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Group Report: Key Sources of Variability in Coping

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INTRODUCTION

Animals respond to environmental challenge by activating a variety of coping systems (see Broom, this volume), which include aspects of the autonomic nervous system, the immune system, behavioral and emotional systems, as well as neuroendocrine systems such as the hypothalamic-pituitary-adrenal (HPA) axis. Indeed, coping systems are an essential part of biological function. There is an understandable tendency to focus on coping systems of laboratory animals and domestic animals, and while most of our discussion centers around these animals we wish to emphasize that the coping systems have evolved in nature, as responses to natural challenges, and their function has to be understood in that context.

There is a variation in coping patterns, both between and within individuals, as well as between species. Part of the variation seen might be due to differences in the early experiences of the individual; however, it is also possible that there are different stable coping strategies that coexist within a population, strategies that are largely innate. When presented with a series of stressors, it is possible that an individual responds by either altering his perception of the stressor or by altering the response to the stressor. Variations in circumstances surrounding the stressing event are also important, not least the presence or absence of social support. An important final aspect on the variation of stress responses is the cost of the different responses, costs that probably vary from one individual to another. This chapter discusses these different sources of variability and what can be learned from them. Both from a basic and an applied point of view, for example, an animal welfare perspective, it is important to understand not only the different coping systems per se, but also the sources of variability in coping.

Both of the two important stress axes, the HPA axis and the sympathetic-adrenal-medullary (SAM) axis, can be activated independently of each other, especially in chronic stress. Discussion in this chapter, however, focuses on the HPA axis, in part because

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of the depth of information about this system currently available but also because it appears to function to promote "self-remodeling" within the other peripheral and central coping systems. By this we mean that glucocorticoids are secreted into the peripheral circulation and, being lipophilic, generally pass easily into cells and the brain (cf. section on WHAT ARE THE CONSEQUENCES AND DYNAMICS OF THE ORGANIZATION OF THE HPA AXIS?). Thus, in addition to their role in regulating the availability of energy sources, glucocorticoids can coordinate the actions and subsequent sensitivity of disparate systems through the induction of plasticity resulting from their regulation of gene expression. Plasticity occurs throughout life and is the basis of development and adaptation. However, the degree of plasticity and permanence of stimulus-induced plasticity varies with a species-typical age dependence. In general, this plasticity is more permanent when it occurs in the perinatal period, becoming less permanent after the age of weaning in mammals, and essentially becoming state- or context-dependent past the pubertal stage. With regard to coping, it is suggested that stimulus-induced activation of the HPA axis leads to an increase in circulating glucocorticoids, which in turn functions to influence the selection of one of a subset of coping strategies. The important general principles here are that the particular outcome of glucocorticoid exposure is highly dependent upon the stage of central nervous system (CNS) development. Glucocorticoid targets shift with the increasing maturity of the CNS and via alterations in gene expression profiles and provide the underlying mechanism for this plasticity in neurocircuit structure and function. Of course, it is also important to acknowledge that the individual's genotype sets the limits of possible plasticity and coping strategies.

The specific questions considered in this chapter are:

- How do early experiences influence brain development and coping abilities of individuals?
- What are the consequences and dynamics of the organization the HPA axis?
- Is it possible to interpret part of the variation seen as the animal reacting with either a perceptive and evaluative system or a response system?
- How well defined are the coping responses or strategies?
- What is social support and what are its consequences?
- What are the costs associated with different coping systems?

HOW DO EARLY EXPERIENCES INFLUENCE BRAIN DEVELOPMENT AND COPING ABILITIES OF INDIVIDUALS?

Organisms are constantly challenged by internal and external stressors. The maintenance of the internal milieu within a normal physiological range (i.e., homeostasis) is very important for the normal pre- and postnatal development. Both pre- and postnatal manipulations have profound and persistent effects on the development of the neural organization, neuroendocrine processes, and behavior, and will consequently affect the coping abilities of the individuals (Ward 1972; Herrenkohl 1979; Sachser and Kaiser 1996; Koehl et al. 1999). Thus, early experiences are one key source of individual variability in coping.

Although evidence on the prenatal influence of substance abuse is extensive, for the purpose of this discussion we focus on the effects of pre- and postnatal stress, in particular postnatal disruptions of mother–infant relationships. Effects of both pre- and postnatal stress

have been documented in rodents, primates, and humans, but very little has been done in other species, including farm animals.

The effects of pre- and postnatal stress can be very different. The description of early experiences has therefore been divided into a section on prenatal and one on postnatal stress.

Prenatal Stress

In mammals, the internal environment of the pregnant animal constitutes the embryo's external environment; it is therefore reasonable to suspect that the former can affect the fetal development. The term prenatal stress is taken here to mean both any stress imposed directly on the embryo and any stress imposed on the pregnant female.

Prenatal stress can have long-term effects not only on behavior but also on reproductive functions, immune as well as endocrine system, and on brain development (Herrenkohl 1979; Klein and Rager 1995; Koehl et al. 1999; Anderson et al. 1985; Kay et al. 1998). Increasing evidence indicates that the degree of activation of the HPA axis and the duration of the response can be altered permanently by prenatal stress (cf. Weinstock 1997). Changes in the reactivity of the HPA axis may be associated with an increase in corticotrophin-releasing hormone (CRH) in the amygdala and a decrease in the number of glucocorticoid receptors in the hippocampus. This indicates long-term alterations in the control of glucocorticoids on neuronal functioning. In view of the role of these limbic brain structures in emotional behavior and in learning and memory processes, this implies a long-term change in coping processes as well.

