Editorial

Dear Colleagues,

In spite of its apparent simplicity, the word "stress" can convey a wide variety of meanings and connotations in modern psychiatry.

- As a symptom, stress expresses the reaction to external circumstances (stressful events), or to internal factors (for instance when the individual's psychological and somatic defense mechanisms are overwhelmed, such as in angina pectoris).
- Stress can also be understood as a disorder. Post-traumatic stress disorder was covered in a previous issue of *Dialogues in Clinical Neuroscience* (DCNS Vol 2, No 1).
- Stress can trigger complex psychiatric disorders (for instance, recurring episodes of a mood disorder, or acute delusional episodes, etc).
- Through an effect on neuroplasticity, the accumulation of stress can predispose to depressive disorders. The elucidation of this pathogenetic mechanism has led to new hypotheses and treatment options in depression.

In addition to defining stress and its clinical consequences, it is also important to understand the biological mechanisms that are responsible for or associated with stress.

Research into the etiology of stress involves genetics, and the study of structures such as the prefrontal cortex, the amygdala, and the hypothalamo-pituitary-adrenal axis, which have been shown to play key roles in the genesis of stress.

The discovery of the interaction between the accumulation of stress and disturbances of neuroplasticity was one of the key scientific advances in recent years, and it paved the way for the development of new hypotheses and treatment methods in depression.

We felt that it was important to dedicate an issue of DCNS to the question of "stress." This issue was coordinated by David Rubinow (University of North Carolina, USA). We are grateful to him for bringing together such an outstanding panel of experts, and would like to thank all the authors for their brilliant contributions.

Sincerely yours,

Jean-Paul Macher, MD Marc-Antoine Crocq, MD

Dialogues in Clinical Neuroscience is a quarterly publication that aims to serve as an interface between clinical neuropsychiatry and the neurosciences by providing state-of-the-art information and original insights into relevant clinical, biological, and therapeutic aspects. Each issue addresses a specific topic, and also publishes free contributions in the field of neuroscience as well as other non–topic-related material. All contributions are reviewed by members of the Editorial Board and submitted to expert consultants for peer review.

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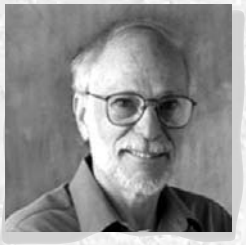

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In this issue...

It is empirical truth for most that stress is not good for us, and, further, we now recognize that stress comes in different flavors and cannot be considered a unitary phenomenon. Nothing new there. What is new, however, is the recognition that the stress response is not simply an amplifier of behavioral and affective symptoms but is instead critical to their development and expression. What are the isomorphs between stress maladaptation and psychophathology, how does stress change how the brain learns, and what are the molecules and circuits of the stress response? In this issue of *Dialogues in Clinical Neuroscience*, these questions are answered by authors who both decompose the stress response, identifying its chemical and neural mediators, and demonstrate the centrality of stress adaptation to compromised as well as resilient psychological functioning.

In the ***State of the art*** opening article, Bruce McEwen describes the brain as not only the director of the stress response, but also its target. The cumulative demands of everyday life combine with the efficiency of one's management of stressors to generate what Dr McEwen calls "allostatic overload," the consequences of which include both chemical and structural remodeling of the brain. Current knowledge of this process is sufficient to argue for the implementation of societal policies to reduce allostatic overload.

In the first ***Basic research*** article, Sean Smith and Wylie Vale deconstruct the stress response by first describing the pharmacology of its hormonal components, particularly the corticotropin-releasing factor (CRF) family of peptides, largely discovered through the work of Dr Vale and colleagues. The authors then clearly and comprehensively describe the neuroendocrine and neuronal regulation of the hypothalamic-pitutary-adrenal (HPA) axis. With this background, the diversity of the stress response becomes clear, as different stressors activate different neurocircuitry, with different behavioral and physiological consequences.

In the second ***Basic research*** article, Steven Maier and colleagues, in a tour de force, describe the central role of the medial prefrontal cortex in the perception of control and in the subsequent inhibition of the adverse consequences of stress. Further, they demonstrate that the activation of the medial prefrontal cortex, rather than the controllability of the stressor, is what determines both the acute response to stressor and the response to subsequent stressors. These "behavioral immunization" studies provide a unique framework for understanding the development and expression of resilience or psychopathology in the face of repeated exposure to traumatic stressors.

In the third ***Basic research*** article, Jay Schulkin redirects our attention from the prefrontal cortex to the amygdala. Known for years to be central to the fear response, the amygdala has increasingly been implicated in a variety of psychiatric disorders, including depression and post-traumatic stress disorder (PTSD). Dr Schulkin first describes the anatomical complexity of the amygdala and the implications of the differential "wiring." He then suggests how stress-induced glucocorticoid secretion may, in the proper genetic context, increase corticotropin-releasing hormone (CRH) expression in the amygdala and, by so doing, result in exaggerated subsequent amygdala responses to stress, with concomitant alterations in both the perception of and response to life events.

Some of the ambiguities in stress research are explained in the fourth ***Basic research*** article by Vladimir and Alexandre Patchev, who systematically review existing animal models for the stress response. These authors first describe the multitude of outcome measures that have been employed and then the variety of experimental approaches to stress induction .While no perfect model exists, appreciation of the major sources of variance permits the integration of what otherwise might be viewed as disparate findings.

In the first ***Clinical research*** article, Marcus Ising and Florian Holsboer review the heritability and genetic association studies of the stress response before arguing that the study of stress-related disorders—hypertension, coronary artery disease, and affective illness (bipolar and unipolar)—reveals genes relevant to the stress response that would not otherwise be identified. The authors describe how burgeoning technical capabilities must be dovetailed with clinical investigations that assess gene-gene and gene-environment interactions if we are to understand the role of genetic context in the etiopathogenesis of the stress response.

In the second ***Clinical research*** article, J. Douglas Bremner uses brain imaging data to trace the neurocircuitry of the response to (and consequences of) traumatic stress in humans. The brain regions so identified are then exam-

In this issue...

ined in PTSD as mediators of learning, targets of stress-related hormones, and possible sites of action of pharmacotherapies. Of particular note are the neural consequences of early abuse as well as the different expression of these consequences (eg, deficient activation of the medial prefrontal cortex) in the presence and absence of PTSD.

Neurosteroids are neuroactive metabolites of the stress-activated hormone deoxycorticosterone and progesterone. These hormones are powerful modulators of γ-aminobutyric acid (GABA)-mediated chloride ion channel activity and, hence, behavior. In the third ***Clinical research*** article, Leslie Morrow and colleagues present a strong argument for the role of neuroactive steroids in the behavioral response to alcohol and in the susceptibility to alcoholism. Data presented in this article suggest the possible therapeutic use of neurosteroids in alcohol withdrawal or relapse prevention.

Finally, in the ***Poster***, Rebecca Shansky and Amy Arnsten present an elegant example of modulation of the stress response, namely estradiol-dependent increased sensitivity to the detrimental effects of stress on the prefrontal cortex (PFC). These findings may, in part, explain both the increased sensitivity to stress-induced PFC dysfunction in female rats and the increased susceptibility in women to stress-related disorders (eg, depression).

David Rubinow, MD

Protective and damaging effects of stress mediators: central role of the brain

Bruce S. McEwen, PhD

"Stress" is a commonly used word that generally refers to experiences that cause feelings of anxiety and frustration because they push us beyond our ability to successfully cope. "There is so much to do and so little time!" is a common expression. Besides time pressures and daily hassles at work and home, there are stressors related to economic insecurity, poor health, and interpersonal conflict. More rarely, there are situations that are life-threatening—accidents, natural disasters, violence—and these evoke the classical "fight or flight"

The mind involves the whole body, and two-way communication between the brain and the cardiovascular, immune, and other systems via neural and endocrine mechanisms. Stress is a condition of the mind-body interaction, and a factor in the expression of disease that differs among individuals. It is not just the dramatic stressful events that exact their toll, but rather the many events of daily life that elevate and sustain activities of physiological systems and cause sleep deprivation, overeating, and other health-damaging behaviors, producing the feeling of being "stressed out." Over time, this results in wear and tear on the body, which is called "allostatic load," and it reflects not only the impact of life experiences but also of genetic load, individual lifestyle habits reflecting items such as diet, exercise, and substance abuse, and developmental experiences that set life-long patterns of behavior and physiological reactivity. Hormones associated with stress and allostatic load protect the body in the short run and promote adaptation by the process known as allostasis, but in the long run allostatic load causes changes in the body that can lead to disease. The brain is the key organ of stress, allostasis, and allostatic load, because it determines what is threatening and therefore stressful, and also determines the physiological and behavioral responses. Brain regions such as the hippocampus, amygdala, and prefrontal cortex respond to acute and chronic stress by undergoing structural remodeling, which alters behavioral and physiological responses. Translational studies in humans with structural and functional imaging reveal smaller hippocampal volume in stress-related conditions, such as mild cognitive impairment in aging and prolonged major depressive illness, as well as in individuals with low self-esteem. Alterations in amygdala and prefrontal cortex are also reported. Besides pharmaceuticals, approaches to alleviate chronic stress and reduce allostatic load and the incidence of diseases of modern life include lifestyle change, and policies of government and business that would improve the ability of individuals to reduce their own chronic stress burden.

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Selected abbreviations and acronyms

ACTH	*acetylcholine*
BDNF	*brain-derived neurotrophic factor*
CRS	*chronic restraint stress*
CRF	*corticotropin-releasing factor*
CRH	*corticotropin-releasing hormone*
NCAM	*neural cell adhesion molecule*

response. In contrast to daily hassles, these stressors are acute, and yet they also usually lead to chronic stress in the aftermath of the tragic event.

The most common stressors are therefore ones that operate chronically, often at a low level, and that cause us to behave in certain ways. For example, being "stressed out" may cause us to be anxious and or depressed, to lose sleep at night, to eat comfort foods and take in more calories than our bodies need, and to smoke or drink alcohol excessively. Being stressed out may also cause us to neglect to see friends, or to take time off or engage in regular physical activity as we, for example, sit at a computer and try to get out from under the burden of too much to do. Often we are tempted to take medications—anxiolytics, sleep-promoting agents—to help us cope, and, with time, our bodies may increase in weight…

The brain is the organ that decides what is stressful and determines the behavioral and physiological responses, whether health-promoting or health-damaging. And the brain is a biological organ that changes under acute and chronic stress, and directs many systems of the body—metabolic, cardiovascular, immune—that are involved in the short- and long-term consequences of being stressed out. What does chronic stress do to the body and brain? This review summarizes some of the current information, placing emphasis on how the stress hormones can play both protective and damaging roles in brain and body, depending on how tightly their release is regulated, and it discusses some of the approaches for dealing with stress in our complex world.

Definition of stress, allostasis, and allostatic load

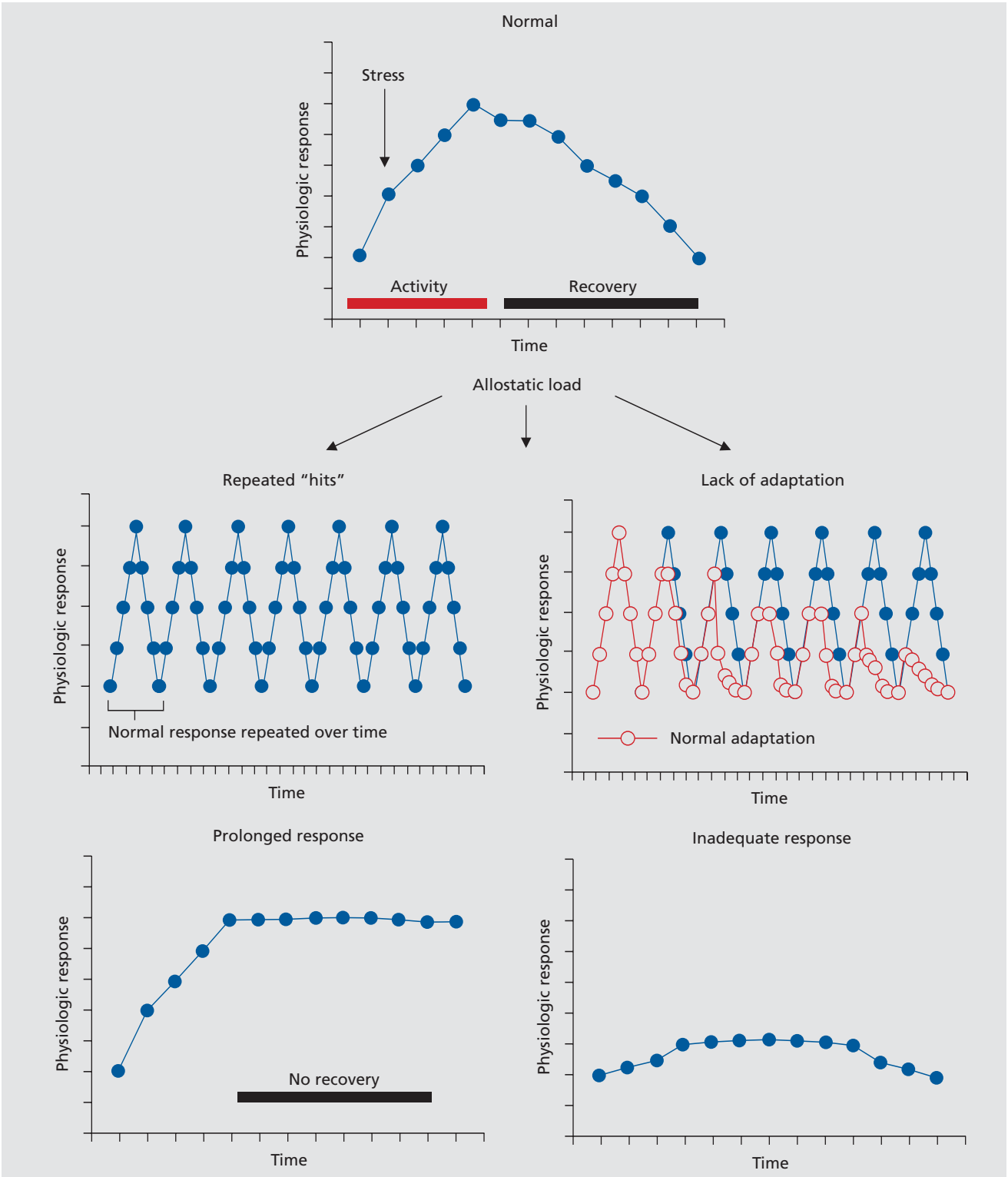
"Stress" is an ambiguous term, and has connotations that make it less useful in understanding how the body handles the events that are stressful. Insight into these processes can lead to a better understanding of how best to intervene, a topic that will be discussed at the end of this article. There are two sides to this story[1]: on the one hand, the body responds to almost any event or challenge by releasing chemical mediators—eg, catecholamines that increase heart rate and blood pressure—that help us cope with the situation; on the other hand, chronic elevation of these same mediators—eg, chronically increased heart rate and blood pressure—produce chronic wear and tear on the cardiovascular system that can result, over time, in disorders such as strokes and heart attacks. For this reason, the term "allostasis" was introduced by Sterling and Eyer[2] to refer to the active process by which the body responds to daily events and maintains homeostasis (allostasis literally means "achieving stability through change"). Because chronically increased allostasis can lead to disease, we introduced the term "allostatic load or overload" to refer to the wear and tear that results from either too much stress or from inefficient management of allostasis, eg, not turning off the response when it is no longer needed.[1,3,4] Other forms of allostatic load are summarized in *Figure 1*, and involve not turning on an adequate response in the first place, or not habituating to the recurrence of the same stressor, and thus dampening the allostatic response.

Protection and damage as the two sides of the response to stressors

Thus, protection and damage are the two contrasting sides of the physiology involved in defending the body against the challenges of daily life, whether or not we call them "stressors." Besides adrenaline and noradrenaline, there are many mediators that participate in allostasis, and they are linked together in a network of regulation that is nonlinear *(Figure 2)*, meaning that each mediator has the ability to regulate the activity of the other mediators, sometimes in a biphasic manner.

Glucocorticoids produced by the adrenal cortex in

Figure 1. Four types of allostatic load. The top panel illustrates the normal allostatic response, in which a response is initiated by a stressor, sustained for an appropriate interval, and then turned off. The remaining panels illustrate four conditions that lead to allostatic load: **top left**—repeated "hits" from multiple stressors; **top right**—lack of adaptation; **bottom left**—prolonged response due to delayed shut down; and **bottom right**—inadequate response that leads to compensatory hyperactivity of other mediators (eg, inadequate secretion of glucocorticoid, resulting in increased levels of cytokines that are normally counter-regulated by glucocorticoids). Reproduced from reference 1: McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med*. 1998; 338:171-179. Copyright © Massachusetts Medical Society 1998.


Normal
Stress
Physiologic response
Activity
Recovery
Time
Allostatic load
Repeated "hits"
Physiologic response
Normal response repeated over time
Time
Lack of adaptation
Physiologic response
Normal adaptation
Time
Prolonged response
Physiologic response
No recovery
Time
Inadequate response
Physiologic response
Time

response to acetylcholine (ACTH) from the pituitary gland is the other major "stress hormone." Pro- and anti-inflammatory cytokines are produced by many cells in the body; they regulate each other and are, in turn, regulated by glucocorticoids and catecholamines. Whereas catecholamines can increase proinflammatory cytokine production, glucocorticoids are known to inhibit this production.[5] Yet, there are exceptions—proinflammatory effects of glucocorticoids that depend on dose and cell or tissue type.[6,7] The parasympathetic nervous system also plays an important regulatory role in this nonlinear network of allostasis, since it generally opposes the sympathetic nervous system and, for example, slows the heart and also has anti-inflammatory effects.[8,9]

What this nonlinearity means is that when any one mediator is increased or decreased, there are compensatory changes in the other mediators that depend on time course and level of change of each of the mediators. Unfortunately, we cannot measure all components of this system simultaneously, and must rely on measurements of only a few of them in any one study. Yet the nonlinearity must be kept in mind in interpreting the results.

Stress in the natural world

The operation of allostasis in the natural world provides some insight into how animals use this response to their own benefit or for the benefit of the species. As an example of allostasis, in spring, a sudden snowstorm causes stress to birds and disrupts mating, and stress hormones are pivotal in directing the birds to suspend reproduction, to find a source of food, and to relocate to a better mating site, or at least to delay reproduction until the weather improves.[10] As an example of allostatic load, bears preparing to hibernate for the winter eat large quantities of food and put on body fat to act as an energy source during the winter.[11] This accumulation of fat is used, then, to survive the winter and provide food for gestation of young; this is in contrast to the fat accumulation that occurs in bears that are captive in zoos and eating too much, partially out of boredom, while not exercising.[4] The accumulation of fat under these latter conditions can be called "allostatic overload," referring to a condition that is associated with pathophysiology. However, allostatic overload can also have a useful pur-

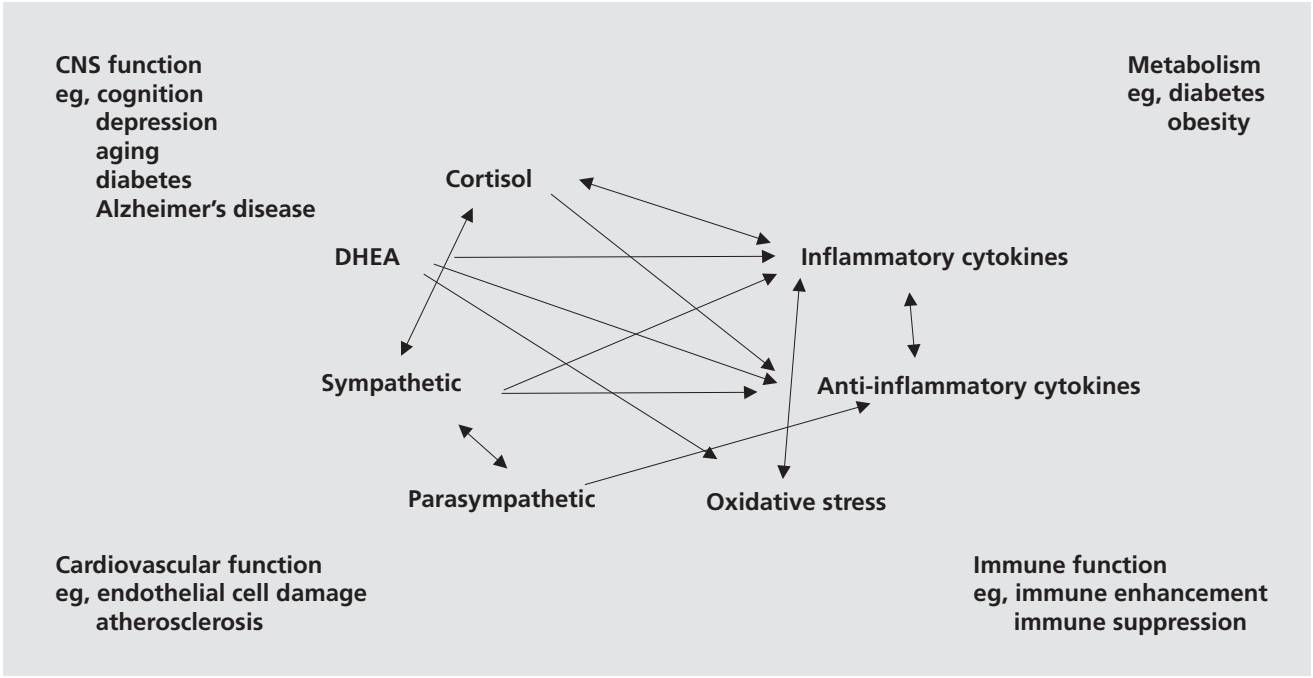


Figure 2. Nonlinear network of mediators of allostasis involved in the stress response. Arrows indicate that each system regulates the others in a reciprocal manner, creating a nonlinear network. Moreover, there are multiple pathways for regulation—eg, inflammatory cytokine production is negatively regulated via anti-inflammatory cytokines as well as via parasympathetic and glucocorticoid pathways, whereas sympathetic activity increases inflammatory cytokine production. Parasympathetic activity, in turn, restrains sympathetic activity. DHEA, dehydroepiandrosterone

pose for the preservation of the species, such as in migrating salmon or the marsupial mouse, which die of excessive stress after mating—the stress, and allostatic load, being caused for salmon, in part, by the migration up the rapidly flowing rivers, but also because of physiological changes that represent accelerated aging.[12-14] The result is freeing up food and other resources for the next generation. In the case of the marsupial mouse, it is only the males that die after mating, due apparently to a response to mating that reduces the binding protein, corticosteroid-binding globulin (CBG), for glucocorticoids and renders them much more active throughout the body.[15]

Being "stressed out," especially sleep deprivation and its consequences

The common experience of being "stressed out" has as its core the elevation of some of the key systems that lead to allostatic load—cortisol, sympathetic activity, and proinflammatory cytokines, with a decline in parasympathetic activity. Nowhere is this better illustrated than for sleep deprivation, which is a frequent result of being "stressed out." Sleep deprivation produces an allostatic overload that can have deleterious consequences.

Sleep restriction to 4 hours of sleep per night increases blood pressure, decreases parasympathetic tone, increases evening cortisol and insulin levels, and promotes increased appetite, possibly through the elevation of ghrelin, a proappetitive hormone, and decreased levels of leptin.[16-18] Proinflammatory cytokine levels are increased, along with performance in tests of psychomotor vigilance, and this has been reported to result from a modest sleep restriction to 6 hours per night.[19] Reduced sleep duration was reported to be associated with increased body mass and obesity in the NHANES study.[20]

Sleep deprivation also causes cognitive impairment. The brain is the master regulator of the neuroendocrine, autonomic, and immune systems, along with behaviors that contribute to unhealthy or health lifestyles, which, in turn, influence the physiological processes of allostasis *(Figure 3)*.[2] Alterations in brain function by chronic stress can, therefore, have direct and indirect effects on the cumulative allostatic overload. Allostatic overload resulting from chronic stress in animal models causes atrophy of neurons in the hippocampus and prefrontal cortex, brain regions involved in memory, selective attention, and executive function, and causes hypertrophy of neurons in the amygdala, a brain region involved in fear and anxiety, as well as aggression.[21] Thus, the ability to learn and remember and make decisions may be compromised by chronic stress, and may be accompanied by increased levels of anxiety and aggression.

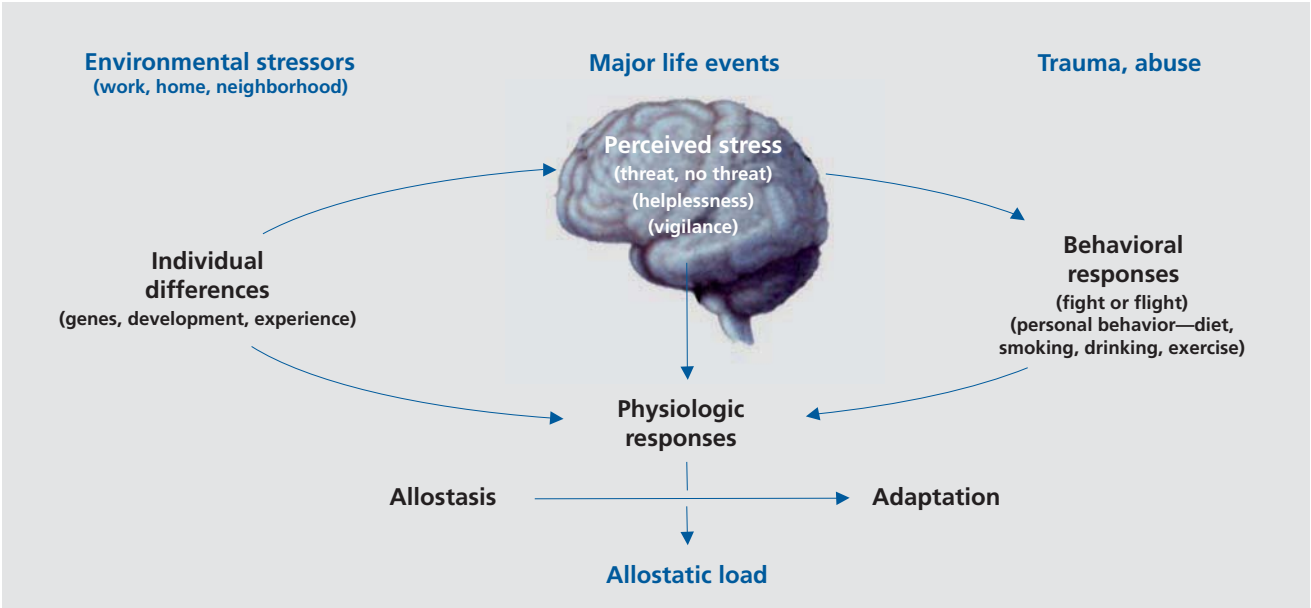


Figure 3. Central role of the brain in allostasis and the behavioral and physiological response to stressors. Reproduced from reference 1: McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med*. 1998;338:171-179. Copyright © Massachusetts Medical Society 1998.

Although sleep deprivation has not yet been studied in terms of all these aspects, there is increasing evidence, not only for cognitive impairment resulting from sleep restriction, but also for altered levels of cytokines, oxidative stress markers, glycogen levels, and structural changes in the form of reduced dentate gyrus neurogenesis.

With respect to proinflammatory cytokines, IL-1β messenger ribonucleic acid (mRNA) levels in brain are reported to increase following sleep deprivation by gentle handling and to be higher in daytime (during the normal sleep period in rodents) than in darkness (during the normal activity time for rodents).[22] Closely related to inflammatory processes through the actions of reduced nicotinamide adenine nucleotide phosphate (NADPH) oxidase[23,24] is oxidative stress involving the generation of free radicals. Sleep deprivation in mice for 72 hours by the "flowerpot" or platform method has been reported to increase oxidative stress in hippocampus, as measured by increased lipid peroxidation and increased ratios of oxidized to reduced glutathione.[25]

Another noteworthy effect of sleep deprivation is regulation of the level of glycogen, found predominantly in white matter, which is reported to decrease by as much as 40% in rats deprived of sleep for 24 hours by novelty and gentle handling, and reversed by recovery sleep.[26,27] It is noteworthy that glycogen in astrocytes is able to sustain axon function during glucose deprivation in central nervous system (CNS) white matter.[28]

Sleep deprivation in rats using a treadmill for 96 hours has been reported to decrease proliferation of cells in the dentate gyrus of the hippocampal formation by as much as 50%.[29] A similar effect has also been reported by keeping rats in a slowly rotating drum, but here again, there is a question of how much physical activity and physical stress may have contributed to the suppression of cell proliferation.[30] Nevertheless, sleep restriction by novelty exposure, a more subtle method, prevented the increased survival of new dentate gyrus neurons promoted by spatial training in a Morris water maze.[31]

Indeed, with respect to memory and cognitive performance, there are numerous reports of impairments following sleep deprivation. For example, sleep deprivation by the platform (or flowerpot) method resulted in impaired retention of passive avoidance memory, a context-dependent fear memory task,[25] as well as impaired performance of spatial memory in the Morris water maze[32] and a reduction in long-term potentiation in the CA1 region of the hippocampus.[33]

Sleep deprivation by gentle stimulation or novelty in the aftermath of contextual fear conditioning has been reported to impair memory consolidation.[34] Moreover, a 6-hour period of total sleep deprivation by novelty exposure impaired acquisition of a spatial task in the Morris water maze.[35] Furthermore, a 4-hour period of sleep deprivation by gentle stimulation impaired the late-phase long-term potentiation (LTP) in the dentate gyrus 48 hours later, but had the opposite effect of enhancing late-phase LTP in the prefrontal cortex.[36]

Sleep deprivation has also been associated with increases in fighting behavior after deprivation of rapid eye movement (REM) sleep;[37] there is also a report of increased aggression in the form of muricide after phencyclidine administration after sleep deprivation.[38] These findings may be related to the finding of increased aggression among cagemates in rats subjected to 21 days of 6 hours per day of chronic restraint stress during the resting period when some sleep deprivation may occur.[39] Interestingly, a 12-hour sleep deprivation that is applied by using a slowly rotating drum which minimizes physical stress, but does produce locomotor activity, reversed the decreased open-field behavior induced by a single social defeat.[40]

Key role of the brain in response to stress

The brain is the key organ of the stress response because it determines what is threatening, and therefore, stressful, and also controls the behavioral and physiological responses that have been discussed earlier in this article (see *Figure 3*). There are enormous individual differences in the response to stress, based upon the experience of the individual early in life and in adult life. Obviously, positive or negative experiences in school, at work, or in romantic and family interpersonal relationships can bias an individual towards either a positive or negative response in a new situation. For example, someone who has been treated badly in a job by a domineering and abusive supervisor and/or has been fired will approach a new job situation quite differently than someone who has had positive experiences in employment.

Early life experiences perhaps carry an even greater weight in terms of how an individual reacts to new situations. Early life physical and sexual abuse imposes a lifelong burden of behavioral and pathophysiological problems.[41,42] Cold and uncaring families produce long-lasting emotional problems in children.[43] Some of these effects

are seen on brain structure and function, and in the risk for later depression and post-traumatic stress disorder (PTSD).[44-46]

Animal models have been useful in providing insights into behavioral and physiological mechanisms. Early life maternal care in rodents is a powerful determinant of life-long emotional reactivity and stress hormone reactivity, and increases in both are associated with earlier cognitive decline and a shorter lifespan.[47,48] Effects of early maternal care are transmitted across generations by the subsequent behavior of the female offspring as they become mothers, and methylation of deoxyribonucleic acid (DNA) on key genes appears to play a role in this epigenetic transmission.[49] Furthermore, in rodents, abuse of the young is associated with an attachment, rather than an avoidance, of the abusive mother, an effect that increases the chances that the infant can continue to obtain food and other support until weaning.[50] Moreover, other conditions that affect the rearing process can also affect emotionality in offspring. For example, uncertainty in the food supply for rhesus monkey mothers leads to increased emotionality in offspring and possibly an earlier onset of obesity and diabetes. [51]

So far, we have emphasized the important role of the environment and experiences of individuals in the health outcomes, but clearly genetic differences also play an important role. Different alleles of commonly occurring genes determine how individuals will respond to experiences. For example, the short form of the serotonin transporter is associated with a number of conditions such as alcoholism, and individuals who have this allele are more vulnerable to respond to stressful experiences by developing depressive illness.[52] In childhood, individuals with an allele of the monoamine oxidase A gene are more vulnerable to abuse in childhood and more likely to themselves become abusers and to show antisocial behaviors compared with individuals with another commonly occurring allele.[53] Yet another example is the consequence of having the Val66Met allele of the brain-derived neurotrophic factor (BDNF) gene on hippocampal volume, memory, and mood disorders.[54-57]

The brain as a target of stress

The hippocampus

One of the ways that stress hormones modulate function within the brain is by changing the structure of neurons. The hippocampus is one of the most sensitive and malleable regions of the brain, and is also very important in cognitive function. Within the hippocampus, the input from the entorhinal cortex to the dentate gyrus is ramified by the connections between the dentate gyrus and the CA3 pyramidal neurons. One granule neuron innervates, on the average, 12 CA3 neurons, and each CA3 neuron innervates, on the average, 50 other CA3 neurons via axon collaterals, as well as 25 inhibitory cells via other axon collaterals. The net result is a 600-fold amplification of excitation, as well as a 300-fold amplification of inhibition, that provides some degree of control of the system.[58]

As to why this type of circuitry exists, the dentate gyrus (DG)-CA3 system is believed to play a role in the memory of sequences of events, although long-term storage of memory occurs in other brain regions.[59] However, because the DG-CA3 system is so delicately balanced in its function and vulnerability to damage, there is also adaptive structural plasticity, in that new neurons continue to be produced in the dentate gyrus throughout adult life, and CA3 pyramidal cells undergo a reversible remodeling of their dendrites in conditions such as hibernation and chronic stress.[58,60,61] The role of this plasticity may be to protect against permanent damage. As a result, the hippocampus undergoes a number of adaptive changes in response to acute and chronic stress.

One type of change involves replacement of neurons. The subgranular layer of the dentate gyrus contains cells that have some properties of astrocytes (eg, expression of glial fibrillary acidic protein) and which give rise to granule neurons.[62,63] After BrdU administration to label DNA of dividing cells, these newly born cells appear as clusters in the inner part of the granule cell layer, where a substantial number of them will go on to differentiate into granule neurons within as little as 7 days. In the adult rat, 9000 new neurons are born per day, and survive with a half-life of 28 days.[64] There are many hormonal, neurochemical, and behavioral modulators of neurogenesis and cell survival in the dentate gyrus including estradiol, insulin-like growth factor (IGF)-1, antidepressants, voluntary exercise, and hippocampal-dependent learning.[65-67] With respect to stress, certain types of acute stress and many chronic stressors suppress neurogenesis or cell survival in the dentate gyrus, and the mediators of these inhibitory effects include excitatory amino acids acting via *N*-methyl-D-aspartate (NMDA) receptors and endogenous opioids.[68]

Another form of structural plasticity is the remodeling of dendrites in the hippocampus. Chronic restraint stress

causes retraction and simplification of dendrites in the CA3 region of the hippocampus.[58,69] Such dendritic reorganization is found in both dominant and subordinate rats undergoing adaptation of psychosocial stress in the visible burrow system, and it is independent of adrenal size.[70]
What this result emphasizes is that it is not adrenal size or presumed amount of physiological stress per se that determines dendritic remodeling, but a complex set of other factors that modulate neuronal structure. Indeed, in species of mammals that hibernate, dendritic remodeling is a reversible process, and occurs within hours of the onset of hibernation in European hamsters and ground squirrels, and it is also reversible within hours of wakening of the animals from torpor.[60,61,71] This implies that reorganization of the cytoskeleton is taking place rapidly and reversibly, and that changes in dendrite length and branching are not "damage," but a form of structural plasticity.
Regarding the mechanism of structural remodeling, adrenal steroids are important mediators of remodeling of hippocampal neurons during repeated stress, and exogenous adrenal steroids can also cause remodeling in the absence of an external stressor. The role of adrenal steroids involve many interactions with neurochemical systems in the hippocampus, including serotonin, γ-aminobutyric acid (GABA), and excitatory amino acids.[21,58] Probably the most important interactions are those with excitatory amino acids such as glutamate. Excitatory amino acids released by the mossy fiber pathway play a key role in the remodeling of the CA3 region of the hippocampus, and regulation of glutamate release by adrenal steroids may play an important role.[58]
Among the consequences of restraint stress is the elevation of extracellular glutamate levels, leading to induction of glial glutamate transporters, as well as increased activation of the nuclear transcription factor, phosphoCREB.[72] Moreover, 21d chronic restraint stress (CRS) leads to depletion of clear vesicles from mossy fiber terminals and increased expression of presynaptic proteins involved in vesicle release.[73-75] Taken together with the fact that vesicles that remain in the mossy fiber terminal are near active synaptic zones and that there are more mitochondria in the terminals of stressed rats, this suggests that CRS increases the release of glutamate.[73]
Extracellular molecules play a role in remodeling. Neural cell adhesion molecule (NCAM) and its polysialated-NCAM (PSA-NCAM), as well as L1 are expressed in the dentate gyrus and CA3 region, and the expression of both NCAM, L1, and PSA-NCAM are regulated by 21d CRS.[76]
Tissue plasminogen activator (tPA, see below) is an extracellular protease and signaling molecule that is released with neural activity and is required for chronic stress-induced loss of spines and NMDA receptor subunits on CA1 neurons.[77]
Within the neuronal cytoskeleton, the remodeling of hippocampal neurons by chronic stress and hibernation alters the acetylation of microtubule subunits that is consistent with a more stable cytoskeleton,[78] and alters microtubule associated proteins, including the phosphorylation of a soluble form of tau, which is increased in hibernation and reversed when hibernation is terminated.[71]
Neurotrophic factors also play a role in dendritic branching and length in that BDNF +/- mice show a less branched dendritic tree and do not show a further reduction of CA3 dendrite length with chronic stress, whereas wild-type mice show reduced dendritic branching (Magarinos and McEwen, unpublished data). However, there is contradictory information thus far concerning whether CRS reduces BDNF mRNA levels, some reporting a decrease[79] and other studies reporting no change.[80,81] This may reflect the balance of two opposing forces, namely, that stress triggers increased BDNF synthesis to replace depletion of BDNF caused by stress.[82] BDNF and corticosteroids appear to oppose each other—with BDNF reversing reduced excitability in hippocampal neurons induced by stress levels of corticosterone.[83]
Corticotropin-releasing factor (CRF) is a key mediator of many aspects related to stress.[84] CRF in the paraventricular nucleus regulates ACTH release from the anterior pituitary gland, whereas CRF in the central amygdala is involved in control of behavioral and autonomic responses to stress, including the release of tPA that is an essential part of stress-induced anxiety and structural plasticity in the medial amygdala.[85] CRF in the hippocampus is expressed in a subset of GABA neurons (Cajal-Retzius cells) in the developing hippocampus, and early life stress produces a delayed effect that reduces cognitive function and the number of CA3 neurons, as well as decreased branching of hippocampal pyramidal neurons.[86,87] Indeed corticotropin-releasing hormone (CRH) inhibits dendritic branching in hippocampal cultures in vitro.[88]

Prefrontal cortex and amygdala

Repeated stress also causes changes in other brain regions, such as the prefrontal cortex and amygdala. Repeated stress causes dendritic shortening in medial

prefrontal cortex.[89-95] but produces dendritic growth in neurons in amygdala,[95] as well as in orbitofrontal cortex.[96] Along with many other brain regions, the amygdala and prefrontal cortex also contain adrenal steroid receptors; however, the role of adrenal steroids, excitatory amino acids, and other mediators has not yet been studied in these brain regions. Nevertheless, in the amygdala, there is some evidence regarding mechanism, in that tPA is required for acute stress to activate not only indices of structural plasticity but also to enhance anxiety.[97] These effects occur in the medial and central amygdala and not in basolateral amygdala, and the release of CRH acting via CRH1 receptors appears to be responsible.[85]

Acute stress induces spine synapses in the CA1 region of hippocampus[98] and both acute and chronic stress also increases spine synapse formation in amygdala,[95-99] but chronic stress decreases it in hippocampus.[77] Moreover, chronic stress for 21 days or longer impairs hippocampal-dependent cognitive function[58] and enhances amygdala-dependent unlearned fear and fear conditioning,[100] which are consistent with the opposite effects of stress on hippocampal and amygdala structure. Chronic stress also increases aggression between animals living in the same cage, and this is likely to reflect another aspect of hyperactivity of the amygdala.[39] Behavioral correlates of remodeling in the prefrontal cortex include impairment in attention set shifting, possibly reflecting structural remodeling in the medial prefrontal cortex.[95]

Translation to the human brain

Much of the impetus for studying the effects of stress on the structure of the human brain has come from the animal studies summarized thus far. Although there is very little evidence regarding the effects of ordinary life stressors on brain structure, there are indications from functional imaging of individuals undergoing ordinary stressors, such as counting backwards, that there are lasting changes in neural activity.[101] Moreover, the study of depressive illness and anxiety disorders has also provided some insights. Life events are known to precipitate depressive illness in individuals with certain genetic predispositions.[52,102,103] Moreover, brain regions such as the hippocampus, amygdala, and prefrontal cortex show altered patterns of activity in positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), and also demonstrate changes in volume of these structures with recurrent depression: decreased volume of hippocampus and prefrontal cortex and amygdala *(Figure 4)*.[104-106] Interestingly, amygdala volume has been reported to increase in the first episode of depression, whereas hippocampal volume is not decreased.[107,108] It has been known for some time that stress hormones, such as cortisol, are involved in psychopathology, reflecting emotional arousal and psychic disorganization rather than the specific disorder per se.[109] We now know that adrenocortical hormones enter the brain and produce a wide range of effects upon it.

