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Sex, stress and the hippocampus: allostasis, allostatic load and the aging process

Bruce S. McEwen*

Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology, Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

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Abstract

The adaptive responses of the body that maintain homeostasis in response to stressors can be called "allostasis", meaning "achieving stability through change". Mediators produced by the immune system, autonomic nervous system (ANS) and hypothalamo–pituitary–adrenal(HPA) axis produce allostasis. The brain also shows allostasis, involving the activation of nerve cell activity and the release of neurotransmitters. When the individual is challenged repeatedly or when the allostatic systems remain turned on when no longer needed, the mediators of allostasis can produce a wear and tear on the body and brain that has been termed "allostatic load". Examples of allostatic load include the accumulation of abdominal fat, the loss of bone minerals and the atrophy of nerve cells in the hippocampus. Studies of the hippocampus as a target of stress and sex hormones have revealed a considerable degree of structural plasticity and remodeling in the adult brain that differs between the sexes. Three forms of hippocampal structural plasticity are affected by circulating hormones: (1) repeated stress causes remodeling of dendrites in the CA3 region; (2) different modalities of stress suppress neurogenesis of dentate gyrus granule neurons; (3) ovarian steroids regulate synapse formation during the estrous cycle of female rats. All three forms of structural remodeling of the hippocampus are mediated by hormones working in concert with excitatory amino acids (EAA) and NMDA receptors. EAA and NMDA receptors are also involved in neuronal death that is caused in pyramidal neurons by seizures, by ischemia and by severe and prolonged psychosocial stress. The aging brain seems to be more vulnerable to such effects, although there are considerable individual differences in vulnerability that can be developmentally determined. Moreover, the brain retains considerable resilience in the face of stress, and estrogens appear to play a role in this resilience. "Resilience is an example of successful allostasis in which wear and tear is minimized, and estrogens exemplify the type of agent that works against the allostatic load associated with aging." This review discusses the current status of work on underlying mechanisms for protection and damage.
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1. Introduction

Individual differences in the aging process can be conceptualized as an accumulation of wear and tear of daily experiences and major life stressors which interact with the genetic constitution and predisposing early life experiences [52,164,175]. The neuroendocrine system, autonomic nervous system (ANS) and immune system are mediators of adaptation to challenges of daily life, referred to as "allostasis", meaning "maintaining stability through change" [178]. Physiological mediators such as adrenalin from the adrenal medulla, glucocorticoids from the adrenal cortex and cytokines from cells of the immune system act upon receptors in various tissues and organs to produce effects that are adaptive in the short run but can be damaging if the mediators are not shut off when no longer needed. When release of the mediators is not efficiently terminated, their effects on target cells are prolonged, leading to other consequences that may include receptor desensitization and tissue damage. This process has been named "allostatic load" [121,127], and it refers to the price the tissue or organ pays for an overactive or inefficiently managed allostatic response. Therefore, allostatic load refers to the "cost" of adaptation.

The brain is the master controller of the three systems noted above and is also a target of these systems, subject to both protection and damage. Allostasis also applies not only to circulating hormones but also to organs and tissues of the body. In the nervous system, neurotransmitters are released by neuronal activity, and they produce effects locally to either propagate or inhibit further neural activity. Neurotransmitters and hormones are usually released during a discrete

* Tel.: +1-212-327-8624; fax: +1-212-327-8634.
E-mail address: mcewen@rockefeller.edu (B.S. McEwen).

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period of activation and then are shut off, and the mediators themselves are removed from the intracellular space by reuptake or metabolism in order not to prolong their effects. When that does not happen, however, there is allostatic load and the brain is at increased risk for damage [103].

The processes of allostasis and allostatic load have been described and measured for metabolic and cardiovascular changes that are associated with obesity, Type 2 diabetes and cardiovascular disease [171]. However, the same type of elevated and prolonged secretion of glucocorticoids during aging has also been associated with impairment of cognitive function in rodents [92,93,165] and in humans [104,105,170]. Moreover, the endogenous excitatory amino acid neurotransmitters appear to play a major role in these changes [165] even though they are also an essential part of normal synaptic neurotransmission and plasticity. Their actions lead to the formation of excess free radicals that can damage nerve cells, leading to the search for agents that can interfere with free radical production or enhance free radical quenching. The "glucocorticoid cascade hypothesis" of aging [94,120,165,166] is an example of a theory of aging that emphasizes the pivotal nature of aging of key brain structures such as the hippocampus, a brain region involved in key aspects of episodic, declarative, spatial and contextual memory and also in regulation of autonomic, neuroendocrine and immune responses. Agents that are protective against accelerated aging should be judged for their ability to protect key brain structures such as the hippocampus from the effects of a variety of insults, many of which involve excitotoxicity and damage from reactive oxygen species and free radicals. The "glucocorticoid cascade hypothesis" of aging is a prime example of allostatic load since it recognizes feed forward mechanism that gradually wears down a key brain structure, the hippocampus, while the gradually disregulated HPA axis promotes pathophysiology on tissues and organs throughout the body.

In spite of its vulnerability to allostatic load, the brain retains considerable resilience in the face of challenges to adapt through allostasis. Studies on the hippocampus reveal a number of types of structural plasticity, ranging from neurogenesis in the dentate gyrus to remodelling of dendrites to the formation and replacement of synapses. These changes, along with compensatory neurochemical and neuroendocrine responses, provide the brain with a considerable amount of resilience. This has led to a search for agents that help the brain maintain its resilience as it ages.

This article discusses allostasis and allostatic load in the brain in relation to the aging process and a number of brain disorders in which there is overactivity of stress mediators that causes brain dysfunction. Specifically, this article summarizes research on the protective and damaging effects of adrenal steroids and estrogens on the brain, particularly on the hippocampus. It also discusses the topic of neuroprotection and the potential value of estrogens and flavonoids as anti-oxidants in promoting allostasis and enhancing resilience and countering the allostatic load promoted by excitatory amino acids and other agents that promote the generation of free radicals such as the β-amyloid protein. "Resilience is an example of successful allostasis in which wear and tear is minimized, and estrogens exemplify the type of agent that works against the allostatic load associated with aging." *Before entering full-force into this discussion*, it is important to review briefly the role of the biological mediators of stress in the protective and damaging aspects of stress on the brain and body and to discuss the terms "allostasis" and "allostatic load".

2. Protective and damaging effects of stress mediators: homeostasis and allostasis

Before discussing the brain and individual differences in the cumulative wear and tear during the aging process, it is important to clarify ambiguities in some key terms. In common usage, *stress* usually refers to an event or succession of events that challenges homeostasis and causes a response, often in the form of "distress" but also, in some cases, referring to a challenge that leads to a feeling of exhilaration, as in "good" stress [54]. But, the term "stress" is often used to mean the event (stressor) or the response (stress response). Furthermore, it is frequently used to describe a chronic state of imbalance in the response to stress. Here we use *stress* to mean the physiological and behavioral *responses* to a "stressor", defined as a challenge to the individual that *either* perturbs homeostasis and requires an adaptive response *or* that is interpreted as threatening and results in a hormonal or behavioral response even if physiological homeostasis is not compromised.

In this latter case, stress hormones and/or some type of behavior are produced and results in consequences for the individual that may create additional problems. For example, compensatory or displacement behaviors by a stressed individual, such as eating junk food, smoking, drinking alcohol, may add to the physiological burden through elevated levels of catecholamines and glucocorticoids. Moreover, elevated levels of glucocorticoids and catecholamines in the absence of a physiological challenge may exacerbate pathophysiological processes such as abdominal fat deposition, hypertension, muscle wasting and bone mineral loss.

The types of perceived stress that leads to such responses are largely the result of the individual's interpretation that there is a threat, and it is the body's reaction to the perceived threat that causes the problems, particularly if it becomes chronic. Contrary to the late Hans Selye, who emphasized physical stressors [174], psychological and experiential factors are among the most powerful of stressors, e.g. novelty, withholding of reward, and anticipation of punishment rather than the punishment itself are among the most potent activators of HPA and ANS activity [114,116].

