

Neurobiology of Depression

Review

Eric J. Nestler,¹ Michel Barrot, Ralph J. DiLeone,
Amelia J. Eisch, Stephen J. Gold,
and Lisa M. Monteggia

Department of Psychiatry and Center for
Basic Neuroscience
The University of Texas Southwestern Medical
Center
5323 Harry Hines Boulevard
Dallas, Texas 75390

Current treatments for depression are inadequate for many individuals, and progress in understanding the neurobiology of depression is slow. Several promising hypotheses of depression and antidepressant action have been formulated recently. These hypotheses are based largely on dysregulation of the hypothalamic-pituitary-adrenal axis and hippocampus and implicate corticotropin-releasing factor, glucocorticoids, brain-derived neurotrophic factor, and CREB. Recent work has looked beyond hippocampus to other brain areas that are also likely involved. For example, nucleus accumbens, amygdala, and certain hypothalamic nuclei are critical in regulating motivation, eating, sleeping, energy level, circadian rhythm, and responses to rewarding and aversive stimuli, which are all abnormal in depressed patients. A neurobiologic understanding of depression also requires identification of the genes that make individuals vulnerable or resistant to the syndrome. These advances will fundamentally improve the treatment and prevention of depression.

Mood disorders are among the most prevalent forms of mental illness. Severe forms of depression affect 2%–5% of the U.S. population, and up to 20% of the population suffer from milder forms of the illness. Depression is almost twice as common in females than males. Another roughly 1%–2% are afflicted by bipolar disorder (also known as manic-depressive illness), which affects females and males equally. Mood disorders are recurrent, life threatening (due to the risk for suicide), and a major cause of morbidity worldwide (Blazer, 2000).

Depression has been described by mankind for several millennia. The term melancholia (which means black bile in Greek) was first used by Hippocrates around 400 B.C. (Akiskal, 2000). Most of the major symptoms of depression observed today were recognized in ancient times, as were the contributions of innate predispositions and external factors in causing the illness. The ancients also recognized a large overlap of depression with anxiety and excessive alcohol consumption, both of which are well established today. Indeed, similarities between ancient descriptions of depression and those of the modern era are striking, yet it wasn't until the middle part of the 19th century that the brain became

the focus of efforts to understand the pathophysiology of this disorder.

Diagnosis of Depression

Since the 1960s, depression has been diagnosed as “major depression” based on symptomatic criteria set forth in the Diagnostic and Statistical Manual (DSMIV, 2000) (Table 1). Milder cases are classified as “dysthymia,” although there is no clear distinction between the two. It is obvious from these criteria (summarized in Table 1) that the diagnosis of depression, as opposed to most diseases of other organ systems (diabetes, cancer, chronic obstructive pulmonary disease, to name a few), is not based on objective diagnostic tests (serum chemistry, organ imaging, or biopsies), but rather on a highly variable set of symptoms. Accordingly, depression should not be viewed as a single disease, but a heterogeneous syndrome comprised of numerous diseases of distinct causes and pathophysiologies. Attempts have been made to establish subtypes of depression defined by certain sets of symptoms (Table 2) (see Akiskal, 2000; Blazer, 2000). However, these subtypes are based solely on symptomatic differences and there is as yet no evidence that they reflect different underlying disease states.

Genetic and Environmental Causes of Depression

Epidemiologic studies show that roughly 40%–50% of the risk for depression is genetic (Sanders et al., 1999; Fava and Kendler, 2000). This makes depression a highly heritable disorder, at least as heritable as several common complex medical conditions (type II diabetes, hypertension, asthma, certain cancers), which are often thought of as genetic. Yet, the search for specific genes that confer this risk has been frustrating, with no genetic abnormality being identified to date with certainty. The difficulty in finding depression vulnerability genes parallels the difficulty in finding genes for other psychiatric disorders and, in fact, for most common complex diseases. There are many reasons for this difficulty, which are reviewed elsewhere (Burmeister, 1999), including the fact that depression is a complex phenomenon with many genes possibly involved. Thus, any single gene might produce a relatively small effect and would therefore be difficult to detect experimentally. It is also possible that variants in different genes may contribute to depression in each family, which further complicates the search for depression genes.

In addition, vulnerability to depression is only partly genetic, with nongenetic factors also being important. Nongenetic factors as diverse as stress and emotional trauma, viral infections (e.g., Borna virus), and even stochastic (or random) processes during brain development have been implicated in the etiology of depression (Akiskal, 2000; Fava and Kendler, 2000). Depressive syndromes—indistinguishable from major depression defined by DSMIV and by their response to standard antidepressant treatments—occur in the context of innumerable medical conditions such as endocrine disturbances

¹Correspondence: eric.nestler@utsouthwestern.edu

Table 1. Diagnostic Criteria for Major Depression

Depressed mood
Irritability
Low self esteem
Feelings of hopelessness, worthlessness, and guilt
Decreased ability to concentrate and think
Decreased or increased appetite
Weight loss or weight gain
Insomnia or hypersomnia
Low energy, fatigue, or increased agitation
Decreased interest in pleasurable stimuli (e.g., sex, food, social interactions)
Recurrent thoughts of death and suicide

A diagnosis of major depression is made when a certain number of the above symptoms are reported for longer than a 2 week period of time, and when the symptoms disrupt normal social and occupational functioning (see DSMIV, 2000).

(hyper- or hypocortisolemia, hyper- or hypothyroidism), collagen vascular diseases, Parkinson's disease, traumatic head injury, certain cancers, asthma, diabetes, and stroke.

The role of stress warrants particular comment. Depression is often described as a stress-related disorder, and there is good evidence that episodes of depression often occur in the context of some form of stress. However, stress per se is not sufficient to cause depression. Most people do not become depressed after serious stressful experiences, whereas many who do become depressed do so after stresses that for most people are quite mild. Conversely, severe, horrendous stress, such as that experienced during combat, rape, or physical abuse, does not typically induce depression, but instead causes post-traumatic stress disorder (PTSD) that is distinct from depression based on symptomatology, treatment, and longitudinal course of illness. This underscores the view that depression in most people is caused by interactions between a genetic predisposition and some environmental factors, which makes the mechanisms of such interactions an important focus of investigation.

Treatment of Depression

In contrast to our limited understanding of depression, there are many effective treatments. The large majority (~80%) of people with depression show some improve-

ment with any of several antidepressant medications or electroconvulsive seizures (ECS). In addition, several forms of psychotherapy (in particular, cognitive and behavioral therapies) can be effective for patients with mild to moderate cases, and the combination of medication and psychotherapy can exert a synergistic effect.

The treatment of depression was revolutionized about 50 years ago, when two classes of agents were discovered—entirely by serendipity—to be effective antidepressants: the tricyclic antidepressants and the monoamine oxidase inhibitors. The original tricyclic agents (e.g., imipramine) arose from antihistamine research, whereas the early monoamine oxidase inhibitors (e.g., iproniazid) were derived from work on antitubercular drugs. The discovery that depression can be treated with these medications provided one of the first clues into the types of chemical changes in the brain that regulate depressive symptoms. Indeed, much depression research over the last half-century was based on the notion that understanding how these treatments work would reveal new insight into the causes of depression.

The acute mechanisms of action of antidepressant medications were identified: inhibition of serotonin or norepinephrine reuptake transporters by the tricyclic antidepressants, and inhibition of monoamine oxidase (a major catabolic enzyme for monoamine neurotransmitters) by monoamine oxidase inhibitors (see Frazer, 1997). These discoveries led to the development of numerous second generation medications (e.g., serotonin-selective reuptake inhibitors [SSRIs] and norepinephrine-selective reuptake inhibitors) which are widely used today. The availability of clinically active antidepressants also made it possible to develop and validate a wide range of behavioral tests with which to study depression-like phenotypes in animal models. Moreover, these medications and behavioral tests represent important tools with which to study brain function under normal conditions and to identify a range of proteins in the brain that might serve as targets for novel antidepressant treatments.

That's the good news. The bad news is that progress in developing new and improved antidepressant medications has been limited. The SSRIs, for example, have a better side effect profile for some patients, and are easier for physicians to prescribe, compared with the older agents. This explains their astonishing financial

Table 2. Examples of Proposed Subtypes of Depression

Depression Subtype	Main Features
Melancholic depression ^a	Severe symptoms; prominent neurovegetative abnormalities
Reactive depression ^b	Moderate symptoms; apparently in response to external factors
Psychotic depression	Severe symptoms; associated with psychosis: e.g., believing depression is a punishment for past errors (a delusion) or hearing voices that depression is deserved (a hallucination)
Atypical depression	Associated with labile mood, hypersomnia, increased appetite, and weight gain
Dysthymia	Milder symptoms, but with a more protracted course

These subtypes are based on symptoms only and may not describe biologically distinct entities. The subtypes also cannot generally be distinguished by different responses to various subclasses of antidepressant medications.

^aMelancholic depression is similar to a syndrome classified as "endogenous depression," based on the speculation that it is caused by innate factors.

^bReactive depression is similar to a syndrome classified as "exogenous depression," based on the speculation that it is caused by external factors.

success, now a world-wide market of >\$10 billion a year in sales. However, these newer medications have essentially the same mechanism of action as the older tricyclic antidepressants and, as a result, the efficacy of the newer agents and the range of depressed patients they treat are no better than the older medications. Today's treatments remain sub-optimal, with only ~50% of all patients demonstrating complete remission, although many more (up to 80%) show partial responses.