In Cushing's disease, there are depressive symptoms that can be relieved by surgical correction of the hypercortisolemia.[110,111] Both major depression and Cushing's disease are associated with chronic elevation of cortisol that results in gradual loss of minerals from bone and abdominal obesity. In major depressive illness, as well as in Cushing's disease, the duration of the illness, and not the age of the subjects, predicts a progressive reduction in volume of the hippocampus, determined by structural magnetic resonance imaging.[103,112] Moreover, there are a variety of other anxiety-related disorders, such as PTSD[113,114] and borderline personality disorder,[115] in which atrophy of the hippocampus has been reported, suggesting that this is a common process reflecting chronic imbalance in the activity of adaptive systems, such as the hypothalamo-pituitary-adrenocortical (HPA) axis, but also including endogenous neurotransmitters, such as glutamate.

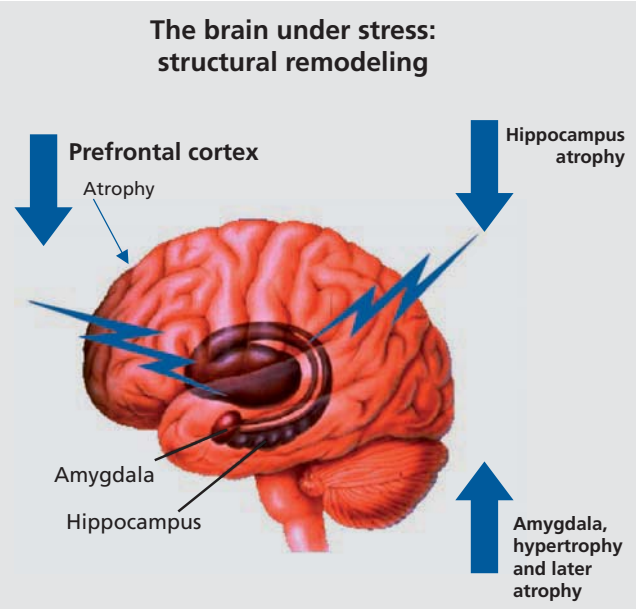


Figure 4. Brain regions that are involved in perception and response to stress, and which show structural remodeling as a result of stress.

Another important factor in hippocampal volume and function is glucose regulation. Poor glucose regulation is associated with smaller hippocampal volume and poorer memory function in individuals in their 60s and 70s who have "mild cognitive impairment" (MCI),[116] and both MCI and type 2, as well as type 1, diabetes are recognized as risk factors for dementia.[117-119]

Positive affect, self-esteem, and social support

Having a positive outlook on life and good self-esteem appear to have long-lasting health consequences,[120] and good social support is also a positive influence on the measures of allostatic load.[121] Positive affect, assessed by aggregating momentary experiences throughout a working or leisure day, was found to be associated with lower cortisol production and higher heart rate variability (showing higher parasympathetic activity), as well as a lower fibrinogen response to a mental stress test.[122]

On the other hand, poor self-esteem has been shown to cause recurrent increases in cortisol levels during a repetition of a public speaking challenge in which those individuals with good self-esteem are able to habituate, ie, attenuate their cortisol response after the first speech.[123] Furthermore, poor self-esteem and low internal locus of control have been related to a 12% to 13% smaller volume of the hippocampus, as well as higher cortisol levels during a mental arithmetic stressor.[124,125]

Related to both positive affect and self-esteem is the role of friends and social interactions in maintaining a healthy outlook on life. Loneliness, often found in people with low self-esteem, has been associated with larger cortisol responses to wakening in the morning and higher fibrinogen and natural killer cell responses to a mental stress test, as well as sleep problems.[126] On the other hand, having three or more regular social contacts, as opposed to zero to two such contacts, is associated with lower allostatic load scores.[121]

Conclusions: what can one do about being stressed out?

If being stressed out has such pervasive effects on the brain as well as the body, what are the ways that individuals, as well as policymakers in government and business, can act to reduce the negative effects and enhance the ability of the body and brain to deal with stress with minimal consequences? The answers are simple and obvious, but often difficult to achieve.

From the standpoint of the individual, a major goal should be to try to improve sleep quality and quantity, have good social support and a positive outlook on life, maintain a healthy diet, avoid smoking, and have regular moderate physical activity. Concerning physical activity, it is not necessary to become an extreme athlete, and seemingly almost any amount of moderate physical activity helps.[127,128] Regarding self-esteem, although this is still early in the story, efforts to build self-esteem in individuals might have long-term benefits for physical as well as mental health.

From the standpoint of policy, the goal should be to create incentives at home and in work situations and build community services and opportunities that encourage the development of the beneficial individual lifestyle practices.

As simple as the solutions seem to be, changing behavior and solving problems that cause stress at work and at home is often difficult, and may require professional help on the personal level, or even a change of job or profession. Yet these are important issues because the prevention of later disease is very important for full enjoyment of life, and also to reduce the financial burden on the individual and on society.

Nevertheless, many people often lack the proactive, long-term view of themselves and/or feel that they must maintain a stressful lifestyle and, if they deal with these issues at all, they want to treat their problems with "a pill." Are there any medications to treat being stressed out? In fact, there are many useful pharmaceutical agents: sleeping pills, anxiolytics, β-blockers, and antidepressants are all drugs that are used to counteract some of the problems associated with being stressed out. Likewise, drugs that reduce oxidative stress or inflammation, block cholesterol synthesis or absorption, and treat insulin resistance or chronic pain can help deal with the metabolic and neurologic consequences of being stressed out. All are valuable to some degree, and yet each one has its side effects and limitations that are based in part on the fact that all of the systems that are dysregulated in allostatic overload are also systems that interact with each other and perform normal functions when properly regulated. Because of the nonlinearity of the systems of allostasis, the consequences of any drug treatment may be either to inhibit the beneficial effects of the systems in question or to perturb other systems that interact with it in a direction that

promotes an unwanted side effect. So the best solution would seem to be not to rely solely on such medications and find ways to change lifestyle in a positive direction.

Being able to change lifestyle and associated behavior is not just an individual matter, and might become easier with changes via another level of intervention, namely, policies in government and business. The Acheson Report[129] from the United Kingdom in 1998 recognized that no public policy should be enacted without considering the implications for health of all citizens. Thus, basic education, housing, taxation, setting of a minimum wage, and policies and programs addressing occupational health and safety and environmental pollution regulations are all likely to affect health via a myriad of mechanisms. At the same time, providing higher-quality food and making it affordable and accessible in poor as well as affluent neighborhoods is necessary for people to eat better, providing they also learn what types of food to eat. Likewise, making neighborhoods safer and more congenial and supportive[130] can improve opportunities for positive social interactions and increased recreational physical activity.

However, governmental policies are not the only way to reduce allostatic load. For example, businesses that encourage healthy lifestyle practices among their employees are likely to gain reduced health insurance costs and possibly a more loyal workforce.[131-133] Above all, policymakers and business leaders need to be made aware of their broader issues of improving health and preventing disease and the fact that they make economic sense as well as being "the right thing to do."

Finally, there are programs in existence that combine some of the key elements just described, namely, education, physical activity and social support, along with one other ingredient that is hard to quantify: namely, finding meaning and purpose in life. One such program is the Experience Corps which takes elderly volunteers and trains them as teachers' assistants for younger children in the neighborhood schools.[134] Not only does this program improve the education of the children, it also benefits the elderly volunteers and improves their physical and mental health.[135] This program has now been adopted as a key part of a political campaign for the governorship of the state of Maryland.[136] One can only hope that politicians and business leaders will listen to and heed the advice of science, which often is reinforcing common sense, in helping to address the pervasive problems of stress in our world. ❑

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Efectos protectors y dañinos de los mediatores del estrés: papel central del cerebro

La mente se extiende a todo el cuerpo y a la comunicación bilateral entre el cerebro y los aparatos cardiovascular, sistema inmunitario y otros a través de mecanismos neurales y endocrinos. El estrés es un estado de interacción entre la mente y el cuerpo e interviene en la expresión diferente de la enfermedad entre las personas. No son únicamente los sucesos estresantes más llamativos los que cuestan más, sino más bien los múltiples episodios de la vida cotidiana que elevan y sostienen la actividad de los sistemas fisiológicos y determinan una privación del sueño, sobrealimentación y otras conductas dañinas para la salud que producen una sensación de "agotamiento por estrés". Con el tiempo, el organismo desgasta por la llamada "carga alostática", que refleja no sólo el impacto de las experiencias vitales sino también de la carga genética, de los hábitos personales de vida —que traducen aspectos como la alimentación, el ejercicio y el abuso de sustancias— y de las experiencias del desarrollo que fijan los patrones duraderos de conducta y reactividad fisiológica. Las hormonas asociadas al estrés y a la carga alostática protegen el organismo a corto plazo y fomentan la adaptación a través de un proceso llamado alostasia pero, a la larga, la carga alostásica determina cambios corporales que pueden causar enfermedades. El cerebro es el órgano destinatario del estrés, la alostasia y la carga alostática, porque decide qué información resulta amenazadora y, en consecuencia, estresante y determina, además, las respuestas fisiológicas y conductuales. Las regiones cerebrales, como el hipocampo, la amígdala (núcleo amigdalino) y la corteza prefrontal, responden al estrés agudo y crónico sometiéndose a una remodelación estructural que modifica las respuestas comportamentalesl y fisiológicas. Los estudios translacionales de imágenes estructurales y funcionales de seres humanos revelan un volumen hipocámpico más reducido en los estados de estrés, por ejemplo una ligera alteración cognitiva con el envejecimiento y el trastorno depresivo mayor prolongado, así como entre las personas que se subestiman. Se han descrito también alteraciones de la amígdala y de la corteza prefrontal. Además del enfoque farmacéutico, las medidas para aliviar el estrés crónico y reducir la carga alostásica así como la incidencia de las enfermedades de la vida moderna se basan en cambios en los hábitos de vida y políticas gubernamentales y empresariales para mejorar la capacidad del individuo y reducir la carga propia y crónica del estrés.

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Effets protecteurs et délétères des médiateurs du stress : rôle central du cerveau

L'esprit implique le corps entier, et il existe une intercommunication entre le cerveau, les systèmes cardiovasculaire, immunitaire et d'autres, par des mécanismes neuraux et endocriniens. Le stress est la manifestation d'une interaction entre l'esprit et le corps et un facteur d'expression de maladies qui diffère selon les individus. Les événements de la vie quotidienne, plus que les stress aigus ou intenses de la vie, élèvent et entretiennent les niveaux d'activité des systèmes physiologiques, entraînant privation de sommeil, boulimie et autres comportements néfastes pour la santé qui donnent le sentiment d'être « dépassé par les événements ». Avec le temps, ceci entraîne une usure du corps appelée « charge allostatique », qui reflète non seulement l'impact des expériences de la vie mais aussi la charge génétique, les habitudes de vie quotidienne comme le régime, l'exercice, l'usage de drogues et le vécu au cours du développement qui mettent en place tout au long de la vie des schémas de comportement et de réactivité physiologique. Les hormones associées au stress et à la charge allostatique protègent le corps à court terme et favorisent l'adaptation par un procédé nommé allostase mais à long terme, les modifications somatiques dues à la charge allostatique peuvent entraîner l'apparition d'une maladie. Le cerveau est l'organe clé du stress, de l'allostase et de la charge allostatique car il détermine ce qui est menaçant et donc stressant ainsi que les réponses physiologiques et comportementales. Les régions cérébrales comme l'hippocampe, l'amygdale et le cortex préfrontal répondent au stress aigu et chronique par un remodelage structural qui modifie les réponses physiologiques et comportementales. Des études réalisées chez l'homme par imagerie structurale et fonctionnelle montrent que le volume de l'hippocampe est diminué dans des situations de stress comme il l'est dans les déficits cognitifs légers dus à l'âge, les dépressions majeures prolongées et les individus qui se sous-estiment. Des altérations de l'amygdale et du cortex préfrontal sont aussi rapportées. Outre les traitements pharmacologiques, les modifications du mode de vie, les politiques gouvernementales et de travail pouvant améliorer la capacité individuelle à réduire la charge de stress chronique de chacun, sont autant d'approches visant à alléger le stress chronique et réduire la charge allostatique et l'incidence des maladies liées à la vie moderne.

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The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress

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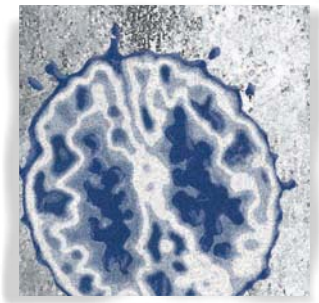

Animals respond to stress by activating a wide array of behavioral and physiological responses that are collectively referred to as the stress response. Corticotropin-releasing factor (CRF) plays a central role in the stress response by regulating the hypothalamic-pituitary-adrenal (HPA) axis. In response to stress, CRF initiates a cascade of events that culminate in the release of glucocorticoids from the adrenal cortex. As a result of the great number of physiological and behavioral effects exerted by glucocorticoids, several mechanisms have evolved to control HPA axis activation and integrate the stress response. Glucocorticoid feedback inhibition plays a prominent role in regulating the magnitude and duration of glucocorticoid release. In addition to glucocorticoid feedback, the HPA axis is regulated at the level of the hypothalamus by a diverse group of afferent projections from limbic, midbrain, and brain stem nuclei. The stress response is also mediated in part by brain stem noradrenergic neurons, sympathetic andrenomedullary circuits, and parasympathetic systems. In summary, the aim of this review is to discuss the role of the HPA axis in the integration of adaptive responses to stress. We also identify and briefly describe the major neuronal and endocrine systems that contribute to the regulation of the HPA axis and the maintenance of homeostasis in the face of aversive stimuli.

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Stress is commonly defined as a state of real or perceived threat to homeostasis. Maintenance of homeostasis in the presence of aversive stimuli (stressors) requires activation of a complex range of responses involving the endocrine, nervous, and immune systems, collectively known as the stress response.[1,2] Activation of the stress response initiates a number of behavioral and physiological changes that improve an individual's chance of survival when faced with homeostatic challenges. Behavioral effects of the stress response include increased awareness, improved cognition, euphoria, and enhanced analgesia.[1,3] Physiological adaptations initiated by activation of this system include increased cardiovascular tone, respiratory rate, and intermediate metabolism, along with inhibition of general vegetative functions such as feeding, digestion, growth, reproduction, and immunity.[4,5] Due to the wide array of physiologic and potentially pathogenic effects of the stress response, a number of neuronal and endocrine systems function to tightly regulate this adaptive process.

Anatomy of the stress response

The anatomical structures that mediate the stress response are found in both the central nervous system and peripheral tissues. The principal effectors of the stress response are localized in the paraventricular

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Selected abbreviations and acronyms

ACTH	*adrenocorticotropic hormone*
BNST	*bed nucleus of stria terminalis*
cAMP	*cyclic adenosine monophosphate*
CeA	*central nuclei of amygdala*
CNS	*central nervous system*
CRF	*corticotropin-releasing factor*
DMH	*dorsomedial hypothalamic nucleus*
GR	*glucocorticoid receptor*
HPA	*hypothalamic-pituitary-adrenal*
LC	*locus coeruleus*
LS	*lateral septum*
MeA	*medial nuclei of the amygdala*
NTS	*nucleus of solitary tract*
POA	*preoptic area*
PVN	*paraventricular nucleus*
SFO	*subfornical organ*

nucleus (PVN) of the hypothalamus, the anterior lobe of the pituitary gland, and the adrenal gland. This collection of structures is commonly referred to as the hypothalamic-pituitary-adrenal (HPA) axis *(Figure 1)*. In addition to the HPA axis, several other structures play important roles in the regulation of adaptive responses to stress. These include brain stem noradrenergic neurons, sympathetic andrenomedullary circuits, and parasympathetic systems.[5-7]

The HPA axis

Hypophysiotropic neurons localized in the medial parvocellular subdivision of the PVN synthesize and secrete corticotropin-releasing factor (CRF), the principle regulator of the HPA axis.[8,9] In response to stress, CRF is released into hypophysial portal vessels that access the anterior pituitary gland. Binding of CRF to its receptor on pituitary corticotropes induces the release of adrenocorticotropic hormone (ACTH) into the systemic circulation. The principal target for circulating ACTH is the adrenal cortex, where it stimulates glucocorticoid synthesis and secretion from the zona fasciculata. Glucocorticoids are the downstream effectors of the HPA axis and regulate physiological changes through ubiquitously distributed intracellular receptors.[10,11] The biological effects of glucocorticoids are usually adaptive; however, inadequate or excessive activation of the HPA axis may contribute to the development of pathologies.[10,12]

The CRF family of peptides

Corticotropin-releasing factor is a 41 amino acid peptide that was originally isolated from ovine hypothalamic tissue in 1981.[8] Since this initial identification, CRF has been shown to be the primary regulator of ACTH release from anterior pituitary corticotropes[9] and has also been implicated in the regulation of the autonomic nervous system, learning and memory, feeding, and reproduction-related behaviors.[13-19] CRF is widely expressed through-

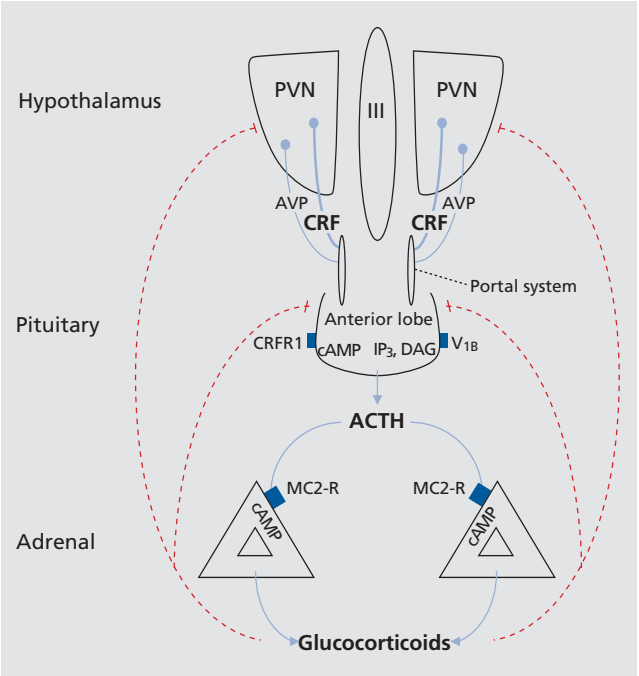

Figure 1. Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis. Hypophysiotropic neurons localized in the paraventricular nucleus (PVN) of the hypothalamus synthesize corticotropin-releasing factor (CRF) and vasopressin (AVP). In response to stress, CRF is released into hypophysial portal vessels that access the anterior pituitary gland. Binding of CRF to the CRF type 1 receptor (CRFR1) on pituitary corticotropes activates cyclic adenosine monophosphate (cAMP) pathway events that induce the release of adrenocorticotropic hormone (ACTH) into the systemic circulation. In the presence of CRF, AVP elicits synergistic effects on ACTH release that are mediated through the vasopressin V_{1b} receptor. Circulating ACTH binds to the melanocortin type 2 receptor (MC2-R) in the adrenal cortex where it stimulates glucocorticoid synthesis and secretion into the systemic circulation. Glucocorticoids regulate physiological events and inhibit further HPA axis activation (red lines) through intracellular receptors that are widely distributed throughout the brain and peripheral tissues. IP3, inositol triphosphate; DAG, diacylglycerol

out the central nervous system (CNS) and in a number of peripheral tissues. In the brain, CRF is concentrated in the medial parvocellular subdivision of the PVN and is also localized in the olfactory bulb, bed nucleus of the stria terminalis (BNST), medial preoptic area, lateral hypothalamus, central nucleus of the amygdala, Barington's nucleus, dorsal motor complex, and inferior olive.[20] In the periphery, CRF has been detected in the adrenal gland, testis, placenta, gastrointestinal tract, thymus, and skin.[21-23]

Three additional members of the CRF peptide family have recently been identified. These include urocortin (Ucn) 1[24] and the recently cloned Ucn 2[25] and Ucn 3,[26] which are also known as stresscopin-related peptide and stresscopin,[27] respectively. In the mammalian brain, Ucn 1 is predominantly expressed in the Edinger-Westphal nucleus[24] and Ucn 2 expression is restricted to the PVN and locus coeruleus.[25] Ucn 3 has a wider distribution in the brain and is localized in the perifornical area of the hypothalamus, BNST, lateral septum (LS), and amygdala.[28] The widespread anatomical distribution of CRF and the urocortins correlates well with the diverse array of physiological functions associated with this peptide family.

CRF receptors

The physiological actions of the CRF family of peptides are mediated through two distinct receptor subtypes belonging to the class B family of G-protein coupled receptors.[29] The CRF type 1 receptor (CRFR1) gene encodes one functional variant (α) in humans and rodents along with several nonfunctional splice variants.[30-32] The CRF type 2 receptor (CRFR2) has three functional splice variants in human (α, β, and γ) and two in rodents (α and β) resulting from the use of alternate 5' starting exons.[33,34]

CRFR1 is expressed at high levels in the brain and pituitary and low levels in peripheral tissues. The highest levels of CRFR1 expression are found in the anterior pituitary, olfactory bulb, cerebral cortex, hippocampus, and cerebellum. In peripheral tissues, low levels of CRFR1 are found in the adrenal gland, testis, and ovary.[35,36] In contrast, CRFR2 is highly expressed in peripheral tissues and localized in a limited number of nuclei in the brain.[37] In rodents, the CRF type 2α splice variant is preferentially expressed in the mammalian brain and is localized in the lateral septum, BNST, ventral medial hypothalamus, and mesencephalic raphe nuclei.[36] The CRF type 2β variant is expressed in the periphery and is concentrated in the heart, skeletal muscle, skin, and the gastrointestinal tract.[29,38,39]

Radioligand binding and functional assays have revealed that CRFR1 and CRFR2 have different pharmacological profiles. CRF binds to the CRFR1 with higher affinity than to CRFR2.[29,33] Ucn1 has high affinity for both CRFR1 and CRFR2 and is more potent than CRF on CRFR2.[24,33] Ucn 2 and Ucn 3 are highly selective for CRFR2 and exhibit low affinities for CRFR1. In addition, Ucn 2 and Ucn 3 minimally induce cyclic adenosine monophosphate (cAMP) production in cells expressing either endogenous or transfected CRFR1.[25-27]

The neuroendocrine properties of CRF are mediated through CRFR1 in the anterior pituitary. Binding of CRF to the type 1 receptor results in the stimulation of adenylate cyclase and a subsequent activation of cAMP pathway events that culminate with the release of ACTH from pituitary corticotropes.[29,39,40] The integral role of CRFR1 in the regulation of ACTH release was confirmed by the phenotype of CRFR1-deficient mice. Mice deficient for CRFR1 have a severely attenuated HPA response to stress and display decreased anxiety-like behaviors.[41,42] The role of CRFR2 in the regulation of the HPA axis and adaptive responses to stress is less clear. Mice deficient for CRFR2 have an amplified HPA response to stress and display increased anxiety-like behaviors.[43-45] However, administration of CRFR2 agonists and antagonists into discrete brain regions reveal both anxiolytic and anxiogenic roles for CRFR2.[45]

Vasopressin

Vasopressin (AVP) is a nonapeptide that is highly expressed in the PVN, supraoptic (SON), and suprachiasmatic nuclei of the hypothalamus.[46,47] Magnocellular neurons of the PVN and SON project to the posterior lobe of the pituitary and release AVP directly into the systemic circulation to regulate osmotic homeostasis.[48,49] In addition to magnocellular neurons, parvocellular neurons of the PVN synthesize and release AVP into the portal circulation, where this peptide potentiates the effects of CRF on ACTH release from the anterior pituitary.[7,50,51]

The synergistic effects of AVP on ACTH release are mediated through the vasopressin V_{1b} (also known as V_3) receptor on pituitary corticotropes.[52] Binding of AVP to

the V_{1b} receptor activates phospholipase C by coupling to Gq proteins. Activation of the phospholipase C stimulates protein kinase C, resulting in the potentiation of ACTH release.[53] Several investigators have reported that the expression of AVP in parvocellular neurons of the PVN and V_{1b} receptor density in pituitary corticotropes is significantly increased in response to chronic stress.[54-58] These findings support the hypothesis that AVP plays an important role in the stress response by maintaining ACTH responsiveness to novel stressors during periods of chronic stress.

Adrenocorticotropic hormone

Pro-opiomelanocortin (POMC) is a prohormone that is highly expressed in the pituitary and the hypothalamus. POMC is processed into a number of bioactive peptides including ACTH, β-endorphin, β-lipotropic hormone, and the melanocortins.[59-61] In response to CRF, ACTH is released from pituitary corticotropes into the systemic circulation where it binds to its specific receptor in the adrenal cortex. ACTH binds to the melanocortin type 2 receptor (MC2-R) in parenchymal cells of the adrenocortical zona fasciculata. Activation of the MC2-R induces stimulation of cAMP pathway events that induce steroidogenesis and the secretion of glucorticoids, mineralcorticoids, and androgenic steroids.[62,63] Specifically, ACTH promotes the conversion of cholesterol into δ-5 pregnenolone during the initial step of glucocorticoid biosynthesis.[61,64]

Glucocorticoids

Glucocorticoids, cortisol in humans and corticosterone in rodents, are a major subclass of steroid hormones that regulate metabolic, cardiovascular, immune, and behavioral processes.[3,4] The physiological effects of glucocorticoids are mediated by a 94kD cytosolic protein, the glucocorticoid receptor (GR). The GR is widely distributed throughout the brain and peripheral tissues. In the inactive state, the GR is part of a multiprotein complex consisting of several different molecules of heat shock proteins (HSP) that undergo repeated cycles of dissociation and ATP-dependent reassociation.[11,65,66] Ligand binding induces a conformational change in the GR, resulting in the dissociation of the receptor from the HSP complex and translocation into the nucleus. Following translocation, the GR homodimer binds to specific DNA motifs termed glucocorticoid response elements (GREs) in the promoter region of glucocorticoid responsive genes and regulates expression through interaction with transcription factors.[11,67,68] The GR has also been shown to regulate activation of target genes independent of GRE-binding through direct protein-protein interactions with transcription factors including activating protein 1 (AP-1) and nuclear factor-κB (NF-κB).[69-71]

Endocrine regulation of the HPA axis

Activation of the HPA axis is a tightly controlled process that involves a wide array of neuronal and endocrine systems. Glucocorticoids play a prominent role in regulating the magnitude and duration of HPA axis activation.[72] Following exposure to stress, elevated levels of circulating glucocorticoids inhibit HPA activity at the level of the hypothalamus and pituitary. The HPA axis is also subject to glucocorticoid independent regulation. The neuroendocrine effects of CRF are also modulated by CRF binding proteins that are found at high levels in the systemic circulation and in the pituitary gland.[73,74]

Glucocorticoid negative feedback

The HPA axis is subject to feedback inhibition from circulating glucocorticoids.[72] Glucocorticoids modulate the HPA axis through at least two distinct mechanisms of negative feedback. Glucocorticoids have traditionally been thought to inhibit activation of the HPA axis through a delayed feedback system that is responsive to glucocorticoid levels and involves genomic alterations. There is increasing evidence for an additional fast nongenomic feedback system that is sensitive to the rate of glucocorticoid secretion; however, the exact mechanism that mediates rapid feedback effects has not yet been characterized.[11,72,75]

The delayed feedback system acts via transcriptional alterations and is regulated by GR localized in a number of stress-responsive brain regions.[76] Following binding of glucocorticoids, GRs modulate transcription of HPA components by binding to GREs or through interactions with transcription factors.[11,72] Glucocorticoids have a low nanomolar affinity for the GR and extensively occupy GRs during periods of elevated glucocorticoid secretion that occur following stress.[77] Mineralocorticoid receptors (MRs) have a subnanomolar affinity for glucocorticoids, a restricted expression pattern in the brain, and bind glu-

cocorticoids during periods of basal secretion.[76,77] The distinctive pharmacologies of these two receptors suggest that MRs regulate basal HPA tone while GRs mediate glucocorticoid negative feedback following stress.[75,78,79]
GRs are widely expressed in the brain, and thus the precise anatomical locus of glucocorticoid negative feedback remains poorly defined. However, two regions of the brain appear to be key sites for glucocorticoid feedback inhibition of the HPA axis. High levels of GR are expressed in hypophysiotropic neurons of the PVN, and local administration of glucocorticoids reduce PVN neuronal activity and attenuate adrenalectomy-induced ACTH hypersecretion.[80-83] These findings suggest that the PVN is an important site for glucocorticoid feedback inhibition of the HPA axis. The hippocampus has been implicated as a second site for glucocorticoid negative feedback regulation of the HPA axis. The hippocampus contains a high concentration of both GR and MR, and infusion of glucocorticoids into this structure reduces basal and stress induced glucocorticoid release.[84-86]

CRF binding proteins

Two soluble proteins have been identified that bind the members of the CRF family of peptides with high affinity. The CRF binding protein (CRF-BP) is a highly conserved 37kD glycoprotein that binds both CRF and Ucn 1 with high affinity.[74,87,88] The CRF-BP was originally identified in maternal plasma where it functions to inhibit HPA axis activation stemming from the elevated circulating levels of placenta-derived CRF.[89,90] The CRF-BP is highly expressed in the pituitary, and recombinant CRF-BP attenuates CRF-induced ACTH release from dispersed anterior pituitary cells in culture.[74] These findings suggest the CRF-BP may function to sequester CRF at the level of the pituitary and reduce CRFR activity.
Our laboratory has recently identified a transcript that encodes a soluble splice variant of the CRFR2 receptor (sCRFR2α) in the mouse brain.[73] Soluble CRFR2α is a predicted 143 amino acid protein generated from a predicted 143 amino acid protein generated from exons 3-5 of the extracellular domain of *CRFR2α* gene and a unique 38 amino acid hydrophilic C-terminal tail. High levels of sCRFR2α expression are found in the olfactory bulb, cortex, and midbrain regions that have been shown to express CRFR1.[36] Recombinant sCRFR2α binds CRF with low nanomolar affinity and inhibits cellular responses to both CRF and Ucn 1 in signal transduction assays,[73] suggesting that sCRFR2α may function as a decoy receptor for the CRF family of peptides.

Neuronal regulation of the HPA axis

Hypophysiotropic neurons in the PVN are innervated by a diverse constellation of afferent projections from multiple brain regions. The majority of afferent inputs to the PVN originate from four distinct regions: brain stem neurons, cell groups of the lamina terminalis, extra-PVN hypothalamic nuclei, and forebrain limbic structures.[20,91] These cell groups integrate and relay information regarding a wide array of sensory modalities to influence CRF expression and release from hypophysiotropic neurons of the PVN *(Figure 2)*.

Brain stem neurons

Brain stem catecholaminergic centers play an important role in the regulation of the HPA axis. Neurons of the nucleus of the solitary tract (NTS) relay sensory information to the PVN from cranial nerves that innervate large areas of thoracic and abdominal viscera. The NTS also receives projections from limbic structures that regulate behavioral responses to stress including the medial prefrontal cortex and the central nucleus of the amygdala.[92] Accordingly, neuronal populations in the NTS are activated following lipopolysaccharide injection,[93,94] hypotension,[95] forced swim, and immobilization stress paradigms.[96]
Stress-receptive neurons in the A2/C2 region of the NTS densely innervate the medial parvocellular subdivision of the PVN.[97,98] Findings from both in vivo and in vitro studies demonstrate that catecholaminergic input represents a major excitatory drive on the HPA axis and induces CRF expression and protein release through an α-1 adrenergic receptor-dependent mechanism.[99-101] Nonaminergic NTS neurons also innervate the PVN and contribute to HPA axis regulation. Glucagon-like peptide 1 containing neurons in the NTS are activated by physiological stressors and have been shown to induce ACTH release in vivo.[102,103] The neuropeptides somatostatin, substance P, and enkephalin are also expressed in NTS neurons that innervate the PVN and have been shown to have regulatory effects on the HPA axis.[104-106]

The lamina terminalis

A series of interconnected cell groups including the subfornical organ (SFO), median preoptic nucleus (MePO), and the vascular organ of the lamina terminalis are localized on the rostral border of the third ventricle and make up the lamina terminalis.[107] Cell groups of the lamina terminalis lie outside of the blood-brain barrier and relay information concerning the osmotic composition of blood to the PVN.[108] The medial parvocellular subdivision of the PVN receives rich innervation from the SFO and to a lesser extent from the OVLT and MePO.[109] Neurons in the SFO that project to the PVN are angiotensinergic, and promote CRF secretion and biosynthesis.[110,111] This afferent pathway has parallel input to the magnocellular division of the PVN, and had been hypothesized to serve as a link between HPA and neurohypophysial activation.[112-114]

Hypothalamus

The medial parvocellular subdivision of the PVN receives afferent projections from γ-aminobutyric acid (GABA)-ergic neurons of the hypothalamus.[115] Hypophysiotropic neurons of the PVN express GABA-A receptor subunits[116] and hypothalamic injection of the GABA-A receptor agonists inhibit glucocorticoid secretion following exposure to stressors.[117,118] These studies suggest that GABA plays a prominent role in hypothalamic stress integration.

Hypothalamus: DMH and POA

GABAergic neurons in the dorsomedial hypothalamic nucleus (DMH) and preoptic area (POA) project to the medial parvocellular division of the PVN, and are activated following exposure to stressors.[115,117] Lesions of

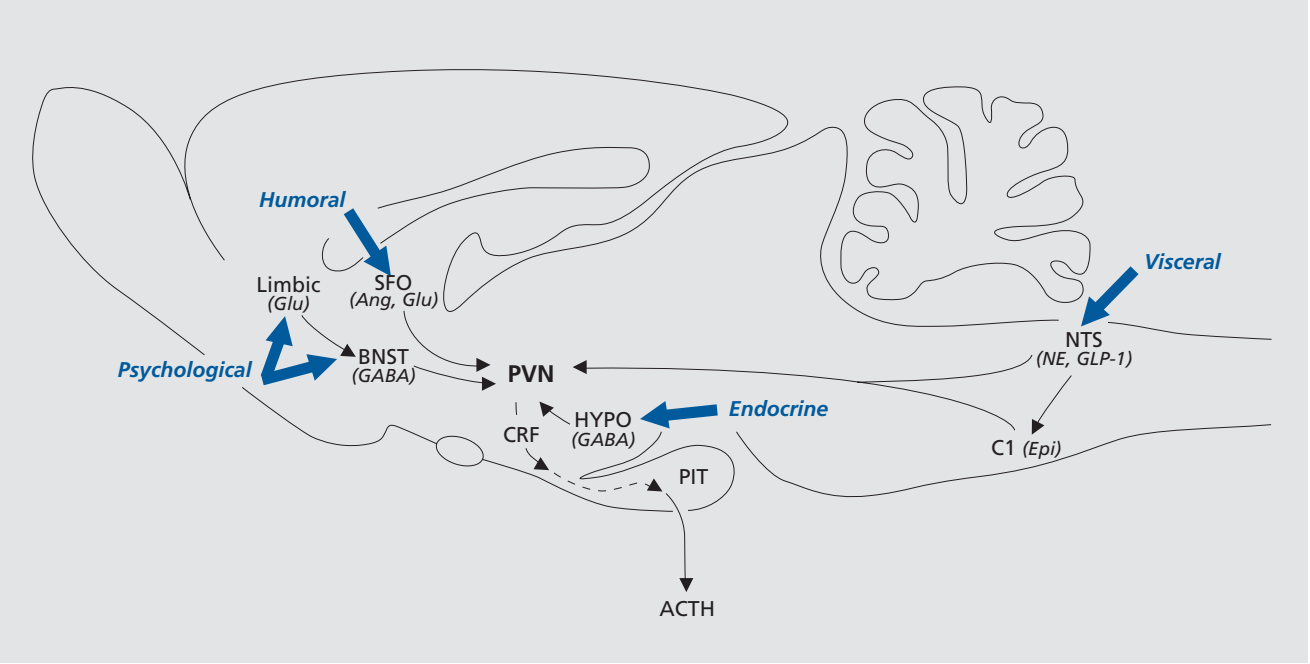

Figure 2. Depiction of the major brain regions and neurotransmitter groups that supply afferent innervation to the medial parvocellular zone of the paraventricular nucleus (PVN). Cell groups of the nucleus of the solitary tract (NTS) and ventral medulla (C1) relay visceral information to the PVN though noradrenergic (NE), adrenergic (Epi), and glucagon-like peptide 1 (GLP-1)-containing neurons. Hypothalamic nuclei (HYPO) encode information from endocrine systems and send mainly γ-aminobutyric acid (GABA)-ergic (GABA) projections to the PVN. Cell groups of the lamina terminalis relay information concerning the osmotic composition of blood to the PVN through glutamatergic (Glu) and angiotensinergic (Ang) neurons. Limbic structures including the hippocampus, prefrontal cortex, and the amygdala contribute to the regulation of PVN neurons through intermediary neurons of the bed nucleus of the stria terminalis (BNST). PIT, pituitary
Adapted from reference 20: Sawchenko PE, Imaki T, Potter E, Kovacs K, Imaki J, Vale W. The functional neuroanatomy of corticotropin-releasing factor. *Ciba Found Symp*. 1993;172:5-21; discussion 21-29. Copyright © John Wiley and Sons 1993.

hypothalamic regions encompassing the DMH and the POA amplify HPA responses to stress.[119,120] Furthermore, glutamate microstimulation of DMH neurons produces inhibitory postsynaptic potentials in hypophysiotropic neurons of the PVN,[121] and stimulation of the POA attenuates the excitatory effects of medial amygdalar stimulation of glucocorticoid release.[122] The POA is a potential site of integration between gonadal steroids and the HPA axis. Accordingly, neurons of the POA are activated by gonadal steroids and express high levels of androgen, estrogen, and progesterone receptors.[123,124]

Hypothalamus: feeding centers

Hypothalamic centers involved in the regulation of energy homeostasis directly innervate PVN neurons. Neurons in the arcuate nucleus are sensitive to circulating levels of glucose, insulin, and leptin These cells also synthesize neuropeptide Y (NPY), agouti-related peptide (AGRP), α-melanocyte stimulating hormone (αMSH), and cocaine- and amphetamine-regulated transcript (CART) which play critical roles in the regulation of feeding behaviors.[125-127] In addition to their roles in energy homeostasis, arcuate neuropeptides have significant effects on HPA axis activity. Central injection of the orexigenic factor NPY results in HPA axis activation[128,129] and infusion of AGRP significantly increases CRF release from hypothalamic explants.[130] The anorectic peptides αMSH and CART have been reported to increase circulating levels of ACTH and corticosterone,[130-132] induce cAMP binding protein phosphorylation in CRF neurons,[133] and stimulate CRF release from hypothalamic neurons.[130,134] These studies suggest that the HPA axis is activated in response to positive and negative states of energy balance.