The brain is the master controller of the interpretation of what is stressful and the behavioral and physiological responses that are produced. The brain is also a target of stress,

along with the immune system, metabolic and cardiovascular systems and other systems of the body. Stress hormones play a major role in mediating both adaptive and maladaptive responses, and they do so by interacting with specific aspects of the physiology of each tissue.

What is often overlooked is that the stress hormones are protective in the short run and yet can participate in damage when they are overproduced or not shut off when no longer needed. Thus, although stress is often thought about as bad and damaging, recent studies paint a different picture as far as the brain and also the immune system are concerned. The main point is that the brain appears to handle repeated stress over weeks by showing adaptive plasticity in which local neurotransmitters, as well as systemic hormones, interact to produce structural as well as functional changes. This will be discussed further, but first we discuss some of the key concepts and terms that will be used in the rest of the article.

2.1. Stress response

The most commonly studied physiological systems that respond to stress are the hypothalamo–pituitary–adrenal(HPA) axis and the ANS, particularly the sympathetic response of the adrenal medulla and sympathetic nerves. These systems respond in daily life according to stressful events as well as to the diurnal cycle of rest and activity. Thus, these systems do more than respond to "stressors" even though they are frequently identified as "stress response systems". Behaviorally, the response to stress may consist of fight or flight reactions but it also may include potentially health-related behaviors such as eating, alcohol consumption, smoking and other forms of substance abuse. Another type of reaction to a potentially stressful situation is an increased state of vigilance, accompanied, at least in our own species, by enhanced anxiety and worrying, particularly when the threat is ill-defined or imaginary and when there is no clear alternative behavioral response that would end the threat. The behavioral responses to stress and these states of anxiety are both capable of exacerbating and potentiating the production of the physiological mediators of health outcomes.

Homeostasis, in a strict sense, applies to a limited number of systems like pH, osmolarity, body temperature and oxygen tension, components of the internal milieu, that are truly essential for life and are, therefore, maintained over a narrow range, as a result of their critical role in survival. These systems are not activated or varied in order to help the individual adapt to its environment. In contrast, systems that show "variation to meet perceived/anticipated demands" [178] characterize the state of the organism in a changing world and reflects the operation of most body systems in meeting environmental challenges, e.g. through fluctuating hormones, heart rate and blood pressure, cytokines of the immune system, and other tissue mediators like neurotransmitters and hormones. Those mediators are most certainly not held constant, and their levels will operate over a wide range and they participate in processes leading to adaptation as well as contributing to pathophysiology when they are produced insufficiently or in excess, i.e. outside of the normal range.

Allostasis is a term introduced by Sterling and Eyer [178] to characterize how blood pressure and heart rate responses vary with experiences and time of day and also to describe changes in the set point of these parameters in hypertension. The change in set point was used by them as the primary example that distinguishes allostasis from homeostasis. Yet there is a much broader implication of what they wrote. In their paper, Sterling and Eyer state: "Allostasis emphasizes that the internal milieu varies to meet perceived and anticipated demand". This led us [127] to define allostasis more broadly than the idea of a changing set point, namely, as the process for actively maintaining homeostasis. This is important because, in our view, the systems that vary according to demand, like the HPA axis and ANS, actually help maintain those systems that are truly homeostatic. Moreover, large variations in the HPA axis and ANS do not lead directly to death as would large deviations in oxygen tension, osmolarity and pH.

Therefore, we propose that allostasis is a much better term for physiological coping mechanisms than is homeostasis, which should be reserved for the parameters that are essentially maintained for survival. Therefore, allostasis is the process that keeps the organism alive and functioning, i.e. maintaining homeostasis or "maintaining stability through change" and promoting adaptation and coping, at least in the short run [119,121,126,154].

We note, however, that another view of homeostasis is that it can also mean the operation of coordinated physiological processes which maintain most of the steady states of the organism [20]. In this interpretation, homeostasis and allostasis might seem to mean almost the same thing. The problem with this use of "homeostasis" is that it does not distinguish between those systems essential for life and those that maintain them.

What are some examples of allostasis? Sterling and Eyer [178] used variations in blood pressure as an example: in the morning, blood pressure rises when we get out of bed and blood flow is maintained to the brain when we stand up in order to keep us conscious. This type of allostasis helps to maintain oxygen tension in the brain. There are other examples: catecholamine and glucocorticoid elevations during physical activity mobilize and replenish, respectively, energy stores needed for brain and body function under challenge. These adaptations maintain essential metabolism and body temperature.

Examples of allostasis go beyond the immediate control of body temperature and pH to broader aspects of individual survival, e.g. threats from pathogens or physical danger. In the immune system, we will see afterwards that acute stress-induced release of catecholamines and glucocorticoids facilitates the movement of immune cells to parts of the body where they are needed to fight an infection or to produce other immune responses [34]. Finally, in the

brain, glucocorticoids and catecholamines act in concert to promote the formation of memories of events of potentially dangerous situations so that the individual can avoid them in the future [162]. Yet, each of these adaptive processes has a potential cost to the body when allostasis is either called upon too often or is inefficiently managed, and that cost is referred to as "allostatic load".

2.2. Allostatic load

Whereas allostasis refers to the process of adaptation to challenges, "allostatic load" refers to the price the body pays for being forced to adapt to adverse psychosocial or physical situations, and it represents either the presence of too much allostasis or the inefficient operation of the allostasis response systems, which must be turned on and then turned off again after the stressful situation is over. What are the damaging, as well as the adaptive effects, in different systems? For example, glucocorticoids, so-named because of their ability to promote conversion of protein and lipids to usable carbohydrates, serve the body well in the short run by replenishing energy reserves after a period of activity, like running away from a predator. Glucocorticoids also act on the brain to increase appetite for food and to increase locomotor activity and food seeking behavior [98], thus regulating behaviors which control energy input and expenditure. This is very useful when we do manual labor or play active sports, but it is not beneficial when we grab a pizza and a beer while watching television or writing a paper, particularly when these activities may also be generating psychological stress, e.g. watching distressing news or worrying about getting the paper done in time. Inactivity and lack of energy expenditure creates a situation where chronically-elevated glucocorticoids that may result from either poor sleep, ongoing stress, or as side effects of rich diet can impede the action of insulin to promote glucose uptake. One of the results of this interaction is that insulin levels increase, and, together, insulin and glucocorticoid elevations promote the deposition of body fat and this combination of hormones also promotes the formation of atherosclerotic plaques in the coronary arteries [15]. Thus, whether psychological stress or sleep deprivation or a rich diet is increasing the levels of glucocorticoids, the consequences in terms of allostatic load are the same—insulin resistance and increased risk for cardiovascular disease.

For the heart, we see a similar paradox. Getting out of bed in the morning requires an increase in blood pressure and a reapportioning of blood flow to the head, so we can stand up and not faint [178]. Our blood pressure rises and falls during the day as physical and emotional demands change, providing adequate blood flow as needed. Yet, repeatedly-elevated blood pressure promotes the generation of atherosclerotic plaques, particularly when combined with a supply of cholesterol and lipids and oxygen free radicals that damage the coronary artery walls [109]. Beta adrenergic receptor blockers are known to inhibit this cascade of events and to slow down the atherosclerosis that is accelerated in dominant male cynomologus monkeys exposed to an unstable dominance hierarchy [110]. Thus, catecholamines and the combination of glucocorticoids and insulin can have dangerous effects on the body, besides their important short-term adaptive roles [15].