Furthermore, the mechanism of action of antidepressant medications is far more complex than their acute mechanisms might suggest. Inhibition of serotonin or norepinephrine reuptake or catabolism would be expected to result in enhanced actions of these transmitters. However, all available antidepressants exert their mood-elevating effects only after prolonged administration (several weeks to months), which means that enhanced serotonergic or noradrenergic neurotransmission per se is not responsible for the clinical actions of these drugs. Rather, some gradually developing adaptations to this enhanced neurotransmission would appear to mediate drug action. Important progress has been made in the search for such drug-induced plasticity, as will be seen below, but definitive answers are still out of reach. Moreover, several generations of research have failed to provide convincing evidence that depression is caused by abnormalities in the brain's serotonin or norepinephrine systems. This is consistent with the ability of "antidepressant" medications to treat a wide range of syndromes, far beyond depression, including anxiety disorders, PTSD, obsessive-compulsive disorder, eating disorders, and chronic pain syndromes. It also is consistent with the fact that many medications used in general medicine work far from the molecular and cellular lesion underlying a disease.

The remainder of this review provides a progress report of what is known about depression and antidepressant treatments. We first discuss briefly the neural circuitry of normal mood and of depression. We then describe the leading animal models that are available today to study mechanisms of depression and antidepressant action. We end by presenting three working hypotheses of the neurobiology of depression, which highlight both the advances that have been made in understanding this disorder, but also the tremendous amount of work that is still needed to establish the neurobiologic mechanisms of depression.

Neural Circuitry of Depression

While many brain regions have been implicated in regulating emotions, we still have a very rudimentary understanding of the neural circuitry underlying normal mood and the abnormalities in mood that are the hallmark of depression. This lack of knowledge is underscored by the fact that even if it were possible to biopsy the brains of patients with depression, there is no consensus in the field as to the site of the pathology, and hence the best brain region to biopsy. This is in striking contrast to other neuropsychiatric disorders (e.g., Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis) where pathologic lesions have been identified in specific regions of the central nervous system.

It is likely that many brain regions mediate the diverse symptoms of depression. This is supported by human brain imaging studies—still in relatively early stages—which have demonstrated changes in blood flow or related measures in several brain areas, including regions of prefrontal and cingulate cortex, hippocampus, striatum, amygdala, and thalamus, to name a few (Drevets, 2001, Liotti and Mayberg, 2001). Similarly, anatomic studies of brains of depressed patients obtained at autopsy have reported abnormalities in many of these same brain regions (Zhu et al., 1999; Manji et al., 2001; Drevets, 2001; Rajkowska, 2000). Much work remains, however, since some of the imaging and autopsy studies have yielded contradictory findings; still, this work has underscored the need to investigate mechanisms of mood regulation and dysregulation in numerous brain areas.

Knowledge of the function of these brain regions under normal conditions suggests the aspects of depression to which they may contribute. Neocortex and hippocampus may mediate cognitive aspects of depression, such as memory impairments and feelings of worthlessness, hopelessness, guilt, doom, and suicidality. The striatum (particularly the ventral striatum or nucleus accumbens [NAc]) and amygdala, and related brain areas, are important in emotional memory, and could as a result mediate the anhedonia (decreased drive and reward for pleasurable activities), anxiety, and reduced motivation that predominate in many patients. Given the prominence of so-called neurovegetative symptoms of depression, including too much or too little sleep, appetite, and energy, as well as a loss of interest in sex and other pleasurable activities, a role for the hypothalamus has also been speculated. Of course, these various brain regions operate in a series of highly interacting parallel circuits, which perhaps begins to formulate a neural circuitry involved in depression (Figure 1).

Animal Models of Depression

A major impediment in depression research is the lack of validated animal models. Many of the core symptoms of depression (e.g., depressed mood, feelings of worthlessness, suicidality) cannot be easily measured in laboratory animals. Also, the lack of known depression vulnerability genes means that genetic causes of depression cannot be replicated in animals. As a result, all available animal models of depression rely on one of two principles: actions of known antidepressants or responses to stress (Table 3) (see Willner, 1995; Hitzemann, 2000; Porsolt, 2000; Lucki, 2001). Some of these tests (in particular, the forced swim test) have been very effective at predicting the antidepressant efficacy of new medications. They also provide potentially useful models in which to study the neurobiologic and genetic mechanisms underlying stress and antidepressant responses. One caveat is that these tests have not yet resulted in the introduction of medications with truly novel (i.e., non-monoamine-based) mechanisms, although this may reflect difficulties with clinical trials and the FDA approval process as much as limitations of the animal models. Another caveat is that the medications are active in the animal tests after acute administration, while their clinical efficacy requires chronic administration. As

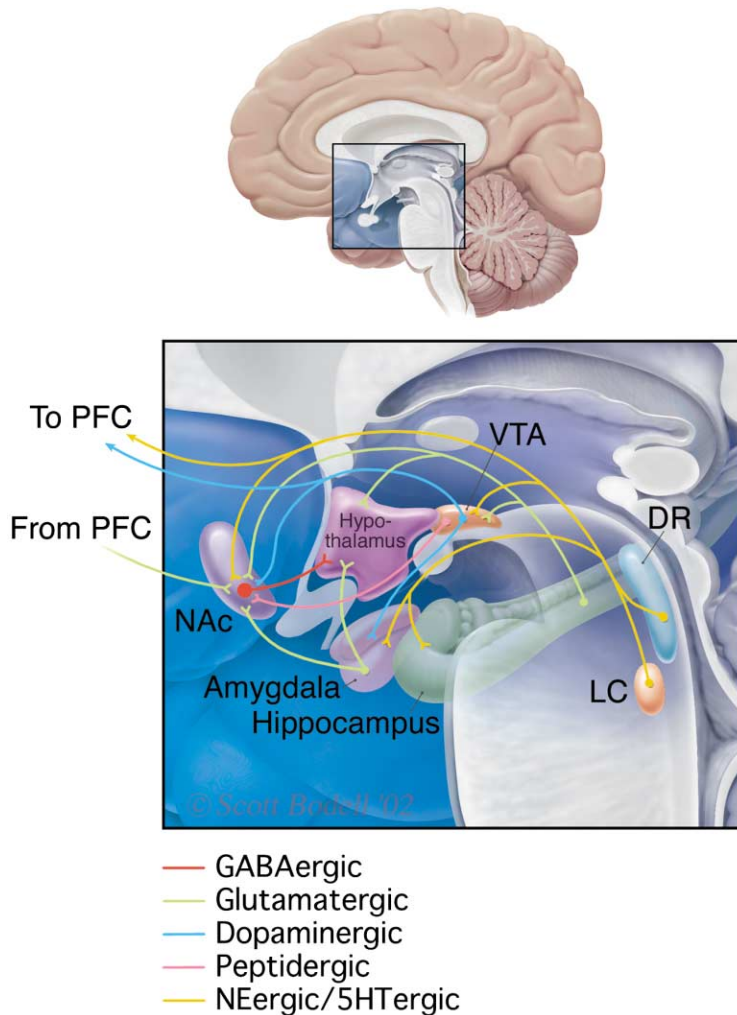


Figure 1. Neural Circuitry of Depression

The figure shows a highly simplified summary of a series of neural circuits in the brain that may contribute to depressive symptoms. While most research in the depression field has focused on hippocampus (HP) and frontal cortex (e.g., prefrontal cortex [PFC]), there is the increasing realization that several subcortical structures implicated in reward, fear, and motivation are also critically involved. These include the nucleus accumbens (NAc), amygdala, and hypothalamus. The figure shows only a subset of the many known interconnections among these various brain regions. The figure also shows the innervation of several of these brain regions by monoaminergic neurons. The ventral tegmental area (VTA) provides dopaminergic input to the NAc, amygdala, PFC, and other limbic structures. Norepinephrine (from the locus coeruleus or LC) and serotonin (from the dorsal raphe [DR] and other raphe nuclei) innervate all of the regions shown in the figure. In addition, there are strong connections between the hypothalamus and the VTA-NAc pathway.

a result, it is not known whether the tests are sensitive to the true mood-elevating changes that the drugs cause in the brain or to some other epiphenomena.

Another weakness of available animal models of depression is that they utilize normal mice, while depression probably requires a genetic vulnerability in most cases. Ultimately, bona fide animal models will only be developed once the etiology and pathophysiology of human depression are identified. This becomes a catch-22: we need animal models of depression to better understand the disorders, but such models can only be developed after we understand the human disorder! Depression vulnerability genes will eventually be identified. During this interim period, one approach would be to make increasing use of animal models of particular aspects of depression, for example, cognitive or attentional impairments, or abnormalities in psychomotor activity, responses to pleasurable stimuli, and eating and sleeping behavior (Table 3). These behavioral tests have not normally been utilized in depression research and may offer new insights into the neurobiologic mechanisms involved.

Despite the pitfalls of available animal models of depression, these models have enabled the field to formulate several hypotheses by which depression may occur

and antidepressant treatments may work. The three hypotheses presented below are not comprehensive of the field, but provide representative examples of recent approaches toward understanding depression and antidepressant action; they also highlight the work that still remains.

