Chapter 12 The Role of the Stress Axis in Life-History Adaptations

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The neuroendocrine system is a major pathway in vertebrates that integrates environmental change and through which life-history decisions to reproduce, to grow, or to put energy into storage are implemented (McEwen 2001; Ricklefs and Wikelski 2002; Boonstra 2005). The goal of individuals is to maximize lifetime reproductive fitness, and the functioning of the stress axis plays a central role in the neuroendocrine system in making this happen. At the individual level, the stress axis plays a key role in allowing animals to cope with change and challenge in the face of both environmental certainty and uncertainty. At the species level, the stress axis plays a central role in evolutionary adaptations to particular ecological pressures, and an understanding of differences among species is essential to life-history adaptations. In this chapter, we discuss the basic mammalian response to stressors and how this response is modified at both the individual and species levels in response to different ecological pressures.

The stress axis is multitasking throughout the life of an organism. The stress axis is composed of the limbic system (dentate gyrus and hippocampus) and the hypothalamic-pituitary-adrenocortical axis (HPA), and is pivotal for successful adaptation for four reasons. The first three focus on common responses of individuals within a species; the fourth, on between-species differences to the basic pattern. First, the stress axis is involved in normal day-to-day activities associated with the diurnal cycle of waking, such as increased locomotion, exploratory behavior, increased appetite, and food-seeking behavior (reviewed in Wingfield and Romero 2001). Second, the stress axis permits short-term adjustment to maintain survival in the face of acute environmental stressors. This response is the classic "flight or fight" reaction, and is a generalized response to a wide variety of stressors, such as bouts of severe weather, physical stressors such as attacks by a conspecific or a predator, or psychological stressors such as the fear of an imminent attack. Though we focus on the limbic system and the HPA axis here, it is only one part of the stress response, which includes other hormones, neurotransmitters, opioid peptides, cytokines, and brain functions (Sapolsky et al. 2000). Third, the stress axis can be permanently programmed during development because of stressors affecting the mother, and this may adapt the individual to new conditions it may experience during its lifetime (Matthews 2002). Fourth, the stress axis is subject to evolutionary modification, and equips species to succeed under different ecological contexts. Rodents span the gamut of life-history variation, with many species showing high reproductive rates, rapid development, and short life spans, while other species show the opposite traits. In the former, the stress axis functions to trade off survival for reproduction, whereas in the latter, the opposite occurs (Boonstra et al. 2001c; Boonstra 2005).

In this chapter we draw on evidence from natural populations of rodents, but supplement this with research from the laboratory, as this research is generally more in-depth (examining molecular and cellular changes), can provide a guide to indicate what is potentially occurring in nature, and is investigating areas such as neurogenesis, which field studies are only beginning to tackle. However, caution must be exercised when making extrapolations from the labora-

tory to the field, to avoid oversimplification and artifacts. Laboratory rodents are often less aggressive, less aware of their environment, explore less, are more social, and respond more to stressors than their natural counterparts (Künzl et al. 2003; see also Wolff 2003c).

The Stress Response

An external stressor sets off a rapid cascade of responses in vertebrates to respond to the stressor and then to reestablish homeostasis (fig. 12.1). The first line of defense, occurring within seconds of the stressor, is that the sympathetic nervous system causes the adrenal medulla to release catecholamines (epinephrine and norepinephrine) into the general circulation. The second line of defense also occurs immediately, starting with the paraventricular nucleus of the hypothalamus releasing primarily corticotropic-releasing hormone. These hormones cause the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the general circulation and, within minutes, the adrenal cortex releases glucocorticoids (GCs) into the blood. In some rodents (voles and mice) the GC that is released is corticosterone, and in others (chipmunks and squirrels) it is cortisol, or a mixture of cortisol and corticosterone. The HPA axis signals the body to mobilize energy, inhibit physiological processes not required to deal with the stressor, and return the body to homeostasis after the stressor has passed. Immediate catabolic effects result in the mobilization of glucose for the muscles, the stimulation of hepatic gluconeogenesis (the breakdown of other body tissues, such as protein), and the shunting of energy resources away from peripheral tissues not needed for short-term survival. Cardiovascular tone is increased, immune function is stimulated, reproductive physiology and behavior inhibited, feeding and appetite is decreased, and cognition is sharpened (Sapolsky 2002). Under conditions where the stressor is acute, GCs exert feedback at three levels in the brain (fig. 12.1) to return the body back to the preactivation state. Key to this feedback is the intracellular GC receptors (mineralocorticoid receptors [MR] and glucocorticoid receptors [GR]) in the critical brain areas, especially the hippocampus, which regulates the overall functioning of the HPA axis (fig. 12.1; de Kloet et al. 1999).

The GC plasma carrier protein, corticosteroid binding globulin (CBG), plays a major role in allowing mammals to cope with stressors, but it changes rapidly as a function of reproduction or of chronic stressors. The mammalian body is typically buffered from the immediate impact of GCs in the blood because they are tightly bound to CBG. Only about 5 to 10% of GCs are unbound and free, and only the free GCs are biologically active (Rosner 1990). CBG is thought to act as a reservoir of GC, so that GCs can be rapidly released in response to an environmental challenge. CBG concentrations are affected both by the stress axis and the gonadal axis. Chronic stressors, lasting for as little as 24 h, result in a marked reduction in CBG (Schlechte and