2.3. Allostatic state

Whereas "allostatic load" refers to the cumulative cost to the body of adapting repeatedly to demands placed upon it [121,127], an "allostatic state" refers to the elevated or disregulated activity of the mediators of allostasis (circulating hormones, tissue mediators) that causes "allostatic load" [88]. Fig. 1 illustrates temporal profiles of allostasis mediators such as cortisol and catecholamines that lead to allostatic load. Shown in the figure are four scenarios in which the body is exposed to greater-than-normal levels of these mediators: repeated "hits", lack of adaptation, prolonged response, and inadequate response. For example, repeated stress may change the temporal response profiles and lead, for example, to impaired shut off or to an elevated or reduced diurnal rhythm of cortisol or catecholamine production. These response profiles may be altered by genetic factors, early developmental influences or the effects of life style. Thus, the first step in many cases may be that the production of mediators becomes disregulated and the set-point for their regulation is changed, so that they are produced in elevated or reduced levels and/or according to an abnormal temporal pattern. If this perturbation becomes a chronic condition, it can be referred to as an "allostatic state" [88]. Prolongation of the allostatic state can produce tissue pathophysiology, referred to as *allostatic load*. In other words, there are circumstances in which the number of stressful events may not be excessive but in which the body fails to efficiently manage the response to challenges or maintain a normal diurnal rhythm, and some examples of allostatic states are illustrated in the four panels in Fig. 1.

The middle-right panel illustrates a failure to habituate to repeated stressors of the same kind. Measurement of cortisol in a repeated public speaking challenge has revealed individuals who do not habituate, and these individuals, who lack self-confidence and self-esteem, may well be overexposing their bodies to stress hormones under many circumstances in daily life that do not overtly disturb other individuals [85].

The bottom-left panel in Fig. 1 refers to a failure to turn off each stress response efficiently or to show a normal diurnal rhythm. For example, individuals with a genetic load, i.e. two parents who are hypertensive, show prolonged elevation of blood pressure in the aftermath of a psychological stressor [51]. Another example of the failure to shut off a response takes us into the realm of the housekeeping function of the mediators of allostasis, namely, the diurnal rhythm. Reduced amounts of sleep for a number of days results in elevated cortisol levels during the evening hours [99,177]. Sleep deprivation and elevated diurnal levels of cortisol are

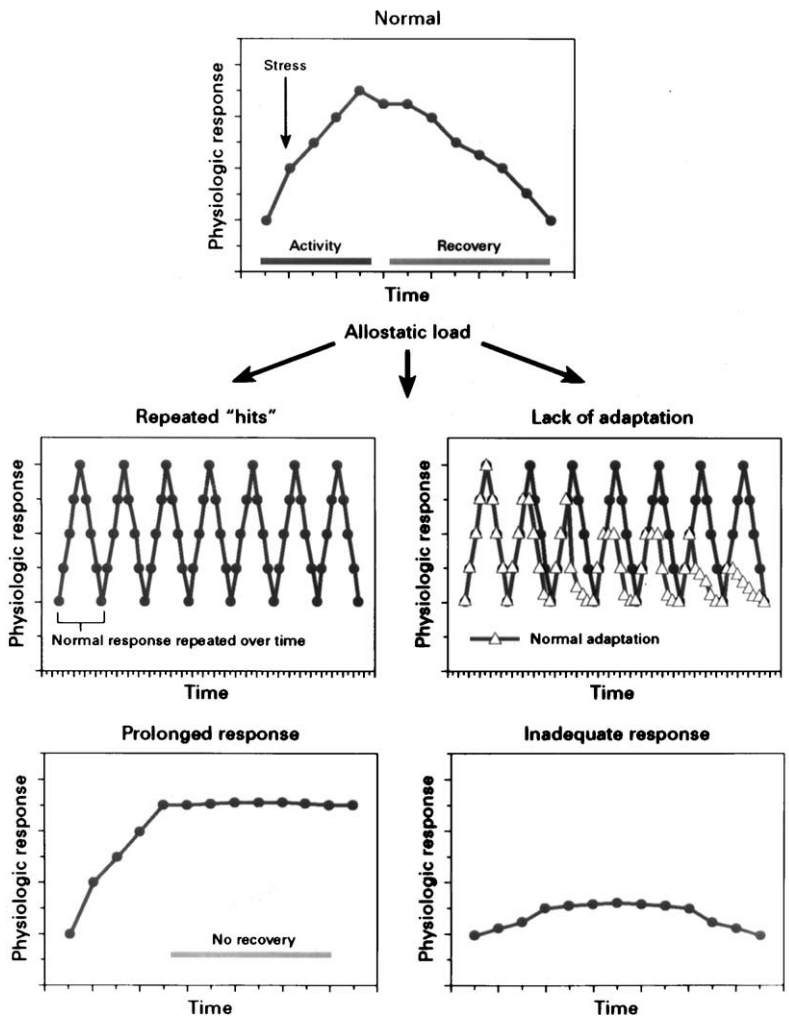

Fig. 1. Four types of response patterns of allostatic mediators. The top panel illustrates the normal allostatic response, in which a response is initiated by a stressor, sustained for an appropriate interval, and then turned off. The remaining panels illustrate four conditions that lead to allostatic load: (1) repeated "hits" from multiple novel stressors, which may or may not change the response profiles of the mediators, leading to an allostatic state; (2) an example of an allostatic state involving lack of adaptation of the mediator to repeated presentations of the same situation; (3) example of an allostatic state involving a prolonged response due to delayed shut down of the mediator in the aftermath of a stress or failure to show a normal diurnal rhythm; (4) an example of an allostatic state involving the inadequate response of one mediator that leads to compensatory hyperactivity of other mediators, e.g. inadequate secretion of glucocorticoid, resulting in increased levels of cytokines that are normally counter-regulated by glucocorticoids. Figure drawn by Dr. Firdaus Dhabhar, Rockefeller University. Reprinted from [121] by permission.

also features of major depression. One reason that elevation of cortisol in the evening is bad is that it has greater effects in causing a delayed hyperglycemic state than does cortisol elevation in the morning [148]. In depressive illness, loss of bone mineral density has been reported that is linked to elevated diurnal glucocorticoid levels [132]. The loss of bone minerals and muscle protein are two of the recognized consequences of chronic elevation of glucocorticoids [166].

The final example of an allostatic state comes from the notion that an acute response of a mediator of allostasis should be of sufficient magnitude to produce an adaptive response, and, if it is not, the systems that are affected by these mediators can themselves malfunction by over-reacting. The bottom-right panel in Fig. 1 describes a situation in which the glucocorticoid response is inadequate to the needs of the individual genotype, resulting in excessive activity of other allostatic systems such as the inflammatory cytokines, which are normally contained by elevated levels of cortisol and catecholamines. The Lewis rat illustrates a genetic contribution to this condition, having less corticosterone than the virtually syngenic Fischer rat. Lewis rats are vulnerable to inflammatory and autoimmune disturbances, which are not found in Fischer rats, and yet these can be overcome by giving exogenous glucocorticoids [179]. Comparable human disorders in which lower-than-needed cortisol may play a role include fibromyalgia and chronic fatigue syndrome [16,27,107,152,188]. This will be discussed further afterwards.

Thus, the distinction between protection and damage, as far as hormonal or tissue mediators of allostasis are concerned, is related to the dynamics of the mediator response, as will be illustrated for excitatory amino acids in brain. But first, we will consider a different use of the concept of allostasis, namely, in the measurement of cumulative physiologic burden in studies on human populations.

3. The measurement of allostasis, allostatic states and allostatic load

How can we measure allostasis and its consequences in terms of allostatic states and allostatic load, particularly when it comes to following the events that lead to disease over the life course in individual human subjects and groups of individuals? This is a major goal of the biologist in working with social scientists and epidemiologists in attempting to answer questions such as the relationship between working, living environments and socioeconomic conditions and health or disease [4]. And it is one of the main reasons that the definition of terms should be made more precise. The distinction between allostatic states and allostatic load provides two different types of endpoints that can be measured, at least in principle. On the one hand, allostatic states refer to the response profiles of the primary mediators themselves. On the other hand, allostatic load focuses on the tissues and organs that show the cumulative effects of over exposure to the mediators of allostasis, either because of too much stress or because of different allostatic states (see Fig. 1). This section will briefly consider the challenges and opportunities in measuring allostatic states and allostatic load.