Dysregulation of the Hippocampus and Hypothalamic-Pituitary-Adrenal Axis

A prominent mechanism by which the brain reacts to acute and chronic stress is activation of the hypothalamic-pituitary-adrenal (HPA) axis (Figure 2). Neurons in the paraventricular nucleus (PVN) of the hypothalamus secrete corticotropin-releasing factor (CRF), which stimulates the synthesis and release of adrenocorticotropin (ACTH) from the anterior pituitary. ACTH then stimulates the synthesis and release of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal cortex. Glucocorticoids exert profound effects on general metabolism and also dramatically affect behavior via direct actions on numerous brain regions.

The activity of the HPA axis is controlled by several brain pathways, including the hippocampus (which exerts an inhibitory influence on hypothalamic CRF-containing neurons via a polysynaptic circuit) and the amy-

Table 3. Examples of Animal Models Used in Depression Research

Model	Main Features
Forced swim test	Antidepressants acutely increase the time an animal struggles in a chamber of water; lack of struggling thought to represent a state of despair.
Tail suspension test	Antidepressants acutely increase the time an animal struggles when suspended by its tail; lack of struggling thought to represent a state of despair.
Learned helplessness	Animals exposed to inescapable footshock take a longer time to escape, or fail to escape entirely, when subsequently exposed to escapable foot shock; antidepressants acutely decrease escape latency and failures.
Chronic mild stress	Animals exposed repeatedly to several unpredictable stresses (cold, disruption of light-dark cycle, footshock, restraint, etc.) show reduced sucrose preference and sexual behavior; however, these endpoints have been difficult to replicate, particularly in mice.
Social stress	Animals exposed to various types of social stress (proximity to dominant males, odors of natural predators) show behavioral abnormalities; however, such abnormalities have been difficult to replicate, particularly in mice.
Early life stress	Animals separated from their mothers at a young age show some persisting behavioral and HPA axis abnormalities as adults, some of which can be reversed by antidepressant treatments.
Olfactory bulbectomy	Chemical or surgical lesions of the olfactory bulb cause behavioral abnormalities, some of which can be reversed by antidepressant treatments.
Fear conditioning	Animals show fear-like responses when exposed to previously neutral cues (e.g., tone) or context (cage) that has been associated with an aversive stimulus (e.g., shock).
Anxiety-based tests ^a	The degree to which animals explore a particular environment (open space, brightly lit area, elevated area) is increased by anxiolytic drugs (e.g., benzodiazepines).
Reward-based tests ^b	Animals show highly reproducible responses to drugs of abuse (or to natural rewards such as food or sex) in classical conditioning and operant conditioning assays.
Cognition-based tests ^c	The ability of animals to attend, learn, and recall is measured in a variety of circumstances.

Most of these tests are available in rats and mice; the tail suspension test is used in mice only.

^a Examples include open field, dark-light, and elevated plus maze test.

^b Examples include conditioned place preference, drug self-administration, conditioned reinforcement, and intra-cranial self-stimulation assays.

^c Examples include test of spatial memory (Morris water maze, radial arm maze), working memory (T-maze), and attention (5 choices serial test).

dala (which exerts a direct excitatory influence) (Figure 2). Glucocorticoids, by potently regulating hippocampal and PVN neurons, exert powerful feedback effects on the HPA axis. Levels of glucocorticoids that are seen under normal physiological circumstances seem to enhance hippocampal inhibition of HPA activity. They may also enhance hippocampal function in general and thereby promote certain cognitive abilities. However, sustained elevations of glucocorticoids, seen under conditions of prolonged and severe stress, may damage hippocampal neurons, particularly CA3 pyramidal neurons. The precise nature of this damage remains incompletely understood, but may involve a reduction in dendritic branching and a loss of the highly specialized dendritic spines where the neurons receive their glutamatergic synaptic inputs (Figure 3) (McEwen, 2000; Sapolsky, 2000). Stress and the resulting hypercortisolemia also reduce the birth of new granule cell neurons in the adult hippocampal dentate gyrus (Fuchs and Gould, 2000). Such hippocampal neurogenesis is proposed to contribute to memory formation, but this remains controversial. Regardless of the nature of the damage, it would be expected to reduce the inhibitory control that

the hippocampus exerts on the HPA axis, which would further increase circulating glucocorticoid levels and subsequent hippocampal damage.

Such a positive feedback process with pathological consequences has been implicated in a subset of depression. Abnormal, excessive activation of the HPA axis is observed in approximately half of individuals with depression, and these abnormalities are corrected by antidepressant treatment (Sachar and Baron, 1979; De Kloet et al., 1988; Arborelius et al., 1999; Holsboer, 2001). Some patients exhibit increased cortisol production, as measured by increases in urinary free cortisol and decreased ability of the potent synthetic glucocorticoid, dexamethasone (see Figure 2), to suppress plasma levels of cortisol, ACTH, and β -endorphin (which is derived from the same peptide precursor as ACTH). There also is direct and indirect evidence for hypersecretion of CRF in some depressed patients (Arborelius et al., 1999; Holsboer, 2001; Kasckow et al., 2001). ACTH responses to intravenously administered CRF are blunted, and increased concentrations of CRF have been found in cerebrospinal fluid. A small number of postmortem studies of depressed individuals have reported increased levels of

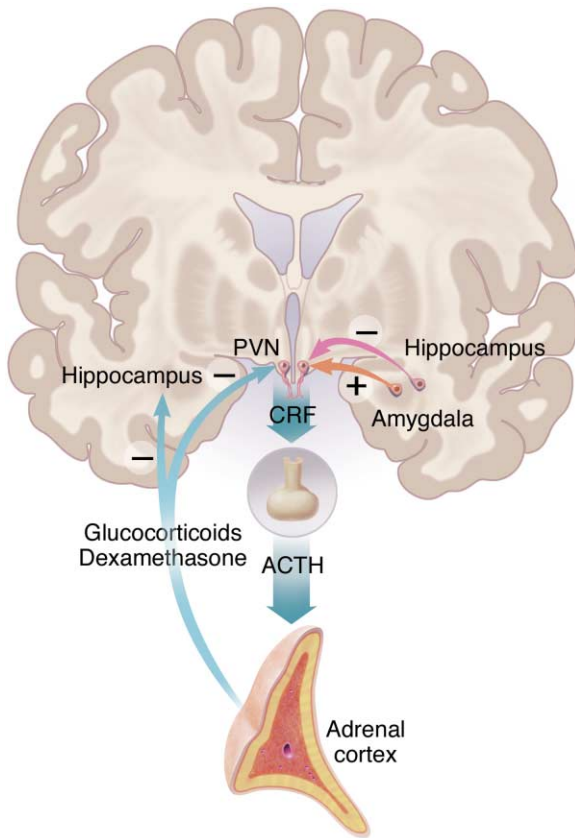


Figure 2. Regulation of the Hypothalamic-Pituitary-Adrenal Axis
CRF-containing parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVN) integrate information relevant to stress. Prominent neural inputs include excitatory afferents from the amygdala and inhibitory (polysynaptic) afferents from the hippocampus, as shown in the figure. Other important inputs are from ascending monoamine pathways (not shown). CRF is released by these neurons into the hypophyseal portal system and acts on the corticotrophs of the anterior pituitary to release ACTH. ACTH reaches the adrenal cortex via the bloodstream, where it stimulates the release of glucocorticoids. In addition to its many functions, glucocorticoids (including synthetic forms such as dexamethasone) repress CRF and ACTH synthesis and release. In this manner, glucocorticoids inhibit their own synthesis. At higher levels, glucocorticoids also impair, and may even damage, the hippocampus, which could initiate and maintain a hypercortisolemic state related to some cases of depression.

CRF in the PVN of the hypothalamus, whereas levels of CRF receptors are downregulated perhaps as a response to elevated CRF transmission. Consistent with these human data are the observations that rodents separated from their mothers early in life show abnormalities in HPA axis function, which resemble those seen in some depressed humans (De Kloet et al., 1988; Francis and Meaney, 1999; Heim and Nemeroff, 2001). These abnormalities can persist into adulthood and be corrected by antidepressant treatments.

How might a hyperactive HPA axis contribute to depression? Current hypotheses focus on cortisol and CRF. The levels of cortisol seen in some depressed patients, particularly over sustained periods of time, might be high enough to be toxic to hippocampal neu-

rons, as described above. Based on the normal functions subserved by hippocampus, impaired hippocampal function might be expected to contribute to some of the cognitive abnormalities of depression. Antidepressant treatments would work, then, by reversing these abnormalities, although the molecular and cellular mechanisms by which prolonged enhancement of monoamine transmission would produce such actions are not known.

Stress-induced changes in hippocampus (e.g., reduction in dendritic arborizations or birth of new neurons) seen in animal models could be related to the small reductions in hippocampal volume documented in some patients with depression (Sheline et al., 1999; Bremner et al., 2000). However, it is not known whether these reduced hippocampal volumes are a result of depression or an antecedent cause. In animal models, several classes of antidepressants reverse the stress-induced reductions in dendritic arborizations of hippocampal pyramidal neurons (Kuroda and McEwen, 1998; Norrholm and Ouimet, 2001) as well as increase neurogenesis in the dentate gyrus (Malberg et al., 2000; Duman et al., 2001; Manji et al., 2001). However, there is currently no direct evidence to link dendritic morphology or neurogenesis either to the human brain imaging findings or to the symptomatology of depression in humans or animal models.