The limbic system

Limbic structures of the forebrain contribute to the regulation of the HPA axis. Neuronal populations in the hippocampus, prefrontal cortex, and amygdala are the anatomical substrates for memory formation and emotional responses, and may serve as a link between the stress system and neuropsychiatric disorders.[86,135] The hippocampus, prefrontal cortex, and amygdala have significant effects on glucocorticoid release and behavioral responses to stress.[84,136,137] However, these limbic structures have a limited number of direct connections with hypophysiotropic neurons of the PVN and are thought to regulate HPA axis activity through intermediary neurons in the BNST, hypothalamus, and brain stem.[20,138,139]

Limbic system: hippocampus

The hippocampus plays an important role in the terminating HPA axis responses to stress.[84,139] Stimulation of hippocampal neurons decreases neuronal activity in the parvocellular division of the PVN and inhibits glucocorticoid secretion.[140-142] Hippocampal lesions produce elevated basal levels of circulating glucocorticoids,[143,144] increase parvocellular CRF and AVP expression,[145] and prolong ACTH and corticosterone release in response to stress.[141,146]

The regulatory effects of the hippocampus on the HPA axis are mediated through a multisynaptic pathway and appear to be stressor-specific.[139] Hippocampal outflow to the hypothalamus originates in the ventricle subiculum and CA1 regions of the hippocampus.[139,147] These regions send afferent projections to GABAergic neurons of BNST and the peri-PVN region of the hypothalamus that directly innervate the parvocellular division of the PVN.[139,147,148] Hippocampal lesions encompassing the ventral subiculum produce exaggerated HPA responses to restraint and open field exposure, but not to hypoxia or ether exposure, suggesting that hippocampal neurons respond to distinct stress modalities.[146,149,150]

Limbic system: prefrontal cortex

The prefrontal cortex also regulates HPA responses to stress. Neurons of the medial prefrontal cortex are activated and release catecholamines following exposure to acute and chronic stressors.[117,151,152] Bilateral lesions of the anterior cingulate and prelimbic cortex increase ACTH and glucocorticoid responses to stress,[85,153] demonstrating that the prefrontal cortex has inhibitory effects on the HPA axis. Anatomic tracing studies reveal that the there is an intricate topographic organization of prefrontal cortex output to HPA regulatory circuits. Afferents from the infralimbic cortex project extensively to the BNST, amygdala, and the NTS.[154,155] In contrast, the prelimbic/anterior cingulate cortex projects to the POA and the DMH but fails to synapse with the BNST, NTS, or amygdalar neurons.[139,154,155]

The prefrontal cortex may also play a role in glucocorticoid feedback inhibition of the HPA axis. High densities of GR are expressed in layers II, III, and VI of the

prefrontal cortex.[156] Infusion of glucocorticoids into the medial prefrontal cortex attenuates ACTH and corticosterone responses to restraint stress, but has no significant effect on HPA responses to ether.[85,157] Similarly to the hippocampus, it appears that neurons of the prefrontal cortex are subject to modality-specific regulation of glucocorticoid feedback inhibition of the HPA axis.[139]

Limbic system: amygdala

In contrast to the hippocampus and the prefrontal cortex, the amygdala is thought to activate the HPA axis. Stimulation of amygdalar neurons promotes glucocorticoid synthesis and release into the systemic circulation.[158,159] The medial (MeA) and central (CeA) nuclei of the amygdala play a key role in HPA axis activity and contribute the majority of afferent projections from the amygdala to cortical, midbrain, and brain stem regions that regulate adaptive responses to stress.[160,161] The MeA and CeA respond to distinct stress modalities and are thought to have divergent roles in HPA regulation.[139] Neurons in the MeA are activated following exposure to "emotional" stressors including predator, forced swim, social interaction, and restraint stress paradigms.[117,162-165] In contrast, the CeA appears to be preferentially activated by "physiological" stressors, including hemorrhage and immune challenge.[166,167]

The CeA exerts its regulatory effects on the HPA axis through intermediary neurons in the brain stem.[139] Afferent projections from the CeA densely innervate the NTS and parabrachial nucleus.[92,168] The MeA sends a limited number of direct projections to the parvocellular division of the PVN[169]; however, this subnucleus innervates a number of nuclei that directly innervate the PVN. Neurons of the MeA project to the BNST, MePO, and ventral premammillary nucleus.[169]

The amygdala is a target for circulating glucocorticoids and the CeA and MeA express both GR and MR. In contrast to the effects on hippocampal and cortical neurons, glucocorticoids increase expression of CRF in the CeA and potentiate autonomic responses to chronic stressors. Glucocorticoid infusion into the CeA does not acutely effect HPA activation but may play a feed-forward role to potentiate HPA responses to stress.[139,157,170]

Sympathetic circuits and the stress response

Activation of brain stem noradrenergic neurons and sympathetic andrenomedullary circuits further contribute to the body's response to stressful stimuli. Similarly to the HPA axis, stress-evoked activation of these systems promotes the mobilization of resources to compensate for adverse effects of stressful stimuli.[3,171] The locus coeruleus (LC) contains the largest cluster of noradrenergic neurons in the brain and innervates large segments of the neuroaxis.[172] The LC has been implicated in a wide array of physiological and behavioral functions including emotion, vigilance, memory, and adaptive responses to stress.[173-175] A wide array of stressful stimuli activate LC neurons, alter their electrophysiological activity, and induce norepinephrine release.[176-178] Stimulation of the LC elicits several stress-associated responses including ACTH release,[179] anxiogenic-like behaviors,[180] and suppression of immune functions.[181] In addition, there are interactions between CRF and NE neurons in the CNS. Central administration of CRF alters activity of LC neurons and NE catabolism in terminal regions.[13,182] Finally, dysfunction of catecholamergic neurons in the LC has been implicated in the pathophysiology of affective and stress-related disorders.[183,184]

Conclusions

Maintenance of homeostasis in the presence of real or perceived challenges requires activation of a complex range of responses involving the endocrine, nervous, and immune systems, collectively known as the stress response. Inappropriate regulation of the stress response has been linked to a wide array of pathologies including autoimmune disease, hypertension, affective disorders, and major depression. In this review we briefly discussed the major neuronal and endocrine systems that contribute to maintenance of homeostasis in the presence of stress. Clearly deciphering the role of each of these systems and their regulatory mechanisms may provide new therapeutic targets for treatment and prophylaxis of stress-related disorders including anxiety, feeding, addiction, and energy metabolism. ❑

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Función del eje hipotálamo-hipofisis-suprarenal en las respuestas endocrinas al estrés

Los animales responden al estrés, activando una amplia gama de respuestas comportamentales y fisiológicas que se conocen, de forma genérica, como respuesta al estrés. El factor liberador de corticotropina (CRF) desempeña una misión cardinal en la respuesta al estrés, al regular el eje hipotálamo-hipófisis-suprarrenal (HHS). En respuesta al estrés, el CRF inicia una cascada de acontecimientos que culminan con la liberación de glucocorticoides por la corteza suprarrenal. Como consecuencia del elevado número de efectos fisiológicos y conductuales inducidos por los glucocorticoides, han surgido varios mecanismos para controlar la activación del eje HHS e integrar la respuesta al estrés. La inhibición por retroalimentación de los glucocorticoides contribuye decisivamente a regular la magnitud y la duración de su liberación. Además de esta retroalimentación glucocorticoidea, el eje HHS está regulado en el hipotálamo por un grupo diverso de proyecciones aferente de los núcleos límbicos, mesencefálicos y del tronco cerebral. La respuesta al estrés está mediada también, en parte, por las neuronas noradrenérgicas del tronco cerebral, los circuitos adrenomedulares simpáticos y los sistemas parasimpáticos. En resumen, el objetivo de esta revisión es exponer la importancia del eje HHS en la integración de las respuestas adaptativas al estrés. Asimismo, se señalan y describen brevemente los principales sistemas neuronales y endocrinos que contribuyen a la regulación del eje HHS y al mantenimiento de la homeostasis frente a los estímulos adversos.

Rôle de l'axe hypothalamo-hypophyso-surrénalien dans les réponses neuro-endocriniennes au stress

Les animaux répondent au stress en activant un large panel de réponses comportementales et physiologiques, collectivement considérés comme constituant la réponse au stress. Le facteur de libération de corticotrophine (CRF) joue un rôle central dans la réponse au stress en régulant l'axe hypothalamo-hypophyso-surrénalien (HPA). Dans la réponse au stress, le CRF déclenche une cascade d'événements qui aboutissent à la libération de glucocorticoïdes à partir du cortex surrénalien. Etant donné le grand nombre d'effets physiologiques et comportementaux produits par les glucocorticoïdes, plusieurs mécanismes se sont développés afin de contrôler l'activation de l'axe HPA et intégrer les réponses au stress. Le rétrocontrôle inhibiteur des glucocorticoïdes joue un rôle essentiel dans l'ampleur et la durée de leur libération. En plus de ce rétrocontrôle, l'axe HPA est régulé au niveau hypothalamique par différentes projections afférentes provenant du système limbique, du mésencéphale et des noyaux du tronc cérébral. La réponse au stress est également transmise en partie par les neurones noradrénergiques du tronc cérébral, les circuits sympathiques adrénomédullaires et le système parasympathique. En résumé, cet article a pour but d'examiner le rôle de l'axe HPA dans l'intégration des réponses adaptatives au stress. Nous avons aussi identifié et brièvement décrit les principaux systèmes neuronaux et endocriniens qui participent à la régulation de l'axe HPA et au maintien de l'homéostasie face à des agressions.

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Behavioral control, the medial prefrontal cortex, and resilience

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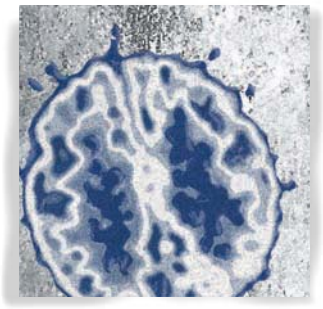

The degree of control that an organism has over a stressor potently modulates the impact of the stressor, with uncontrollable stressors producing a constellation of outcomes that do not occur if the stressor is behaviorally controllable. It has generally been assumed that this occurs because uncontrollability actively potentiates the effects of stressors. Here it will be suggested that in addition, or instead, the presence of control actively inhibits the impact of stressors. At least in part, this occurs because (i) the presence of control is detected by regions of the ventral medial prefrontal cortex (mPFCv); and (ii) detection of control activates mPFCv output to stress-responsive brain stem and limbic structures that actively inhibit stress-induced activation of these structures. Furthermore, an initial experience with control over stress alters the mPFCv response to subsequent stressors so that mPFCv output is activated even if the subsequent stressor is uncontrollable, thereby making the organism resilient. The general implications of these results for understanding resilience in the face of adversity are discussed.

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The experience of traumatic life events is an important factor in the development of a number of clinical conditions, ranging from anxiety disorders such as post-traumatic stress disorder (PTSD) to drug addiction. However, not all individuals who encounter stressful life events develop these disorders, and so there is considerable interest in understanding what makes an individual vulnerable, and what makes an individual resilient to the deleterious effects of traumatic events.[1] Genetic factors doubtlessly play a role, but aspects of the stress experience and complex cognitive factors regarding how the individual appraises or views that experience have been argued to be key. In humans, most studies of resilience have included the individual's perceived self-efficacy,[2] perceived ability to cope,[3] or actual ability to exert control over the stressor[4] as key variables. Furthermore, other factors, such as religious faith[5] and sociopolitical effectiveness,[3] have been argued to produce resilience because they induce a sense of control.

It is difficult to study variables such as these in animals, yet it is in animals that detailed neurobiological mechanisms can be explored. The stressor controllability paradigm, however, is one of the few that allows isolation of this type of process. Here, animals that receive stressors that are physically identical are compared, with one group having behavioral control over an aspect of the stressor (its termination), and the other group having no control. In our version of this paradigm, rats are placed in small boxes with a wheel mounted on the front. The

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Selected abbreviations and acronyms

5-HT	*serotonin*
CE	*central nucleus of amygdala*
CS	*conditioned stimulus*
DRN	*dorsal raphe nucleus*
ES	*escapable shock*
IS	*inescapable shock*
LC	*locus coeruleus*
mPFCv	*medial prefrontal cortex*
mPFC	*ventral medial prefrontal cortex*
PTSD	*post-traumatic stress disorder*
US	*unconditioned stimulus*

rat's tail extends from the rear of the box so that electrodes can be directly fixed to the tail. For one group of rats ("escape") each of a series of tailshocks terminate when the rat turns the wheel with its paws. Thus, this group has behavioral control over the termination of each tailshock. Each member of a second group ("yoked") is paired with one of the escape group and simply receives tailshocks of the same durations as determined by its partner; turning the wheel has no consequence. There are other stressors whose sequelae may well be due to the uncontrollability of the stressor (eg, social defeat), but since controllability cannot be readily manipulated in these paradigms, this cannot be determined. Indeed, this is why shock is used in our studies. We know of no other aversive event whose controllability can readily be manipulated in such a way that the subjects with and without control experience identical physical events.

Research conducted by numerous laboratories has revealed a constellation of behavioral changes that follow uncontrollable, but not controllable, shocks. Thus, rats exposed to uncontrollable shock later fail to learn to escape shock in a different situation (the so-called "learned helplessness" effect), are inactive in the face of aversive events (so-called "behavioral depression"), become less aggressive and show reduced social dominance, behave anxiously in tests of "anxiety" such as the social interaction test, are neophobic, develop ulcers, respond in exaggerated fashion to drugs of abuse, etc.[6] None of these outcomes follow if the organism is able to exert control over the stressor.

Prior research has focused on the neural mechanism(s) by which uncontrollable stress (inescapable shock, IS) leads to the above behavioral outcomes. Indeed, this can be said of most stress research in animals, since the stressors that are used (restraint, social defeat, cold water, etc) have almost always been uncontrollable. There has been very little work directed at understanding the mechanism(s) by which control confers protection from the effects of the stressor. Indeed, most experiments studying the neurobiology of stress do not even contain a group for whom the stressor is controllable—the typical comparison is between a group exposed to an uncontrollable stressor and a home cage control group. What is known is that uncontrollable stress produces sequelae that are not produced by physically identical controllable stress. It has been implicitly assumed that this difference occurs because the organism detects/learns/perceives that the uncontrollable stressor is uncontrollable, and that this sets in motion the neural cascade that mediates the behavioral outcomes. The unstated assumption has been that stress per se produces neural consequences that are then magnified by the detection/learning/perception of uncontrollability. That is, it has been assumed that uncontrollability is the "active ingredient." From this point of view, controllable stressors fail to produce outcomes such as exaggerated anxiety simply because they lack the active uncontrollability element. However, it is also possible that instead the *presence* of control is the "active ingredient." Here, the detection/learning/perception of control would *inhibit* neural responses to stressors. Of course, both could be true. As will become clear, this is not merely a semantic difference.

The purposes of the present paper are to review recent work suggesting that the presence of control does actively inhibit limbic and brain stem reactions to a stressor, and the mechanisms whereby this inhibition is achieved. It will be argued that the research that will be described provides insights into mechanisms that produce resilience in the face of adversity.

Serotonin and the dorsal raphe nucleus

As noted above, most of the research on stressor controllability has been directed at understanding how uncontrollable stress produces its behavioral outcomes, such as poor escape learning and exaggerated fear/anxiety. Different laboratories have focused on different brain regions and neurotransmitter systems. We have concentrated our efforts on the dorsal raphe nucleus (DRN). The DRN is the largest of the raphe nuclei and provides serotonergic (5-HT) innervation to much of the forebrain, as well as other structures. We originally stud-

ied the DRN as a potential critical mediator of the behavioral effects of IS because it projects to structures that are the proximate neural mediators of many of the behavioral sequelae of IS, and elevated 5-HT within these structures seemed to produce the appropriate behaviors. For example, the dorsal periaqueductal gray is a proximate mediator of escape behavior,[7] and it is innervated by the DRN. Moreover, stimulation of the DRN interferes with escape.[8] Analogous neural arrangements existed for many of the other behavioral consequences of IS, and so it seemed, a priori, as if the known behavioral consequences of IS would occur if IS were to differentially activate DRN 5-HT neurons. The DRN has proved to have a complex subnuclear organization, with different regions of the DRN receiving discrete sets of afferents and having different efferent projections.[9] Our work has implicated mid and caudal regions of the DRN as being critical to IS effects. All that needs to be noted here is that this work, as well as recent research from other laboratories,[10] has delineated a 5-HT system, projecting to a number of mesolimbic structures, that appears to be important in the mediation of anxiety-like behavior.[11] We[12] have argued that the changes produced by IS are much more related to anxiety than depression, and so the argument that what is involved is an exaggerated 5-HT response is not problematic.

The most relevant findings are the following: (i) IS produces a much greater activation of 5-HT neurons in the mid and caudal DRN than do exactly equal amounts and distributions of escapable tailshock (ES). This has been assessed both by an examination of Fos in 5-HT-labeled cells[13] as well as measurement of 5-HT efflux within the DRN[14] and projection regions of the DRN15 with in vivo microdialysis; (ii) This intense activation of 5-HT neurons leads to the accumulation of high extracellular levels of 5-HT within the DRN. This high concentration of 5-HT desensitizes/downregulates inhibitory somatodendritic 5-HT_{1A} receptors within the DRN for a number of days[16]; (iii) 5-HT_{1A} desensitization/downregulation within the DRN sensitizes DRN 5-HT neurons since this normal source of tonic inhibition is now reduced. Thus, for a number of days, stimuli that normally produce little or no 5-HT response now induce large 5-HT activation.[15] Behavioral testing conditions such as escape training, fear conditioning, etc, now lead to exaggerated 5-HT release in projection regions of the DRN, the proximate cause of the behavioral outcomes. It is known that DRN 5-HT activity is a cause of the behavioral outcomes of IS because lesion of the DRN[17] and selective pharmacological inhibition of 5-HT DRN neurons at the time of behavioral testing[18] completely block the behavioral effects of IS. In addition, pharmacological inhibition of DRN 5-HT activity at the time of IS prevents the usual behavioral outcomes of IS from occurring.[18] Finally, simply activating DRN 5-HT neurons, in the absence of any IS, produces the same behavioral outcomes as does IS.[19]

This focus on the DRN is not meant to suggest that other structures are not involved. For example, the work of J. Weiss (eg, ref 20) clearly implicates the locus coeruleus (LC). However, the behavioral effects of IS and other uncontrollable stressors must be mediated by a complex neural circuit, and the DRN is likely but one, albeit critical, part of the circuit. We believe that the DRN is a key integrative site on the efferent end of the circuit and receives inputs from multiple key structures. The LC can be viewed as one of these inputs.[21]

The medial prefrontal cortex

Although the work summarized above clearly implicates the DRN as a key site in the mediation of the behavioral effects of uncontrollable stress, the concept that it must be part of a more extended circuit naturally suggests the question of whether the DRN (or LC) could be the structure that detects/learns/perceives whether a stressor is, or is not, under behavioral control. The DRN is a small brain stem structure consisting of perhaps 30 000 neurons in the rat. Moreover, the DRN does not receive direct somatosensory input. Thus, it would appear to have neither the inputs required, nor the "processing power," to compute whether a stressor is controllable or uncontrollable. The circuitry that performs this analysis must have available to it information concerning exactly when motor responses occur and when the stressor begins and ends. Further, it must be able to compute the correlation between the two. We thus determined inputs to the DRN that mediate the effects of uncontrollable stress, and uncovered several (locus coeruleus, lateral habenula, and likely the bed nucleus of the stria terminalis [BNST]). However, none were themselves sensitive to stressor controllability—they simply provided excitatory drive to the DRN whenever a stressor was present, controllable or uncontrollable.[22]

In any case, the detection/computation of degree of control would seem likely to be a cortical function, and so

it is of interest to inquire into which regions of cortex provide monosynaptic inputs to the DRN. Interestingly, the DRN receives all, or virtually all, of its cortical inputs from infralimbic (IL) and prelimbic (PL) regions of the medial prefrontal cortex (mPFC).[23] The mPFC is involved with mediating "executive functions"[24]; functions that are consistent with behavioral control detection. Furthermore, the mPFC has been shown to be a key site in "contingency learning" as opposed to habit formation,[25] a process very close to control learning.

IL and PL regions, which comprise the ventral mPFC (mPFCv) send excitatory glutamatergic projections to the DRN.[26] However, within the DRN these pyramidal glutamatergic projections synapse preferentially onto γ-aminobutyric acid (GABA)-ergic interneurons that inhibit the 5-HT cells.[26] As would be expected from this anatomy, electrical stimulation of regions of the mPFCv that contain output neurons to the DRN leads to *inhibition* of 5-HT activity within the DRN.[27,28]

The fact that activation of mPFCv output to the DRN actively inhibits DRN 5-HT activity immediately suggests that if the mPFCv is indeed involved in control/lack of control detection, then perhaps it is really control that is the active ingredient, leading to mPFCv-mediated active inhibition of the DRN when it is present. Here the idea is that aversive stimulation per se drives the DRN, and when the presence of behavioral control is detected by the mPFCv, the DRN, and perhaps other stress-responsive limbic and brain stem structures (see below) are actively inhibited.

In our first attempt to test the role of the mPFCv, we inactivated the mPFCv during exposure to IS and ES by microinjecting muscimol into the region.[29] Muscimol is a GABA agonist, and so inhibits the activity of cells that express GABA receptors, such as the pyramidal output neurons. Inactivating the mPFCv did indeed eliminate the differential effects of controllability—that is, IS and ES now produced the same outcomes. However, mPFCv inactivation eliminated the IS-ES in a particular way. The presence of control was no longer protective, and now ES as well as IS produced later escape learning failure and exaggerated fear conditioning. Furthermore, ES now activated the DRN to the same degree as did IS. Inactivating the mPFCv did not make IS better or worse; it acted only in ES subjects to eliminate the protective effect of control. It is important to note that muscimol microinjection did not retard the learning of the wheel-turn escape response during ES by the ES subjects. That is, the ES subjects turned the wheel and terminated the tailshocks, but did not benefit from the experience. This is in keeping with data indicating that the mPFC is not involved in the learning of habits or motor responses, but rather in more complex cognitive aspects of behavior. Thus, when the mPFCv was inactivated the animals learned to turn the wheel, but this now did not lead to inhibition of the DRN. The DRN acted as if the stressor was uncontrollable, even though the rats turned the wheel and escaped normally!

The foregoing suggests that what is important is whether the mPFCv is activated during a stressor, not whether the stressor is actually controllable or not. To further test this idea, we directly activated the mPFCv during IS and ES. The mPFCv was activated by microinjection of the GABA antagonist picrotoxin, a procedure that has been shown to activate mPFCv output.[30] *Figure 1* shows the results of shuttlebox escape testing administered 24 hours after the ES and IS sessions, or home cage control treatment. Escape trials terminated automatically after 30 sec if the subject failed to escape on that trial, and so group means near 30 seconds indicate that most of the rats in the group completely failed to escape. In vehicle-injected subjects, IS interfered with later shuttlebox escape and ES did not, as is typical. Dramatically, IS produced no interference with escape at all if the mPFCv was activated during the IS with picrotoxin. These animals did not have a means to control shock during the initial stress

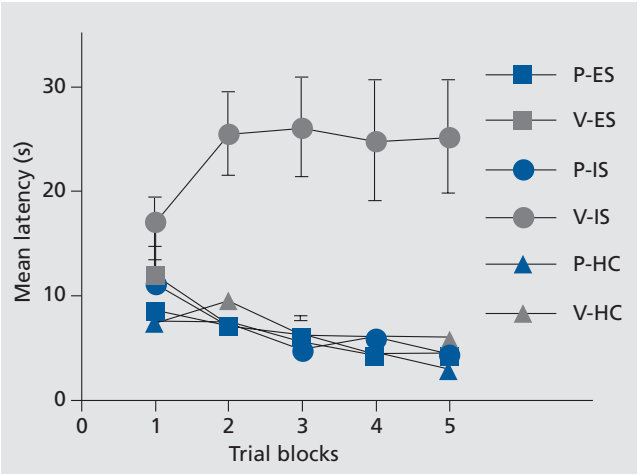

Figure 1. Mean latency to escape across blocks of five shuttlebox trials 24 h after experimental treatment. Experimental treatments were escapable shock (ES), yoked inescapable (IS), or home cage control (HC). P, picrotoxin before experimental treatment; V, vehicle

experience, but simply activating the mPFC during the stressor protected them. Importantly, the DRN was now not activated—it responded as if the shock was controllable (these data are not shown).

Behavioral immunization, resilience, and the mPFCv

In both humans and animals, an individual's early or initial experiences with stressors can determine how that individual reacts to subsequent stressful life experiences.[31] Many years ago, it was reported that an initial experience with controllable shock blocks the typical behavioral effects of a later exposure to uncontrollable shock, even if the two experiences occur in very different environments.[32,33] That is, an initial experience with control seemed to "immunize" the rat subjects.

This immunization phenomenon is very different than the usual effects of control that have been studied. In the typical experiment, the presence of control blunts the impact of the stressor that is occurring at that time. However, in the immunization paradigm, an initial experience with control blunts the impact of an uncontrollable stressor occurring at a later period of time.

This immunization phenomenon has not been studied at the neurobiological level. Clearly, the initial exposure to controllable stress would activate the mPFCv. It is our hypothesis that there is plasticity in this system so that mPFCv activity becomes associated with or "tied" to the stressor or some aspect of the stress experience such as fear/anxiety (see below). If this were so, then the mPFCv would become activated during the later uncontrollable stressor, thereby inhibiting the DRN and protecting the organism from outcomes that depend on DRN activation. During the past year we have begun to test this admittedly speculative hypothesis. *Figure 2* shows the results of an experiment in which rats received either ES, IS, or HC treatment on Day 1, and IS in a different environment 7 days later. Shuttlebox escape testing occurred 24 hours after the Day 8 IS. Either intra-mPFCv muscimol or vehicle microinjection preceded the Day 1 treatment. As is evident, the experience of ES 7 days before IS completely blocked the behavioral effect of IS. That is, behavioral immunization occurred. However, mPFCv inactivation during ES blocked the ability of ES to produce immunization. In a separate experiment, the mPFCv was inactivated at the time of the Day 8 IS rather than during ES on Day 1. This manipulation also blocked immunization (data not shown in the *Figure*). Thus, mPFCv activity is necessary for immunization, both at the time of the initial experience with control and the later exposure to the uncontrollable stressor for protection to occur.

The hypothesis being considered suggests that, as above, it is not control per se that is critical, but rather whether the mPFCv is activated during the initial experience with the aversive event. Thus, we conducted an identical experiment to the one just described, but activated the mPFCv with picrotoxin during the Day 1 stress session. *Figure 3* shows the shuttlebox escape latencies. ES, of course, produced immunization. Activating the mPFCv by itself, without the presence of a stressor (P-HC/IS) did not confer protection against the effects of IS. However, the combination of picrotoxin and IS produced immunization. That is, the experience of uncontrollable stress actually protected the organism if the mPFCv was activated during the experience.

Finally, if it is true that after an initial experience with control now even IS would activate the mPFCv, then the DRN should be inhibited during IS. *Figure 4* shows extracellular levels of 5-HT within the DRN during IS in animals that had received either IS, ES, or HC 7 days earlier. IS produced a large increase in 5-HT as usual, but this effect was virtually eliminated by prior ES. Here, the DRN acted as if the stressor were controllable. This result is analogous to an "illusion of control" at the neuro-

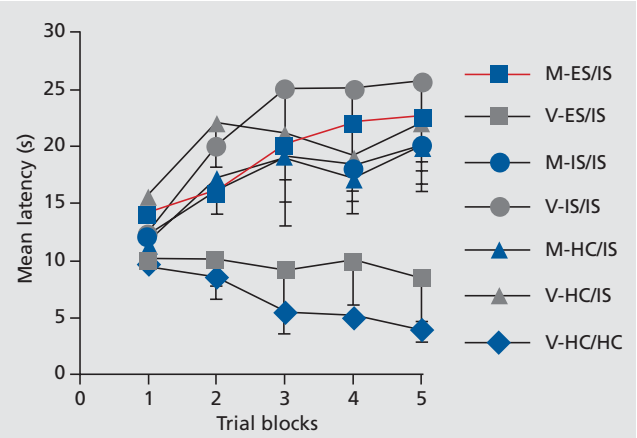

Figure 2. Mean latency to escape across blocks of five shuttlebox trials. Day 1 treatments were escapable shock (ES), yoked inescapable (IS), or home cage control (HC). All animals received inescapable shiock (IS) on Day 8. Escape testing occurred on Day 9. M, muscimol before day 1 treatment; V, vehicle

chemical level. Clearly, an initial experience with control promotes resilience in the face of later aversive stimulation, and does so by activating the mPFCv.

Fear conditioning and the amygdala

To this point we have focused on the interaction between the mPFCv and the DRN, with control leading to protection against the effects of aversive events by increasing mPFCv inhibition of the DRN. However, the mPFCv projects to other stress-responsive structures as well. The amygdala is of special interest in this regard. The amygdala is a key site in the mediation of fear and anxiety. Its role in fear conditioning is well known, and fear conditioning has been argued to be a key process in the development of a number of anxiety disorders.[34] The work of numerous investigators has suggested the following scenario (see ref 35 for a review). Inputs from neutral stimuli (the conditioned stimulus [CS], eg, a tone) and aversive stimulation (the unconditioned stimulus [US], eg, a footshock) converge in the lateral amygdala (LA) where the association between the CS and US is formed by an *N*-methyl-D-aspartate (NMDA)/long-term potentiation (LTP)-dependent process. Expression of conditioned fear involves CS transmission to the LA, connections from the LA to the central nucleus of the amygdala (CE) either directly or indirectly via the basal nucleus, and then output connections from the CE to regions of the brain that are the proximate mediators of the specific aspects of fear responses (autonomic, endocrine, and behavioral). This is an oversimplified scheme (eg, 36, 37), but it nevertheless captures a large amount of data.

In the present context, it is interesting to note that the mPFCv projects to the amygdala,[38] and stimulation of the mPFCv has been reported to inhibit the increase in electrical activity in the LA produced by an already conditioned fear stimulus, as well as the fear response to that stimulus, and to prevent the association between CS and US when they are paired.[39] Similarly, Quirk et al[40] found that mPFCv stimulation reduces output from the CE in response to electrical stimulation of input pathways to the CE, and Milad et al[41] found mPFCv stimulation to reduce fear responses produced by a fear CS. Although the exact projections of the mPFCv to the amygdala responsible for the inhibition of fear conditioning and fear responses resulting from mPFCv stimulation are unclear, the mPFCv does project to the intercalated cell mass (ITM) within the amygdala. These cells are almost all GABAergic, and project to the CE, providing an obvious pathway by which mPFCv activation could inhibit the CE.[42] Indeed, Berretta et al[30] found that stimulation of the mPFCv with picrotoxin increases Fos expression in the GABAergic cells of the ITM.

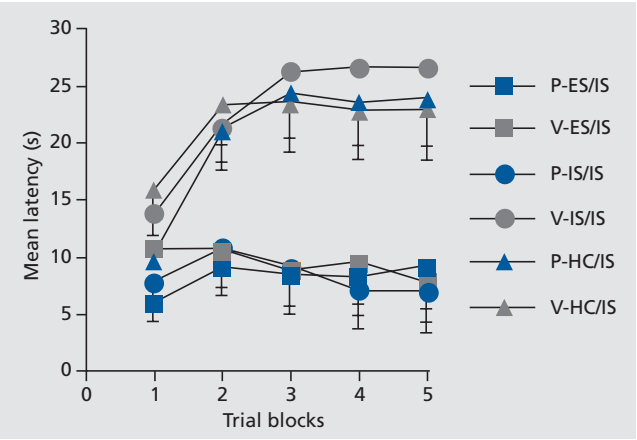

Figure 3. Mean latency to escape across blocks of five shuttlebox trials. Day 1 treatment s were escapable shock (ES), yoked inescapable (IS), or home cage control (HC). All animals received inescapable shock (IS) on Day 8. Escape testing occurred on Day 9. P, picrotoxin before experimental treatment; V, vehicle

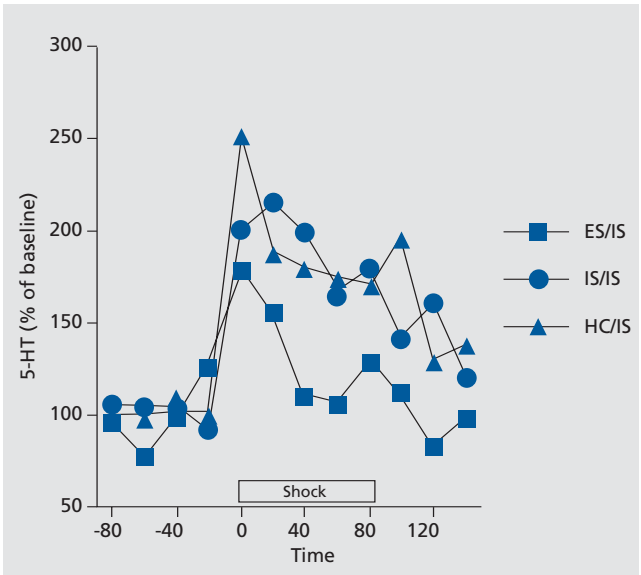

Figure 4. Extracellular levels of serotonin (5-HT) within the dorsal raphe nucleus (DRN), as a percentage of baseline, before, during, and after inescapable shock (IS). Separate groups received either escapable shock (ES), yoked inescapable (IS), or home cage control (HC) 7 days earlier.

The foregoing suggests that any factor that increases mPFCv output to the amygdala should reduce fear. We have reviewed research that suggests that behavioral control increases mPFCv output to the DRN, thereby reducing DRN-driven behavioral changes. Perhaps this phenomenon is more general, and control also increases mPFCv output to the amygdala, thereby inhibiting CE function and fear. Consistent with this possibility, it is already known that ES leads to the conditioning of less fear to cues that are present than does IS. However, the possibility being considered here makes an even stronger prediction. Recall that an initial experience with ES protected the organism against the effects of subsequent IS, the argument having been that the original experience led the later IS to now activate the mPFCv. The idea was that the initial ES experience "tied" mPFCv activation to shock, or to something associated with or produced by shock. What if that "something" is fear? If this were so, then an initial experience with ES should actually interfere with fear conditioning conducted some time later in a different environment.

To begin to explore these ideas, we first gave rats ES or yoked IS in wheel turn boxes, or HC treatment. Seven days later the rats received fear conditioning in a standard gridbox chamber. A tone was paired with gridshock, and the level of conditioning to the tone and to the environmental context was measured 2 days later. Freezing to the context was used as the measure of conditioning to the context. The rats were simply placed in the fear conditioning chamber for 5 min and freezing assessed. To assess fear conditioned to the tone, the rats were placed in a novel chamber and freezing measured for 3 min. The tone was then sounded for 3 min. *Figure 5* shows the results. First, it should be stated that there was virtually no freezing at all on the conditioning day before the first footshock. Thus, the freezing observed on the test day was the result of conditioning, not some aftereffect of the earlier IS or ES. The results for fear conditioned to the context are on the left. IS 7 days before fear conditioning exaggerated fear conditioning, a result that was already known.[43] In contrast, prior ES *retarded* fear conditioning. The results for conditioning to the tone, shown on the right, were similar. These results are dramatic, as ES is itself quite "stressful" and is not somehow "negative stress." Indeed, the ES conditions used here produce a hypothalamo-pituitary-adrenal response that is as large as that produced by IS.[44,45] We know of no other position that would predict, or even explain, how exposure to a highly stressful event could retard the later development of fear.

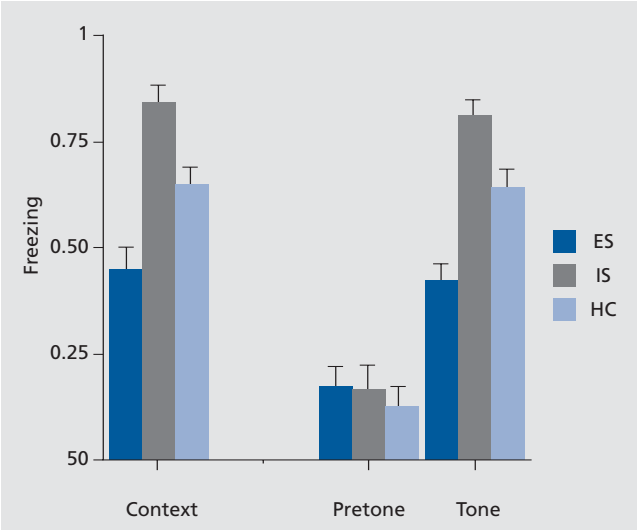

Figure 5. Percentage of the observation intervals on which freezing occurred during testing for fear conditioning. Testing was 24 h after conditioning. Groups received either escapable shock (ES), yoked inescapable (IS), or home cage control (HC) 7 days before fear conditioning. Data on the left shows freezing in the context in which conditioning had occurred. Data on the right shows freezing before and during the tone that had been paired with shock, with testing occurring in a novel context.

Clearly, much more work is needed, but it may be that experiences of control produce resilience in the face of circumstances that induce fear. The amygdala is importantly involved in fear-related processes that go beyond the conditioning of fear to anxiety more generally. It thus may be that experiences of control, and other circumstances that might activate the mPFCv, confer resistance to the development of anxiety.

Conclusions and clinical implications

The general conclusion to be reached is that control is not detected or computed by brain stem structures such as the DRN, but rather by circuitry within the mPFCv. Stress or aversive stimulation per se would seem to activate structures such as the DRN, with this activation then being inhibited by input from the mPFCv if behavioral control is present. This arrangement might make good evolutionary sense. Primitive organisms possess only a limited behavioral capacity to deal with threats, and in such species adaptations and responses to threats are largely physiological in nature. For these types of species

behavioral control and other methods of psychological coping are largely irrelevant, and so it may make sense that more primitive parts of the brain that are involved in responding to threats are themselves insensitive to dimensions such as behavioral controllability. As organisms became more complex, behavioral methods of coping became possible. Under circumstances in which a threat can be dealt with behaviorally, it would be adaptive to inhibit or reduce the more physiological adaptive mechanisms since they can be costly in various ways.[46] Of course, more recently evolved "higher" regions of the brain such as the mPFC would have taken this function. It is also possible that a lack of control might weaken the inhibitory control exerted by the mPFC. The experiments discussed above were not well suited to detecting effects in this direction given possible "ceiling effects." Indeed, we have some evidence that uncontrollability might exert this sort of effect, but it is too preliminary to present.

Although our evidence is limited, it further suggests that initial experiences with stressors can bias the system such that the mPFCv responds to later stressors as it did to earlier stressors. If this plasticity proves to be real, then this would constitute a mechanism of resilience. The fear conditioning data presented above suggests that this mechanism may generalize broadly, with control over tailshock generalizing to fear conditioning. Thus, experiences with control may be broadly protective. Of course, there is no reason to believe that behavioral control is unique, and there are likely other aspects of experience that would activate mPFCv inhibition of stress-responsive limbic and brain stem structures.

The research and theorizing presented here articulates well with the recent clinical literature. Abnormalities in mPFC function have been detected in disorders ranging from depression[47] to PTSD.[48] Imaging studies of PTSD are especially illuminating in the present context, since they typically measure both amygdala and mPFC function. Not surprisingly, PTSD patients show substantial amygdala activation to stimuli related to the events that caused the disorder. Thus, combat veterans with PTSD show exaggerated amygdala activation to war scenes, relative to non-PTSD controls.[48] Interestingly, they also show exaggerated amygdala activity to fear stimuli unrelated to combat, such as fearful faces.[49] However, PTSD patients have *reduced* mPFC activity in response to these stimuli,[48-50] and this often correlates with the degree of disorder. It is possible that there is exaggerated amygdala activation in PTSD because there has been a loss of mPFC inhibition of the amygdala. Many of the events that induce PTSD are ones over which the individual has little behavioral control. Not all of the individuals who experience these events develop PTSD, and it may be that earlier experiences with control or other forms of coping protect against the development of the disorder by biasing the mPFC to respond actively, thereby maintaining inhibition of the amygdala, and perhaps other stress-responsive structures. ❑

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Control comportamental, corteza prefrontal medial y resiliencia

El grado de control que ejerce un organismo sobre un factor estresante modula poderosamente la repercusión de éste; los elementos incontrolables generadores de estrés determinan una constelación de resultados que no se daría si esos factores pudieran controlarse comportamentalmente. En general, se ha admitido que esto ocurre porque la falta de control potencia de una manera activa los efectos de los elementos estresantes. Aquí se propone como tesis complementaria o alternativa que la presencia del control inhibe activamente la repercusión de estos elementos. Esto sucede, al menos en parte, porque i) las regiones de la corteza prefrontal ventromedial detectan el control e ii) la detección del control activa las eferencias de la corteza prefrontal ventromedial hacia el tronco encefálico y las estructuras límbicas, que responden al estrés lo que inhibe fuertamente la activación de estas estructuras inducida por el estrés. Es más, la experiencia inicial de control del estrés modifica la respuesta de la corteza prefrontal ventromedial a los factores estresantes subsiguientes, de manera que las eferencias de la corteza prefrontal ventromedial se activan, aun cuando el elemento estresante posterior resulte incontrolable, con lo que el organismo adquiere resiliencia. Se comentan las implicaciones generales de estos resultados para entender la resiliencia frente a la adversidad.

Contrôle comportemental, cortex médian préfrontal et résilience

Le degré de contrôle qu'un organisme exerce sur un facteur de stress module fortement l'impact de ce dernier. Les facteurs de stress incontrôlables engendrent un cortège de comportements qui ne se produiraient pas si le facteur de stress pouvait être maîtrisé. L'absence de contrôle est connue pour potentialiser fortement les effets des facteurs de stress. A contrario, ainsi qu'il l'est suggéré dans cet article, la présence d'un contrôle inhibe de manière active l'impact des facteurs de stress. Ceci survient au moins du fait de deux facteurs 1) la présence du contrôle est détectée au niveau des régions du cortex préfrontal médioventral (mPFCv) ; et 2) cette détection active les efférences du mPFCv vers le tronc cérébral et les structures limbiques sensibles au stress inhibant fortement leur activation due au stress. De plus, une première expérience de stress contrôlé modifie la réponse du mPFCv face aux agressions ultérieures, si bien que l'efférence du mPFCv est activée même si le facteur de stress suivant reste incontrôlable, rendant de ce fait l'organisme résilient. Les implications générales de ces résultats pour comprendre la résilience face aux agressions vont être examinées dans cet article.

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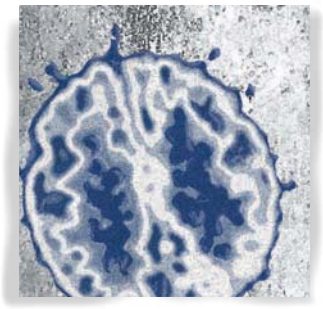
Angst and the amygdala

Jay Schulkin, PhD



Fear is an adaptation to danger, but excessive fear underlies diverse forms of mental anguish and pathology. One neural site linked to a sense of adversity is the amygdala, and one neuropeptide, corticotropin-releasing hormone (CRH), is localized within the central nucleus of the amygdala. Glucocorticoids enhance the production of CRH in this region of the brain, resulting in increased attention to external events and, when sustained for longer periods of time, perhaps contributing to anxious depression.

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Fear, as the perception of danger, is an adaptive response, and fundamental in problem-solving and survival. In fact, fear is an emotion that likely evolved as part of problem-solving.[1] Appraisal mechanisms which discern danger become overactive, leading to increased perception of fear, which then leads to anxious thought, and perhaps to endless gloom.[2,3] In psychological terms, both anxious and depressive states have a common core of heightened negative affect,[4] a product of overactivity of the neural systems that underlie fear[3,5] and that contribute to a number of affective disorders.[6] While fear is a central state of the brain, changes in heart rate, blood pressure, respiration, facial muscles, and catecholamines, both peripheral and central, all influence the state of fear.[3,5]

One should note at the outset that fear, of which there are several kinds (conditioned fear, fear of unfamiliar objects, fear to sensory stimuli, etc[7]), is more than amygdala function, and amygdala function is more than fear[8,9]; however, fear is one thing in which the amygdala participates, and exaggerated amygdala activation creates a vulnerability to affective disorders.[6,10,11]

Anatomical considerations about the amygdala

Regions of the amygdala receive and send information from both cortical and subcortical regions.[12-14] More specifically, the basolateral complex is comprised of the lateral, basal, and accessory basal nuclei, which are richly innervated by neocortical and subcortical uni- and polymodal sensory regions,[13-15] which then relay information to the central nucleus of the amygdala.[16] Intra-amygdala connectivity is widespread.[13,14]

Selected abbreviations and acronyms

ACTH	*adrenocorticotropic hormone*
BNST	*bed nucleus of stria terminalis*
CRH	*corticotropin-releasing hormone*
HPA	*hypothalamic-pituitary-adrenal*
PTSD	*post-traumatic stress disorder*
PVN	*paraventricular nucleus*

The central nucleus projects to numerous nuclei in the midbrain and brain stem to orchestrate the rapid and primary behavioral, autonomic, and endocrine responses to threat and danger.[3,5,17] The central nucleus also receives visceral information from brain stem sites that include the solitary and parabrachial nuclei[18] and reciprocally projects to these brain stem regions (eg, ref 19). Regions of the amygdala directly project to the nucleus accumbens, which led investigators[20,21,22] to suggest an anatomical route by which motivation and motor control action are linked in the organization of active behavior (see also refs 21-25).