For determining different allostatic states (e.g. Fig. 1) in human subjects, the choice of which mediators to measure depends, in large part, upon where in the body one is able to measure them as non-invasively as possible. This can be done most easily by collecting urine or saliva, but, if necessary, blood and cerebrospinal fluid can be obtained. The choice is dictated by such factors as the size of the study, cost of the assays, and not wanting to disrupt the lives of the subjects under study more than absolutely necessary in order to insure cooperation and minimize added stress and anxiety that can influence the secretion of the mediators being measured. This means measuring the circulating mediators such as glucocorticoids, dehydroepiandrosterone (DHEA), catecholamines and certain cytokines (see Table 1). Salivary assays are particularly attractive. For any of these mediators, the question arises as to how to sample over time to get an adequate representation of a dynamic system, since the levels of the mediators may fluctuate during the day and night. This is a topic unto itself and has been the subject of a number of methodological studies (see website for MacArthur SAS and Health Research Network: www.macses.ucsf.edu/). Portable monitoring of blood pressure and heart rate provide complementary information to the measurement of mediators such as catecholamines in body fluids. The ease of such measurements also explains why the study of cardiovascular function as an endpoint of disease has progressed so far relative to other systems of the body that are sensitive to stress and show allostatic load.

Table 1
Primary mediators of allostasis

Systemic mediators
Glucocorticoids
DHEA
Catecholamines (epinephrine, norepinephrine)
Cytokines (e.g. IL-6, IL-1, TNF-α)
Many systemic hormones (e.g. thyroid hormone, insulin, insulin-like growth factors, leptin)
Many pituitary hormones (e.g. prolactin, ACTH, growth hormone)
Tissue mediators
CRF
Excitatory amino acids
Monoamines (e.g. serotonin, norepinephrine, epinephrine, histamine)
Other neurotransmitters (e.g. GABA, glycine)
Other neuropeptides (e.g. neuropeptide Y, cholecystokinin, enkephalin, dynorphin, substance P)
Many cytokines (e.g. TNF-α, IL-1, IL-6, IL-4, IL-10, IFN-γ)
Some pituitary hormones (e.g. prolactin, POMC)

As far as assessing allostatic load, it is necessary to distinguish between the primary mediators discussed above, which permit the assessment of allostatic states, and secondary outcomes, or consequences of the actions of those mediators (see Table 2). A fundamental issue is determining the extent to which the mediators of allostasis are a significant part of the causal chain leading to the secondary pathophysiological outcomes that represent allostatic load. Fortunately, for some of the most commonly-measured mediators that can easily be measured in human subjects, there is considerable evidence that they are involved in diverse forms of allostatic load. Table 2 presents a list of some endpoints, or secondary outcomes, that can be used for cumulative assessment of allostatic load in different systems of the body.

Some progress in validating the organizing concept of allostatic load through relatively inexpensive and non-invasive measures has been possible with the markers that were developed during the MacArthur Successful Aging Study. These markers were chosen for a somewhat different purpose, but they have found utility, nevertheless [89,126,169–171]. Available data from the MacArthur Successful Aging Study provided information on the following parameters [171] which represent a combination of primary mediators and secondary outcomes:

- systolic and diastolic blood pressure, indices of cardiovascular activity;
- waist–hip ratio, an index of more chronic levels of metabolism and adipose tissue deposition, thought to be influenced by increased glucocorticoid activity;
- serum HDL and total cholesterol, related to the development of atherosclerosis—increased risks being seen with

Table 2
Some primary mediators and secondary outcomes

Primary mediators—assessment of allostatic states
Elevated levels of inflammatory cytokines
Elevated and flattened diurnal cortisol rhythms
Elevated overnight urinary cortisol
Low DHEA:cortisol ratio
Elevated levels of overnight urinary catecholamines
Note: autonomic nervous system activity is also assessed indirectly by measuring blood pressure
Abnormal insulin levels (also assessed indirectly as abnormal glucose levels)
Secondary outcomes—measures of allostatic load
Brain: atrophy of brain regions, cognitive impairment
Cardiovascular: atherosclerosis, left-ventricular hypertrophy, clotting factors, homocysteine, oxidative stress markers
Immune system: impaired wound healing, retarded immunization response, suppressed delayed-type hypersensitivity, chronic pain and fatigue reflecting imbalance of immune system regulators in the CNS
Metabolic: glycosylated hemoglobin, HDL:LDL, cholesterol, abdominal fat deposition, as measured by the waist–hip ratio, bone mineral density

Definitions: Primary mediators are circulating hormonal agents that produce a variety of effects upon diverse target tissues throughout the body. These mediators interact with each other in producing primary and secondary effects and in regulating their own production. Secondary outcomes refer to biological parameters or functional states that are the products of the interactions of primary mediators (often more than one primary mediator) with tissue substrates. They reflect parameters that are themselves indicators of pathophysiological processes.

higher levels in the case of total cholesterol and lower levels in the case of HDL;

- blood plasma levels of glycosylated hemoglobin, an integrated measure of glucose metabolism over several days time;
- serum dehydroepiandrosterone-sulfate (DHEA-S), a functional HPA axis antagonist;
- over-night urinary cortisol excretion, an integrated measure of 12-h HPA axis activity;
- overnight urinary norepinephrine and epinephrine excretion levels, integrated indices of 12-h SNS activity.

The initial compilation of an "allostatic load score" was created by summing across indices of subjects' status with respect to these 10 components of allostatic load. For each of the 10 indicators, subjects were classified into quartiles based on the distribution of scores in the high function cohort (see [171]). The decision to use distributions in the high function cohort was based on the fact that analyses of relationships between allostatic load and health outcomes were based on longitudinal data for this latter group. Allostatic load was measured by summing the number of parameters for which the subject fell into the "highest" risk quartile (i.e. top quartile for all parameters except HDL cholesterol and DHEA-S for which membership in the lowest quartile corresponds to highest risk).

Several alternative criteria for calculating allostatic load were also examined and are discussed elswhere [126,169]. However, the original measures and original "allostatic load score" has provided useful information that supports the usefulness of this type of measurement in predicting changes over time and influences of such factors as social support, income, education and positive or negative influences of relationships and occupation during the life course [169,172].

It should be noted that the allostatic load score is limited by the choice of measures and by the reliance on a combination of primary mediators and secondary outcomes. The additional markers described in Table 1 offer the possibility of expanding the list of markers somewhat further to include measure related to oxidative stress and immune function. Now we return to mechanistic studies of allostasis and allostatic load in the brain that may lead us to the development of protective treatments, as well as to a greater appreciation of resilience of the aging brain.

4. Allostasis and allostatic load in the brain

As defined earlier in this article, allostasis is the process of adaptation to challenge that maintains stability, or homeostasis, through an active process [178], and allostatic load is the wear and tear produced by the repeated activation of allostatic, or adaptive, mechanisms, frequently involving allostatic states of chronically elevated or disregulated activity of key tissue and hormonal mediators [121,127]. Four types of allostatic states leading to allostatic load have been identified and are summarized in Fig. 1. As we have noted earlier in this article, these consist of (1) repeated challenges, (2) failure to habituate with repeated challenges, (3) failure to shut off the response after the challenge is past, and (4) failure to mount an adequate response. In the hippocampus, we can recognize at least two types of allostatic load involving excitatory amino acid release, namely, (1) the potential to cause damage with repeated stressful challenges and (2) the failure in aging rats to shut off glutamate release after stress.