There also are striking parallels between some aspects of the stress response, severe depression, and the effects of centrally administered CRF. These include increased arousal and vigilance, decreased appetite, decreased sexual behavior, and increased heart rate and blood pressure (Arborelius et al., 1999; Holsboer, 2001). This has led to the proposal that a hyperactive HPA axis may contribute to depression not only via hypercortisolemia, but also via enhanced CRF transmission in the hypothalamus and other brain regions that are innervated by these neurons.

Despite the compelling model outlined above, it is still unknown whether HPA axis abnormalities are a primary cause of depression or, instead, secondary to some other initiating cause. Nevertheless, a strong case can be made for its role in the generation of certain symptoms of depression, and for an impact on the course of the disease and its somatic sequelae. Such observations have provided a clear rationale for the use of glucocorticoid or CRF receptor antagonists as novel antidepressant treatments. There is growing evidence that glucocorticoid receptor antagonists, such as mifepristone (RU486), may be useful in treating some cases of depression (Belanoff et al., 2001). Intense attention is being given to antagonists of the CRF₁ receptor, the major CRF receptor in brain, although agents directed against CRF₂ receptors are also of interest (Arborelius et al., 1999; Holsboer, 2001). CRF₁ receptor antagonists exert clear antidepressant-like effects in several stress-based rodent models of depression. These drugs may treat depression by limiting hypercortisolemia through actions on the HPA axis (see Figure 2).

In addition, an action with potentially greater impact on depression, assuming the drugs prove clinically effective, may be inhibition of the CRF system in many other brain regions, independent of the PVN and the HPA axis. For example, in amygdala and several related

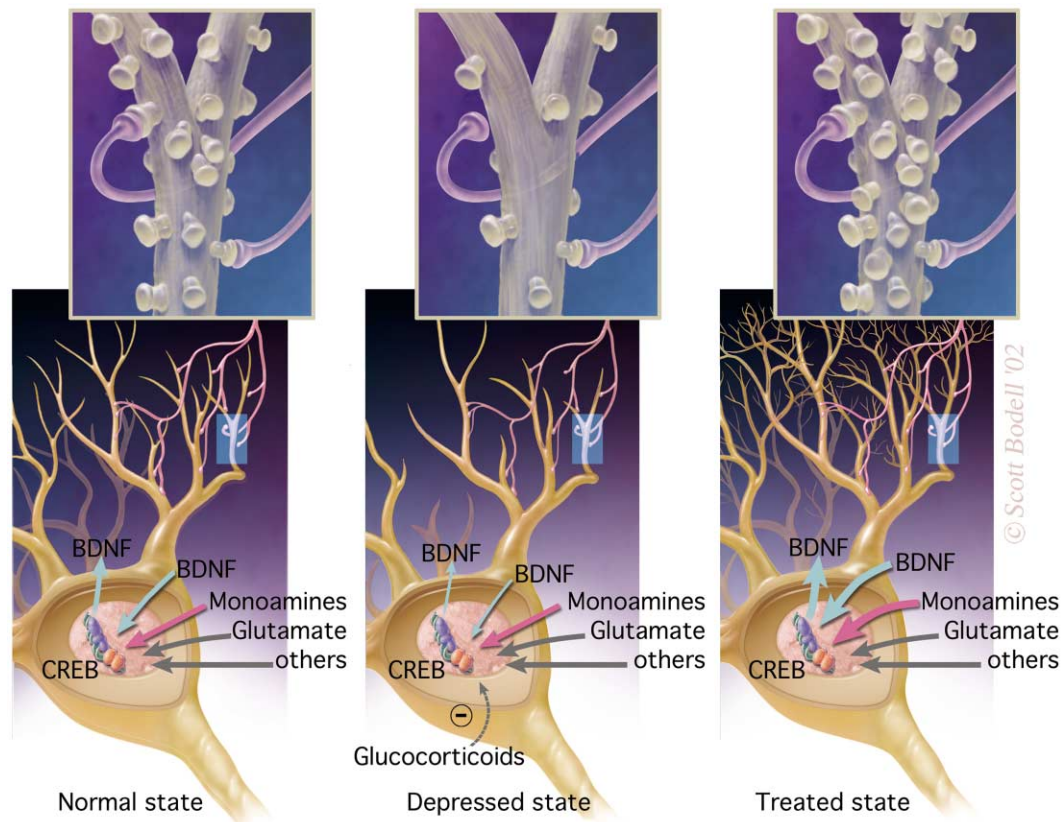


Figure 3. Neurotrophic Mechanisms in Depression

The panel on the left shows a normal hippocampal pyramidal neuron and its innervation by glutamatergic, monoaminergic, and other neurons. Its regulation by BDNF (derived from hippocampus or other brain areas) is also shown. Severe stress causes several changes in these neurons, including a reduction in their dendritic arborizations, and a reduction in BDNF expression (which could be one of the factors mediating the dendritic effects). The reduction in BDNF is mediated partly by excessive glucocorticoids, which could interfere with the normal transcriptional mechanisms (e.g., CREB) that control BDNF expression. Antidepressants produce the opposite effects: they increase dendritic arborizations and BDNF expression of these hippocampal neurons. The latter effect appears to be mediated by activation of CREB through the types of pathways shown in Figure 4. By these actions, antidepressants may reverse and prevent the actions of stress on the hippocampus, and ameliorate certain symptoms of depression.

brain areas, as will be seen below, CRF is a critical mediator of fear conditioning and other forms of emotional memory to both aversive and rewarding stimuli.

Impairment of Neurotrophic Mechanisms

The pathologic effects of stress on hippocampus, described above, have contributed to another recent hypothesis, one that proposes a role for neurotrophic factors in the etiology of depression and its treatment (Duman et al., 1997; Altar, 1999). Neurotrophic factors were first characterized for regulating neural growth and differentiation during development, but are now known to be potent regulators of plasticity and survival of adult neurons and glia. The neurotrophic hypothesis of depression states that a deficiency in neurotrophic support may contribute to hippocampal pathology during the development of depression, and that reversal of this deficiency by antidepressant treatments may contribute to the resolution of depressive symptoms.

Work on this hypothesis has focused on brain-derived neurotrophic factor (BDNF), one of the most prevalent

neurotrophic factors in adult brain. Acute and chronic stress decreases levels of BDNF expression in the dentate gyrus and pyramidal cell layer of hippocampus in rodents (Smith et al., 1995a). This reduction appears to be mediated partly via stress-induced glucocorticoids and partly via other mechanisms, such as stress-induced increases in serotonergic transmission (Smith et al., 1995a; Vaidya et al., 1997). Conversely, chronic (but not acute) administration of virtually all classes of antidepressant treatments increases BDNF expression in these regions (Nibuya et al., 1995), and can prevent the stress-induced decreases in BDNF levels. There is also evidence that antidepressants increase hippocampal BDNF levels in humans (Chen et al., 2001b). Antidepressant induction of BDNF is at least partly mediated via the transcription factor CREB (cAMP response element binding protein), as described below. Together, these findings raise the possibility, illustrated in Figure 3, that antidepressant-induced upregulation of BDNF could help repair some stress-induced damage to hippocampal neurons and protect vulnerable neurons from further damage. Moreover, since BDNF is reported to enhance

long-term potentiation and other forms of synaptic plasticity in hippocampus (Korte et al., 1996; Patterson et al., 1996; Kang et al., 1997), increased BDNF levels induced by antidepressants may promote hippocampal function. The findings could also explain why an antidepressant response is delayed: it would require sufficient time for levels of BDNF to gradually rise and exert their neurotrophic effects.

Despite the appeal and heuristic value of this hypothesis, direct evidence linking BDNF function in hippocampus to depression is still limited. The most compelling evidence comes from a recent study, where administration of BDNF or a related neurotrophin (neurotrophin-3) into the dentate gyrus or CA3 region of hippocampus causes antidepressant-like effects in the forced swim and learned helplessness tests (Shirayama et al., 2002). On the other hand, there is a report that the ability of an antidepressant to reverse the dendritic changes in CA3 pyramidal neurons caused by stress is not mediated via induction of BDNF (Kuroda and McEwen, 1998). Mice lacking CREB, which do not show antidepressant induction of BDNF in hippocampus, still show normal responses to antidepressants in the forced swim test (Conti et al., 2002).

One limitation in the ability to test the BDNF hypothesis is that mice lacking BDNF die shortly after birth from peripheral complications. Recently, a conditional knock-out of BDNF has been achieved, where the loss of BDNF occurs late in embryonic development; these mice survive into adulthood (Rios et al., 2001). The mice show increased anxiety-like behavior, as well as obesity, but no obvious depression-like syndrome has yet been reported. On the other hand, this may relate to current limitations in animal models of depression as mentioned earlier.