In nonhuman primates, exposure to prenatal stressors is associated with long-term changes in their behavioral regulation, endocrine function, and central neurocircuits. Placing pregnant females in a stressful situation was associated with an increase in miscarriage rates (Sackett 1981) and an increased dermatoglyphic asymmetry, which is indicative of altered fetal development and is associated with increased perinatal mortality (Newell-Morris et al. 1989). Studies by Schneider (1992a, b, c) support the existence of periods of increased vulnerability during early gestation (E45–90) versus mid-to-late gestation (E90–145) with respect to the occurrence of attentional deficits and neuromotor maturation. In addition, both HPA axis function (Clarke et al. 1994) and cerebrospinal fluid concentrations of biogenic amines (Schneider et al. 1998) were affected in these animals. It is interesting to note that the changes in the HPA axis observed after prenatal stress are similar to those seen in humans with endogenous depression.

Prenatal stress in rats also induces enhanced activation of the sympathetic nervous system in response to foot shock stress (Weinstock et al. 1998). It has also been reported that the secretion of different neurotransmitters in specific brain areas (e.g., acetylcholine, dopamine and noradrenaline) is influenced by perinatal exposure (e.g., Moyer et al. 1978).

Ward (1972) showed that prenatal stress (immobilization combined with bright light) had a distinct effect on the behavioral development of males. She showed that prenatal stress in rats leads to a demasculinization and feminization of the behavior of the male offspring. After Ward's fundamental work, numerous studies were conducted which describe different effects of prenatal stress on various aspects of male behavior (e.g., Harvey and Chevins 1985; Kinsley and Svare 1986). In contrast, only a few studies show that prenatal stress also affects the behavior of the female offspring. In one such study, female guinea pigs lived during

pregnancy in an unstable social environment, in which the group composition was changed every third day. This had distinct effects on the behavior of the female offspring. Prenatally stressed female guinea pigs showed a behavioral masculinization: they displayed male-typical courtship and play behavior more often than control females (Sachser and Kaiser 1996). Alonso et al. (1991) describe behavioral suppression in stressful environments during adulthood of prenatally stressed female rats. Such animals were less able to cope in aversive situations. Clinical observations on humans suggest that exposure to stress during pregnancy results in changes in the behavior of the children. In particular, hyperactivity, hyperanxiety, and disturbed social behavior are described (Weinstock 1997).

In summary, the animal's prenatal stress experiences induces permanent changes in a variety of physiological systems (neuroendocrine, neurochemical) involved in the coping response indicating a permanent alteration in the later ability to cope with stressors.

There is controversy in the literature as to whether different stressors acting on the same species also have the same outcome or whether the effects are specific for the stressor. As mentioned above, in rats, immobilization combined with bright light leads to a feminization and demasculinization of the behavior of the male offspring (Ward 1972). Using overcrowding as a prenatal stressor, a feminization was also found. However, no demasculinization occurred (Dahlöf et al. 1977). Quite often these different outcomes can be explained by the animals having been stressed at different times. It is important—when comparing the effects of different stressors in the same species—to expose the animals to these stressors at exactly the same time, which results in the animals being at the same developmental stage. On the other hand when comparing the effects of the same stressor in different species, it is necessary to know the different developmental processes of the species. For example, the sexual differentiation of rats starts shortly before birth and continues until ten days after birth (most of it having occurred by three days postpartum), whereas in guinea pigs it occurs around day 36–45 of pregnancy (total duration about 68 days; MacLusky and Naftolin 1981). One method translating neurodevelopmental time across mammalian species involves determining peak times of neurogenesis in developing neuronal systems, as described by Finlay and Darlington (1995) and Clancy and colleagues (2001). Thus, using the same stressor to the same time (e.g., shortly before birth) will lead to a different outcome regarding the behavior, endocrine system, and brain development of the offspring. In conclusion there are many variables that have to be taken into account in the investigation of the effects of perinatal stressors.

Postnatal Stress

The effects of early postnatal manipulations, such as maternal separation, are extensive and range from altering the responsiveness of the adult rodent HPA axis to changing behavioral responses related to fear and anxiety. The effects of maternal separation, however, are dependent upon the number of variables such as the age at which the separation occurs, the duration of the separation period, and the number of repeated separations. The effects of a single 24-hour separation during the initial neonatal week, or shorter repeated maternal separations beginning early in the first neonatal week and continuing through the second neonatal week, have behavioral and HPA axis effects that are opposite to those in response to handling (which also entails a brief maternal separation). The functional significance of early manipulations

are extensive and permanent and range from altering the responsiveness of the adult rodent HPA axis to changing behavioral responses related to fear and anxiety. Future research should focus on determining more precisely the extent and nature of the changes occurring in the developing brain that are responsible for the functional changes which have been observed as well as on the particular aspects of maternal behavior and its encoding by the neonate that mediate these changes.

The continuous interplay of genetic and environmental factors "programs" the developing neurocircuitry. These processes influence how an organism perceives and responds to stimuli in its environment, yielding individual differences. Among environmental factors, adverse early experience including abuse, neglect, and severe childhood illness, is a major risk factor during development and may influence the vulnerability to a variety of physio- and psychopathologies later in life. A Long Evans rat model of neonatal maternal separation (180 min daily, during postnatal days 2–14) has been used to explore the consequences of adverse early experience on individual responsiveness (Caldji et al. 2001; Ladd et al. 2000). These animals were compared to animals reared under normal animal facility conditions or animals exposed to brief handling and 15 minutes of maternal separation. As adults, animals in each of these rearing groups displayed a distinct and stable phenotype. The maternally separated rats exhibited enhanced anxiety-like behavior, moderate anhedonia, alcohol preference, resistance to glucocorticoid-mediated negative feedback, and HPA axis hyperresponsiveness to psychological stressors. Underlying these features were regionally specific changes at the level of gene expression, neurochemistry, electrophysiology, and morphology.

Overall, neonatal maternal separation at an early age resulted in rats exhibiting a depression-like syndrome with morbid anxiety that could be reversed by neonatal cross fostering or by chronic antidepressant treatment as adults. Perhaps as important, these animals also exhibited reduced arterial contractility, an increased rate of diabetes with aging, and markers of compromised immune function.