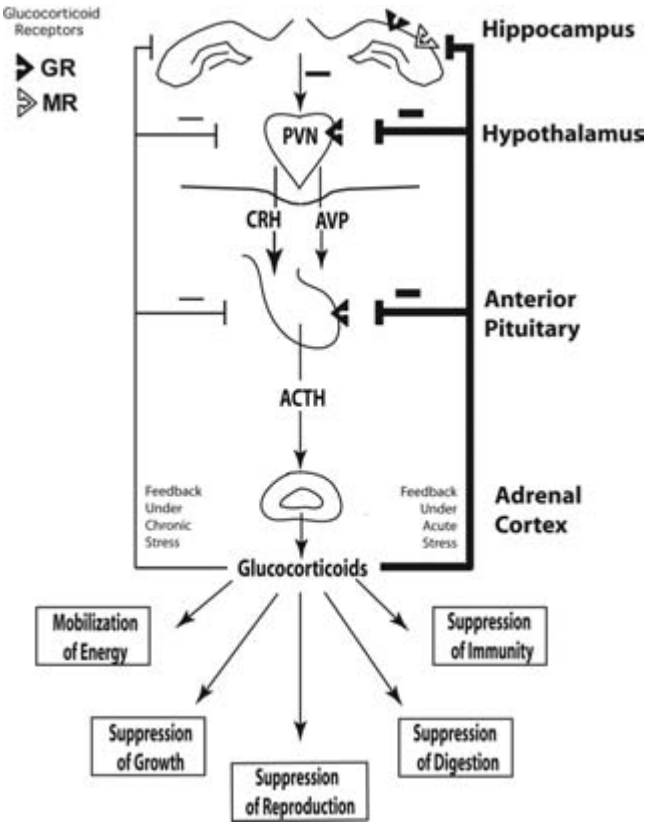

Figure 12.1 The hippocampus and the hypothalamic-pituitary-adrenal (HPA) axis, the major impacts on body processes, and the glucocorticoid (GC) feedback in the mammalian brain. The hippocampus regulates the overall functioning of the HPA. A stressor causes the hypothalamic paraventricular nucleus (PVN) to release corticotropin releasing hormone (CRH) and vasopressin (AVP), and this causes the anterior pituitary to release adrenocorticotropic hormone (ACTH). ACTH initiates the synthesis and release of glucocorticoids (GCs, corticosterone in some rodents, cortisol in others) from the adrenal cortex. GCs act at multiple sites within the body to maintain homeostasis, but because of the damaging effects of extended exposure to GCs, the HPA axis is tightly regulated through feedback (inhibition indicated by -) on glucocorticoid receptors to inhibit further HPA activity. GCs feed back on the hypothalamus and pituitary to cause a rapid inhibition of CRF release. Under conditions where the stressor is acute, feedback mechanisms operate efficiently and the system rapidly returns to normal, resulting in effects on body processes that are only short term. Under conditions where the stressor is chronic, feedback signals are weak and the system remains activated for longer periods, resulting in effects on body processes that can be long term and detrimental. Short-term effects result in suppressive impacts on body processes; long-term chronic effects result in inhibitory impacts on body processes. Glucocorticoid (GR) and mineralocorticoid receptors (MR) occur in the limbic system (hippocampus and dentate gyrus) and GRs occur in the PVN and anterior pituitary. In the brain, MRs have a higher affinity than do GRs for GCs, and at basal concentrations of cortisol, MRs are occupied whereas GRs remain largely unoccupied. During periods of stress and elevated plasma GCs there is increased occupation of GRs. Hippocampal MRs may be primarily involved in feedback regulation during basal secretion, whereas GRs become important during periods of increased GC secretion (from de Kloet et al. 1999; Matthews 2002; Sapolsky 2002).

Hamilton 1987; Boonstra et al. 1998, 2001c), and hence an increase in free GCs. During pregnancy and lactation, females have higher CBG levels; breeding males have lower levels.

The stress response and the homeostatic set-point are not fixed, lifelong species-dependent characteristics, but are modified by experience, by development, and by the annual pattern of life-history changes. First, experience may alter the stress response. The stress response functions well when the stressor is acute (minutes to hours); thereafter, the negative, inhibitory effects of chronic stress become evident and intensify. Laboratory evidence in rodents indicates that the ACTH response is desensitized when the animal is repeatedly exposed to certain types of stressors (e.g., cold exposure) and not to others, but that entirely new stressors continue to elicit a typical stress response (Aguilera 1998). Under conditions of chronic stress (days to months), concentrations of free GCs increase and the normal suppressive effects of GCs grade into inhibition (fig. 12.1). The net result is potentially deleterious, affecting long-term survival and fitness through infertility, impaired resistance to disease, and inhibition of growth. Second, pre-, and postnatal periods of development are particularly vulnerable to permanent modification by stressors affecting the mother (Welberg and Seckl 2001; Matthews 2002). Offspring born to mothers who experienced a high level of stress during gestation, or offspring that experienced high levels of stress during postnatal development are programmed to have a hyperactive stress axis. An interplay also occurs between changes in the stress axis and the reproductive axis (Wartella et al. 2003) that ultimately translates into changes in adult fitness. Finally, in mammals living in seasonal environments, the annual cycle of reproduction, migration, and coping with winter may require the stress axis to be modulated in different ways at different times to optimize reproduction, survival, or both in the face of environmental challenges (Wingfield and Romero 2001). Challenges that are recurrent and predictable, such as the direct male-male aggression associated with breeding, would, if the animal did not evolve a modifying solution, inhibit reproduction.

Impact on Reproduction

The stress axis plays a key role in the entire reproductive process, as it is a transducer of how competition for resources and mates limits or augments reproduction of individuals. In turn, reproduction may result in the progressive deterioration of the stress axis with age, and thus there is a complex interaction between the stress axis and the gonadal axis (Meites and Lu 1994; McEwen 2001). Stressors cause a disruption of reproductive behavior and physiology because of the general suppressive actions of glucocorticoids (Wingfield and Sapolsky 2003). However, the negative impacts of stress do not necessarily occur, with evolutionary adaptations allowing reproduction to proceed in spite of chronic stressors. In this section we examine some of these adaptations.

Breeding frequency in males

Organisms must successfully integrate time and space to maximize their breeding success (Southwood 1977). The breeding choices organisms must make with respect to time is whether they do it "now" or "later," and with respect to space is whether they do it "here" or "elsewhere." The dichotomy in life-history characteristics between those mammals that are semelparous and those that are iteroparous is a reflection of integrating these two dimensions. Semelparity tends to be found in those animals in which adults face low or variable probabilities of survival (Roff 1992; Stearns 1992), or in which juveniles face higher survival in one season than another (Braithwaite and Lee 1979).

