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NEUROBIOLOGICAL BASIS OF ANXIETY DISORDERS

DENNIS S. CHARNEY
WAYNE C. DREVETS

The 1990s witnessed tremendous progress in the acquisition of knowledge about the molecular, cellular, and anatomic correlates of fear and anxiety. Advances in neuropharmacology and molecular biology have enabled elucidation of multiple chemical neurotransmitter systems that play roles in fear and anxiety behavior. The anatomic circuits where these transmitters participate in mediating and modulating fear and anxiety are also being illuminated through improvements in neurotoxic techniques, which have enhanced the selectivity of lesion analyses in experimental animals, and by advances in neuroimaging technology, which have permitted mapping of the neurophysiologic correlates of emotion in humans. The findings of these investigations have informed the design and interpretation of clinical neuroscience approaches aimed at investigating how dysfunction within these neurochemical and anatomic systems may result in psychiatric conditions such as panic, posttraumatic stress, and phobic disorders. This chapter reviews the preclinical and clinical data regarding the neural mechanisms underlying normal and pathologic anxiety and discusses their implications for guiding development of novel treatments for anxiety disorders.

NEUROANATOMIC CIRCUITS SUPPORTING FEAR AND ANXIETY

Fear and anxiety normally comprise adaptive responses to threat or stress. These emotional-behavioral sets may arise in response to exteroceptive visual, auditory, olfactory, or somatosensory stimuli or to interoceptive input through the viscera and the endocrine and autonomic nervous systems. Anxiety may also be produced by cognitive processes mediating the anticipation, interpretation, or recollection of perceived stressors and threats.

Emotional processing in general can be divided into evaluative, expressive, and experiential components (1). Evaluation of the emotional salience of a stimulus involves appraisal of its valence (e.g., appetitive versus aversive), its relationship with previous conditioning and behavioral reinforcement experiences, and the context in which it arises (2,3). Emotional expression conveys the range of behavioral, endocrine, and autonomic manifestations of the emotional response, whereas emotional experience describes the subjective feeling accompanying the response. To optimize their capacity for guiding behavior, all these aspects of emotional processing are modulated by complex neurobiological systems that prevent them from becoming persistent, excessive, inappropriate to reinforcement contingencies, or otherwise maladaptive.

The emotional processes pertaining to fear and anxiety that have been most extensively studied (largely because of their amenability to experimental manipulation) have involved pavlovian fear conditioning and fear-potentiated startle (4,5). These types of "fear learning" have been shown to comprise experience-dependent forms of neural plasticity in an extended anatomic network that centers around the critical involvement of the amygdala (1,6). The structures that function in concert with the amygdala during fear learning include other mesiotemporal cortical structures, the sensory thalamus and cortices, the orbital and medial prefrontal cortex (mPFC), the anterior insula, the hypothalamus, and multiple brainstem nuclei (1,5,7). Much of this network appears to participate in the general process of associating a conditioned stimulus (CS) or operant behavior with an emotionally salient unconditioned stimulus (US) (see Fig. 63.1 on p. 905) (5,8–11).

Role of the Amygdala in Fear Learning and Expression

The anatomic systems supporting fear learning are organized to permit both rapid responses to simple perceptual

Dennis S. Charney: Mood and Anxiety Disorder Research Program, National Institute of Mental Health, Bethesda, Maryland.

W. C. Drevets: Section on Mood and Anxiety Disorders Imaging, Molecular Imaging Branch, National Institute of Mental Health, Bethesda, Maryland.

elements of potentially threatening stimuli and longer-latency responses to more highly processed information about complex sensory stimuli and environmental contexts. The former processes depend on monosynaptic projections from the sensory thalamus to the amygdala, whereas the latter involve projections from sensory association cortices and mesiotemporal cortical structures to the amygdala (1, 12). These neural networks also respond to visceral input received both directly through the nucleus paragigantocellularis and the nucleus tractus solitarius (NTS) of the vagus nerve and indirectly through the locus ceruleus (LC), the anterior insula, and the infralimbic and prelimbic cortices (4,7,13). Finally, neural activity within the amygdala is modulated by cortisol, norepinephrine (NE), and other neurotransmitters and by mnemonic input related to previous conditioning and reinforcement experiences conveyed by projections from mesiotemporal and prefrontal cortical structures (14–18).

The lateral nucleus of the amygdala (LA) comprises the primary sensory interface of the amygdala and receives synaptic input representing sensory information from the sensory thalamus and cortex (4). Single neurons within the LA are responsive to auditory, visual, and somatic stimuli, thus enabling the LA to serve as a locus of convergence for information about CS and US (19). Olfactory input, in contrast, directly projects to the periamygdaloid cortex from the olfactory bulb through the olfactory tract (20). The olfactory tract also sends projections to the pyriform cortex and the entorhinal cortex, areas with reciprocal connections to the amygdala (20). Although the periamygdaloid cortex neurons project to deeper amygdaloid nuclei, the specific pathways conveying olfactory information through the amygdala have not been delineated.

In addition to its role in conditioning to explicit sensory stimuli, the amygdala is involved in the development of emotional responses to environmental context. The projections from the hippocampal formation to the amygdala through the fornix have been specifically implicated in spatial contextual conditioning (21,22). Thus, lesioning these projections specifically prevents fear conditioning to the chamber or the position within a maze in which aversive stimulation previously occurred (22–25). Other structures that participate in the modulation of contextual fear include the rostral perirhinal cortex and the ventrolateral PFC/ anterior (agranular) insula. Lesions of the latter regions reduce fear *reactivity* to contextual stimuli, but they do not affect CS acquisition or response extinction (26). In contrast, lesions placed in the rostral perirhinal cortex *after* fear conditioning interfere with the expression of conditioned fear responses elicited by visual and auditory stimuli when these stimuli are presented in contexts that differ from the initial conditioning context (27). Notably, genetic studies in mice identified a quantitative trait locus for contextual conditioning (28,29) that was associated with mouse "emotionality" in another study (30), although the molecular genetic, neurochemical, and functional anatomic correlates of this trait have not been established.

The projections from sensory thalamus to the LA are thought to support rapid conditioning to simple visual and auditory features, presumably accounting for fear responses below the level of conscious awareness (31). Thus, lesioning the *auditory cortex* before conditioning does not prevent conditioning to single auditory tones. In contrast, projections to the LA from the primary sensory and sensory association cortices appear to be essential for some aspects of conditioned responding to more complex sensory stimuli (4, 32). These relationships are modality specific. For example, disruption of the projections from the auditory thalamus and auditory cortex to the LA specifically prevents acquisition of fear conditioning to auditory stimuli and fear-conditioned responses to previous auditory CSs (33–35).

After sensory input enters the LA, the neural representation of the stimulus is distributed in parallel to various amygdaloid nuclei, where it may be modulated by diverse functional systems, such as those mediating memories from past experiences or knowledge about ongoing homeostatic states (36). The most extensive extranuclear projections of the LA are composed of reciprocal projections to the basal and accessory basal nuclei and the central nucleus of the amygdala (CE) (37,38). Lesions of either the LA or the CE—but not of other amygdala nuclei—disrupt fear conditioning to a tone CS, a finding suggesting that this direct projection from LA to CE is sufficient to mediate conditioning to simple sensory features (4).

The projections from LA to the basal amygdaloid nuclei also participate in forming long-lasting memory traces for fear conditioning (2,15,39). Functional inactivation of the lateral and basal amygdaloid nuclei before pavlovian fear conditioning interferes with acquisition of learning, whereas inactivation immediately after conditioning has no effect on memory consolidation (40). The basal nuclei have widespread intranuclear connections and also project to other amygdalar nuclei, including the CE and the LA (41). They also share extensive, reciprocal projections with the orbital and mPFC (43). The basal nuclei are thus anatomically positioned to modulate neuronal responses in both the LA and the PFC (42,43).

The plasticity within the amygdala that constitutes memory for conditioning experiences has been shown to involve long-term potentiation–like associative processes (6). Plasticity related to fear learning also occurs in cortical areas, presumably making possible the establishment of explicit or declarative memories about the fear-related event through interactions with the medial temporal lobe memory system (44,45). The influence of the amygdala on cortically based memories has been most clearly characterized with respect to *late* plastic components of the auditory cortex neuronal responses to a CS. Single-unit recordings during fear conditioning indicate that some auditory cortex neurons, which before conditioning did not respond to the CS tone, develop

late-conditioned responses (i.e., 500 to 1,500 milliseconds after CS onset) that anticipate the US and show extinction-resistant memory storage (46). These late-conditioned auditory cortical neuronal responses take more trials to learn and respond more slowly than LA neurons within trials, and their late development is prevented by amygdala lesions. Thus, whereas rapid conditioning of fear responses to potentially dangerous stimuli depends on plasticity in the amygdala, learning involving higher cognitive (i.e., mnemonic and attentional) processing of fear experiences may depend on plasticity involving cortical neurons that is influenced by neural transmission from the amygdala to the cortex.

Other auditory cortex neurons show an early (less than 50 milliseconds of stimulus onset) plastic component during fear conditioning, in which the preexisting electrophysiologic responses of auditory cortex neurons to the CS become enhanced by conditioning (46). This short-latency plasticity within the auditory cortex appears to depend on input from the auditory thalamus and is unaffected by amygdala lesions. Nevertheless, such short-latency responses are extinguished more quickly (during repeated exposure to the CS alone) in animals with amygdala lesions, a finding implying that the amygdala is involved in preventing extinction of these responses.

In human neuroimaging studies, hemodynamic activity in the amygdala increases during initial exposures to fear-conditioned stimuli (47,48). However, during repeated, unreinforced exposures to the same stimulus, single-trial functional magnetic resonance imaging (fMRI) studies show that this initial elevation of hemodynamic activity attenuates and subsequently decreases to less than baseline (47). This observation suggests that synaptic input into the amygdala may be actively reduced during the extinction process (49), although the level at which this suppression of afferent synaptic activity into (or within) the amygdala is being suppressed during nonreinforced exposures to the CS has not been established.

Activation of the amygdala during an emotional event enhances the strength of long-term memory for emotional stimuli represented in other cortical memory circuits as well (16,50,51). These circuits presumably involve the medial temporal lobe memory system, which has extensive anatomic connections with the amygdala and presumably provides a neuroanatomic substrate for the interaction between storage and explicit recall of affectively salient memories (16). For example, as healthy humans read stories, the magnitude of physiologic activation in the amygdala correlates both with the negative emotional intensity and with the subsequent recall performance of the story's content (52, 53). Physiologic activity in the amygdala and the hippocampus measured during memory encoding reportedly correlates with enhanced episodic memory for pleasant as well as aversive visual stimuli (54), and the amygdala's role in modulating emotional memory may depend more generally on the degree of arousal or the behavioral salience associated with verbally conveyed information (9,16).

Human neuroimaging and electrophysiologic and lesion analysis studies have also demonstrated that the amygdala is involved in the recall of emotional or arousing memories (4,53,55). In humans, bursts of electroencephalographic activity have been recorded in the amygdala during recollection of specific emotional events (56). Moreover, electrical stimulation of the amygdala can evoke emotional experiences (especially fear or anxiety) (57) and the recollection of emotionally charged life events from remote memory (58).

Role of the Amygdala in Organizing Emotional Expression

The amygdaloid output nuclei, especially the CE, receive convergent information from multiple amygdala regions and generate behavioral responses that are thought to reflect the sum of neuronal activity produced by different amygdaloid nuclei (36). The CE comprises the interface between the amygdala and the motor, autonomic, and neuroendocrine systems involved in expressing fear behavior (4,5). The CE projects to nuclei in the hypothalamus, midbrain, and medulla that mediate the neuroendocrine, autonomic, and behavioral responses associated with fear and anxiety. For example, the amygdala facilitates stress-related corticotropin-releasing hormone (CRH) release by both intrinsic CRH-containing neurons and bisynaptic (double γ-aminobutyric acid–ergic [GABAergic]) anatomic projections to the paraventricular nucleus (PVN) of the hypothalamus (59). Electrical stimulation of the CE produces responses similar to those elicited by fear-conditioned stimuli (60,61), and lesions of the CE prevent the expression of fear responses of various types (4,62,63). In contrast, lesioning of specific structures efferent to the CE, such as the lateral hypothalamus or periaqueductal gray (PAG), produces selective deficits in cardiovascular or somatomotor behavioral fear responses, respectively (1,64).

The amygdala also sends projections to the thalamus, the nucleus accumbens, the ventromedial caudate, and parts of the ventral putamen that participate in organizing motor responses to threatening stimuli (65). For example, activation of the amygdalar projections to the ventral striatum arrests goal-directed behavior in experimental animals (66), a finding suggesting a possible neural mechanism for the cessation of motivated or reward-directed behavior during anxiety and panic. The amygdala may also influence motor behavior by projections through the hypothalamus and PAG (1). For example, in experimental animals, stimulation of the lateral PAG produces defensive behaviors, sympathetic autonomic arousal, and hypoalgesia, whereas stimulation of the ventrolateral PAG produces social withdrawal and behavioral quiescence, as in response to deep injury or visceral pain (67).

Other Roles of the Amygdala in Fear Processing

The amygdala also appears to play important roles in mediating innate fear and in processing affective elements of social interactions (68). Amygdala lesions cause rats to lose their fear of cats and monkeys to lose their fear of snakes (reviewed in ref. 4). In monkeys, amygdala lesions reduce aggression as well as fear and cause animals to become more submissive to dominant animals (69). In humans, blood flow increases in the amygdala as subjects view faces expressing fear or sadness (70,71), and amygdala lesions impair the ability to recognize fear or sadness in facial expression (55, 72) and fear and anger in spoken language (73).

Bed Nucleus of the Stria Terminalis: Hypothesized Role in Anxiety

The hypothalamic and brainstem structures that mediate the expression of emotional behavior can also be activated directly by the bed nucleus of the stria terminalis (BNST) (5). Anxiety-like responses elicited either by exposure to a threatening environment for several minutes or by intraventricular administration of CRH appear to be specifically mediated by the BNST, rather than the CE (5). This system is thus hypothesized to play a role in mediating anxiety during exposure to less explicit, or less well defined, sensory cues or to contexts that occur over a longer duration.

Other Temporal Cortical Structures

The perirhinal cortex shares reciprocal anatomic connections with the amygdala (74), and it is thought to play a role in conveying information about complex visual stimuli to the amygdala during presentation of fear-conditioned visual stimuli. Lesions of the anterior perirhinal cortex, the basolateral nucleus of the amygdala, or the CE can each completely eliminate fear-potentiated startle during exposure to some conditioned visual stimuli (75,76). In contrast, complete removal of the entire visual cortex, insular cortex, mPFC, and posterior perirhinal cortex produces no significant effect on the magnitude of fear-potentiated startle, and lesions of the frontal cortex only partly attenuate fear-potentiated startle. The perirhinal cortex receives input regarding conditioned visual stimuli from the lateral geniculate nucleus, and lesions of this structure can also block fear-potentiated startle (77). Finally, the anterior perirhinal cortex receives afferent projections from the visual cortices as well as from the anterior cingulate cortex (ACC), the infralimbic cortex, and the parietal cortex (74), structures implicated in modulating behavioral responses to fear-conditioned stimuli.

The temporopolar cortex has been implicated in modulating autonomic aspects of emotional responses and in processing emotionally provocative visual stimuli. Electrical stimulation of various sites within the temporopolar cortex can alter a variety of autonomic responses (reviewed in ref. 1). In humans with simple phobias or posttraumatic stress disorder (PTSD), physiologic activity increases in the anterior temporopolar cortex during experimentally induced exacerbations of anxiety involving visual exposure to phobic stimuli or word scripts describing traumatic events, respectively (78,79). Blood flow also increases in the anterior temporopolar cortex of healthy humans during exposure to emotionally provocative visual stimuli, whether the stimuli convey "sad," "disgusting," or "happy" content, relative both to conditions involving exposure to emotionally "neutral" visual stimuli and to conditions in which corresponding emotional states are elicited by recall of autobiographic information (80,81). Portions of the temporopolar cortex may thus function as sensory association areas that participate in evaluating the emotional salience of actual or anticipated stimuli and in modulating autonomic responses to such stimuli.

Neuroendocrine and Autonomic Responses during Fear or Stress

The peripheral hormonal and autonomic responses to threat mediated by the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic and parasympathetic autonomic nervous systems also play adaptive roles in responding to threat or stress (5). Stimulation of the lateral nucleus of the hypothalamus by afferent projections from the CE of the amygdala, the BNST, or the ventral striatum (82) activates the sympathetic system and produces increases in blood pressure and heart rate, sweating, piloerection, and pupillary dilation. Stress stimulates release of CRH from the PVN of the hypothalamus and amygdala. The CRH secretion from the PVN, in turn, increases peripheral adrenocorticotropic hormone (ACTH) levels, and this stimulates the adrenal glands to secrete cortisol. The ACC, anterior insula, and posterior orbital cortex send anatomic projections to the hypothalamus that participate in modulating or inhibiting cardiovascular and endocrine responses to threat and stress (1,43,83).

The vagus and splanchnic nerves constitute the major efferent projections of the parasympathetic nervous system to the viscera. The vagal nuclei receive afferent projections from the lateral hypothalamus, the PVN, the LC, the amygdala, the infralimbic cortex, and the prelimbic portion of the ACC (43,84). The splanchnic nerves receive afferent connections from the LC. The innervation of the parasympathetic nervous system from these limbic structures is thought to mediate visceral symptoms associated with anxiety, such as gastrointestinal and genitourinary disturbances (Fig. 63.1).

Role of Prefrontal Cortical Structures in Modulating Fear and Anxiety Behavior

Multiple areas of the medial and orbital PFC appear to play roles in modulating anxiety and other emotional behaviors.

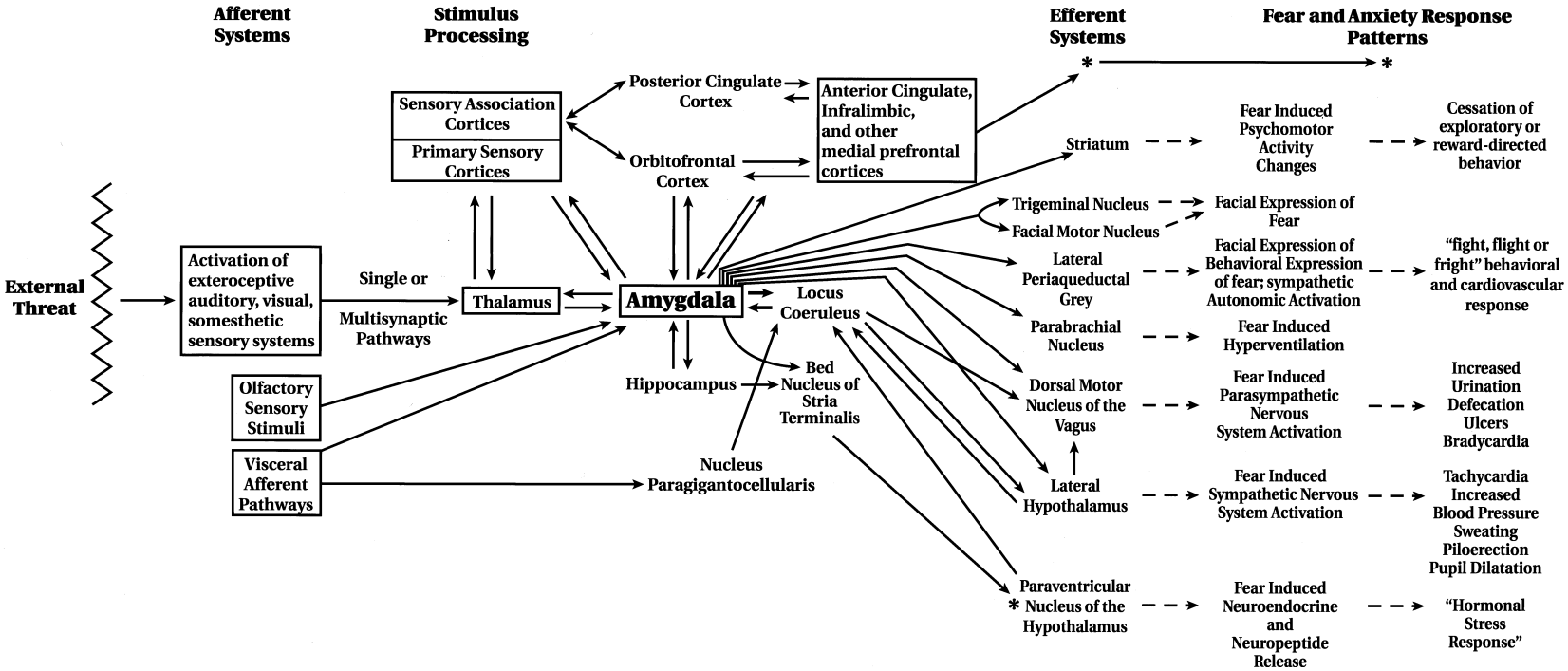

FIGURE 63.1. The innervation of the parasympathetic nervous system from limbic structures is thought to mediate visceral symptoms associated with anxiety.