Under restraint stress, rats show increased extracellular levels of glutamate in hippocampus, as determined by microdialysis, and adrenalectomy markedly attenuates this

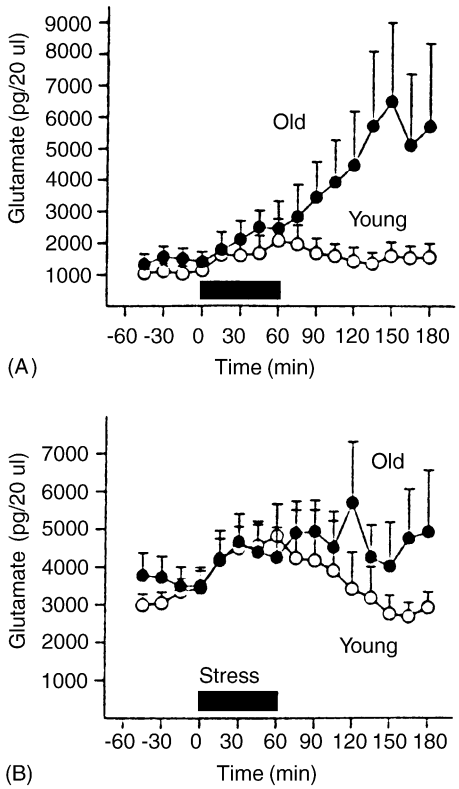

Fig. 2. Effect of 1 h immobilization stress on extracellular levels of excitatory amino acids in the hippocampus (A) and medial prefrontal cortex (B) of young (3–4-month-old) adult and aging (22–24-month-old) rats. Note that both young and old rats released glutamate during stress, but that during the 2 h post-stress period, old rats continued to release glutamate whereas young rats did not. Reprinted from [100] by permission.

elevation [102]. Glucocorticoids appear to be involved in potentiating the increased extracellular levels of excitatory amino acids under stress [134]. Similar results have been reported using lactography, a method that is based upon the stimulation of glucose metabolism by increased neuronal activity [29,168]. The consequences of this increased level of extracellular glutamate will be discussed in terms of hippocampal dendritic remodelling, which is an example of Type 1 allostatic load. In aging rats, hippocampal release of excitatory amino acids during restraint stress is markedly potentiated [103], and this constitutes an example of Type 3 allostatic load in the brain, i.e. the failure to shut off the production and/or removal of a mediator of neuronal activation (see Fig. 2).

Free radical formation is a by-product of excitatory amino acid release and a consequence of the activation of second messenger systems [118,159]. A key factor in regulating production of free radicals is the maintenance of homeostasis of calcium ions [77]. When excitatory amino acid neurotransmitters are released, calcium ions are mobilized via activation of NMDA and AMPA receptors, and second messenger systems are activated leading to a cascade of effects including the long-term potentiation (LTP) and long-term depression (LTD) that are believed to be related to information storage mechanisms [13,112].

The reuptake and rebuffering of calcium ions is an active process [24,77]. If the calcium ions are not removed and put back into intracellular stores rapidly and efficiently, the cascade of events is potentiated and can result in the increased accumulation of free-radicals as by-products of lipid peroxidation that produce an allostatic load on brain and cardiovascular cells [77,118]. There is a link between stressful events and the production of the free radicals, namely, that acute stress increases the production of free radicals in the brain and other organs [101]. However, the role of glucocorticoids in this process is not known. Glucocorticoids may play a role in the process by facilitating the activity of NMDA receptors [6,192], by impairing glucose uptake and reducing intracellular energy supplies [159,165] and by increasing calcium currents (see above), and individual differences in glucocorticoid secretion over the life-course may thus make a contribution. What are these individual differences in glucocorticoid secretion and how may they come about?

5. Age-related shifts of calcium homeostasis and its consequences

The hippocampus is a brain region that is very important for declarative and spatial learning and memory, and yet is a particularly vulnerable and sensitive region of the brain that expresses high levels of receptors for adrenal steroid “stress” hormones [30,44]. Hippocampal neurons are vulnerable to seizures, strokes and head trauma, as well as responding to stressful experiences [30,123,166]. At the same time these neurons show remarkable and paradoxical plasticity, involving long-term synaptic potentiation and depression, dendritic remodeling, synaptic turnover and neurogenesis in the case of the dentate gyrus [18,122,124]. This will be discussed further.

Studies in animal models have shown that the hippocampus undergoes progressive changes with age in calcium homeostasis, in the plasticity of response to glucocorticoids, and in the expression of markers related to neuroprotection and damage. The activity of L-type calcium channels increases in hippocampal CA1 pyramidal neurons of aging rats and results in an increased after-hyperpolarization [94]. Some of this can be mimicked in a cell culture system. In embryonic hippocampal neurons that are maintained for 28 days in cell culture, there is enhanced calcium channel activity and increased after-hyperpolarization that are accompanied by decreased neuronal survival; blocking L-type calcium channels increased neuronal survival [151]. It is interesting to note that the increased after-hyperpolarization is associated with alterations of two important neurophysiologic responses in CA1 pyramidal neurons of the hippocampus, namely, enhanced induction of LTD and an impaired induction of LTP [139]. Thus, insofar as LTP and LTD may be related to synaptic plasticity during learning [111], these

age-related changes suggest a possible basis for cognitive impairment in aging rats [139].

Glucocorticoids enhance calcium channel activity and after-hyperpolarization [71,94], and hippocampal glucocorticoid receptor expression shows a progressive failure of negative feedback regulation in old versus young rats. In young rats, repeated stress causes a down-regulation of glucocorticoid receptor levels, thus decreasing glucocorticoid efficacy on various target genes, whereas this down-regulation is lost with increasing age, thus potentiating glucocorticoid actions, some of which may be destructive to brain cells [83]. Therefore, there is a natural mechanism in the young hippocampus for resilience in the face of repeated stress that acts to reduce the magnitude of the glucocorticoid feedback signal and thus reduce the impact of glucocorticoids on calcium channel activity, among other effects. This may be protective, insofar as increased calcium channel activity contributes to free radical generation and other processes that may damage neurons [101,118]. With the loss of stress-induced down-regulation of glucocorticoid receptors, older rats appear to lose this protective device and may be more vulnerable to increased levels of glucocorticoids, particularly in cognitively-impaired rats [83].

It is still unclear whether outright neuronal loss is a major event in the aging hippocampus of cognitively-impaired rats ([156,157]; see [115] for review). Nevertheless, there are indications that gene products associated with neurodegeneration and damage are differentially regulated in the aging-impaired brain compared to unimpaired aging rats and young rats, although the interpretation of the results is very complex [180]. In aging, cognitively-impaired rats, the levels of mRNA for the 695 amino acid form of the β-amyloid precursor protein (βAPP) and for the magnesium-dependent superoxide dismutase (Mg-SOD) were both elevated throughout the hippocampus compared with young rats; at the same time the levels of the βAPP protein and Mg-SOD protein were both depressed [180]. Levels of mRNA for glial fibrillary acidic protein (GFAP), a marker of astrocytes which increases with damage, were elevated in the hippocampus of aging, cognitively impaired rats, although the level of the GFAP protein was not elevated [180]. Since βAPP gives rise to both a toxic β-amyloid protein and a protective secreted form, the reduced levels of βAPP expression in aging, cognitively impaired rats is difficult to interpret without a separate measurement of the two forms of the protein. On the other hand, lower Mg-SOD protein is consistent with a lower capacity for free-radical scavenging and an increased risk for free-radical induced neural damage [23], although another interpretation of lower SOD is that there may be less production of free radicals and thus less need for scavenging.

Next, although the role of glucocorticoids in promoting these changes is still under investigation, it is important to consider how progressive changes with age in these indices of damage fit into a broader view of the role of the HPA axis in individual differences in the aging process.

6. Developmental determinants of individual differences in allostatic load

The vulnerability of many systems of the body to stress is influenced by experiences early in life. In animal models, unpredictable prenatal stress causes increased emotionality and increased reactivity of the HPA axis and ANS and these effects last throughout the lifespan [193]. Postnatal handling in rats, a mild stress involving brief daily separation from the mother, counteracts the effects of prenatal stress and results in reduced emotionality and reduced reactivity of the HPA axis and ANS [3,67,100]. "Taken together, these types of studies provide a basis in a relatively simple animal model for study of human conditions in which early life events such as parental loss, neglect and even abuse in childhood exert long-lasting influences on behavior, emotionality and health [46,63,184] and in which nurturing and social support can ameliorate at least some of the negative outcomes of inexperienced or poor parenting [42,43,117]."