Boonstra and Boag (1992) proposed a model to account for differences in the hormonal and physiological responses between species with semelparous males and those with iteroparous males. Semelparous males employ the "adaptive stress response" and trade off survival for reproduction by maximizing the energy available for a brief period of intense reproduction. This strategy results in the failure of normal feedback mechanisms of the stress axis, causing the males to die from immunosuppression, gastric ulceration, and anti-inflammatory responses. Iteroparous males employ the "homeostasis stress response," in which reproductive effort was spread out over a longer breeding season or multiple breeding seasons. This strategy results in normal feedback mechanisms of the stress axis to remain intact throughout the breeding season. Recent evidence indicates a continuum in the suite of physiological and hormonal adaptations occurring between the extremes of semelparity and iteroparity, reflecting the continuum of life histories that mammals experience (Boonstra et al. 2001c; Woods and Hellgren 2003). The only truly semelparous species are found in marsupials of the dasyurid and the didelphid families (Bradley 2003). However, partial semelparity, in which many but not all of the males die after one mating period, occurs in many mammals, including rodents. We will examine the adaptations of the stress axis in rodents under scenarios of partial semelparity and of iteroparity and discuss some of the environmental constraints that select for these life-history traits.

Partial semelparity

The arctic ground squirrel (*Spermophilus parryii*) is found throughout the alpine and arctic areas of northern North

America. They are obligate hibernators, emerging above ground from a 7–8 month hibernation in early to mid-April (Buck and Barnes 1999). Females only have sufficient time for one litter during the brief northern summer. Virtually all yearlings are reproductively mature and thus almost the entire population breeds each year. During the synchronized 2–3 wk mating period, males compete intensely for access to females, roaming widely, sustaining severe injuries, eating less, and losing more weight than females (fig. 12.2). The detrimental impact of breeding on male survival is dramatic, being immediate in some males and delayed in others. In the boreal forest, 48% of the males disappear during the mating season (Boonstra et al. 2001c), whereas in the adjacent alpine area, 28% die (Gillis 2003). The mortality rate is age dependent, with older males (i.e., those who had gone through at least two breeding seasons—i.e., > 2 yrs) bearing it differentially. Older males expend more effort on reproduction (more severe wounding and greater loss of body weight) than yearling males (Gillis 2003). For those males that do survive the mating period, summer survival is then high. However, despite apparent recovery in summer, over half of the males die while hibernating the next winter (Gillis 2003; Hubbs and Boonstra 1997), suggesting that there are long-term consequences of the severe conflict of the previous spring. Again, older males tend to survive more poorly than yearling males at this time. The net result is that about 80% of breeding male arctic ground squirrels die each year (Gillis 2003).

Figure 12.2 Photographs of the same male arctic ground squirrel from an alpine site in the Ruby Mountain Range in SW Yukon in 2002. The first was taken in early April, just after the adult breeding male emerged from its hibernaculum and before the onset of the intense mating frenzy; snow still covered the alpine meadows. The second was taken three weeks later, when virtually all females had been mated and vegetation was being to appear. Note the radiocollar under its neck. He was now haggard and worn out from his mating activity and was found dead several days later. Photographs courtesy of T. J. Karels.

Breeding males in spring are chronically stressed during the mating period and have deficits in a number of areas, but not others (fig. 12.3). To assess the responsiveness of the stress axis, a brief explanation is needed to understand the hormonal-challenge protocol employed. Injections of hormones, or analogues of them that are part of the normal stress response, are used to measure an animal's stress response over a series of blood samples. Two steps were involved: the dexamethasone suppression test, followed by the adrenocorticotropic hormone (ACTH) stimulation test. Dexamethasone, a synthetic glucocorticoid agonist, should inhibit GC secretion through negative feedback mechanisms at the level of the brain by causing a reduction in ACTH release. The ACTH stimulation test is a method to directly probe the responsiveness of adrenals. Arctic ground squirrel breeding males have the highest concentrations of free cortisol as a result of the lowest CBG concentrations relative to abdominal adult males from August (Boonstra et al. 2001c). Dexamethasone resistance is modest, and thus negative feedback regulation remains largely intact (fig. 12.3). The ACTH challenge results in a rapid rise in free cortisol concentrations that exceeds those of nonbreeding males. Unlike the situation in most other species, in which both dexamethasone and ACTH inhibit testosterone secretion, the testosterone concentrations in breeding male arctic ground squirrels remain high (fig. 12.4). ACTH injections actually cause testosterone levels to increase, not decrease, reaching higher concentrations than basal levels. This pattern is unique in mammals. Stress-induced immunosuppression is pronounced, being reflected in those individuals with the poorest ability to respond to the foreign antigen challenge and the lowest number of white blood cells. Thus, the intensity of male-male competition during the mating season chronically stresses males and increases their mortality rate dramatically. However, unlike the total and immediate death of males found in the semelparous marsupials, the mortality is partial, age-dependent, and graded over time.

Iteroparity

Iteroparity occurs in most mammals, with males having multiple mating opportunities over their adult lives, either