In nonhuman primates, experimental disruption of the maternal–infant dyad by various means, including maternal separation, introduction of social instability to the colony, or presentation of food in an unpredictable manner, has been used as a probe of the effects of postnatal stress. The effects studied include the development of the CNS, endocrine function, and behavioral regulation. Although few studies have been reported on the heritability of individual differences in stress responsiveness in monkeys, a recent report by Lyons et al. (1999) addressed this issue in squirrel monkeys (*Saimiri sciureus*). Combining paternal half-sibling analysis with several postnatal rearing protocols that altered maternal availability, they reported significant postnatal rearing effects with the lowest heritabilities for peak elevations of cortisol 24 hours following removal of the mother in otherwise undisturbed groups. In contrast, individual differences in cortisol 3–7 days later revealed the highest heritabilities with negligible postnatal rearing effects. These studies illustrate the interaction between extrinsic and intrinsic factors in determining individual stress responsiveness.

In the bonnet macaque (*Macaca radiata*), imposition of a variable foraging demand protocol in which the manner of food presentation is altered between periods requiring strenuous foraging with periods of free access to food, has profound consequences on the offspring as a result of altered social and maternal behavior of the mother (Rosenblum and Andrews 1994). Normal physical development of the offspring is observed, but progeny are more fearful and responsive to stressful stimuli and less socially competent. Interestingly, the offspring exhibit

persistent alterations in cerebrospinal fluid levels of CRH, somatostatin, and biogenic amines even 4–6 years after this experience (Coplan et al. 1996, 1998). They also exhibit a reduced growth hormone response to the alpha-adrenergic agonist clonidine (Coplan et al. 2000). Elevations in cerebrospinal fluid corticotropin-releasing factor (CRF) levels and a blunted growth hormone response to clonidine challenge are frequently observed in patients with mood and anxiety disorders (Heim et al. 1997).

Rearing rhesus (*Macaca mulatta*) infants under conditions in which animals have auditory, visual, and olfactory but not tactile contact has profound long-term consequences on the animals (e.g., the occurrence of autistic-like behaviors, the inability to develop positive social relationships within the social group, hyperaggressiveness, disoriented sexual behavior, and lack of recognition of social cues). However, if animals are exposed to a social environment at any time prior to 6 months of age, the species-typical repertoire of social and nonsocial behaviors develop, even in the infants reared in total isolation (Harlow et al. 1971).

Adverse early experience such as maternal separation also has long-lasting effects on ingestive behaviors (e.g., polydipsia and polyphagia), immune system function (Lubach et al. 1995), the sympathetic response to stressful stimuli (Martin et al. 1988), central catecholamine levels or innervation patterns (Ginsberg et al. 1993; Higley et al. 1992; Lewis et al. 1990), and cognitive development (Beauchamp et al. 1991; Gluck and Sackett 1976; Sanchez et al. 1998). These cognitive impairments include a higher error rate during the learning phase, which indicates a potential attentional or motivational deficit and strong perseveration, suggesting a failure of systems involved in the cessation and inhibition of ongoing behavior. The neurochemical and neuroanatomical changes underlying such impairments remain unclear.

Structural magnetic resonance imaging (MRI) has been used to evaluate gross neuroanatomical changes in 18-month-old rhesus monkeys separated from the mother at two months of age and nursery reared under conditions of partial isolation (Sanchez et al. 1998). Partial isolation rearing was not associated with differences in the total brain, cerebellar or hippocampal volumes, or in the size of the anterior commissure. MRI scans did reveal a reduction in the midsagittal size of the corpus callosum, in parallel with a decrease in white, but not gray, matter content in the parietal and prefrontal cortices, suggesting the presence of specific reductions in cross-hemispheric projections, possibly resulting from a reduction in the number of callosal axons or in their myelination and growth. Interestingly, these alterations occurred in parallel with cognitive impairments and were not reversed by introduction of these animals into a peer group for six months.

Gender-specific effects of neonatal stress exposure in rodents have been difficult to document as a result of the numerous variations in stressor protocols with respect to age of initiation, duration and number of repetitions of the event, age at testing, and type of testing as well as the tendency for these types of studies to be performed primarily in male progeny. Nonetheless, there are reports of gender-specific effects on neuroendocrine, behavioral, and CNS function in the rodent. Matthews et al. (1996) found that females, but not males, which had experienced repeated neonatal maternal separation, exhibited an attenuation of the acquisition of a conditioned anticipatory locomotor response to the presentation of food. All separated animals displayed enhanced weight gain, a blunted locomotor response to a novel environment, and a blunting of the response to both negative and positive contrast effects. When tested on an elevated plus maze, female Sprague-Dawley rats exposed to neonatal 3

hours of daily maternal separation from postnatal days 1–21 exhibited less anxiety than nonhandled, gender-matched controls (McIntosh et al. 1999). In addition, only the feeding suppressant response to the satiety peptide cholecystokinin (CCK), but not bombesin or amylin, was affected by the early life experience with it being more pronounced in handled and maternally separated males. Wigger and Neumann (1999) found a twofold greater index of anxiety in adult male rats than in females that had experienced three hours of daily neonatal maternal separation from postnatal days 3–10. Neuroendocrine parameters were also altered in these maternally separated rats in a gender-dependent manner. Neither basal adrenocorticotropic hormone (ACTH) or corticosterone levels differed among progeny from the different rearing groups; however, the ACTH response to an emotional stressor was higher in male, but not female, rats of the separation group versus their respective controls. Overall, these studies support the thesis of gender-specific effects of adverse early experience during the neonatal period via mechanisms not yet elucidated.