These PFC structures are thought to participate in interpreting the higher-order significance of experiential stimuli, in modifying behavioral responses based on competing reward versus punishment contingencies, and in predicting social outcomes of behavioral responses to emotional events (8, 11,85,86). These areas share extensive, reciprocal projections with the amygdala, through which the amygdala can modulate PFC neuronal activity and the PFC can modulate amygdala-mediated responses to emotionally salient stimuli (17,18,42,43).

Areas within the orbital and mPFC and the anterior insula also participate in modulating peripheral responses to stress, including heart rate, blood pressure, and glucocorticoid secretion (13,17,43,87). The neuronal activities within these areas are, in turn, modulated by various neurotransmitter systems that are activated in response to stressors and threats. For example, the noradrenergic, dopaminergic, and serotonergic systems play roles in enhancing vigilance, modulating goal-directed behavior, and facilitating decision making about probabilities of punishment versus reward by modulating neuronal activity in the PFC (86,88–90).

Medial Prefrontal Cortex

The mPFC areas implicated in anxiety and fear-related behavior in humans and experimental animals *include* the infralimbic cortex, the ACC located ventral (''subgenual'') and anterior (''pregenual'') to the genu of the corpus callosum, and a more dorsal mPFC region that extends from the rostral ACC (BA 24, 32) toward the frontal pole (91). The reciprocal projections between the amygdala and the mPFC are hypothesized to play critical roles in attenuating fear responses and extinguishing behavioral responses to fear-conditioned stimuli that are no longer reinforced (17,18). Lesions of the ACC in rats resulted in enhanced freezing to a fear-conditioned tone, a finding suggesting that this mPFC region may be involved in fear reduction (17). In addition, neurons in the rat prelimbic cortex (thought to be homologous to subgenual PFC) reduce their spontaneous firing activity in the presence of a conditioned, aversive tone to an extent that is inversely proportional to the magnitude of fear (42). This suppression of prelimbic cortex neuronal firing activity is inversely correlated with increases in amygdala neuronal activity. Finally, lesions of the infralimbic cortex specifically interfere with the recall of extinction processes after long delays between the acquisition of extinction learning and reexposure to the initial CS (18). Extinction does not appear to occur by erasing memory traces of the CS-US association, but rather by new learning through which the behavioral response to the CS is actively inhibited (31).

In humans, the pregenual ACC shows areas of elevated hemodynamic activity during a variety of anxiety states elicited in healthy or anxiety-disordered subjects (reviewed in ref. 49). Electrical stimulation of this region elicits fear, panic, or a sense of foreboding in humans and vocalization in experimental animals (reviewed in ref. 7). Nevertheless, physiologic activity also increases in the ACC during the generation of positive emotions in healthy humans (92,93) and during depressive episodes in some subtypes of major depressive disorder (MDD) (94,95).

The subgenual ACC has been implicated in healthy sadness, MDD, mania, and PTSD (90,96,97). In patients with familial unipolar and bipolar depression, reductions in cerebral blood flow (CBF) and metabolism were associated with left-lateralized reductions in the volume of the corresponding cortex (96,98,99). The subgenual PFC activity shows a mood state dependency in which the metabolism is higher in the depressed than the remitted phase of MDD, consistent with the findings that blood flow increases in this region in healthy, nondepressed humans during experimentally induced sadness (85,100,101) and in persons with PTSD during internally generated imagery of past trauma (97).

Both the subgenual and the pregenual ACC share reciprocal anatomic connections with areas implicated in emotional behavior such as the posterior orbital cortex, amygdala, hypothalamus, nucleus accumbens, PAG, ventral tegmental area (VTA), raphe, LC, and NTS (Fig. 63.1) (102,103). Humans with mPFC lesions that include the pregenual and subgenual ACC show abnormal autonomic responses to emotionally provocative stimuli, inability to experience emotion related to concepts, and inability to use information regarding the probability of aversive social consequences versus reward in guiding social behavior (104). In rats, bilateral or *right*-lateralized lesions of the ventral mPFC composed of infralimbic, prelimbic, and ACC cortices *attenuate* corticosterone secretion, sympathetic autonomic responses, and gastric stress disorders during restraint stress or exposure to fear-conditioned stimuli (17,83,105). In contrast, *left*-sided lesions of this cortical strip *increase* sympathetic arousal and corticosterone responses to restraint stress (105). Finally, the ventral ACC contains glucocorticoid receptors that, when stimulated, inhibit stress-induced corticosterone release in rats (87).

Physiologic activity also increases in more dorsal mPFC areas in healthy humans as they perform tasks that elicit emotional responses or require emotional evaluations (81, 106,107). During anxious anticipation of an electrical shock, CBF increases in the rostral mPFC (vicinity of anterior BA24, BA32, and rostral BA9), and the magnitude of ΔCBF correlates inversely with changes in anxiety ratings and heart rate (107). In rats, lesions of the rostral mPFC result in exaggerated heart rate responses to fear-conditioned stimuli, and stimulation of these sites attenuates defensive behavior and cardiovascular responses evoked by amygdala stimulation (83). In primates, whereas BA24 and 32 have extensive reciprocal connections with the amygdala through which they may modulate emotional expression, the BA9 cortex has only sparse projections to the amygdala. Nevertheless, all three areas send extensive efferent projections to the PAG and the hypothalamus through which cardiovascu-

lar responses associated with emotional behavior can be modulated (43,108).

In the depressed phase of MDD and bipolar disorder, metabolic activity is abnormal in the dorsomedial and dorsal anterolateral PFC (in the vicinity of rostral BA9) (91,109). Postmortem studies of these regions have shown abnormal reductions in the size of glia and neurons in MDD (110). Given the preclinical and neuroimaging evidence presented earlier, indicating that this area may modulate anxiety, it may be hypothesized that dysfunction of this mPFC area contributes to the development of anxiety symptoms in mood disorders.

Orbital and Anterior Insular Cortex

Other areas of the PFC that are implicated in studies of fear or anxiety in human and nonhuman primates are the posterior and lateral orbital cortex, the anterior (agranular) insula, and the ventrolateral PFC (1,43). Physiologic activity increases in these areas during experimentally induced anxiety states in healthy subjects and in subjects with obsessive-compulsive disorder (OCD), simple phobia, and panic disorder (PD) (49,111). (See Chapter 65) The baseline metabolic activity is also abnormally elevated in these regions in unmedicated study subjects with primary MDD (91) and OCD (112) scanned while resting with eyes closed. The elevated activity in these areas in both MDD and OCD appears state dependent, and effective antidepressant or antiobsessional treatment results in decreases in CBF and metabolism in the medicated-improved relative to the unmedicated-symptomatic phase (112–114).

A complex relationship exists between anxiety-depressive symptoms and physiologic activity in the orbital cortex and the ventrolateral PFC. In MDD, whereas CBF and metabolism increase in these areas in the depressed relative to the remitted phase, the magnitude of these measures correlates inversely with ratings of depressive ideation and severity (115,116). Similarly, posterior orbital cortex flow increases in OCD and animal phobic subjects during exposure to phobic stimuli and in healthy subjects during induced sadness, but this change in CBF correlates inversely with changes in obsessive thinking, anxiety, and sadness, respectively (114,117,118).

These data appear consistent with electrophysiologic and lesion analysis data showing that the orbital cortex participates in modulating behavioral and visceral responses associated with fearful, defensive, and reward-directed behavior as reinforcement contingencies change. Nearly one-half of the orbital cortex pyramidal neurons alter their firing rates during the delay period between stimulus and response, and this firing activity relates to the presence or absence of reinforcement (11). These cells are thought to play roles in extinguishing unreinforced responses to aversive or appetitive stimuli (7,11,66). The posterior and lateral orbital cortex and the amygdala send projections to each other and to overlapping portions of the striatum, hypothalamus, and PAG through which these structures modulate each other's neural transmission (Fig. 63.1) (42,66,108,119). For example, the defensive behaviors and cardiovascular responses evoked by electrical stimulation of the amygdala are attenuated or ablated by concomitant stimulation of orbital sites, which, when stimulated alone, exert no autonomic effects (120).

Humans with orbital cortex lesions show impaired performance on tasks requiring application of information related to punishment or reward, perseverate in behavioral strategies that are unreinforced, and exhibit difficulty in shifting intellectual strategies in response to changing task demands (11,121). Likewise, monkeys with surgical lesions of the lateral orbital cortex and ventrolateral PFC demonstrate "perseverative interference," characterized by difficulty in learning to withhold prepotent responses to nonreinforced stimuli as reinforcement contingencies change (122). Activation of the orbital cortex during anxiety or obsessional states may thus reflect endogenous attempts to attenuate emotional expression or to interrupt unreinforced aversive thought and emotion (91). Conversely, dysfunction of the orbital cortex may contribute to pathologic anxiety and obsessional states by impairing the ability to inhibit nonreinforced or maladaptive emotional, cognitive, and behavioral responses to social interactions and sensory or visceral stimuli.

Posterior Cingulate Cortex

Many functional imaging studies report that exposure to aversive stimuli of various types increases physiologic activity in the retrosplenial cortex and other portions of the posterior cingulate gyrus (reviewed in ref. 123). Posterior cingulate cortical flow and metabolism have also been found abnormally elevated in some studies of depressed subjects with MDD (reviewed in ref. 91). In contrast, Mayberg et al. reported that script-driven sadness resulted in decreased posterior cingulate activity in healthy subjects, and flow was decreased in depressed relative to remitted subjects with MDD, findings raising the possibility that this large region is functionally heterogenous with respect to emotional behavior (101). The posterior cingulate cortex appears to serve as a sensory association cortex and may participate in processing the affective salience of sensory stimuli. The posterior cingulate cortex sends a major anatomic projection to the ACC, through which it may relay such information into the limbic circuitry (124).

FUNCTIONAL ANATOMIC CORRELATES OF SPECIFIC ANXIETY DISORDERS

Neuroimaging studies have assessed neurophysiologic abnormalities in anxiety-disordered samples in the baseline,

"resting" condition and during symptom provocation. These data converge with those obtained from studies of healthy subjects and of experimental animals to implicate the limbic, paralimbic, and sensory association areas reviewed earlier in the functional anatomy of emotional behavior. Nevertheless, the results of most of the imaging studies reviewed herein await replication, and the data they provide do not clearly establish whether differences between anxiety-disordered and control subjects reflect physiologic correlates of anxiety symptoms or traitlike biological abnormalities underlying the vulnerability to anxiety syndromes.

Panic Disorder

The baseline state in PD is characterized by mild to moderate levels of chronic anxiety (termed *anticipatory anxiety*). In this state, abnormalities of CBF and glucose metabolism have been reported in the vicinity of the hippocampus and parahippocampal gyrus. Reiman et al. initially reported an abnormal resting asymmetry (left less than right) of blood flow and oxygen metabolism in a region of interest placed over the parahippocampal gyrus (125). Nordahl et al. similarly found that glucose metabolism measured over the hippocampus-parahippocampal gyrus was asymmetric and concluded that this abnormality reflected an abnormal metabolic elevation on the right side (126). Bisaga et al. also found abnormal metabolism in this vicinity, but with the opposite laterality (i.e., elevated metabolism in the left hippocampal-parahippocampal area) in lactate-sensitive PD study subjects relative to healthy controls (127). In contrast, De Cristofaro et al. reported that resting perfusion, measured using single photon emission computed tomography (SPECT) and [^{99m}Tc]hexamethylpropyleneamineoxime (HMPAO), was abnormally decreased in the hippocampus, bilaterally, in lactate-sensitive PD study subjects relative to controls (128).

Each of these studies employed region-of-interest based approaches that were incapable of localizing the center of mass of the abnormality in this region. Reanalysis of some of these data using a voxel-by-voxel approach suggested that the abnormal radioactivity in the vicinity of the mesiotemporal cortex may actually reflect elevated metabolism in the adjacent midbrain (111). This midbrain region, which may reflect the lateral PAG, has been implicated in lactate-induced panic (129), other acute anxiety states (130), and animal models of panic attacks (67).

Study subjects with PD have also been imaged during panic elicited using a variety of chemical challenges. Panic attacks induced by intravenous sodium lactate infusion were associated with regional CBF increases in the anterior insula, the anteromedial cerebellum, and the midbrain (129); areas of increased CBF may also exist in the temporal polar cortex, but these findings were confounded by corresponding increases in the adjacent facial muscles during severe anxiety (115). Blood flow also increased in these regions in animal phobic subjects during exposure to phobic stimuli and in healthy subjects during the threat of a painful electrical shock, findings suggesting that these CBF changes reflect the neurophysiologic correlates of fear processing in general (111,130). Consistent with this hypothesis, anxiety attacks induced in healthy humans using cholecystokinin tetrapeptide (CCK-4) were also associated with CBF increases in the insular-amygdala region and the anteromedial cerebellum (131).

Indirect evidence suggests that the neurophysiologic responses in the PFC during panicogen challenge may differ between PD subjects and healthy controls. For example, panic attacks induced using CCK-4 were associated with CBF increases in the ACC in healthy humans (131), but flow did not significantly change in the ACC in subjects with PD during lactate-induced panic (129). The ACC was also a region where flow significantly increased in healthy subjects but not in subjects with PD during fenfluramine challenge in a study in which fenfluramine induced panic attacks in 56% of subjects with PD but in only 11% of control subjects (132). Finally, Cameron et al. found that normalized medial frontal CBF increased in healthy controls after yohimbine administration (i.e., after normalizing to remove effects on whole brain CBF) (133), whereas Woods et al. found that the relative prefrontal cortical flow was decreased in PD relative to control subjects following yohimbine challenge (134).

Structural MRI studies have begun to investigate whether morphometric or morphologic abnormalities may exist in PD. Ontiveros et al. reported qualitative abnormalities of temporal lobe structure in PD (135), although these findings have not been replicated. Vythilingam et al. reported that hippocampal volume did not differ between PD and healthy control subjects (136).

Phobias

In simple animal phobias, phobic anxiety was imaged by acquiring blood flow scans during exposures to the feared animal. During the initial fearful scans, flow increased in the lateral orbital-anterior insular cortex, bilaterally, the pregenual ACC, and the anteromedial cerebellum (78,111), areas where CBF also increases in other anxiety states (see earlier). During the development of habituation to phobic stimuli, the magnitude of the hemodynamic responses to the phobic stimulus diminished in the anterior insula and the medial cerebellum, but it increased in the left posterior orbital cortex in an area where flow had not changed during exposures that preceded habituation (117). The magnitude of the CBF increase in this latter region was inversely correlated with the corresponding changes in heart rate and anxi-

TABLE 63.1. EVIDENCE OF ALTERED CATECHOLAMINERGIC FUNCTION IN ANXIETY DISORDERS

	PTSD	Panic Disorder
Increased resting heart rate and blood pressure	+/–	+/–
Increased heart rate and blood pressure response to traumatic reminders/panic attacks	+++	++
Increased resting urinary NE and E	+	+/–
Increased resting plasma NE or MHPG	–	–
Increased plasma NE with traumatic reminders/panic attacks	+	+/–
Increased orthostatic heart rate response to exercise	+	+
Decreased binding to platelet α2 receptors	+	+/–
Decrease in basal and stimulated activity of cAMP	+/–	+
Decrease in platelet MAO activity	+	NS
Increased symptoms, heart rate and plasma MHPG with yohimbine noradrenergic challenge	++	+++
Differential brain metabolic response to yohimbine	+	+

–, One or more studies did not support this finding (with no positive studies), or the majority of studies do not support this finding; +/–, an equal number of studies support this finding and do not support this finding; +, at least one study supports this finding and no studies do not support the finding, or the majority of studies support the finding; ++, two or more studies support this finding, and no studies do not support the finding; +++, three or more studies support this finding, and no studies do not support the finding; cAMP, cyclic adenosine 3′, 5′-monophosphate; E, epinephrine; MAO, monoamine oxidase; MHPG, 3-methoxy-4-hydroxyphenylglycol; NE, norepinephrine; NS, not studied; PTSD, posttraumatic stress disorder.

ety ratings. As discussed earlier, the posterior orbital cortex was a site where CBF increased in subjects with OCD during exposure to phobic stimuli, with the increase in flow inversely correlated with obsessional ratings (114).

In social anxiety disorder, an aversive conditioning paradigm (in which the US was an aversive odor and the CS was a picture of a human face) showed that hemodynamic activity decreased in the amygdala and the hippocampus during presentations of the CS in healthy controls, but it increased in social phobic subjects (137). Interpretation of these data was confounded by the problem that both human faces and aversively CSs normally activate the amygdala, so it remained unclear which of the stimuli produced abnormal responses in social phobia. Nevertheless, these data appear conceptually intriguing, given the role of hippocampal-amygdalar projections in mediating contextual fear and the possibility that deficits in the transmission of information regarding context may be involved in the pathogenesis of phobias (21).

Posttraumatic Stress Disorder

PTSD is hypothesized to involve the emotional-learning circuitry associated with the amygdala, because the traumatic event constitutes a fear-conditioning experience, and subsequent exposure to sensory, contextual, or mnemonic stimuli that recall aspects of the event elicits psychological distress and sympathetic arousal. Potentially consistent with this expectation, some studies demonstrated activation of the amygdala as patients with PTSD listened to auditory scripts describing the traumatic event (79) or to combat sounds (in combat-related PTSD) (138) or generated imagery related to the traumatic event without sensory cues (139). However, other studies found no significant changes in amygdala CBF as patients with PTSD listened to scripts describing the traumatic event or viewed trauma-related pictures, and studies comparing CBF responses with trauma-related stimuli have not shown significant differences in the amygdala between patients with PTSD and trauma-matched, non-PTSD control subjects (97,139–141). The extent to which these negative findings reflect limitations in statistical sensitivity or in positron emission tomography (PET) temporal resolution must be addressed in provocation studies involving larger subject samples and employing fMRI instead of PET. In this regard, it is noteworthy that a preliminary fMRI study found exaggerated hemodynamic changes in the amygdala in patients with PTSD relative to trauma-matched, non-PTSD control subjects during exposure to pictures of fearful faces presented using a backward-masking technique (142). If replicated, this finding may suggest that the emotional dysregulation associated with PTSD may involve amygdalar responses to emotional stimuli of various types.

Other limbic and paralimbic cortical structures have also been implicated in provocation studies of PTSD. In both patients with PTSD and trauma-matched, non-PTSD control subjects, CBF increases in the posterior orbital cortex, anterior insula, and temporopolar cortex during exposure to trauma-related stimuli, but these changes have generally not differentiated PTSD and control samples (79,139,140). In contrast, the pattern of CBF changes elicited in the mPFC by traumatic stimuli may differ between PTSD and control subjects. During exposure to trauma-related sensory stimuli, flow decreased in the left (97,140) but increased in the right pregenual ACC in PTSD (79,138), a finding potentially consistent with the evidence reviewed earlier that the role of the mPFC in emotional behavior is lateralized (105). However, CBF in the right pregenual ACC increased significantly more in non-PTSD, trauma-matched control subjects than in patients with PTSD (139). Moreover, in the infralimbic cortex, CBF *decreased* in patients with combat-related PTSD but *increased* in combat-matched, non-PTSD

control subjects during exposure to combat-related visual and auditory stimuli (141).