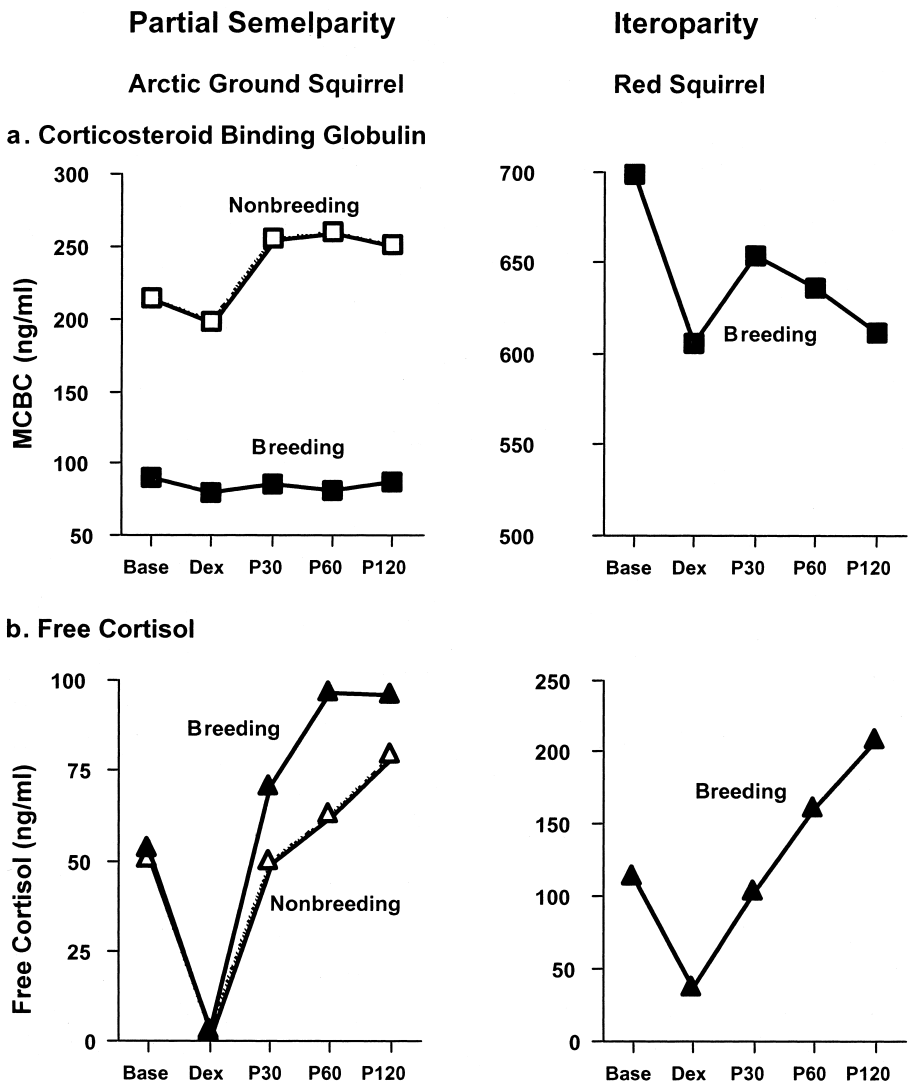

Figure 12.3 Life history variation in breeding frequency in male squirrels from the Yukon and the associated glucocorticoid plasma changes. The hormonal challenge protocol was used in both squirrels: Base levels indicate initial values, Dex indicates values 2 h after the dexamethasone injection, and P30, P60, and P120 indicate values 30, 60, and 120 min after the ACTH (adrenocorticotropic hormone) injection. In arctic ground squirrels, breeding (scrotal) males were trapped in May and nonbreeding (abdominal) males were adults trapped in August. In red squirrels, breeding males were trapped in May. Means (but not standard errors—see original publications) are presented. The data for arctic ground squirrels are from Boonstra et al. (2001) and for the red squirrel are from Boonstra and McColl (2000). Corticosteroid binding globulin measured as maximum corticosteroid binding capacity (MCBC).

during one breeding season for species that live < 1 yr (e.g., voles and mice), or over multiple breeding seasons for long-lived species (e.g., marmots). We will examine the stress axis in two wild rodents.

The meadow vole *(Microtus pennsylvanicus)* is a short-lived iteroparous small rodent found throughout grasslands of central and northern North America. Meadow voles have a promiscuous mating system, are short-lived (< 1 year), and breed continuously (ca. 3–5 litters per summer) throughout the summer and often into winter; young born early in the breeding season can mature and breed in that season, and males are not territorial (see references in Boonstra 1994). Free GC concentrations in breeding males are usually low, with CBG concentrations always exceeding total plasma corticosterone concentrations by about 3–4 times (Boonstra and Boag 1992). In breeding males, androgen concentrations are not correlated to CBG concentrations.

The red squirrel *(Tamiasciurus hudsonicus)* is an arboreal tree squirrel whose distributional range covers the en-

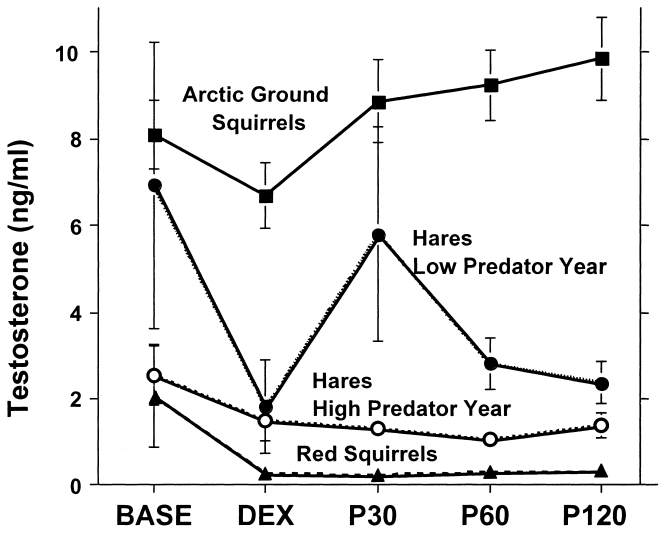

Figure 12.4 Changes in testosterone concentrations in response to the hormonal challenge protocol in adult breeding arctic ground squirrels and red squirrels (see fig.12.3 for description of hormonal challenge protocol). Changes in snowshoe hares, an iteroparous species, are included for comparison. In squirrels, males were trapped in May 1996. In hares, the males were captured in two different years: (a) when predation risk was low (1994) and the stress response was indicative of unstressed animals; and (b) when predation risk was high (1991) and the stress response was indicative of chronically stressed animals. Means are given ± SE.

tire boreal forest of North America. The red squirrel is highly territorial, asocial except during mating, has one or two litters per summer, has a sex ratio skewed toward males in the older age classes, and is long-lived (4–7 yrs; Obbard 1987). During the breeding season males have very high concentrations of cortisol and CBG (five and seven times, respectively, those of arctic ground squirrels (fig. 12.3, Boonstra and McColl 2000). Red squirrels are dexamethasone resistant, with cortisol concentrations declining only to 33% of those at baseline, whereas in nonresistant species, GC concentrations decline to ca. 5% or less. Certain rodent species are naturally dexamethasone resistant (e.g., guinea pigs [*Cavia aperea*], Keightley and Fuller 1996; and prairie voles [*M. ochrogaster*], Taymans et al. 1997), and this resistance is associated with elevated levels of GCs. Thus, red squirrels appear to be in this group. However, they do respond to ACTH with an increase in cortisol concentrations. As in most iteroparous species, the gonadal axis is very sensitive to the inhibitory effects of glucocorticoids. At the baseline levels, testosterone concentrations are negatively correlated to free cortisol concentrations (Boonstra unpublished data) and then decline markedly with the dexamethasone injection and remain low with the ACTH injection (fig. 12.4). There is no correlation between testosterone and CBG concentrations (Boonstra unpublished data). Immunologically, breeding male red squirrels have four times the numbers of white blood cells of arctic ground squirrels.