For prenatal stress and postnatal handling, once the emotionality and the reactivity of the adrenocortical system are established by events early in life, it is the subsequent actions of the HPA axis in adult life, as discussed above, that are likely to contribute to the rate of brain and body aging. Rats with increased HPA reactivity show early decline of cognitive functions associated with the hippocampus [31] as well as increased propensity to self-administer drugs such as amphetamine and cocaine [33,147]. In contrast, rats with a lower HPA reactivity as a result of neonatal handling have a slower rate of cognitive aging and a reduced loss of hippocampal function [22,130,131]. Thus, life-long patterns of adrenocortical function, determined by early experience, contribute to rates of brain aging, at least in experimental animals.

Evidence for a human counterpart to the story of individual differences in rat HPA activity and hippocampal aging is very limited. Individual differences in human brain aging that are correlated with cortisol levels have been recognized in otherwise healthy individuals that are followed over a number of years [104,105,170]. In the most extensive investigation, healthy elderly subjects were followed over a 4-year-period, and those who showed a significant and progressive increase in cortisol levels, during yearly exams, over the 4 years, and had high basal cortisol levels in year 4, showed deficits on tasks measuring explicit memory as well as selective attention, compared to subjects with either decreasing cortisol levels over 4 years or subjects with increasing basal cortisol but moderate current cortisol levels [104]. Using MRI, they also showed a hippocampus that was 14% smaller than age-matched controls who did not show progressive cortisol increases and were not cognitively impaired [105]. "In these studies, the influence of early life events was not investigated. Such studies are very much needed but are clearly difficult because of the problems of accurately accessing early life events through retrospection."

7. Adaptive plasticity—another role for excitatory amino acids and hormones

The hippocampus is not only a vulnerable brain structure to damage but is also a very plastic region of the brain and expresses high levels of adrenal steroid receptors. Adrenal steroids, which, as we have noted, have a bad reputation as far as their role in exacerbating these forms of damage [165], are also involved in three types of adaptive plasticity in the hippocampal formation. "Adaptive plasticity is a form of allostasis that enables the brain to respond to a changing environment and adapt in a way that helps the individual to survive the immediate challenge. This adaptive plasticity takes a number of forms."

First, *adrenal steroids* reversibly and biphasically modulate excitability of hippocampal neurons and influence the magnitude of LTP, as well as producing LTD [30,82, 144–146]. These effects may be involved in biphasic effects of adrenal secretion on excitability and cognitive function and memory during the diurnal rhythm and after stress [5,28,35,36]. In particular, adrenal steroids facilitate fear-motivated learning [26,163] whereas acute non-painful novelty stress inhibits primed-burst potentiation and spatial memory [36,37] and post-training shock stress inhibits recall of a spatial memory task that depends on the hippocampus [32].

Second, adrenal steroids participate along with excitatory amino acids in regulating neurogenesis of dentate gyrus granule neurons [18], in which acute stressful experiences can suppress the ongoing neurogenesis [48]. These effects may be involved in fear-related learning and memory, because of the anatomical and functional connections between the dentate gyrus and the amygdala [69], a brain area important in memory of aversive and fear-producing experiences [96].

Third, adrenal steroids participate along with excitatory amino acids in a reversible stress-induced atrophy, or remodeling, of dendrites in the CA3 region of hippocampus of male rats and tree shrews [122], a process that affects only the apical dendrites and results in cognitive impairment in the learning of spatial and short-term memory tasks [122]. Although this type of plasticity does impair cognitive function at least temporarily, it may be beneficial to the brain in the long run if the remodeling of dendrites reduces the amount of excitatory synaptic input and, thereby, reduces the impact of excitatory amino acids and glucocorticoids in causing more permanent damage. This is a hypothesis that remains to be rigorously tested.

Besides what stress does to change hippocampal structure, there are other forms of plasticity in the hippocampus, including reversible synaptogenesis that is regulated by ovarian steroids in female rats and occurs in the CA1 region [195] and a very rapid and reversible atrophy of dendrites of CA3 neurons during hibernation in ground squirrels and hamsters [149,150]. The estrogen-regulated CA1 synaptic plasticity is also a rapid event, occurring during the female rats' 5-day estrous cycle, with the synapses taking several days to be induced under the influence of estrogens and endogenous glutamic acid, and then disappearing within 12 h under the influence of the proestrus surge of progesterone [125,128].

In view of the discussion above, concerning excitatory amino acids and NMDA receptors, it is important to note that the above-mentioned hormone effects on morphology and function of the hippocampus do not occur alone but rather in the context of ongoing neuronal activity. In particular, excitatory amino acids and NMDA receptors play an important role in the adaptive functional and structural changes produced in the hippocampal formation by steroid hormones. This includes not only the estradiol-induced synaptogenesis [125] but also the effects of adrenal steroids to produce atrophy of CA3 pyramidal neurons [122], as well as the actions of adrenal steroids to contain dentate gyrus neurogenesis [17]. Blocking NMDA receptors prevents atrophy as well as estrogen-induced synaptogenesis [108,196], and NMDA receptors are induced by estrogens on CA1 neurons [50,191] and by glucocorticoids throughout the hippocampus [192]. At the same time, as we have already noted, excitatory amino acids and NMDA receptors are involved in free radical generation leading to neural damage, and one of the challenges for future research is to understand what triggers the transition from adaptive plasticity to permanent damage.

8. Adaptive plasticity and the concept of resilience

We have noted that the young brain is resilient and able to withstand challenges and adapt, and the structural plasticity noted above is an example of this resilience and adaptability. *The term allostasis means adaptation and coping and implies resilience*. Allostasis operates most efficiently when the body is doing its best to maintain homeostasis without doing harm. As noted and illustrated above, allostatic load is the cost of adaptation, reflecting both the overuse of the system by repeated stressors as well as the inefficient management of allostasis—failure to shut off or habituate; failure to turn on when needed. Here we consider how structural plasticity is related to resilience and how this plasticity may be lost as the brain ages.

Neurogenesis in the dentate gyrus provides an excellent example of resilience in the adult brain [56]. Production of new cells is increased by voluntary exercise [190], by an enriched environment [81] and by estrogens, as noted above. Dendritic remodeling by repeated stress provides another example of resilience, since it is a reversible process that may protect nerve cells from permanent damage [25,122]. Down-regulation of glucocorticoid receptors in response to repeated stress [45] is another example of a protective response, since glucocorticoids exacerbate permanent damage to hippocampal nerve cells [165].

An important mediator of resilience in brain is IGF-1. Circulating IGF-1 is another stimulator of neurogenesis [1,140].

IGF-1 is a 7.5 kDa protein and, yet, it is taken up into CSF by a process that is independent of IGF receptors or binding proteins [155]. In rats, voluntary running in a running wheel has been reported to increase neurogenesis in the dentate gyrus [190]. Such exercise increases the uptake of IGF-1 from the blood and activates c-fos expression in dentate gyrus and other brain regions in a manner that is mimicked by IGF-1 administration into the circulation [21]. Moreover, immunoneutralization of IGF-1 blocks the effects of exercise to enhance neurogenesis [186]. Receptors for IGF-1, IGF-2 and insulin are expressed in the hippocampus [39], with IGF-1 receptors undergoing a decrease after adrenalectomy [70]. Although IGF-1, IGF-2 and insulin binding does not decrease with age in the rat hippocampus [39], the level of IGF-1 mRNA undergoes a small but selective decrease in some hippocampal fields [91]. Exogenous IGF-1 ameliorates memory deficits in aging rats [113] and enhances glucose uptake in the aging hippocampus [106] as well as having neuroprotective actions [38,53,181].