Possible Adaptiveness of the Perinatal Stress Response

An exciting possibility is that some of the stress responses of the infants might be due to the infants allocating resources based on information gathered from the mother, just as food preferences may be influenced by what the mother eats perinatally (Bilko et al. 1994). A period of starvation during pregnancy might, for example, tell the infant that food is probably going to be scarce in the future. A prerequisite for this adaptive explanation, however, is that the stress response of the infant is specific to what the stressor might predict in the future. If the infant reacts to all stressors in the same way, it does not seem probable that the responses given by the infants are adaptive.

A consideration of the ways in which experimental manipulations can result in a shift in the trajectory of fetal and infant development raises the obvious question of whether these observations are relevant to animals under natural conditions. Here it should be noted that pregnant dams in nature are often exposed to more extreme periods of privation than would ever be employed by researchers. Such challenges may include periods of gestation during extreme climatic conditions (cold or hot), as well as times of limited food availability. In this regard, it is of interest that many seasonally breeding species orient the timing of the birth period towards the optimal time of the year. Often it has been assumed that this temporal orientation has been adapted for the postnatal needs of the infant, but it is equally important to organize the timing around the needs of the pregnant female. For example, as the pregnant female squirrel monkey approaches term, she may drink up to half her body weight of water each day. Thus, the birth of her infant in the rainy season in the Amazon is probably not coincidental.

An example of what might be indicative of the types of patterns we should expect to see if the stress reaction of the offspring were adaptive in the long term can be seen in humans. Population level surveys in several countries have indicated that periods of growth retardation during fetal development, as reflected by birth weight, may be significant predictors for several adult diseases, including cardiovascular illness and diabetes, which are of course not adaptive (Phillips et al. 2000; Godfrey and Barker 2000). Interestingly enough, however, one of the consequences of starvation during pregnancy is obesity in the infant, which is what one would expect from a functional point of view. If food is scarce during the pregnancy of the

mother, the risk is that it will be scarce in the future as well. It therefore makes sense for the infant to attempt to build up a fat reserve against further food shortages. The proposed mechanism for these persistent effects into adulthood is an alteration in set points for various aspects of basic metabolism (e.g., glucoregulation, adiposity, blood pressure).

In conclusion, there is little evidence that the majority of the consequences shown, which have been caused by perinatal stress, are adaptive in the long term. It should be noted however, that most of the studies cited here were not done to test this hypothesis explicitly, and that further studies might very well yield different results.

Implications and Conclusions

Overall, the knowledge of existing mechanisms of plasticity and its species-typical age dependence suggests avenues for development of new strategies for management of environmental, including pathological, challenges to promote good welfare of domesticated species, laboratory animals, and humans. Some implications and questions raised by current animal research on glucocorticoid-induced plasticity and the central importance of mother–infant interactions on human and animal welfare include:

1. What genes are involved in conferring susceptibility or resistance to the effects of perinatal experience?
2. How can we translate this knowledge of perinatal plasticity into beneficial practice in animal husbandry as well as in human clinical care? For instance, what does this imply about long-term treatment consequences of current practice in the care of premature infants and neonates, or cow/calf separations in dairy cattle? What might the consequences be of the general failure to acknowledge and prevent the experience of pain in premature infants?
3. When interventions are available, do they promote actual reversal of the stimulus-induced plastic changes in brain circuitry structure or function, or are they associated with a new structure or function on top of the existing adaptation? What might the consequences be of such a "patch" in terms of constraining the range available in coping systems?

WHAT ARE THE CONSEQUENCES AND DYNAMICS OF THE ORGANIZATION OF THE HPA AXIS?

Much research effort is aimed at the HPA axis as the main mediator of the stress-induced changes in behavioral, neuroendocrine, neurochemical, and immunological systems involved in coping. The brain is responsible for the regulation of the HPA axis. Activation of specific neural pathways are required for the neuroendocrine cascade that results in changes in the paraventricular nucleus (PVN), which controls the output of CRH, the major secretagogue for ACTH, and subsequently the secretion and synthesis of glucocorticoids. The neural pathways that are activated and that subsequently activate CRH are, in many cases, stimulus dependent. However, most stimuli that activate the neural pathways result in the activation of multiple neural systems. The brain is a target organ for the glucocorticoids. Although there is controversy concerning which specific glucocorticoid, cortisol, or

corticosterone freely enters the brain, the glucocorticoids act on specific structures that result in changes both at the level of the CNS and at the periphery. Three major sites in the CNS appear particularly sensitive to an increased level of the adrenal steroids: the hypothalamus, hippocampus, and amygdala. These areas are rich in steroid receptors. Glucocorticoids act by binding to mineralocorticoid receptors (MR, or "type 1" receptors) or glucocorticoid receptors (GR or "type 2" receptors), which are primarily located in the cytoplasm, evoking activation, dimerization, translocation to the nucleus, and binding to specific sites on the chromatin. The functional action of GR complexes depends upon interaction with concurrently activated intracellular signaling cascades thus imparting a degree of regional specificity of action. It is important to be aware that the distribution patterns of these adrenal steroid receptors may vary among species. In the hypothalamus these steroids serve to reduce the production of CRH effectively, and thus the production of ACTH and glucocorticoids are turned off and return to basal levels. The hippocampus contains a large population of both GR and MR. MR appear to be involved in maintaining basal levels and regulating circadian rhythms. In contrast GR, which are primarily occupied during stress when high levels of circulating glucocorticoids are present, seem to regulate the stress levels of these hormones. In the amygdala, glucocorticoids appear to increase CRH gene expression. CRH in the amygdala has been implicated in the regulation of behavioral expression of emotion and anxiety.

There is a circadian rhythm in the level of cortisol, with a peak at the beginning of the active period of the animal. Cortisol concentrations during the trough of the rhythm are maintained at very low levels because of feedback action of cortisol through MR (high affinity). This trough cortisol level is sufficiently low so that there is not appreciable occupancy of the lower affinity GR at circadian peak. Cortisol feedback regulates CRF secretion through occupancy of both MR and GR.

