPA receptors are a subfamily of ionotropic glutamate receptors that mediate fast excitatory transmission in the central nervous system (*87*, *90*). As with most other ligand-gated ion channels, AMPA receptors have multiple allosteric modulatory sites that represent targets for "fine-tuning" the activity of the receptor by pharmacologic means. In addition to their ionotropic properties, AMPA receptors have been functionally coupled to various signal transduction events involving Src-family kinases, heterotrimeric GTP-binding proteins (G proteins), and MAPK (*91*). This raises the possibility that more subtle modulation of AMPA receptors may be a useful strategy to activate MAPK neurotrophic cascades. One class of compounds—the "AMPA receptor potentiators" (ARPs)—dramatically reduce the rate of receptor desensitization and/or deactivation (*92*). Preclinical studies have shown that structurally dissimilar ARPs enhance ERK activation, increase BDNF expression (*92*–*94*), and reduce the extent of synaptic and neuronal degeneration resulting from excitotoxic insults, even when infused after the insult (*91*).

It has recently been demonstrated that chronic stress (21 days of foot-shock) induced a pronounced and persistent hyperphosphorylation of ERK1/2 in dendrites of the higher prefrontal cortical layers, whereas phospho-CREB was reduced in several cortical regions including frontal cortex (*95*). Because CREB is a target of ERK1/2 activation, this may implicate another signaling pathway in the reduction in CREB phosphorylation, and the subsequent down-regulation of Bcl-2 and BDNF transcription, following chronic stress. In this context, it is noteworthy that a recent study demonstrated that severe stress exacerbates stroke outcome by suppressing Bcl-2 expression (*96*). In this study, the stressed mice expressed ~70% less *bcl-2* mRNA than unstressed mice after ischemia. Furthermore, stress greatly exacerbated infarct in control mice, but not in transgenic mice, that constitutively expressed increased neuronal Bcl-2. Finally, high corticosterone concentrations were significantly correlated with larger infarcts in wild-type mice, but not in Bcl-2–overexpressing transgenic mice. Thus, enhanced Bcl-2 expression appears to be capable of offsetting the potentially deleterious consequences of stress-induced neuronal endangerment, which suggests that pharmacologically induced up-regulation of Bcl-2 may have considerable utility in the treatment of various disorders associated with endogenous or acquired impairments of cellular resilience (vide infra). Overall, it is clear that the neurotrophic factor–ERK1/2–MAPK–Bcl-2 signaling cascade plays a critical role in cell survival in the CNS and that there is a fine balance maintained between the levels and activities of cell survival and cell death factors.