Summary

Iteroparous males do not exhibit the symptoms of chronic stress during the breeding season, whereas partially semelparous species exhibit some of them. Iteroparous species show the following: the gonadal axis is inhibited by high GC concentrations, resulting in declines in testosterone; high testosterone concentrations do not drive down CBG levels; dexamethasone resistance, though it may occur under chronic stress conditions, is not the rule under normal conditions; and immunosuppression does not occur as a normal condition. Thus the negative feedback system continues to function well. The negative feedback system also continues to function in the partially semelparous arctic ground squirrel, but the gonadal axis becomes insensitive to the inhibitory effects of high GCs and testosterone levels remain high. The consequences are that free GCs increase. Immunosuppression is particularly pronounced in the partially semelparous species.

There are four factors that may select for a partially semelparous life history, the first being an ultimate one, and the other three being proximate ones. First, high adult mortality, particularly during the nonbreeding season (Roff 1992; Stearns 1992), may be a key factor. In the short-lived arctic ground squirrel, only 17–50% of the males from high latitudes survive winter (Hubbs and Boonstra 1997; Gillis 2003). In contrast, in the long-lived Columbian ground squirrel (*S. columbianus*) from the midlatitude mountain areas, over 90% survive winter (Neuhaus and Pelletier 2001). Second, a high degree of seasonality occurs, with the length of time during a year when reproduction is favorable being only sufficient for one litter per year. In arctic ground squirrels, 60–75% of a female's active season is occupied with pregnancy and rearing her litter. Third, mating occurs at a time that is optimal for females, but not for males. As a result, insufficient food is available for males, to sustain them or to replenish energy expended on reproduction. High GC concentrations permit the replacement of external food resources with internal body reserves through the mobilization of energy by gluconeogenesis. In arctic ground squirrels, mating in the alpine occurs when snow still covers all or most of the ground, and thus males must rely either on body stores or underground caches (Gillis 2003). Fourth, the mating system is one in which intense, direct aggression occurs among males for access to females, and male territoriality either is not present or breaks down. These latter three factors may be necessary, but not sufficient, for a partially semelparous life history, as there are examples of species in which the males are long-lived and iteroparous in

spite of living under these constraints (Sherman and Morton 1984).

Socially-induced reproductive suppression

Reproductive suppression is common in many species of rodents (see reviews in Solomon and French 1997), being found both in those with social systems characterized by dominant-subordinate hierarchies (e.g., marmots and mole-rats) as well as those characterized by simple bonding relationships (e.g., microtines). In females, suppression could occur in any of the following ways: delay of puberty, suppression of estrus, suppression of ovulation, or failure of implantation (Faulkes et al. 1990). Direct aggression or chemical signals from conspecifics may be the causal mechanism resulting in suppression. Because of the inhibitory impact of the stress axis on the gonadal axis (Wingfield and Sapolsky 2003), the stress axis is often implicated as playing a deciding role. However, because of the reciprocal interactions between the two axes, simply showing that nonreproductive subordinates have higher GC concentrations than reproductive dominants is not sufficient to conclude that the latter inhibits the former. Testosterone acts to inhibit HPA function, and estrogen to enhance HPA function (Handa et al. 1994). Thus, in lab rodents, nonreproductive males have much higher concentrations of CBG and GCs than reproductive males, and pregnant and lactating females have higher CBG and GCs than nonreproductive females. Moreover, the response to stressors can vary with the stage of the estrous cycle (Viau and Meaney 1991) and of pregnancy (Neuman et al. 1998). In wild populations of rodents, the effects of reproduction may not have the same effect on the stress axis as in lab rats, but major differences among reproductive classes with respect to GCs are generally present (e.g., meadow voles: Boonstra and Boag 1992; degus [*Octodon degus*]: Kenagy et al. 1999; arctic ground squirrels: Boonstra et al. 2001b; Columbian ground squirrels: Hubbs et al. 2000; golden-mantled ground squirrels [*S. lateralis*]: Boswell et al. 1994; and yellow-pine chipmunks [*Tamias amoenus*]: Kenagy et al. 2000). Thus the critical requirement in studies on reproductive suppression is to compare animals of the same reproductive age under different social conditions and population densities. In addition to this caveat, a number of field studies also indicate that high levels of GCs do not invariably compromise the ability to reproduce (Creel 2001). In both territorial male alpine marmots (Arnold and Dittami 1997) and breeding male Arctic ground squirrels (Boonstra et al. 2001c) high concentrations of androgens occur, in spite of high GC concentrations.

Although surprisingly few field studies in rodents have teased out the impact of the stress axis on reproductive suppression, stress may play a crucial role. The naked mole-rat *(Heterocephalus glaber)* is a highly social species living in large underground colonies of up to almost 300 individuals, where only a single dominant female and 1 to 3 dominant males breed (see references in Faulkes et al. 1990, 1991; Faulkes and Bennett, chap. 36, this volume). Changes in reproductive hormones have been closely monitored. In nonbreeding females, lutenizing hormone (LH) from the pituitary is much lower than in breeding females, ovulation is suppressed, and progesterone levels are usually undetectable. This block is removed when these females are isolated from the colony and paired with a male (Faulkes et al. 1990). The relationship to the stress axis is not entirely clear. As expected, urinary GCs are lowest in breeding females and high in suppressed females (Faulkes and Abbott 1997), and these subordinate females are subjected to shoving matches by the dominant female. However, when the latter are removed from the colony, their GC levels remain high. These high levels may be a reflection of endocrine changes occurring as a function of endogenous reproductive changes (see previous citations on the effects of reproduction on GC levels). In males, although spermatozoa are present in both breeding and nonbreeding males, testosterone levels are much lower in nonbreeding males, and they are much less sensitive to injections of gonadotropin releasing hormone (GnRH), which is released from the hypothalamus and stimulates the pituitary to release LH (this hormone stimulates the Leydig cells of the testes to produce testosterone; Faulkes et al. 1990). Thus, both nonbreeding males and females have marked endocrine deficiencies. The link to the stress axis may be as follows: GCs have effects at the level of the brain, decreasing hypothalamic GnRH release and thus GnRH stimulated release of LH from the pituitary (reviewed in Sapolsky et al. 2000). In addition, GCs also have direct inhibitory effects on the gonads, reducing responsiveness to LH and reducing the concentrations of LH receptors.