Although we do not have examples for each type of structural plasticity described above for the aging brain, a number of examples exist to show that *adaptive plasticity, i.e. resilience*, is frequently lost in the aging brain. This is the case for neurogenesis, in which there is reduced neurogenesis in aging rats that can be restored by bilateral adrenalectomy [19]. It is also the case for the down-regulation of glucocorticoid receptors in the hippocampus, in which 24-month-old rats lose the capacity to show stress-induced down-regulation of glucocorticoid receptors [94]; the lack of receptor down-regulation makes the hippocampus vulnerable to the potentially-damaging effects of elevated glucocorticoids in the aging brain. Finally, estrogen induction of new synapses in hippocampus is deficient in aging female rats while at the same time estrogen treatment induces NMDA receptors, which are placed in existing dendritic spines and may actually increase the vulnerability of those synapses for excitotoxic actions of glutamate [2]. We do not know yet if dendritic remodeling is lost with age.

9. Stress and estrogen interactions affecting brain function

There are a number of points of interaction with ovarian hormones that indicate that estrogens may have a neuroprotective role in relation to stress and glucocorticoid secretion and actions in the brain. For example, estrogens stimulate neurogenesis in the female dentate gyrus [182]. Moreover, female rats appear to be resistant to the stress-induced atrophy of hippocampal dendrites seen in male rats [47]. Because there are developmentally programmed structural and functional sex differences in the hippocampus [57,74,161,194], it is not clear whether the estrogen-stimulation of neurogenesis or the lack of atrophy in the hippocampus after stress are processes that would occur in males if they were castrated and given estrogens as adults, or if these effects reflect programming earlier in life during sexual differentiation.

Extrapolation of these findings to humans is difficult because of a lack of comparable studies on the human brain. However, there is evidence for increased vulnerability of postmenopausal women to declines in hippocampal dependent cognitive function that is correlated with elevated urinary cortisol [170]. Moreover, HPA activity in women tends to increase postmenopausally as increased levels across the diurnal cycle and a flattening of the rhythm, although there were considerable individual differences [189]. Furthermore, another study showed that women have an age-dependent increase in cortisol secretion in response to a laboratory challenge test that is greater than that for men [173]. “Although in these two studies it was not clear which women, if any, were receiving estrogen replacement therapy, a recent study indicates that ERT does reduce both HPA reactivity and sympathetic nervous system reactivity [86] (see also [173] for other references), both measures indicating that estrogens may reduce allostatic load that can exacerbate cardiovascular disease, hypertension and abdominal obesity [121].”

This is a new area of study and much more needs to be done, but the hypothesis that arises from the available data is that estrogens work to contain the HPA axis and to counteract some of the potentially damaging actions of glucocorticoids on nerve cells. Recent support for this latter notion comes from studies showing that estrogens reduce excitotoxic damage and that glucocorticoids increase it [55]. In vivo studies have shown that estrogens reduce damage produced by ischemia in an animal model of stroke, in which excitotoxicity is involved [40,41,62] and in which it is known that glucocorticoids exacerbate ischemic damage [167].

10. Resilience of the brain in the face of stress and allostatic load

We have seen that stress and glucocorticoids act in concert with excitatory amino acids to modulate the branching of dendrites in the hippocampus of experimental animals and the replacement of neurons in the dentate gyrus [122]. Atrophy of dendrites and inhibition of neurogenesis caused by stress compromises cognitive functions that depend on the hippocampus, such as spatial, declarative and contextual memory. However, these effects are reversible, along with the morphological changes, as long as the stress is terminated after a number of weeks—much longer periods of stress may cause permanent damage to the hippocampus [187]. Thus, the brain is resilient and capable of adaptive plasticity, and we must consider the role of endogenous mediators of resilience. “Such mediators include estrogens and other substances that can reduce the allostatic load generated by excitatory amino acids and they also include genes that afford neuroprotection.”

10.1. Estrogens, flavonoids and neuroprotection

Besides the protective effects of estrogen treatment on ischemic damage, noted above, there is evidence that estrogens reduce the risk for Alzheimer's disease [64,76,183]. Thus, the search for neuroprotective mechanisms has intensified, and multiple mechanisms have been uncovered for estrogen action in the brain. The variety of estrogen effects has been expanded to include rapid actions on excitability of neuronal and pituitary cells, the activation of cyclic AMP and mitogen-activated protein kinase (MAP kinase) pathways, effects on calcium channels and calcium ion entry, and protection of neurons from damage by excitotoxins and free radicals [97,124] (see Table 3 and Fig. 3). These estrogen actions occur through at least two types of intracellular receptors, ERα and ERβ, as well as a handful of other mechanisms for which receptor sites are not clearly identified [97,124]. Indeed, for a number of processes, there are conflicting reports, based upon estrogen structure–activity studies and the actions of estrogen antagonists, as to whether intracellular receptors are involved. Thus, for estrogen actions on some aspects of calcium homeostasis, certain aspects of second messenger systems and some features of neuroprotection, a novel receptor mechanism is implicated, in which stereospecificity for 17β-estradiol over 17α-estradiol is replaced by a broader specificity for the 3 hydroxyl group on the A ring (for review, see [97,124]). Some of these actions of estrogens appear to reduce the production of or actions of free radicals in causing cell damage and promoting cell death through apoptosis [55,59].

Flavonoids are potentially useful exogenous agents in protecting the aging brain and other organs and tissues of the body against free-radical induced damage [12]. Flavonoids include substances that are estrogenic [90] and estrogenic substances have neuroprotective effects against free-radical damage which may occur via another mechanism, in addition to the traditional intracellular estrogen receptors, based upon the structure–activity profile [8,9,11,58,135]. Thus, the further study of flavonoids and their neuroprotective effects may have some overlap with the continuing investigation of neuroprotection by estrogen-replacement therapy towards Alzheimer's disease [10,65,66,76,142,183,198]. Indeed, flavonoids, along with estradiol and other anti-oxidants, may be useful agents to protect the brain without preventing the normal plasticity that the same systems, involving NMDA receptors, calcium ions and circulating glucocorticoids, are mediating. Other strategies, involving direct interference with NMDA receptors or calcium channels, or glucocorticoid secretion or action, may have the effect of disrupting the normal processes and impairing cognitive and other important functions even if they are effective in retarding permanent damage.

One major weakness with the story of flavonoids and other anti-oxidants is that there is very little data collected in vivo on animal models or human subjects. However, recent data on a rat aging model lends support to the efficacy of plant anti-oxidant compounds [73]. In this particular study, treatment of 6-month-old rats for 8 months with a number of fruit or vegetable extracts or Vitamin E conferred some protection against age-related decline of cognitive function and a number of neurochemical processes [73]. Flavonoids are known to be among the anti-oxidant substances in such extracts. Moreover, a recent report on human subjects indicates that an extract of *Gingko biloba*, which contains flavonoids, stabilized and even improved the cognitive performance and social functioning of demented patients for 6 months to 1 year [95].

Table 3
Actions of estrogens related to excitability and cell membrane events

Membrane binding sites—identified but not well-characterized in pituitary, liver and endometrium, but not in brain (see [124])
Genomic effects on membrane events
Induction of the MINK potassium channel in pituitary via genomic mechanism [14]
Calcium channel expression in pituitary and hippocampus [72,160]
Rapid non-genomic actions, e.g. rapid excitation of electrical activity in hypothalamus, cerebellum, hippocampus, amygdala, striatum and cerebral cortex. Effects occur within seconds and are unlikely to involve a transcriptional activation, e.g. [61,78,137,176]
Second messenger activation
CREB phosphorylation: genomic vs. non-genomic mechanism unclear
MAP kinase activation: possible novel receptor pathway or involvement of classical ER in a novel signaling pathway, e.g. [185]
MAP kinase dependent activation of IGF-1 receptors via ERα [75]
G protein coupling, e.g. rapid actions to suppress GnRH release via K^+ channel; rapid actions to suppress GTPγS binding [133]
Calcium homeostasis [124]
Rapid inhibition of L-type calcium channels in smooth muscle, striatal neurons
Possible genomic actions: delayed and sustained increase in calcium channel activity
Neuroprotection
Rapid actions: 17β-estradiol is as potent as 17α-estradiol vs. oxidative damage
Genomic actions: 17β-estradiol is more potent; anti-estrogen blockade

For detailed summaries, see [79,80,97,124].