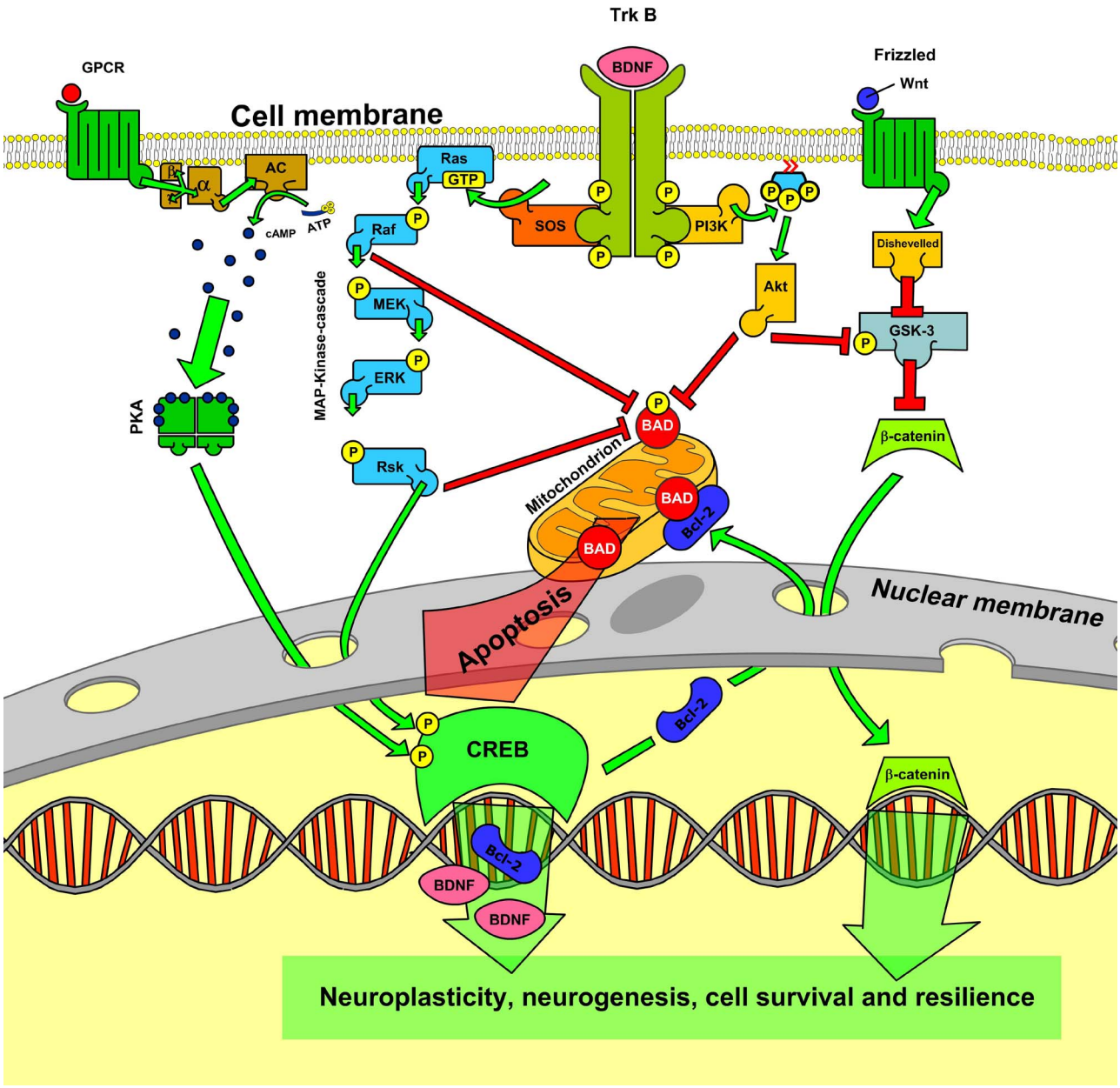

Fig. 1. Multiple pathways converge on CREB to regulate the expression of plasticity genes. A growing body of evidence suggests that the cellular effects of various antiapoptotic signals are mediated by several signaling cascades, including the PI3K-Akt, the ERK1/2-MAPK, the Ca^{2+}-CaMK, and the cAMP-PKA signaling pathways, that converge on the CREB family of transcription factors, which bind as homo- or heterodimers to the CRE, a cis-acting enhancer element in the regulatory region of various genes, including *BDNF* and *bcl-2*, and are activated by phosphorylation. Cell survival depends on neurotrophic factors, such as BDNF and NGF, and the expression of these factors can be induced by synaptic activity. The influence of neurotrophic factors on cell survival is mediated by activation of the MAPK cascade. Activation of neurotrophic factor receptors, also referred to as Trks, results in activation of the MAPK cascade through several intermediate steps, including phosphorylation of the adaptor protein SHC and recruitment of the guanine nucleotide exchange factor SOS (Son of sevenless). This results in activation of the small guanosine triphosphate-binding protein Ras, which leads to activation of a cascade of serine-threonine kinases, including Raf, MAP kinase kinase (MEK), and MAPK (also referred to as extracellular-signal regulated kinase, or ERK). One target of the MAP kinase cascade is Rsk, which influences cell survival in at least two ways. Rsk phosphorylates and inactivates the proapoptotic factor BAD. Rsk also phosphorylates CREB and thereby increases the expression of *BDNF*, *Bcl-2*, and other genes implicated in mediating plasticity. Bcl-2 translocates to the mitochondria and inhibits BAD. Neurotrophins like BDNF also regulate the activity of the Wnt signaling pathway through the PI3K-Akt cascade. Phosphorylation and inhibition of glycogen synthase kinase 3 (GSK-3) leads to an increase in β-catenin levels. β-Catenin translocates to the nucleus to regulate the expression of critical plasticity genes. AC, adenylyl cyclase, BAD, Bcl-2/Bcl-XL-associated death promotor. GPCR, G protein–coupled receptor.

Dysregulation of the BDNF–ERK1/2–CREB–Bcl-2 cascade may be a key mechanism by which prolonged stress induces atrophy of selective subpopulations of vulnerable neurons, distal dendrites, or both. It is likely that dysregulation of this cascade reduces the probability of neuronal survival; however, the differential survival is likely modulated not only by the region-specific expression of protective factors, but also by the network properties of vulnerable structures (*83*). The dynamics of the impairments of cellular plasticity and resilience is thus also likely to be determined by intrinsic properties of the affected areas.