In addition to projections from the central nucleus of the amygdala to midbrain and brain stem targets important for mounting quick behavioral, autonomic, and endocrine responses to danger, the amygdala projections to the cortex and subcortical structures are also quite extensive.[13,14] In rat, the sources are the lateral, basal, and accessory basal nuclei, and their projections are fairly restricted to the multisensory temporal lobe structures (perirhinal, pyriform, and entorhinal cortices) and prefrontal cortex.[26] In primate brain, the primary visual cortex also receives input from the amygdala.[12] These cortical structures also contribute the heaviest cortical input to the amygdala, suggesting that many of the connections between the amygdala and cortex are reciprocal. This is particularly the case with the amygdala and prefrontal cortex, both anatomically[12,26] and functionally (for review see refs 27, 28).

In addition to the basolateral nucleus of the amygdala, the central nucleus of the amygdala also plays a unique role in conditioned fear.[3,5] The basolateral complex of the amygdala, with its rich afferents from the thalamus and cortical regions, is neuroanatomically situated to connect information about neutral stimuli with those that produce pain or are harmful.

The central nucleus can orchestrate behavioral responses related to fear via its direct connections to numerous midbrain and brain stem regions and circuits instantiating various fear-related behaviors.[17,29-31] Thus, the central nucleus of the amygdala, via its projections to lower brain, orchestrates behavioral (freezing[5,17]), autonomic, and endocrine responses to fear, while efferents of the basal nucleus of the amygdala participate in active avoidance behaviors to fear,[23,32,33] likely through basal ganglia.

The bed nucleus of the stria terminalis (BNST) is anatomically linked to the central/medial amygdala[34] and is also distinguished from the basolateral complex as being part of an autonomic brain system.[25] Importantly, the central nucleus and the BNST are not only the major efferent sources of input to midbrain and brain stem targets controlling autonomic responses to fear, but are the main recipients of autonomic information from the nucleus of the solitary tract and parabrachial nucleus.[13,19,35]

Corticotropin-releasing hormone (CRH) is one of the cell groups (neuropeptides) richly expressed in the central nucleus of the amygdala and in the lateral BNST, and therefore is of special interest, as it is tied to all of these behavioral and autonomic events (see below).

There are reasonable conceptual issues of what defines the amygdala,[25,36] and the ultimate basis for deciding what is amygdala is still open to investigation (eg, the extent to which the amygdala is part of the striatum and/or the larger cortical areas, the link to the BNST). There is little doubt that the amygdala is importantly involved in diverse forms of motivated behaviors (eg, fear) and their aberration during pathological states.

Fear, uncertainty, unfamiliar objects, and the amygdala

Humans with damage to the amygdala have impaired fear-related behavior and autonomic responses to conditioned stimuli (eg, refs 37-41). Also, positron emission tomography (PET) imaging studies in normals have shown greater activation of the amygdala during fear and anxiety-provoking stimuli than during presentation of neutral stimuli.[42] Such PET studies have revealed that the amygdala is activated when presented with fearful, unfamiliar, and uncertain faces.[2,43,44] With the use of functional magnetic resonance imaging (fMRI), it has further been shown that the amygdala is activated and then habituates when subjects are shown fearful faces but not when they are shown neutral or happy faces[45,46]; however, the amygdala is also responsive to a variety of facial responses.[47,48] A number of studies have also demonstrated that anxiety disorder patients have excessive activation in the amygdala when presented with stimuli that provoke anxiety attacks.[6,10,27]

CRH expression and the brain

One cell group within the amygdala (and the primary focus of this review) and elsewhere in the brain is CRH,[24,49,50] which is well known to be both a peptide that regulates pituitary and adrenal function and an extrahypothalamic peptide hormone linked to a number of behaviors, including behavioral expressions of fear.[51-53]
CRH cell bodies are widely distributed in the brain.[49,50] The majority of CRH neurons within the paraventricular nucleus (PVN) are clustered in the parvicellular division. Other regions with predominant CRH-containing neurons are the lateral BNST and the central division of the central nucleus of the amygdala.[49,54] To a smaller degree, there are CRH cells in the lateral hypothalamus and the prefrontal and cingulate cortex. In brain stem regions, CRH cells are clustered near the locus coeruleus (Barrington's nucleus), parabrachial region, and regions of the solitary nucleus.[49,50,55,56]
The CRH family has at least two receptors, CRH_1 and CRH_2, localized in rodent and primate brain (eg, refs 57-60). Activation of both the CRH_1 and CRH_2 receptors is linked to a G protein, and activates adenylate cyclase cascade and an increase in intracellular cyclic adenosine monophosphate (cAMP) and calcium levels; CRH appears to bind primarily to CRH_1 receptors.[60,61]
The distribution of CRH_1 receptor sites includes regions of the hippocampus, septum, and amygdala (medial and lateral region) and neocortex, ventral thalamic, and medial hypothalamic sites; sparse receptors are located in the PVN and the pituitary gland. The distribution is widespread in cerebellum in addition to brain stem sites such as major sensory nerves and the solitary nucleus.[62,63]
The distribution of CRH_2 receptors is more limited than that of CRH_1 receptors and is found primarily in subcortical regions including the amygdala, septum, BNST, and PVN and ventral medial nucleus of the hypothalamus.[63,64]

Differential regulation of CRH by glucocorticoids

Glucocorticoids are importantly involved in the restraint of CRH production in regions of the PVN.[65,66] This negative feedback is a fundamental way in which the hypothalamic-pituitary-adrenal (HPA) axis is restrained during stress and activity.[67] Glucocorticoids directly control neuronal excitability.[68] Some of the glucocorticoid effects on the brain are quite rapid, suggesting that corticosterone has nongenomic membrane effects via γ–aminobutyric acid(GABA)-ergic mechanisms.[69] Neurons within the lateral BNST and within the PVN may activate or inhibit PVN function via GABAergic mechanisms.[70,71]
While the profound effect of inhibition is indisputable, there are neuronal populations within the PVN that project to the brain stem that are not inhibited by glucocorticoids, and the activity of which is actually enhanced.[66,72] That is, CRH neurons en route to the pituitary are restrained by glucocorticoids, but CRH en route to other regions of the brain appears not to be restrained.[66,73-75] Moreover, the activity of extrahypothalamic regions of the brain in which CRH is expressed (central nucleus of the amygdala or lateral BNST) is actually increased by glucocorticoid hormones.[54,66,75,76]

CRH, glucocorticoids, and fear-related behaviors

Central CRH activation has been consistently linked to the induction of fear, uncertainty, unfamiliarity, and uncontrollability in animal studies.[9,52,53,77-79] Central infusions of CRH induce or potentiate a number of fear-related behavioral responses,[80] and infusion of CRH antagonists both within and outside the amygdala reduce fear-related responses.[52,81] One study, for example, reported that injection of a CRH antagonist into the basolateral complex of the amygdala, one of the regions in the amygdala which contains glucocorticoid receptors,[82] immediately following footshock diminished retention of aversive conditioning in an inhibitory avoidance task.[32] It was also shown in this study that the expression of CRH in the central nucleus of the amygdala increased 30 minutes following footshock. The results indicated that, similar to glucocorticoids and norepinephrine magnifying memory,[33] CRH in the amygdala modulated learning and memory for aversive events.[83]
While glucocorticoids are essential in the development of fear,[84] perhaps by the induction of central CRH, glucocorticoids, and CRH both play a larger role in the organization of behavior.[85-87] Nonetheless, glucocorticoids are secreted under a number of experimental conditions in which fear, anxiety, novelty, and uncertainty are experimental manipulations.[9,78,88-90] In contexts where there is loss of control, or the perception of a loss of control (worry is associated with the loss of control), glucocorticoids are secreted. This holds across a number of

species, including humans; perceived control reduces the levels of glucocorticoids.[88] These findings are congruent with those of Curt Richter[91] who observed an enlarged adrenal gland in stressed, fearful wild rats when compared with unstressed laboratory analogs.

Glucocorticoids in the basolateral complex of the amygdala appear to be necessary for aversive and fear conditioning. For example, injection of the glucocorticoid receptor antagonist RU-486 into the basolateral complex of the amygdala will reduce the consolidation of aversive conditioning[92] in addition to other forms of conditioning, including contextual fear.[93] Other experiments have shown that glucocorticoid injections into the amygdala can facilitate aversive conditioning.[33] Experiments like these, which use post-training injection procedures, demonstrate that glucocorticoids are necessary for consolidation of the memory of aversive conditioning and may facilitate the memory process.[94,95]

Glucocorticoid levels impact on learned fear.[94-97] For example, in one study rats received conditioning trials in which the unconditioned stimulus (footshock) was presented concurrently with the conditioned stimulus (auditory tone). For several days after conditioning the rats were treated with corticosterone; conditioned fear-induced freezing was enhanced.[96]

Corticosterone, by the induction of central CRH expression, facilitates fear-related behavioral responses.[76] Thus, in one study looking at contextual fear conditioning, groups of rats that were chronically treated with corticosterone displayed more fear conditioning than the vehicle-treated rats. Glucocorticoid antagonists disrupt contextual fear conditioning.[94,95] Thus, the data suggest that repeated high levels of corticosterone can facilitate the retention of contextual fear conditioning, perhaps by the induction of CRH gene expression in critical regions of the brain such as the amygdala.

Importantly, amygdala infusion of corticosterone aimed at the central nucleus also increases milder forms of anxiety as measured with rats in the elevated plus maze.[98] Shepard et al have, furthermore, demonstrated that implants of corticosterone resulted in an increase in CRH expression in the central nucleus of the amygdala. In addition, the corticosterone implants to the central nucleus of the amygdala increased levels of CRH expression in the dorsal lateral BNST[99] and administration of the type 1 CRH receptors decreased this fear-related response.[100] In other tests, pretreatment with the type-l receptor CRH antagonist ameliorated fear-inducing events, or reactivity to the events,[100] (see also refs 101-103 for the role of the CRH type-1 receptor; and 104, 105 for the role of the type II receptor).

Furthermore, Cook demonstrated that the CRH response in the amygdala of sheep to a natural (dog) and unnatural (footshock) adversity is regulated by glucocorticoids.[106] Following acute exposure to the dog, for example, amygdala CRH had a large increase during exposure to the dog and a second peak corresponding to the increase in cortisol. Administration of a glucocorticoid receptor antagonist blocked the second CRH peak in the amygdala without affecting the first peak.

There is a body of evidence suggesting that the BNST may be important for unconditioned fear[107] and that perhaps CRH plays an important role.[83] Lesions of the BNST do not interfere with conditioned fear-related responses, unlike lesions of regions of the amygdala which interfere with fear-potentiated startle or conditioned freezing.[108,109] However, inactivation of the BNST can interfere with unconditioned startle responses[109] and with longer-term CRH effects on behavior.[109] High chronic plasma levels of corticosterone in adrenally intact rats facilitated CRH-induced startle responses.[110] Perhaps what occurs normally is that the glucocorticoids, by increasing CRH gene expression, increase the likelihood that something will be perceived as a threat, which results in a startle response.

Lesions of the BNST also interfere with unconditioned freezing of rats to a fox odor,[111] while amygdala lesions do not.[11,112] Corticosterone can potentiate freezing to predator odor,[113] (Rosen et al, unpublished observations). Perhaps the BNST may be linked to CRH-facilitated unconditioned adaptive anxiety and to general anxiety associated with drug abuse and to symptoms associated with pathological generalized anxiety disorder.[114-116]

Depression, anxiety, CRH, cortisol, brain

A genetic predisposition for a hyperactive amygdala has long been thought to result in a vulnerability to exaggerated fear and perhaps anxiety/depression.[11,117] There is a substantial number of findings of increased activity in the amygdala of depressive patients.[27,44,118] correlating with negative affect in other medication-free depressives[119] and patients suffering from a number of anxiety disorders.[2] In addition, a finding in depressive patients, particularly in those with comorbid anxiety, is hypercortisolemia.[120-122] Interestingly, antiglucorticoids are, in a

number of contexts, reported to ameliorate depressive symptoms,[123,124] which perhaps results in a reduction in central CRH expression. Importantly, depressive patients tend to have higher levels of CRH in cerebrospinal fluid than normal controls.[125-129] There is some evidence that TYPE 1 receptor regulation can impact on depression.[130]

One study has found a significant positive correlation between activity in the amygdala measured by PET and plasma cortisol levels in both unipolar and bipolar depressives.[118] Interestingly, patients with major depression show exaggerated responses in the left amygdala to sad facial expressions.[131,132] Acute infusions of cortisol in normal patients resulted in exaggerated amygdala responses to sad faces.[46]

This correlation may reflect either the effect of amygdala activity on CRH secretion or cortisol actions directly in amygdala. It is intriguing to speculate that the findings that patients with a first episode of depression have an enlarged amygdala[133] may be due to increased chronic levels of glucocorticoids and blood flow in the amygdala.[134] Interestingly, fearful anxious children in whom cortisol was elevated in development[117,135] also display a hyperactive amygdala to social performance as adults.[11]

Importantly, there is evidence of increased dendritic hybridization in amygdala and decreased dendritic hybridization of the hippocampus in animals under duress.[136] Glucocortiocoids are known to produce morphological changes in brain, typically decreases in hippocampal and prefrontal neurons' dendritic trees.[137,138] Moreover, studies have linked increased glucocorticoid production to changes in neuronal morphology in the basolateral complex of the amygdala following repeated stress[136,139] and such changes in plasminogen activator in cell bodies within the amygdala promotes corticotropin-releasing factor (CRF) activity; the administration of antalarmin, a CRF TYPE 1 antagonist, does the converse.[140]

An fMRI study reported that, whereas the amygdala in both normals and depressives responded to aversive stimuli, the amygdala response of normals habituated quickly while the familial depressives' amygdala remained active significantly longer.[141] Whether CRH and cortisol are involved in the sensitized responses awaits further study. We do know that in animal studies, increased CRH increases the salience of familiar incentives[9,87,142] and perhaps glucocorticoids magnify the CRH effect.[83,85,142]

Data on anxiety also indicate that the amygdala and cortisol are interactive in several anxiety disorders and for which cortisol, and the return to normal function, may be therapeutic.[143] Although the research has developed along two separate paths, activity in the amygdala in a number of different anxiety disorders has been shown to be highly reactive to triggers that evoke anxious reactions[2,6] and the HPA axis is hyper-responsive in anxiety disorders, particularly post-traumatic stress disorder (PTSD).[144-46] PTSD patients also have high norepinephrine/cortisol ratios[144,147] In research on cortisol measures, PTSD patients have basal hypocortisolemia but increased reactivity of the HPA axis to cortisol, suggesting that CRH and adrenocorticotropic hormone (ACTH)-secreting cells are sensitized to cortisol in PTSD patients.[145] Indeed, CRH has been found to be elevated in cerebrospinal fluid of PSTD patients.[147,148]

PTSD patients have normal resting (nonprovoked) levels of amygdala activity, but the amygdala is highly responsive to anxiety provocation.[149-152] While most of these studies do not demonstrate an abnormal response of the amygdala per se, particularly because normal humans also demonstrate increased amygdala activity to fearful or aversive stimuli (however, they do suggest that the amygdala has a lower threshold for responding to fearful stimuli in anxiety disorder patients).[153]

While focus here has been on the amygdala and, to a lesser extent, on the BNST, a fundamental part of fear circuitry is the prefrontal cortex (eg, refs 27,154,155). The medial prefrontal cortex (mPFC), for example, plays a role in inhibition of fear responses and extinction.[154,156]

There is evidence that regions of the prefrontal cortex regulate glucocorticoid responses to duress.[157-159] The prefrontal cortex has relatively dense expression of glucocorticoid receptors in most regions, including the infralimbic cortical areas and CRH neurons are also located in most regions of the prefrontal cortex,[49,50] Rosen and Schulkin, unpublished data. Chronic glucocorticoid treatment has been shown to alter apical dendrites of medial prefrontal neurons.[137]

Conclusions

Although the amygdala has been known to be involved in the emotion of fear since the seminal studies of Kluver and Bucy[160] showed a taming effect of amygdala lesions in monkeys, research in the last two decades has produced great advances in determining the neuroanatomy of fear

circuits. Not only has the amygdala been found to be critical for many types of fear, but fear circuits that connect the amygdala to many other brain regions have been described, which suggests that these circuits have evolved to function as neurobehavioral systems for particular kinds of cognitive and behavioral strategies. Understanding the neural circuitry that underlies fear/anxiety leads one to be in a better position for clinical judgment about treatment for states such anxious depression.

Normal fear is an adaptation to danger; chronic anxiety and depression are the overexpression of the neural systems involved in adaptation to danger. Coping with anxious depression is metabolically expensive; expectations of adversity predominate. Moreover, anxious depression is a condition in which there can be both high systemic cortisol and elevated CRH in the cerebrospinal fluid[118,125,161,162] Anxious depressed patients also tend to have increased glucose metabolic rates in the amygdala.[118,134] The cortisol that regulates CRH gene expression in the amygdala may underlie the fear and anxiety of the anxiously depressed person.[3,85] The exaggerated amygdala response that can occur because of life events and genetic predisposition (eg, refs 11, 77, 90, 129) contributes to the anxious/depressed person's altered perception and experience of the world, leading to a chronic sense of anticipatory angst. ❑

Angustia existencial y la amìgdala

El miedo es una adaptación al peligro pero el miedo excesivo es la expresión de diversas formas de angustia y enfermedad mentales. Una localización neural relacionada con el sentido de la adversidad es la amígdala; el neuropéptido hormona liberadora de corticotropina (CRH), se localiza en el núcleo central del cuerpo amigdalino. Los glucocorticoides refuerzan la producción de CRH en esta región cerebral, con lo que aumenta la atención a los acontecimientos externos y, si se sostiene durante largos períodos, puede contribuir a la depresión ansiosa.

Angoisse existentielle et amygdale

La peur est une adaptation au danger, mais une peur excessive est à l'origine de diverses formes d'angoisse et de pathologies. L'amygdale est un site cérébral traitant le concept d l'adversité. La CRH (corticotropin-releasing hormone) est un neuropeptide situé dans le noyau central de l'amygdale. Les glucocorticoïdes augmentent la sécrétion de CRH dans cette région du cerveau, conduisant ainsi à une attention accrue aux événements extérieurs. En se pérennisant sur de plus longues périodes, cette sécrétion pourrait contribuer au trouble anxio-dépressif.

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Experimental models of stress

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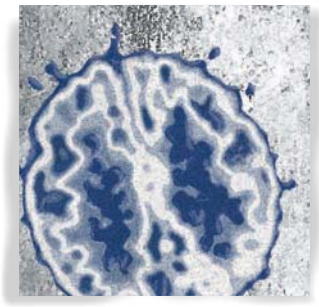

Illustrating the complexity of the stress response and its multifaceted manifestations is the leading idea of this overview of experimental paradigms used for stress induction in laboratory animals. The description of key features of models based on naturalistic stressors, pharmacological challenges, and genomic manipulations is complemented by comprehensive analysis of physiological, behavioral, neurochemical, and endocrine changes and their appropriateness as outcome readouts. Particular attention has been paid to the role of sex and age as determinants of the dynamics of the stress response. Possible translational applications of stress-inducing paradigms as models of disease are briefly sketched.

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Stress comprises mobilization of basic physiological repertoires for coping with adversity and restoring homeostasis; inappropriate strain on this arsenal, with respect to either magnitude or duration of the response, precipitates measurable pathological aberrations in several systems of the organism.[1-4]

After more than six decades of research, virtually every aspect of the organism's responses to stress has been addressed, and numerous end-point parameters have been proposed as descriptors of general and specific reactions to stressful stimuli. Stress-induced changes in perception, behavior, thermoregulation, social interactions, sleep, cognition, endocrine secretions, neurotransmission, reproductive competence, immune defense, cardiovascular and gastrointestinal function, metabolic outcome, and susceptibility to noxious impact have shown rather concurrent patterns across mammalian species and, therefore, have become reliable indices of both stress exposure and stress-coping ability. However, these universal responses to homeostatic disturbance are beset by certain "original sins": (i) their activation results in overcorrection of vital parameters that may linger for some time before the status quo is reinstalled; (ii) mobilization of the "full standard repertoire" mostly exceeds the strict demand for the counterbalance of occasional or solitary shifts in homeostasis; (iii) the magnitude and dynamics of response depend not solely on the intensity of the stressful challenge, but also on numerous codeterminant variables, such as stimulus duration and context, sex, age, health condition, and previous experience of the individual, to name only a few.

From the perspective of stress modeling, three important consequences of the temporal dimension should be taken into consideration: the time point of assessment of indicators of the stress, the duration of the stressful challenge, and the phenomenon of habituation. Systems involved in

Selected abbreviations and acronyms

ACTH	*adrenocorticotropic hormone*
AVP	*vasopressin*
CRH	*corticotropin-releasing hormone*
DMH	*dorsomedial hypothalamic nucleus*
GABA	*γ-aminobutyric acid*
GR	*glucocorticoid receptor*
LHPA	*limbic-hypothalamic-pituitary-adrenal*
POMC	*pro-opiomelanocortin*
PVN	*paraventricular nucleus*

the organism's response to stress have different activation latencies; accordingly, measurable end-point changes occur at different intervals upon the challenge. Further, these systems act within physiological limits (described by, eg, synthetic and secretory capacity, feedback regulation within the system, consistency with key vital functions, etc) and cannot indefinitely maintain a maximal level of performance. Thus, changes in measurable end points vary depending on the duration of the stimulus, its perceived homeostatic threat, and the efficacy of the individually selected coping strategy (see below), but also due to output readjustment or exhaustion of the involved system. Finally, repeated exposure to homotypic stressors has been shown to produce gradual decline in the magnitude of several, but not all, commonly used indices of physiological response to stress. The omnipresence of this phenomenon is debatable, though there may be controversy based on species and paradigm differences. Habituation to repeated homotypic stress has a plausible teleological explanation: it is supposed to ensure the ability of a system involved in stress response to discriminate and adequately meet novel incoming challenges. Here, another important feature of the stress response, referred to as cross-sensitization, should be mentioned. It has been recognized that, despite habituation to repeated homotypic challenge, stress-responsive systems retain and, more importantly, even augment, their ability to react to challenges of a different modality. Several substrates of this phenomenon have been identified,[5] and its importance in the pathogenesis of stress-related disorders is generally recognized.[1,2,4]

Experimental modeling of stress requires clear definition of the research objectives, and consideration of numerous factors that may modify individual aspects of the stress response. Investigation of the magnitude and temporal course of a particular stress-responsive parameter to a single challenge of limited duration has substantial diagnostic value in several medical disciplines. Ensuring truly "baseline" conditions for the variable of interest by minimization of confounding input from the environment and consideration of sex- and age-related response deviations are usually sufficient prerequisites for obtaining reliable results. However, tasks which aim at the examination of the resistance of a stress-responsive physiological system under the influence of long-term or superimposed challenges, pharmacological treatment, or coexisting pathology, are by far more demanding. In such cases, careful evaluation of the condition and response capacity of the targeted system, alterations in its basal function resulting from each individual influence, and the time course of response must be added to the former requirements.

End points for assessment of the response to stress

Stress induces mobilization of a broad array of reactions which involve virtually every physiological system, albeit with different time courses. Accordingly, numerous parameters can be used for response monitoring in models of stress, under the provision that their temporal profiles and the changes possibly occurring in the course of habituation/sensitization are sufficiently defined.

Behavioral end points

The original description of the response to stress as a "fight-or-flight" reaction and evidence that arousal activation is invariably associated with this response implies that observation of general behavior can reliably disclose symptoms of stress. Assessment of the explorative activity by means of well-established quantifiable parameters is a frequently used behavioral descriptor of the response to stress in laboratory rodents.[6] As in most species exposure to novelty is a stressor per se, monitoring of stress-induced effects in this experimental condition should be preceded by careful baseline definition. Although outcome may vary depending on the characteristics and duration of the challenge, decreased exploratory activity is considered to be a reliable behavioral consequence of stress exposure. In its extreme expression, this response is described as "freezing," a period of time during which locomotion and exploration are completely abolished. The freezing response is reproducibly evoked in several stress paradigms, and protocols for its quantification have

been developed.[7] Behavioral deficits known as acquired immobility, behavioral despair, and learned helplessness can be viewed as alterations specifically associated with severe stress; however, a learning component has a leading role in the manifestation of these phenomena.

Behavioral responses to stress are frequently linked with anxiety, and there is a substantial overlap of neurochemical mechanisms activated by stressful challenges and those involved in the control of anxiety. Evaluation of anxiety belongs to the standard arsenal for the assessment of behavioral effects of stress, and offers a direct possibility to disclose stress-associated neuropathological consequences. Since habituation may rapidly occur in some experimental paradigms used for evaluation of anxiety,[6] caution applies to their repeated use for the examination of long-term effects.

Elicitation of defensive behavior is a core component of the stress response, and can be perceived as a continuum of altered anxiety. Assessment of manifestation of aggression and changes in its prestress degree of expression (especially within an established group hierarchy) is a recommended approach for the monitoring of stress effects,[8] and substantial correlation between behavioral and neurochemical end points has been established.

Analysis of audible and, especially, ultrasonic vocalization is a well-established method for the assessment of stress in pain- and fear-based paradigms,[9] especially in infant rats whose endocrine responses are subject to developmental inconsistency (see below). In juvenile animals, ultrasonic vocalization reliably indicates anxiety, but can be specifically modulated by maternal contact or predator cues.[10]

Stress exerts profound effects on the acquisition, retention, and retrieval of new behavioral repertoire. As this process is an integral part of the formation of strategies for coping with stress and correlations with morphological and neurochemical measures have been established, assessment of learning and memory can be used for the evaluation of transient and persistent consequences of stress. The emphasis, however, should be put on "persistent," as behavioral acquisition is associated with the mobilization of several stress-responsive neurochemical mechanisms, and the outcome depends on their "reverberation," especially considering factors such as stress duration, crosstalk between neurochemical systems, and the organism's adequate coping with the challenge. Several publications on this subject note dichotomous effects: short and controllable stress facilitates acquisition, whereas severe chronic stress interferes with memory consolidation and retrieval. Activation of monoaminergic transmission and arousal is a plausible explanation of the former phenomenon, while biphasic effects of glucocorticoids, also in conjunction with their secondary influence on neurotransmission, have been implicated in the interpretation of shifts in learning and memory performance under stressful conditions.[11] To make this issue even more complicated, significant contribution of sex and age to this outcome should be noted. The concise message in the context of this review is that the impairment of acquisition, consolidation, and retrieval can serve as descriptors of detrimental consequences of poorly controlled chronic stress.

Physiological end points

Cardiovascular responses, such as changes in heart rate and arterial blood pressure, were recognized early as essential components of the response to stress, and are causally associated with the activation of the autonomic nervous system. With the increasing popularity of telemetric recording equipment, monitoring of cardiovascular end points has become a useful research tool in stress models.[12]

The capacity of stress to trigger pain suppression has been known for a long time, and the involved neurochemical mechanisms have been comprehensively elucidated.[13] Measurement of stress-induced analgesia belongs to the standard repertoire of methods for monitoring of stress and pharmacological assessment of involved neurotransmitter and neuromodulator systems.

Transient increase in body core temperature is a well-established physiological correlate of stress. Although the proper nature of stress-induced hyperthermia is still a matter of debate, its time course and several contributing neuropharmacological mechanisms have been extensively studied, and the reliability of the method confirmed in various experimental settings.[14]

Several stressful challenges significantly influence feeding behavior, and investigations of the underlying neurochemical mechanisms have revealed the involvement of some stress-responsive systems in this phenomenon. Changes in the amount and pattern of food intake have been sporadically used for stress monitoring per se, whereas exposure to stress has advanced to a modeling approach of eating disorders.[15]

Stress-induced changes in sleep architecture in experimental animals have been comprehensively described[16]

and used for monitoring in different models; invasive interventions and sophisticated equipment have limited their widespread application.

Metabolic end points

Stress triggers distinct metabolic alterations, most of which are readily discernible. The "prototypic" metabolic response to acute stress consists of rapid and strong elevation of plasma concentrations of glucose, insulin, glycerol, and ketone bodies. The latter effects probably reflect the stimulation of adipose tissue lipase by circulating catecholamines. Activation of the autonomic nervous system has been also associated with stress-induced stimulation of glucagon secretion. Changes associated with repeated stress are also of catabolic nature, but less dramatic and, in some aspects (insulin) inconsistent. Both acute and chronic stress regimens decrease triacylglycerol levels, whereas reports on changes in cholesterol fractions are controversial.[17]

Neurochemical end points

Increased sympathoadrenal outflow in the periphery and activation of monoaminergic neurotransmission in the brain were among the first described neurochemical correlates of the stress response, and their importance for the elicitation of several allostatic reactions in the organism is beyond doubt. Measurement of circulating levels of catecholamines and/or their metabolites, as well as their content, release, and biosynthesis in discrete brain regions[18] have become standard approaches for stress response monitoring. Continuous microdialysis of discrete projection areas, in combination with morphological and histochemical techniques, has provided comprehensive description of the neuronal populations and pathways affected by stress, as well as of their distinct responsiveness to specific stressors.[3] Meticulous studies on the role of catecholamines in stress have shown that the morphofunctional heterogeneity of peripheral and central monoaminergic systems ensures discriminative responses to individual stress modalities.

Early experimental evidence for stress-induced changes in serotonergic neurotransmission has been extensively corroborated in subsequent pharmacological studies.[19] Monitoring of serotonin synthesis, release, and receptor expression have provided valuable insight into the role of this transmitter in certain aspects of the behavioral and neuroendocrine response to stress and the pathogenesis of stress-related disorders.

Evidence for global activation of dopaminergic neurotransmission under stressful conditions and links to stress-related pathology suggests possible use of changes in this system for stress monitoring. These include morphological and functional heterogeneity of dopaminergic pathways, intricate involvement of dopaminergic transmission in selective information transfer, and motivation, integration, and adjustment of central nervous system (CNS) responses to novelty and aversion[20]; however, the appropriateness of dopamine-related end points in stress research requires careful evaluation. It should be noted that individual dopaminergic projections display differential degree of activation following stress, with the mesoprefrontal pathway being particularly vulnerable,[21] and the character of changes in dopaminergic transmission might heavily depend on the context of stress and cross-modulation by multiple convergent neurotransmitter input and endocrine variables. Stress-induced changes in reward-mediating neurotransmitters and their interaction with other neurohumoral constituents of the stress response entail the possibility of using liability to addiction as a measure for the assessment of behavioral impact of stress.

Activation of cerebral cholinergic transmission by stress has been documented, and its established roles in arousal, motivation, and cognition are suggestive of an involvement in the processing of stressful stimuli. Probably due to differential regional and temporal release patterns, as well as discordant observations on their coincidence with other physiological end points,[22] changes in acetylcholine release are less frequently used as end points for stress evaluation.

Dramatic stress-induced increase in extracellular levels of glutamate, the major excitatory amino acid transmitter, have been reported in numerous brain regions. Glutamate efflux in the prefrontal cortex has been implicated in the modulation of the dopamine response to stress, and an array of potential pathological consequences was outlined.[23] Interactions between adrenocortical secretions and glutamate signaling in the hippocampus have prompted strong interest in the role of this neurotransmitter in long-term consequences of stress and their projections to various aspects of neuro- and psychopathology, as well as therapeutic strategies.[24]

Measurements of the synthesis and release of γ-aminobutyric acid (GABA) in the course of stress response have

a long history; however, results are burdened by controversy, and the relevance of this end point in stress monitoring has been questioned.[25] On the other hand, pharmacological modification of GABA-ergic transmission and measurement of changes in GABA receptor properties convincingly demonstrate a substantial involvement of GABA in the control of the stress response. The importance of GABA has been increasingly associated with anxiety and related defensive responses, as well as regulation of stress-specific neuroendocrine circuits.[26] It is pertinent to note that several aspects of GABA-ergic neurotransmission can be obscured by endogenous steroid hormone derivatives, which act as allosteric ligands of the GABA-A receptor, and whose synthesis is increased following stress. These compounds have been shown to influence several aspects of the behavioral and neuroendocrine response to stress.

Antinociceptive effects of endocannabinoids, evidence for stress-related changes in their release in discrete brain areas, and localization of cannabinoid receptors in neuronal populations that participate in the behavioral and endocrine response to stress have stimulated the interest in monitoring the activity of this system. Although the current prevailing view is that endocannabinoids play a pivotal role in the modulation of the stress response and neuroprotection, several contentious issues on the dynamics of these modulatory effects remain to be resolved.[27]

The causal involvement of endogenous opioids in stress-induced analgesia has been the starting point for extensive research on the global role of opioidergic transmission in stress. Ample evidence supports the view that opioidergic systems are profoundly affected by stress, and their secretory products participate in several aspects of the organism's response. Alterations in the endogenous opioid tone are implicated in stress-related endocrine and autonomic responses.[28] Anatomical and neurochemical heterogeneity of endogenous opioidergic systems, however, has made pharmacological paradigms a preferential approach for the investigation of stress-related changes in opioid neurotransmission.

Observations of rapid induction of proto-oncogenes in distinct brain regions by various stress modalities led to the adoption of *c-fos* expression as a firm morpho-functional marker of stress exposure. Monitoring of *c-fos* induction is a reliable tool for the identification of neuronal populations affected by stress,[29] and has significantly contributed to the delineation of neural pathways involved in the stress response.[3] The applicability of this method is, however, restricted to post-mortem examination; it should be also noted that signs of habituation of this response have been described, and controversy exists as to whether its magnitude reflects the stressfulness and intensity of the challenge. Nonetheless, monitoring of proto-oncogene induction may become an essential approach to the elucidation of spatiotemporal patterns in novel and less familiar models of stress.

It should be mentioned that several neuropeptide systems in the brain are substantially affected by stress[30] and, upon characterization of their distinct expression patterns in the selected paradigm, might eventually enrich the palette of neurochemical indicators.

Endocrine end points

Activation of the limbic-hypothalamo-pituitary-adrenal (LHPA) neuroendocrine axis is not only a "constant companion" of the stress response, but also provides the most reliable neurohumoral substrate for the assessment of its magnitude, dynamics and, ultimately, the capacity of the organism to overcome the present and meet subsequent challenges. As comprehensive work of reference has addressed the structural and functional organization and the regulation of the LHPA axis under stressful conditions,[31] here we will focus on the conclusiveness of individual measures of its activity in models of stress.

Input from stress-responsive neural circuits onto the hypothalamic paraventricular nucleus (PVN) induces the release of neuropeptide secretagogues of adrenocorticotropin (ACTH). Although stress-related fluctuations in corticotropin-releasing hormone (CRH) blood levels have been reported, its measurement in the systemic circulation has not attained widespread appreciation in laboratory animals. Monitoring of CRH concentrations in hypophyseal portal blood and, especially, perfusates and dialysates from defined brain regions is considered more reliable, and enables the distinction of CRH release from individual neuronal populations.[3] The most popular approach, however, is the direct assessment of CRH neurons by either the "output" of the hypophyseotropic population to the median eminence or the "steady state" of the CRH gene expression. The latter gained importance also in view of evidence for multiple neurotropic effects of intracerebral projections of CRH neurons, beyond those involved in the neuroendocrine response to stress.[32] CRH-coding transcripts in the parvocellular compart-

ment of the PVN are a good descriptor of LHPA axis activity under basal and stress-related conditions.

Measurements of circulating vasopressin (AVP) levels have been used for assessment of stress responses; however, caution applies to their interpretation, due to the heterogeneity of the neuronal populations that produce AVP found in the circulation.[33] Peripheral AVP originates mainly from the posterior pituitary terminals of magnocellular neurons of the supraoptic and the posterior-lateral portion of the paraventricular nucleus, and the involvement of these neuronal populations in the control of the LHPA axis is ambivalent.[34] Thus, quantification of AVP expression in anatomically defined neuronal clusters, which make up the adenohypophyseal projection of the PVN, appears to be the method of choice for assessment of the contribution of vasopressin to the endocrine response to stress. Extensive research in the past has shown that stress-associated changes in CRH and AVP expression in the PVN follow distinct temporal patterns, with AVP "coming into action" with certain delay or in the course of chronic stress load.[35]

Oxytocin and angiotensin also deserve mention as auxiliary peptidergic ACTH secretagogues. Like AVP, oxytocin is produced in heterogeneous neuronal populations, and is released in response to various stressors in the systemic and adenohypophyseal portal circulation. Induction of oxytocin synthesis and secretion have been documented in various stress paradigms, and its role seems to extend beyond that of mere "booster" of CRH and AVP. However, while oxytocin is clearly a stress-responsive hormone, the interpretation of its "net" effect compels consideration of dissociated secretory activity of hypophyseotropic and intracerebral projections, subject's sex and physiological condition, stress modality, and other interacting factors.[36] Changes in angiotensin secretion represent an established component of the neuroendocrine response to stress, with multiple involvements in several aspects of allostasis.[37]

Increased concentrations of ACTH in the systemic circulation and its precursor peptide pro-opiomelanocortin (POMC) in the anterior pituitary are a typical consequence of stress exposure. While in acute stress ACTH responses fairly reflect the activity level of CRH neurons, chronic stress and continuous CRH hypersecretion result in desensitization of pituitary CRH receptors and blunted ACTH release. This dissociation between CRH hyperactivity and refractory corticotrophin responsiveness is a pathognomonic feature of stress-associated neuroendocrine dysregulation.

Systemic glucocorticoid levels under quiescent conditions (eg, at the nadir and zenith of circadian activity), the amplitude of the acute stress-induced increase (albeit influenced by sex, age and diurnal time point of examination), and the sensitivity of the hypothalamo-pituitary unit for glucocorticoids (as defined by the swiftness of reinstatement of basal secretions after stress cessation or the capacity of exogenously administered glucocorticoids to subdue the diurnal secretory peak) comprehensively characterize the status of the LHPA axis *(Figure 1)*.

Stress profoundly affects reproductive function and gonadal secretions; however, changes in sex hormone levels following acute stress are not among the widely used monitoring end points. While there is unambiguous evidence that stress exposure impairs gonadal function and reproductive activity, the reserved use of measurements of gonadal secretions for the assessment of acute stress consequences is based on the complexity of neural mechanisms which control the key variable, the pulsatile discharge of gonadotropin-releasing hormone (GnRH)-producing neurons.[38] On the other hand, decreased gonadotropin levels, suppressed secretion of gonadal steroids, disruption of the ovarian cycle, and inhibition of sexual behavior are consistent outcomes of chronic and

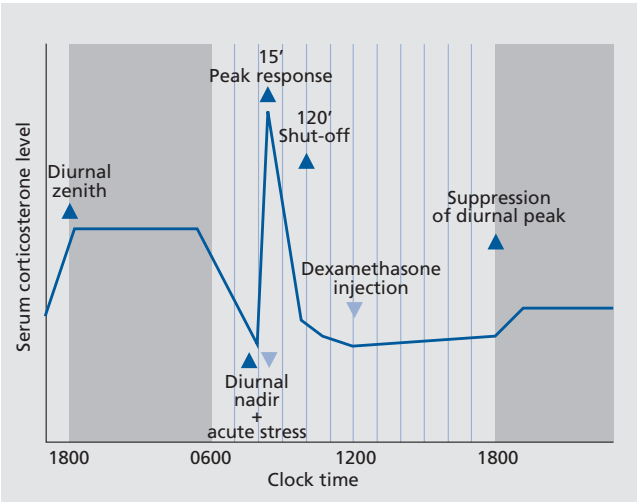

Figure 1. Algorithm for the assessment of basal and stress-induced LHPA activity and its sensitivity to glucocorticoid negative feedback in the rat. The curve depicts the course of changes in serum corticosterone levels. Shaded areas indicate diurnal dark phases; bold and light symbols denote time points of blood sample collection and experimental interventions, respectively. LHPA, limbic-hypothalamic-pituitary-adrenal

insuperable stress.[39] Circulating prolactin levels promptly increase with acute stress[40] and are a reliable endocrine end point, even if one abstains from reflective elaboration on the multiplicity of pathophysiological projections of stress-related hyperprolactinemia. Growth hormone secretion is altered by stress[40]; however, the pattern of changes may vary depending on the stress modality and require sophisticated evaluation.

Alterations in thyroid axis function and hormone secretion following stress exposure have been described in various experimental settings. The reported consequences of acute stress are somewhat contradictory, as both activation and inhibition have been described. Suppression by chronic or uncontrollable stress[41] is in line with the prevailing view of thyroid axis hypofunction in stress-related disorders; however, conflicting data exist also on this aspect.

Immunological end points

The immune system is unequivocally influenced by stress, and changes in various aspects of the inflammatory/immune response have been extensively documented. Exposure to infectious agents or antigenic challenge are stressful stimuli per se, and trigger a cascade of reactions within an intricate network which encompasses several components of the humoral stress response. The changes in immunological parameters following nonimmune stressful stimuli, however, are mostly considered consequences of the activation of two fast-acting stress-responsive systems, the sympatho-adrenomedullary and the hypothalamo-pituitary-adrenocortical.[42,43] In general, immunosuppression is an obvious and understandable effect of acute stress, whereas persistent activation of the LHPA axis under the condition of chronic stress is accompanied with substantial shift in the quality of the immune response.

Experimental approach to stress induction

Physiological responses directed to restoration of the homeostasis and encompassing changes in several of the above-listed end points can be elicited by a myriad of environmental challenges and perturbations of the *milieu intérieur*. For the purpose of modeling, however, it is essential to demonstrate that a given challenge engenders traceable changes in (preferably, more than one) end points indicative of the occurrence of an allostatic response.

The most widely used classification of stress-inducing paradigms operates with two principal categories: systemic (physical) and neurogenic (psychoemotional), with conscious processing of the stimulus being the leading separation criterion.[31] While adhering to this taxonomy, we will take the liberty to introduce, for didactic reasons, subcategories based upon the procedural features of the stress model.

Naturalistic models of survival threat

Deprivation paradigms

Food deprivation (not to be confused with caloric restriction) produces alterations in numerous descriptors of the humoral and behavioral response to stress. While demonstration of rapid-onset responses requires consideration of species-specific circadian activity patterns, prolonged food deprivation produces long-term consequences which are compatible with those seen in chronic exposure to stress.[44]

Water deprivation and ensuing dehydration has been shown to elicit humoral changes suggestive of stress-induced LHPA axis activation.[45] Similar effects can be rapidly triggered by osmotic challenge using intraperitoneal injections of hypertonic saline. Osmotic challenge is a reliable paradigm of stress induction, and repeated application is reportedly not accompanied by signs of response desensitization. Since dehydration selectively activates neuronal populations with a primary role in osmoregulation and only auxiliary contributions to the LHPA axis stimulation, explanation of mechanisms involved in the hormonal response suffers from a certain inconsistency.