This discussion highlights the need for additional experimental tools to establish a link between hippocampal BDNF levels and the formation of depressive symptoms and their resolution with antidepressant administration. There also is the need to examine the possible involvement of many other types of neurotrophic factors in stress- and antidepressant-induced changes in hippocampal function, and to evaluate the influence of neurotrophic mechanisms outside the hippocampus. Indeed, stress decreases BDNF expression in neocortex and amygdala, like it does in hippocampus, but increases it elsewhere (e.g., hypothalamus) (Smith et al., 1995b). In addition, infusion of BDNF into the midbrain causes antidepressant-like effects (Siuciak et al., 1997), similar to those seen upon intra-hippocampal administration.

The BDNF hypothesis predicts that agents that promote BDNF function might be clinically effective antidepressants. Currently, no such compounds are available, but the development of small molecules that regulate neurotrophic factors or their signaling cascades is a major focus of drug development efforts. Another approach would be to intervene earlier in the process, that is, in the mechanisms by which antidepressants induce BDNF expression. There is now considerable evidence that CREB is involved. The BDNF gene is induced in vitro and in vivo by CREB (Tao et al., 1998; Conti et al., 2002). Moreover, virtually all major classes of antidepressants increase levels of CREB expression and function in several brain regions including hippocampus (Ni-

buca et al., 1996; Thome et al., 2000). Levels of CREB are reportedly reduced in temporal cortex (which presumably included hippocampus) in depressed patients studied at autopsy (Dowlatshahi et al., 1998). Increased CREB activity in the hippocampal dentate gyrus, achieved by injection of a viral vector encoding CREB directly into this brain region, exerts an antidepressant-like effect in the forced swim and learned helplessness tests (Chen et al., 2001a). A palliative effect of CREB could be related to CREB's ability to promote long-term memory in hippocampus (Mayford and Kandel, 1999; Silva and Murphy, 1999).

While these effects of CREB could be mediated via numerous target genes in addition to BDNF, it does illustrate novel strategies by which to influence hippocampal function in the context of depression. One positive lead along these lines are inhibitors of phosphodiesterases, the enzymes that degrade cAMP. Several groups have reported that chronic antidepressant treatment upregulates the functioning of the cAMP pathway in hippocampus and neocortex (see Nestler et al., 1989; Duman et al., 1997; Thome et al., 2000). (Drug-induced increases in CREB mentioned above would be one component of this upregulation.) Consistent with the possibility that upregulation of the cAMP pathway is relevant to the therapeutic efficacy of antidepressants is the preliminary clinical observation that rolipram, a type 4 phosphodiesterase inhibitor, which would be expected to increase cAMP levels, reduces the symptoms of depression (see Duman et al., 1997). However, rolipram is poorly tolerated by humans, due to its many side effects. The recent cloning of numerous subtypes of type 4 phosphodiesterase, and the demonstration of their region-specific expression in brain (Takahashi et al., 1999; Houslay, 2001), holds promise for the future development of more selective agents that are effective antidepressants with fewer side effects.

Impairment of Brain Reward Pathways

As is evident from the above discussion, most preclinical studies have focused on the hippocampus as the site involved in the generation and treatment of depression. However, while the hippocampus is undoubtedly involved, it is unlikely that it accounts completely for these phenomena. The hippocampus is best understood for its role in declarative memory and spatial learning. Symptoms affecting learning and memory are certainly seen in depression, but in many patients such symptoms do not represent the overwhelming presentation of the illness. Indeed, as mentioned earlier, brain imaging and autopsy studies have suggested abnormalities in several brain areas of depressed individuals well beyond the hippocampus. In recent years, there has been increasing recognition of the role played by particular subcortical structures (e.g., NAc, hypothalamus, and amygdala) in the regulation of motivation, sleep, appetite, energy level, circadian rhythms, and responses to pleasurable and aversive stimuli, domains which are prominently affected in most depressed patients (see Table 1). Such regions have begun to be explored for a role in normal mood and depression.

Nucleus Accumbens

The NAc is a target of the mesolimbic dopamine system, which arises in dopaminergic neurons in the ventral teg-

mental area (VTA) of the midbrain. These VTA neurons also innervate several other limbic structures, including the amygdala and limbic regions of neocortex (Figure 1). The NAc, and its dopaminergic inputs, play critical roles in reward. Virtually all drugs of abuse increase dopaminergic transmission in the NAc, which partly mediates their rewarding effects (Koob et al., 1998; Wise, 1998). Some drugs produce their rewarding effects in the NAc also via dopamine-independent mechanisms. For example, opiates activate dopaminergic transmission in the NAc via actions in the VTA, but can also directly activate μ opioid receptors on NAc neurons. In addition, increasing evidence suggests that similar mechanisms in the VTA and NAc mediate responses to natural reinforcers under normal conditions as well as compulsive responses under pathological conditions (e.g., over-eating, pathological gambling, etc.). Recent work in nonhuman primates suggests that the firing patterns of VTA dopamine neurons are sensitive readouts of reward expectations: new rewards activate the cells, whereas the absence of an expected reward inhibits the cells (Schultz, 2000). A major gap in knowledge is the means by which altered firing of the dopamine cells, and the consequential altered activity of NAc and other limbic neurons, mediates “reward.” This will ultimately require a circuit level of understanding that is not yet available.

The possible involvement of the VTA-NAc pathway in mood regulation and depression is not well studied. There have been sporadic publications reporting an association between the two over the past several decades (e.g., Willner, 1995; DiChiara et al., 1999; Brown and Gershon, 1993; Pallis et al., 2001; Yacid et al., 2001). However, research in the depression field has focused largely on serotonergic and noradrenergic mechanisms in other brain circuits (e.g., hippocampus and neocortex), while research of the VTA-NAc pathway and of dopaminergic mechanisms has largely focused on addiction. These distinctions are clearly artificial, and there is now the need to systematically examine the role of the VTA-NAc reward pathway in mood regulation.

One approach would be to use behavioral models of drug reward in depression research (see Table 3). One of the best examples is a paradigm called intra-cranial self-stimulation (ICSS) (Hall et al., 1977; Wise, 1996; Macey et al., 2000). Animals work (press a lever) to electrically stimulate particular brain areas, including the mesolimbic dopamine system. Drugs of abuse decrease the stimulation threshold (intensity of the electrical stimulus) for which animals will work, whereas aversive conditions (e.g., drug withdrawal states, severe stress) have the opposite effect. It is possible that ICSS provides a novel measure of an animal’s affective state, which is not easily inferable from more traditional models of depression.

Another approach would be to examine molecular and cellular changes, which occur in the VTA-NAc pathway upon exposure to drugs of abuse, in the context of depression models. Recent studies of CREB illustrate this point. Drugs of abuse have been shown to activate CREB in the NAc (Berke and Hyman, 2000; Nestler, 2001; Shaw-Lutchman et al., 2002), and increased CREB function in this region has been shown to decrease rewarding responses to drugs of abuse whereas decreased CREB function has the opposite effect (Carlezon et al., 1998;

Pliakas et al., 2001; M. Barrot et al., submitted). Based on these findings, we have recently found that CREB-mediated transcription is also induced in the NAc in response to acute and chronic stress (Pliakas et al., 2001; M. Barrot et al., submitted). Interestingly, increased CREB function in this brain region decreases an animal’s sensitivity to several types of aversive stimuli, including anxiogenic and nociceptive stimuli, while decreased CREB function increases that sensitivity (M. Barrot et al., submitted). Thus, it would appear that CREB in the NAc controls the behavioral responsiveness of an animal to emotional stimuli in general, such that the increase in CREB seen after stress or drug exposure may contribute to symptoms of emotional numbing or anhedonia, which are seen in some forms of depression, in PTSD, and in drug withdrawal states. The opioid peptide dynorphin may be one target gene through which CREB produces this behavioral phenotype (Figure 4) (Carlezon et al., 1998; Pliakas et al., 2001).

It is important to note that the proposed action of CREB in the NAc is very different from that proposed for CREB in hippocampus, where it is implicated in induction of BDNF and antidepressant-like responses in animal models (see above). This may explain why mice deficient in CREB show overall normal responses to antidepressants in certain behavioral tests (Conti et al., 2002). Thus, a given molecule can exert different (and even opposing) effects on complex behavior in distinct brain regions, based on different targets—and therefore different effects—of the molecule in distinct types of neurons and on distinct circuits in which the neurons operate. This highlights the need to view molecular changes that occur in the brain within the context of the neural circuitry involved.

Another illustration of this principle is BDNF. BDNF in hippocampus is implicated in antidepressant action, as described above. In the VTA-NAc pathway, BDNF dramatically potentiates drug reward mechanisms (Horger et al., 1999), while preliminary studies have found that BDNF in the VTA-NAc produces a *depression-like* effect in the forced swim test (E.J.N. and A.J.E., unpublished observations). These data implicate BDNF within the mesolimbic dopamine system in the regulation of mood, motivation, and, possibly, depression, and underscore the need to examine neural circuits outside the hippocampus for a complete understanding of these phenomena.

Hypothalamus

The hypothalamus has long been known to mediate many neuroendocrine and neurovegetative functions. It is a highly complex structure; numerous micronuclei have been characterized by standard histologic techniques, yet the neurotransmitters (and particularly the peptide transmitters) expressed by these various nuclei are just now being delineated. The hypothalamus has been studied in the context of depression, although most of this work has focused on the HPA axis (as outlined above) or other neuroendocrine functions such as the hypothalamic-pituitary-thyroid axis. Other hypothalamic functions and nuclei have remained largely unexplored in depression research, despite the fact that these nuclei and their peptide transmitters are crucial for appetite, sleep, circadian rhythms, and interest in sex, which are abnormal in many depressed patients.