Neurotrophic Signaling Cascades in the Pathophysiology and Treatment of Depression

As discussed more extensively earlier, endogenous neurotrophic factors are necessary for the survival and functioning of neurons (*73*). They increase cell survival by providing necessary trophic support for growth, but also by exerting inhibitory effects on cell death cascades (*77*). There is emerging evidence–primarily from postmortem studies–that supports a role for abnormalities in neurotrophic signaling pathways in depression. Decreased levels of CREB, BDNF, and the TrkB receptor have been reported in suicide victims (*97–99*).

Genetic abnormalities in CREB and BDNF may also occur in depression. Sequence variations in the CREB1 gene have been reported to cosegregate with depressive disorders in women (*9*). A BDNF coding variant may be associated with the personality trait of neuroticism, which is a risk factor for depression (*100*). In addition, two recent studies (*101*, *102*) suggest that a polymorphism in the pro-BDNF molecule has been associated with bipolar disorder (a condition in which depressive episodes alternate with manic episodes). This polymorphism has been associated with alterations in BDNF trafficking and secretion in vitro and with alterations in hippocampal working memory in humans (*103*). Thus, there is the opportunity to study the interactions of life stress, signal transduction–related genes, neuroimaging abnormalities consistent with deficient structural plasticity, and vulnerability to depression.

Neurotrophic Signaling Molecules are Targets for the Long-Term Actions of Medications Used to Treat Depression

A growing body of evidence suggests that antidepressants may regulate neurotrophic signaling cascades. Antidepressant treatment in rats increases CREB phosphorylation and CREB-mediated gene expression in mice limbic brain regions (*104*). Different classes of long-term antidepressant treatments–including norepinephrine (NE) reuptake inhibitors, selective serotonin reuptake inhibitors (SSRIs), and electroconvulsive seizure–up-regulate CREB and BDNF expression, which indicates that CREB and BDNF are common postreceptor targets of these therapeutic agents (*105*, *106*); furthermore, the increase was only seen with chronic use, which corresponds to the onset of action of these medications. More evidence that links up-regulation of these pathways and antidepressant activity comes from behavioral models (*107*). Thus, it was observed that CREB overexpression in the dentate gyrus or BDNF injection results in an antidepressant-like effect in the learned-helplessness paradigm and the forced swim test model of antidepressant efficacy in rats (*108–110*). Chronic, but not acute, antidepressant treatment also increases the neurogenesis of dentate gyrus granule cells (*111–113*). Lithium, one of the most effective antidepressant potentiating agents, also increases neurogenesis in the dentate gyrus (*114*). In contrast, increased neurogenesis is not observed in response to long-term administration of nonantidepressant psychotropic drugs. Studies demonstrating that neurogenesis is increased by conditions that stimulate neuronal activity (such as enriched environment, learning, and exercise) suggest that this process is also positively regulated by, and may even depend on, neuronal plasticity (*115*).

The enhancement of hippocampal neurogenesis by antidepressants serves to highlight the degree to which these treatments can regulate long-term neuroplastic events in the brain. In view of the opposite effects of stress and antidepressants on hippocampal neurogenesis, it is quite plausible that alterations in hippocampal neurogenesis are fundamental to the clinical syndrome of depression (*111–113*, *115*). To further investigate this possibility, Santarelli and colleagues (*116*) recently conducted an important series of experiments. Mice were administered various antidepressants or vehicle for 28 days, and their responses on a novelty-suppressed feeding test were investigated. The authors noted a 35% improvement in the speed for retrieving food or water in mice taking antidepressants. In a second experiment, they found a 60% increase in cells with bromodeoxyuridine (BrdU, a marker of cell division) in the dentate gyrus after 11 to 28 days of treatment with fluoxetine. To test whether hippocampal neurogenesis was necessary for the antidepressants' behavioral effects, the authors exposed mice to x-rays directed at the hippocampus, which led to an 85% reduction in BrdU-positive cells in the subgranular zone. These mice were then treated with fluoxetine, imipramine, or vehicle for 28 days. The previously noted effect of antidepressants on the novelty-suppressed feeding test was not seen in irradiated mice, which suggested that these behavioral effects of chronic antidepressants may be mediated by new neuronal growth in the hippocampus. However, novelty-suppressed feeding behavior is generally regarded as a test of anxiety and also responds to benzodiazepines (generally not regarded as having antidepressant efficacy); thus, it may be premature to infer that an inhibition of the antidepressant effect of these drugs occurs as a result of the suppression of neurogenesis. Furthermore, Vollmayr and associates (*117*) have undertaken a series of studies to determine whether behavioral deficits in the inescapable foot-shock learned-helplessness model are associated with reduced hippocampal proliferation, and—this is key—if the changes occur in a time frame consistent with the behavioral deficits. They did not find a significant reduction in cell proliferation at a time when the helpless behavior was first observed (*117*). In follow-up studies, these authors used restraint stress to decrease the rate of cell proliferation ~40%; subsequent investigation showed that these animals did not develop learned helplessness at a higher rate than the control animals. Also pertinent are the observations that recent large-scale clinical studies have shown lack of antidepressant efficacy of a neurokinin-1 (NK1) antagonist. These findings are noteworthy, because rodents administered NK1 antagonists and NK1 knockout mice show increased neurogenesis (*118*, *119*); furthermore, the administration of an NK1 antagonist attenuates the effects of stress on dentate cytogenesis (*120*). Although many factors may have contributed to the lack of demonstrable antidepressant effects in the clinical study (*120*), these results do suggest a dissociation between rodent hippocampal neurogenesis and clinical antidepressant efficacy.