Deprivation of rapid eye movement (REM) sleep by different procedures is a recognized method of stress induction. There is firm evidence that prolonged sleep deprivation affects several physiological parameters in a fashion indicative of severe stress.[46] In this paradigm initial responses can be largely ascribed to the encounter with a highly adverse and novel environment, whereas changes seen in the course of long-term exposure also reflect progressive exhaustion of adaptation-relevant systems.

Restriction of the freedom of locomotion and exploration, better known and referred to as restraint or immobilization, is probably the most widespread method of stress induction (as judged by its reported use in more than 2000 publications). In any mode of application (single short-term, intermittent, chronic), restraint is per-

ceived as a severe stressor, and robustly induces the entire spectrum of known allostatic responses.[47]

Exposure to adverse environmental stimuli

Cold exposure (also cold-water swimming) causes noticeable activation of several stress-responsive systems.[48] The magnitude of some changes suggests that cold environment is not a powerful stressor in adult rats, but is a reliable method of stress induction in neonates. Cold stress is consistently associated with activation of the thyroid axis, which probably serves thermogenesis.

Significant neurochemical and endocrine responses have been documented in laboratory rodents following exposure to a hot environment.[49] While the magnitude of changes seems to correlate with the abruptness of transition and the ambient temperature, their temporal dynamics is rather sluggish.

Acute hemorrhage is a powerful signal for the activation of allostatic mechanisms. Induction of neurohumoral and endocrine responses by this systemic stressor has been extensively documented,[50] whereas behavioral and metabolic alterations have not been systematically examined.

Even if not associated with specific adverse stimuli, exposure to novel environment is a well-recognized naturalistic stressor, and changes in brain catecholamines and pituitary and adrenal secretions have been demonstrated. Less congruous are data concerning the dynamics of the hormonal response following repeated exposure and the direction of changes in hypothalamic peptide stimulators of ACTH release.[51,52]

Several environmental signals acting through different sensory modalities (auditory, visual, tactile) have been shown to elicit stress responses. Audiogenic stress (noise exposure) is a well-characterized paradigm, with response profiles of individual parameters having been thoroughly examined.[53] Exposure to bright light or abrupt alteration of illumination rhythms are naturalistic stressors in laboratory rodents, and endocrine responses have been documented,[54] though some mechanisms require elucidation. Responses induced by modification of the illumination regimen may be obscured by interference with established circadian and ultradian activity patterns of the involved physiological systems.

The capacity of olfactory stimuli to elicit pronounced stress reactions is best exemplified by studies employing the paradigm of exposure to odors originating from either a predator or a stressed cospecific individual. Odor-induced stress responses do not completely overlap with those seen after realistic encounter with a predator.[55] The importance of olfactory stressors in experimental routine should be taken into consideration: whenever animals are sequentially stressed, the odor of the "predecessor" must be eliminated after completion of the test.

Pain paradigms

Nociceptive stimuli are among the most powerful inducers of stress responses. Although concerns of animal welfare have gradually diminished the use of pain-based paradigms, painful manipulations, such as electric footshock, tail pinch, and pharmacologically-induced hyperalgesia (formalin, carrageenan), have served for decades as fundamental approaches for stress induction and dependable manifestation of most of the known stress-associated reactions of the organism. Chronic pain of inflammatory or neuropathic origin produces consequences that show extensive similarities and share several mediators with chronic stress.[56]

Fear-and anxiety-based paradigms

Exposure to a predator is a prototypic example for fear-mediated stress induction, and the response profiles of several systems have been comprehensively elucidated.[55] Intriguingly, repeated predator stress appears to promote a homotypic sensitization of neuroendocrine response mechanisms, with little evidence for a primary involvement of hypothalamic corticotropin secretagogue-producing neuronal populations.[57]

Albeit with certain exaggeration, the generic term *neophobia* summarizes the anxiogenic potential of a host of stimuli emerging from either the natural environment or the laboratory setting[58] and their capacity to evoke measurable behavioral, neurochemical, endocrine, and metabolic stress responses. This intrinsic conflict between the drive for exploration of a novel environment and the assessment of the threatening potential of nonfamiliar stimuli is exploited for the generation of standard methods of fear- and anxiety-based stress induction.[59]

Conditioned anticipation of fearful experience is also a powerful tool for the induction of stress responses, and there is substantial overlapping of the anatomical substrates involved in unconditioned and conditioned fear. However, quantitative and, to a lesser degree, qualitative

differences in the activation of distinct neural populations have been revealed,[60] and the LHPA axis appears to have a crucial role in the emergence of conditioned fear. It should be mentioned that the degree of stress response resulting from the first (and, sometimes, also subsequent) exposure to experimental devices and procedures must be meticulously characterized and, if possible, minimized by handling, in order to avoid bias while measuring the "proper" outcome of a stress model.

Models of social conflict and disruption

Interactions within a cospecific group (population) are probably the most persistent source of stressful stimuli; however, in a colony of highly domesticated laboratory animals their impact often remains unaccounted, especially when using them as subjects in stress experiments. The baseline characteristics and the response profiles of end points used for stress assessment may critically depend on the individual's status within the rapidly formed social group hierarchy and/or his or her previous experience in this environment. Models based on social conflicts exploit either the aggravation of existing, or the *de novo* creation of, stressful interactions in the course of establishing and maintaining of hierarchic relationships of dominance or subordination. Specific conflict-producing experimental settings, such as territory defense (resident-intruder paradigm, colony overcrowding), hierarchy formation (social defeat, visible burrow system), offspring protection, and social instability are comprehensively reviewed.[61] These paradigms produce strong alterations in several indicators of the stress response and, upon chronic application, the outcome may mimic the features of human pathological conditions. In rats there are pronounced sex differences in the liability to social stress, with females being generally refractory to paradigms of hierarchy formation, but responsive to conditions of social instability.[62]

Social isolation (solitary housing) has been considered an appropriate method for stress induction[63]; however, some caveats of this model merit consideration. Social isolation implies long-term deprivation of the familiar environment and, accordingly, immediate effects of separation can be ascribed to novelty and experimental procedures (eg, handling, restraint). Most consequences of social isolation become manifest after longer exposure periods. Finally, alterations in stress-related end points may be indicative of increased sensitivity to superimposed challenges rather than persistent activation of stress-responsive systems.

Disruption of social contacts during early ontogeny, mostly referred to as maternal separation/deprivation, is a powerful stressor in several species. The reputation of this paradigm is based on its capacity to evoke long-lasting alterations in the function of several adaptation-relevant systems and their susceptibility to stress.[64] A few marginal notes appear appropriate with regard to the practical use of this model. While immediate behavioral correlates (eg, vocalization) have been routinely used for monitoring the effects of maternal separation, the time course of endocrine responses to this stressor indicates that significant changes become apparent only after 2 to 4 hours of exposure, and their amplitude may vary depending on the age of the animals.[65] Thus, although maternal deprivation is a recognized stressor, caution applies to the selection of parameters and timepoints for the assessment of its early consequences.

Pharmacological models

Accumulation of knowledge on neurohumoral systems, which participate in the processing of stressful stimuli and induction of related physiological reactions, enables the use of appropriate pharmacological agents to modify the activity of individual response cascade fragments and bring about changes in end-point indicators even in the absence of a prototypic stressor. Conceivably, drug-induced alterations in the initial "links" of stress-reactive chains would result in a broader spectrum of "downstream" responses; however, as systems of allostatic regulation operate through closed-loop mechanisms, pharmacological modifications that interfere with feedback circuits are also capable of changing the activity level of several interconnected response cascades.

Several pharmacological challenges are able to activate individual stress-responsive systems (eg, the LHPA axis). However, since stress is a complex and multipronged response, the list of pharmacological agents that can simultaneously influence several systems is rather short. The concomitant occurrence of pharmacologically induced responses in multiple systems involved in adaptation is exemplified by the effects of ether inhalation. This stressor produces behavioral agitation (before anesthesia takes place) and affects brain monoamine metabolism, and CRH and AVP biosynthesis and release. Likewise, glucoprivation induced by either insulin or 2-deoxyglucose

administration results in distinct stress-like behavioral, neurochemical, and neuroendocrine alterations.

Abundant experimental evidence shows that pharmacological modulation of the major neurotransmitter systems that inaugurate the response to stressful stimuli can mimic several behavioral and endocrine responses to stress. Approaches aiming at the activation of distinct aspects of monoaminergic neurotransmission have been impressively summarized[66] and their efficacy convincingly demonstrated. The established role of GABA-ergic signaling as a major tonic inhibitor of stress responses provides plausible explanation for the capacity of GABA/benzodiazepine antagonists to induce several behavioral and endocrine correlates of stress or augment the responsiveness to systemic and emotional challenges.[67]

Although endogenous opioids definitely contribute to several aspects of the response to stress, divergent effects of opioid administration on neuroendocrine parameters, also due to intricate interactions with other neurotransmitter systems, appear to be somewhat at odds with the reigning opinion that opioids tonically suppress the LHPA axis.[68] It is thus helpful to consider that the issue discussed herein concerns pharmacological effects with abrupt onset, which are not expected to produce immediately dramatic shifts in what is called "opioidergic tone." An abridged statement in the context of this paper summarizes that (i) acute administration of morphine or receptor-selective opioid agonists results in distinct stress-like changes of neuroendocrine end points and (ii) similar phenomena occur after spontaneous or antagonist-precipitated withdrawal from chronic opioid treatment. As with several other opioid-sensitive systems, development of tolerance is accompanied by attenuated responsiveness of the LHPA axis to subsequent opioid administration. The effects of psychomotor stimulants, as exemplified by cocaine[69] and amphetamine,[70] include stress-like symptoms of behavioral disruption and defensive withdrawal and stimulation of hypothalamo-pituitary-adrenal secretions. Most of these effects and the stress-contrasting suppression of prolactin release are ascribed to their agonistic influence on central monoaminergic transmission. Elevation of circulating ACTH and glucocorticoid concentrations has been demonstrated following intracerebral cannabinoid treatment; however, the involvement of drug-specific signaling mechanisms remains unclear, as specific cannabinoid receptor antagonists have produced biphasic effects. Alcohol administration powerfully stimulates the LHPA axis[71] and potentiates defensive responses. As with opioids, endocrine changes in the course of chronic treatment are suggestive of the development of selective tolerance.

In view of its essential role in the initiation and integration of behavioral, autonomic, and endocrine responses to stress, exogenous CRH dependably mimics several consequences of stressful stimuli. It should be added, however, that the stressogenic action of CRH is warranted following intracerebral administration, while some divergence (eg, in cardiovascular effects) may occur following systemic application.[72] Despite compelling evidence for the involvement of vasopressin in several aspects of the stress response,[73] administration of exogenous vasopressin has produced, at best, modest stress-like symptoms. Concerning the endocrine response, these observations are in agreement with the auxiliary role of vasopressin in the control of the LHPA axis. Continuing interest in the involvement of neuropeptides other than ACTH secretagogues in stress and emerging availability of selective analogues suggests novel possibilities for the use of such agents in pharmacological stress modeling.[30,74]

Persistent hypercorticalism has been shown to result in deterioration of neuroendocrine circuits that control the basal activity of the LHPA axis and its responsiveness to stressful challenges.[4] This outcome can be brought about pharmacologically by long-term administration of supraphysiological doses of glucocorticoids. Although this approach is confined to the LHPA axis and manifestation of stress-related symptoms in other systems has not been meticulously examined, distinct signs of basal hyperactivity and exaggerated endocrine responses to stress persist in this model for several weeks upon cessation of the glucocorticoid treatment.[75]

A typical example of pharmacologically induced activation of several stress-reactive systems is represented by peptide mediators/integrators of the inflammatory and immune responses. The most frequently used agents are tumor necrosis factor α, interleukin-1 and interleukin-6, or their sequential releaser, bacterial lipopolysaccharide (LPS). Endotoxin- or cytokine-induced effects involve a complex of typical defensive behavioral responses, referred to as "sickness behavior," with vagal afferentation playing an essential role.[76] Alterations in central and peripheral neurotransmission largely resemble those evoked by physical and neurogenic stress modalities,[77] and activation of the LHPA axis is a firmly

established consequence.[78] Suppression of reproductive functions as part of the "sickness behavior," and in terms of endocrine secretions[79] has been demonstrated; it seems that cytokine-mediated disruption of the gonadal axis employs mechanisms which are independent of those involved in the general stress response. The reports on changes in growth hormone and prolactin secretion upon cytokine challenge are ambivalent.

The list of drugs with stressogenic properties becomes considerably longer if LHPA axis activation is considered a solitary symptom of stress. Association of thyreotoxicosis with symptoms of hypercorticalism has prompted experimental studies showing that chronic administration of thyroid hormones leads to activation of the LHPA axis.[80] Increased secretion of ACTH and glucocorticoids has been also seen following treatment with cholinomimetics, adenosine and histamine agonists, phosphodiesterase inhibitors, free fatty acids, and a high-fat diet. However, convincing evidence is still lacking that these agents are able to elicit a full-scale stress response.

Genetic models

Since stress is a transient condition, and its enduring presence is incompatible with survival, the following subject should be understood as models of increased stress responsiveness resulting from genetic manipulations or selective breeding.

Breeding strategies aiming at the consolidation of behavioral traits suggestive of increased vulnerability to stress have yielded interesting models; however, concordant changes in multiple end points were not always observable. Thus, several rat strains which are typified by enhanced anxiety and dysproportionate behavioral responsiveness to stress displayed inconsistent signs of increased (Fawn-Hooded, Maudsley reactive, Roman high avoidance) or, even, paradoxically subdued (Syracuse low avoidance) LHPA axis activity. The behavioral repertoire of the Flinders Sensitive line reveals several symptoms of aberrant responsiveness, but abnormal hormonal reactions could be evoked only by specific pharmacological challenges. Similarly, animals selected for their predisposition to learned helplessness upon stress exposure are fulfilling several behavioral and neurochemical criteria,[81] but establishment of endocrine correlates seems to depend on additional challenges during early ontogeny. Recent reports indicate that selective breeding based on the manifestation of enhanced anxiety produces a phenotype that is characterized by dominance of defensive responses to novelty, increased ultrasonic vocalization, and amplified endocrine reactivity. In this rat line, increased activity of the LHPA axis appears to result from vasopressin overexpression and hypersecretion, and the phenotype apparently correlates with distinct signs of polymorphism in the vasopressin gene promoter.[82]

The most advanced approach to stress liability modeling is the targeted modifications of the expression of genes encoding individual components of stress-responsive cascades. Overexpression of monoamine-synthesizing enzymes, even in brain regions of specific importance, was not associated with a stress-prone phenotype.[83] More successful were genetic modifications of mechanisms involved in the control of endogenous catecholamine release and metabolism. Genomic disruption of α_2-adrenoceptors resulted in behavioral and neurochemical phenotypes that resemble those seen following stress exposure or pharmacological interventions,[84] but copresent endocrine alterations have not been reported. Similarly, increased behavioral responsiveness to stressful stimulin animals deficient for monoamine oxidase A[85] and catechol-*o*-methyltransferase[86] is not accompanied by corresponding changes in endocrine end points. Overexpression of inflammatory cytokines (interleukin-6, leukemia inhibitory factor) and growth hormone has resulted in distinct symptoms of LHPA axis activitation which, however, have been ascribed to either altered adrenocortical sensitivity or improper pituitary development.

The most compelling data have been obtained in studies with transgenics overexpressing CRH. The phenotype of these animals recapitulates most of the effects seen following CRH administration, such as increased anxiety and defensive behavior, impaired autonomic functions, immunosuppression, reproductive impairment, and LHPA axis hyperactivity under basal and post-challenge conditions.[87] Genetic elimination of the CRH-binding protein resulted in behavioral symptoms compatible with increased CRH bioavailability, but failed to alter pituitary-adrenal secretions under basal and stress-related conditions.[88]

The crucial role of glucocorticoid receptor (GR) signaling in the tonic restraint and dynamic feedback control of the magnitude and duration of the neuroendocrine stress response, as well as its involvement in virtually every aspect of allostasis and adaptation,[43] has prompted

numerous investigations on the outcome of GR genetic modifications. The results have produced more questions than answers, thus illustrating the intricacy of neuroendocrine control of stress responsiveness. Partial or complete disruption of GR expression in the brain has consistently led to increased LHPA axis output; however, surprisingly, this was not accompanied by behavioral alterations (as disclosed by measures of anxiety)[89]; some signs of coincident behavioral and neuroendocrine impairment following targeted GR disruption were reported only recently.[90] Brain-specific overexpression of GR had anxiogenic effects, but failed to alter the activity of the LHPA axis under both basal and stressful conditions.[91] An elegant explanation of these confounding observations suggests that proper GR signaling in the brain not only controls the expression of stressogenic neuropeptides, but also ensures the correct detection of stress-induced adrenocortical output and its translation into defensive behavioral responses.[92]

The importance of sex and age

Sex-related dichotomy has been recognized and extensively studied with regard to virtually every aspect of the stress response. Sympathoadrenal responses to stress[93] and basal or stress-induced LHPA axis activity are higher in females, as long as physiological gonadal secretions are maintained (for review see ref 94). The neurobiological foundations for this dichotomy appear to be laid down during early ontogeny under the organizing influence of perinatal sex hormone levels.[95] Glucocorticoid-sensing mechanisms in the female brain operate at lower discrimination thresholds, and female sex steroids seem to deflect the loss of sensitivity induced by autologous downregulation.[94] Most of the listed differences are abolished by gonadectomy and reinstalled by hormone replacement, thus underlining the role of activating effects of physiological gonadal secretions.[94,96]

Interestingly, sex-specific differences in the magnitude of neurochemical and neuroendocrine responses do not correlate with the expression of defensive behavior. Several studies using various experimental paradigms indicate that stress-induced behavioral suppression and anxiety are rather a "male privilege." Experimental data on sex differences in stress-related analgesia reveal that this phenomenon is predominantly expressed in males, and generally matches gender differences seen in the responsiveness to analgesic drugs. The abovementioned sex differences in neuroendocrine responses to stress are not necessarily in accordance with observations in humans. Data from clinical studies are suggestive of stronger responsiveness in males,[97] and these sex-specific profiles persisted under the condition of simulated hypogonadism.[98]

The robust female-specific response to stress in laboratory rodents is significantly attenuated during pregnancy, parturition, and lactation. Extensive research in the past has elucidated the joint causal contribution of various neurochemical and neuroendocrine mechanisms to this stress-protective phenomenon.[99]

During a defined phase of early ontogeny (between postnatal days 3 and 14) rats and mice display blunted pituitary-adrenal responsiveness to several stressors that are perfectly effective in adult animals. The mechanisms underlining this stress-hyporesponsive period have been exhaustively elucidated. Briefly, subdued hormonal secretions following stress are believed to reflect the immaturity of pituitary corticotropin synthesis,[100] sluggish mobilization of adrenocortical steroidogenesis, and tight, pituitary-focused glucocorticoid-mediated control of the LHPA axis.[101] Stress hyporesponsiveness during early ontogeny is not absolute, as it can be breached by cytokine, endotoxin, and pharmacological challenges or pre-exposure to maternal separation. There are changes in proto-oncogene expression in relevant areas, and the neonatal brain reacts to several stressful stimuli,[102] but neuronal activation is apparently not translated into commensurate endocrine responses. The behavioral repertoire in infant animals is relatively poor, and does not provide many end point choices for the assessment of the stress response. Nonetheless, ultrasonic vocalization, a reliable sign of behavioral distress, is manifest also during the stress-hyporesponsive period.

The LHPA axis function in senescent animals displays aberrations that are attributed to dwindling efficacy of GR-mediated feedback control. While age-dependent differences in the magnitude of the stress-induced secretory response occasionally become apparent after a single challenge, deficits in its termination can be readily disclosed in both acute and chronic paradigms. Impaired signal discrimination in glucocorticoid-sensing mechanisms is considered the principal cause for protracted duration of the secretory response to stress in aged animals. A few debatable issues affecting the use of aged subjects in models of stress should be mentioned. Data on LHPA function under basal conditions are contradic-

tory,[103,104] and there is little evidence that disinhibition of this endocrine axis becomes apparent during its circadian acrophase. Age-associated changes in the adrenocortical sensitivity and expression/secretion of CRH and AVP are also arguable. Although some discordance exists as to the response profiles of the sympatho-adrenomedullary system and brain monoamines in aged animals, the majority of published data suggests exaggerated and, in some cases, protracted increases, with possible aberrations depending on the stressor modality.[105] Observations of reduced neophobia and anxiety (but also locomotion and exploration) in aged rodents[106] is a further illustration of the difficulties on the way to an all-embracing view of age-associated control of stress responsiveness.

Translational aspects: models of stress as models of disease

Assessment of individual aspects of the response to acute stress provides valuable information on the integrity of the major systems of vital importance for adaptation, as well as on the perception of a stimulus as a homeostatic threat. Usually, response deficiency is interpreted as a clue for the search of organic damage in the challenged system or, alternatively, a sign of negligible aversive property/hazard potential of the stressful stimulus. Rather than by its magnitude, the physiological dimension of a response to stress is defined by the organism's ability to terminate it upon cessation of the stimulus or by the implementation of adequate means to control it or avoid repeated exposure. Elimination of the latter prerequisites is readily achieved in stress paradigms employing enduring, variable, and nonpredictable challenges, whose common outcome is persistent activation and, ultimately, insuperable allostatic load. Rheostasis (set-point shifting) may postpone, but not prevent, exhaustion of adaptive capacity, and is probably the best indicator of the transition from norm to pathology. Achievement of persistent shift in set points of signal reading and thresholds of response initiation, and the resulting formation of self-potentiating vicious circuits describes the objectives of the generation of stress-based models of disease. These objectives can be achieved in several paradigms under the conditions of chronic, unpredictable, and uncontrollable exposure, but also by exploiting sex- and age-dependent set-point differences or their pharmacological or genetic modification.

The list of stress-related models that have been successfully used to establish approximate correlates of human disease is long and steadily growing. Evidence for the role of stress as (at the minimum) precipitating factor in depression and has encouraged the extensive transfer of stress paradigms into models of this disease. Posttraumatic stress disorder is another major area for the translational application of experimental stress models. Stress-based paradigms have a firm place in the arsenal of methods for realistic modeling of alcohol and drug addiction, withdrawal, and relapse. Knowledge accumulated in stress research has been implicated in models of eating disorders, aggression, and self-destructive behavior. Increasing understanding of specific stress-related consequences in vital physiological systems has opened new possibilities for the modeling of cardiovascular, gastrointestinal, and, more recently, metabolic conditions. The profound projections of stress to the regulation of the immune responsiveness and reproduction form a solid rationale for the use of stress paradigms in investigations of the pathogenesis of inflammatory/immune disorders and reproductive disturbance.

Conclusions: the perfect model

Under laboratory conditions, stress can be readily emulated through numerous modalities. Nevertheless, stress modeling is associated with considerable problems casting doubts on the quality of results and the validity of conclusions.
Several essential features of allostatic responses, such as variable amplitude, sensitization, and habituation, and complex interactions between their mechanisms preclude the existence of perfect models. Besides adherence to general precautions that guarantee the reproducibility of experimental data (eg, animal strain, sex, age, source, ambient conditions, staff skills, etc), preemptive consideration of certain issues may improve the design and performance of animal models of stress. What is the temporal profile of the selected outcome? Is the stressor capable of eliciting coincident changes in several systems? Are there confounding interactions between simultaneously activated responses? Can effects be obscured by physiological oscillations of the baseline of the selected parameter? Are the responses of interest subject to rapidly evolving habituation or cross-sensitization? What are the physiological limits of the system used for response monitoring? This catalogue can be extended depending on the experimental objective and investigator's concerns.

Research areas with a long and successful history, such as the biology of stress, persuade scientists to rely unreservedly on the validity and reliability of frequently used "hallmark" techniques and experimental models. One of our intentions was to underline that the complexity of the stress response may produce variable outcomes, even in models that have been established for decades. Thus, adherence to the rule *Sapiens nihil affirmat quod non probat* may prove more useful than recommendations in favor of, or dissuasion from, the use of specific models and end points. ❑

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Modelos experimentales de estrés

La complejidad de la respuesta al estrés y sus manifestaciones polifacéticas son la linea conductora de esta revisión de paradigmas experimentales empleados para inducir estrés en animales de laboratorio. La descripción de las características fundamentales de los modelos, que están basados en los elementos estresantes naturales, provocaciones farmacológicas y manipulaciones genómicas se completa con un análisis extenso de los cambios fisiológicos, de comportamento, neuroquímicos y endocrinos y su idoneidad como criterios de evaluación. Se ha prestado especial atención a la importancia del sexo y la edad como determinantes de la dinámica de la respuesta al estrés. Se esbozan de forma sucinta las posibles aplicaciones translacionales de los paradigmas inductores del estrés como modelos de enfermedad.

Modèles expérimentaux du stress

Cette vue d'ensemble des paradigmes expérimentaux utilisés pour l'induction du stress chez les animaux de laboratoire a pour but d'illustrer la complexité de la réponse au stress et la multiplicité de ses manifestations. La description des caractéristiques clés concernant les différents modèles sont décrits, basés sur des stresseurs nature, sur des tests pharmacologiques ou sur des manipulations du génome, et sont complétés par une analyse détaillée des variations physiologiques, comportementales, neurochimiques et endocriniennes et de leur intérêt pour les résultats qui en découlent. Le rôle du sexe et de l'âge, en tant que déterminants de la dynamique de la réponse au stress, a été particulièrement étudié. La possibilité d'appliquer ces paradigmes d'induction du stress aux modèles pathologiques est brièvement évoquée.

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Genetics of stress response and stress-related disorders

Marcus Ising, PhD; Florian Holsboer, MD, PhD

Recent advances in molecular genetics have stimulated basic and clinical research, and opened up access to hypothesis-driven and unbiased genetic approaches. With knowledge of the genes involved in complex basic functions like the stress response, and of multifactorial diseases like stress-related disorders, we can improve our understanding of the mechanisms and moderators involved in the biology of normal and altered stress response, which in turn will help to identify new drug targets and interventions for stress-related disorders.

Stress response and stress-related disorders

Though there is no generally accepted definition, stress is usually defined as a state of disturbed homeostasis evoking a multiplicity of somatic and mental adaptive reactions, which are summarized as stress response aim-

The major findings regarding the genetics of stress response and stress-related disorders are: (i) variations in genes involved in the sympathetic system or in the hypothalamic-pituitary-adrenocortical axis are associated with altered stress responses; (ii) genes related to the renin-angiotensin-aldosterone system or inflammation/immune response show associations with cardiovascular disorders; (iii) genes involved in monoaminergic neurotransmitter systems are associated with bipolar disorder and unipolar depression. The vast majority of these association studies followed a conventional hypothesis-driven approach, restricting the gene selection to established candidates. This very conservative approach retarded our understanding of the complex interplay between genetic factors, stress response, and stress-related disorders. Chip-based whole-genome technologies will open up access to new unbiased and statistically efficient approaches that will help to identify new candidate genes, which should be thoroughly validated in clinical and preclinical confirmatory studies. This, together with the use of new text- and information-mining tools, will bring us closer to integrating all the findings into sophisticated models delineating the pathways from genes to stress response and stress-related disorders.

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Keywords: *stress; cardiovascular disorder; bipolar disorder; unipolar depression; genetics; sympathetic system; hypothalamic-pituitary-adrenocortical axis; renin-angiotensin-aldosterone system*

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Selected abbreviations and acronyms

ACTH	*adrenocorticotropic hormone, corticotropin*
AVP	*(arginin)-vasopressin*
CRH	*corticotropin-releasing hormone*
DEX	*dexamethasone*
GR	*glucocorticoid receptor*
HPA	*hypothalamic-pituitary-adrenocortical*
MR	*mineral corticoid receptor*
RAAS	*renin-angiotensin-aldosterone system*
TSST	*Trier Social Stress Test*

ing to reconstitute the initial homeostasis or allostasis,[1] ie, a new level of homeostasis after successful adaptation.[2] The pioneer of stress research, Hans Selye, claimed a stimulus-independent nonspecificity of the stress response[3,4] which has been criticized by others.[1,5,6] Nevertheless, different kinds of stressors, physical and psychosocial, lead equivocally to a rapid activation of the sympathetic nervous system followed by a stimulation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Successful coping with stress implies an appropriate regulation of the stress response and an effective termination when the stress is over or the individual has adapted to the new conditions.

The perception of a stressful situation activates a large number of neuronal circuits in the prefrontal cortex and limbic system, including the hypothalamus, where the sympathetic nervous system is activated; this in turn leads to a widespread release of noradrenalin from the postganglionic fibers and to the release of adrenalin (and noradrenalin) from the adrenal medulla. Additionally, the parvocellular neurons of the hypothalamus are stimulated to secrete the neuropeptides corticotropin-releasing hormone (CRH) and vasopressin (AVP) into the portal vessel system to activate the synthesis and release of corticotropin (ACTH) from the anterior pituitary. ACTH, in turn, stimulates the adrenal cortex to synthesize and release glucocorticoids, in particular cortisol (in humans). These hormones have a multiplicity of functions, which are necessary for the adaptation to acute stress, but can be pathogenic when the organism is persistently exposed. Therefore, a fine-tuned regulation of the sympathetic system and of the HPA axis is essential to avoid the development of a pathological dysregulation that can progress to stress-related disorders, which can be defined as illnesses whose causation, onset, or development is substantially influenced by stress and its neurobiological correlates. Among others, cardiovascular dis-

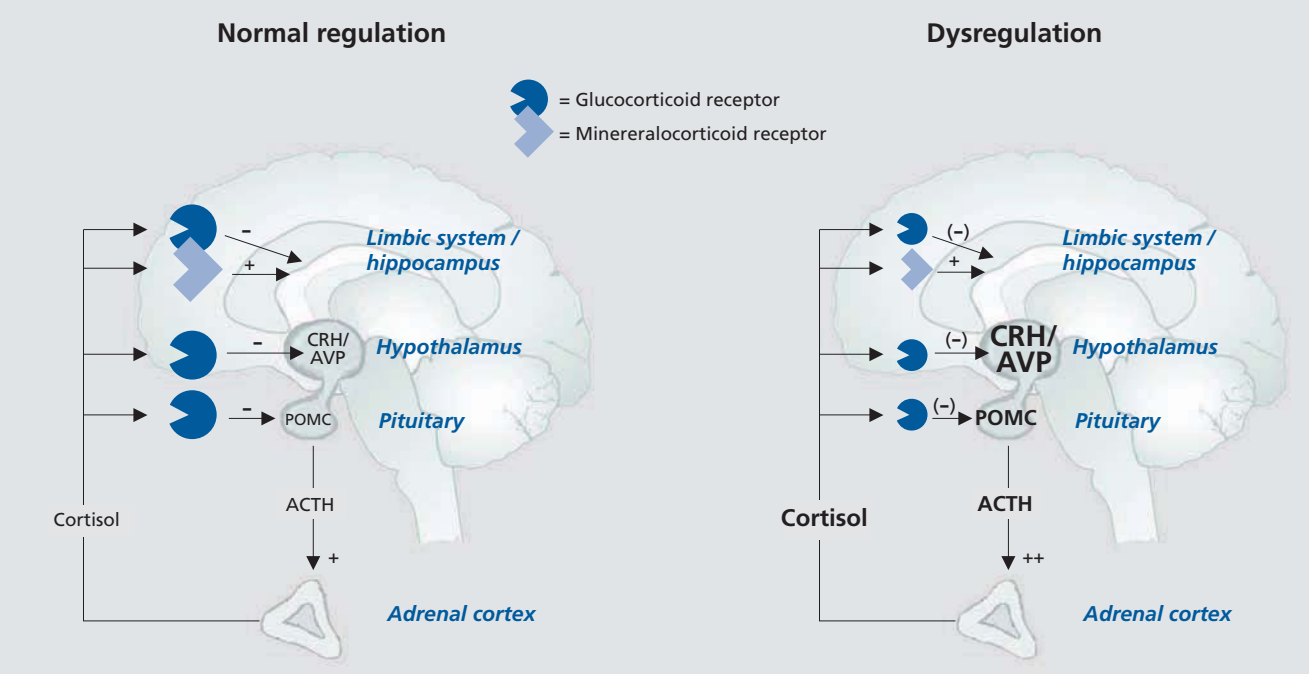

Figure 1. Model for normal and impaired regulation of the HPA axis. HPA, hypothalamic-pituitary-adrenocortical; CRH, corticotropin-releasing hormone; AVP, arginin-vasopressin; POMC, pro-opiomelanocortin; ACTH, adrenocorticotropic hormone

orders such as hypertension and coronary artery disease, as well as psychiatric diseases such as bipolar disorder and unipolar depression, are examples of stress-related disorders that will be discussed in this review.

The main central structure for the regulation of the autonomic nervous system is the hypothalamus, which receives input from cortical and subcortical structures, as well as from peripheral receptors and organs. The primary regulatory elements of the HPA axis are the corticosteroid receptors, glucocorticoid receptors (GR), and mineral corticoid receptors[7] (for details see ref 8).

As indicated in the left panel of *Figure 1*, activation of the HPA axis leads to the secretion of cortisol (in humans), which induces a negative feedback inhibition to CRH and AVP (at the level of the hypothalamus) and to ACTH (at the level of the anterior pituitary). Impaired corticosteroid signaling results in an attenuation of the negative feedback inhibition, which could result in the failure to sufficiently suppress CRH and AVP release from the hypothalamus and ACTH from the anterior pituitary, which in turn leads to chronically elevated levels of cortisol (*Figure 1*, right panel). The attenuated negative feedback inhibition can be most sensitively diagnosed with a neuroendocrine challenge test of the HPA axis, the combined dexamethasone (dex)/CRH test.[9] In this test, the stimulating effects of 100 µg intravenous human CRH upon ACTH and cortisol are examined under the suppressive action of 1.5 mg of dexamethasone.[10,11] This test is sensitive to impaired GR signaling at the pituitary level, as well as to the effects of increased secretion of the hypothalamic neuropeptides CRH and AVP, which is a consequence of impaired central GR signaling.[8,12,13]

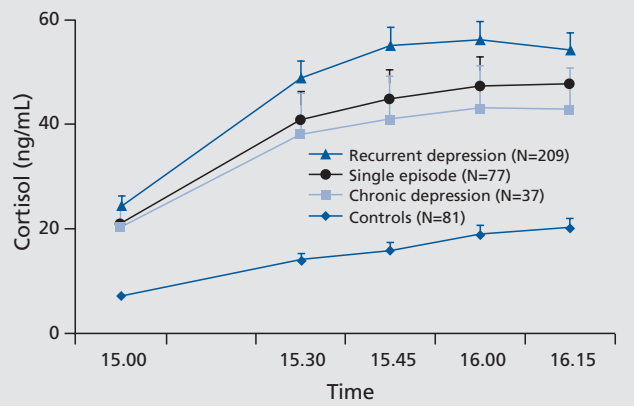

Figure 2. Cortisol response to the combined dex/CRH test is elevated in depression (AUC, *P*<.001) suggesting dysregulation of the HPA axis due to impaired glucocorticoid signaling. Dex, dexamethasone; CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenocortical; AUC, area under the curve

Impaired HPA axis regulation during an acute episode is the most consistent laboratory finding in depression and bipolar disorder (see refs 13 to 15 for reviews), which corresponds to the concept of stress-related disorders. Accordingly, the majority of depressed patients exhibit an exaggerated ACTH and cortisol response to the combined dex/CRH test *(Figure 2)*.

These alterations were shown to normalize after successful antidepressant treatment,[11,16-18] suggesting that altered HPA axis regulation and its normalization is involved in the pathogenesis of and recovery from depression, respectively.

Genetics of stress response

Evidence for heritability is a prerequisite for the involvement of genetic factors. The most efficient way for evaluating heritability is twin studies comparing phenotypical similarity between monozygotic and dizygotic twins. Twin data are available for the Trier Social Stress Test (TSST),[19] which is a standardized procedure for the assessment of the psychosocial stress response. Briefly, this test comprises a public speaking task involving a mock job interview and a mental arithmetic task. Subjects are asked to prepare a presentation for promoting their candidacy for a position that is tailored to their education. After the preparation time, subjects give their presentation in front of a panel of judges who are evaluating the talk. After 5 minutes, subjects are requested to perform an unexpected mental arithmetic task for a further 5 minutes. HPA axis activity (plasma ACTH and cortisol and/or salivary cortisol) is evaluated before and after the tasks as well as during recovery. Federenko and coworkers[20] reported a heritability estimate (h^2) of 0.32 for the plasma cortisol response to the TSST in 33 monozygotic and 25 dizygotic twin pairs, suggesting moderate heritability, but this increased up to 0.98 in two repetitions of the test. Heritability estimates for ACTH and salivary cortisol were distinctly smaller in the first test session, but increased markedly in the repeated test sessions. A previous study by Kirschbaum and coworkers[21] with 13 monozygotic and 11 dizygotic twin pairs also reported only marginal heritability for the salivary cortisol response to a single administration of the

TSST. High heritability was observed for salivary cortisol after stimulation with 100 µg human CRH (without dex suppression) and no heritability was found for the salivary cortisol response to strenuous physical exercise (ergometer activity).[21]

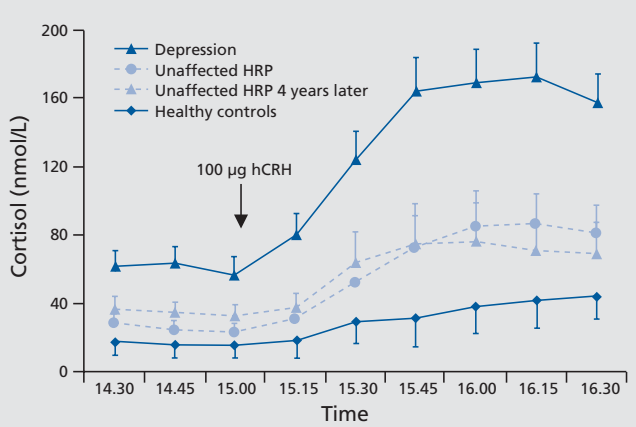

Figure 3. Cortisol response to the combined dex/CRH test is moderately elevated in high risk probands for affective disorders (AUC, *P*<.05), which was stable over time at the group level (AUC, *P*=.758) as well as at the individual level (Pearson correlation, *r*=.51, *P*<.05) in a follow-up investigation 4 years later. Dex, dexamethasone; CRH, corticotropin-releasing hormone; AUC, area under the curve

No heritability data are available for the combined dex/CRH test. However, in the Munich Vulnerability Study,[22,23] the combined dex/CRH test was conducted in healthy first-degree relatives of patients with a major depressive disorder, who are assumed to carry a genetic vulnerability for affective disorders. These so-called high-risk probands (HRPs) are characterized by a moderately elevated hormonal response to the combined dex/CRH test, which was significantly higher compared with controls without a personal or familial history of psychiatric disorders, but less pronounced compared with the response in acutely depressed patients. Modell and coworkers[24] replicated these findings in still unaffected HRPs who were re-examined in a follow-up investigation about 4 years later *(Figure 3)*, suggesting that this trait-like impaired regulation of the HPA system could reflect the genetic vulnerability for affective disorders in these subjects.

Despite the statistical evidence for a considerable heritability of the stress response, the number of significant genetic findings is small, and the conclusiveness rather limited. The findings are summarized in *Table I*. Due to the importance of the HPA system for the stress response, which is primarily regulated by GR, the GR gene has been proposed as the primary candidate for the

Genes	Chromosomal position	Results
Psychosocial stress response		
Glucocorticoid receptor *(GR, NR3C1)*	5q31.3	Combined BclI and N363S polymorphisms associated with salivary cortisol response to psychosocial stress (Trier Social Stress Test, TSST) in male mono- and dizygotic twins[25]; replicated in male unrelated subjects but not in female subjects (Kumsta and Wüst, 2006; personal communication)
GABA(A) α6 receptor subunit *(GABRA6)*	5q34	T1521C polymorphism associated with ACTH, cortisol, and blood pressure response to psychosocial stress (TSST) in healthy subject[26]
Opioid receptor µ1 *(OPRM1)*	6q24-q25	A118G polymorphism associated with cortisol response to psychosocial stress (modified TSST) in healthy subjects[27]
Endocrine HPA challenge tests		
Glucocorticoid receptor *(GR, NR3C1)*	5q31.3	BclI and N363S polymorphisms associated with ACTH and cortisol suppression after oral low-dose dexamethasone (dexamethasone suppression test) in elderly subjects[28,29]
Angiotensin-converting enzyme *(ACE)*	17q23.3	Insertion/deletion polymorphism associated with hormonal response to the combined dexamethasone suppression/CRH stimulation test in acute major depression[30,31]
Brain-derived neurotrophic factor *(BDNF)*	11p13	Val66Met polymorphism associated with ACTH and cortisol response to the combined dexamethasone suppression/CRH stimulation test in acute depression[23]

Table I. Genetic associations with stress response in human paradigms. GABA, γ-aminobutyric acid; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal

genetic association studies. Significant associations between GR and psychosocial stress response were reported, but only when a haplotype approach is applied[25] or when male subjects are separately analyzed (Kumsta and Wust, 2006; personal communication). Further genetic associations, not yet replicated, are reported for the γ-aminobutyric acid (GABA) A 6 receptor subunit gene[26] and for an nonsynonymous exon single-nucleotide polymorphism (SNP) of the micro-opioid receptor 1 (MOR) gene.[27]

Additional evidence for an involvement of the GR gene in the genetics of the stress response has been provided by two other studies *(Table I)* employing a low-dose dex suppression test in elderly subjects.[28,29] In this test, plasma cortisol levels after oral administration of dex are interpreted as an indicator for GR sensitivity, which is the major regulator of the stress hormone activity at the pituitary level. Two other studies in patients suffering from major depression[30,31] reported associations between the angiotensin-converting enzyme (ACE) gene and the hormonal response to the combined dex suppression/CRH stimulation test, which is the most sensitive challenge test for evaluating stress hormone regulation. ACE is involved in the so-called renin-angiotensin cascade of water regulation, which in turn affects blood volume and blood pressure. A recent study observed an association between the combined dex/CRH test and brain-derived neurotrophic factor (BDNF) in depressed patients, which has been interpreted as evidence for an involvement of a reduced neuroplasticity in the development of disturbed HPA axis regulation.[23]

Taken together, there are only a limited number of studies examining the association between candidate genes and the stress response. Besides genes involved in the sympathetic (ACE) or HPA axis-mediated (GR) stress response, further genes constituting different biological systems implicated in emotional regulation[26] and neuroplasticity (BDNF) have been examined. However, the results show only moderate effect sizes, although heritability estimates suggest a strong involvement of genetic factors. Further evidence for genes involved in the regulation of the stress response could be provided by clinical studies investigating genetic vulnerability factors for stress-related disorders. These genetic risk factors are assumed to be responsible for an inappropriate response to repeated and/or continuous stress and thus for mediating the vulnerability for stress-related disorders.