Given evidence that the ACC and the infralimbic cortex play roles in extinguishing fear-conditioned responses (17, 18), the observation that patients with PTSD fail to activate these structures to a similar extent as traumatized, non-PTSD control subjects during exposure to traumatic cues (139,141) suggests that neural processes mediating extinction to trauma-related stimuli may be impaired in PTSD. Compatible with this hypothesis, PTSD samples have been shown to acquire *de novo* conditioned responses more readily and to extinguish them more slowly than control samples (143,144). Such an impairment could conceivably be related to the vulnerability to developing PTSD, because PTSD occurs in only 5% to 20% of individuals exposed to similar traumatic events.

Structural MRI studies of PTSD have identified subtle reductions in the volume of the hippocampus in PTSD samples relative to healthy or traumatized, non-PTSD control samples (145-148). Although limitations existed in these studies in the matching of alcohol use or abuse between PTSD and control samples, the reductions in hippocampal volume did not correlate with the extent of alcohol exposure in the PTSD samples, and no volumetric differences were found between PTSD and control samples in the amygdala, entire temporal lobe, caudate, whole brain, or lateral ventricles. Although the magnitude of the reduction in hippocampal volume only ranged from 5% to 12% in the PTSD samples relative to trauma-matched controls, these abnormalities were associated with short-term memory deficits in some studies (145,147). It remains unclear whether the difference in hippocampal volume may reflect a result of the chronic stress associated with PTSD (e.g., from sustained exposure to elevated glucocorticoid concentrations) or a biological antecedent that may confer risk for developing PTSD (149,150).

Obsessive-Compulsive Disorder

The anatomic circuits involved in the production of obsessions and compulsions have been elucidated by converging evidence from functional neuroimaging studies of OCD, analysis of lesions resulting in obsessive-compulsive symptoms, and observations regarding the neurosurgical interventions that ameliorate OCD (113,114,151). PET studies of OCD have shown that "resting" CBF and glucose metabolism are abnormally increased in the orbital cortex and the caudate nucleus bilaterally in primary OCD (reviewed in ref. 112). With symptom provocation by exposure to relevant phobic stimuli (e.g., skin contact with "contaminated" objects for patients with OCD who have germ phobias), flow increased further in the orbital cortex, ACC, caudate, putamen, and thalamus (114). During effective pharmacotherapy, orbital metabolism decreased toward normal, and both drug treatment and behavioral therapy were associated with a reduction of caudate metabolism (112). The baseline areas of hypermetabolism in the orbital cortex and the caudate may thus reflect physiologic concomitants of obsessive thoughts or chronic anxiety, and, conversely, the reduction in caudate metabolism associated with effective (but not ineffective) treatment may reflect a physiologic correlate of symptom resolution rather than a primary mechanism of treatment.

Based on the evidence reviewed earlier from electrophysiologic and lesion analysis studies indicating that the orbital cortex participates in the correction of behavioral responses that become inappropriate as reinforcement contingencies change, posterior orbital areas may be specifically activated as an endogenous attempt to interrupt patterns of nonreinforced thought and behavior in OCD (11,91). Compatible with this hypothesis, the posterior orbital cortex CBF increases during symptom provocation in OCD, but the magnitude of this increase correlates *inversely* with the corresponding rise in obsession ratings ($r = -0.83$) (114). In contrast, flow also increases in an area of the right anterior orbital cortex implicated in a variety of types of mnemonic processing, and the change in CBF in this region correlates positively with changes in obsession ratings (114,152).

The neurologic conditions associated with the development of secondary obsessions and compulsions also provide evidence that dysfunction within circuits formed by the basal ganglia and the PFC may be related to the pathogenesis of OCD. Such conditions involve lesions of the globus pallidus and the adjacent putamen: Sydenham chorea (a poststreptococcal autoimmune disorder associated with neuronal atrophy in the caudate and putamen), Tourette syndrome (an idiopathic syndrome characterized by motoric and phonic tics that may have a genetic relationship with OCD), chronic motor tic disorder, and lesions of the ventromedial PFC (151–154). Several of these conditions are associated with complex motor tics (repetitive, coordinated, involuntary movements occurring in patterned sequences in a spontaneous and transient manner). It is conceivable that complex tics and obsessive thoughts may reflect homologous, aberrant neural processes manifested within the motor and cognitive-behavioral domains, respectively, because of their origination in distinct portions of the cortical-striatal-pallidal-thalamic circuitry (113,155).

In contrast to the regional metabolic abnormalities found in primary OCD, imaging studies of obsessive-compulsive syndromes arising in the setting of Tourette syndrome or basal ganglia lesions have not found elevated blood flow and metabolism in the caudate and in some cases have found reduced metabolism in the orbital cortex in such subjects relative to controls (111,151). The differences in the functional anatomic correlates of primary versus secondary OCD are consistent with a neural model in which dysfunction arising at various points within the ventral prefrontal cortical-striatal-pallidal-thalamic circuitry may result in pathologic obsessions and compulsions. This circuitry ap-

pears to be generally involved in organizing internally guided behavior toward a reward, switching of response strategies, habit formation, and stereotypic behavior (66, 155).

These circuits have also been implicated in the pathophysiology of MDD, another illness in which intrusive, distressing thoughts recur to an extent that the ability to switch to goal-oriented, rewarding cognitive-behavioral sets is impaired (91). Although MDD and OCD appear distinct in their course, prognosis, genetics, and neurochemical concomitants, substantial comorbidity exists across these syndromes. Major depressive episodes occur in about one-half of patients with OCD, pathologic obsessions can arise in primary MDD, and the pharmacologic interventions that ameliorate OCD can also effectively treat MDD. Moreover, the neurosurgical procedures that are effective at reducing both obsessive-compulsive and depressive symptoms in intractable cases of OCD and MDD interrupt white matter tracts carrying neural projections between the frontal lobe, the basal ganglia, and the thalamus (155). The clinical comorbidity across these two disorders may thus reflect involvement of an overlapping neural circuitry by otherwise distinct pathophysiologic processes.

NEUROCHEMICAL BASIS OF FEAR AND ANXIETY

The neuroanatomic circuits that support fear and anxiety behavior are modulated by a variety of chemical neurotransmitter systems. These include the peptidergic neurotransmitters, CRH, neuropeptide Y (NPY), and substance P, the monoaminergic transmitters, NE, serotonin (5-hydroxytryptamine or 5-HT), and dopamine (DA), and the amino acid transmitters, GABA and glutamate. The neurotransmitter systems that have been best studied in association with responses to stress or threat involve the HPA axis and the central noradrenergic system. These neurochemical systems subserve important adaptive functions in preparing the organism for responding to threat or stress, by increasing vigilance, modulating memory, mobilizing energy stores, and elevating cardiovascular function. Nevertheless, these biological responses to threat and stress can become maladaptive if they are chronically or inappropriately activated. Additional neurochemical systems that play important roles in modulating stress responses and emotional behavior include the central GABAergic, serotonergic, dopaminergic, opiate, and NPY systems. The preclinical and clinical literature regarding these neurochemical concomitants of stress and fear and their potential relevance to the pathophysiology of anxiety disorders are reviewed in the following sections.

Role of the Central Noradrenergic System in Fear and Anxiety

Exposure to stressful stimuli of various types increases central noradrenergic function. Thus, exposure to fear-conditioned stimuli, immobilization stress, foot shock, or tail pinch increases NE turnover in the LC, the hypothalamus, the hippocampus, the amygdala, and the cerebral cortex (156). The firing activity of LC neurons also increases during exposure to fear-conditioned stimuli and other stressors or threats (157–159). For example, the firing activity of NE neurons in the cat LC increases two- to threefold during confrontation with a dog or an aggressive cat, but it remains unchanged during exposure to other novel stimuli or to nonaggressive cats (160). However, repeated exposure to severe stressors from which the animal cannot escape results in the behavioral pattern termed *learned helplessness,* which is associated with depletion of NE, possibly reflecting a point at which NE synthesis cannot keep pace with NE release (161,162).

Acquisition of fear-conditioned responses requires an intact central noradrenergic system, a finding suggesting that NE release plays a critical role in fear learning (157,163, 164). For at least some types of emotional learning, memory consolidation depends on noradrenergic stimulation of β- and α_1-adrenoreceptors in the basolateral nucleus of the amygdala (15). The activation of NE release in such models may, in turn, depend on effects of stress hormones on noradrenergic neurons (15).

The responsiveness of LC neurons to future novel stressors can be enhanced by chronic exposure to some stressful experiences. In rats, the amount of NE synthesized and released in the hippocampus and the mPFC in response to a novel stressor or to local depolarization is increased after repeated exposure to chronic cold stress (165–167). This effect may result from a stress-mediated alteration in the sensitivity of presynaptic α_2-adrenoreceptors, which inhibit NE synthesis and release. In the native state, administration of the α_2-adrenoreceptor antagonists, idazoxan or yohimbine, increases the electrophysiologic response of LC neurons to stressful stimuli (without altering their basal firing rates) and increases NE release and synthesis, whereas administration of the α_2-adrenoreceptor agonist, clonidine, decreases NE release and synthesis (167,168). In chronically cold-stressed rats, idazoxan administration produces a greater increase in NE release and synthesis, and clonidine administration produces a blunted attenuation of NE release and synthesis relative to naive rats (167). Consistent with these observations, Torda et al. found that cold immobilization stress decreases the α_2-adrenoreceptor density in the hippocampus and the amygdala (169).

The effect of chronic stress on noradrenergic responses to subsequent, novel stressors may constitute a form of "behavioral sensitization," a process by which single or repeated exposures to aversive stimuli or pharmacologic agents can

increase the behavioral sensitivity to subsequent stressors (reviewed in ref. 170). Such phenomena are hypothesized to account for clinical observations that patients with anxiety disorders report experiencing exaggerated sensitivity to psychosocial stress. Neural models for the pathogenesis of anxiety disorders built on sensitization phenomena thus hold that *repeated* exposure to traumatic stress comprises a risk factor for the subsequent development of anxiety disorders, particularly PTSD.

Noradrenergic Function in Anxiety Disorders

The recurrent symptoms of anxiety disorders, such as panic attacks, insomnia, exaggerated startle, and chronic sympathetic autonomic arousal, may conceivably reflect elevated noradrenergic function (171–173). Patients with PTSD and PD show evidence of heightened peripheral sympathetic nervous system arousal that, because of the correlation between peripheral sympathetic activity and central noradrenergic function, is compatible with the hypothesis of increased central NE activity in these disorders (174,175). Moreover, patients with PD, PTSD, and phobic disorders report that their hyperarousal symptoms and intrusive memories are attenuated by alcohol, benzodiazepines (BZDs), and opiates, agents known to decrease LC neuronal firing activity, but are exacerbated by cocaine, which increases LC neuronal firing. The risk of abuse of these substances appears increased in patients with anxiety disorders, a finding raising the possibility that such patients are "self-medicating" anxiety symptoms with these agents. It remains unclear, however, whether alterations in noradrenergic function play a primary, etiologic role in the pathogenesis of anxiety disorders, or instead reflect secondary, compensatory changes in response to disorders in other systems.

PD has been specifically associated with elevations of α_2-adrenoreceptor sensitivity and nocturnal urinary NE excretion (176), although β-adrenoreceptor function, baseline heart rate and blood pressure, and other measures reflecting central NE secretion have not been consistently altered in PD (see Table 63.1) (177). Altered α_2-adrenoreceptor sensitivity is evidenced by findings that administration of the α_2-adrenoreceptor agonist, clonidine, results in greater hypotension and larger reductions in plasma 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in PD relative to control subjects (178–181). In addition, administration of the α_2-adrenoreceptor antagonist, yohimbine (which stimulates NE release by antagonizing presynaptic α_2-adrenoreceptors) produces exaggerated anxiogenic and cardiovascular responses and enhanced plasma MHPG and cortisol increases in PD relative to control subjects (133,172,173, 182–186). Finally, yohimbine administration resulted in reduced relative frontal cortex flow in patients with PD that did not occur in control subjects, as measured with SPECT and [^{99m}Tc]HMPAO (134); it remains unclear, however, whether this difference reflected a differential physiologic sensitivity to yohimbine or an effect of greater anxiety in the patients with PD, because all the patients with PD but only one control subject developed increased anxiety in response to yohimbine. The sensitivity of α_2-adrenoreceptors also appears increased in PTSD. Patients with combat-related PTSD show increased behavioral, chemical, and cardiovascular responses to yohimbine, relative to healthy controls (187–189).

Considerable evidence also indicates that noradrenergic function is abnormal in PTSD (see Table 63.1). Women with PTSD secondary to childhood sexual abuse showed elevated 24-hour urinary excretion of catecholamines and cortisol (190). In addition, men—but not women—with PTSD resulting from a motor vehicle accident exhibited elevated urinary levels of epinephrine, NE, and cortisol 1 month after the accident and still had higher epinephrine levels 5 months later (191). Similarly, maltreated children with PTSD excreted greater amounts of urinary DA, NE, and cortisol over 24 hours than controls, with the urinary catecholamine and cortisol output positively correlated with the duration of PTSD trauma and the severity of PTSD symptoms (192). Exposure to traumatic reminders (e.g., combat films or sounds) produced greater increases in plasma, epinephrine, NE, and cortisol in patients with PTSD than in control subjects (191,193,194), although baseline concentrations of catecholamines are not consistently altered in combat-related PTSD (188,189). Geracioti et al. found that cerebrospinal fluid (CSF) NE concentrations are abnormally elevated in PTSD (195). Finally, platelet α_2-adrenoreceptor density (196), platelet basal adenosine, isoproterenol, forskolin-stimulated cyclic adenosine monophosphate signal transduction (197), and basal platelet monoamine oxidase activity (198) were decreased in PTSD, findings hypothesized to reflect compensatory responses to chronically elevated NE release.

In study subjects with specific phobias, plasma NE and epinephrine concentrations, heart rate, blood pressure, and subjective anxiety ratings increase in response to exposure to phobic stimuli (199). Subjects with social anxiety disorder show greater increases in plasma NE during orthostatic challenge than healthy subjects or those with PD (200).The growth hormone response to intravenous clonidine (a marker of central α_2-adrenoreceptor function) is blunted in social anxiety disorder (201), although the density of lymphocyte β-adrenoreceptors has not differed between social anxiety–disordered and control samples (202) (Table 63.1).

Finally, Gerra et al. reported that, plasma NE concentrations increased to a greater extent in male peripubertal patients with generalized anxiety disorder than in controls in response to a psychological stress test (203). However, the

pretest baseline NE concentrations did not differ between the anxious and control subjects.

Hypothalamic-Pituitary-Adrenal Axis and Corticotropin-Releasing Hormone

Exposure to acute stress of various types results in release of CRH, ACTH, and cortisol. This HPA-axis activation during acute stress can produce a transient elevation of the plasma cortisol concentration and partial resistance to feedback inhibition of cortisol release that persists during and shortly after the duration of the stressful stimulus. This phenomenon may involve a rapid down-regulation of glucocorticoid receptors, because elevated glucocorticoid levels such as those elicited by acute stress decrease the number of hippocampal glucocorticoid receptors, with a resulting increase in corticosterone secretion and feedback resistance (204). After stress termination, as glucocorticoid levels decrease (presumably because the limbic drive on CRH release diminishes), glucocorticoid-receptor density increases, and feedback sensitivity normalizes (204).

During some types of *chronic* stress, adaptive changes in ACTH and corticosterone secretion occur such that the plasma ACTH and corticosterone concentrations achieved are lower than those seen in response to acute stress (205). In contrast, other types of chronic stress are associated with enhanced corticosterone secretion in rats (206). Moreover, Dallman and Jones showed that the experience of prior stress can result in augmented corticosterone responses to subsequent stress exposure (207). The factors that determine whether adaptation or sensitization of glucocorticoid activity occurs after chronic stress remain poorly understood.

Some stressors experienced within critical periods of neurodevelopment exert long-term effects on HPA-axis function. In rats exposed to either severe prenatal *(in utero)* stress or early maternal deprivation stress (208,209), the plasma concentrations of corticosterone achieved in response to subsequent stressors are increased, and this tendency to show exaggerated glucocorticoid responses to stress persists into adulthood. Early postnatal adverse experiences such as maternal separation are associated with long-lasting alterations in the basal concentrations of hypothalamic CRH mRNA, hippocampal glucocorticoid-receptor mRNA, median eminence CRH, and in the magnitude of stress-induced CRH, corticosterone, and ACTH release (210–212). In nonhuman primates, adverse early experiences induced by variable maternal foraging requirements reportedly result in alterations in juvenile and adult social behavior, such that animals are more timid, less socially interactive, and more subordinate (213). Adult monkeys who were raised in such a maternal environment are also hyperresponsive to yohimbine and have elevated CRH concentrations and decreased cortisol levels in the CSF, findings that parallel those in humans with PTSD (213).

Conversely, positive early-life experiences during critical developmental periods may have beneficial long-term consequences on the ability to mount adaptive responses to stress or threat. For example, daily postnatal handling of rat pups by human experimenters within the first few weeks of life has been shown to produce persistent (throughout life) increases in the density of type II glucocorticoid receptors. This increase was associated with enhanced feedback sensitivity to glucocorticoid exposure and reduced glucocorticoid-mediated hippocampal damage in later life (214, 215). These effects are hypothesized to comprise a type of "stress inoculation" induced by the mothers' repeated licking of the pups after they were handled by humans. Taken together with the data reviewed in the preceding paragraph, these data indicate that a high degree of plasticity exists in stress-responsive neural systems during the prenatal and early postnatal periods that "programs" future biological responses to stressful stimuli (210).

Regional differences in the regulation of CRH function by glucocorticoid-receptor stimulation and stress may play major roles in the mediation of fear and anxiety (216). The feedback inhibition of CRH function by glucocorticoids (to suppress HPA-axis activity) occurs at the level of the PVN of the hypothalamus, where systemically administered glucocorticoids reduce CRH expression, and the anterior pituitary, where glucocorticoids decrease CRH *receptor* expression (217–220). The regulation of CRH *receptor* mRNA expression shows a regional specificity that becomes altered when stress occurs concomitantly with elevated glucocorticoid concentrations. After both short-term and long-term corticosterone (CORT) administration, the CRH *receptor* RNA expression decreases in the PVN and the anterior pituitary (219). However, after acute or repeated immobilization stress sufficient to produce a large increase in plasma CORT levels, the CRH mRNA expression decreases in the anterior pituitary, but *increases* in the PVN. In contrast, neither CORT administration nor restraint stress alters the CRH *receptor* expression in the CE of the amygdala or the BNST. Furthermore, CRH secretion is not constrained by glucocorticoids in the CE or the lateral BNST, and CRH mRNA expression *increases* in these areas during systemic CORT administration (217,218,220). It is thus conceivable that the positive feedback of glucocorticoids on extrahypothalamic CRH function in the amygdala or the BNST may contribute to the production of anxiety symptoms (216, 221).

Another level through which the CRH-glucocorticoid system maintains homeostasis and provides mechanisms for modulating mechanism over stress or anxiety responses involves functional differences between CRH-receptor subtypes. The CRH_1 and CRH_2 receptors appear to play reciprocal roles in mediating stress responsiveness and anxiety-like behaviors (221). Mice genetically deficient in CRH_1-receptor expression exhibit diminished anxiety and

stress responses to threat or stress (222,223). In contrast, mice deficient in CRH_2 receptors display heightened anxiety in response to stress (224,225). The affinity of CRH is higher for CRH_1 than CRH_2 receptors, a finding consistent with evidence that CRH elicits anxiogenic effects either when exogenously administered to native animals or when endogenously released in mice genetically altered to overexpress CRH (221). Also consistent with the hypothesis that CRH_1-receptor stimulation facilitates anxiety responses, oral administration of the CRH_1-receptor antagonist, antalarmin, inhibits the behavioral, sympathetic autonomic, and neuroendocrine responses (i.e., attenuating increases in the CSF CRH concentration and in the pituitary-adrenal and adrenal-medullary activity) to acute social stress in monkeys (226).