The role of the stress axis in reproductive suppression has been most clearly worked out in alpine marmots (*Marmota marmota;* Arnold and Dittami 1997; Hackländer et al. 2003). This species is also a highly social, cooperative breeder, with one dominant pair and several subordinate offspring of up to 5 years old, not all of which are related. Reproductive suppression is complete in subordinate females, but not in subordinate males. In males, dominants predominantly attack unrelated subordinates that show higher GC concentrations and androgen suppression, but sons above a critical age and mass are left alone (Arnold and Dittami 1997). All adult females breed, but only the dominant female carries the pregnancy through to parturition. The breeding subordinates are subject to aggression by the dominant, resulting in significantly higher GC concentrations in the former and falling progesterone levels (fig. 12.5, Hack-

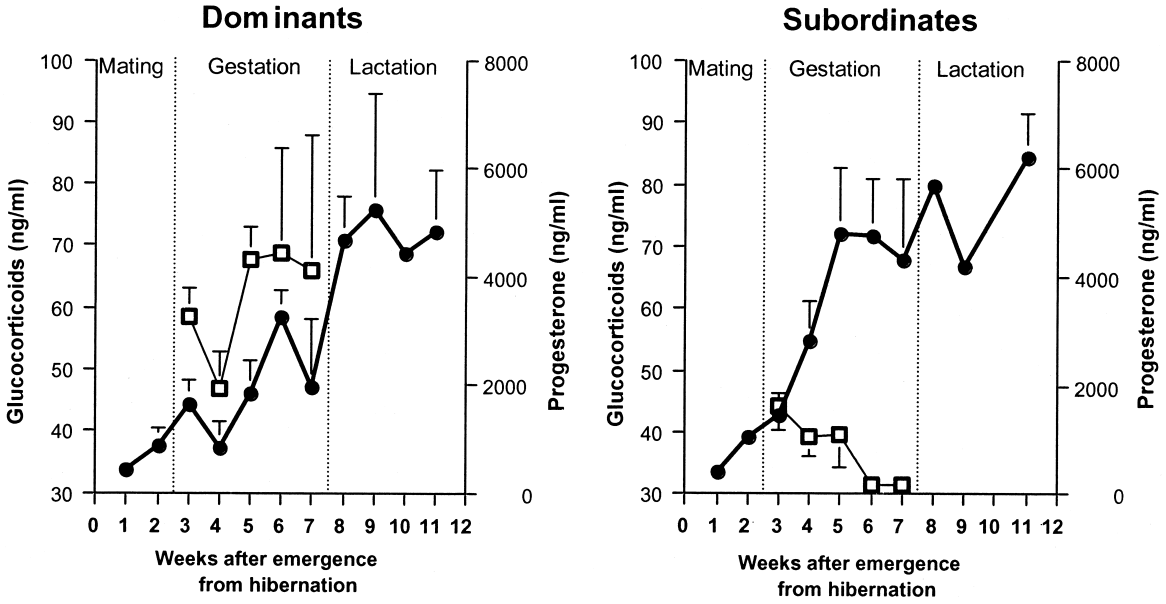

Figure 12.5 Changes in concentrations of glucocorticoids (●) and progesterone (□) in dominant and subordinate female alpine marmots during the reproductive period. Progesterone, which is needed to sustain pregnancy, was measured only during the gestation period. Parous, dominant females were those that subsequently gave birth; nonparous, subordinate females were inseminated, but pregnancy failed either before or immediately after implantation. Means are given ± SE. Adapted from Hackländer et al. (2003).

länder et al. 2003). Thus high GC concentration in the subordinates may inhibit GnRH release which results in lower progesterone levels, causing the pregnancy to terminate before or shortly after implantation (Hackländer et al. 2003).

We do not know exactly how suppression occurs in less social species, such as the voles, but recent experiments suggest that mothers do not suppress reproduction in their daughters (Wolff et al. 2001). Strangers, operating through increased social conflict at high density, may be one possible mechanism. Boonstra and Boag (1992) found that GC concentrations in the adult meadow voles were directly correlated to population density, although they did not continue the study long enough to relate this to reproductive suppression in the young. In the greater gerbil *(Rhombomys opimus)*, a more social small mammal, high density was also correlated with higher GC concentrations, probably related to increased social interactions (Rogovin et al. 2003).

Predation-induced reproductive suppression

Coping with predators is a key problem for virtually all organisms. Predators affect prey both directly (by killing them), thus potentially influencing population dynamics, and indirectly, by affecting prey behavior, foraging patterns, physiology, and reproduction, thus affecting fitness (Hik 1995; Boonstra et al. 1998; Lima 1998). Thus, predation is a major evolutionary force shaping the adaptations of the prey. In this section, we focus primarily on chronic stress that results from sustained high levels of predation risk. There are basically two responses prey can make to chronically high predation risk: either continue to perceive it and show a chronic activation of the stress axis, resulting in the host of suppressive effects, or ignore it, at least physiologically, and get on with reproduction and the other necessities of life.

The immediate effect of predator risk on the stress axis in rodents has only been examined in the laboratory because of logistical constraints. In rats, fox odor elicits an acute stress response, causing an increase in corticosterone concentrations (e.g., Tanapat et al. 2001). When rats are chronically stressed by visual exposure to a cat (a potential predator) for 20 days, they do not habituate, even though the cat never presses home an attack (Blanchard et al. 1998). These rats show all the evidence of being chronically stressed, including higher basal corticosterone concentrations, adrenal hypertrophy, and reduced thymus weights. Some of these rats also show an enhanced stress response when challenged with an acute stressor, possibly related to a failure of the feedback system. In a laboratory study on rodents from natural populations, Eilam et al. (1999) measured both the acute stress response and the behavioral response in voles *(Microtus socialis)* and spiny mice *(Acomys cahirinus)* to owl calls. Individuals of both species showed a stress response, with higher GC concentrations. However, only the voles showed a behavioral response. Thus lack of a behavioral response is not necessarily indicative that the prey is not stressed by the threat.