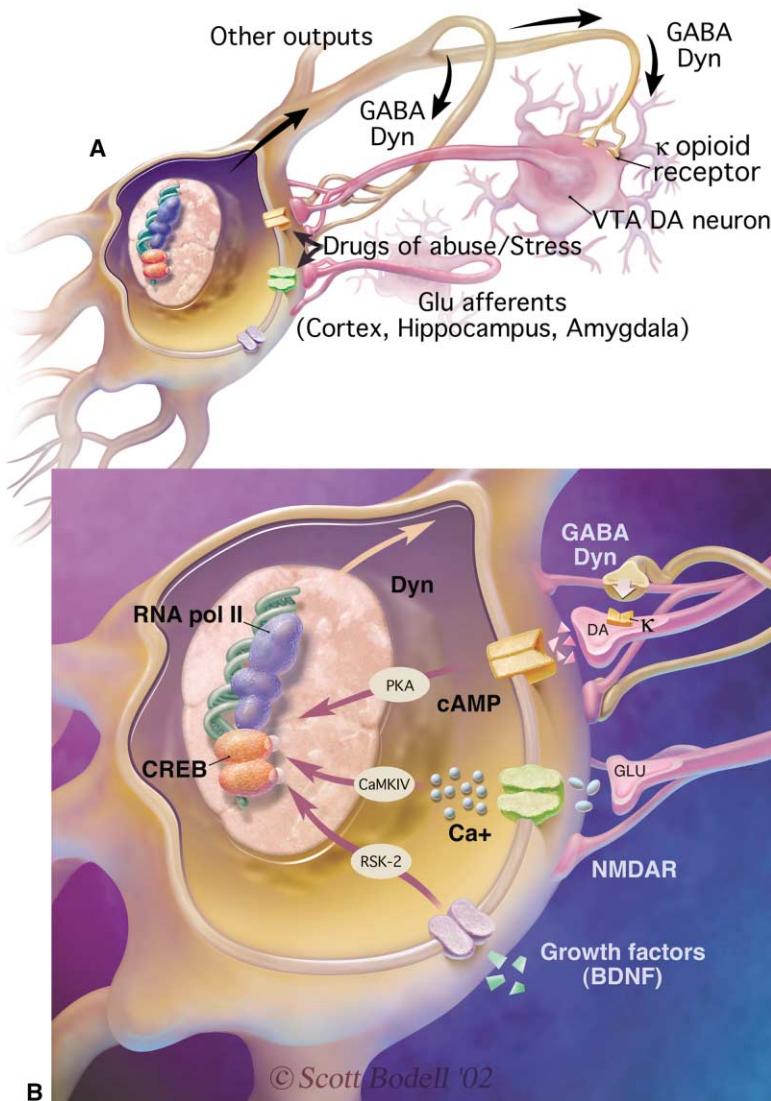


Figure 4. Regulation of NAc Function by CREB and Dynorphin

The figure shows a VTA dopamine (DA) neuron innervating a class of NAc GABAergic projection neuron that expresses dynorphin (Dyn). Dynorphin serves a negative feedback mechanism in this circuit: dynorphin, released from terminals of the NAc neurons, acts on κ opioid receptors located on nerve terminals and cell bodies of the DA neurons to inhibit their functioning. Regulation of NAc neurons by glutamate (via projections from frontal cortex, amygdala, and hippocampus) and by BDNF (derived from glutamatergic or dopaminergic neurons) is also shown. Exposure to drugs of abuse or to several forms of stress upregulates the activity of the dynorphin feedback loop via activation of CREB and induction of dynorphin gene expression. Such activation of CREB could be mediated via any of the diverse mechanisms shown in the figure, all of which lead to the phosphorylation of CREB on ser133 and to its activation. Activation of CREB and induction of dynorphin seems to reduce an animal's sensitivity to both rewarding and aversive stimuli and could contribute to certain symptoms of depression. NMDAR, NMDA glutamate receptor; PKA, protein kinase A; CaMKIV, Ca²⁺/calmodulin-dependent protein kinase type IV; RSK-2, ribosomal S6 kinase-type 2; RNA pol, RNA polymerase II complex.

Hypothalamic mechanisms also could contribute to the greatly increased risk of depression among females.

A possible relationship between hypothalamic peptides implicated in feeding, and those involved in the regulation of reward and mood, is particularly striking. CRF, part of the stress-responsive HPA axis, is also a potent anxiogenic and anorexigenic signal (Arborelius et al., 1999; Holsboer, 2001; Ahima and Osei, 2001). Orexin (also known as hypocretin), expressed in the lateral hypothalamus, regulates sleep and alertness as well as eating (Willie et al., 2001) and potently activates VTA dopamine neurons (Uramura et al., 2001). Melanin concentrating hormone (MCH), also expressed in the lateral hypothalamus, is another potent orexigenic peptide (Ahima and Osei, 2001), and increases sexual behavior and reduces anxiety-like behavior as well (Gonzalez et al., 1996; Monzon et al., 2001). The MCH receptor (MCH₁R) is highly enriched within the NAc (Saito et al., 2001). Melanocortin (MC or α -MSH), expressed in medial hypothalamus, is an anorexigenic peptide and also increases anxiety-like behavior. Interestingly, one of the major melanocortin receptors in brain, MC₄R, is highly

enriched in the NAc and dorsal striatum, where activation of the receptor dramatically antagonizes the rewarding effects of cocaine (R. Hsu et al., submitted). CART (cocaine- and amphetamine-regulated transcript), as its name implies, was first identified in the NAc based on its drug regulation, but is even more enriched in the lateral hypothalamus where it functions as a potent anorexigenic peptide (Kuhar and Dall Vechia, 1999; Ahima and Osei, 2001). Expression of these various feeding peptides is controlled by the peripheral hormone leptin, and leptin itself has been shown to dramatically diminish ICSS of the lateral hypothalamus (Fulton et al., 2000), which provides yet another link between the hypothalamus and reward and affective state. A systematic examination of these hypothalamic factors in depression models, at the molecular, cellular, and behavioral levels, is now warranted.

Amygdala

The amygdala is best studied for its role in conditioned fear (Davis, 1998; Cahill et al., 1999; LeDoux, 2000). It mediates the ability of previously nonthreatening stimuli, when associated with naturally frightening stimuli (e.g,

exposure to a predator or other severe stresses), to elicit a wide range of stress responses. Fear-related information enters the amygdala via its basal and lateral nuclei, which also appear to be the site of the plasticity where these associations are encoded. These nuclei project to the central nucleus, from which projection fibers (containing glutamate and in some cases CRF) to numerous brain regions produce the diverse physiological and behavioral effects characteristic of fear responses. These projection regions include the central gray (e.g., periaqueductal gray), lateral hypothalamus, PVN of hypothalamus, and several monoaminergic nuclei. Other brain regions, such as septal nuclei and the bed nucleus of the stria terminalis, which are functionally and anatomically related to the amygdala, are also important for fear and anxiety-like responses. These same brain regions are implicated in the aversive symptoms seen during withdrawal from drugs of abuse (Koob et al., 1998).

The amygdala is equally important for conditioned responses to rewarding stimuli, including drugs of abuse and natural rewards (Everitt et al., 1999). In fact, some view the amygdala as part of a larger circuit—termed the extended amygdala—which also includes the NAc, bed nucleus of the stria terminalis, and other brain regions (de Olmos and Heimer, 1999). It is proposed that the circuits formed by these structures are critical for emotional memory, that is, in establishing the emotional valence of a memory (aversive versus rewarding) as well as its strength and persistence.

The molecular basis of the plasticity that occurs in the amygdala and is important for emotional memory is not as well studied as that in the hippocampus or Nac; however, some of the same molecular mediators have been implicated. The cAMP pathway and CREB in the amygdala appear to promote the formation of both aversive and rewarding associations (Hall et al., 2001; Joselyn et al., 2001; Jentsch et al., 2002). Stress decreases the expression of BDNF in the amygdala (Smith et al., 1995b), as seen in hippocampus, although the mechanisms involved, and the functional consequences of this regulation, remain poorly understood.

The amygdala and its related structures have been the focus of a great deal of work in the anxiety, PTSD, and drug addiction fields, but have received relatively little attention in depression. This despite the fact that symptoms of anxiety and fear, and abnormal responses to pleasurable stimuli, are prominent in many depressed individuals.

It would be interesting to use behavioral tests that focus on the amygdala (e.g., cue-elicited fear responses, conditioned aversion or reinforcement assays), as well as direct manipulations of specific proteins in the amygdala (e.g., CREB and BDNF, among many others), to explore the role played by these circuits in depression and antidepressant action.

Future Directions

It is clear from this discussion that one of the major needs in the field of depression research is a better understanding of the neural circuits in the brain that control mood under normal circumstances and mediate abnormalities in mood that are seen in depression. Given

the pervasive symptoms of depression, it is likely that the pathophysiology of the disorder, and the mechanisms by which currently available treatments reverse its symptoms, involve numerous brain regions. Recent work has begun to incorporate studies of amygdala, striatal, and hypothalamic circuits with studies of hippocampus and neocortex to formulate a more complete neural circuitry of mood and depression. The neocortex, in particular, is likely critical for features of depression that would appear to be peculiarly human (feelings of worthlessness, hopelessness, guilt, suicidality, etc.), yet the molecular, cellular, and circuit basis of these complex behaviors remains almost completely obscure.