Chronic stress will elevate through cortisol if mild (by overriding MR-mediated feedback). As the intensity of the stress is increased, the level of trough cortisol will progressively increase until the rhythm flattens finally out at high level. It is the increased GR occupancy (duration and magnitude) that does the damage.

There is a difference between species in the proportions of different steroid hormones produced. For instance, guinea pigs and primates, including humans, have cortisol as their predominant glucocorticoid while rats and most mice have corticosterone. Dogs, on the other hand, produce an approximate 50/50 mixture of cortisol and corticosterone. Among the common farm species, poultry have corticosterone, whereas cattle have cortisol, and finally pigs produce some corticosterone and much cortisol. The functional significance of these species-typical patterns is unknown.

These glucocorticoids act by binding to MR or GR which are primarily located in the cytoplasm, evoking activation, dimerization, translocation to the nucleus, and binding to specific sites on the chromatin. The functional action of these GR complexes depends upon interaction with concurrently activated intracellular signaling cascades, thus imparting a degree of regional specificity of action. It is important to be aware that the distribution patterns of these adrenal steroid receptors may vary among species.

It has long been recognized that the ability of specific glucocorticoids to reach the brain differs; for instance, penetration into the brain of the synthetic glucocorticoid dexamethasone appears to be restricted relative to that of the endogenous rat glucocorticoid, corticosterone (De Kloet et al. 1975). One mechanism regulating glucocorticoid access to the brain appears

to be the multidrug-resistant gene product P-glycoprotein (Van Kalken et al. 1993) which is expressed by endothelial cells at the blood-brain-barrier (Cordon-Cardo et al. 1989). The multidrug-resistant P-glycoprotein restricts access of dexamethasone and cortisol to the brain but not access to the pituitary or to peripheral tissues. Genetically engineered mice lacking this protein exhibit penetration of both dexamethasone and corticosterone into the brain (Meijer et al. 1998). This has important implications as it means that treating rodents and primates (including humans) with dexamethasone will saturate peripheral glucocorticoid receptors, shutting down the pituitary-adrenal axis via glucocorticoid-mediated negative feedback, while at the same time possibly creating an apparent glucocorticoid deficit in the CNS akin to an adrenalectomized state. Perhaps the most interesting and controversial findings about the multidrug-resistant P-glycoprotein is that it also appears to restrict access of the primary nonhuman primate and human adrenal glucocorticoid, cortisol, to the brain (Karssen et al. 2000; Pariante et al. 2000). The physiological significance of this finding is unknown but would imply that cortisol may not be the major steroid hormone mediating CNS actions of glucocorticoids in primates, guinea pigs, and other animals in which cortisol is the primary adrenal glucocorticoid product. However, it must be understood that the multidrug-resistant pump does not entirely exclude these substances from the brain but merely impedes their entry. Thus, it is possible for dexamethasone to enter the brain, although its entry is restricted relative to that of corticosterone. Similarly, cortisol does enter the brain and, considering the concentration difference between human adrenal cortisol production versus the minor adrenal steroids, it may still function as the primary glucocorticoid. Further studies are required to assess the importance of minor glucocorticoid products of the adrenal (see Kage et al. 1982) as well as the intricacies of their regulation. Overall, this suggests that it may be important to measure these "minor" steroids in those species primarily producing cortisol if one wishes to use the level of glucocorticoids as a parameter in assessing stress or poor welfare. This is clearly an important area for both basic and clinical research.

IS VARIATION CAUSED BY THE ANIMAL RESPONDING WITH EITHER A PERCEPTIVE OR A RESPONSE SYSTEM?

It is possible to conceptualize variability in coping responses as either being due to differences in the way in which an organism appraises and evaluates the situation or due to differences in the responses that it is able to show, or both (see Mendl, this volume, Figure 14.1). For example, differences between proactive and reactive copers (Koolhaas et al., this volume) may be the result of differences in the range of response patterns that are available to the individuals or due to differences in the way in which they assess and evaluate a challenging situation. This idea of splitting coping responses into (a) appraisal and evaluation and (b) response components is worth exploring more generally, because it has potential relevance for how we interpret data on coping responses in terms of welfare.

In general, when attempting to assess stress or welfare, we measure the response or "readout" component. There are a number of examples in which a lowering of, or decreased reactivity of, a response to a challenge is observed. For instance, one of the actions of oxytocin appears to be to reduce the reactivity of the HPA system to challenges (Carter, this volume).

Several domesticated species appear to show lower HPA and SAM responsiveness to challenges in comparison with their ancestral species. For example, when compared with its wild ancestor, the cavy (*Cavia aperea*), the domestic guinea pig (*Cavia aperea* f. *porcellus*), shows lower reactivities of the HPA and SAM axis (Künzl and Sachser 1999).

When faced with repeated exposure to a predator stimulus (e.g., a model bird of prey flying overhead), a prey animal in a cage may show a lessening of its escape response. What we observe in this example is a change in the activity of the response system. Our interpretation of this change may be that the organism has adjusted or adapted to the challenge or is now less disturbed by the challenge. We could conclude from this that its welfare has been only temporarily poorer. However, this conclusion would probably be strengthened if we found that the change was primarily brought about by an alteration in the appraisal and evaluation system, i.e., the organism no longer evaluates the challenge as a threat. For example, a human may no longer be psychologically disturbed by the experience of flying after becoming used to it and therefore behaves calmly when entering the plane. On the other hand, if the change is primarily in the response system, then our conclusions would be very different. A human may still be terrified of flying but has learned that resisting entry to the plane is a useless strategy and thus behaves calmly when entering the plane. The habituation of the escape behavior of the prey animal in the cage may likewise be explained in these two different ways.

Similarly, the effect of oxytocin on the reactivity of the HPA system may be primarily through local actions in the PVN on CRH synthesis and release, and hence by altering the activity of the response and readout system, or the "final common path" for the HPA system. Alternatively, it may involve some changes to sensory systems or reward systems which could be thought of as part of the appraisal and evaluation component. We might interpret the latter effects as more likely to result in decreased danger or stress being perceived, and thus as having a more beneficial effect on welfare.