The best evidence for the suppressive impact of chronic

stress of predators on natural rodent populations comes from work on ground squirrels. They are known to be extremely sensitive to predation risk, modifying their behavior both in response to direct evidence of predator presence (visual, olfactory, auditory, and tactile stimuli coming from predators), and to indirect evidence, which corresponds to the increased likelihood of encountering predators (e.g., increased foraging distance from burrows or trees, or increased visual obstructions). The only field study (to our knowledge) to measure both acute and chronic stress responses under seminatural conditions is that of Hubbs et al. (2000). Reproducing female Columbian ground squirrels were exposed to a dog (the model predator) over 8 wks. Predator-challenged females had higher levels of total and free cortisol than controls, with evidence of a heightened stress response occurring only after about one month of exposure. Nonreproductive arctic ground squirrels living in the predator-rich boreal forest exhibit evidence of chronic stress relative to those in the adjacent predator-poor alpine area (Hik et al. 2001). These squirrels exhibit lower levels of basal-free cortisol levels, dexamethasone resistance in females (but not males), reduced ability to respond to an ACTH challenge, and lower corticosteroid-binding globulin levels. Furthermore, evidence suggests that chronic physical and psychological stressors inhibit reproduction in arctic ground squirrels. In the same boreal forest as the Hik et al. (2001) study, a long-term experimental manipulation was carried out in which mammalian predators were excluded from a 1 km^2 area. Litter sizes and weaning rates were generally higher within the predator exclosure (Karels et al. 2000). This evidence is consistent with the hypothesis that reproduction is suppressed under conditions of chronically high predation risk and with similar findings on snowshoe hares (*Lepus americanus;* Boonstra et al. 1998). Thus, reproduction is suppressed in some species in response to the chronic stress of high predation risk.

In contrast, some species may have evolved to not be stressed by their predators. Initially, microtines (voles and lemmings) were predicted to exhibit reproductive suppression under conditions of high predation risk, particularly of weasels. This was the basis of the predator-induced breeding suppression hypothesis, which postulated that it was adaptive to delay reproduction until such time as predator density declined (Ylönen and Ronkainen 1994). Though GC concentrations were not measured, evidence in favor of this hypothesis came largely from laboratory studies using weasel odor. This odor produced suppression of reproduction in pairs of voles and delayed maturation in young females (Ylönen and Ronkainen 1994). However, most field studies using mustelid odor have failed to corroborate these findings (Wolff and Davis-Born 1997; Mappes et al. 1998), and thus predator-induced breeding suppression appears to be an artifact of the laboratory (Wolff 2003c). In contrast, a recent field study by Fuelling and Halle (2004) reports evidence in favor of the breeding suppression hypothesis in northern Norway. We think that methodological problems (performed only for one month in mid-late August, and the possibility of a neophobic response of young born prior to the treatment avoiding traps during the treatment) call the conclusion into question. Theoretical modeling indicates that delayed reproduction is only optimal when the number of future offspring produced by not breeding exceeds that of breeding immediately (Kokko and Ranta 1996). For microtines, it may never pay to delay reproduction in the face of predation, given their short life spans and seasonal breeding.

Impact on Aging

Senescence is defined as an age-related increase in mortality rate that can be attributed to physiological deterioration (Rose 1991). Some argue that the rate of extrinsic mortality is so high in natural populations from competition, predation, parasites, and environmental stressors that animals never live long enough to experience an age-related physiological deterioration, and thus senescence would not evolve (Hayflick 2000). However, this flies in the face of theory and of most evidence from a wide variety of taxa. An age-related increase in mortality rate has been detected in many long-lived mammals (e.g., Packer et al. 1998 [*Papio anubis, Panthera leo*], Loison et al. 1999 [*Capreolus capreolus, Ovis canadensis, Rupicapra pyrenaica*]) and birds (McDonald et al. 1996 [*Aphelocoma coerulescens*]). Evidence also suggests that free-ranging animals experience age-related declines in reproduction (Packer et al. 1998, Coltman et al. 1999 [*Ovis aries*], Ericsson et al. 2001 [*Alces alces*]). Moreover, a few papers have integrated measures of survival and reproduction and have demonstrated that animals experience age-related declines in fitness (e.g., reproductive value: Newton and Rothery 1997; Møller and de Lope 1999; Ericsson et al. 2001).

In rodents, however, the evidence is mixed. Senescence was not found in five grassland species from Kansas (Slade 1995). In contrast, evidence for senescence was found in meadow voles (Boonstra and Mihok unpublished data). In the former case, uncertainty of age was a problem, whereas in the latter case, exact age was known. However, known-aged, female yellow-bellied marmots *(M. flaviventris)* apparently did not exhibit an increase in mortality rate with age but showed a total lack of reproduction in the last four years of life, suggesting reproductive senescence (Schwartz et al. 1998).

For natural populations, we do not know the nature of the physiological changes that cause an age-related increase in mortality rate, and thus we rely on evidence from labo-

ratory populations as to the possibilities. There are three periods where the stress axis is sensitive to permanent organizational changes likely to affect age-dependent mortality: the prenatal-postnatal period, the juvenile-adult period, and periods of chronic stress. First, stressors experienced during pregnancy and the postnatal period, and a reduced level of maternal care following birth, result in nongenetic, lifetime programming of stress axis, which increases susceptibility to disease (Meaney 2001). Such offspring are more fearful, respond more strongly to stressors, and recover from them more slowly (Meaney 2001; Matthews 2002). The inherent plasticity to permit this programming may be adaptive, because it allows the environmental factors experienced by the mother to program the offspring to perform optimally for conditions that it will likely face (Meaney 2001; Welberg and Seckl 2001). For example, if the mother is in an environment where predation is unusually high, it may be beneficial for her offspring to be programmed with an extremely active HPA axis so that they are hypervigilant to predators. However, programming hypervigilant offspring means that they are likely to experience the costs of a much higher GC exposure, which may affect survival. In laboratory rodents and humans, the high levels of stress hormones that result from programming are associated with stress-related diseases in later life. There is no direct evidence from natural populations of rodents indicating that pre- or postnatal programming affects the stress axis. However, indirect evidence suggests that maternal effects may operate this way. This evidence relies on the negative reciprocal interaction between the stress axis and the reproductive axis (Wingfield and Sapolsky 2003). In cycling microtines, declining populations often show extremely low rates of survival and/or reproduction (see review in Boonstra 1994). When meadow voles from declining and low populations are brought into the lab, females and their laboratory-born progeny continue to breed poorly compared with those collected from increasing populations (Mihok and Boonstra 1992; see Sinclair et al. 2003 for comparable evidence from snowshoe hares). These intrinsic effects may be the result of stressors experienced during the decline, resulting in programming of the HPA axis that then has negative effects on the gonadal axis.