Regional differences in the anatomic distribution of CRH_1 and CRH_2 receptors likely play a role in balancing facilitatory versus modulatory effects of CRH-receptor stimulation on stress responses. In monkeys, the CRH_1-receptor density is high in most amygdaloid nuclei, the cingulate cortex, the PFC, the insular cortex, the parietal cortex, the dentate gyrus, and the entorhinal cortex, and it is moderate in the CE and the LC. The CRH_2-receptor density is high in the cingulate cortex, the mPFC, the CE, the CA-1 region of the hippocampus, and the PVN and supraoptic nucleus of the hypothalamus. An important avenue of future research will involve assessments of the homeostatic balance between CRH_1- and CRH_2-receptor systems in anxiety disorders.

HPA-Axis Function and CRH Release in Anxiety Disorders

The anxiety disorder for which abnormalities of CRH or HPA-axis function has been most commonly reported is PTSD. Nevertheless, the nature of such abnormalities has been inconsistent across studies, because basal plasma or 24-hour urine cortisol concentrations have been reported to be abnormally decreased (227–229), not different (230, 231), or abnormally increased (190,192,232,233) in PTSD samples relative to healthy or trauma-matched control samples. Differences across these studies may reflect effects of gender, age of illness onset (i.e., childhood versus adult), trauma type or duration, or physiologic variation relative to illness phase. For example, Hawk et al. showed that 24-hour urine cortisol concentrations were elevated in males but not females with PTSD, and that this abnormality in males was evident at 1 month but not 6 months after the traumatic event (191).

The HPA-axis responses to behavioral or pharmacologic challenge have also been assessed in PTSD. During provocation of PTSD symptoms by exposure to combat sounds, the *changes* in plasma cortisol and ACTH concentrations did not differ between patients with combat-related PTSD and either healthy or combat-matched, non-PTSD control subjects (232). In response to dexamethasone administration, cortisol suppression was found to be normal (234) or enhanced (228,235,236) in PTSD, with the latter result particularly found in response to low-dose (0.25 and 0.5 mg) dexamethasone. Yehuda et al. also observed that patients with PTSD have an increased density of glucocorticoid receptors on peripheral lymphocytes (228). This finding, together with the observations that patients with PTSD show hypersensitivity to low-dose dexamethasone, led Yehuda et al. to hypothesize that an increase in hypothalamic glucocorticoid-receptor function results in enhanced feedback sensitivity to cortisol, leading to decreased peripheral cortisol levels (237). Preliminary data suggest that a reduced cortisol response after trauma exposure may predict PTSD development, a finding raising the possibility that enhanced feedback sensitivity to cortisol may arise acutely or may even antedate illness onset in some patients with PTSD (229, 238).

The central release of CRH in PTSD was examined in two studies of CSF concentrations, both of which found abnormally *increased* in chronic, combat-related PTSD (239,240). Potentially consistent with this observation, PTSD samples show a blunted ACTH response to CRH relative to control samples (241,242). Although these observations would appear most consistent with findings that basal cortisol secretion and excretion are abnormally increased in PTSD (190,192,232,233), they do not clearly contradict the findings of normal or reduced peripheral cortisol concentrations in PTSD because hypothalamic and extrahypothalamic CRH secretion are independently regulated (216).

Nevertheless, the studies that either identified reductions or were unable to identify elevations in peripheral cortisol concentrations in PTSD present a challenge to the hypothesis that the reduced hippocampal volume found in MRI studies of PTSD (reviewed earlier) are accounted for by cortisol hypersecretion (150). This hypothesis may still be reconciled with the peripheral cortisol measures associated with chronic PTSD if the cortisol secretion was elevated near the time of the stressor (191,243). Longitudinal studies in male patients who developed PTSD after motor vehicle accidents suggest that cortisol secretion is elevated 1 month after the trauma, but it is normal when measured 6 months after the trauma (191). In rats, the atrophy of pyramidal cell apical dendrites that occurs in response to stress-induced corticosterone secretion is reversible when the exposure to elevated glucocorticoid concentrations is terminated early, but it can become irreversible if the elevated corticosterone concentration persists beyond a critical time period (149). Hippocampal damage may thus conceivably occur in PTSD during a period of excessive cortisol secretion that follows the traumatic event and is prolonged enough so that hippocampal neuronal atrophy becomes irreversible. An alternative hypothesis for the reduction of hippocampal volume in PTSD, however, is that this abnormality antedates the

TABLE 63.2. EVIDENCE OF ALTERATIONS IN CRF-HPA AXIS FUNCTION IN ANXIETY DISORDERS[a]

	PTSD	Panic Disorder
Alteration in urinary cortisol	+/–[a]	+/–
Altered plasma cortisol with 24-hour sampling	+ (dec.)	+ (inc.)/–
Supersuppression with DST	++[b]	–
Blunted ACTH response to CRF	++	+/–
Elevated CRF in CSF	++	–
Increased lymphocyte glucocorticoid receptors	++	NS

[a]Findings of decreased urinary cortisol in older male combat veterans and holocaust survivors and increased cortisol in younger female abuse survivors may be explainable by differences in gender, age, trauma type, developmental epoch at the time of the trauma, or timing within illness course.
[b]Pertains specifically to response to low-dose dexamethasone (0.25 and 0.5 mg).
–, One or more studies did not support this finding (with no positive studies), or the majority of studies do not support this finding; +/–, an equal number of studies support this finding and do not support this finding; +, atleast one study supports this finding and no studies do not, or the majority of studies support the finding; ++, two or more studies support this finding, and no studies do not support the finding; +++, three or more studies support this finding, and no studies do not; ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; CSF, cerebrospinal fluid; dec., decrease; DST, dihydrostreptomycin; HPA, hypothalamic pituitary adrenal axis; inc., increase; NS, not studied; PTSD, posttraumatic stress disorder.

development of PTSD and may comprise a risk factor for developing PTSD in response to traumatic stress.

In PD, the results of studies examining CRH-receptor and HPA-axis function have been less consistent (Table 63.2). Elevated plasma cortisol levels were reported in one study (244), but not in another (245), and the results of studies assessing urinary free cortisol have been similarly inconsistent (177,246). In a study of 24-hour secretion of ACTH and cortisol, PD subjects had subtle elevations of nocturnal cortisol secretion and greater amplitude of ultraradian secretory episodes relative to control subjects (247), but these findings await replication. Both normal and elevated rates of cortisol nonsuppression after dexamethasone administration have been reported in PD (248). After combined dexamethasone-CRH challenge, the HPA-axis response was higher in PD subjects than in healthy controls, but the magnitude of this abnormality was less than that seen in depressed samples (249,250). The ACTH response to CRH was blunted in some studies (249,250), but not in others (250), in PD relative to control samples, although CSF levels of CRH did not differ between PD and control samples (251). The extent to which pathophysiologic heterogeneity within PD samples may account for the inconsistency of these findings remains unclear.

Functional Interactions among Noradrenergic, HPA, and CRH Systems

Coordinated functional interactions between the HPA axis and the noradrenergic systems play major roles in producing adaptive responses to stress, anxiety, or fear. The secretion of CRH increases LC neuronal firing activity and results in enhanced NE release in a variety of cortical and subcortical regions (252,253). Conversely, NE release stimulates CRH secretion in the PVN (the nucleus containing most of the CRH-synthesizing neurons in the hypothalamus). During chronic stress in particular, the LC is the brainstem noradrenergic nucleus that appears preferentially to mediate NE release in the PVN (254). Conversely, as CRH release in the PVN stimulates ACTH secretion from the pituitary and thereby increases cortisol secretion from the adrenal glands, the rise in plasma cortisol concentrations acts through a negative feedback pathway to decrease both CRH and NE synthesis at the level of the PVN. Glucocorticoid-mediated inhibition of NE-induced CRH stimulation may be evident primarily during stress, rather than under resting conditions, as an adaptive response that restrains stress-induced neuroendocrine and cardiovascular effects mediated by the PVN (254). NE, cortisol, and CRH thus appear tightly linked as a functional system that offers a homeostatic mechanism for responding to stress.

A clinical phenomenon of anxiety disorders that may be specifically regulated by interactions between NE and glucocorticoid secretion involves the acquisition and consolidation of traumatic memories. A characteristic feature of PTSD and PD is that memories of the traumatic experience or the original panic attack, respectively, persist for decades and are recalled in response to multiple stimuli or stressors. In experimental animals, alterations of both brain catecholamine and glucocorticoid levels affect the consolidation and retrieval of emotional memories (50,51). Glucocorticoids influence memory storage by activation of glucocorticoid receptors in the hippocampus, whereas NE effects are mediated in part through β-adrenoreceptor stimulation in the amygdala (255). In humans, adrenocortical suppression blocks the memory-enhancing effects of amphetamine and epinephrine (256), and propranolol impairs memory for an emotionally provocative story, but not for an emotionally "neutral" story (257). These data suggest that the acute release of glucocorticoids and NE in response to trauma may modulate the encoding of traumatic memories. It is conceivable that long-term alterations in these systems may account for memory distortions seen in PTSD, such as the memory fragmentation, hypermnesia, and deficits in declarative memory.

Central Benzodiazepine-GABA–Receptor System

Several lines of preclinical and clinical evidence have established that BZD-receptor agonists exert anxiolytic effects

and have suggested that BZD-receptor function may be altered in anxiety disorders. Central BZD receptors are expressed are present throughout the brain, but they are most densely concentrated in the cortical gray matter. The BZD and $GABA_A$ receptors form parts of the same macromolecular complex, and although they constitute distinct binding sites, they are functionally coupled and regulate each other in an allosteric manner (258). Central BZD-receptor agonists potentiate and prolong the synaptic actions of the inhibitory neurotransmitter, GABA, by increasing the frequency of GABA-mediated chloride channel openings (258, 259). Microinjection of BZD-receptor agonists in limbic and brainstem regions such as the amygdala and the PAG exert antianxiety effects in animal models of anxiety and fear (260). Conversely, administration of BZD-receptor *inverse* agonists, such as β-carboline-3-carboxylic acid ethylester, produces behaviors and increases in heart rate, blood pressure, plasma cortisol, and catecholamines similar to those seen in anxiety and stress (261,262), effects that can be blocked by administration of BZD-receptor agonists (263).

Transgenic mouse studies have identified behavioral roles for specific $GABA_A$-receptor subunits. The anxiolytic action of diazepam appears absent in mice with α_2 subunit point mutations, but it is present in mice with α_1 or α_3 subunit point mutations (264,265). These data suggest that the anxiolytic effect of BZD agonists is at least partly mediated by the $GABA_A$-receptor α_2 subunit, which is largely expressed in the limbic system, but not by the α_3 subunit, which is predominately expressed in the reticular activating system, or the α_1 subunit, which is implicated in mediating the sedative, amnestic, and anticonvulsive effects of BZDs (265,266). These findings hold clear implications for investigations of the pathophysiology of anxiety disorders and for the development of anxioselective BZD-receptor agonists.

Some other agents with anxiolytic effects appear to modulate the function of the $GABA_A$/BZD-receptor–chloride ionophore complex by mechanisms distinct from those of the BZD agonists. The neurosteroid, allopregnenolone, exerts antianxiety effects in conflict paradigms that serve as putative animal models of anxiety. The anticonflict effects of allopregnenolone are reversed by either isopropylbicyclophosphate, which binds at the picrotoxinin site on the $GABA_A$ receptors, or RO15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-α]-[1,4]benzodiazepine-3-carboxylate), a BZD-receptor inverse agonist that inhibits $GABA_A$-activated chloride flux in neuronal membranes. In contrast, administration of the BZD-receptor antagonist flumazenil (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-α]-[1,4]benzodiazepine-3- carboxylate) does not block allopregnenolone's anxiolytic-like effects, a finding indicating that allopregnenolone does not bind at the BZD site. Allopregnenolone may thus exert anxiolytic-like effects by stimulating the chloride channel in $GABA_A$ receptors by binding at the picrotoxinin site or at a site specific for RO15-4513.

The antianxiety effects of antidepressant drugs with primary effects on monoamine reuptake may also be partly mediated through a GABAergic mechanism. These agents are effective for the treatment of a spectrum of anxiety disorders including social anxiety disorder, generalized anxiety disorder, PD, and PTSD. One of the multiple secondary effects of these agents involves potentiation of GABAergic function. For example, in rats, the effective dose of phenelzine (15 mg/kg) on the elevated plus maze administered produces a more than twofold increase in whole-brain level GABA concentrations, whereas an ineffective dose of phenelzine (5.1 mg/kg) does not significantly alter GABA levels (267). Moreover, the *N*-acetylated metabolite of phenelzine, *N*-2-acetylphenelzine, which potently inhibits monoamine oxidase but does not change whole-brain GABA concentrations, does not produce anxiolytic effects in the elevated plus-maze test (267). Phenelzine's anxiolytic effects in the plus-maze model may thus depend on elevating brain GABA concentrations, in contrast to the mechanism of the classic BZDs, which instead increase the affinity of $GABA_A$ receptors for GABA.

Effects of Stress on Benzodiazepine-$GABA_A$ Receptors

BZD- and GABA-receptor function can be altered by exposure to stress in some brain regions. In experimental animals exposed to inescapable stress in the form of cold swim or foot shock, the BZD-receptor binding decreases in the frontal cortex, with less consistent reductions occurring in the hippocampus and hypothalamus, but no changes in the occipital cortex, striatum, midbrain, thalamus, cerebellum, or pons (268). Chronic stress in the form of repeated foot shock or cold water swim resulted in decreased BZD-receptor binding in the frontal cortex and hippocampus, and possibly in the cerebellum, midbrain, and striatum, but not in the occipital cortex or pons (268–270). These reductions in BZD-receptor binding were associated with deficits in maze escape behaviors that may have reflected alterations in mnemonic processing (269,270). Some of these stress effects may be mediated by glucocorticoids, because chronic exposure to stress levels of CORT alters mRNA levels of multiple $GABA_A$-receptor subunits (271). Consistent with the effects of chronic stress on BZD-receptor expression, the *Maudsley "genetically fearful" rat strain* shows decreased BZD-receptor density relative to other rats in several brain structures including the hippocampus (272).

Stressors arising early in life may also influence the development of the GABAergic system. In rats, early-life adverse experiences such as maternal separation result in decreased $GABA_A$-receptor concentrations in the LC and the NTS, reduced BZD-receptor sites in the LC, the NTS, the frontal cortex, and the CE and the LA of the amygdala, and reduced mRNA levels for the γ2 subunit of the $GABA_A$-receptor complex in the LC, the NTS, and the amygdala (273). The extent to which these developmental responses to early-life

stress may alter the expression of fear and anxiety in adulthood remains unclear.

Benzodiazepine-GABA–Receptor Function in Anxiety Disorders

The central BZD receptor has been implicated in anxiety disorders on the basis of the anxiolytic and anxiogenic properties of BZD agonists and inverse agonists, respectively, and by the evidence that the BZD-receptor sensitivity to BZD agonists is reduced in some anxiety-disordered subjects (21,274,275). Hypotheses advanced regarding the role of $GABA_A$-BZD–receptor function in anxiety disorders have proposed either that changes in the $GABA_A$-BZD macromolecular complex conformation or that alterations in the concentration or properties of an endogenous ligand account for the pathologic anxiety symptoms seen in anxiety disorders. However, these hypotheses have not been conclusively tested by *in vivo* or postmortem studies of anxiety-disordered humans.

In PD, oral (276) and intravenous (274) administration of the BZD-receptor antagonist, flumazenil, produces panic attacks and increases anticipatory anxiety in some subjects with PD, but not in healthy controls. In addition, the sensitivity to the effects of diazepam on saccadic eye movement velocity is abnormally reduced in PD, a finding implying that the functional sensitivity of the $GABA_A$-BZD supramolecular complex is attenuated in brainstem regions controlling saccadic eye movements (275). Subjects with PD also show abnormally reduced sensitivity to the suppressant effects of diazepam on plasma NE, epinephrine, and heart rate (see Table 63.3 on p. 920) (277).

Receptor imaging studies using PET and SPECT have assessed central BZD-receptor binding in anxiety disorders. SPECT studies have reported reduced uptake of the selective BZD-receptor radioligand, [^{123}I]iomazenil, in the frontal (278–280), temporal (278,279), and occipital (278) cortices in subjects with PD relative to control subjects. However, interpretation of these results was limited by the absence of medication-free PD study subjects and of healthy controls (278,279) or by the dependence on nonquantitative methods for estimating BZD-receptor binding. A SPECT-iomazenil study that quantitated BZD-receptor binding by derivation of distribution volumes found reduced binding in the left hippocampus and precuneus in unmedicated PD relative to healthy control samples and reported an inverse correlation between panic anxiety ratings and frontal cortex iomazenil binding (281). Another SPECT-iomazenil study reported lower distribution volumes for BZD receptors in the dorsomedial PFC in PTSD relative to control samples (281a). These findings appeared consistent with the evidence cited earlier that stress downregulates BZD-receptor binding in the frontal cortex and the hippocampus of experimental animals.

Central BZD-receptor binding has also been assessed in PD using PET and [^{11}C]flumazenil. Malizia et al. reported a global reduction in BZD site binding in seven study subjects with PD relative to eight healthy controls, with the most prominent decreases evident in the right orbitofrontal cortex and the right insula (areas consistently activated during normal anxiety processing) (282). In contrast, Abadie et al. found no differences in the B_{max}, K_d or bound/free values for [^{11}C]flumazenil in any brain region in ten *unmedicated* PD study subjects relative to healthy controls (283).

Dopaminergic System

Acute stress increases DA release and turnover in multiple brain areas. The dopaminergic projections to the mPFC appear particularly sensitive to stress, because brief or low-intensity stressors (e.g., exposure to fear-conditioned stimuli) increase DA release and turnover in the mPFC in the absence of corresponding changes in other mesotelencephalic dopaminergic projections (284). For example, in rats, low-intensity electric foot shock increases tyrosine hydroxylase activity and DA turnover in the mPFC, but not in the nucleus accumbens or the caudate-putamen (285). In contrast, stress of greater intensity or longer duration additionally enhances DA release and metabolism in other areas as well (285). The regional sensitivity to stress appears to follow a pattern in which dopaminergic projections to the mPFC are more sensitive to stress than the mesoaccumbens and nigrostriatal projections, and the mesoaccumbens dopaminergic projections are more sensitive to stress than the nigrostriatal projections (284).

Thus far, there is little evidence that dopaminergic dysfunction plays a primary role in the pathophysiology of human anxiety disorders. In PD, Roy-Byrne et al. found a higher plasma concentration of the DA metabolite, homovanillic acid (HVA), in patients with high levels of anxiety and frequent panic attacks relative to controls (286). Patients with PD were also shown to have a greater growth hormone response to the DA-receptor agonist, apomorphine, than depressed controls (287). However, Eriksson et al. found no evidence of alterations in the CSF HVA concentrations in patients with PD or for correlations between CSF HVA and anxiety severity or panic attack frequency (288). In addition, genetic studies examining associations between PD and gene polymorphisms for the DA D4 receptor and the DA transporter have produced negative results (289).

In social phobia, two preliminary SPECT imaging studies involving small subject samples reported abnormal reductions in DA-receptor binding. Tiihonen et al. found a significant reduction in β-CIT binding in the striatum in social phobic relative to healthy control samples (290), presumably reflecting a reduction in DA-transporter binding. Schneier et al. reported reduced uptake of the DA D2/D3-receptor radioligand, [^{123}I]IBZM, in social phobic subjects relative to healthy control subjects (291). Both findings await replication.