It would be useful to develop techniques that could tease apart effects on appraisal and evaluation and on response and readout components. At the neurobiological level, this may be very difficult to do because of the inherent self-adjusting nature of the many neural systems and processes that coordinate the expression of the final (behavioral and neuroendocrine) responses to challenge.

Behavioral techniques, however, may help. For example, an organism faced with repeated exposure to challenge A shows a habituation of its HPA response. If it is then exposed to challenge B, a strong HPA response would indicate that the adjustment to challenge A was occurring at the level of some appraisal system rather than because of a general decrease in activity of the (HPA) response component. However, a continued weak HPA response to challenge B would indicate the opposite.

Another approach would be to use a technique such as aversion learning. For example, if an animal is exposed to a cue predicting an aversive stimulus, it will learn this association and may show an HPA response when the cue is presented. If oxytocin is administered during training, we might predict that the animal shows a smaller HPA response. The animal can then be tested with the cue only in the absence of oxytocin. If its HPA response remains small, this would indicate that the oxytocin minimized the perceived unpleasantness of the stimulus during training and so when the conditioned cue is presented, this is perceived as relatively harmless. However, if the HPA response is increased in this test, this would indicate that the main role of the oxytocin during training was to act directly on the HPA response without affecting

perception or appraisal. Once the oxytocin is removed, the perceived danger can then be fully expressed by a large HPA response.

While some of the techniques described above have already been used, there are still very few studies dealing with this specific question.

HOW WELL DEFINED ARE COPING STRATEGIES?

Many coping responses come in packages, which we term as coping strategies, with several responses acting in concert. In some cases this might be explained by the evolutionary inertia of animal's physiology, while in other cases an adaptive explanation seems more likely.

The studies on coping strategies in mice originated in the early 1970s from an intensive collaboration between behavioral geneticists and behavioral ecologists in studies on the role of genetic variation for aggressive behavior in the population dynamics of wild house mice (Koolhaas et al., this volume). Evidence was obtained that the cyclic nature of feral populations of house mice was due to a disruptive selection for high levels of aggressive behavior. The genetic nature of this was confirmed by the successful development of genetic selection lines for high and low levels of aggressive behavior. Subsequently, these lines were studied by behavioral physiologists. A whole range of basically correlative studies resulted in a further characterization of these selection lines by a set of behavioral, neuroendocrine, and neurobiological parameters. Recent behavioral studies suggest that the differences can be reduced to a differential degree of feedforward versus feedback control of behavior. This led to the idea that the two lines in fact represent two different ways of coping with environmental challenges. Over the course of the years, similar individual differences were found in a variety of animal species. Some studies considered the stability of these characteristics over time, while others focused on the consistency across situations. In general, however, apart from rats, mice, and pigs, these aspects need further experimentation in many species. Ideally, the issue of trait characteristics needs a multi-factorial statistical approach to determine the number of dimensions that might explain the individual variation observed. There is evidence in rats and mice that the proactive and reactive coping strategies are also expressed in females.

Recent publications in the field of evolutionary ecology indicate that disruptive selection for behavioral traits such as aggression may occur in other species as well. These findings support the idea that it is possible to generalize the coping strategies shown in the house mice to other taxa (see Koolhaas et al., this volume).

There are a number of studies of farm animals in which the results support some of the predictions of the proactive/reactive strategies. In studies by Ruis et al. (2000), it was shown that pigs differing in behavioral resistance in a so-called "back test" (piglet is put on its back and manually restrained for one minute and the number of escape attempts are measured) at 2–4 days of age also differed in several other situations. Extreme responding gilts (roughly the top and bottom 25% of the distribution) were classified as high resistant (HR) and low resistant (LR). HR gilts showed more aggression in a group-feeding competition test at ten weeks of age. Also, in the competition for the most productive teats at the anterior, a predominant position of HR piglets at this side was observed during the suckling period. In a novel environment test at ten weeks (but not when this was repeated at 24 weeks) LR pigs hesitated longer to leave their home pens and to contact a human. LR pigs had a higher reactivity of the HPA axis, as shown by higher cortisol responses to the first novel environment test, to routine

weighing at 25 weeks of age, and to administration of a high dose of ACTH. In another study of Ruis et al. (in preparation) HR and LR pigs also responded differently to social isolation. LR animals showed a higher initial cortisol response, more explorative behavior and less escape behavior. These data give support to consistency of coping strategy over context.

There are, however, also data from a number of other studies on several other species that fail to show either the bimodal distribution or the expected correlations found in the house mice (e.g., pigs: Erhard and Mendl 1999, Forkman et al. 1995; dogs: Scott and Fuller 1965; cattle: Hopster 1998).

There are several probable reasons why the results from these studies are ambiguous and why the results from house mice may be difficult to replicate in domestic species. It is possible that there are domestication effects, with a strong, directed selection for one type of coping strategy and therefore a strong reduction of the individual variation. Another possible explanation involves the ecological situation of the wild ancestors of our domestic species. Although wild populations of many animals show density cycles, the rodent cycles of the temperate zone are extreme in comparison with those of the wild boar or the wild ancestor of cattle. Hence the selection pressure might be disruptive for mice, but stabilizing for other species. This would explain why many other studies that have used factor analyses to look for personality traits have found other patterns of responding (for a review, see Gosling and John 1999). These explanations, however, do not hold for the findings of Hansson (1996). He found that the tendency of bank voles to either freeze or be passive in a stressful situation strongly correlated to age, a result not expected from the data on house mice, in which the coping strategy is relatively inflexible. Additional information, both on the coping patterns of rodents that do not show cyclical fluctuations as well as cyclical nonrodent species, would be helpful to explain this controversial issue.