Genetics of stress-related disorders

A large number of diseases can be understood as stress-related disorders, and most of them are characterized by an at least moderate heritability. In this review, we focus on the most prevalent stress-related disorders, hypertension and coronary artery disease, as examples of cardiovascular disorders, and on bipolar disorder and unipolar depression as examples of psychiatric disorders.

Cardiovascular disorders are the leading cause of mortality in the Western world, and are projected to become the leading cause of disease burden worldwide in 2020.[32] Essential hypertension is the most common cardiovascular disorder, with a lifetime prevalence of above 50% in most western communities, affecting approximately 1 billion individuals worldwide[33]; heritability estimates around 30% have been reported.[34] Myocardial infarction is a serious outcome of coronary artery disease. Twin studies suggest that the risk for myocardial infarction is fairly heritable, with a heredity estimate of 60% in females and 26% in males.[35]

A large number of case-control association studies in essential hypertension are available *(Table IIa)* focussing on a number of candidate gene systems. The majority of findings have been obtained with candidates from the sympathetic system, including adrenergic genes, genes of the renin-angiotensin-aldosterone system (RAAS), and genes involved in vascular regulation. Despite the large number of studies, only a few associations can be regarded as convincing, including the associations with the angiotensinogen *(AGT)*, aldosterone synthase *(CYP11B2)*, and with the renin *(REN)* gene, all involved in the RAAS.

Several studies report gene x gene interaction effects, eg, between the endothelin 1 *(EDN1)* and serotonin receptor 2a *(5HTR2A)* genes,[69] and between the *ACE*, aldosterone synthase *(CYP11B2)*, and α adductin *(ADD1)* genes.[42] Several candidate genes from other biological systems (eg, *DRD2*, *GNB3*, *ACSM3*) have been proposed, but no unambiguous conclusion can yet be drawn from the findings from these studies.

As for hypertension, a large number of genetic association studies have also been conducted for coronary artery disease. However, the results are more difficult to interpret than in hypertension, since different clinical conditions, including myocardial infarction and arteriosclerosis/stenosis, are integrated as coronary artery disease. Most candidate genes showing replicable associations

have been derived from the concept of inflammation as a major risk factor for coronary heart disease. Convincing evidence for genetic associations has been reported for genes involved in innate immunity or genes moderating the inflammatory reaction, such as leukotrienes and lymphotoxins *(Table IIb)*.

The number of positive results outweighs the negative findings, and most effect sizes were in an at least moderate range. Nevertheless, not all candidate genes derived from potent endophenotypes show convincing associations. One example of this divergence is lipoprotein A, which has been identified as a potent vulnerability factor for coronary artery disease,[98] even though there is only a little evidence for a genetic association of the lipoprotein A *(LPA)* gene. Further gene candidates have been derived from studies in mendelian disorders involving premature coronary artery diseases such as familial hypercholesterolemia, familial defective apolipoprotein B (APOB), sitosterolemia, and Tangier disease. An overview of these findings is provided by Watkins and Farrall.[99] However, the translation of these findings to multifactorial cardiovascular disorders is limited.

Besides cardiovascular diseases, bipolar disorder and unipolar depression are further examples of burdensome stress-related disorders with a distinct heritability and a high prevalence in the general population, especially unipolar depression, which is projected to become the second leading cause for disease burden in 2020.[32] Lifetime prevalence of bipolar disorder is around 1% according to population-based epidemiological studies in Europe[100] as well as in the US,[101] while lifetime prevalence of unipolar depression is distinctly higher, with a similar rate of 17% in Europe and in the USA. Twin studies suggest a high heritability for bipolar disorder, with heritability estimates, h^2, ranging between 80% and 90%, and a moderate heritability for unipolar depression with h^2 between 33% and 42%.[102]

Most candidate genes for association studies with bipolar disorder and unipolar depression have been derived from neurotransmitter systems involved in antidepres-

Genes	Chromosomal position	Results
Adrenergic system		
β_2-adrenoceptor *(ADRB2)*	5q31-q32	Significant associations reported in Caucasian[36,37] and Asian populations,[38] but also several negative findings[39]
β_3-adrenoceptor *(ADRB3)*	8p12-p11.2	Significant associations reported in Caucasian population[40] and in male type 2 diabetics[41]
Renin-angiotensin-aldosterone system		
Angiotensin-converting enzyme *(ACE)*	17q23.3	Significant small to moderate effects,[42-45] but also several negative reports[40,46-48]
Angiotensinogen *(AGT)*	1q42-q43	Largest number of positive studies,[47,49,50] but also some negative findings[51]
Aldosterone synthase *(CYP11B2)*	8q21-q22	More positive[52-56] than negative[57] reports
Angiotensin *(AT1)* receptor *(AGTR1)*	3q21-q25	Mixed results, positive findings[49] as well as negative reports[44]
α Adductin *(ADD1)*	4p16.3	Mixed results, positive findings[58] as well as negative reports[51]
Atrial natriuretic peptide *(NPPA, NPPB)*	1p36.2	Less positive findings[59] than negative reports[60,61]
Renin *(REN)*	1q32	Predominance of positive findings[62-64]
11β-hydroxisteroid dehydrogenase 2 *(HSD11B2)*	16q22	Weak positive effects are reported[65,66]
Vascular system		
Endothelin 1 *(EDN1)*	6p24.1	Significant association with blood pressure in obese subjects;[67,68] some evidence for association with hypertension[69]; in interaction with 5-HTR2A
Nitric oxide synthase *(NOS3)*	7q36	Less positive findings[70] than negative reports[71,72]
Other genes		
D_2 receptor *(DRD2)*	11q23	Associated with hypertension[73] and with elevated blood pressure in personality disorder[74]
G protein β3 subunit *(GNB3)*	12p13	Less positive findings[75] than negative reports[51,54,76]
SAH *(ACSM3)*	16p13.11	Mixed results, positive findings[77] as well as negative reports[78]

Table IIa. Replicated findings of genetic associations with hypertension. 5-HT, serotonin; SAH, SA hypertension-associated homolog

sant drug action. Only some of the findings could be consistently replicated, including associations between the monoaminoxidase A *(MAOA)*[103] and catechol-o-methyltransferase *(COMT)* gene and bipolar disorder and tryptophan hydroxilase 2 *(TPH2)* gene and unipolar depression *(Table III)*. Further conclusive evidence exists for an involvement of the D-aminoacidoxidase activator DAOA (G72)/G30 locus in the susceptibility for bipolar disorder, but also for schizophrenia. A large number of studies have examined the genetic associations between

Genes	Chromosomal position	Results
Innate immunity		
CD14 molecule *(CD14)*	5q31.1	Significant associations with myocardial infarction,[79-81] but also negative reports[82,83]
Toll-like receptor 4 *(TLR4)*	9q32-q33	Significant associations reported for acute coronary events[84] and myocardial infarction[85,86] but not with coronary stenosis[87]
Leukotrienes		
Arachidonate 5-lipoxygenase-activating protein *(ALOX5AP)*	13q12	Evidence for an association with myocardial infarction[88,89] and arteriosclerosis[90]
Leukotriene A4 hydrolase *(LTA4H)*	12q22	Significant association with ethnicity-specific risk for myocardial infarction in different ethnic samples[91]
Other genes		
Lymphotoxine α *(LTA)*	6p21.3	Significant association with myocardial infarction in Japanese populations[92,93] as well with arteriosclerosis in Caucasians,[94] but also negative reports[95,96]
Galectin 2 *(LGALS2)*	22q13.1	Associated with myocardial infarction[97]; protein interacts with LTA

Table IIb. Replicated findings of genetic associations with coronary artery disease.

Genes	Chromosomal position	Results
Bipolar disorder		
Monoaminoxidase A *(MAOA)*	5q31.3	Significant associations with a modest effect size confirmed by meta-analyses[103,104] suggesting greatest effects in female patients
Catechol-o-methyltransferase *(COMT)*	22q11.21	Meta-analysis revealed a modest effect size[105,106] and has been suggested as a common susceptibility gene for bipolar disorder and schizophrenia[107]
5-HT transporter *(SLC6A4)*	17q11.1-q12	A number of positive studies[108-111] confirmed in meta-analyses,[112,113] but also negative studies for 5-HTTLPR,[114] one negative meta-analysis[105]
D-aminoacidoxidase activator *DAOA (G72) / G30*	13q33-q34	Several positive reports with polymorphisms in the proximity of these nested genes,[7,115-117] but also with schizophrenia, suggesting a common susceptibility locus[118]
Brain-derived neurotrophic factor *(BDNF)*	11p13	Family-based association studies showed significant effects[119,120] but most replication studies were negative[121-124]; one study suggested association with a subgroup of patients displaying rapid cycling[124]
P2X ligand-gated ion channel 7 *(P2RX7)*	12q24	Significant associations reported[125,126]
Unipolar depression		
Tryptophan hydroxilase 2 *(TPH2)*	12q21.1	Significant associations with major depression[127,128] and suicide[129]
5-HT transporter *(SLC6A4)*	17q11.1-q12	More depressive symptoms in carriers of the short 5-HTTLPR allele,[130,131] but also negative reports[114,132]
Glucocorticoid receptor *(NR3C1)*	5q31.3	BclI and ER22/23EK polymorphisms associated with susceptibility to recurrent unipolar depression[133]
P2X ligand-gated ion channel 7 *(P2RX7)*	12q24	Significant associations with unipolar depression reported[134,135]

Table III. Replicated findings of genetic associations with bipolar disorder and unipolar depression. 5-HT, serotonin

polymorphisms in the serotonin (5-HT) transporter *(SLC6A4)* gene and bipolar disorder and unipolar depression. Most attention focused on a functional insertion/deletion polymorphism in the promoter region to *SLC6A4*, known as 5HTTLPR. Despite several positive results, the number of negative replications is increasing, and the relevance of this polymorphism for the susceptibility to bipolar disorder or unipolar depression is meanwhile being challenged.

Besides *SLC6A4*, P2X ligand-gated ion channel 7[125] is the only gene showing replicated effects for susceptibility to both bipolar disorder and unipolar depression. This gene codes for a cation-selective ion channel expressed in central glial cells as well as in neurons, and is assumed to regulate immune function and neurotransmitter release.[136,137]

In summary, genetic association studies in stress-related disorders have provided evidence for an involvement of several other genes not identified by basic genetic studies on stress response. Since an inappropriate response to repeated and/or continuous stress mediates the susceptibility to stress-related disorders, these genes are also assumed to moderate the stress response. We have reviewed genetic association studies in hypertension, coronary artery disease, bipolar disorder, and unipolar depression. Due to the large and rapidly increasing number of publications, it is impossible to provide a complete overview. However, we have tried to summarize the most consistent and most frequently discussed findings. It is important to note that different classes of candidate genes have been investigated in the four diagnostic groups reported in this review, despite their common relationship to stress and inappropriate stress response. While candidate genes in hypertension and coronary artery disease are primarily related to the RAAS and to inflammation/immune response, respectively, the majority of candidate genes in bipolar disorder and unipolar depression are derived from monoaminergic neurotransmitter systems. This makes it clear that our actual knowledge of the complex interplay between genetic factors, altered stress response, and stress-related disorders is still limited, and that further research and new approaches are required to improve our understanding of these complex functions.

Conclusion and outlook

The summarized findings do not provide an exhaustive and satisfying answer about the genetics of stress response and stress-related disorders. Many single findings are still unconnected, and the restriction of the gene selection to established candidates has retarded our understanding of the complex interplay between genetic factors, stress response, and stress-related disorders. Sophisticated models, especially those aiming to integrate the findings from basic and clinical research as well as from the different types of stress-related disorders, are required to close the gap in our knowledge. The new chip-based whole-genome technologies, Affymetrix GeneChip and Illumina Genotyping BeadChip, are powerful tools for this endeavor. With this technology, the advantages of an unbiased approach as provided by linkage analysis, and the statistical power of association studies are combined to identify new candidate genes. However, results from unbiased approaches are always preliminary, and require validation in confirmatory studies. This means that independent replication studies are needed, but also clinical studies taking gene x gene and gene x environment interactions into account. For causal inferences, preclinical experiments are required, including (conditional) genetic modification and the development of specific compounds as research tools for the protein targets. Finally, text- and information-mining tools, which are already available but have to be further developed, will be very helpful to integrate all findings into sophisticated models delineating the pathways from genes to stress response and stress-related disorders. There is still a long way to go—but the prerequisites for success are more present than ever. ❑

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Genetica de la respuesta al estrés y de los trastornos relacionados con el estrés

Los descubrimentos principales en la genética de y de los trastornos relacionados con el estrés son: i) las variaciones de los genes implicados en el sistema simpático o en el eje hipotálamo-hipófisis-corteza suprarrenal están asociadas con respuestas alteradas al estrés; ii) los genes relacionados con el sistema renina-angiotensina-aldosterona o la respuesta inflamatoria/inmunitaria están asociados a los trastornos cardiovasculares; iii) los genes implicados en los sistemas neurotransmisores monoaminérgicos están asociados al trastorno bipolar y a la depresión unipolar. La inmensa mayoría de estos estudios de asociación siguieron un enfoque convencional, impulsado por hipótesis, lo que restringe la selección de genes candidatos conocidos. Este método tan conservador ha retrasado el conocimiento de la interrelación compleja entre los factores genéticos, la respuesta al estrés y los trastornos relacionados con éste. Las tecnologías de chip para el estudio de todo el genoma abrirán las puertas a métodos nuevos, objectivos y eficaces, lo que estadísticamente permitirá identificar nuevos genes candidatos que serán validados minuciosamente en estudios confirmatorios clínicos y preclínicos. Todo ello, sumado al uso de nuevos instrumentos para la explotación de texto e información, nos ayudará a integrar todos los datos dentro de modelos complejos que delimiten las vías desde los genes hasta la respuesta al estrés y los trastornos relacionados con el estrés.

Génétique de la réponse au stress et des troubles liés au stress

Voici les principaux résultats sur la génétique de la réponse au stress et des troubles liés au stress: 1) les variations des gènes impliqués dans le système sympathique ou l'axe hypothalamo-hypophyso-surrénalien sont associées à des anomalies de la réponse au stress ; 2) les gènes liés au système rénine-angiotensine-aldostérone ou à une la réponse inflammatoire/immune sont associés avec des maladies cardiovasculaires ; 3) les gènes impliqués dans les systèmes de neurotransmission monoaminergiques sont associés aux troubles bipolaires et à la dépression unipolaire. La grande majorité de ces études d'association a suivi une approche conventionnelle hypothético-déductive, limitant donc la sélection des gènes aux candidats établis. Cette approche très conservatrice a retardé notre compréhension des interactions complexes entre les facteurs génétiques, la réponse au stress et les troubles liés au stress. Les technologies de puce à ADN sur le génome entier ouvriront la voie à de nouvelles approches non biaisées et statistiquement efficaces qui permettront d'identifier de nouveaux gènes candidats. Ces derniers devront être minutieusement validés dans des études cliniques et précliniques de confirmation. Ces technologies, associées à de nouveaux outils d'analyse des textes et des informations, nous permettront d'intégrer plus facilement tous les résultats dans des modèles sophistiqués précisant les voies qui vont des gènes à la réponse au stress et aux troubles liés au stress.

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Traumatic stress: effects on the brain

J. Douglas Bremner, MD

Brain areas implicated in the stress response include the amygdala, hippocampus, and prefrontal cortex. Traumatic stress can be associated with lasting changes in these brain areas. Traumatic stress is associated with increased cortisol and norepinephrine responses to subsequent stressors. Antidepressants have effects on the hippocampus that counteract the effects of stress. Findings from animal studies have been extended to patients with post-traumatic stress disorder (PTSD) showing smaller hippocampal and anterior cingulate volumes, increased amygdala function, and decreased medial prefrontal/anterior cingulate function. In addition, patients with PTSD show increased cortisol and norepinephrine responses to stress. Treatments that are efficacious for PTSD show a promotion of neurogenesis in animal studies, as well as promotion of memory and increased hippocampal volume in PTSD.

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Effects of traumatic stress

Traumatic stressors such as early trauma can lead to post-traumatic stress disorder (PTSD), which affects about 8% of Americans at some time in their lives,[1] as well as depression,[2,3] substance abuse,[1,4] dissociation,[5] personality disorders,[6,7] and health problems.[8] For many trauma victims, PTSD can be a lifelong problem.[9] The President's New Freedom Commission Report highlights the importance of providing services for mental disorders related to early trauma.[10-12] However, the development of effective treatments is limited by gaps in knowledge about the underlying neurobiological mechanisms that mediate symptoms of trauma-related disorders like PTSD. This paper reviews preclinical and clinical studies on the effects of traumatic stress on the brain.

Normal development of the brain across the lifespan

To understand how traumatic stress occurring at different stages of the life cycle interacts with the developing brain, it is useful to review normal brain development. The normal human brain undergoes changes in structure and function across the lifespan from early childhood to late life. Understanding these normal developmental changes is critical for determining the difference between normal development and pathology, and how normal development and pathology interact.

Although the bulk of brain development occurs in utero, the brain continues to develop after birth. In the first 5 years of life there is an overall expansion of brain volume related to development of both gray matter and white

Selected abbreviations and acronyms

ACTH	*adrenocorticotropic hormone*
BDNF	*brain-derived neurotropic factor*
BPD	*bipolar disorder*
CRF	*corticotropin-releasing factor*
CS	*conditioned stimulus*
FDG	*fluorodeoxyglucose*
HPA	*hypothalamic-pituitary-adrenal*
MRI	*magnetic resonance imaging*
mRNA	*messenger ribonucleic acid*
NAA	N-*acetyl aspartate*
PET	*positron emission tomography*
PTSD	*post-traumatic stress disorder*
US	*unconditioned stimulus*

matter structures; however, from 7 to 17 years of age there is a progressive increase in white matter (felt to be related to ongoing myelination) and decrease in gray matter (felt to be related to neuronal pruning) while overall brain size stays the same.[13-16] Gray matter areas that undergo the greatest increases throughout this latter developmental epoch include frontal cortex and parietal cortex.[17,18] Basal ganglia decrease in size, while corpus callosum,[19,20] hippocampus, and amygdala[21-23] appear to increase in size during childhood, although there may be developmental sex-laterality effects for some of these structures.[24] Overall brain size is 10% larger in boys than girls during childhood.[24]

During the middle part of life (from age 20 to 70) there is a gradual decrease in caudate,[25] diencephalon,[25] and gray matter,[25,26] which is most pronounced in the temporal[27] and frontal cortex,[26] with enlargement of the ventricles[26,27] and no change in white matter.[25,26] Studies have not been able to document changes in hippocampal volume in normal populations during this period.[27] After menopause in women at about the age of 50, however, there are changes in reproductive hormones, such as decreased levels of estrogen. Since estrogen promotes neuronal branching in brain areas such as the hippocampus,[28] a loss of estrogen may lead to changes in neuronal structure. Although the effects of menopause on the brain have not been well studied, it is known that sex hormones also affect brain function and circuitry[29]; therefore, the changes in sex hormones with menopause will presumably affect brain function, as well as possibly structure. There is some evidence in super-elderly individuals (age >70) for modest reductions in hippocampal volume with late stages of aging.[27,30] More robust findings have included increased ventricular volume and reduction in gray matter, temporal lobe, and cerebellum volumes with normal aging, that begins before the age of 70.[25,27,31-33]

Therefore, trauma at different stages in life will presumably have different effects on brain development. The few studies that have looked at this issue do suggest that there are differences in the effects of trauma on neurobiology, depending on the stage of development at which the trauma occurs. Studies in this area, however, have been limited.

Neurobiology of PTSD

PTSD is characterized by specific symptoms, including intrusive thoughts, hyperarousal, flashbacks, nightmares, and sleep disturbances, changes in memory and concentration, and startle responses. Symptoms of PTSD are hypothesized to represent the behavioral manifestation of stress-induced changes in brain structure and function. Stress results in acute and chronic changes in neurochemical systems and specific brain regions, which result in long-term changes in brain "circuits," involved in the stress response.[34-37] Brain regions that are felt to play an important role in PTSD include hippocampus, amygdala, and medial prefrontal cortex. Cortisol and norepinephrine are two neurochemical systems that are critical in the stress response *(Figure 1)*.

The corticotropin-releasing factor (CRF)/hypothalamic-pituitary-adrenal (HPA) axis system plays an important role in the stress response. CRF is released from the hypothalamus, with stimulation of adrenocorticotropic hormone (ACTH) release from the pituitary, resulting in glucocorticoid (cortisol in man) release from the adrenal, which in turn has a negative feedback effect on the axis at the level of the pituitary, as well as central brain sites including hypothalamus and hippocampus. Cortisol has a number of effects which facilitate survival. In addition to its role in triggering the HPA axis, CRF acts centrally to mediate fear-related behaviors,[38] and triggers other neurochemical responses to stress, such as the noradrenergic system via the brain stem locus coeruleus.[39] Noradrenergic neurons release transmitter throughout the brain; this is associated with an increase in alerting and vigilance behaviors, critical for coping with acute threat.[40-42]

Studies in animals showed that early stress has lasting effects on the HPA axis and norepinephrine. A variety of early stressors resulted in increased glucocorticoid

response to subsequent stressors.[43-45] Maternally deprived rats had decreased numbers of glucocorticoid receptors in the hippocampus, hypothalamus, and frontal cortex.[46] Stressed animals demonstrated an inability to terminate the glucocorticoid response to stress,[47,48] as well as deficits in fast-feedback of glucocorticoids on the HPA axis, which could be related to decreased glucocorticoid receptor binding in the hippocampus.[49] Early postnatal adverse experiences increase hypothalamic CRF messenger ribonucleic acid (mRNA), median eminence CRF content, and stress-induced glucocorticoid[50] and ACTH release.[46] These effects could be mediated by an increase in synthesis of CRH mRNA following stress.[51] In nonhuman primates, adverse early experiences resulted in long-term effects on behaviors, as well as elevated levels of CRF in the cerebrospinal fluid.[52] Exposure to chronic stress results in potentiation of noradrenergic responsiveness to subsequent stressors and increased release of norepinephrine in the hippocampus and other brain regions.[42]

Preclinical and clinical studies have shown alterations in memory function following traumatic stress,[53] as well as changes in a circuit of brain areas, including hippocampus, amygdala, and medial prefrontal cortex, that mediate alterations in memory.[54] The hippocampus, a brain area involved in verbal declarative memory, is very sensitive to the effects of stress. Stress in animals is associated with damage to neurons in the CA3 region of the hippocampus (which may be mediated by hypercortisolemia, decreased brain-derived neurotrophic factor (BDNF), and/or elevated glutamate levels) and inhibition of neurogenesis.[55-60] High levels of glucocorticoids seen with stress were also associated with deficits in new learning.[61,62]

Antidepressant treatments have been shown to block the effects of stress and/or promote neurogenesis.[58,63-66] Animal studies have demonstrated several agents with

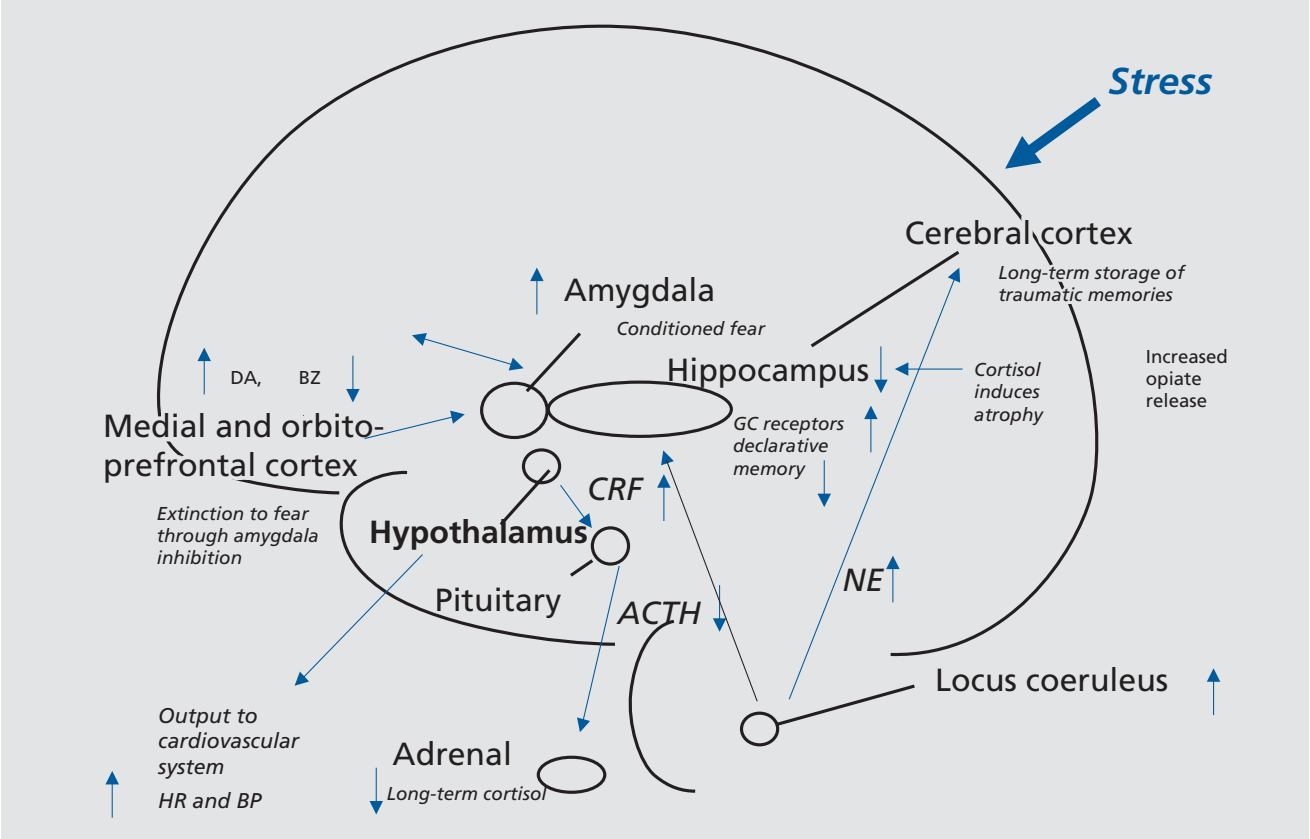

Figure 1. Lasting effects of trauma on the brain, showing long-term dysregulation of norepinephrine and cortisol systems, and vulnerable areas of hippocampus, amygdala, and medial prefrontal cortex that are affected by trauma. GC, glucocorticoid; CRF, corticotropin-releasing factor; ACTH, adrenocorticotropin hormone; NE, norepinephrine; HR, heart rate; BP, blood pressure; DA, dopamine; BZ, benzodiazapine; GC, glucocorticoid

potentially beneficial effects on stress-induced hippocampal damage. It has been found that phenytoin blocks the effects of stress on the hippocampus, probably through modulation of excitatory amino acid-induced neurotoxicity.[67] Other agents, including tianeptine, dihydroepiandosterone (DHEA), and fluoxetine have similar effects.[63,64,66,68-73] These medications may share a common mechanism of action through upregulation of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) that may lead to regulation of expression of specific target genes involved in structural modeling of the hippocampus. Such treatment effects on BDNF and trkB messenger ribonucleic acid (mRNA), can have long-term effects on brain structure and function. There is new evidence that neurogenesis is necessary for the behavioral effects of antidepressants,[74,75] although this continues to be a source of debate.[72,76]

The hippocampus demonstrates an unusual capacity for neuronal plasticity and regeneration. In addition to findings noted above related to the negative effects of stress on neurogenesis, it has recently been demonstrated that changes in the environment, eg, social enrichment or learning, can modulate neurogenesis in the dentate gyrus of the hippocampus, and slow the normal age-related decline in neurogenesis.[77,78] Rat pups that are handled frequently within the first few weeks of life (picking them up and then returning them to their mother) had increased type II glucocorticoid receptor binding which persisted throughout life, with increased feedback sensitivity to glucocorticoids, and reduced glucocorticoid-mediated hippocampal damage in later life.[79] These effects appear to be due to a type of "stress inoculation" from the mothers' repeated licking of the handled pups.[80] Considered together, these findings suggest that early in the postnatal period there is a naturally occurring brain plasticity in key neural systems that may "program" an organism's biological response to stressful stimuli. These findings may have implications for victims of childhood abuse.

Long-term dysregulation of the HPA axis is associated with PTSD, with low levels of cortisol found in chronic PTSD in many studies[81-86] and elevations in CRF.[82,87] Not all studies, however, have found lower cortisol levels in PTSD.[88-91] Exposure to a traumatic reminder appears to be associated with a potentiated release of cortisol in PTSD.[92]

The few studies of the effects of early stress on neurobiology conducted in clinical populations of traumatized children have generally been consistent with findings from animal studies. Research in traumatized children has been complicated by issues related to psychiatric diagnosis and assessment of trauma.[93] Some studies have not specifically examined psychiatric diagnosis, while others have focused on children with trauma and depression, and others on children with trauma and PTSD. Sexually abused girls (in which effects of specific psychiatric diagnosis were not examined) had normal baseline cortisol and blunted ACTH response to CRF,[94] while women with childhood abuse-related PTSD had hypercortisolemia.[95] Another study of traumatized children in which the diagnosis of PTSD was established showed increased levels of cortisol measured in 24-hour urines.[96] Emotionally neglected children from a Romanian orphanage had elevated cortisol levels over a diurnal period compared with controls.[97] Maltreated school-aged children with clinical-level internalizing problems had elevated cortisol compared with controls.[98] Depressed preschool children showed increased cortisol response to separation stress.[99] Adult women with a history of childhood abuse showed increased suppression of cortisol with low-dose (0.5 mg) dexamethasone.[100] Women with PTSD related to early childhood sexual abuse showed decreased baseline cortisol based on 24-hour diurnal assessments of plasma, and exaggerated cortisol response to stressors (traumatic stressors[101] more than neutral cognitive stressors).[102] We also found that patients with PTSD had less of an inhibition of memory function with synthetic cortisol (dexamethasone) than normal subjects.[103] Adult women with depression and a history of early childhood abuse had an increased cortisol response to a stressful cognitive challenge relative to controls,[104] and a blunted ACTH response to CRF challenge.[105] These findings show long-term changes in stress responsive systems. Early in development, stress is associated with increased cortisol and norepinephrine responsiveness, whereas with adulthood, resting cortisol may be normal or low, but there continues to be increased cortisol and norepinephrine responsiveness to stressors. In addition, early stress is associated with alterations in hippocampal morphology which may not manifest until adulthood, as well as increased amygdala function and decreased medial prefrontal function.

Cognitive function and brain structure in PTSD

Studies in PTSD are consistent with changes in cognition and brain structure. Multiple studies have demonstrated verbal declarative memory deficits in PTSD.[53,106-108]

Patients with PTSD secondary to combat[109-113] and childhood abuse[114,115] were found to have deficits in verbal declarative memory function based on neuropsychological testing. Studies, using a variety of measures (including the Wechsler Memory Scale, the visual and verbal components of the Selective Reminding Test, the Auditory Verbal Learning Test, Paired Associate Recall, the California Verbal New Learning Test, and the Rivermead Behavioral Memory Test), found specific deficits in verbal declarative memory function, with a relative sparing of visual memory and IQ.[109-113,115-124] These studies have been conducted in both patients with PTSD related to Vietnam combat,[109-113,116,119-121,123] rape,[117] the Holocaust,[124-126] adults with early childhood abuse,[115] and traumatized children.[118] One study in adult rape survivors showed that verbal declarative memory deficits are specifically associated with PTSD, and are not a nonspecific effect of trauma exposure.[117] Another study of women with early childhood sexual abuse in which some, but not all, of the patients had PTSD, showed no difference between abused and nonabused women,[127] while another study was not able to show a difference between Vietnam veterans with and without PTSD.[128] Other types of memory disturbances studied in PTSD include gaps in memory for everyday events (dissociative amnesia),[129] deficits in autobiographical memory,[130] an attentional bias for trauma-related material,[131-140] and frontal lobe-related impairments.[141] These studies suggest that traumas such as early abuse with associated PTSD result in deficits in verbal declarative memory. It is not clear if cognitive deficits in early abuse survivors are specific to PTSD and are not related to the nonspecific effects of abuse.

These effects were specific to verbal (not visual) memory, and were significant after controlling for IQ. Some of these studies used neuropsychological tests of declarative memory, such as the Wechsler Memory Scale (WMS) and Selective Reminding Test (SRT), that have been validated as sensitive to loss of neurons in the CA3 region of the hippocampus in epileptics who underwent hippocampal resection.[142,143] Vietnam veterans with PTSD were originally shown by us to have 8% smaller right hippocampal volume based on magnetic resonance imaging (MRI) relative to controls matched for a variety of factors such as alcohol abuse and education ($P<0.05$); smaller volume was correlated with deficits in verbal declarative memory function as measured with the Wechsler Memory Scale.[144] A second study from our group showed a 12% reduction in left hippocampal volume in 17 patients with childhood abuse-related PTSD compared with 17 case-matched controls, that was significant after controlling for confounding factors.[145] Smaller hippocampal volume was shown to be specific to PTSD within the anxiety disorders, and was not seen in panic disorder.[146] Gurvits et al[147] showed bilateral hippocampal volume reductions in combat-related PTSD compared with combat veterans without PTSD and normal controls. Combat severity was correlated with volume reduction. Stein et al[148] found a 5% reduction in left hippocampal volume. Other studies in PTSD have found smaller hippocampal volume and/or reductions in *N*-acetyl aspartate (NAA), a marker of neuronal integrity.[149-153] Studies in childhood[154-156] and new-onset[157,158] PTSD did not find hippocampal volume reduction, although reduced NAA (indicating loss of neuronal integrity) was found in medial prefrontal cortex in childhood PTSD.[159] In a recent meta-analysis we pooled data from all of the published studies and found smaller hippocampal volume for both the left and the right sides, equally in adult men and women with chronic PTSD, and no change in children.[160] More recent studies of holocaust survivors with PTSD did not find a reduction in hippocampal volume, although PTSD patients who developed PTSD in response to an initial trauma had smaller hippocampal volume compared with those who developed PTSD after repeated trauma, suggesting a possible vulnerability of smaller hippocampal volume.[161] Two independent studies have shown that PTSD patients have deficits in hippocampal activation while performing a verbal declarative memory task,[149,162] although it is unclear if this is a deficit in activation or higher hippocampal blood flow at baseline. Both hippocampal atrophy and hippocampal-based memory deficits reversed with treatment with the selective serotonin reuptake inhibitor (SSRI) paroxetine, which has been shown to promote neurogenesis (the growth of neurons) in the hippocampus in preclinical studies.[163] In addition, treatment with the anticonvulsant phenytoin led to an improvement in PTSD symptoms[164] and an increase in right hippocampal and right cerebral volume.[165] We hypothesize that stress-induced hippocampal dysfunction may mediate many of the symptoms of PTSD which are related to memory dysregulation, including both explicit memory deficits as well as fragmentation of memory in abuse survivors. It is unclear at the current time whether these changes are specific to PTSD, whether certain common environmental events (eg, stress) in different disorders lead to similar brain changes, or whether common genetic traits lead to similar outcomes.

The meaning of findings related to deficits in memory and the hippocampus in PTSD, and questions related to the relative contribution of genetic and environmental factors, has become an important topic in the field of PTSD and stress research. There are three possible models, taking into account genetic or environmental factors, which have been proposed to explain smaller hippocampal volume in PTSD: Model A (Environment), Model B (Environment and Genetic), and Model C (Genetic).[166-169] In Model C (Genetic), smaller hippocampal volume represents a premorbid risk factor for PTSD. In support of this model Pitman and colleagues[170] have demonstrated that lower premilitary IQ is associated with combat-related PTSD, as well as finding a correlation between PTSD symptoms and hippocampal volume in twin brothers.[151] Model A (Environment) states that stress leads to damage or inhibition of neurogenesis via hypercortisolemia, decreased BDNF, or increased glutamate. Model B (Environment/Genetic) states that a combination of environmental and genetic factors leads to deficits in hippocampal function and structure. Showing that an intervention like medication changes hippocampal volume and cognition would provide support for at least a partial contribution of the environment to the outcomes of interest.

In addition to the hippocampus, other brain structures have been implicated in a neural circuitry of stress, including the amygdala and prefrontal cortex. The amygdala is involved in memory for the emotional valence of events, and plays a critical role in the acquisition of fear responses. The medial prefrontal cortex includes the anterior cingulate gyrus (Brodmann's area [BA] 32) and subcallosal gyrus (area 25) as well as orbitofrontal cortex. Lesion studies demonstrated that the medial prefrontal cortex modulates emotional responsiveness through inhibition of amygdala function. Conditioned fear responses are extinguished following repeated exposure to the conditioned stimulus in the absence of the unconditioned (aversive, eg, electric shock) stimulus. This inhibition appears to be mediated by medial prefrontal cortical inhibition of amygdala responsiveness.

Animal studies also show that early stress is associated with a decrease in branching of neurons in the medial prefrontal cortex.[171] Rauch and colleagues found smaller volume of the anterior cingulate based on MRI measurements in PTSD[172]; we have replicated these findings in women with abuse and PTSD.[160] An important question is whether these effects are reversible with treatment.

Neural circuits in PTSD

Brain imaging studies have shown alterations in a circuit including medial prefrontal cortex (including anterior cingulate), hippocampus, and amygdala in PTSD. Many of these studies have used different methods to trigger PTSD symptoms (eg, using traumatic cues) and then look at brain function. Stimulation of the noradrenergic system with yohimbine resulted in a failure of activation in dorsolateral prefrontal, temporal, parietal, and orbitofrontal cortex, and decreased function in the hippocampus.[173] Exposure to traumatic reminders in the form of traumatic slides and/or sounds or traumatic scripts was associated with an increase in PTSD symptoms, decreased blood flow, and/or failure of activation in the medial prefrontal cortex/anterior cingulate, including Brodmann's area 25, or subcallosal gyrus, area 32 and 24, as measured with positron emission tomography (PET) or functional MRI (fMRI).[174-183] Other findings in studies of traumatic reminder exposure include decreased function in hippocampus,[176] visual association cortex,[176,180] parietal cortex,[176,179,180,184] and inferior frontal gyrus,[176,179,180,184] and increased function in amygdala,[181,184] posterior cingulate,[174,176,177,180] and parahippocampal gyrus.[174,176,178] Shin and colleagues found a correlation between increased amygdala function and decreased medial prefrontal function with traumatic reminders,[181] indicating a failure of inhibition of the amygdala by the medial prefrontal cortex that could account for increased PTSD symptoms with traumatic reminders. Other studies found increased amygdala and parahippocampal function and decreased medial prefrontal function during performance of an attention task,[182] increased posterior cingulate and parahippocampal gyrus and decreased medial prefrontal and dorsolateral prefrontal function during an emotional Stroop paradigm,[185] and increased amygdala function with exposure to masked fearful faces.[186] Retrieval of emotionally valenced words[187] (eg "rape-mutilate") in women with PTSD from early abuse resulted in decreases in blood flow in an extensive area which included orbitofrontal cortex, anterior cingulate, and medial prefrontal cortex (BA 25, 32, and 9), left hippocampus, and fusiform gyrus/inferior temporal gyrus, with increased activation in posterior cingulate, left inferior parietal cortex, left middle frontal gyrus, and visual association and motor cortex.[188] Another study found a failure of medial prefrontal cortical/anterior cingulate activation, and decreased visual association and parietal

cortex function, in women with abuse and PTSD relative to women with abuse without PTSD, during performance of the emotional Stroop task (ie, naming the color of a word such as "rape").[189] We recently found increased amygdala activation with classical fear conditioning (pairing a shock and a visual stimulus), and decreased medial prefrontal cortex function with extinction, in abuse-related PTSD.[190] The findings described above point to a network of related regions mediating symptoms of PTSD, including medial prefrontal cortex, anterior cingulate, hippocampus, amygdala, posterior cingulate, parietal, visual association, and dorsolateral prefrontal cortex.[191]

Fewer brain imaging studies have been performed in children with PTSD. Several studies have shown alterations in electroencephalogram (EEG) measures of brain activity in children with a variety of traumas who were not selected for diagnosis compared with healthy children. About half of the children in these studies had a psychiatric diagnosis. Abnormalities were located in the anterior frontal cortex and temporal lobe and were localized to the left hemisphere.[192,193] Two studies have found reductions in brain volume in children with trauma and PTSD symptoms.[154,155] One group did not find reductions in hippocampal volume, either at baseline or over a longitudinal period,[154,156] while another group found an 8.5% reduction in hippocampal volume that was not significant after controlling for smaller brain volumes in the PTSD group.[155] One study used single-voxel proton magnetic resonance spectroscopy (proton MRS) to measure relative concentration of NAA and creatinine (a marker of neuronal viability) in the anterior cingulate of 11 children with maltreatment-related PTSD and 11 controls. The authors found a reduction in the ratio of NAA to creatinine in PTSD relative to controls.[159] Studies have also found smaller size of the corpus callosum in children with abuse and PTSD relative to controls.[154] as well as larger volume of the superior temporal gyrus.[194] In a study of abused children in whom diagnosis was not specified, there was an increase in T2 relaxation time in the cerebellar vermis, suggesting dysfunction in this brain region.[195] The reason for differences in findings between adults and children are not clear; however, factors such as chronicity of illness or interaction between trauma and development may explain findings to date.