Serotonergic System

Exposure to various stressors including restraint stress, tail shock, tail pinch, and high-level (but not low-level) foot shock results in increased 5-HT turnover in the mPFC, nucleus accumbens, amygdala, and lateral hypothalamus in experimental animals (285). During exposure to fear-conditioned stimuli, the 5-HT turnover in the mPFC appears particularly sensitive to the severity of stress, increasing as the aversiveness of the US and the magnitude of the conditioned fear behavioral response increases (285). However, exposure to repeated electric shocks sufficient to produce learned helplessness is associated with reduced *in vivo* release of 5-HT in the frontal cortex (292), a finding possibly reflecting a state in which 5-HT synthesis is outpaced by release. Preadministration of BZD-receptor agonists or tricyclic antidepressant drugs prevents stress-induced reductions in 5-HT release and interferes with the acquisition of learned helplessness, whereas infusion of 5-HT into the frontal cortex after stress exposure *reverses* learned-helplessness behavior (292,293). Finally, administration of 5-HT–receptor antagonists produces behavioral deficits resembling those of the learned helplessness seen after inescapable shock during animal stress models that do not ordinarily result in learned helplessness (293).

The effect of stress in activating 5-HT turnover may stimulate both anxiogenic and anxiolytic pathways within the forebrain, depending on the region involved and the 5-HT–receptor subtype that is predominantly stimulated. For example, microinjection of 5-HT into the amygdala appears to enhance conditioned fear, whereas 5-HT injection into the PAG inhibits unconditioned fear (260). Graeff et al. hypothesized that the serotonergic innervation of the amygdala and the hippocampus mediates anxiogenic effects by 5-HT_{2A}–receptor stimulation (260), whereas serotonergic innervation of hippocampal 5-HT_{1A} receptors suppresses formation of new CS-US associations and provides resilience to aversive events. Potentially compatible with this hypothesis, 5-HT_{1A}–receptor knockout mice exhibit behaviors consistent with increased anxiety and fear, and long-term administration of 5-HT_{1A}–receptor partial agonists exerts anxiolytic effects in generalized anxiety disorder (295).

Notably, stress and glucocorticoids exert major effects on the genetic expression of 5-HT_{1A} and 5-HT_{2A} receptors. Postsynaptic 5-HT_{1A}–receptor gene expression is under tonic inhibition by adrenal steroids in the hippocampus and possibly other regions where mineralocorticoid receptors are expressed (reviewed in ref. 296). Thus, 5-HT_{1A}–receptor density and mRNA levels decrease in response to chronic stress or CORT administration and increase after adrenalectomy (296–299). The stress-induced down-regulation of 5-HT_{1A}–receptor expression is prevented by adrenalectomy, a finding showing the importance of circulating adrenal steroids in mediating this effect (296). Although both mineralocorticoid-receptor stimulation and glucocorticoid-receptor stimulation are involved in mediating this effect, the former is most potent, and 5-HT_{1A} mRNA levels markedly decrease within hours of mineralocorticoid-receptor stimulation (296). Conversely, 5-HT_{2A}–receptor expression is up-regulated during chronic stress and CORT administration, and it is down-regulated in response to adrenalectomy (298,300). In view of evidence that 5-HT_{1A} and 5-HT_{2A} receptors may play reciprocal roles in mediating anxiety, it is conceivable that these corticosteroid mediated effects on 5-HT_{1A} and 5-HT_{2A} expression may be relevant to the pathophysiology of anxiety.

Serotonergic Function in Anxiety Disorders

The literature regarding serotonergic function in anxiety disorders is in disagreement (see Table 63.3). In PD, platelet 5-HT uptake has been reported to be abnormally elevated (301), normal (302), or abnormally reduced (303). Platelet imipramine binding (to a site related to the 5-HT transporter site), did not differ in PD relative to control samples (304,305). Another study reported reduced concentrations of circulating 5-HT in PD relative to control samples (306), although this finding has not been replicated.

Pharmacologic challenge studies involving 5-HT have been similarly unable to establish a primary role for 5-HT in the pathophysiology in PD. Neuroendocrine responses to challenge with the 5-HT precursors, L-tryptophan and 5-hydroxytryptophan (5-HTP), did not differentiate PD study subjects from healthy controls (307,308). Moreover, tryptophan depletion did not prove anxiogenic in unmedicated PD study subjects (309). Nevertheless, challenge with the 5-HT releasing agent, fenfluramine, produced greater increases in anxiety, plasma prolactin, and cortisol in PD compared with control subjects (131,310). Fenfluramine challenge also resulted in reduced CBF in the left posterior parietal-superior temporal cortex in PD study subjects relative to healthy controls (131), although it was unclear whether this abnormality reflected an abnormality of serotonergic function or a physiologic correlate of fenfluramine-induced anxiety, because more PD study subjects (56%) developed panic attacks than did control subjects (11%).

Preliminary data regarding the sensitivity of specific 5-HT–receptor subtypes appear more promising, particularly because the elevation of plasma ACTH and cortisol and the hypothermic responses to the 5-HT_{1A} partial agonist, ipsapirone, were blunted in PD relative to healthy control samples (311). Finally, increases in anxiety and plasma cortisol in PD relative to control samples have been reported after oral (312), but not intravenous, administration of the 5-HT_2–receptor agonist, m-chloromethylpiperazine (mCPP) (313).

Samples with combat-related PTSD have been shown to have decreased paroxetine binding in platelets relative to controls, a finding suggesting alterations in the 5-HT trans-

porter (314). Southwick et al. observed that a subgroup (five of 14 subjects) with PTSD experienced panic anxiety and "flashbacks" after mCPP challenge (189). Thus, a subgroup of patients with PTSD may have abnormal sensitivity to serotonergic provocation.

Cholecystokinin

CCK is an anxiogenic neuropeptide present in both the brain and the gastrointestinal tract. CCK-containing neurons are found in high density in the cerebral cortex, amygdala, hippocampus, midbrain PAG, substantia nigra, and raphe. Iontophoretic administration of CCK has depolarizing effects on pyramidal neurons and stimulates action potential formation in the dentate gyrus of the hippocampus (reviewed in ref. 315).

The CCK-receptor agonist, CCK-4, is anxiogenic in a variety of animal models of anxiety, whereas CCK-receptor antagonists exert anxiolytic effects in the same models (315). CCK has important functional interactions with other systems implicated in anxiety and fear (noradrenergic, dopaminergic, BZD). For example, the panicogenic effect of CCK-4 in PD is attenuated by administration of the β-adrenoreceptor antagonist, propranolol, and by long-term imipramine treatment, which down-regulates β-adrenoreceptors (316).

Study subjects with PD or PTSD are more sensitive to the anxiogenic effects of CCK-4 than are control subjects (317,318). For example, Strohle et al. found that of 24 PD study subjects tested, 15 experienced a panic attack after CCK-4 administration (319). Although the mechanism underlying the enhanced sensitivity to CCK-4 has not been elucidated, it is noteworthy that CSF concentrations of CCK are lower in PD study subjects than in healthy controls (320).

The neuroendocrine effects associated with CCK-4 induced panic appear to differ between PD and PTSD. In PTSD, CCK-4–induced panic was associated with a *lower* ACTH response in the PTSD study subjects than in healthy controls, and cortisol concentrations increased in both the PTSD and control groups (318). The elevation in the cortisol concentrations attenuated more rapidly in the PTSD group than in the control group.

In contrast to the findings in PTSD, ACTH secretion was higher in subjects with PD who developed panic attacks in response to CCK-4 than in those who did not, although even the latter subjects showed brief, less pronounced increases in ACTH concentrations (319). Neither PD subgroup showed significant changes in the plasma cortisol concentration after CCK-4 administration. The elevation of ACTH concentrations suggested that CRH secretion increases in CCK-4–induced panic in PD (consistent with preclinical evidence regarding the role of CRH in stress and anxiety and the interaction of CRH and CCK in modulating anxiety) (221).

The CCK receptors are classified into CCK-A and CCK-B subtypes. Kennedy et al. reported a significant association between PD and a single nucleotide polymorphism found in the coding region of the CCK-B–receptor gene (321). In contrast, genetic polymorphisms for the CCK-A–receptor gene and the CCK-pre-pro hormone genes showed no association with PD (321). If confirmed by replication, these data would suggest that a CCK-B–receptor gene variation may be involved in the pathogenesis of PD.

Pande et al. assessed the efficacy of the selective CCK-B–receptor antagonist, CI-988, for preventing panic attacks in PD (322). No differences in the rate of panic attacks were seen between the active drug and placebo treatment groups. Nevertheless, because of the limited bioavailability of oral CI-988, studies involving this drug may not have sufficiently tested the hypothesis that CCK-B–receptor antagonism produces antipanic effects in PD.

Other Neuropeptides

Opioid Peptides

Acute, uncontrollable shock increases secretion of opiate peptides and decreases μ-opiate–receptor density (323, 324). The elevation of opioid peptide secretion may contribute to the analgesia observed after uncontrollable stress and exposure to fear-conditioned stimuli (325). This analgesic effect shows evidence of sensitization, because subsequent exposure to less intense shock in rats previously exposed to uncontrollable shock also results in analgesia (326).

Potentially consistent with these data, Pitman et al. found that patients with PTSD showed reduced pain sensitivity compared with veterans without PTSD after exposure to a combat film (327), an effect that was reversed by the opiate antagonist naloxone (a finding suggesting mediation by endogenous opiate release during symptom provocation). In the baseline state, the CSF β-endorphin levels were abnormally elevated in PTSD relative to control samples (328). However, Hoffman et al. found *lower* morning and evening *plasma* β-endorphin levels in a PTSD group versus healthy control samples (329). Another study found no differences in plasma methionine-enkephalin concentrations between PTSD subjects and control subjects, although this compound's degradation half-life was higher in the PTSD group (330).

During opiate administration, Bremner et al. reported that some patients with combat-related PTSD experience an attenuation of their hyperarousal symptoms (331). Because preclinical studies in experimental animals have shown that opiates potently suppress central and peripheral noradrenergic activity, these data appear compatible with the hypothesis that some PTSD symptoms are mediated by noradrenergic hyperactivity (discussed earlier). Conversely, during opiate withdrawal noradrenergic activity increases, and it

has been noted that some symptoms of PTSD resemble those of opiate withdrawal (170).

Neuropeptide Y

NPY administered in low doses intraventricularly attenuates experimentally induced anxiety in a variety of animal models (332). Consistent with these data, transgenic rats that overexpress hippocampal NPY show behavioral insensitivity to restraint stress and absent fear suppression of behavior in a punished drinking task (333). In healthy humans subjected to uncontrollable stress during military training exercises, plasma NPY levels increased to a greater extent in persons rated as having greater stress resilience (334). During stress exposure, the NPY plasma levels were positively correlated with plasma cortisol concentrations and behavioral performance, and they were negatively correlated with dissociative symptoms (334).

In humans with PD, plasma NPY concentrations were abnormally elevated, and this finding, given NPY's putative anxiolytic effects, may reflect an adaptive response to anxiety symptoms (335). In contrast, patients with combat-related PTSD had *lower* plasma NPY concentrations both at baseline and in response to yohimbine challenge than healthy controls (336). In the PTSD group, the baseline NPY levels were inversely correlated with PTSD and panic symptoms and with yohimbine-induced increases in MHPG and systolic blood pressure (336). If this finding proves reproducible, it suggests that a deficit in endogenous NPY secretion may be involved in the generation of anxiety and sympathetic autonomic symptoms in PTSD.

Thyrotropin-Releasing Hormone and the Thyroid Axis

In the early twentieth century, Graves described cases in which thyroid hormone hypersecretion was associated with anxiety, palpitations, breathing difficulties, and rapid heart rate in persons recently exposed to traumatic stress. Nevertheless, systematic epidemiologic studies of the relationship between stress and thyroid disease have not been conducted. Although few studies have looked at thyroid function in anxiety disorders, Mason et al. found elevated levels of triiodothyronine in patients with combat-related PTSD (337) (Table 63.3), a finding consistent with evidence that stress results in long-lasting elevations of thyroid hormone secretion (338).

TABLE 63.3. EVIDENCE OF ALTERATION IN OTHER NEUROTRANSMITTER SYSTEMS IN ANXIETY DISORDERS

	PTSD	Panic Disorder
Benzodiazepine		
Increased symptomatology with benzodiazepine antagonist	–	++
Decreased number of benzodiazepine receptors using SPECT-iomazenil or PET-flumazenil binding	+	+++/–
Opiate		
Naloxone-reversible analgesia	+	NS
Reduced plasma β-endorphin	+	NS
Elevated levels of CSF β-endorphin	+	–
Serotonin		
Decreased serotonin reuptake site binding in platelets	++	+/–
Decreased serotonin transmitter in platelets	–	+/–
Blunted endocrine response to 5-HT_{1A} probe	–	+
Altered serotonin effect on cAMP in platelets (5-HT_{1A} probe)	–	NS
Increased anxiogenic responses to 5-HT agonists	+	+/–
Thyroid		
Increased baseline indices of thyroid function	+	–
Increased TSH response to TRH	+	–
Somatostatin		
Increased somatostatin levels at baseline in CSF	+	–
Cholecystokinin		
Increased anxiogenic responses to CCK agonists	+	+++

–, One or more studies did not support this finding (with no positive studies), or the majority of studies do not support this finding; +/–, an equal number of studies support this finding and do not support this finding; +, at least one study supports this finding and no studies do not support the finding, or the majority of studies support the finding; ++, two or more studies support this finding, and no studies do not support the finding; +++, three or more studies support this finding, and no studies do not support the finding; +++/–, three or more studies support this finding, and one study does not support the finding; cAMP, cyclic adenosine 3′, 5′-monophosphate; CCK, cholecystokinin; CSF, cerebrospinal fluid; NS, not studied; PTSD, posttraumatic stress disorder; SPECT, single photon emission computed tomography; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

Respiratory System Dysfunction in Panic Disorder

Associations between respiratory perturbation and acute anxiety have been demonstrated in PD, in which various forms of respiratory stimulation consistently produce panic anxiety and alterations in parameters of respiratory physiology (339–342). The most straightforward forms of respiratory stimulation that produce panic anxiety produce elevations of carbon dioxide pressure (hypercapnia). Thus, panic attacks can be consistently induced in patients with PD by rebreathing air, inhaling 5% to 7% carbon dioxide in air (343,344), or inhaling a single deep breath of 35% carbon dioxide (345,346). Other panicogenic chemical challenges have also been hypothesized to induce anxiogenic effects

through respiratory stimulation (340,341,347). Although the panicogenic mechanism of intravenous administration of sodium lactate remains unclear, it may also involve respiratory stimulation (339,340).

The evidence that respiratory parameters index risk for panic anxiety includes data showing the following: (a) asymptomatic adult relatives of patients with PD have abnormally increased sensitivity to respiratory stimulation by carbon dioxide inhalation; (b) among PD samples, stronger family loading for PD is found among persons with evidence of respiratory dysregulation; and (c) the respiratory indices associated with PD are heritable, a finding suggesting a shared genetic vulnerability for panic attacks and respiratory dysregulation (reviewed in Chapter 61). Nevertheless, these data partly depend on subjective ratings of dyspnea during stress or respiratory stimulation, and the mechanisms underlying this sensitivity remain unclear. One possibility is that this hypersensitivity reflects an overall sensitivity to somatic sensations, because high degrees of anxiety sensitivity are linked to future panic attacks (348).

The associations between respiratory perturbation and acute anxiety are not specific to PD. Exaggerated sensitivity to respiratory perturbation has also been reported in anxiety-disordered patients with some simple phobias, limited symptom panic attacks, childhood separation anxiety disorder, or limited-symptom anxiety attacks and in nonpsychiatrically ill subjects with high ratings on anxiety sensitivity scales. (See Chapter 61) For example, children with separation anxiety disorder exhibit greater changes in somatic symptoms during carbon dioxide inhalation that positively correlate with increases in respiratory rate, tidal volume, minute ventilation, end-tidal carbon dioxide pressure, and irregularity in respiratory rate during room-air breathing (349).

CONCLUDING REMARKS

The inconsistency in the results of biological investigations of anxiety disorders highlights the importance of addressing the neurobiological heterogeneity inherent within criteria-based, psychiatric diagnoses. Understanding this heterogeneity will be facilitated by the continued development and application of genetic, neuroimaging, and neurochemical approaches that can refine anxiety disorder phenotypes and can elucidate the genotypes associated with these disorders. Application of these experimental approaches will also facilitate research aimed at elucidating the mechanisms of anti-anxiety therapies.

The knowledge reviewed herein regarding the neurobiology of fear and anxiety already suggests themes along which the development of new therapeutic approaches can be organized. In general, anxiolytic treatments appear to inhibit neuronal activity in the structures mediating fear expression and behavioral sensitization and facilitate endogenous mechanisms for modulating the neural transmission of information about aversive stimuli and responses to such stimuli. Novel treatments being developed to exploit the former type of mechanisms include pharmacologic agents that selectively target subcortical and brainstem pathways supporting specific components of emotional expression (e.g., CRH-receptor antagonists). In contrast, nonpharmacologic treatments for anxiety may augment the brain's systems for modulating anxiety responses, by facilitating the extinction of putative fear-conditioned responses or directing the reinterpretation of anxiety-related thoughts and somatic sensations (so they produce less subjective distress). Informed by increasingly detailed knowledge about the pathophysiology of specific anxiety disorders and the neural pathways involved in anxiety and fear processing, the development of therapeutic strategies that combine both types of approaches may ultimately provide the optimal means for reducing the morbidity of anxiety disorders.