While data on animals often indicate a strong consistency over time for different personality traits (e.g., Goddard and Beilharz 1986; Visser et al. 2001; Netto and Planta 1997), some studies on humans show less such consistencies. Rather then discussing this in detail, we have considered the possibility that the coping strategies, as observed in various animal species, might be related to the behavioral inhibition theory formulated to characterize stable temperaments in humans. Research on behavioral inhibition with humans has treated social withdrawal and reticence as individual characteristics reflective of a stable temperament (see Kagan et al. 1988). Studies of the stability of these behavioral characteristics, however, have demonstrated mixed results. Although statistically significant correlations have been reported, these correlations tend to be reported only when the groups selected are at the extremes of the distribution during the first measurement stage. When unselected samples are tested at different times, the correlations are usually very small and often approach 0. Similarly, attempts to identify neurophysiological correlates of these behavioral styles have resulted in weak findings. However, although weak, the findings tend to suggest that the behaviorally inhibited individuals express a different autonomic profile with features reflecting sympathetic-adrenal excitation. The basic issue is whether the behavioral typology is useful as a measure of stable, individual differences that might have a genotypic substrate, or is the typology reflecting a more fluid physiological state that can be readily manipulated to potentiate either social withdrawal or social engagement. Research by Coplan et al. (1994) has demonstrated behavioral strategies of children remaining on the periphery of socially interactive children. These reticent children, when measured in the laboratory, appear to have

autonomic and EEG response profiles that distinguish them from more spontaneous, engaging children (Schmidt et al. 1999). However, these distinctions in neurophysiological and psychophysiological profiles do not ensure that the differences represent changes in morphology or even neurophysiology. It is possible that research strategies providing the children with methods to regulate neurophysiological state (e.g., methods to increase vagal regulation of heart) might result in a change in behavior, since it has been proposed that manipulation of the behavior state will result in a shift in the profile of emergent behaviors (Porges, this volume). The emphasis here is on the neural regulation of state and the neural mechanisms that can rapidly alter or buffer neurophysiological control of visceral systems (e.g., heart rate, HPA, SAM), which often can be modulated by vagal efferent actions on visceral organs, as well as on the HPA, SAM, and immune systems.

Although the differentiation in coping strategies relates vulnerability to a variety of stress-related diseases, the relevance of the concept of coping strategies for individual fitness and welfare needs further experimentation.

WHAT IS SOCIAL SUPPORT AND WHAT ARE ITS CONSEQUENCES?

Social support can be defined as the degree to which an individual receives "emotional support" (e.g., attention, help) from other individuals (e.g., partners, relatives, friends). Social support appears to have stress-buffering effects, which significantly affect and dampen the effects of stress on behavior, endocrine, and immune functions and disease outcome.

Examples from Work on Humans

Social support given during an acute stress situation reduced the cortisol response to psychological stressors in young healthy subjects (18–20 years old). These stressors consisted of public speaking and mental arithmetic (Kirschbaum et al. 1995; Roy et al. 1998, 2001). Not all persons could function as social supporters, however. In this study, both men and women received a stronger effect of the social support if the supporter was a woman rather than a man.

In an experimental setting, it has been demonstrated that human subjects with a high degree of social support have had the lowest rate of upper respiratory infection and virus load compared to subjects with a low degree of social support (Cohen et al. 1997). Similarly, a prospective, longitudinal study (6 months) demonstrated associations between the degree of social support and disease exacerbation in patients with lupus erythematosus (SLE): the higher the degree of social support, the lower the disease activity (Pawlak et al., submitted).

In addition to experimental work, there is also much literature on social support and psychiatric disorders, particularly depression. Distinctions have been made between wider support from a social network and close support from intimate relationships. There are also distinctions between perceived and actual support. For humans, what is perceived as supportive is in the end likely to be the most important. Social support partly acts by buffering effects of acute stress, and partly its absence may have an independent effect. Mechanisms behind social support still need to be worked out fully. They may include practical help, benefits in cognitive rehearsal with others of solving strategies, and more directly emotional benefits.

Examples from Work on Nonhuman Animals

Several studies in animals show the same pattern. Social animals that are kept alone develop higher frequencies of abnormal behavior (e.g., Broom and Johnson 1993) and infections (e.g., Lewis et al. 2000). In the same way, there are studies showing which rats that have suffered a recent defeat will endure less serious consequences of the defeat, measured as the level of glucocorticoids, if they have access to a companion (Ruis et al. 1999). Just as with humans, not all individuals can give this support. For example, in the guinea pig, young juveniles separated from their mother will accept any other female as a supporting individual. As they age, however, this general acceptance changes so that subadults only accept support from specific known individuals (Sachser et al. 1998).

In animal studies, however, there are several other possible explanations. A rat that receives an electrical shock will show lower levels of ACTH if it has the possibility of attacking a conspecific (Levine et al. 1979). Despite this, it would be wrong to call the role of the attacked individual supportive.

Future research on the effects of social support on behavior, endocrine and immune functions as well as disease outcome should analyze the "acting components" of social support (e.g., strength of relationship, duration, frequency). In addition, the neuroendocrine mechanisms and pathways through which social support affects immune functions and disease outcome need to be analyzed.

There are many clinical and practical applications of this field of research. As soon as the stress-buffering effects of social support are known in more detail, social support strategies can, for example, be precisely "tailored" as intervention strategies to improve welfare in animals and humans.

WHAT ARE THE COSTS ASSOCIATED WITH DIFFERENT COPING SYSTEMS?

Since the various coping systems have evolved, it can reasonably be assumed that they must have a net benefit in fitness terms and, when the individual is selecting among the various possible coping strategies, the cost of the coping attempt as well as the probability that coping will be successful should ideally be taken into account. This would mean that on average in the population, costs which are measured by increased mortality, delayed breeding, or reduced number of offspring produced would be outweighed by benefits. However, any particular attempt to cope may have costs to the individual and, in selecting one coping strategy over another, these costs might be taken into account.