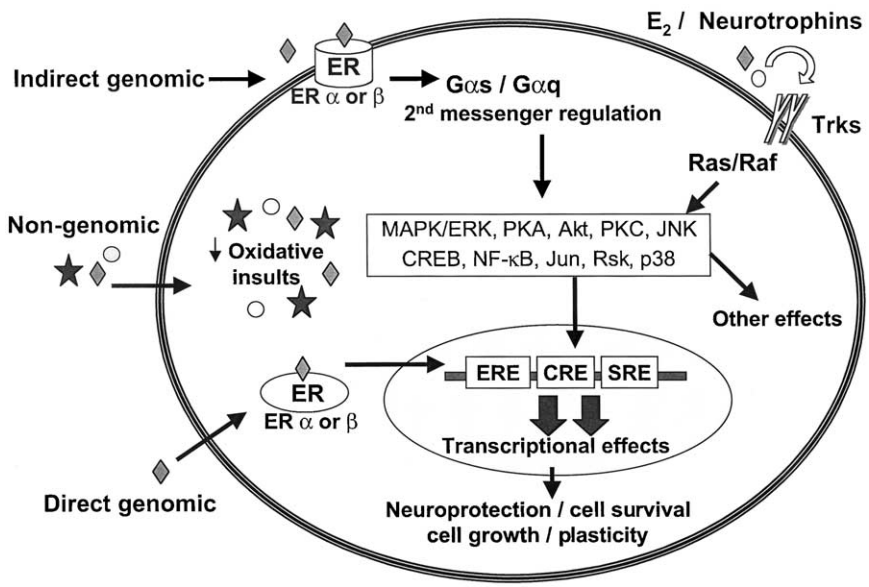

Fig. 3. Putative mechanisms of estrogen action. In the direct genomic mechanism, the nuclear form of ERα or ERβ associates with either the ERE or fos/jun heterodimers that bind, in turn, to AP-1 sites. Indirect genomic mechanisms include the activation of an ER linked to second messenger systems, such as AC/PKC, cAMP/PKA and MAPK/ERK, converging with the genomic pathway. In one of these pathways, Ras activates Raf, which leads to sequential phosphorylation and activation of MAPK/ERK. Activated ERK then translocates into the nucleus to interact directly with nuclear transcription factors (e.g. CREB, cfos/cjun), and indirectly through the activation of intermediary signaling proteins (e.g. Rsk, p38, JNK) to bind to the DNA regulatory regions CRE and SRE. Neurotrophins and estrogens may influence each other's actions by regulating receptors and/or ligand availability through reciprocal regulation at the genomic level. Non-genomic estrogen effects at high concentrations involve anti-oxidant effects not mediated by known intracellular ERs. ERE, AP-1, SRE, and CRE are regulatory regions in DNA sequences that are recognized by specific gene-regulatory proteins. ERE is recognized by estrogen–ER complexes; AP-1 is recognized by fos/jun heterodimers; CRE is recognized by phospho-CREB (phoshorylated by PKA in response to a rise in cAMP levels); SRE is recognized by SRF–Elk-1 complex phosphorylated by MAPK/ERK. The MAPK/ERK migrates from the cytoplasm to the nucleus and phosphorylates Elk-1, thereby activating it to turn on the transcription of the *fos* gene. MAPK/ERK and PKC can phosphorylate jun protein, which combines with the newly formed fos to form heterodimers that ultimately bind to AP-1. (◊), Estriol; (★), 17α-estradiol; (○), 17β-estradiol, ERE, estrogen response element; CRE, cAMP response element; SRE, serum response element. See text for other abbreviations. Reprinted from [97] by permission, from the *Annual Review of Pharmacology and Toxicology*, Vol. 41, 2001 by *Annual Reviews* (www.AnnualReviews.org).

Nevertheless, caution is in order since flavonoids may have potentially deleterious actions that may be related to their partial agonist/antagonist profile, re: estrogen receptors and their demonstrated ability to exert opposite effects to that of estradiol [143]. Another recent report indicates that they lower circulating levels of estradiol in women [138]. Finally, in recent animal studies, a flavonoid, coumestrol, with a higher affinity for ERβ over ERα, showed antagonistic effects to those of estradiol on locomotor activity and reduced fearfulness in a fear conditioning paradigm under conditions in which estradiol had no effect [49].

10.2. Genes and vulnerability

In spite of the need to develop exogenous neuroprotective strategies to treat brain damage associated with aging, it is important to note that the brain is normally resilient in the face of acute and repeated stress, indicating that there are protective factors that promote resilience of brain cells over the life-span and in the face of stressful challenges. How can these protective factors be identified and studied, and how can a resilient brain be made vulnerable to allostatic load? This is an important question for study of animal models, and there are a number of approaches. For example, transgenic mice are beginning to be used in identifying factors that promote vulnerability or protection. Among these are the *p53* tumor suppressor genes that triggers apoptosis and death of cells when DNA damage is large; knock out of the *p53* gene protects brain cells from epileptic and other damage, both in vivo and in vitro [136,197]. On the other hand, deletion of the superoxide dismutase gene increases vulnerability of the hippocampus to ischemia-induced damage [23,87]. Another important gene is *bcl-2* which plays a key role in maintaining mitochondrial calcium homeostasis (see [159] for review). Thus, one strategy in studying protective factors is to manipulate genes that are likely to provide protection, such as the neurotrophins or superoxide dismutase; mice with deficiencies in these genes should be more vulnerable to age- and stress-induced damage of hippocampal structure and function, and studies are underway to test the validity of this strategy. Another approach is to manipulate metabolic factors, e.g. by making rats diabetic or by stressing animals that have genetic risk for either Type 1 or Type 2 diabetes. Some initial results indicate that diabetes may accelerate stress-induced dendritic atrophy in hippocampus

and promote stress-induced neuronal damage [158,197]. A third approach is to use hippocampal cell culture models and study the interaction of androgens, estrogens, glucocorticoids and excitatory amino acids in producing excitotoxic damage [60,151,153,165]. As noted above, the vulnerability to excitotoxicity in hippocampal neurons has been related to increased calcium channel activity that develops with increasing age in culture [151]. The cell culture approach has been extended recently to demonstrate the protective effects of another steroid that declines with age in humans, namely, DHEA, towards NMDA-induced neurotoxicity [7,84]. A fourth strategy is to use targeted delivery of genetic material in viral vectors in order to overcome the restrictions of energy supply in the face of excitotoxic challenge using local enhancement of glucose transporter activity [68,129,141].

11. Conclusions

In conclusion, allostatic states and the cumulative wear and tear (allostatic load) that the body experiences as a result of daily life experiences, differences in individual life style, major life events and socioeconomic status is a highly individual matter, dependent on genotype, early experience and the types of experiences throughout life. Initial attempts to measure allostatic states and allostatic load have been successful enough to encourage further development of methods for measuring biological parameters in order to predict later vulnerability for disease. The use of such measures to assess "predisease pathways" [175] offers hope of encouraging early interventions to delay or prevent diseases later in life.

The only way that assessment of predisease pathways is possible is through new knowledge pertaining to mechanisms leading to diseases that increase in prevalence with age. We strongly suspect that stress hormones play a role in determining the rate of brain and body aging and that they do so in part by exacerbating processes involving the generation of excess free radicals *that cause damage to tissues and organs, including cardiac smooth muscle cells and brain cells*. "Yet, at the same time, there are natural processes and agents such as estrogens and flavonoids that have neuro- and cardioprotective effects and enhance allostasis while minimizing allostatic load." Moreover, the brain and body have the capacity for considerable resilience in the face of stressful challenges, and we need to appreciate more the ways in which this resilience can be harnessed to improve individual trajectories of aging.

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