REFERENCES

1. LeDoux JE. Emotion. In: Mills J, Mountcastle VB, Plum F, et al., eds. *Handbook of physiology: the nervous system V*. Baltimore: Williams & Wilkins, 1987:373–417.
2. LeDoux, Joseph E. Emotion: clues from the brain. *Annu Rev Psychol* 1995;46:209–235.
3. Izard CE. Four systems for emotion activation: cognitive and noncognitive processes. *Psychol Rev* 1993;100:68–90.
4. LeDoux J. Fear and the brain: where have we been, and where are we going? *Biol Psychiatry* 1998;44:1229–1238.
5. Davis M. Are different parts of the extended amygdala involved in fear versus anxiety? *Biol Psychiatry* 1998;44:1239–1247.
6. Rogan MT, Staubli UV, LeDoux JE. Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 1997;390:604–607.
7. Price JL, Carmichael ST, Drevets WC. Networks related to the orbital and medial prefrontal cortex: a substrate for emotional behavior? *Prog Brain Res* 1996;107:523–536.
8. Baxter MG, Parker A, Lindner CCC, et al. Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. *J Neurosci* 2000;20:4311–4319.
9. Everitt BJ, Cador M, Robbins TW. Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. *Neuroscience* 1989;30:63–75.
10. Nishijo H, Ono T, Nishino H. Single neuron responses in amygdala of alert monkey during complex sensory stimulation with affective significance. *J Neurosci* 1988;8:3570–3583.
11. Rolls ET. A theory of emotion and consciousness, and its application to understanding the neural basis of emotion. In: Gazzaniga MS, ed. *The cognitive neurosciences,* Cambridge, MA: MIT Press, 1995:1091–1106.
12. Doron NN, LeDoux JE. Organization of projections to the lateral amygdala from auditory and visual areas of the thalamus in the rat. *J Comp Neurol* 1999;412:383–409.
13. Neafsey EJ, Terreberry RR, Hurley KM, et al. Anterior cingulate cortex in rodents: connections, visceral control functions, and implications for emotion. In: Vogt BA, Gabriel M, eds. *Neurobiology of cingulate cortex and limbic thalamus.* Boston: Birkhauser, 1993:206–223.
14. Armony JL, Quirk GJ, LeDoux JE. Differential effects of amyg-

dala lesions on early and late plastic components of auditory cortex spike trains during fear conditioning. *J Neurosci* 1998; 18:2592–2601.
15. Ferry B, Roozendaal B, McGaugh JL. Role of norepinephrine in mediating stress hormone regulation of long-term memory storage: a critical involvement of the amygdala. *Biol Psychiatry* 1999;46:1140–1152.
16. Cahill L, McGaugh JL. Mechanisms of emotional arousal and lasting declarative memory. *Trends Neurosci* 1998;21:294–299.
17. Morgan MA, LeDoux JE. Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behav Neurosci* 1995;109: 681–688.
18. Quirk GJ, Russo GK, Barron JL, et al. The role of the ventromedial prefrontal cortex in the recovery of extinguished fear. *J Neurosci* 2000;20:6225–6231.
19. Romanski LM, Clugnet MC, Bordi F., et al. Somatosensory and auditory convergence in the lateral nucleus of the amygdala. *Behav Neurosci* 1993;107:444–450.
20. Carmichael ST, Clugnet M-C, Price JL. Central olfactory connections in the macaque monkey. *J Comp Neurol* 1994;346: 403–434.
21. Gray JA, McNaughton N. The neuropsychology of anxiety: reprise. *Nebr Symp Motiv* 1996;43:61–134.
22. Phillips RG, LeDoux JE. Lesions of the fornix but not the entorhinal or perirhinal cortex interfere with contextual fear conditioning. *J Neurosci* 1995;15:5308–5315.
23. Kim JJ, Fanselow MD. Modality-specific retrograde amnesia of fear. *Science* 1992;256:675–677.
24. Maren S, Aharonov G, Fanselow MS. Neurotoxic lesions of the dorsal hippocampus and pavlovian fear conditioning in rats. *Behav Brain Res* 1997;88:261–274.
25. Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* 1992;106:274–285.
26. Morgan MA, LeDoux JE. Contribution of ventrolateral prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Neurobiol Learn Mem* 1999;72:244–251.
27. Corodimas KP, LeDoux JE. Disruptive effects of posttraining perirhinal cortex lesions on conditioned fear: contributions of contextual cue. *Behav Neurosci* 1995;109:613–619.
28. Caldarone B, Saavedra C, Tartaglia K, et al. Quantitative trait loci analysis affecting contextual conditioning in mice. *Nat Genet* 1997;17:335–337.
29. Wehner JM, Radcliffe RA, Rosmann ST, et al. Quantitative trait locus analysis of contextual fear conditioning in mice [see Comments]. *Nat Genet* 1997;17:331–334.
30. Flint J, Corley R, DeFries JC, et al. A simply genetic basis for a complex psychological trait in laboratory mice. *Science* 1995; 269:1432–1435.
31. LeDoux JE, Romanski L, Xagoratis A. Indelibility of subcortical emotional memories. *J Cogn Neurosci* 1989;1:238–243.
32. Shi C, Davis M. Pain pathways involved in fear conditioning measured with fear-potentiated startle: lesion studies. *J Neurosci* 1999;19:420–430.
33. Campeau S, Davis M. Involvement of subcortical and cortical afferents to the lateral nucleus of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J Neurosci* 1995;15:2312–2327.
34. LeDoux JE, Cicchetti P, Xagoraris A, et al. The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *J Neurosci* 1990;10:1062–1069.
35. Romanski LM, LeDoux JE. Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. *J Neurosci* 1992;12:4501–4509.
36. Pitkanen A, Savander V, LeDoux JE. Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends Neurosci* 1997; 20:517–523.
37. Pitkanen A, Stefanacci L, Farb CR, et al. Intrinsic connections of the rat amygdaloid complex: projections originating in the lateral nucleus. *J Comp Neurol* 1995;356:288–310.
38. Savander V, Miettinen R, LeDoux JE, et al. Lateral nucleus of the rat amygdala is reciprocally connected with basal and accessory basal nuclei: a light and electron microscopy study. *Neuroscience* 1997;77:767–781.
39. Maren S. Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. *Trends Neurosci* 1999;22: 561–567.
40. Welinsky AE, Scafe GE, LeDoux JE. Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory consolidation. *J Neurosci* 1999;19:RC48.
41. Savander V, Go CG, LeDoux JE, et al. Intrinsic connections of the rat amygdaloid complex: projections originating in the basal nucleus. *J Comp Neurol* 1995;361:345–368.
42. Garcia R, Vouimba R-M, Baudry M, et al. The amygdala modulates prefrontal cortex activity relative to conditioned fear. *Nature* 1999;402:294–296.
43. Öngür D, Price JL. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb Cortex* 2000;10:206–219.
44. Murray EA. Memory for objects in nonhuman primates. In: Gazzaniga MS, ed. *The new cognitive neurosciences.* Cambridge, MA: MIT Press, 2000:753–763.
45. Squire LR. Knowlton BJ. Learning about categories in the absence of memory. *Proc Natl Acad Sci USA* 1995;92: 12470–12474.
46. Quirk GJ, Armony JL, LeDoux JE. Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. *Neuron* 1997;19: 481–484.
47. Büchel C, Morris J, Dolan RJ, et al. Brain systems mediating aversive conditioning: an event related fMRI study. *Neuron* 1998;20:947–957.
48. LaBar KS, Gatenby JC, Gore JC, et al. Human amygdala activation during conditioned fear acquisition and extinction: a mixed trial fMRI study. *Neuron* 1998;20:937–945.
49. Drevets WC, Raichle ME. Reciprocal suppression of regional cerebral blood flow during emotional versus higher cognitive processes: implications for interactions between emotion and cognition. *Cogn Emot* 1998;12:353–385.
50. McGaugh JL. Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. *Annu Rev Neurosci* 1989;2:255–287.
51. McGaugh JL. Significance and remembrance: the role of neuromodulatory systems. *Psychol Sci* 1991;1:15–45.
52. Cahill L, Haier RJ, Fallon J, et al. Amygdala activity at encoding correlated with long-term, free recall of emotional information. *Proc Nat Acad Sci USA* 1996;93:8016–8021.
53. Canli T, Zhao Z, Brewer J, et al. Event-related activation in the human amygdala associates with later memory for individual emotional experience. *J Neurosci* 2000;20:RC99.
54. Hamann SB, Ely TD, Grafton ST, et al. Amygdala activity related to enhanced memory for pleasant and aversive stimuli. *Nat Neurosci* 1999;2:289–293.
55. Phelps EA, Anderson AK. Emotional memory: what does the amygdala do? *Curr Biol* 1997;7:R311–314.
56. Halgren E. The amygdala contribution to emotion and memory: current studies in humans. In: Ben-Ari Y, ed. *The amygdaloid complex.* Amsterdam: Elsevier/North Holland Biomedical Press, 1981:395–408.

57. Gloor P, Olivier A, Quesney LF, et al. The role of the limbic system in experiential phenomena of temporal lobe epilepsy. *Ann Neurol* 1982;12:129–144.
58. Brothers L. Neurphysiology of the perception of intentions by primates. In: Gazzaniga MS, ed. *The cognitive neurosciences.* Cambridge, MA: MIT Press, 1995:1107–1116.
59. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* 1997;20:78–84.
60. Iwata J, Chida K, LeDoux JE. Cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus in awake but not anesthetized rats resemble conditioned emotional responses. *Brain Res* 1987;418:183–188.
61. Kapp BS, Gallagher M, Underwood MD, et al. Cardiovascular responses elicited by electrical stimulation of the amygdala central nucleus in the rabbit. *Brain Res* 1982;234:251–262.
62. Hitchcock JM, Davis M. Efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm. *Behav Neurosci* 1991;105:826–842.
63. Kim M, Davis M. Lack of a temporal gradient of retrograde amnesia in rats with amygdala lesions assessed with the fear-potentiated startle paradigm. *Behav Neurosci* 1993;107: 1088–1092.
64. LeDoux JE, Iwata J, Cicchetti P, et al. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear *J Neurosci* 1988;8: 2517–2529.
65. Russchen FT, Bakst I, Amaral DG, et al. The amygdalostriatal projections in the monkey-an anterograde tracing study. *Brain Res* 1985;329:241–257.
66. Mogenson GJ, Brudzynski SM, Wu M, et al. From motivation to action: a review of dopaminergic regulation of limbic nucleus accumbens ventral pallidum pedunculopontine nucleus circuitries involved in limbic-motor integration. In: Kalivas PW, Barnes CD, eds. *Limbic motor circuits and neuropsychiatry.* London: CRC Press, 1993:193–236.
67. Bandler R, Shipley MT. Columnar organization in the midbrain periaqueductal grey: modules for emotional expression? *Trends Neurosci* 1994;17:379–389.
68. Weiskrantz L. Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *JCPP* 1956;49:381–391.
69. Meunier M, Bachevalier J, Murray EA, et al. Effect of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. *Eur J Neurosci* 1999;11:4403–4418.
70. Morris JS, Frith CD, Perrett DI, et al. A differential neural response in the human amygdala to fearful and happy facial expression. *Nature* 1996;383:812–815.
71. Blair RJR, Morris JS, Frith CD, et al. Neural responses to sad and angry expressions. *Brain* 1999;122:883–893.
72. Adolphs R, Tranel D, Damascio H, et al. Fear and the human amygdala. *J Neurosci* 1995;15:5879–5891.
73. Scott SK, Young AW, Calder AJ, et al. Impaired auditory recognition of fear and anger following bilateral amygdala lesions. *Nature* 1997;385:254–257.
74. Krettek JE, Price JL. Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. *J Comp Neurol* 1977;172: 723–752.
75. Rosen, JB, Hitchcock JM, Miserendino MJ, et al. Lesions of the perirhinal cortex but not of the frontal, medial prefrontal, visual, or insular cortex block fear-potentiated startle using a visual conditioned stimulus. *J Neurosci* 1992;12:4624–4633.
76. Sananes CB, Davis M. *N*-methyl-D-aspartate lesions of the lateral and basolateral nuclei of the amygdaloid block fear-potentiated startle and shock sensitization of startle. *Behav Neurosci* 1992;106:72–80.
77. Tischler MD, Davis M. A visual pathway that mediates fear-conditioned enhancement of acoustic startle. *Brain Res* 1983; 276:55–71.
78. Rauch SL, Savage CR, Alpert NM, et al. A positron emission tomographic study of simple phobic symptom provocation. *Arch Gen Psychiatry* 1995;52:20–28.
79. Rauch SI, van der Kolk BA, Fisler RF, et al. A symptom provocation study of posttraumatic stress disorder using positron emission tomography and script driven imagery. *Arch Gen Psychiatry* 1996;53:380–387.
80. Lane RD, Reiman EM, Ahern GL, et al. Neuroanatomical correlates of happiness, sadness, and disgust. *Am J Psychiatry* 1997; 154:926–933.
81. Reiman EM, Lane RD, Ahern GL, et al. Neuroanatomical correlates of externally and internally generated human emotion. *Am J Psychiatry* 1997;154:918–925.
82. Sawchenko PE, Swanson LWW. Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. *Science* 1983;214:685–687.
83. Frysztak RJ, Neafsey EJ. The effect of medial frontal cortex lesions on cardiovascular conditioned emotional responses in the rat. *Brain Res* 1994;643:181–193.
84. Neafsey EJ, Hurley-Gius KM, Arvanitis D. The topographical organization of neurons in the rat medial frontal, insular and olfactory cortex projecting to the solitary nucleus, olfactory bulb, periaqueductal gray and superior colliculus. *Brain Res* 1986;377:261–270.
85. Damasio AR, Grabowski TJ, Bechara A, et al. Neural correlates of the experience of emotions. *Soc Neurosci Abstr* 1998;24:258.
86. Rogers RD, Everitt BJ, Baldacchino A, et al. Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-deleted normal volunteers: evidence for monoaminergic mechanisms. *Neuropsychopharmacology* 1999; 20:322–339.
87. Dioro D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci* 1993;3:3839–3847.
88. Schultz W. Dopamine neurons and their role in reward mechanisms. *Curr Opin Neurobiol* 1997;7:191–197.
89. Usher M, Cohen JD, Servan-Schreiber D, et al. The role of locus coeruleus in the regulation of cognitive performance. *Science* 1999;283:549–554 .
90. Robertson IH, Mattingley JB, Rorden C, et al. Phasic alerting of neglect patients overcomes their spatial deficit in visual awareness. *Nature* 1998;395:169–172.
91. Drevets WC. Neuroimaging studies of mood disorders. *Biol Psychiatry* 2000;48:813–829.
92. Teasdale JD, Howard RJ, Cox SG, et al. Functional MRI study of the cognitive generation of affect. *Am J Psychiatry* 1999;156: 79–88.
93. Northoff G, Richter A, Gessner, et al. Functional dissociation between medial and lateral prefrontal cortical spatiotemporal activation in negative and positive emotions: a combined fMRI/MEG study. *Cereb Cortex* 2000;10:93–107.
94. Drevets WC, Videen TO, Price JL, et al. A functional anatomical study of unipolar depression. *J Neurosci* 1992;12: 3628–3641.
95. Mayberg HS, Brannan SK, Mahurin RK, et al. Cingulate function in depression: a potential predictor of treatment response. *Neuroreport* 1997;8:1057–1061.
96. Drevets WC, Price JL, Simpson JR, et al. Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 1997;386: 824–827.
97. Shin LM, Kosslyn SM, McNally RJ, et al. Visual imagery and perception in posttraumatic stress disorder: a positron emission

tomographic investigation. *Arch Gen Psychiatry* 1997;54: 233–237.
98. Hirayasu Y, Shenton ME, Salisbury DF, et al. Subgenual cingulate cortex volume in first-episode psychosis. *Am J Psychiatry* 1999;156:1091–1093.
99. Öngür D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci USA* 1998;95:13290–13295.
100. George MS, Ketter TA, Parekh PI, et al. Brain activity during transient sadness and happiness in healthy women. *Am J Psychiatry* 1995;152:341–351.
101. Mayberg HS, Liotti M, Brannan SK, et al. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *Am J Psychiatry* 1999;156: 675–682.
102. Carmichael ST, Price JL. Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys, *J Comp Neurol* 1995;363:615–641.
103. Leichnetz GR, Astruc J. The efferent projections of the medial prefrontal cortex in the squirrel monkey *(Saimiri sciureus). Brain Res* 1976;109:455–472.
104. Damasio AR, Tranel D, Damasio H. Individuals with sociopathic behavior caused by frontal damage fail to respond autonomically to social stimuli. *Behav Brain Res* 1990;41:81–94.
105. Sullivan RM, Gratton A. Lateralized effects of medial prefrontal cortex lesions on neuroendocrine and autonomic stress responses in rats. *J Neurosci* 1999;19:2834–2840.
106. Dolan RJ, Fletcher P, Morris J, et al. Neural activation during covert processing of positive emotional facial expressions. *Neuroimage* 1996;4:194–200.
107. Drevets WC, Videen TO, Snyder AZ, et al. Regional cerebral blood flow changes during anticipatory anxiety. *Soc Neurosci Abstr* 1994;20:368.
108. Price JL. Networks within the orbital and medial prefrontal cortex. *Neurocase* 1999;5:231–241.
109. Baxter LR, Schwartz JM, Phelps ME, et al. Reduction of prefrontal cortex glucose metabolism common to three types of depression. *Arch Gen Psychiatry* 1989;46:243–250.
110. Rajkowska G, Miguel-Hidalgo JJ, Wei J, et al. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry* 1999;45:1085–1098.
111. Drevets WC, Botteron, K. Neuroimaging in psychiatry. In: Guze SB, ed. *Adult psychiatry.* St. Louis: CV Mosby, 1997: 53–81.
112. Baxter LR. Neuroimaging studies of human anxiety disorders. In: Bloom FE, Kupfer DJ, eds. *Psychopharmacology: the fourth generation of progress.* New York: Raven, 1995:921–932.
113. Drevets WC. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Prog Brain Res* 2000;126:413–431.
114. Rauch SL, Jenike MA, Alpert NM, et al. Regional cerebral blood flow measured during symptom provocation in obsessive-compulsive disorder using oxygen 15–labeled carbon dioxide and positron emission tomography. *Arch Gen Psychiatry* 1994; 51:62–70.
115. Drevets WC, Videen TO, MacLeod AK, et al. PET images of blood flow changes during anxiety: correction. *Science* 1992; 256:l696.
116. Drevets WC, Spitznagel E, Raichle ME. Functional anatomical differences between major depressive subtypes. *J Cereb Blood Flow Metab* 1995;15:S93.
117. Drevets WC, Simpson JR, Raichle ME. Regional blood flow changes in response to phobic anxiety and habituation. *J Cereb Blood Flow Metab* 1995;15:S856.
118. Schneider F, Gur RE, Mozley LH, et al. Mood effects on limbic blood flow correlate with emotional self-rating: a PET study with oxygen-15 labeled water. *Psychiatry Res* 1995;61:265–283.
119. Carmichael ST, Price JL. Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. *J Comp Neurol* 1995;363:615–641.
120. Timms RJ. Cortical inhibition and facilitation of the defense reaction. *J Physiol (Lond)* 1977;266:98–99.
121. Bechara A, Damasio H, Damasio AR. Emotion, decision making and the orbitofrontal cortex. *Cereb Cortex* 2000;10: 295–307.
122. Iversen SD, Mishkin M. Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity. *Exp Brain Res* 1970;11:376–386.
123. Maddock RJ. The retrosplenial cortex and emotion: new insights from functional neuroimaging of the human brain. *Trends Neurosci* 1999;22:310–316.
124. Van Hoesen GW, Morecraft RJ, Vogt B. Connections of the monkey cingulate cortex. In: Vogt BA, Gabriel M, eds. *Neurobiology of cingulate cortex and limbic thalamus* Boston: Birkhauser, 1993.
125. Reiman E, Raichle MF, Butler K, et al. A focal brain abnormality in panic disorder, a severe form of anxiety. *Nature* 1984; 310:683–685.
126. Nordahl TE, Semple WE, Gross M, et al. Cerebral glucose metabolic differences in patients with panic disorder. *Neuropsychopharmacology* 1990;3:261–271.
127. Bisaga A, Katz JL, Antonini A, et al. Cerebral glucose metabolism in women with panic disorder. *Am J Psychiatry* 1998;155: 1178–1183.
128. De Cristofaro MT, Sessarego A, Pupi A, et al. Brain perfusion abnormalities in drug-naive, lactate-sensitive panic patients: a SPECT study. *Biol Psychiatry* 1993;33:505–512.
129. Reiman EM, Raichle ME, Robins E, et al. Neuroanatomical correlates of a lactate-induced anxiety attack. *Arch Gen Psychiatry* l989;46:493–500.
130. Rauch SL, Savage CR, Alpert NM, et al. The functional neuroanatomy of anxiety: a study of three disorders using positron emission tomography and symptom provocation. *Biol Psychiatry* 1997;42:446–452.
131. Benkelfat C, Bradwejn J, Meyer E, et al. Functional neuroanatomy of CCK4–induced anxiety in normal healthy volunteers. *Am J Psychiatry* 1995;152:1180–1184.
132. Meyer JH, Swinson R, Kennedy SH, et al. Increased left posterior parietal-temporal cortex activation after D-fenfluramine in women with panic disorder. *Psychiatry Res* 2000;98:133–143.
133. Cameron OG, Zubieta JK, Grunhaus L, et al. Effects of yohimbine on cerebral blood flow, symptoms, and physiological functions in humans. *Psychosom Med* 2000;62:549–559.
134. Woods SW, Kosten K, Krystal JH, et al. Yohimbine alters regional cerebral blood flow in panic disorder [Letter]. *Lancet* 1988;2:678.
135. Ontiveros A, Fonaine R, Breton G. Correlation of severity of panic disorder and neuroanatomical changes on magnetic resonance imaging. *J Neuropsychiatry Clin Neurosci* 1989;1: 404–408.
136. Vythilingam M, Anderson GM, Owens MJ, et al. Cerebrospinal fluid corticotropin-releasing hormone in healthy humans: effects of yohimbine and naloxone. *J Clin Endocrinol Metab* 2000; 85:4138–4145.
137. Schneider F, Weiss U, Kessler C, et al. Subcortical correlates of differential classical conditioning of aversive emotional reactions in social phobia. *Biol Psychiatry* 1999;45:863–871.
138. Liberzon I, Taylor SF, Amdur R, et al. Brain activation in PTSD in response to trauma-related stimuli. *Biol Psychiatry*1999;45: 817–826.
139. Shin LM, McNally RJ, Kosslyn SM, et al. Regional cerebral