It may therefore be more appropriate to consider the changes in the context of broader impairments of cellular plasticity and resilience, rather than in that of a limited neurogenesis model. Indeed,

the findings of Santarelli *et al.* (*116*) raise the intriguing possibility that hippocampal neurogenesis may be more related to the effects of antidepressants on anxiety behavior than depression. Although clearly speculative, such a contention receives indirect support from the growing body of data suggesting that SSRIs have very limited antidepressant efficacy in children (*121*), whereas they have robust anxiolytic effects in the same population (*122*).

Therapeutic and Diagnostic Implications

Agents capable of reversing the hypothesized impairments of cellular resilience, reductions in brain volume, and cell death or atrophy in depression have the potential of becoming new therapeutic classes of antidepressant drugs. Novel molecular targets might include phosphodiesterase inhibitors that increase CREB phosphorylation; MAPK phosphatase inhibitors that increase expression of the antiapoptotic protein Bcl-2; presynaptic glutamate receptor subtypes that attenuate glutamate release; AMPA potentiators that increase BDNF expression; and NMDA antagonists that enhance plasticity and cell survival (see Fig. 2).

Life stress affects multiple systems implicated in depression;

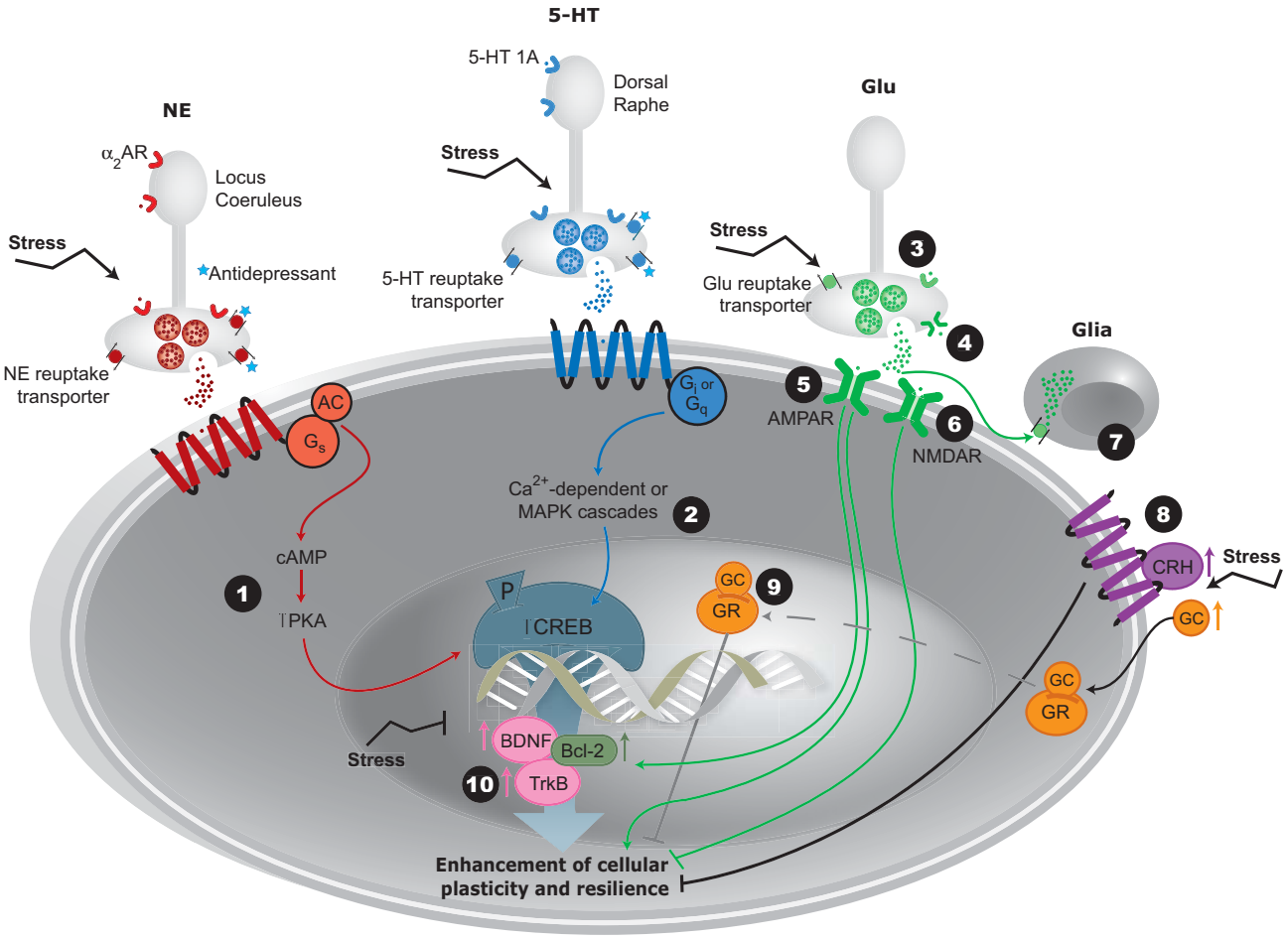

Fig. 2. The functional interactions among monoamine neurotransmitters, glutamate, and neurotrophic signaling cascades. Various sites where stress affects these systems are illustrated. As reviewed in the text, it is likely that genetic factors and life stress both contribute to the neurochemical alterations, impairments in cellular resilience, reductions in brain volume, and cell death and atrophy observed in depression. Novel targets for increasing neuroplasticity and cellular resilience and facilitating new classes of antidepressant medications are numbered as follows. (1) Phosphodiesterase inhibitors increase levels of pCREB; (2) MAPK modulators