Second, the hippocampus is crucial for declarative and spatial leaning and memory, playing an important role in age-related declines of cognition (Eichenbaum 1997). The hippocampus has high concentrations of GC receptors (fig. 12.1), which perceive circulating GC concentrations, setting both basal GC concentrations and terminating the stress-related release of GCs. However, both normal aging and excessive activation of the HPA axis result in hypersecretion of GCs, causing hippocampal damage (dendritic atrophy, loss of GC receptors, and synaptic loss; Pedersen et al. 2001). This damage impairs feedback inhibition of the HPA, leading to further damage caused by elevated GC concentration. Ultimately a positive, self-reinforcing cascade is set up, leading to progressively greater damage and a reduced ability to respond adaptively to stressors (the "glucocorticoid cascade hypothesis" Sapolsky et al. 1986). The studies of lab rodents have provided much of the evidence for our understanding of these changes in the stress response with age. Future studies should attempt to examine these changes in the natural world.

Third, long-term, chronic stressors also accelerate the rate of aging. In rats, chronic stress accelerates electrophysiological and morphological changes in the hippocampus, which have been correlated with altered HPA activity and impaired cognitive function (Pedersen et al. 2001). In addition, chronic stress in rodents causes neuron damage as well as a reduction in the GC receptor levels in the hippocampus, with the net result that negative feedback mechanisms are inhibited and the stress axis hyperactivated with age (Nichols et al. 2001). In nature, situations of long-term chronic stress are likely to be less common, though they may occur under times of high predation risk or high population density when there is competition for access to resources.

The preceding evidence all deals with changes in the stress axis that potentially occurs at the individual level to affect the rate of aging, but there is little evidence that different rodent species are programmed to age at different rates contingent on their life history. This is, however, a reasonable expectation, given that such evidence is becoming available or suggested in other groups, such as salmon and semelparous marsupial dasyurids (Finch 1990; Bradley 2003).

Impact on Neurogenesis

Conventional wisdom assumed that mammalian brains did not generate new neurons after early development (Gross 2000). However, we now know this is wrong, and that neurogenesis is a ubiquitous feature occurring in all mammals until death. Laboratory studies on rodents (primarily on mice and rats, but also on hamsters—Huang et al. 1998; eastern gray squirrels [*Sciurus carolinensis*]—Lavenex et al. 2000; and meadow voles—Galea and McEwen 1999) have played a key role in our understanding of the process, elucidating what factors increase or decrease levels of neurogenesis. However, we do not know the adaptive and functional significance of neurogenesis for animals in the natural world, nor do we know how stressors affect neurogenesis in nature (Boonstra et al. 2001a). Only three studies have examined neurogenesis in free-living rodents (Sivalingam

2002; Amrein et al. 2004; Barker et al. 2005). To put our understanding on a firm foundation, neurogenesis must ultimately be related to the ecology and evolutionary biology of species in the natural world.

In the hippocampus, neurogenesis appears to be related to spatial memory. Increased or decreased levels of neurogenesis are correlated with improved or impaired spatial memory, respectively (e.g., Lee et al. 1998). In one study involving spatial memory and neurogenesis in natural populations (Barker et al. 2005), eastern gray squirrels (a scatter-hoarding species) and yellow-pine chipmunks (a larder-hoarding species) were studied during the autumn, when memory of food storage locations would be critical. Squirrels had much higher rates of cell birth than chipmunks (as predicted), but not of early neuron survival. In the olfactory bulb, neurogenesis appears to be related to memory of odors (Carleton et al. 2003), particularly the discrimination of different odors.

Stressors at all life stages in laboratory rodents adversely affect the rate of neurogenesis. Prenatal and early postnatal stress reduces hippocampal neurogenesis in male rats (Schmitz et al. 2002), but this may be ameliorated later in life by an enriched environment and by an enhanced opportunity for learning (Ehninger and Kempermann 2003). Young rat pups show increased corticosterone concentrations and decreased rates of neurogenesis in response to the odors of adult male rats that are known to commit infanticide (Tanapat et al. 1998). In adult rats and mice, stressors produce high corticosterone concentrations that then decrease the rate of granule cell production in the hippocampus (Cameron and Gould 1994). Predator odor also inhibits neurogenesis in adult rats (Tanapat et al. 2001). Finally, stressors induced by the social situation can influence the rate of neurogenesis (Lu et al. 2003). Group housing of mice (the normal, unstressed situation in the lab) increases the number of newly generated neurons in the dentate gyrus, whereas rearing rats in isolation decreases neurogenesis, despite having no effect on endogenous GC concentrations. If the presence of conspecifics involves aggressive interactions, group housing can be stressful and decrease hippocampal neurogenesis (Czéh et al. 2002). Thus, rates of neurogenesis are heavily dependent on environmental conditions and appear to be readily altered, and, because of the potential implications on memory, may alter individual fitness in the natural world. We do not know whether stressors in nature will differentially affect rates of neurogenesis in wild rodents contingent on their life histories, though that is a reasonable expectation.

Summary

Coping with change is a key requirement for survival and reproduction, from both a short-term, ecological perspective and from a long-term, evolutionary perspective. The stress axis is a key control mechanism of the neuroendocrine system, which plays a central role in life-history adaptations that deal with change. At the individual level, the stress axis is integral to the regular changes associated with body function over the daily and seasonal cycles of life, and to dealing with stressors that threaten the homeostasis of the organism. However, at the individual level the axis shows a high degree of plasticity and sensitivity, and is subject to either long-term or permanent change as a result of either chronic stressors or of stressors occurring during development that permanently programs the axis at the level of the brain. At the species level, the functioning of the stress axis is not fixed, but is subject to evolutionary modification and habitat that sets the context for that modification. Differences in the functioning of the stress axis are seen most clearly in the area of reproduction (impact of mating in males and suppression of reproduction by either conspecifics or predators). We also expect, but have no evidence for at present, that differences among species in the rates of aging and in the need for neurogenesis will constrain how the stress axis responds to challenges.

Acknowledgments

The Natural Sciences and Engineering Research Council of Canada supported this research. We thank Jim Kenagy for helpful comments on an earlier draft of this

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