blood flow during script-driven imagery in childhood sexual abuse-related PTSD: a PET investigation. *Am J Psychiatry* 1999; 156:575–584.
140. Bremner JD, Narayan M, Staib LH, et al. Neural correlates of memories of childhood sexual abuse in women with and without posttraumatic stress disorder. *Am J Psychiatry* 1999;156: 1787–1795.
141. Bremner JD, Staib LH, Kaloupek D, et al. Neural correlates of exposure to traumatic pictures and sounds in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study. *Biol Psychiatry* 1999;45: 806–816.
142. Rauch SL, Whalen PJ, Shin LM, et al. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biol Psychiatry* 2000;47:769–776.
143. Orr SP, Metzger LJ, Lasko NB, et al. Pitman RK. *De novo* conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *J Abnorm Psychol* 2000;109: 290–298.
144. Peri T, Ben-Shakhar G, Orr SP, et al. Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. *Biol Psychiatry* 2000;47:512–519.
145. Bremner JD, Randall P, Scott TM, et al. MRI-based measurement of hippocampal volume in combat-related posttraumatic stress disorder. *Am J Psychiatry* 1995;152:973–981.
146. Bremner J.D, Randall P, Vermetten E, et al. MRI-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse: a preliminary report. *Biol Psychiatry* 1997;41:23–32.
147. Gurvits TG, Shenton MR, Hokama H, et al. Magnetic resonance imaging study of hippocampal volume in chronic combat-related posttraumatic stress disorder. *Biol Psychiatry* 1996; 40:192–199.
148. Stein MB, Koverola C, Hanna C, et al. Hippocampal volume in women victimized by childhood sexual abuse. *Psychol Med* 1997;27:951–959.
149. McEwen BS. Stress and hippocampal plasticity. *Annu Rev Neurosci* 1999;22:105–122.
150. Sapolsky RM. Why stress is bad for your brain. *Science* 1996; 273:749–750.
151. Laplane D, Levasseur M, Pillon B, et al. Obsessive-compulsive and other behavioural changes with bilateral basal ganglia lesions. *Brain* 1989;112:699–725.
152. Buckner RL, Petersen SE, Ojemann JG, et al. Functional anatomical studies of explicit and implicit memory retrieval tasks. *J Neurosci* 1995;15:12–29.
153. Swedo SE, Rapoport JL, Cheslow DL, et al. High prevalence of obsessive-compulsive symptoms in patients with Sydenham's chorea. *Am J Psychiatry* 1989;146:246–249.
154. Eslinger PJ, Damasio AR. Severe disturbance of higher cognition after bilateral frontal lobe ablation: patient EVR. *Neurology* 1985;35:1731–1741.
155. Nauta WJH. Reciprocal links of the corpus striatum with the cerebral cortex and limbic system: a common substrate for movement and thought? In: Mueller J, ed.*Neurology and psychiatry: a meeting of minds.* New York: Karger, 1989.
156. Cassens G, Kuruc A, Roffman M, et al. Alterations in brain norepinephrine metabolism and behavior induced by environmental stimuli previously paired with inescapable shock. *Behav Brain Res* 1981;2:387–407.
157. Rasmussen K, Marilak DA, Jaclobs BI. Single unit activity of the locus coeruleus in the freely moving cat. I. During naturalistic behaviors and in response to simple and complex stimuli. *Brain Res* 1986;371:324–334.
158. Redmond DF Jr. Studies of the nucleus locus coeruleus in monkey and hypotheses for neuropsychopharmacology. In: Meltzer HY, ed. *Psychopharmacology: the third generation of progress.* New York: Raven, 1987:967–975.
159. Abercrombie ED, Jacobs BL. Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. I. Acutely presented stressful and nonstressful stimuli. *J. Neurosci* 1987;7:2837–2843.
160. Levine ES, Litto WJ, Jacobs BL. Activity of cat locus coeruleus noradrenergic neurons during the defense reaction. *Brain Res* 1990;531:189–195.
161. Bremner JD, Krystal JH, Southwick SM, et al. Noradrenergic mechanisms in stress and anxiety. I. Preclinical studies. *Synapse* 1996;23:28–38.
162. Bremner JD, Krystal JH, Southwick SM, et al. Noradrenergic mechanisms in stress and anxiety. II.Clinical studies. *Synapse* 1996;23:39–51.
163. Cose BJ, Robbins TW. Dissociable effects of lesions to dorsal and ventral noradrenergic bundle on the acquisition, performance, and extinction of aversive conditioning. *Behav Neurosci* 1987;101:476–488.
164. Charney DS, Deutch A. A functional neuroanatomy of anxiety and fear: implications for the pathophysiology and treatment of anxiety disorders. *Crit Rev Neurobiol* 1996;10:419–446.
165. Finlay JM, Abercrombie ED. Stress induced sensitization of norepinephrine release in the medial prefrontal cortex. *Soc Neurosci Abstr* 1991;17:151.
166. Nisenbaum LK, Zigmund MJ, Sved AF, et al. Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. *J Neurosci* 1991;11:1473–1484.
167. Nisenbaum LK, Abercrombie ED. Presynaptic alterations associated with enhancement of evoked release and synthesis of NE in hippocampus of chronically cold stressed rats. *Brain Res* 1993; 608:280–287.
168. Simson PE, Weiss JM. Altered activity of the locus coeruleus in an animal model of depression.*Neuropsychoparmacology* 1988; 1:287–295.
169. Torda T, Kvetnansky R, Petrikova M. Effect of repeated immobilization stress on rat central and peripheral adrenoceptors. In: Usdin E, Kvetnansky R, Axelrod J, eds. *Stress: the role of catecholamines and other neurotransmitters.* New York: Gordon & Breach, 1984:691–701.
170. Charney DS, Deutch AY, Krystal JH, et al. Psychobiologic mechanisms of posttraumatic stress disorder. *Arch Gen Psychiatry* 1993;50:294–299.
171. Grillon C, Morgan CA. Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *Biol Psychiatry* 1998;44:990–997.
172. Charney DS, Heninger GR, Breier A. Noradrenergic function in panic anxiety: effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. *Arch Gen Psychiatry* 1984;41:751–763.
173. Charney DS, Woods SW, Goodman WK, et al. Neurobiological mechanisms of panic anxiety: biochemical and behavioral correlates of yohimbine-induced panic attacks. *Am J Psychiatry* 1987; 144:1030–1036.
174. Aston-Jones G, Shipley MT, Chouvet G, et al. Afferent regulation of locus coeruleus neurons: anatomy, physiology and pharmacology. *Prog Brain Res* 1991;88:47–75.
175. Prins A, Kaloupck DG, Keane TM. Psychophysiological evidence for autonomic arousal and startle in traumatized adult populations. In: Friedman MJ, Charney DS, Deutch AY, eds. *Neurobiological and clinical consequences of stress: from normal adaptation to PTSD* New York: Raven, 1995:291–314.
176. Bandelow B, Sengos G, Wedekind D, et al. Urinary excretion of cortisol, norepinephrine, testosterone, and melatonin in panic disorder. *Pharmacopsychiatry* 1997;30:113–117.

177. Wilkinson D, Thompson JM, Lambert GW, et al. Sympathetic activity in patients with panic disorder at rest, under laboratory mental stress, and during panic attacks. *Arch Gen Psychiatry* 1998;55:511–520.
178. Uhde T, Joffe RT, Jimerson DC, et al. Normal urinary free cortisol and plasma MHPG in panic disorder: clinical and theoretical implications. *Biol Psychiatry* 1988;23:575–585.
179. Nutt DJ. Altered alpha$_2$-adrenoceptor sensitivity in panic disorder. *Arch Gen Psychiatry* 1989;46:165–169.
180. Coplan JD, Pine D, Papp L, et al. Uncoupling of the noradrenergic-hypothalamus-pituitary adrenal axis in panic disorder patients. *Neuropsychopharmacology* 1995;13:65–73.
181. Coplan JD, Papp LA, Martinez MA, et al. Persistence of blunted human growth hormone response to clonidine in fluoxetine-treated patients with panic disorder. *Am J Psychiatry* 1995;152: 619–622.
182. Gurguis GNM, Uhde TW. Plasma 3–methoxy-4hydroxyphenylethylene glycol (MHPG) and growth hormone responses in panic disorder patients and normal controls. *Psychoneuroendocrinology* 1990;15:217–227.
183. Gurguis GN, Vitton BJ, Uhde TW. Behavioral, sympathetic and adrenocortical responses to yohimbine in panic disorder patients and normal controls. *Psychiatry Res* 1997;71:27–39.
184. Albus M, Zahn TP, Brier A. Anxiogenic properties of yohimbine: behavioral, physiological and biochemical measures. *Eur Arch Psychiatry* 1992;241:337–344.
185. Charney DS, Woods SW, Krystal JH, et al. Noradrenergic neuronal dysregulation in panic disorder: the effects of intravenous yohimbine and clonidine in panic disorder patients. *Acta Psychiatr Scand* 1992;86:273–282.
186. Yeragani VK, Berger R, Pohl R, et al. Effects of yohimbine on heart rate variability in panic disorder patients and normal controls: a study of power spectral analysis of heart rate. *J Cardiovasc Pharmacol* 1992;20:609–618.
187. Bremner JD, Innis RB, Ng CK, et al. PET measurement of central metabolic correlates of yohimbine administration in posttraumatic stress disorder. *Arch Gen Psychiatry* 1997;54: 246–256.
188. Southwick SM, Krystal JH, Morgan CA, et al. Abnormal noradrenergic function in posttraumatic stress disorder. *Arch Gen Psychiatry* 1993;50:266–274.
189. Southwick SM, Krystal JH, Bremner JD, et al. Noradrenergic and serotonergic function in posttraumatic stress disorder. *Arch Gen Psychiatry* 1997;54:749–758.
190. Lemieux AM, Coe CL. Abuse-related PTSD: evidence for chronic neuroendocrine activation in women. *Psychosom Med* 1995;57:105–115.
191. Hawk LW, Dougall AL, Ursano RJ, et al. Urinary catecholamines and cortisol in recent-onset posttraumatic stress disorder after motor vehicle accidents. *Psychosom Med* 2000;62: 423–434.
192. DeBellis MD, Baum AS, Birmaher B, et al. A.E. Bennett Research Award: developmental traumatology. I. Biological stress systems. *Biol Psychiatry* 1999;45:1259–1270.
193. McFall ME, Veith RC, Murburg MM. Basal sympathoadrenal function in posttraumatic stress disorder. *Biol Psychiatry* 1992; 31:1050–1056.
194. Blanchard EB, Kolb LC, Prins A, et al. Changes in plasma norepinephrine to combat-related stimuli among Vietnam veterans with post traumatic stress disorder. *J Nerv Ment Dis* 1991; 179:371–373.
195. Geracioti TD, Baker DG, Ekhator NN, et al. Csf norepinephrine concentrations in posttraumatic stress disorder. *Am J Psychiatry* 2001;158:1227–30.
196. Perry GD, Giller EL, Southwick SM. Altered platelet alpha$_2$ adrenergic binding sites in posttraumatic stress disorder. *Am J Psychiatry* 1987;144:1511–1512.
197. Lerer B, Ebstein RP, Shestatsky M, et al. Cyclic AMP signal transduction in posttraumatic stress disorder. *Am J Psychiatry* 1987;144:1324–1327.
198. Davidson J, Lipper S, Kilts CD, et al. Platelet MAO activity in posttraumatic stress disorder. *Am J Psychiatry* 1985;142: 1341–1343.
199. Nesse RM, Curtis GC, Thyer BA, et al. Endocrine and cardiovascular responses during phobic anxiety. *Psychosom Med* 1985; 47:320–332.
200. Stein MB, Tancer ME, Uhde TW. Heart rate and plasma norepinephrine responsivity to orthostatic challenge in anxiety disorders: comparison of patients with panic disorder and social phobia and normal control subjects. *Arch Gen Psychiatry* 1992;49: 311–317.
201. Tancer ME, Stein MB, Uhde TW. Effects of thyrotropin-releasing hormone on blood pressure and heart rate in phobic and panic patients: a pilot study. *Biol Psychiatry* 1990;27:781–783.
202. Stein MB, Huzel LL, Delaney SM. Lymphocyte α-adrenoreceptors in social phobia. *Biol Psychiatry* 1993;34:45–50.
203. Gerra G, Zaimovic A, Zambelli U, et al. Neuroendocrine responses to psychological stress in adolescents with anxiety disorder. *Neuropsychobiology* 2000;42:82–92.
204. Sapolsky RM, Plotsky PM. Hypercortisolism and its possible neural bases. *Biol Psychiatry* 1990;27:937–952.
205. Kant GJ, Leu JR, Anderson SM, et al. Effects of chronic stress on plasma corticosterone, ACTH and prolactin. *Physiol Behav* 1987;40:775–779.
206. Irwin J, Ahluwalia P, Zacharko RM, et al. Central norepinephrine and plasma corticosterone following acute and chronic stressors: influence of social isolation and handling. *Pharmacol Biochem Behav* 1986;24:1151–1154.
207. Dallman MF, Jones MT. Corticosteroid feedback control of ACTH secretion: effect of stress-induced corticosterone secretion on subsequent stress responses in the rat. *Endocrinology* 1973;92:1367–1375.
208. Stanton ME, Gutierrez YR, Levine S. Maternal deprivation potentiates pituitary-adrenal stress responses in infant rats. *Behav Neurosci* 1988;102:69–70.
209. Levine S, Wiener SG, Coe CL. Temporal and social factors influencing behavioral and hormonal responses to separation in mother and infant squirrel monkey. *Psychoneuroendocrinology* 1993;18:297–306.
210. Liu D, Diorio J, Tannenbaum B, et al. Maternal care, hippocampal glucocorticoid receptors and hypothalamic-pituitary-adrenal responses to stress. *Science* 1997;277:1654–1662.
211. Plotsky PM, Meaney MJ. Early postnatal experience alters hypothalamic corticotropin-releasing factor mRNA medial CRF context, and stress induced release in adult rats. *Mol Brain Res* 1993; 18:195–200.
212. Heit C, Woens MJ, Plotsky PM, et al. Persistent changes in corticotropin releasing factor systems due to early life stress: relationship to pathophysiology of major depression and posttraumatic stress disorder. *Psychopharmacol Bull* 1997;33: 185–192.
213. Coplan JD, Andrews MW, Rosenblum LA, et al. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci USA* 1996;93:1619–1623.
214. Meaney MJ, Airken DH, vanBerkel C, et al. Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science* 1988;239:766–768.
215. Meaney MJ, Aitken DH, Sharma S, et al. Neonatal handling alters adrenocortical negative feedback sensitivity and hippo-

campal type II glucocorticoid receptor binding in the rat. *Neuroendocrinology* 1989;50:597–604.
216. Shulkin J, Gold PW, McEwen BS. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology* 1998;23:219–243.
217. Makino S, Gold PW, Schulkin J. Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. *Brain Res* 1994;640:105–112.
218. Makino S, Gold PW, Schulkin J. Effects of corticosterone on CRH mRNA and content in the bed nucleus of the stria terminalis; comparison with the effects in the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. *Brain Res* 1994;657:141–149.
219. Makino S, Schulkin J, Smith MA, et al. Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. *Endocrinology* 1995;136:4517–4525.
220. Swanson LW, Simmons DM. Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *J Comp Neurol* 1989;285:413–435.
221. Koob GF, Heinrichs SC. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. *Brain Res* 1999;848:141–152.
222. Smith GW, Aubry JM, Dellu F, et al. Corticotropin releasing factor receptor 1––deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* 1998;20:1093–1102.
223. Timpl P, Spanagel R, Sillaber I, et al. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor. *Nat Genet* 1998;19:162–166.
224. Bale TL, Contarino A, Smith GW, et al. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat Genet* 2000;24:410–414.
225. Kishimoto T, Radulovic J, Radulovic M, et al. Delection of *Crhr2* reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. *Nat Genet* 2000;24;415–419.
226. Habib KE, Weld KP, Rice KC, et al. Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. *Proc Natl Acad Sci USA* 2000;97:6079–6084.
227. Mason JW, Giller EL, Kosten TR, et al. Urinary free-cortisol levels in post-traumatic stress disorder patients. *J Nerv Ment Dis* 1986;174:145–149.
228. Yehuda R, Boisoneau D, Lowy MT, et al. Dose-response changes in plasma cortisol and lymphocyte glucocorticoid receptors following dexamethasone administration in combat veterans with and without posttraumatic stress disorder. *Arch Gen Psychiatry* 1995;52:583–593.
229. Yehuda R, Bierer LM, Schmeidler J, et al. Low cortisol and risk for PTSD in adult offspring of holocaust survivors. *Am J Psychiatry* 2000;157:1252–1259.
230. Pitman RK, Orr SP. Twenty-four-hour urinary cortisol and catecholamine excretion in combat-related post traumatic stress disorder. *Biol Psychiatry*1990;27:245–247.
231. Yehuda R, Lowy MT, Southwick SM, et al. Lymphocyte glucocorticoid receptor number in posttraumatic stress disorder. *Am J Psychiatry* 1991;148:499–504.
232. Liberzon I, Abelson JL, Flagel SB, et al. Neuroendocrine and psychophysiological responses in PTSD: a symptom provocation study. *Neuropsychopharmacology* 1999;21:40–50.
233. Maes M, Lin A, Bonaccorso S, et al. Increased 24-hour urinary cortisol excretion in patients with post-traumatic stress disorder and patients with major depression, but not in patients with fibromyalgia. *Acta Psychiatr Scand* 1998;90:328–325.
234. Kosten TR, Wahby V, Giller EL, et al. The dexamethasone suppression test and thyrotropin-releasing hormone stimulation test in posttraumatic stress disorder. *Biol Psychiatry* 1990;28:657–664.
235. Yehuda R, Southwick SM, Krystal JH, et al. Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. *Am J Psychiatry* 1993;150:83–86.
236. Stein MB, Yehuda R, Koverola C, et al. Enhanced dexamethasone suppression of plasma cortisol in adult women traumatized by childhood sexual abuse. *Biol Psychiatry* 1997;42:680–686.
237. Yehuda R, Teicher MH, Trestman RL, et al. Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. *Biol Psychiatry* 1996;40:79–88.
238. Delahanty DL, Raimonde AJ, Spoonster E. Initial posttraumatic urinary cortisol levels predict subsequent PTSD symptoms in motor vehicle accident victims. *Biol Psychiatry* 2000;48:940–947.
239. Bremner JD, Licinio J, Darnell A, et al. Elevated CSF corticotropin-releasing factor concentrations in posttraumatric stress disorder. *Am J Psychiatry* 1997;154:624–629.
240. Baker D, West SA, Orth DN, et al. Cerebrospinal fluid corticotropin-releasing hormone and adrenal cortical activity in post traumatic stress disorder. *Am J Psychiatry* 1999;156:585–588.
241. Smith MA, Davidson J, Ritchie JC, et al. The corticotropin-releasing hormone test in patients with PTSD. *Biol Psychiatry* 1989;26:349–355.
242. Heim C, Newport DJ, Heit S, et al. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA* 2000;284:592–597.
243. Sapolsky RM, Uno H, Rebert CS, Finch CE. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *J. Neurosci* 1990;10:2897–2902.
244. Goldstein S, Halbreich U, Asnis G, et al. The hypothalamic pituitary-adrenal system in panic disorder. *Am J Psychiatry* 1987;144:1320–1323.
245. Holsboer F, vonBardeleben U, Buller R, et al. Stimulation response to corticotropin-releasing hormone (CRH) in patients with depression, alcoholism an panic disorder. *Horm Metab Res* 1987;16[Suppl]:80–88.
246. Kathol RG, Anton R, Noyes R, et al. Relationship of urinary free cortisol levels in patients with panic disorder to symptoms of depression and agoraphobia. *Psychiatry Res* 1988;24:211–221.
247. Abelson JL, Curtis GC. Hypothalamic-pituitary-adrenal axis activity in panic disorder. *Arch Gen Psychiatry* 1996;53:323–332.
248. Coryell W, Noyes R. HPA axis disturbance and treatment outcome in panic disorder. *Biol Psychiatry* 1988;24:762–755.
249. Roy-Byrne PP, Uhde TW, Post RM, et al. The corticotropin-releasing hormone stimulation test in patients with panic disorder. *Am J Psychiatry* 1986;143:896–899.
250. Rapaport MH, Risch SC, Golshan S, et al. Neuroendocrine effects of ovine corticotropin-releasing hormone in panic disorder patients. *Biol Psychiatry* 1989;26:344–348.
251. Jolkkonen J, Lepola V, Bissette G, et al. CSF corticotropin-releasing factor is not affected in panic disorder. *Biol Psychiatry* 1993;33:136–138.
252. Curtis AL, Lechner SM, Pavcovich LA, et al. Activation of the locus coeculeus noradrenergic system by intracoerulear microinfusion of corticotropin releasing factor: effects on discharge, rate, cortical norepinephrine levels, and cortical electroencephalographic activity. *J Pharmacol Exp Ther* 1997;281:163–172.
253. Smagin GN, Swiergiel AH, Dunn AJ. Corticotropin releasing factor administered in the locus coeruleus, but not the para-