In summary, dysfunction of a circuit involving the medial prefrontal cortex, dorsolateral prefrontal cortex, and possibly hippocampus and amygdala during exposure to traumatic reminders may underlie symptoms of PTSD. These studies have primarily assessed neural correlates of traumatic remembrance, while little has been done in the way of utilizing cognitive tasks as probes of specific regions, such as memory tasks as probes of hippocampal function.

MRI assessment of brain abnormalities in PTSD and trauma spectrum disorders

Findings of smaller hippocampal volume appear to be associated with a range of trauma related psychiatric disorders, as long as there is the presence of psychological trauma. We have used MRI to show smaller hippocampal volume in PTSD,[144,145,149,196] depression,[197] depression with early abuse,[198] borderline personality disorder (BPD) with early abuse,[199] and Dissociative Identity Disorder (DID) with early abuse.[200] The greatest magnitude of difference was seen in the DID patients, who had unusually severe early childhood sexual abuse histories. We did not find changes in hippocampal volume in patients with panic disorder without a history of abuse (suggesting that findings are not generalized to other anxiety disorders).[201] We found smaller amygdala volume in BPD with early abuse[199] and increased amygdala volume in depression.[197,202] Patients with depression had smaller orbitofrontal cortex volume with no changes in anterior cingulate (BA 32) or medial prefrontal cortex (BA 25).[203] More recently, we found smaller anterior cingulate volume in women with abuse and PTSD relative to controls.[204]

Neural circuits in women with abuse and PTSD

We have used PET to study neural circuits of trauma-related disorders in women with early abuse and a variety of trauma spectrum mental disorders. Initially we studied women with abuse and PTSD.[54,205-208] We initially measured brain activation with a paragraph-encoding task in conjunction with PET O-15 water measurement of brain blood flow. Women with abuse and PTSD showed a failure of hippocampal activation during the memory task relative to controls.[149] Women with abuse and PTSD in this study also had smaller hippocampal volume measured with MRI relative to both women with abuse without PTSD and nonabused non-PTSD women. The failure of hippocampal activation was significant

after controlling differences in hippocampal volume as well as accuracy of encoding. In another study we measured neural correlates of exposure to a personalized script of childhood sexual abuse. Women with abuse and PTSD showed a failure of medial prefrontal and hippocampal activation relative to abused women without PTSD.[176] Women with abuse and PTSD also showed a failure of medial prefrontal and hippocampal function during recall of paired word associates with traumatic-emotional content (eg, "rape-mutilate"),[188] and decreased medial prefrontal function during an emotional Stroop task with trauma-content words.[209] Other studies showed a failure of medial prefrontal activation in women with BPD and early abuse during an abandonment script.[210] Women with BPD and abuse had increased psychophysiological responses to abandonment scripts relative to trauma scripts, while women with PTSD and abuse had the opposite pattern,[211] indicating differential responding in those two disorders in spite of the common exposure to early abuse.

In another project we studied 19 physically healthy women including women with a history of severe childhood sexual abuse and the diagnosis of current PTSD (N=8) and women without childhood abuse or PTSD (N=11).[212] All subjects underwent PET measurement of cerebral blood flow and psychophysiology measurement of heart rate and skin conductance during habituation, acquisition, and extinction conditions, on a single day, with scanning during a control condition on another day separated by 1 week from the active condition. Subjects were randomly assigned to undergo either the active condition or the control condition first (ie, active-control or control-active). Subjects were told at the beginning of the study that they would be exposed to electric shocks and viewing images on a screen during collection of PET and psychophysiology data. During habituation subjects were exposed to a blue square on a screen (conditioned stimulus [CS]), 4 seconds in duration, followed by 6 seconds of a blank screen. CS exposure was repeated eight times at regular intervals over 80 seconds in two separate blocks separated by 8 minutes. One PET image of brain blood flow was obtained starting from the beginning of each of the blocks. During active fear acquisition exposure to the blue square (CS) was paired with an electric shock to the forearm (unconditioned stimulus [UCS]). Subjects had 8 paired CS-UCS presentations at 10-second intervals for each of two blocks. With extinction subjects were again exposed to the blue squares (CS) without shock ("active" extinction). On a second day subjects went through the same procedure with electric shocks delivered randomly when the blue square was not present (unpaired CS-UCS) (an equal number as on day 1) during scans 3 and 4, which served as a control for active fear acquisition.

PTSD subjects had increased symptoms of anxiety, fear, dissociation, distress, substance use disorders (SUDs), and PTSD at all time points during both study days relative to non-PTSD. Acquisition of fear was associated with increased skin conductance (SC) responses to CS exposure during the active versus the control conditions in all subjects. There was increased SC for PTSD during the first CS-UCS presentation. Extinction of fear was associated with increased skin conductance (SC) responses to CS exposure during the active versus the control conditions in all subjects. When PTSD and non-PTSD subjects were examined separately, SC levels were significantly elevated in non-PTSD subjects undergoing extinction following the active compared with the control condition during session one.

PTSD subjects showed activation of the bilateral amygdala during fear acquisition compared with the control condition. Non-PTSD subjects showed an area of activation in the region of the left amygdala. When PTSD subjects and control subjects were directly compared, PTSD subjects showed greater activation of the left amygdala during the fear conditioning condition (pairing of US and CS) relative to the random shock control than healthy women. Other areas that showed increased activation with fear acquisition in PTSD included bilateral superior temporal gyrus (BA 22), cerebellum, bilateral inferior frontal gyrus (BA 44, 45), and posterior cingulate (BA 24). Fear acquisition was associated with decreased function in medial prefrontal cortex, visual association cortex, and medial temporal cortex, inferior parietal lobule function, and other areas. Extinction of fear responses was associated with decreased function in the orbitofrontal and medial prefrontal cortex (including subcallosal gyrus, BA 25, and anterior cingulate BA 32), visual association cortex, and other areas, in the PTSD subjects, but not in the controls. Amygdala blood flow with fear acquisition was negatively correlated with medial prefrontal blood flow with fear extinction (increased blood flow in amygdala correlated with decreased blood flow in medial prefrontal cortex) in all subjects (r=-0.48; P<0.05). Increased amygdala blood flow with fear acquisition was positively correlated with PTSD (r=0.45), anxiety (r=0.44) and disso-

ciative (r=0.80) symptom levels in PTSD (but not non-PTSD) subjects. There was a negative correlation between medial prefrontal blood flow during extinction and anxiety as measured with the Panic Attack Symptom Scale (PASS) during extinction in the PTSD group only, which was significant after correction for multiple comparisons (r=-0.90; P=0.006).[190] This study was consistent with increased amygdala function with fear acquisition, and decreased medial prefrontal (anterior cingulate) function during extinction in PTSD. This is consistent with the model of an overactive amygdala and a failure of medial prefrontal cortex to extinguish, or shut off, the amygdala, when the acute threat is no longer present.

Treatment of PTSD

Intervening soon after the trauma is critical for long-term outcomes, since with time traumatic memories become indelible and resistant to treatment.[213] Early treatments are not necessarily effective. For instance, studies have shown that Critical Incident Stress Debriefing (CISD) can be associated with a worsening of outcome relative to no treatment at all.[214] Pharmacological treatment of chronic PTSD has shown efficacy originally for imipramine,[215] amitriptyline,[216] and phenalzine,[215] and later for brofaramine,[217] paroxetine,[218,219] and sertraline.[220] Selective serotonin reuptake inhibitors (SSRIs) and tianeptine are now recommended as first-line treatment for PTSD.[221-226]

The utility of early treatment is also demonstrated by animal studies showing that pretreatment before stress with antidepressants reduces chronic behavioral deficits related to stress.[227,228] Antidepressants, including both norepinephrine and serotonin reuptake inhibitors, as well as gabapentine and phenytoin, promote nerve growth (neurogenesis) in the hippocampus, while stress inhibits neurogenesis.[63,64,66,69,71,75,229] This is important because hippocampal neurogenesis has been shown to be required for antidepressant response.[74]

Few studies have examined the effects of pharmacological treatment on brain structure and function in patients with trauma-related mental disorders. We studied a group of patients with depression and found no effect of fluoxetine on hippocampal volume, although there were increases in memory function[230] and hippocampal activation measured with PET during a memory encoding task. Depressed patients with a history of childhood trauma were excluded, and we subsequently have found hippocampal volume reductions at baseline in women with early abuse and depression but not in women with depression without early abuse;[198] this suggests that the study design of excluding patients with early trauma may account for the negative result. Other studies in depression showed that smaller hippocampal volume was a predictor of resistance to antidepressant treatment.[231] Smaller orbitofrontal cortex volume is associated with depression; one study in geriatric depression found smaller orbitofrontal cortex volume, while length of antidepressant exposure was correlated with larger orbitofrontal volume.[232]

Several studies have looked at functional brain imaging response to antidepressants in depression. Single photon-emission computed tomography (SPECT) blood flow studies in depression showed that antidepressants increased anterior cingulate, right putamen, and right thalamus function.[233] SPECT Xenon-133 studies showed reduced prefrontal function at baseline in depression, with treatment responders showing reduced perfusion in prefrontal cortex compared with nonresponders after treatment.[234] In a fluorodeoxyglucose (FDG) PET study of brain function patients with depression treated with fluoxetine who had a positive response to treatment had limbic and striatal decreases (subgenual cingulate, hippocampus, insula, and pallidum) and brain stem and dorsal cortical increases (prefrontal, parietal, anterior, and posterior cingulate) in function. Failed response was associated with a persistent 1-week pattern and absence of either subgenual cingulate or prefrontal changes.[235] Sertraline resulted in an increase in middle frontal gyrus activity in depression measured with PET FDG, as well as increased function in right parietal lobe and visual association cortex.[236] Successful paroxetine therapy of depression was associated with increased glucose metabolism measured with PET in dorsolateral, ventrolateral, and medial aspects of the prefrontal cortex, parietal cortex, and dorsal anterior cingulate. Areas of decreased metabolism were noted in both anterior and posterior insular regions (left) as well as right hippocampal and parahippocampal regions.[237] In another PET FDG study, at baseline, subjects with depression had higher normalized metabolism than controls in the prefrontal cortex (and caudate and thalamus), and lower metabolism in the temporal lobe. With treatment with paroxetine, subjects with depression had metabolic changes in the direction of normalization in these regions.[238] A PET FDG study of patients with depression and controls showed that at baseline, the mean metabolism was increased in the left and right lateral orbital cortex/ventrolateral prefrontal cortex (PFC), left amygdala,

and posterior cingulate cortex, and decreased in the subgenual anterior cingulate cortex (ACC) and dorsal medial/dorsal anterolateral PFC in depressives relative to controls. Following treatment with antidepressants, metabolism significantly decreased in the left amygdala and left subgenual ACC. The metabolic reduction in the amygdala and right subgenual ACC appeared largely limited to those subjects who both responded to treatment and remained well at 6 months' follow-up.[239] Another study showed that antidepressant treatment of depression resulted in a decrease in amygdala activation with emotional faces as measured with fMRI.[240] In summary, studies show changes in limbic and prefrontal cortical regions with successful antidepressant treatment of depression.

Fewer studies have looked at the effects of pharmacological treatment on the brain in anxiety disorders. One PET FDG study showed that caudate function decreased with treatment of obsessive compulsive disorder with antidepressants.[241] Paroxetine resulted in a decrease in glutamate/glutamine measured with magnetic resonance spectroscopy (MRS) in children with obsessive-compulsive disorder (OCD).[242] Patients with PTSD were shown to have an increase in hippocampal volume and memory function with paroxetine,[163] and increased right hippocampal and right cerebral volume with phenytoin.[165] No published studies have looked at the effects of pharmacological treatment on brain function in PTSD, or on sensitive markers of brain chemistry like NAA.

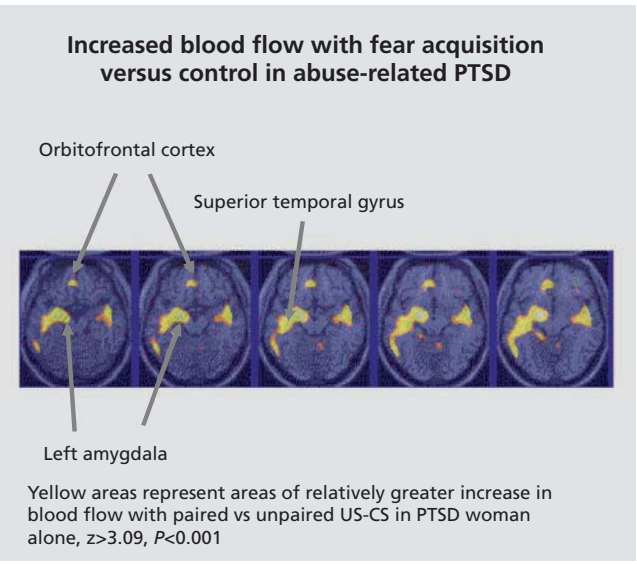

Figure 2. Neural correlates of fear conditioning in women with abuse and PTSD. There was increased amygdala activation with fear acquisition using a classical conditioning paradigm relative to non-PTSD abused women. PTSD, post-traumatic stress disorder

Brain biomarkers like NAA represent an objective marker of neural plasticity. To date psychiatry has relied on subjective reports as the gold standard. However, this is limited by self-reporting and the subjective interpretations of symptoms and response to treatment. Brain markers of antidepressant response may provide a complementary approach to assessing response to treatment, as well as providing insight into the mechanisms of treatment response. Our group is trying to look at mechanisms in the brain underlying treatment response in PTSD.

Effects of pharmacotherapy on brain function and structure in PTSD

We have begun to assess the effects of pharmacotherapy on brain structure and function in PTSD.[243] We recently assessed the effects of phenytoin on brain structure and function. Studies in animals show that phenytoin, which is used in the treatment of epilepsy and is known to modulate glutamatergic function, blocks the effects of stress on the hippocampus.[67] We studied nine patients with PTSD in an open-label function before and after treatment with phenytoin. Phenytoin resulted in a significant improvement in PTSD symptoms.[164] Phenytoin also resulted in increases in both right hippocampal volume and right hemisphere volume.[165] These findings indicate that phenytoin has an effects on PTSD symptoms as well as brain structure in PTSD patients.

We have assessed the effects of open-label paroxetine on memory and the hippocampus in PTSD. Male and female patients with symptoms of PTSD were medication-free for at least 4 weeks before participation in the study. Twenty-eight patients were found to be eligible and started the medication phase. Of the total patient sample five patients did not finish due to noncompliance; 23 patients completed the study.

Before patients started the medication phase, neuropsychological tests were administered, including the *Wechsler Adult Intelligence Scale – Revised*, WAIS-R (arithmetic, vocabulary, picture arrangement, and block design test), two subtests of the *Wechsler Memory Scale-Revised*, WMS-R, including logical memory (free recall of two story narratives, which represents verbal memory) and figural memory (which represents visual memory and involved reproduction of designs after a 6-second presentation); and the verbal and visual components of

the *Selective Reminding Test*, SRT.

Paroxetine was prescribed in the first visit after the pre-treatment assessments. All patients started open-label with a dose of 10 mg daily and were titrated up to 20 mg in 4 days.

Paroxetine treatment resulted in a mean 54% reduction in PTSD symptoms as measured with mean changes from baseline on the CAPS total score ($P<0.005$) among study completers. Improvement was equally strong on all symptom cluster scores (Re-experiencing, Avoidance/Numbing, Hyperarousal). Treatment also resulted in significant improvements in verbal declarative memory as measured with the WMS-R paragraph recall for delayed recall ($P<0.005$) and percent retention (80.2 to 91.1; $P=0.003$), but not immediate recall. Improvements were significant on all subscales of the Verbal Component of the SRT; including long-term recall and delayed recall.

Repeated measures ANOVA with side as the repeated measure showed a main effect for treatment related to a 4.6% increase in mean hippocampal volume (1857.3 mm^3 [SD 225.6] to 1906.2 mm^3, [SD 243.2]) with treatment ($F=8.775$ df=1. 36; $P=0.005$). Increased hippocampal volume was seen for both left (5.6%) (1807.6 mm^3 [SD 255.5] to 1909.3 mm^3 [SD 236.9]) and right (3.7%) (1906.9 mm^3 [SD 195.8] to 1976.7 mm^3 (SD 249.6]) hippocampus. There was no change in whole brain volume with treatment. Increase in hippocampal volume was significant after adding whole brain volume before and after treatment to the model.

Discussion

Traumatic stress has a broad range of effects on brain function and structure, as well as on neuropsychological components of memory. Brain areas implicated in the stress response include the amygdala, hippocampus, and prefrontal cortex. Neurochemical systems, including cortisol and norepinephrine, play a critical role in the stress response. These brain areas play an important role in the stress response. They also play a critical role in memory, highlighting the important interplay between memory and the traumatic stress response. Preclinical studies show that stress affects these brain areas. Furthermore, antidepressants have effects on the hippocampus that counteract the effects of stress. In fact, promotion of nerve growth (neurogenesis) in the hippocampus may be central to the efficacy of the antidepressants. Studies in patients with PTSD show alterations in brain areas implicated in animal studies, including the amygdala, hippocampus, and prefrontal cortex, as well as in neurochemical stress response systems, including cortisol and norepinephrine. Treatments that are efficacious for PTSD show a promotion of neurogenesis in animal studies, as well as promotion of memory and increased hippocampal volume in PTSD. Future studies are needed to assess neural mechanisms in treatment response in PTSD. ❑

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Estrés traumático: efectos en la cerebro

El estrés traumático surte efectos muy diversos sobre la función y la estructura cerebrales. Las regiones cerebrales implicadas en la respuesta al estrés son la amígdala (núcleo amigdalino), el hipocampo y la corteza prefrontal. Los sistemas neuroquímicos, como el cortisol y la noradrenalina, desempeñan una misión crítica en la respuesta al estrés. Estas regiones cerebrales influyen sobre la respuesta al estrés y sobre la memoria, lo que subraya la interrelación entre la memoria y la respuesta al estrés traumático. Los antidepresivos actúan sobre el hipocampo y contrarrestan el efecto del estrés. Los estudios sobre pacientes con trastorno por estrés postraumático (TEPT) revelan alteraciones en las regiones cerebrales implicadas en los estudios con animales como la amígdala, el hipocampo y la corteza prefrontal, así como en los sistemas neuroquímicos de respuesta al estrés, entre ellos el cortisol y la noradrenalina. Los tratamientos con eficacia frente al TEPT promueven la neurogénesis en los estudios con animales y también aumentan la memoria, y el volumen hipocámpico en el TEPT. Se requieren nuevos estudios para evaluar los mecanismos neurales de la respuesta terapéutica en el TEPT.

Effets du stress traumatique sur le cerveau

Le stress traumatique exerce une grande variété d'effets sur la fonction et la structure cérébrales. Les aires cérébrales impliquées dans la réponse au stress comprennent l'amygdale, l'hippocampe et le cortex préfrontal. Les systèmes neurochimiques, incluant le cortisol et la norépinéphrine, jouent un rôle critique dans la réponse au stress. Ces aires cérébrales influent sur la mémoire et sur la réponse au stress traumatique, soulignant ainsi les interactions existant entre les deux. Les effets des antidépresseurs sur l'hippocampe compensent les effets du stress. Les études chez les patients atteints de trouble stress post-traumatique (ESPT) montrent des modifications des aires cérébrales impliquées au cours des études animales, telles l'amygdale, l'hippocampe et le cortex préfrontal, ainsi que des modifications des systèmes neurochimiques de réponse au stress comme le cortisol et la noradrénaline. Les traitements efficaces dans l'ESPT entraînent une activation de la neurogenèse chez l'animal de même qu'une amélioration de la mémoire et une augmentation du volume de l'hippocampe dans l'ESPT. Il faudra d'autres études pour évaluer les mécanismes neuronaux dans la réponse thérapeutique au cours de l'ESPT.

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Hypothalamic-pituitary-adrenal axis modulation of GABAergic neuroactive steroids influences ethanol sensitivity and drinking behavior

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Neuroactive steroids are endogenous neuromodulators that can be synthesized de novo in the brain as well as in the adrenal glands, ovaries, and testes (for review see ref 1). The biosynthetic pathway for these steroids is shown in *Figure 1*. Among these compounds, the metabolites of deoxycorticosterone (DOC) and progesterone, 3α,21-dihydroxy-5α-pregnan-20-one (3α,5α-THDOC or allotetrahydrodeoxycorticosterone) and 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP or allopregnanolone) are the most potent positive modulators of γ-aminobutyric acid type A ($GABA_A$) receptors.[2,3]

Systemic administration of 3α,5α-THDOC and 3α,5α-THP induces anxiolytic, anticonvulsant, and sedative-hypnotic effects, similar to those induced by other $GABA_A$

Activation of the hypothalamic-pituitary-adrenal (HPA) axis leads to elevations in γ-aminobutyric acid (GABA)-ergic neuroactive steroids that enhance GABA neurotransmission and restore homeostasis following stress. This regulation of the HPA axis maintains healthy brain function and protects against neuropsychiatric disease. Ethanol sensitivity is influenced by elevations in neuroactive steroids that enhance the GABAergic effects of ethanol, and may prevent excessive drinking in rodents and humans. Low ethanol sensitivity is associated with greater alcohol consumption and increased risk of alcoholism. Indeed, ethanol-dependent rats show blunted neurosteroid responses to ethanol administration that may contribute to ethanol tolerance and the propensity to drink greater amounts of ethanol. The review presents evidence to support the hypothesis that neurosteroids contribute to ethanol actions and prevent excessive drinking, while the lack of neurosteroid responses to ethanol may underlie innate or chronic tolerance and increased risk of excessive drinking. Neurosteroids may have therapeutic use in alcohol withdrawal or for relapse prevention.

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Selected abbreviations and acronyms

3α-HSD	*3α hydroxysteroid dehydrogenase*
3α,5α-THDOC	*3α,21-dihydroxy-5α-pregnan-20-one*
3α,5α-THP	*3α-hydroxy-5α-pregnan-20-one*
ACTH	*adrenocorticotropic hormone*
CRF	*corticotropin-releasing factor*
DOC	*deoxycorticosterone*
GABA	*γ-aminobutyric acid*
HPA	*hypothalamic-pituitary-adrenal*
PMDD	*premenstrual dysphoric disorder*

receptor positive modulators and ethanol (for review see ref 4). Neuroactive steroids interact with $GABA_A$ receptors via specific binding sites on α subunits[5] that allosterically modulate binding to GABA and benzodiazepine recognition sites.[6] In addition, neuroactive steroids compete for [^{35}S] t-butylbicyclophosphorothionate (TBPS) binding sites.[6] These steroids alter $GABA_A$ receptor function by enhancing GABA-mediated Cl^- conductance and directly stimulating Cl^- conductance in voltage clamp studies and [$^{36}Cl^-$] flux studies.[2,3,7] Neuroactive steroids appear to interact with multiple neurosteroid recognition sites,[8,9] and these sites may differentiate direct gating of Cl^- vs allosteric modulation of GABA-mediated conductance[9] or represent different properties of recognition sites on distinct $GABA_A$ receptor subtypes.[10,11] Studies of the structural requirements for neurosteroid activity at $GABA_A$ receptors include 3α reduction and 5α/5β reduction of the A ring, as well as hydroxylation of C21.[12] The 5β-reduced metabolites of DOC and progesterone, 3α,5β-THDOC and 3α,5β-THP are equipotent modulators of GABAergic transmission.[8,13,14] Humans synthesize these 5β-reduced neuroactive steroids; moreover, the concentrations of 3α,5β-THP are physiologically relevant and comparable to those of 3α,5α-THP in human plasma and cerebrospinal fluid.[15,16] In addition, 3α,5α- and 3α,5β-reduced

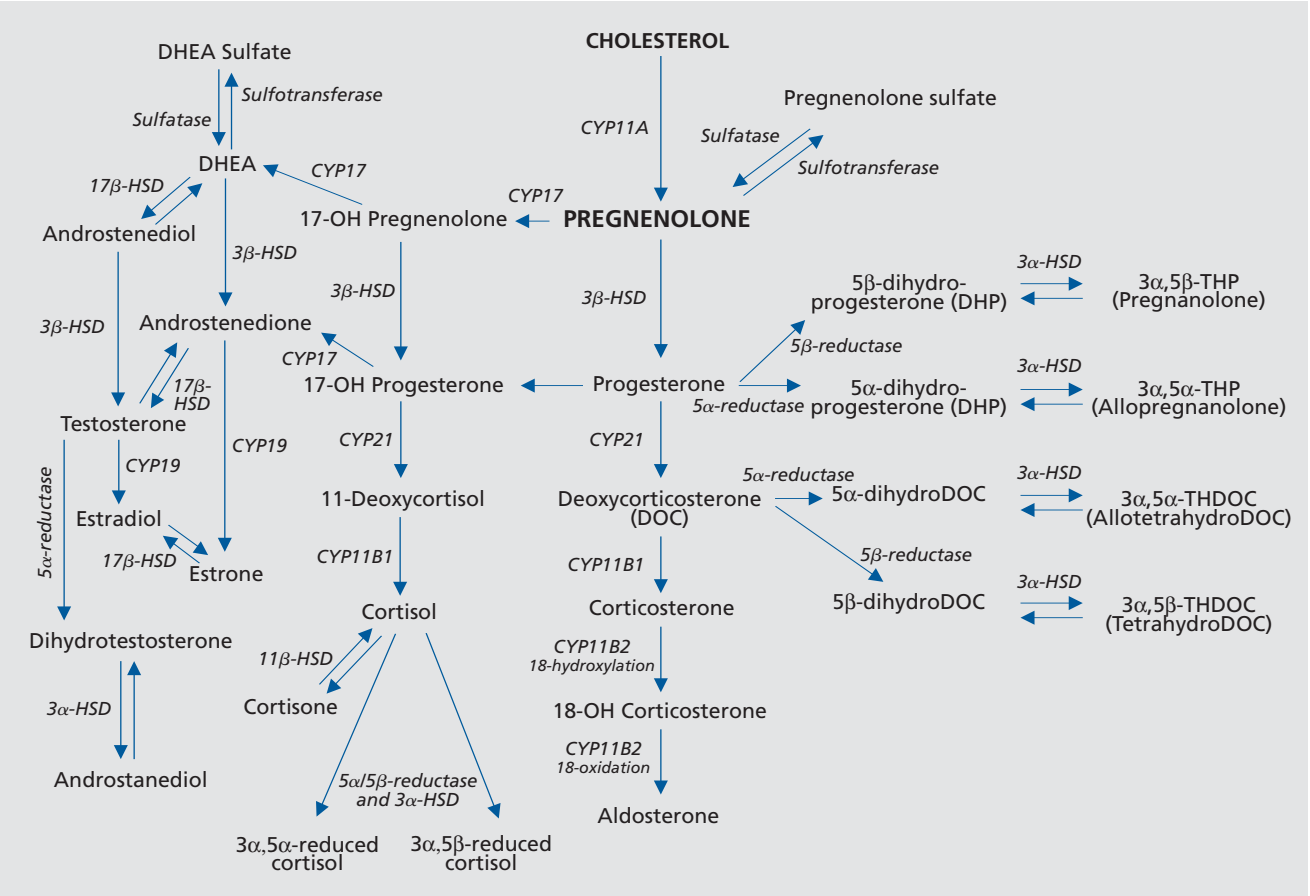

Figure 1. Biosynthetic pathway for neuroactive steroids. DHEA, dehydroepiandrosterone; DOC, deoxycorticosterone

cortisol have antagonist properties at both GABA and neurosteroid recognition sites of $GABA_A$ receptors, and these compounds are the most abundant metabolites of cortisol in human urine.[17] However, to our knowledge, there is no data in the literature on the presence of these metabolites in human brain.

Stress increases plasma and brain levels of GABAergic neuroactive steroids

The brain and plasma concentrations of GABA agonist-like neuroactive steroids are increased by acute stress and ethanol administration in rodents.[18-21] The increase in 3α,5α-THP reaches pharmacologically significant concentrations in brain between 50 and 100 nM that is sufficient to enhance $GABA_A$ receptor activity and produce behavioral effects. Similarly, both stress and acute ethanol administration elevate levels of 3α,5α-THP in human plasma,[22-25] although effects of ethanol in humans are controversial.[26,27] In addition, corticotropin-releasing factor (CRF) infusion increases 3α,5α-THP levels in human plasma.[28] The levels detected in human plasma are lower than rodent plasma and brain. However, 3α,5α-THP levels in post-mortem human brain are similar to rat brain and sufficient to have GABAergic activity.[29] *Table I* summarizes the effects of acute stress on neuroactive steroid levels in rodents, monkeys, and humans.

The increase in neuroactive steroid levels elicited by stressful stimuli, including ethanol administration, appears to be mediated by activation of the hypothalamic-pituitary-adrenal (HPA) axis, since it is no longer apparent in adrenalectomized animals.[18,30,31] Adrenalectomized animals exhibit no circulating concentrations of 3α,5α-THP and 3α,5α-THDOC, but brain levels are still detectable,[18] suggesting that brain synthesis plays an important role in neurosteroid actions. Indeed, brain synthesis of 3α,5α-THP can be increased by ethanol in adrenalectomized immature animals allowed sufficient time for adaptation,[32] suggesting that brain synthesis of neurosteroids may exhibit plasticity in response to physiological challenges.

Neuroactive steroids and the HPA axis

The activation of the HPA axis in response to acute stress increases the release of CRF from the hypothalamus, which stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary; this, in turn, stimulates the adrenal cortex to release glucocorticoids, neuroactive steroid precursors, and GABAergic neuroactive steroids. Glucocorticoids, mainly cortisol in humans and nonhuman primates, and corticosterone in rodents, provide negative feedback on the hypothalamus and pituitary. Likewise, GABAergic neuroactive steroids inhibit CRF production and release, ACTH release, and subsequent corticosterone levels in rodents.[33-35] The ability of neuroactive steroids to reduce HPA axis activation may play an important role in returning the animal to homeostasis following stressful events. This physiological coping response appears to be critical for mental health, since it is dysregulated in various mood disorders, including depression, post-traumatic stress disorder, and premenstrual dysphoric disorder (PMDD).

Neuroactive steroid concentrations are altered in various pathophysiological conditions that involve dysfunction of

	Pregnenolone	Progesterone	3α,5α-THP	DOC	3α,5α-THDOC
Rats					
Acute ethanol	↑	↑	↑	↑	↑
Acute stress	↑	↑	↑	NA	↑
Monkeys					
Acute ethanol	--	NA	NA	--	NA
Acute stress/HPA axis	↑ / --	-- / ↓	NA	↑	NA
Humans					
Acute ethanol	↑	↑ / ↓	↑ / -- / ↓	NA	NA
Acute stress/HPA axis	↑	↑	↑	↑	NA

Table I. Summary of the changes in neuroactive steroids and their precursors in rats, monkeys, and healthy human subjects induced by acute ethanol administration or by acute stress or HPA stimulation. These effects are described and referenced in the text. ↑ = increase; ↓ = decrease; -- = unchanged; na = not assayed; HPA axis: activation by naloxone, CRF, or ACTH; 3α,5α-THDOC, 3α,21-dihydroxy-5α-pregnan-20-one; 3α,5α-THP, 3α-hydroxy-5α-pregnan-20-one; DOC, deoxycorticosterone; HPA, hypothalamic-pituitary-adrenal; CRF, corticotropin-releasing factor; ACTH, adrenocorticotropic hormone

the HPA axis. The HPA axis plays an important role in the pathophysiology of depression: patients with major depression have elevated cortisol levels, a consequence of hypersecretion of CRF due to lowered feedback mechanisms,[36] which also contributes to a blunted dexamethasone response.[37] Some neuroactive steroid concentrations are decreased in patients with major depression as well as in animal models of depression,[15,16,38,39] and administration of antidepressant drugs increases these neuroactive steroids in patients and in rodent brain and plasma.[40-44] This decrease in neuroactive steroids might play a role in the hyperactivity of CRF, since neurosteroids negatively regulate CRF expression and release from the hypothalamus. This increase might be mediated by the HPA axis via an increased serotonin neurotransmission that stimulates the release of CRF (for review see ref 45). While acute fluoxetine administration increases brain levels of 3α,5α-THP, chronic administration of fluoxetine decreases 3α,5α-THP and 3α,5α-THDOC in rat brain and plasma,[43] probably as a consequence of a reduced basal HPA axis activity induced by antidepressant treatments.[36]

Neuroactive steroids are also altered in PMDD, although the literature is controversial, reporting either decrease, no change, or increase in 3α,5α-THP plasma levels.[22,46-53] Differences in analytic methods, diagnostic criteria, or presence of other comorbid psychiatric disorders might account for these discrepancies. Furthermore, PMDD patients had a blunted 3α,5α-THP response to stress[22] and to HPA axis challenges.[53] Women with a history of depression, regardless of PMDD symptoms, also had a blunted 3α,5α-THP response to stress.[54] An altered neuroactive steroid response to stress and acute ethanol administration has been shown in socially isolated animals,[38,55] and this is accompanied by altered HPA axis responsiveness.[56] All this experimental evidence emphasizes the important link between HPA axis function and neuroactive steroid levels in the maintenance of homeostasis and healthy brain function.

Neuroactive steroids have ethanol-like discriminative stimulus properties in rodents and nonhuman primates

The discriminative stimulus paradigm can be used as an in vivo assay of receptor-mediated activity, and may help define the neurotransmitter systems that underlie the behavioral effects of a given dose and class of drug.[57] In addition, drug discrimination can be used as an assay of subjective effects for cross-species comparisons.[58] The relation between subjective effects of a drug and its reinforcing effects is largely asymmetrical: reinforcing effects are discriminable, but not all discriminable effects are reinforcing.[58] For example, ethanol can make a person feel simultaneously drowsy, euphoric, and calm, but only some of these subjective effects will be associated with increased drinking of ethanol.

Neurosteroids such as 3α,5α-THP, 3α,5β-THP, 3β,5β -THP, and 3α,5α-THDOC have been characterized in drug discrimination procedures as similar to other $GABA_A$ receptor positive modulators, including benzodiazepines, barbiturates, and ethanol in rats and mice (reviewed in ref 59). Further, neurosteroids that are negative modulators of $GABA_A$ receptor function, such as pregnenolone sulfate and dehydroepiandrosterone sulfate, do not substitute for the discriminative stimulus effects of ethanol.[60] However, in male rats, the basis for the 3α,5β-THP discrimination also appears to be composed of *N*-methyl-D-aspartate (NMDA) receptor antagonism and serotonin-3 (5-HT3) receptor agonist activity,[61] an effect not found in mice.[59] These results suggest a species difference in the neurotransmitter systems underlying the 3α,5β-THP stimulus cues.

In the macaque monkey, 3α,5α-THP produces a discriminative stimulus effect that is similar to that of ethanol, and sensitivity to these effects is dependent upon the phase of the menstrual cycle, with higher circulating progesterone in the menstrual cycle producing increased sensitivity to ethanol.[62] Furthermore, in male and female monkeys, 3α,5α-THP can produce stimulus effects similar to both a relatively low (1.0 g/kg) and higher (2.0 g/kg) dose of ethanol.[63] The common element in all three species tested (mice, rats, and monkeys) appears to be positive $GABA_A$ receptor modulation.

The neurosteroid 3α,5β-THP substitution for ethanol shows wide individual differences in rats, mice, and monkeys.[59,60,62] This is an unusual finding, because there is extensive training involved in establishing the discrimination, and such overtraining dampens variance across individuals. It has been speculated that the source of such individual variance in sensitivity to neurosteroids is due to the additive effect of experimenter-administered neurosteroids with circulating levels in neurosteroids that differ due to individual variations of HPA axis function.[60] Monkeys also show a wide individual variation in the amount of ethanol they will self-administer, from an average of 1 to 2 drinks/day to an average of over 12

drinks/day. The relationship between sensitivity to ethanol-like effects of neurosteroids and propensity to self-administer ethanol has not been directly tested. However, the suggestion from data showing lower sensitivity to the discriminative stimulus effects of ethanol in the follicular phase of the menstrual cycle (when progesterone and DOC levels are low) and increased alcohol consumption in women during the follicular phase is intriguing.[64] In addition, it has been documented in women who drink heavily and monkeys who self-administer high daily doses of ethanol that their menstrual cycles are disrupted and progesterone levels are very low.[65,66] It will be of interest to first determine sensitivity to the discriminative stimulus effects of ethanol and then allow monkeys to self-administer ethanol to more directly correlate aspects of discriminative stimuli (subjective effects) with risk for heavy drinking.

Neuroactive steroids mediate specific ethanol actions following acute administration in rodents

Systemic administration of moderate doses (1 to 2.5 g/kg) of ethanol increases both plasma and brain levels of 3α,5α-THP and 3α,5α-THDOC.[19,21,31,67,68] Ethanol-induced elevations in neuroactive steroids reach physiologically relevant concentrations that are capable of enhancing GABAergic transmission. The effect of ethanol on neuroactive steroid levels is dose- and time-dependent, and correlates with the time course of some, but not all, effects of ethanol. For example, the motor incoordinating effects of ethanol appear prior to elevations in neuroactive steroids,[69] whereas the anticonvulsant effects of ethanol appear in congruence with elevations of these steroids.[68] A large body of evidence from multiple laboratories suggests that ethanol-induced elevations of GABAergic neuroactive steroids contribute to many behavioral effects of ethanol in rodents. Neuroactive steroids have been shown to modulate ethanol's anticonvulsant effects,[68] sedation,[30] impairment of spatial memory,[4,70] anxiolytic-like,[71] and antidepressant-like[72] actions. Each of these behavioral responses is prevented by pretreatment with the biosynthesis inhibitor finasteride and/or by prior adrenalectomy. The hypnotic effect of ethanol is partially blocked by adrenalectomy. Importantly, administration of the immediate precursor of 3α,5α-THP restores effects of ethanol in adrenalectomized animals, showing that brain synthesis of neuroactive steroids modulates effects of ethanol.[30] However, neuroactive steroids do not appear to influence the motor incoordinating effects of ethanol, since neither finasteride administration or adrenalectomy diminish these actions.[69] Taken together, these studies suggest that elevations in neuroactive steroids influence many of the GABAergic effects of ethanol in vivo and the effects of neuroactive steroids may determine sensitivity to many behavioral effects of ethanol.

Neuroactive steroid precursors are increased by acute ethanol administration in rodents

While several studies have demonstrated that acute ethanol challenges can result in significant increases in neuroactive steroids in plasma and brain, fewer studies have examined in detail the importance of ethanol's effect on their precursors. As early as the 1940s, it was found that DOC acetate and progesterone induced anesthetic effects in rats[73] and both DOC and progesterone had antiseizure effects,[74] probably due to their 3α-reduced metabolites.[75,76] DOC, the precursor of 3α,5α-THDOC, and progesterone, the precursor of 3α,5α-THP, can readily cross the blood-brain barrier and distribute throughout the brain. These precursors of GABAergic neuroactive steroids are synthesized in the adrenals, beginning with cholesterol's metabolism to pregnenolone *(Figure 1)*. While small amounts of these steroids may be formed de novo in the brain, ethanol-induced increases in neuroactive steroids are predominantly formed from adrenal precursors.[77] Plasma and brain concentrations of pregnenolone and progesterone are increased more rapidly than 3α,5α-THP after acute ethanol administration.[31,78] Other studies have also shown increases in both plasma and brain DOC after acute ethanol administration. DOC levels were increased in cerebral cortex, cerebellum, hippocampus, hypothalamus, and olfactory bulb and tubercle, ranging from 28-fold increases in the cerebellum to 38-fold increases in the hypothalamus.[79] A significant increase in DOC levels across many brain regions has also been reported by Kraulis et al following intravenous injections of [1,2-^{3}H]-DOC.[80] A strong correlation exists between plasma and brain levels of DOC. The temporal and regional associations found in these studies suggest that the steroids originate in the adrenals and are transported to the brain. Upon entering the brain the steroids are metabolized by 5α-reductase and 3α-dehydrogenase enzymes. These enzymes display brain

region and cell specific expression[81] that may be responsible for the regional distribution of steroid levels following acute ethanol administration. Furthermore, DOC levels measured in the Khisti et al study are comparable to 3α,5α-THDOC levels measured in plasma and brain,[21] suggesting that DOC formed after acute ethanol administration may be largely converted to the GABAergic neurosteroid 3α,5α-THDOC. Studies of ethanol's effects on neurosteroid precursors are important not only to determine the sources and synthesis of potent metabolites, but also to establish their role in physiological functions.

Effects of neuroactive steroids on drinking behavior in rodents

The GABAergic system is important in regulating ethanol consumption, and neurosteroids can also alter drinking behavior through their actions on $GABA_A$ receptors. 3α,5α-THP dose-dependently increased ethanol self-administration in nondependent ethanol-preferring P rats, while decreasing ethanol administration in ethanol-dependent P rats.[4] This suggests a complex relationship whereby neurosteroids may promote drinking in nondependent animals consuming small amounts of ethanol, while protecting against excessive drinking in dependent animals. This possibility is supported by data in male C57BL/6J mice where 3α,5α-THP dose-dependently modulated ethanol intake in a 2-hour session, with low doses (3.2 mg/kg) increasing ethanol consumption and high doses (24 mg/kg) decreasing ethanol consumption.[82] In addition, at doses of 10 and 17 mg/kg, 3α,5α-THP has been shown to have rewarding properties in mice.[83] However, other studies in nondependent rats have shown that pretreatment with a 3 mg/kg dose of 3α,5α-THP, but not a 1- or 10-mg/kg dose, increases oral self-administration of ethanol.[84] This result suggests that 3α,5α-THP dose-dependently mediates some of the reinforcing effects of ethanol, and its concentration in brain may have an important influence on drinking behavior. Indeed, Sardinian alcohol-preferring rats have larger 3α,5α-THP and 3α,5α-THDOC elevations after ethanol administration than their non-alcohol-preferring counterparts.[21] Other studies have shown that increased ethanol intake after 3α,5α-THP administration is selective for ethanol-reinforced responding, and cannot be attributed to palatability or increased motor activity during the experimental sessions.[85] Furthermore, the ethanol enhanced responses following 3α,5α-THP administration produces the opposite effect of other $GABA_A$ receptor agonists, such as muscimol and barbiturates,[85] suggesting a unique role for $GABA_A$ receptor neurosteroid binding sites in regulating ethanol consumption. Interestingly, ethanol-dependent rats develop tolerance to ethanol-induced increases in neurosteroid levels,[4,79] which may influence the excessive drinking that is observed in ethanol-dependent rats.[86] Together, these data suggest a strong relationship between neurosteroid levels and ethanol consumption that may involve both genetic and environmental factors.