The ability to image, in the living human brain, the various molecules that are implicated in the pathophysiology of depression would represent a dramatic technological breakthrough in the field. Current brain imaging methodologies make it possible to identify gross circuits in the brain that are affected in depression as well as a still small number of neurotransmitter receptors. Imaging BDNF, CREB, various feeding peptides, newly born dentate gyrus neurons, to name a few, is still far out of reach today, but should be feasible as the field progresses.

Another major need in the field is to understand the greater risk for depression in women. The neurobiologic basis for this increased risk is unknown, and could conceivably be related to gender differences in hormonal status or stress response systems, or to sexual dimorphism in any of the several brain areas mentioned above. Perhaps functional brain imaging, which would allow the identification of differential activation of particular brain areas in human patients, will help direct research into the molecular and cellular mechanisms involved.

Ultimately, one key to solving the mystery of depression lies in genetics. Identifying specific genetic variations that confer risk (or resistance) for depression will likely be the essential first step in categorizing depression based on its underlying biology. Knowing these genetic abnormalities will then make it possible to establish bona fide animal models of depression, and begin a long process of delineating, at the molecular, cellular, and neural circuit levels, how the abnormal genes give rise to the behavioral abnormalities that define depression. Such discoveries will also enable us to understand how a host of nongenetic factors interact with genes to cause depressive disorders in vulnerable individuals. These advances will lead to a second revolution in our approach to depression and to the development of definitive treatments and eventually cures and preventive measures.

Acknowledgments

This work was supported by grants from the National Institute of Mental Health and the National Alliance for Research on Schizophrenia and Depression.

References

- Ahima, R.S., and Osei, S.Y. (2001). Molecular regulation of eating behavior: new insights and prospects for therapeutic strategies. *Trends Mol. Med.* 7, 205–213.
- Akiskal, H.S. (2000). Mood disorders: introduction and overview.

- In Comprehensive Textbook of Psychiatry, B.J. Sadock and V.A. Sadock, eds. (New York: Lippincott, Williams & Wilkins), pp. 1284–1298.
- Altar, C.A. (1999). Neurotrophins and depression. *Trends Pharmacol. Sci.* 20, 59–61.
- Arborelius, L., Owens, M.J., Plotsky, P.M., and Nemeroff, C.B. (1999). The role of corticotropin-releasing factor in depression and anxiety disorders. *J. Endocrinol.* 160, 1–12.
- Belanoff, J.K., Flores, B.H., Kalezhan, M., Sund, B., and Schatzberg, A.F. (2001). Rapid reversal of psychotic depression using mifepristone. *J. Clin. Psychopharmacol.* 21, 516–521.
- Berke, J.D., and Hyman, S.E. (2000). Addiction, dopamine, and the molecular mechanisms of memory. *Neuron* 25, 515–532.
- Blazer, D.G. (2000). Mood disorders: epidemiology. In Comprehensive Textbook of Psychiatry, B.J. Sadock and V.A. Sadock, eds. (New York: Lippincott, Williams & Wilkins), pp. 1298–1308.
- Bremner, J.D., Narayan, M., Anderson, E.R., Staib, L.H., Miller, H.L., and Charney, D.S. (2000). Hippocampal volume reduction in major depression. *Am. J. Psychiatry* 157, 115–118.
- Brown, A.S., and Gershon, S. (1993). Dopamine and depression. *J. Neural Trans.* 91, 75–109.
- Burmeister, M. (1999). Basic concepts in the study of diseases with complex genetics. *Biol. Psychiatry* 45, 522–532.
- Cahill, L., Weinberger, N.M., Roozendaal, B., and McGaugh, J.L. (1999). Is the amygdala a locus of “conditioned fear”? Some questions and caveats. *Neuron* 23, 227–228.
- Carlezon, W.A., Jr., Thome, J., Olson, V.G., Lane-Ladd, S.B., Brodtkin, E.S., Hiroi, N., Duman, R.S., Neve, R.L., and Nestler, E.J. (1998). Regulation of cocaine reward by CREB. *Science* 282, 2272–2275.
- Chen, A.C., Shirayama, Y., Shin, K.H., Neve, R.L., and Duman, R.S. (2001a). Expression of the cAMP response element binding protein (CREB) in hippocampus produces an antidepressant effect. *Biol. Psychiatry* 49, 753–762.
- Chen, B., Dowlatshahi, D., MacQueen, G.M., Wang, J.F., and Young, L.T. (2001b). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol. Psychiatry* 50, 260–265.
- Conti, A.C., Cryan, J.F., Dalvi, A., Lucki, L., and Blendy, J.A. (2002). CREB is essential for the upregulation of BDNF transcription, but not the behavioral or endocrine responses to antidepressant drugs. *J. Neurosci.*, in press.
- Davis, M. (1998). Are different parts of the extended amygdala involved in fear versus anxiety? *Biol. Psychiatry* 44, 1239–1247.
- De Kloet, E.R., Rosenfeld, P., Van Eekelen, J.A., Sutanto, W., and Levine, S. (1988). Stress, glucocorticoids and development. *Prog. Brain Res.* 73, 101–120.
- de Olmos, J.S., and Heimer, L. (1999). The concepts of the ventral striatopallidal system and extended amygdala. *Ann. NY Acad. Sci.* 877, 1–32.
- Diagnostic and Statistical Manual IV. (2000). American Psychiatric Press, Washington, D.C.
- Di Chiara, G., Loddo, P., and Tanda, G. (1999). Reciprocal changes in prefrontal and limbic dopamine responsiveness to adverse and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. *Biol. Psychiatry* 46, 1624–1633.
- Dowlatshahi, D., MacQueen, G.M., Wang, J.F., and Young, T.L. (1998). Increased temporal cortex CREB concentrations and antidepressant treatment in major depression. *Lancet* 352, 1754–1755.
- Drevets, W.C. (2001). Neuroimaging and neuropathological studies of depression: Implications for the cognitive-emotional features of mood disorders. *Curr. Opin. Neurobiol.* 11, 240–249.
- Duman, R.S., Heninger, G.R., and Nestler, E.J. (1997). A molecular and cellular hypothesis of depression. *Arch. Gen. Psychiatry* 54, 597–606.
- Duman, R.S., Nakagawa, S., and Malberg, J. (2001). Regulation of adult neurogenesis by antidepressant treatment. *Neuropharmacology* 25, 836–844.
- Everitt, B.J., Parkinson, J.A., Olmstead, M.C., Arroyo, M., Robledo, P., and Robbins, T.W. (1999). Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. *Ann. NY Acad. Sci.* 877, 412–438.
- Fava, M., and Kendler, K.S. (2000). Major depressive disorder. *Neuron* 28, 335–341.
- Francis, D.D., and Meaney, M.J. (1999). Maternal care and the development of stress responses. *Curr. Opin. Neurobiol.* 9, 128–134.
- Frazer, A. (1997). Pharmacology of antidepressants. *J. Clin. Psychopharmacol.* 17 Suppl. 1, 2S–18S.
- Fuchs, E., and Gould, E. (2000). Mini-review: in vivo neurogenesis in the adult brain: regulation and functional implications. *Eur. J. Neurosci.* 12, 2211–2214.
- Fulton, S., Woodside, B., and Shizgal, P. (2000). Modulation of brain reward circuitry by leptin. *Science* 287, 125–128.
- Gonzalez, M.I., Vaziri, S., and Wilson, C.A. (1996). Behavioral effects of alpha-MSH and MCH after central administration in the female rat. *Peptides* 17, 171–177.
- Hall, R.D., Bloom, F.E., and Olds, J. (1977). Neuronal and neurochemical substrates of reinforcement. *Neurosci. Res. Prog. Bull.* 15, 131–314.
- Hall, J., Thomas, K.L., and Everitt, B.J. (2001). Fear memory retrieval induces CREB phosphorylation and Fos expression within the amygdala. *Eur. J. Neurosci.* 13, 1453–1458.
- Heim, C., and Nemeroff, C.B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol. Psychiatry* 49, 1023–1039.
- Hitzemann, R. (2000). Animal models of psychiatric disorders and their relevance to alcoholism. *Alcohol Res. Health* 24, 149–158.
- Holsboer, F. (2001). Stress, hypercortisolism and corticosteroid receptors in depression: implications for therapy. *J. Affect. Disord.* 62, 77–91.
- Horger, B.A., Iyasere, C.A., Berhow, M.T., Messer, C.J., Nestler, E.J., and Taylor, J.R. (1999). Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J. Neurosci.* 19, 4110–4122.
- Houslay, M.D. (2001). PDE4 cAMP-specific phosphodiesterases. *Prog. Nucleic Acid Res. Mol. Biol.* 69, 249–315.
- Jentsch, J.D., Olausson, P., Nestler, E.J., and Taylor, J.R. (2002). Stimulation of protein kinase A activity in the rat amygdala enhances reward-related learning. *Biol. Psychiatry*, in press.
- Josselyn, S.A., Shi, C.J., Carlezon, W.A., Jr., Neve, R.L., Nestler, E.J., and Davis, M. (2001). Long-term memory is facilitated by CREB overexpression in the amygdala. *J. Neurosci.* 21, 2404–2412.
- Kang, H., Welcher, A.A., Shelton, D., and Schuman, E.M. (1997). Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. *Neuron* 19, 653–664.
- Kasckow, J.W., Baker, D., and Geraciotti, T.D., Jr. (2001). Corticotropin-releasing hormone in depression and post-traumatic disorder. *Peptides* 22, 845–851.
- Koob, G.F., Sanna, P.P., and Bloom, F.E. (1998). Neuroscience of addiction. *Neuron* 21, 467–476.
- Korte, M., Staiger, V., Griesbeck, O., Thoenen, H., and Bonhoeffer, T. (1996). The involvement of brain-derived neurotrophic factor in hippocampal long-term potentiation revealed by gene targeting experiments. *J. Physiol. (Paris)* 90, 157–164.
- Kuhar, M.J., and Dall Vechia, S.E. (1999). CART peptides: novel addiction- and feeding-related neuropeptides. *Trends Neurosci.* 22, 316–320.
- Kuroda, Y., and McEwen, B.S. (1998). Effect of chronic restraint stress and tianeptine on growth factors, growth-associated protein-43, and microtubule-associated protein 2 mRNA expression in rat hippocampus. *Mol. Brain Res.* 59, 35–39.
- LeDoux, J.E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184.
- Liotti, M., and Mayberg, H.S. (2001). The role of functional neuroimaging in the neuropsychology of depression. *J. Clin. Exp. Neuropsychol.* 23, 121–136.