increase the expression of *bcl-2*; (3) mGluR II/III receptor agonists modulate the release of excessive levels of glutamate; (4) drugs such as riluzole and felbamate act on Na^+ channels to attenuate glutamate release; (5) AMPA potentiators up-regulate the expression of BDNF; (6) NMDA receptor antagonists such as memantine enhance plasticity and cell survival; (7) drugs that increase glial release of trophic factors and clear excessive glutamate may have antidepressant properties; (8) CRH antagonists may reverse the anxiogenic and depressogenic effects of extrahypothalamic CRH; (9) glucocorticoid antagonists may attenuate the deleterious effects of hypocortisolemia; (10) agents that up-regulate *bcl-2* (such as pramipexole) may have antistress and antidepressant actions. NE, norepinephrine; 5-HT, serotonin; Glu, glutamate; CRH, corticotropin-releasing hormone; BDNF, brain-derived neurotrophic factor; MAPK, mitogen-activated protein kinase; CREB, cyclic adenosine monophosphate response element-binding protein; α_2-AR, α_2-adrenergic receptor; mGluR, metabotropic glutamate receptor; AMPAR, α-amino 3-hydroxy-5-methylisoxazole propionate receptor; NMDAR, *N*-methyl-D-aspartate receptor; GR, glucocorticoid receptor; PKA, protein kinase A. [Modified and reproduced with permission from figures 3 and 5 from Manji *et al.* (*20*)] G_i stands for the family of G protein α subunits that includes G_i and G_o; G_q stands for the family of G protein a subunits that includes G_q and G_{11}.

relevant genotypes are being identified, and clinical research techniques are now capable of defining neurobiological phenotypes. This promises a new generation of research that will clarify the relation among environmental and genetic risk factors, to quantify the risk for the development of depression more precisely. The result will be a radically different diagnostic system based on etiology and the discovery of new approaches to the prevention and treatment of depressive illness, one of the most serious of all medical illnesses.

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