brachial nucleus, stimulates norepinephrine release in the prefrontal cortex. *Brain Res Bull* 1995;36:71–76.
254. Pacak K, Palkovits M, Kopin IJ, et al. Stress induced NE release in the hypothalamic PVN and pituitary-adrenal and sympathoadrenal activity: *in vivo* microdialysis studies. *Front Neuroendocrinol* 1995;16:89–150.
255. Roozendaal B. Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrinology* 2000;25:213–238.
256. Roozendaal B, Carmi O, McGaugh JL. Adrencortical suppression blocks the memory-enhancing effects of amphetamine and epinephrine. *Proc Natl Acad Sci USA* 1996;93:1429–1433.
257. Cahill L, Prins B, Weber M, et al. Beta-adrenergic activation and memory for emotional events. *Nature* 1994;371:702–703.
258. Choi DW, Farb DH, Fischbach GD. Chlordiazepoxide selectively potentiates GABA conductance of spinal cord and sensory neurons in cell culture. *J Neurophysiol* 1981;45:621–631.
259. Study RE, Barker JL. Cellular mechanisms of benzodiazepine action. *JAMA* 1982;247:2147–2151.
260. Graeff FG, Silveira MC, Nogueira RL, et al. Role of the amygdala and periaqueductal gray in anxiety and panic. *Behav Brain Res* 1993;58:123–131.
261. Dorow R, Horowski R, Paschelke G, et al. Severe anxiety induced by FG7142, a beta-carboline ligand for benzodiazepine receptors. *Lancet* 1983;2:98–99.
262. Braestrup C, Schmiechen R, Neef G, et al. Interaction of convulsive ligands with benzodiazepine receptors. *Science* 1982;216:1241–1243.
263. Ninan PT, Insel TM, Cohen RM, et al. Benzodiazepine receptor mediated experimental "anxiety" in primates. *Science* 1982;218:1332–1334.
264. Low K, Crestani F, Keist R, et al. Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 2000;290:131–134.
265. McKernan RM, Rosahl TW, Reynolds DS, et al. Sedative but not anxiolytic properties of benzodiazepines are mediated by the $GABA_A$ receptor $alpha_1$ subtype. *Nat Neurosci* 2000;3:587–592.
266. Rudolph U, et al. Benzodiazepine actions mediated by specific γ-aminobutyric acid a receptor subtypes. *Nature* 1999;410:796–800.
267. Paslawski T, Treit D, Baker GB, et al. The antidepressant drug phenelzine produces antianxiety effects in the plus-maze and increases in rat brain GABA. *Psychopharmacology (Berl)* 1996;127:19–24.
268. Weizman A, Weisman R, Kook KA, et al. Adrenalectomy prevents the stress-induced decrease in *in vivo* [^{3}H]Ro 15–1788 binding to $GABA_A$ benzodiazepine receptors in the mouse. *Brain Res* 1990;519:347–350.
269. Drugan RC, Morrow AL, Weizman R, et al. Stress-induced behavioral depression in the rat is associated with a decrease in GABA receptor-mediated chloride ion flux and brain benzodiazepine receptor occupancy. *Brain Res* 1989;487:45–51.
270. Weizman R, Weizman A, Kook KA, et al. Repeated swim stress alters brain benzodiazepine receptors measured *in vivo. J Pharmacol Exp Ther* 1989;249:701–707.
271. Orchinik M, Weiland NG, McEwen BS. Chronic exposure to stress levels of corticosterone alters GABAA receptor subunit mRNA levels in rat hippocampus. *Brain Res Mol Brain Res* 1995;34:29–37.
272. Robertson HA, Martin IL, Candy JM. Differences in benzodiazepine receptor binding in Maudsley-reactive and non-reactive rats. *Eur J Pharmacol* 1978;50:455–457.
273. Caldji C, Francis D, Sharma S, et al. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. *Neuropsychopharmacology* 2000;22:219–229.
274. Nutt DJ, Glue P, Lawson C, et al. Flumazenil provocation of panic attacks: evidence for altered benzodiazepine receptor sensitivity in panic disorder. *Arch Gen Psychiatry* 1990;47:917–925.
275. Roy-Byrne P, Wingerson DK, Radant A, et al. Reduced benzodiazepine sensitivity in patients with panic disorder: comparison with patients with obsessive compulsive disorder and normal subjects. *Am J Psychol* 1996;153:1444–1449.
276. Woods SW, Charney DS, Silver JM, et al. Behavioral, biochemical, and cardiovascular responses to the benzodiazepine receptor antagonist flumazenil in panic disorder. *Psychiatry Res* 1991;36:115–124.
277. Roy-Byrne PP, Lewis N, Villacres E, et al. Preliminary evidence of benzodiazepine subsensitivity in panic disorder. *Biol Psychiatry* 1989;26:744–748.
278. Schlegel S, Teinert H, Bockisch A, et al. Decreased benzodiazepine receptor binding in panic disorder measured by iomazenil SPECT: a preliminary report. *Eur Arch Psychiatry Clin Neurosci* 1994;244:49–51.
279. Kascka W, Feistel H, Ebert D. Reduced benzodiazepine receptor binding in panic disorders measured by iomazenil SPECT. *J Psychiatry Res* 1995;29:427–434.
280. Kuikka JT, Pitkanen A, Lepola U, et al. Abnormal regional benzodiazepine receptor uptake in the prefrontal cortex in patients with panic disorder. *Nucl Med Commun* 1995;16:273–280.
281. Bremner JD, Innis RB, White T, et al. SPECT [I-123] Iomazenil measurement of the benzodiazepine receptor in panic disorder. *Biol Psychiatry* 2000;47:96–106.
281a. Bremner JD, Innis RB, Southwick SM, et al. Decreased benzodiazepine receptor binding in prefrontal cortex in combat-related posttraumatic stress disorder. *Am J Psychiatry* 2000;157:1120–1126.
282. Malizia AL, Cunningham VJ, Bell CJ, et al. Decreased brain $GABA_A$ –benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. *Arch Gen Psychiatry* 1998;55:715–720.
283. Abadie P, Boulenger JP, Benail K, et al. Relationships between trait and state anxiety and the central benzodiazepine receptor: a PET study. *Eur J Neurosci* 1999;11:1470–1478.
284. Deutch AY, Young CD. A model of the stress-induced activation of prefrontal cortical dopamine systems: coping and the development of post-traumatic stress disorder. In: Friedman MJ, Charney DS, Deutch AY, eds. *Neurobiological and clinical consequences of stress.* Philadelphia: Lippincott–Raven, 1995:163–175.
285. Inoue T, Tsuchiya K, Koyama T. Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. *Pharmacol Biochem Behav* 1994;49:911–920.
286. Roy-Byrne PP, Uhde TW, Sack DA, et al. Plasma HVA and anxiety in patients with panic disorder. *Biol Psychiatry* 1986;21:847–849.
287. Pichot W, Annsseau M, Moreno AG, et al. Dopaminergic function in panic disorder: comparison with major and minor depression. *Biol Psychiatry* 1992;32:1004–1011.
288. Eriksson E, Westberg P, Alling C, et al. Cerebrospinal fluid levels of monoamine metabolites in panic disorder. *Psychiatry Res* 1991;36:243–251.
289. Hamilton SP, Haghighi F, Heiman GA, et al. Investigation of dopamine receptor (DRD4) and dopamine transporter (DAT) polymorphisms for genetic linkage or association to panic disorder. *Am J Med Genet* 2000;96:324–330.
290. Tiihonen J, Kuikka J, Bergstrom K, et al. Dopamine reuptake site densities in patients with social phobia. *Am J Psychiatry* 1997;154:239–242.

291. Schneier FR, Liebowitz MR, Abi-Dargham A, et al. Low dopamine D(2) receptor binding potential in social phobia. *Am J Psychiatry* 2000;157:457–459.
292. Petty F, Kramer G, Wilson L. Prevention of learned helplessness: *in vivo* correlation with serotonin. *Pharmacol Biochem Behav* 1992;43:361–367.
293. Petty F, Kramer GL, Wu J. Serotonergic modulation of learned helplessness. *Ann NY Acad Sci* 1997;821:538–541.
294. Graeff F. Role of 5-HT in defensive behavior and anxiety. *Rev Neurosci* 1993;4:181–211.
295. Ramboz S, Oosting R, Amara DA, et al. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci USA* 1998;95:14476–14481.
296. López JF, Chalmers DT, Little KY, et al. Regulation of serotonin$_{1A}$, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. *Biol Psychiatry*1998;43:547–573.
297. Meijer OC, Van Oosten RV, de Kloet ER. Elevated basal trough levels of corticosterone suppress hippocampal 5-HT1A receptor expression in adrenally intact rats: implication for the pathogenesis of depression. *Neuroscience* 1997;80:419–426.
298. Mendelson SD, McEwen BS. Autoradiographic analyses of the effects of restraint-induced stress on 5-HT1A, 5-HT1C and 5-HT2 receptors in the dorsal hippocampus of male and female rats. *Neuroendocrinology* 1991;54:454–461.
299. Zhono P, Ciaranello R. Transcriptional regulation of hippocampal 5-HT1A receptors by glucocorticoid hormones. *Neuroscience* 1994;20:1161.
300. Watanabe Y, Sakai RR, McEwen BS, et al. Stress and antidepressant effects on hippocampal and cortical 5-HT$_{1a}$ and 5-HT$_2$ receptors and transport sites for serotonin. *Brain Res* 1993;615: 87–94.
301. Norman TR, Judd FK, Gregory M, et al. Platelet serotonin uptake in panic disorder. *J Affect Disord* 1986;11:69–72.
302. Balon R, Poh R, Yeragani V, et al. Platelet serotonin levels in panic disorder. *Acta Psychiatr Scand* 1987;75:315.
303. Pecknold JC, Suranyi-Cadotte B, Chang H, et al. Serotonin uptake in panic disorder and agoraphobia. *Neuropsychopharmacology* 1988;1:173–176.
304. Uhde TW, Berrettini WH, Roy-Byrne PP, et al. Platelet 3H-imipramine binding in patients with panic disorder. *Biol Psychiatry* 1987;22:52–58.
305. Innis RB, Charney DS, Heninger GR. Differential 3H imipramine platelet binding in patients with panic disorder and depression. *Psychiatry Res* 1987;21:33–41.
306. Schneider LS, Munjack D, Severson JA, et al. Platelet H3 imipramine binding in generalized anxiety disorder, panic disorder, and agoraphobia with panic attacks. *Biol Psychiatry* 1987;21: 3–41.
307. Charney DS, Heninger GR. Serotonin function in panic disorders: the effects of intravenous tryptophan in healthy subjects and panic disorder patients before and after alprazolam treatment. *Arch Gen Psychiatry* 1986;43:1059–1065.
308. DenBoer JA, Westenberg HGM. Behavioral, neuroendocrine, and biochemical effects of 5-hydroxytryptophan administration in panic disorder. *Psychiatry Res* 1990;31:367–378.
309. Goddard AW, Sholomskas DE, Augeri FM, et al. Effects of tryptophan depletion in panic disorders. *Biol Psychiatry* 1994; 36:775–777.
310. Targum SD, Marshall LE. Fenfluramine provocation of anxiety in patients with panic disorder. *Psychiatry Res* 1989;28: 295–306.
311. Lesch KP, Wiesmann M, Hoh A, et al. 5-HT1A receptor-effector system responsivity in panic disorder. *Psychopharmacology (Berl)* 1992;106:111–117.
312. Kahn RS, Asnis GM, Wetzler S, et al. Serotonin and anxiety revisited. *Biol Psychiatry* 1988;23:189–208.
313. Charney DS, Woods SW, Goodman WK, et al. Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology (Berl)* 1987;92:14–24.
314. Arora RC, Fichtner CG, O'Connor F, et al. Paroxetine binding in the blood platelets of post-traumatic stress disorder patients. *Life Sci* 1993;53:919–928.
315. Hano J, Vasar E, Bradwejn J. Cholecystokinin in animal and human research on anxiety. *Trends Pharmacol Sci* 1993;14: 244–249.
316. Bradwejn J, Koszycki D. Imipramine antagonism of the panicogenic effects of CCK-4 in panic disorder patients. *Am J Psychiatry* 1994;151:261–263.
317. Bradwejn J, Koszycki D, Couetoux du Tetre A, et al. The panicogenic effects of CCK-4 are antagonized by L-365–260, a CCK receptor antagonist, in patients with panic disorder. *Arch Gen Psychiatry* 1994;51:486–493.
318. Kellner M, Wiedemann K, Yassouridis A, et al. Behavioral and endocrine response to cholecystokinin tetrapeptide in patients with posttraumatic stress disorder. *Biol Psychiatry* 2000;47: 107–111.
319. Strohle A, Holsboer F, Rupprecht R. Increased ACTH concentrations associated with cholecystokinin tetrapeptide–induced panic attacks in patients with panic disorder. *Neuropsychopharmacology* 2000;22:251–256.
320. Lydiard RB, Ballenger JC, Laraia MT, et al. CSF cholecystokinan concentrations in patients with panic disorder and normal comparison subjects. *Am J Psychiatry* 1992;149:691–693.
321. Kennedy JL, Bradwejn J, Koszycki D, et al. Investigation of cholecystokinin system genes in panic disorder. *Mol Psychiatry* 1999;4:284–285.
322. Pande AC, Greiner M, Adams JB, et al. Placebo-controlled trial of the CCK-B antagonist, CI-988 in panic disorder. *Biol Psychiatry* 1999;46:860–862.
323. Madden J, Akil H, Patrick RI, et al. Stress induced parallel changes in central opioid levels and pain responsiveness in the rat. *Nature* 1977;265:358–360.
324. Stuckey J, Marra S, Minor T, et al. Changes in mu opiate receptors following inescapable shock. *Brain Res* 1989;476: 167–169.
325. Fanselow MS. Conditioned fear-induced opiate analgesia: a competing motivational state theory of stress analgesia. *Ann NY Acad Sci* 1986;467:40–54.
326. Maier SF. Stressor controllability and stress induced analgesia. *Ann NY Acad Sci* 1986;467:55–72.
327. Pitman RK, van der Kolk BA, Orr SP, et al. Naloxone-reversible analgesic response to combat-related stimuli in posttraumatic stress disorder. *Arch Gen Psychiatry* 1990;47:541–544.
328. Baker DG, West SA, Orrth DN, et al. Cerebrospinal fluid and plasma beta endorphin in combat veterans with post traumatic stress disorder. *Psychoneuroendocrinology* 1997;22:517–529.
329. Hoffman I, Watsgon PD, Wilson G, et al. Low plasma endorphin in posttraumatic stress disorder. *Aust NZ J Psychiatry* 1989; 23:268–273.
330. Wolf M. Plasma methionine enkephalin in PTSD. *Biol Psychiatry* 1991;29:295 .
331. Bremner JD, Southwick SM, Darnell A, et al. Chronic PTSD in Vietnam combat veterans: course of illness and substance abuse. *Am J Psychiatry* 1996;153:369–375.
332. Heilig M, Koob GF, Ekman R, et al. Corticotropin-releasing factor and neuropeptide Y: role in emotional integration. *Trends Pharmacol Sci* 1994;17:80–85.
333. Thorsell A, Michalkiewicz M, Dumont Y, et al. Behavioral insensitivity to restraint stress, absent fear suppression of behavior

and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. *Proc Natl Acad Sci USA* 2000;97:12852–12857 .
334. Morgan CA III, Wang S, Southwick SM, et al. Plasma neuropeptide-Y concentrations in humans exposed to military survival training. *Biol Psychiatry* 2000;47:902–909.
335. Boulenger J, Jerabek I, Jolicoeur FB. Elevated plasma levels of neuropeptide Y in patients with panic disorder. *Am J Psychiatry* 1996;153:114–116.
336. Rasmusson AM, Hauger RL, Morgan CA, et al. Low baseline and yohimbine-stimulated plasma neuropeptide Y (NPY) levels in combat related PTSD. *Biol Psychiatry* 2000;47:526–539.
337. Mason J, Southwick S, Yehuda R, et al. Elevation of serum free triodothyronine, total triiodothronine, Thyroxine-binding globulin, and total thyroxine levels in combat-related posttraumatic stress disorder. *Arch Gen Psychiatry* 1994;51: 629–641.
338. Mason JW, Mougey FH, Brady JV, et al. Thyroid (plasma butanol-extractable iodine) responses to 72–hr avoidance sessions in the monkey. *Psychosom Med* 1968;30:682–696.
339. Gorman JM, Goetz RR, Dillon E, et al. Sodium d-lactate infusion in panic disorder patients. *Neuropsychopharmacology* 1990; 3:181–190.
340. Klein DF. False suffocation alarms, spontaneous panics, and related conditions. *Arch Gen Psychiatry* 1993;50:306–317.
341. Papp LA, Klein DF, Gorman JM. Carbon dioxide hypersensitivity, hyperventilation, and panic disorder. *Am J Psychiatry* 1993; 150:1149–1155.
342. Pine DS, Coplan JD, Lazlo AP, et al. Ventilatory physiology of children and adolescents with anxiety disorders. *Arch Gen Psychiatry* 1998;55:123–129.
343. Gorman JM, Fyer MR, Goetz R, et al. Ventilatory physiology of patients with panic disorder. *Arch Gen Psychiatry* 1988;45: 31–39.
344. Gorman JM, Battista D, Goetz R, et al. A comparison of sodium bicarbonate and sodium lactate infusion in the induction of panic attacks. *Arch Gen Psychiatry* 1989;46:145–150.
345. Van Den Hout MA, Griez E. Panic symptoms after inhalation of carbon dioxide. *Br J Psychiatry* 1984;144:503–507.
346. Greiz E, Lousberg H, Van Den Hout MA, et al. Carbon dioxide vulnerability in panic disorder. *Psychiatry Res* 1987;20:87–95.
347. Pitts LN, McClure JN. Lactate metabolism in anxiety neuroses. *N Engl J Med* 1967;277:1329–1336.
348. Schmidt NB. Lerew DR. Jackson RJ. Prospective evaluation of anxiety sensitivity in the pathogenesis of panic: replication and extension. *J Abnormal Psychol* 1999;108:532–537.
349. Pine DS, Klein RG, Coplan JD, et al. differential carbon dioxide sensitivity in childhood anxiety disorders and nonill comparison group. *Arch Gen Psychiatry* 2000;57:960–967.