- Lucki, I. (2001). A prescription to resist proscriptio for murine models of depression. *Psychopharmacology (Berl.)* 153, 395–398.
- Macey, D.J., Koob, G.F., and Markou, A. (2000). CRF and corticotropin decreased brain stimulation reward in the rat: reversal by a CRF receptor antagonist. *Brain Res.* 866, 82–91.
- Malberg, J.E., Eisch, A.J., Nestler, E.J., and Duman, R.S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J. Neurosci.* 20, 9104–9110.
- Manji, H.K., Drevets, W.C., and Charney, D.S. (2001). The cellular neurobiology of depression. *Nat. Med.* 7, 541–547.
- Mayford, M., and Kandel, E.R. (1999). Genetic approaches to memory storage. *Trends Genet.* 15, 463–470.
- McEwen, B.S. (2000). Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology* 22, 108–124.
- Monzon, M.E., Varas, M.M., Izquierdo, L.A., Izquierdo, I., Barros, D.M., and de Barioglio, S.R. (2001). Anxiogenesis induced by nitric oxide synthase inhibition and anxiolytic effect of melanin-concentrating hormone (MCH) in rat brain. *Peptides* 22, 1043–1047.
- Nestler, E.J. (2001). Molecular basis of neural plasticity underlying addiction. *Nat. Rev. Neurosci.* 2, 119–128.
- Nestler, E.J., Terwilliger, R.Z., and Duman, R.S. (1989). Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. *J. Neurochem.* 53, 1644–1647.
- Nibuya, M., Morinobu, S., and Duman, R.S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.* 15, 7539–7547.
- Nibuya, M., Nestler, E.J., and Duman, R.S. (1996). Chronic antidepressant administration increases the expression of CREB in rat hippocampus. *J. Neurosci.* 16, 2365–2372.
- Norrholm, S.D., and Ouimet, C.C. (2001). Altered dendritic spine density in animal models of depression and in response to antidepressant treatment. *Synapse* 42, 151–163.
- Pallis, E., Thermos, K., and Spyrali, C. (2001). Chronic desipramine treatment selectively potentiates somatostatin-induced dopamine release in the nucleus accumbens. *Eur. J. Neurosci.* 14, 763–767.
- Patterson, S.L., Abel, T., Deuel, T.A., Martin, K.C., Rose, J.C., and Kandel, E.R. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron* 16, 1137–1145.
- Pliakas, A.M., Carlson, R.R., Neve, R.L., Konradi, C., Nestler, E.J., and Carlezon, W.A., Jr. (2001). Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated CREB expression in the nucleus accumbens. *J. Neurosci.* 21, 7397–7403.
- Porsolt, R.D. (2000). Animal models of depression: utility for transgenic research. *Rev. Neurosci.* 11, 53–58.
- Rajkowska, G. (2000). Histopathology of the prefrontal cortex in major depression: What does it tell us about dysfunctional monoaminergic circuits? *Prog. Brain Res.* 126, 397–412.
- Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R.M., and Jaenisch, R. (2001). Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol. Endocrinol.* 15, 1748–1757.
- Sachar, E.J., and Baron, M. (1979). The biology of affective disorders. *Annu. Rev. Neurosci.* 2, 505–517.
- Saito, Y., Cheng, M., Leslie, F.M., and Civelli, O. (2001). Expression of the melanin-concentrating hormone (MCH) receptor mRNA in the rat brain. *J. Comp. Neurol.* 435, 26–40.
- Sanders, A.R., Detera-Wadleigh, S.D., and Gershon, E.S. (1999). Molecular genetics of mood disorders. In *Neurobiology of Mental Illness*, D.S. Charney, E.J. Nestler, and B.S. Bunney, eds. (New York: Oxford), pp. 299–316.
- Sapolsky, R.M. (2000). Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch. Gen. Psychiatry* 57, 925–935.
- Schultz, W. (2000). Multiple reward signals in the brain. *Nat. Rev. Neurosci.* 1, 199–207.
- Shaw-Lutchman, T.Z., Barrot, M., Wallace, T., Gilden, L., Zachariou, V., Impey, S., Duman, R.S., Storm, D., and Nestler, E.J. (2002). Regional and cellular mapping of CRE-mediated transcription during naltrexone-precipitated morphine withdrawal. *J. Neurosci.*, in press.
- Sheline, Y.I., Sanghavi, M., Mintun, M.A., and Gado, M.H. (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J. Neurosci.* 19, 5034–5043.
- Shirayama, Y., Chen, A.C.-H., Nakagawa, S., Russell, D.S., and Duman, R.S. (2002). Brain derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J. Neurosci.*, in press.
- Silva, A.J., and Murphy, G.G. (1999). cAMP and memory: a seminal lesson from *Drosophila* and *Aplysia*. *Brain Res. Bull.* 50, 441–442.
- Siuciak, J.A., Lewis, D.R., Wiegand, S.J., and Lindsay, R.M. (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol. Biochem. Behav.* 56, 131–137.
- Smith, M.A., Makino, S., Kvetnansky, R., and Post, R.M. (1995a). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J. Neurosci.* 15, 1768–1777.
- Smith, M.A., Makino, S., Kim, S.Y., and Kvetnansky, R. (1995b). Stress increases brain-derived neurotrophic factor messenger ribonucleic acid in the hypothalamus and pituitary. *Endocrinology* 136, 3743–3750.
- Takahashi, M., Terwilliger, R., Lane, C., Mezes, P.S., Conti, M., and Duman, R.S. (1999). Chronic antidepressant administration increases the expression of cAMP-specific phosphodiesterase 4A and 4B isoforms. *J. Neurosci.* 19, 610–618.
- Tao, X., Finkbeiner, S., Arnold, D.B., Shaywitz, A.J., and Greenberg, M.E. (1998). Ca^{2+} influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron* 20, 709–726.
- Thome, J., Sakai, N., Shin, K., Steffen, C., Zhang, Y.J., Impey, S., Storm, D., and Duman, R.S. (2000). cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J. Neurosci.* 20, 4030–4036.
- Uramura, K., Funahashi, H., Muroya, S., Shioda, S., Takigawa, M., and Yada, T. (2001). Orexin-a activates phospholipase C- and protein kinase C-mediated Ca^{2+} signaling in dopamine neurons of the ventral tegmental area. *Neuroreport* 12, 1885–1889.
- Vaidya, V.A., Marek, G.J., Aghajanian, G.K., and Duman, R.S. (1997). 5-HT_{2A} receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J. Neurosci.* 17, 2785–2795.
- Willie, J.T., Chemelli, R.M., Sinton, C.M., and Yanagisawa, M. (2001). To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu. Rev. Neurosci.* 24, 429–458.
- Willner, P. (1995). Animal models of depression: validity and applications. *Advances in Biochem. Psychopharmacol.* 49, 19–41.
- Wise, R.A. (1996). Addictive drugs and brain stimulation reward. *Annu. Rev. Neurosci.* 19, 319–340.
- Wise, R.A. (1998). Drug-activation of brain reward pathways. *Drug Alcohol Depend.* 57, 13–22.
- Yadid, G., Overstreet, D.H., and Zangen, A. (2001). Limbic dopaminergic adaptation to a stressful stimulus in a rat model of depression. *Brain Res.* 896, 43–47.
- Zhu, M.Y., Klimek, V., Dilley, G.E., Haycock, J.W., Stockmeier, C., Overholser, J.C., Meltzer, H.Y., and Ordway, G.A. (1999). Elevated levels of tyrosine hydroxylase in the locus coeruleus in major depression. *Biol. Psychiatry* 46, 1275–1286.