Mechanisms of ethanol-induced elevations of neuroactive steroids in plasma and brain

Ethanol-induced elevations in neuroactive steroids appear to involve activation of the HPA axis to increase circulating levels of neuroactive steroids and their precursors, as well as direct effects of ethanol on brain synthesis. Adrenalectomy completely blocks the effects of ethanol on cerebral cortical 3α,5α-THP concentrations; however, the effect of ethanol on cerebral cortical levels of 3α,5α-THP can be restored by administration of its precursor, 5α-dihydroprogesterone (5α-DHP), to adrenalectomized rats.[30] Since the steroid biosynthetic enzymes are present across brain,[87] it is likely that ethanol-induced increases in brain levels of neuroactive steroids involve brain synthesis that may contribute to effects of ethanol. The first step in steroid synthesis is the translocation of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, where P450scc converts it to pregnenolone. This step is mediated by steroidogenic acute regulatory protein (StAR) and/or the peripheral benzodiazepine receptor. Ethanol rapidly increases the synthesis and translocation of StAR protein from the cytosol to the mitochondria in the adrenal gland.[30] Hence, it is likely that increases in GABAergic neuroactive steroids in adrenals are secondary to ethanol-induced increases in all steroid synthesis initiated by StAR activity.

To determine if ethanol could alter other steroidogenic enzyme activity in rat brain and adrenal minces, Morrow and colleagues investigated the effects of ethanol on 5α-reductase and 3α-hydroxysteroid dehydrogenase (3α-HSD) enzyme activity (unpublished data). Ethanol (10 to 100 mM) did not alter 5α-reductase activity, measured by the conversion of [^{14}C]progesterone to [^{14}C]5α-DHP in tis-

sue minces. In contrast, ethanol (30 to 100 mM) increased the conversion of [^{14}C]5α-DHP to [^{14}C]3α,5α-THP by a maximum of 30 ± 3.6% in the olfactory bulb and tubercle, but had no effect in the adrenal gland. Ethanol did not alter nicotinamide adenine dinucleotide phosphate (NADPH) effects on enzyme activity. Fluoxetine was tested as a positive control since previous studies showed that fluoxetine decreased the K_m of a recombinant 3α-HSD enzyme.[88] Fluoxetine increased the activity of 3α-HSD enzyme in the olfactory bulb and tubercle and adrenal gland and this effect was blocked by the 3α-HSD inhibitor indomethacin. Since the 3α-HSD enzyme possesses bidirectional activity, the effect of ethanol on the oxidative activity of 3α-HSD was determined. Ethanol did not alter the conversion of [^{3}H]3α,5α-THP to [^{3}H]5α-DHP in rat olfactory bulb and tubercle or adrenal gland. An increase in the reductive activity of the 3α-HSD with no change in the oxidative direction would cause a greater conversion of 5α-DHP to 3α,5α-THP. This effect could contribute to ethanol-induced increases in brain 3α,5α-THP levels. Indeed, the increased reductive activity of 3α-HSD would be predicted to increase brain levels of both 3α,5α-THP and other 3α,5α-reduced neuroactive steroids such as 3α,5α-THDOC.

Suppression of neuroactive steroid responses following chronic ethanol exposure in rats

It is well known that chronic stress results in adaptation of the HPA axis, leading to decreased levels of corticosterone in rats.[89] Repeated exposure to alcohol also blunts the response of the HPA axis to a second ethanol challenge.[90] This blunting of the HPA axis is associated with reduction in CRF and ACTH elevations following ethanol challenge.[91] In line with these observations, chronic ethanol consumption in rats results in blunted elevation of cerebral cortical 3α,5α-THP[4] and plasma and brain DOC levels following acute ethanol challenge,[79] compared with pair-fed control rats. These findings suggest that there is tolerance to ethanol-induced increases in neuroactive steroid levels. Since decreases in brain neurosteroid levels were concomitant with decreases in plasma neurosteroid levels, it is likely that the observed decreases in 3α,5α-THP and DOC levels were dependent on blunted HPA axis activity. Thus, adaptations of the HPA axis may contribute to tolerance to effects of ethanol that are mediated by the GABAergic neuroactive steroids.

Chronic ethanol administration to rodents and humans produces tolerance to ethanol and cross-tolerance to benzodiazepines and barbiturates. In contrast, ethanol-dependent rats are sensitized to the anticonvulsant effects of both 3α,5α-THP and 3α,5α-THDOC.[92-94] These studies also show that $GABA_A$ receptor sensitivity to 3α,5α-THP and 3α,5α-THDOC is enhanced in ethanol-dependent rats, likely due to the reduction of ethanol-induced levels in these animals described above. Since ethanol-dependent rats are sensitized to anticonvulsant actions of neuroactive steroids, this class of compounds may be therapeutic during ethanol withdrawal. Indeed, neurosteroid therapy may have advantages over benzodiazepine therapy since benzodiazepines exhibit cross-tolerance with ethanol. Further studies are needed to explore this possibility.

Effects of ethanol on neuroactive steroids in humans

The potential role of neuroactive steroids in alcohol action in humans is relatively unexplored and inconsistent. Recent human studies show that male and female adolescents seen in the emergency room for alcohol intoxication had elevated plasma levels of the neuroactive steroid 3α,5α-THP.[24,25] Furthermore, various subjective effects of ethanol during the rising phase of the blood alcohol curve are diminished by prior administration of the neurosteroid biosynthesis inhibitor finasteride.[95] Finasteride reduces the formation of both 3α,5α-THP and 3α,5α-THDOC by inhibiting the reduction of progesterone and DOC to intermediate precursors. Indeed, finasteride pretreatment blocked subjective effects of alcohol using three different scales to measure the activating, sedating, anesthetic, and peripheral dynamic aspects of alcohol actions. The ability of finasteride to reduce the subjective effects of alcohol was not observed in individuals carrying the $GABA_A$ α2 subunit polymorphism associated with alcoholism, suggesting that individuals carrying this polymorphism have reduced sensitivity to both alcohol and finasteride.[95] Other studies show that 3α,5α-THP levels are decreased during the peak of alcohol withdrawal and return to normal levels upon recovery.[96,97] Likewise, abstinent alcoholics exhibit diminished progesterone levels as well as a lowered ratio of progesterone to pregnenolone.[98] In contrast, laboratory administration of low or moderate doses of ethanol appears to have no effect on plasma 3α,5α-THP levels[26]

or to decrease 3α,5α-THP levels.[27,99] The basis of these conflicting results is unknown, but may involve pharmacologically different ethanol doses, different analytic methods to measure neurosteroids, or environmental factors that influence neurosteroid synthesis in humans. Alternatively, different neuroactive steroids may be elevated in humans vs rodents, or the effects of ethanol on neuroactive steroid levels in humans may be restricted to brain. *Table I* summarizes the different effects of ethanol on neuroactive steroid levels in rodents, monkeys, and humans.

Humans, but not rodents, synthesize multiple 5β-reduced neuroactive steroids including 3α,5β-THP and 3α,5β-THDOC. 3α,5β-THP levels are comparable to those of 3α,5α-THP in human plasma and cerebrospinal fluid.[15,16] These neuroactive steroids also modulate GABAergic transmission,[8,13,14] but have not been measured in humans after ethanol administration. Additionally, the primary stress steroids in humans are cortisol and 11-deoxycortisol, while progesterone and corticosterone are the primary stress steroids in rodents. 3α,5β-reduced cortisol is a negative modulator of $GABA_A$ receptors,[17] and could contribute to the subjective effects of ethanol in humans. Thus, the combined effects of 3α,5α- and 3α,5β-reduced neuroactive steroids may contribute to the effects of ethanol in humans and nonhuman primates. These steroids have never been measured following ethanol, stress, or HPA axis activation in humans or nonhuman primates.

Comprehensive studies of neuroactive steroid levels in humans are needed. While 3α,5α-THP and 3α,5α-THDOC are the primary neuroactive steroids in rodents, other neuroactive steroids may be more relevant in humans. For example, plasma progesterone of adrenal origin is present at much higher levels in rodents than humans, suggesting an explanation for higher levels of plasma 3α,5α-THP in rodents vs humans. Other GABAergic 3α,5α- and 3α,5β-reduced neuroactive steroids, derived from DOC, dehydroepiandrosterone (DHEA), and testosterone, are known GABAergic modulators[100-102] that may be elevated by HPA axis activation in humans. Unfortunately, simple inexpensive analytic methods to measure these steroids are not available. The ability of finasteride to block the subjective effects of ethanol in humans may be due to its ability to prevent the formation of any or all of these neuroactive steroids. Indeed, the combined effects of all steroids regulated by acute or chronic ethanol exposure may contribute to its actions in all species.

Effects of ethanol on neuroactive steroid precursors in nonhuman primates and humans

We have recently shown that acute ethanol challenges in cynomolgus monkeys do not change plasma pregnenolone and DOC levels. Two doses of ethanol, 1.0 and 1.5 g/kg, were tested via intragastric administration, and neither was able to increase neuroactive steroid precursors or circulating cortisol levels despite an average blood ethanol level of 147 mg/dL.[103,104] In contrast, acute ethanol administration increases pregnenolone, progesterone, DOC, and their neuroactive metabolites in rat brain and plasma,[4,31,79,105] and this increase is also prevented by adrenalectomy/orchiectomy, consistent with ethanol activation of the HPA axis.[31,105] These results suggest that higher doses of ethanol might be necessary to stimulate the HPA axis and thus increase pregnenolone and DOC levels in nonhuman primates. Indeed, Williams and collaborators[106] have shown that intravenous administration of ethanol up to 1.9 g/kg failed to increase plasma ACTH levels in rhesus monkeys. Other studies using 2.0 g/kg ethanol have reported increased cortisol levels in monkeys under conditions where monkeys were restrained on a flat surface while receiving ethanol, which may contribute to HPA axis activation.[107] The possibility that pregnenolone, DOC, and their neuroactive metabolites might be differentially regulated in nonhuman primates compared with rodents cannot be ruled out; future studies will be necessary to further address this question.

The effects of ethanol on neuroactive steroid precursors in humans are inconsistent to date. Laboratory administration of moderate doses of ethanol (0.7 to 0.8 g/kg) has recently been reported to increase pregnenolone and DHEA levels and to decrease progesterone levels in healthy human subjects.[27] In contrast, Holdstock et al[26] reported that ethanol administration to healthy volunteers increased progesterone levels in women during the luteal phase, but had no effect during the follicular phase or in men. Low alcohol consumption in premenopausal women was associated with increased estradiol, androstenedione, and testosterone levels throughout the menstrual cycle, while progesterone levels were increased only in the luteal phase.[108] Moreover, abstinent alcoholic women had diminished progesterone levels and a lower progesterone to pregnenolone ratio during the luteal phase.[98] In contrast, others reported that chronic male alcoholics had higher basal progesterone compared with healthy controls.[109]

These variable data suggest that genetic and/or environmental factors may influence effects of ethanol on steroid precursors.

HPA axis modulation in alcohol-dependent humans

Among the neuropsychiatric disorders that show alterations in HPA axis responsiveness is alcoholism. ACTH and cortisol secretion is increased during ethanol intoxication and acute alcohol withdrawal.[110-117] In contrast, an attenuated responsiveness of the HPA axis has been found in both drinking and abstinent alcohol-dependent patients. Alcohol-dependent patients have low cortisol and 11-deoxycortisol basal levels, show a greater suppression in cortisol and ACTH concentrations following dexamethasone test, and have a reduced cortisol response to exogenous ACTH administered after dexamethasone.[118] Moreover, they have attenuated ACTH and cortisol responses after pituitary stimulation by ovine or human CRF[119-122] and an altered ACTH response to naloxone.[123] An altered cortisol and ACTH response to ovine CRF and naloxone have also been found in sons of alcoholics.[124-126] These data are consistent with the idea that HPA axis dysregulation may contribute to altered neurosteroid responses in human alcoholism, though studies showing this consequence of alcoholism are not available to date.

HPA axis modulation of DOC and pregnenolone in cynomolgus monkeys

While stimulation of the HPA axis by acute stress or ethanol administration plays a pivotal role in increasing GABAergic neuroactive steroids and their precursors in rodent brain and plasma, few data are available for nonhuman primates. We have recently demonstrated that plasma DOC and pregnenolone levels in ethanol-naïve cynomolgus monkeys are differentially regulated by various challenges to the HPA axis.[103,104] Plasma DOC levels are sensitive to hypothalamic and pituitary activation of the axis and to negative feedback mechanisms assessed by the dexamethasone test. Thus, administration of naloxone at the doses of 125 and 375 µg/kg increased plasma DOC levels up to 86% and 97%, respectively. This is consistent with data showing an activation of the HPA axis and increased cortisol and ACTH levels in humans and nonhuman primates.[125,127,128] CRF (1 µg/kg) increased plasma DOC levels up to 111%, and this increase was positively correlated with the increase in cortisol levels in the same subject, dexamethasone (130 µg/kg) decreased DOC levels by 42%, in agreement with a suppression of HPA axis activity. In contrast, administration of ACTH (10 ng/kg) 4-6 hours after 0.5 mg/kg dexamethasone had no effect on plasma DOC levels, suggesting that DOC synthesis is independent of ACTH stimulation of the adrenals. Furthermore, changes in DOC levels were correlated with changes in cortisol levels only for some of these challenges, suggesting that other neuroendocrine factors could regulate DOC synthesis in nonhuman primates.[103]

Pregnenolone levels in the same cynomolgus monkey subjects were differentially regulated from DOC. Naloxone administration (125 and 375 µg/kg) increased plasma pregnenolone up to 222 and 216%, respectively. In contrast, CRF (1 µg/kg) and dexamethasone (130 µg/kg) had no effect on pregnenolone levels, while ACTH (10 ng/kg), 4 to 6 hours after 0.5 mg/kg dexamethasone, decreased plasma pregnenolone levels by 43%. CRF and ACTH administration decreased the ratio of plasma pregnenolone:DOC, suggesting increased metabolism of pregnenolone into DOC or other steroids.[104] Thus, circulating pregnenolone levels are subject to complex regulation involving factors other than direct HPA axis modulation. Naloxone could increase pregnenolone levels through mechanisms independent of HPA axis activation, given that exogenous CRF and ACTH had no effect on pregnenolone levels. Opioid receptors are present in peripheral tissue including the adrenals,[129] and a direct action of naloxone on these receptors cannot be ruled out. Opioidergic neurons regulate gonadotropin-releasing hormone (GnRH) secretion,[130] and it is possible that the increase in plasma pregnenolone levels induced by naloxone is due to increased gonadal steroidogenesis via opioid inhibition of GnRH. Furthermore, naloxone could have a direct action on the enzymes involved in steroid biosynthesis. Further studies are needed to investigate these possibilities.

Are neuroactive steroid responses to HPA axis stimulation linked to alcohol drinking?

Neuroactive steroid responses to HPA axis challenges in ethanol-naïve animals may predict future alcohol consumption. Studies have so far focused on nonhuman primates. Dexamethasone suppresses DOC levels in monkey plasma and the degree of dexamethasone suppression measured in ethanol-naïve monkeys was predictive of sub-

sequent alcohol drinking in these monkeys. That is, the highest alcohol drinking was found in the monkeys that showed the lowest suppression of DOC levels in response to dexamethasone.[103] In this study, the monkeys with the lowest response to dexamethasone also developed a pattern of chronic binge drinking, drinking the equivalent of 16 or more drinks in 22 h in approximately 20% of their drinking sessions (Grant et al, submitted). This binge drinking pattern of high quantity of alcohol intake in short time periods persisted throughout 1 year of ethanol self administration (Grant et al, unpublished). In contrast, no other DOC responses to HPA axis stimulation in ethanol-naïve monkeys were predictive of subsequent voluntary drinking or binge drinking. The effect of dexamethasone on plasma DOC levels in monkeys appears to be a trait marker of risk for high alcohol consumption. This trait marker also correlated with alcohol intakes in a small group (n=4) of rhesus monkeys (unpublished data collected in collaboration with David P. Friedman at Wake Forest University). These findings need to be replicated in other primate studies of ethanol self-administration, including cohorts of humans that have not yet started drinking. This adaptation in precursor responses suggests there will also be adaptations in GABAergic neuroactive steroids derived from DOC.

Potential role of neuroactive steroids in ethanol sensitivity and risk for alcoholism: a hypothesis

While the physiological significance is unknown, dysregulation of the HPA axis is associated with ethanol dependence in humans.[118,122] HPA axis suppression in alcohol dependence results in diminished elevations of GABAergic neuroactive steroids in rodents as described above. Diminished elevations of GABAergic neuroactive steroids following ethanol exposure would result in reduced sensitivity to the anxiolytic, sedative, anticonvulsant, cognition-impairing, and discriminative stimulus properties of ethanol. Reduced sensitivity to ethanol is associated with greater risk for the development of alcoholism in individuals with alcoholism in their family.[131,132]

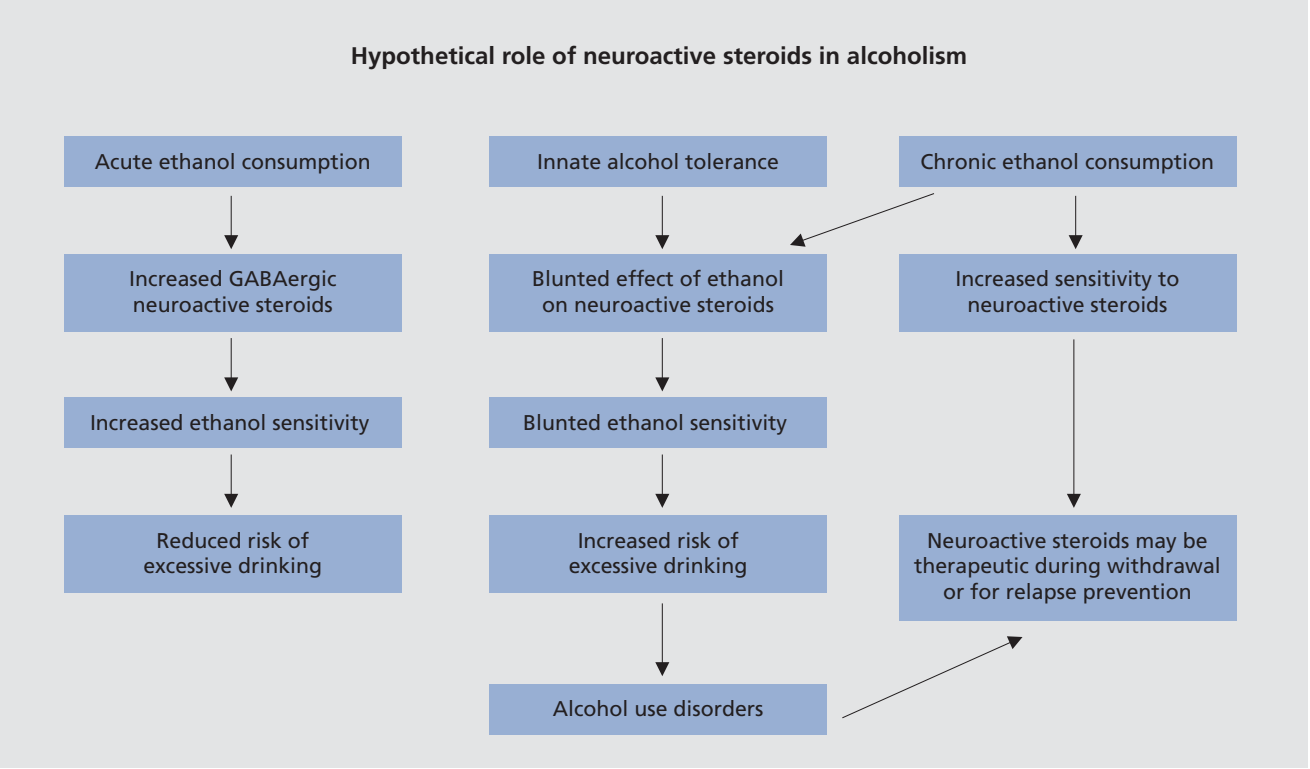

Figure 2. Schematic representation of the hypothetical role of neuroactive steroids in ethanol sensitivity and risk for alcoholism. GABA, γ-aminobutyric acid

Moreover, individuals with the $GABA_A$ receptor α2 subunit polymorphism that is associated with alcohol dependence exhibit substantially reduced sensitivity to the subjective effects of ethanol compared with individuals that lack this polymorphism.[95] Likewise, rats and mice with low sensitivity to various behavioral effects of alcohol tend to self-administer greater amounts of ethanol in laboratory settings. The BXD recombinant inbred strains of mice, PKCγ and PKCε knockout mice, alcohol-preferring P rats, and high-alcohol-drinking (HAD) rats are but a few examples. Taken together, these observations suggest that ethanol-induced elevations of GABAergic neuroactive steroids in brain may underlie important aspects of ethanol sensitivity that may serve to prevent excessive alcohol consumption *(Figure 2)*. The loss of these responses may promote excessive alcohol consumption to achieve the desired effects of ethanol. A deficiency in neurosteroid responses to ethanol intake could result from suppression of the HPA axis or other genetic/environmental factors that inhibit neurosteroid synthesis in brain. Hence, the lack of neurosteroid elevations in response to ethanol could underlie innate ethanol tolerance or ethanol tolerance induced by long-term ethanol use. Indeed, the observation that finasteride did not alter the subjective effects of ethanol in subjects with the $GABA_A$ receptor α2 subunit polymorphism associated with alcohol dependence[95] is consistent with the idea that neurosteroid responses contribute to ethanol sensitivity and risk for alcoholism. Both forms of tolerance may promote excessive alcohol consumption. Excessive alcohol consumption, particularly binge drinking, is a significant risk factor for all alcohol use disorders, including alcohol dependence and alcoholism. The restoration of ethanol sensitivity in ethanol-dependent patients may therefore have therapeutic utility. However, it is unclear at this time whether neuroactive steroid supplementation would reduce excessive alcohol consumption in humans. Indeed, as mentioned above, low doses of neuroactive steroids increased operant ethanol self-administration under some conditions,[84] while neuroactive steroids reduce ethanol consumption at high doses[82] or in ethanol-dependent rats.[4] The relationship between HPA axis response, GABAergic neuroactive steroids, and alcohol drinking deserves further studies in nonhuman primates and humans.

Summary and conclusions

The effects of acute ethanol administration on neuroactive steroid levels found in rodents have not been found in monkeys or humans. Does this mean that neuroactive steroids do not have an important role in ethanol action in these species? We doubt this conclusion, since monkeys exhibit discriminative stimulus properties of ethanol and neuroactive steroids that are indistinguishable.[62] Furthermore, the steroid biosynthesis inhibitor finasteride blocks the subjective effects of ethanol in humans.[95] Primates may synthesize different GABAergic neuroactive steroids in response to ethanol challenge. These steroids may include 3α,5α- and 3α,5β-reduced derivatives of progesterone, DOC, and testosterone, all of which have potent GABAergic activity. Further studies are needed to translate a large body of rodent research on GABAergic neuroactive steroids to better understand the role of endocrine factors in alcohol sensitivity and risk for alcoholism. ❑

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La modulación de los esteroides neuroactivos por el eje hipotálamo-hipofisis-suprarenal, influye en la sensibilidad al etanol y en la conducta frente a la bebida

La activación del eje hipotálamo-hipófisis-suprarrenal (HHS) determina una elevación de los esteroides neuroactivos GABA (ácido Á-aminobutírico)-érgicos que refuerzan la neurotransmisión de GABA y restablecen la homeostasia después del estrés. Esta regulación del eje HHS mantiene sana la función cerebral y la protege frente a las enfermedades neuropsiquiátricas. La sensibilidad al etanol depende de las elevaciones de esteroides neuroactivos que potencian los efectos GABAérgicos del etanol y pueden impidiran el consumo excesivo de alcohol por los roedores y seres humanos. La sensibilidad baja al alcohol se asocia a un mayor consumo de éste, con el riesgo consiguiente de etilismo. De hecho, las ratas dependientes del etanol muestran una respuesta neuroesteroidea a la administración de etanol muy reducida, lo que puede contribuir a la tolerancia etanólica y a la propensión a beber mayores cantidades de alcohol. En esta revisión se ofrecen pruebas que respaldan la hipótesis de que los neuroesteroides contribuyen a las acciones del etanol e impiden un consumo excesivo, mientras que la falta de respuesta neuroesteroidea al etanol podría explicar la tolerancia innata o crónica y el mayor riesgo de excesos en la bebida. Los neuroesteroides podrían tener una utilidad terapéutica en la abstinencia del alcohol o en la evitación de las recaídas.

Le comportement alcoolique la sensibilité à l'éthanol dépendent de la modulation des stéroïdes neuroactifs GABAergiques au niveau de l'axe hypothalamo-hypophyso-surrénalien

L'activation de l'axe hypothalamo-hypophyso-surrénalien (HHS) entraine une élévation de la sécrétion des stéroïdes neuroactifs GABA-ergiques (acide γ-aminobutirique) qui stimulent la neurotransmission GABA et restaurent l'homéostasie après le stress. Cette régulation de l'axe HHS maintient une fonction cérébrale saine et protège des maladies neuropsychiatriques. Les élévations des stéroïdes neuroactifs influent sur la sensibilité à l'éthanol en augmentant ses effets GABAergiques et peuvent ainsi prévenir les consommations alcooliques excessifs chez les rongeurs et chez l'homme. Une faible sensibilité à l'éthanol est associée à une plus grande consommation d'alcool et à un risque d'alcoolisme plus important. Les réponses neurostéroïdes à l'administration d'éthanol chez des rats rendus alcoolodépendants sont donc diminuées, ce qui peut contribuer à une tolérance à l'éthanol et à une propension à en boire de plus grandes quantités. Cette revue de la littérature fournit des arguments en faveur de l'hypothèse d'une contribution des neurostéroïdes aux effets de l'éthanol et à la prévention de sa consommation excessive alors qu'un déficit des réponses neurostéroïdes peut être à la base d'une tolérance innée ou invétérée chronique et d'un risque augmenté de consommation excessive d'alcool. Les neurostéroïdes peuvent avoir une utilité thérapeutique dans le sevrage alcoolique ou dans la prévention d'une rechute.

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Poster

Estrogen enhances stress-induced prefrontal cortex dysfunction: relevance to Major Depressive Disorder in women

It is well documented that exposure to stress can precipitate or exacerbate many mental illnesses,[1,2] including major depressive disorder (MDD) and post-traumatic stress disorder (PTSD). Women are twice as likely as men to develop these disorders,[3,4] as well as most anxiety disorders and phobias,[5] but the biological causes of this discrepancy are poorly understood. Interestingly, there is evidence that the increased prevalence of MDD in women occurs primarily during the childbearing years, when circulating estrogen is present.[6,7] These observations raise questions as to whether men and women have distinct neurobiological responses to stress, and if so, how might estrogen mediate these differences? Attempts to answer these questions in animal models have generated a growing body of literature demonstrating that estrogen can, indeed, modulate the effects of stress in the brain. Moreover, these effects are demonstrable in brain regions relevant to MDD, and are consistent with the idea that estrogen might enhance the stress response, promoting a greater vulnerability to mental illness.

The medial prefrontal cortex (PFC) is widely recognized as a site of dysfunction in patients with stress-related disorders,[8] particularly MDD. Post-mortem studies of suicide victims' brains reveal marked morphological changes—most notably, reduced glia and neuron number in the ventromedial PFC.[9] Similarly, magnetic resonance imaging (MRI) studies demonstrate reduced volume of this area in depressed patients,[10] as well as abnormal activity.[11] The PFC integrates information from multiple brain areas to regulate behavior, thought, and affect[12]—functions that are often compromised in MDD patients.[13] In animal models, the integrity of the PFC is most commonly tested using working memory tasks, which require animals to keep information "online" in the absence of external cues, continually update information, and inhibit inappropriate responses. Exposure to stress has consistently been shown to impair performance on such tasks in nonhuman primates and male rodents,[14] but until recently, neither sex differences nor estrogen effects on this phenomenon had been explored.

The first studies to examine sex differences in the effects of stress on PFC function elicited the stress response in young adult male and female rats with injections of varying doses of the benzodiazepine inverse agonist FG7142. FG7142 is a well-documented anxiogenic drug that is frequently used as a model for stress, given its reliability in producing the biochemical and physiological effects of stress: increased corticosterone release, increased catecholamine turnover, elevated heart rate, and increased blood pressure.[15] Moreover, animals that have been administered FG7142 exhibit classic stress-related behaviors, including defecating, urinating, freezing, and ultrasonic vocalizations.[16]

Following FG7142 administration, animals were tested on a classic measure of working memory—delayed alternation in the T-maze. At high doses of FG7142, all animals displayed impairment on the T-maze. At lower doses, however, only females showed impairment, suggesting that they were more sensitive to the detrimental effects of stress on mPFC function *(Figure 1a)*. To test

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whether fluctuating hormones produced this sex effect, the experiment was repeated while female rats' estrus phase was monitored. It was found that these rats only displayed sensitivity to FG7142 during proestrus, when estrogen levels are highest. Animals in estrus, characterized by low estrogen levels, responded to the low dose of FG7142 in a manner comparable to that of males—that is, showing no impairment at all[17] *(Figure 1b)*. This effect was further replicated using a more conventional stress paradigm, restraint. While 2 hours of restraint stress produced working memory impairments in all groups, only females in proestrus were impaired by 1 hour of restraint as well *(Figure 1c)*.[18] Taken together, these studies suggest that fluctuating hormones can interact with stress systems to modulate PFC function during stress.

This idea was explored further by ovariectomizing a new group of female rats, and implanting a time-release capsule containing either estrogen (OVX + E) or cholesterol (OVX) as a control. These rats were then treated with the same low dose of FG7142 that impaired proestrus females, but not estrus females or males, and then tested on the T-maze task. Strikingly similar results were found—like females in proestrus, the estrogen-treated animals were impaired by this low dose, while OVX animals, like estrus females or males, were unaffected. Collectively, this and the above studies provide compelling evidence that high levels of estrogen, whether occurring naturally or experimentally, can produce a sensitivity to the detrimental effects of stress in the PFC *(Figure 1b)*. The possible mechanisms by which estrogen confers this sensitivity have only just begun to be illuminated.

The PFC receives sizeable afferents from midbrain catecholaminergic nuclei locus coeruleus (LC),[19] the primary source of norepinephrine (NE), and ventral tegmental area (VTA), the primary source of dopamine (DA). The influence of these projections on PFC functioning has been extensively studied, and it is widely accepted that the relationship between catecholamine levels in the PFC and working memory performance is manifest in an "inverted U" curve.[20] Specifically, experimental or age-related catecholamine depletion produces PFC-mediated

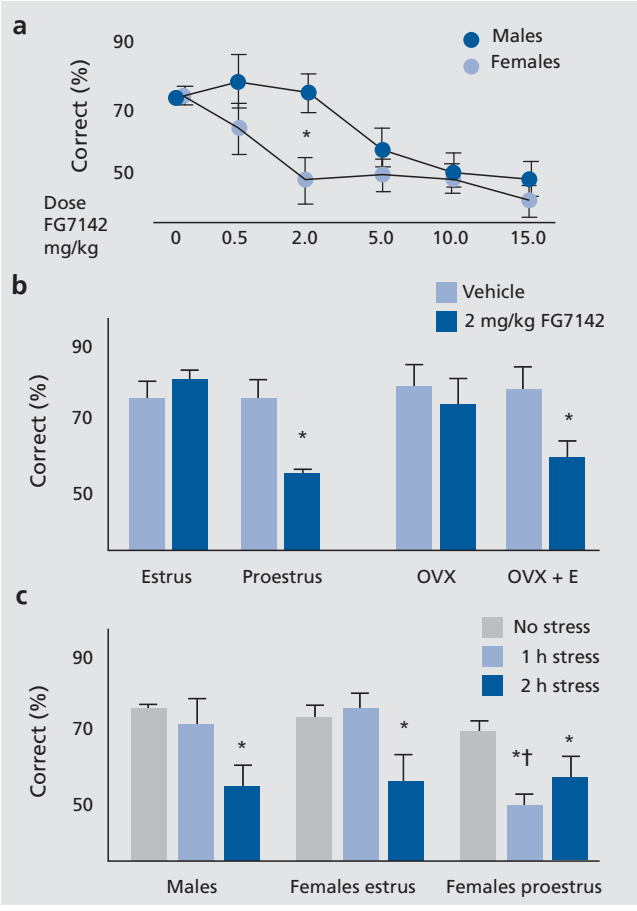

Figure 1. *Sex differences and estrogen effects on stress-induced working memory impairment.* a) Dose-response curve for male and female animals' performance on working memory task delayed alternation after administration of pharmacological stressor FG7142. Mean scores after 0, 0.5, 2, 5, 10, and 15 mg/kg respectively were, for males, 76+/-2.7, 79+/-7.5, 76+/-4.6, 55+/-9, 50+/-7.5, and 48+/-4.2; for females, 69.7+/-3.4, 66+/-8.4, 51.67+/-10, 50+/-8, 50+/-6.1, and 37+/-8. Repeated measures ANOVA revealed a significant sex x drug interaction $F(5,40)=2.4$, $P=0.05$). Post-hoc analysis (test of effects) showed the 2 mg/kg dose to have the most prominent sex difference, where the females were impaired, but the males were not $F(1,8)=6.2$, $P<.04$. b) Working memory performance after 2 mg/kg FG7142 varied according to estrogen levels. Scores after vehicle or 2 mg/kg FG7142, respectively were, for intact females in proestrus, 75+/-6 and 57.7+/-2.3; for intact females in estrus, 74+/-6 and 81+/-4.5; for ovariectomized (OVX) females, 79+/-6.5 and 73+/-6.7; for OVX females with estrogen replacement (OVX + E), 79+/-5.5 and 65+/-4.5. In both experiments, animals with high estrogen levels (proestrus and OVX + E) were significantly impaired by FG7142 ($P<.0002$ and $P<.03$, respectively). c) Working memory performance after restraint stress varied according to estrogen levels. Scores after 0, 1, or 2 h restraint stress, respectively were, for males: 73.3+/-2.3, 76+/-4.5, and 58+/-7.42; females in estrus, 72.3+/-2.1, 75+/- 3.6, and 62.8+/-6.55; for females in proestrus, 69.3+/-2, 50+/-3.7, and 60+/-5.7. Only females in proestrus were significantly impaired by 1 h restraint ($P<.0005$). * = significantly different from self in control conditions, † = significantly different from self in estrus.

cognitive deficits in monkeys and rodents[21] that can be reversed with administration of DA or NE receptor agonists.[22,23] However, extreme *increases* in mPFC catecholamine levels can also have a detrimental effect on PFC function *(Figure 2)*, exerting their actions through the very receptors that restore performance in animals whose catecholamine systems have been compromised. Such increases in catecholamine release are seen with stress,[24] and it has been shown in male rats that stress-induced PFC dysfunction is due in part to binding of the DA D_1 receptor, and the subsequent activation of the protein kinase A (PKA) intracellular signaling pathway.[25,26] Conversely, stress-induced impairments can be *reversed* through stimulation of the NE α-2 receptor,[27] whose activation leads to an inhibition of PKA activity.

To examine whether estrogen's enhancement of stress-induced PFC dysfunction was due to actions at the NE α-2 receptor, OVX and OVX + E animals were coadministered an impairing dose of FG7142 and a dose of the α-2 agonist guanfacine (GFC) known to restore stress-related performance deficits in males, and then tested on the delayed alternation task. Although OVX animals required almost three times as much FG7142 as OVX + E in order to show impairment, OVX showed complete reversal of the impairment with GFC, while OVX + E showed no improvement *(Figure 3)*. These results suggest that estrogen might cause sensitivity to stress-induced PFC dysfunction through suppression of an animal's responsiveness to NE α-2 stimulation. Western Blot analysis showed no difference in PFC NE α-2 protein levels between OVX and OVX + E *(Figure 4)*, indicating that this effect is not due to changes in protein expression, but likely to actions downstream of the receptor. The exact mechanism by which estrogen elicits this effect has yet to be identified. However, estrogen treatment has been shown in hypothalamus to uncouple the NE α-2 receptor from its G-protein,[28] thus rendering it ineffective. If this likewise occurs in the PFC, GFC's inability to rescue working memory function in stressed OVX + E animals could thus be explained.

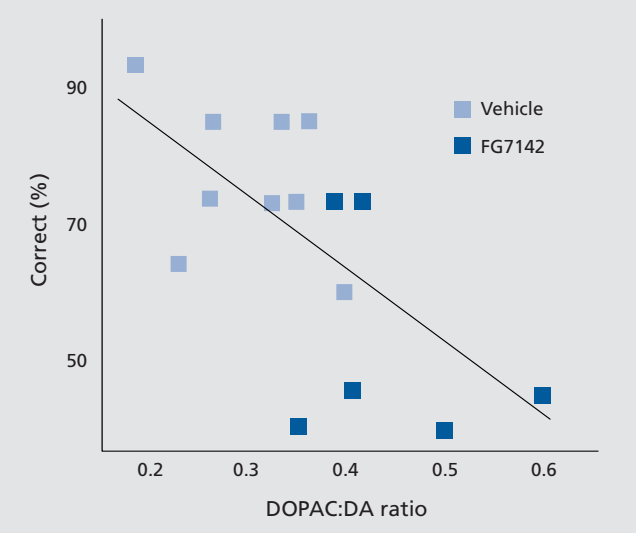

Figure 2. *The correlation between accuracy of delayed alternation performance in the rat and the ratio of DOPAC to dopamine in the prefrontal cortex*. Rats were given vehicle or FG7142 (20 mg/kg) before being tested on delayed alternation, and were sacrificed immediately after testing. Increased dopamine turnover in the prefrontal cortex significantly correlated with impaired performance on the delayed alternation task (r=0.627, *P*<0.01). DOPAC, 3,4-dihydrophenylacetic acid; DA, dopamine

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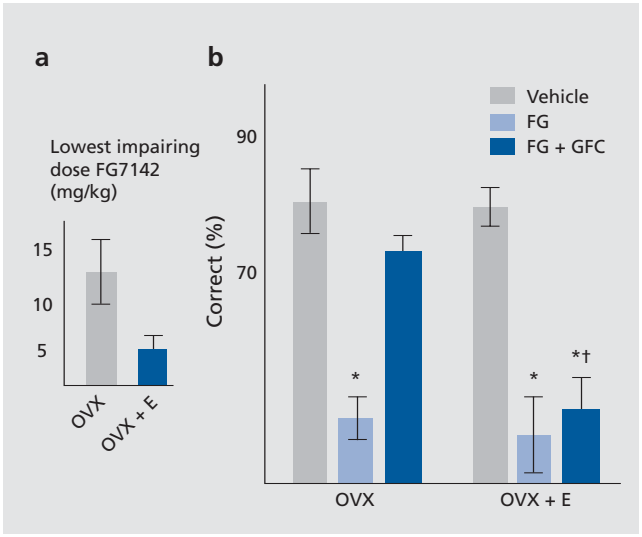

Figure 3. *Estrogen suppresses norepinephrine (NE) α-2 receptor-mediated rescue of working memory function during stress.* a) OVX and OVX + E were administered increasing doses of FG7142 in order to find the lowest impairing dose for each animal. b) Despite receiving higher doses of FG7142 (10+/-3.7 mg/kg vs 3.5+/-1.2 mg/kg), OVX showed full rescue of PFC function with coadministration of NE α-2 agonist guanfacine (GFC), while OVX + E showed no improvement (scores of 74.3+/-3.9 vs 47.5+/-5.5, *P*<.0007). *, significantly different from self in control conditions, †, significantly different from OVX in same condition. PFC, prefrontal cortex

The work described here demonstrates that female rats are more sensitive to stress-induced PFC dysfunction, especially under conditions of high estrogen levels. While this heightened stress response may confer survival value during danger, it may also increase susceptibility for stress-related disorders such as depression. That estrogen also mediated distinct responses to actions at NE α-2 receptors suggests that a more thorough investigation of hormone-intracellular signaling cascade interactions may yield useful information regarding the potential prevention and treatment of stress-induced disorders in women. A better understanding of the neurobiology underlying sex differences in the cognitive response to stress is imperative in forwarding the development of more appropriate therapeutic targets and methods. ❑

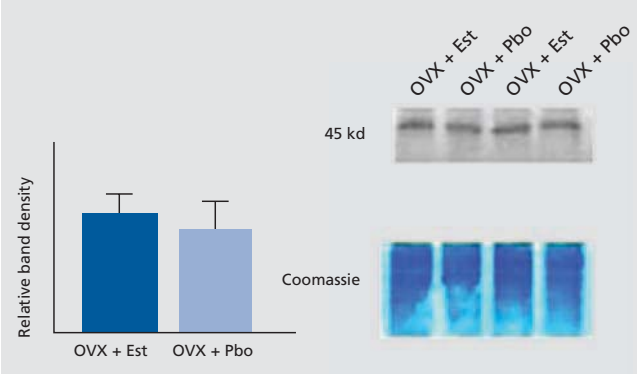

Figure 4. *Estrogen does not affect norepinephrine α-2 receptor expression in the PFC.* As assessed by Western Blot, OVX and OVX + E did not differ in their levels of NE α-2 protein. PFC, prefrontal cortex

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