We have little reliable evidence about the costs of many coping methods. The costs of some coping methods can only be estimated in terms of energy utilization. The actual cost of that energy usage will vary according to the availability of energy to the individual. For an animal with an excess of available food and no constraints on its ingestion and processing (e.g., domestic animals under normal husbandry conditions), a little extra energy consumption is a negligible cost. However, to an animal that is short of food, the same amount of energy consumption could lead to death.

Although in assessing the consequences of any particular challenge, the costs of all of the coping systems in operation should be considered together, it is instructive to evaluate

examples of the various coping systems in relation to their costs. In each case below, where increased energy requirements represents the cost, actual effects on fitness will depend on the availability of energy from food reserves and from acquiring further food. Any extra energy demand, which a debilitated individual could not replenish, would have much greater effect on chances of surviving and reproducing than the same energy demand in an individual that could replenish any energy easily.

Fever and Sickness Behavior

Fever is an increase in body temperature to some upwardly adjusted set point. This requires extra energy. Other possible costs of fever or sickness behavior are reduced digestive efficiency, reduced efficiency of other enzymes, including those with metabolic functions, costs associated with body shutdown, and reduced gametogenesis. The reduction in feeding associated with sickness is necessary, because pre-feeding during sickness can lead to death but the energy loss associated with the inability to feed is costly.

Immune System Function

It has been suggested that at times the immune system may take up a very high proportion of available non-maintenance energy. However, we have very little clear evidence to substantiate this. The immune system experiences a high turnover of cells when it is not actively combating pathogens, so the energy increase resulting from defense against pathogens is not clear but may be very important. In addition to any energetic cost, where leucocytes have to be synthesized at a high rate, the material needed may reduce or render impossible the synthesis of other cells that are important for fitness. One indication of the costs of the immune system is that specific pathogen-free pigs grow considerably faster than normal pigs of the same breed and with the same management.

Brain Activity

The brain is very active during many types of coping response, and brain activity utilizes a much energy that could have been used in other ways. In the same way, resources might be used up within the brain during an activity, and activity within one part of the brain might prevent or reduce activity in another part. There is related evidence that training of dogs by humans makes them more tired than vigorous exercise, perhaps because of the brain activity.

Adrenal Responses

When glucocorticoids or catecholamines are produced, energy is expended. After production, they affect the modification of food reserves, which involve enzyme activity and which might result in an inefficient use of glucose and other products. Hence, energy may be wasted because of adrenal responses.

A second consequence of adrenal cortex activity is the increased risk of various potential problems, some of them very severe. The effects of glucocorticoids on the immune system are to some extent regulatory, but high and especially prolonged glucocorticoid activity has a

substantial suppressive effect on cell-mediated immunity. In addition, high levels of glucocorticoids can disturb water balance, suppress reproductive function, lead to brain remodeling, and perhaps in certain rare circumstances cause brain damage.

Behavioral Responses

All behavior requires some energy, and some behavioral responses to challenge are vigorous or continue for long periods. When a behavioral response to adversity occurs, there is likely to be inhibition of other, potentially useful, behaviors, and there may be harmful consequences of the behavior. It could be directly damaging or could increase risks such as those of predation.

Heart Rate Responses

If an individual responds to challenge by lowering its heart rate (e.g., in the course of a freezing response), there is a risk of dying because of the heart beat control mechanism. If the heart rate increases, to prepare the individual for vigorous activity, myocardial infarction or other heart damage is a possibility. Other cardiovascular responses can also increase risks.

Responses to Poison Ingestion

An immediate consequence of the detection of a noxious substance in the mouth or stomach is retching. This results in loss of ingested food and may also have significant side effects, such as an increased risk of predation. Many ingested toxins can be detoxified, but such processes have a significant energetic cost. Learning about toxins can be somewhat inefficient and lead to the future rejection of nutritious food because of its perception as potentially toxic.

Summary

The individual should select its coping method well because of the costs associated with each method. In some cases there are no alternatives (e.g., when there is too much carbon dioxide in inhaled air, the immediate reaction is straightforward). However, some problems in life can be solved in various ways and the selection, whether or not cognitive processes are involved, has major consequences for fitness. This whole area is not well understood and studies on the costs of coping mechanisms are needed. In practice, overall costs of a variety of methods used at one time would often have to be measured.

A further aspect of this area is the welfare of individuals using the various coping systems to different extents. The welfare (*sensu* Fraser and Broom 1990) of an individual is worse if its fitness is impaired, for example, in the sense that it is more likely to die rapidly. However, welfare is also poor if the individual experiences much difficulty in coping. In many cases that poor welfare is associated with the set of bad feelings that we describe as suffering. Whenever there are alternative coping strategies, we should endeavor to assess the extent of any poor welfare or good welfare using a range of measures such as those of abnormal behavior, emergency physiological responses, injury, etc. One example of this is the work of Duncan and Filshie (1979), which shows that hens disturbed by humans may either show

extensive behavioral escape responses or no behavior change but a high increase in heart rate. Where degree of aversion has to be measured in a preference test, such differences in coping methods used should also be taken into account.

CONCLUSIONS AND IMPLICATIONS

Early stressful experiences can influence the development of the brain and consequently also the behavior of the individual as an adult. Stressors affecting the mother while she is pregnant can have a clear effect on the fetus, and later on the adult individual. Precisely how these changes take place and what neurocircuits are modified remains unclear. To what extent these changes are adaptive, i.e., the presence of a stressor is used by an infant to predict its future environment, is unknown. It is clear, however, that there are a large number of studies, the results of which cannot be explained by this hypothesis.

Recent research has shown that the multiple drug-resistant pump may regulate entry of cortisol into the brain. As a consequence, the influence of other adrenal steroids may be of more importance in the primate brain than previously appreciated, at least under certain circumstances. It is not known whether this holds true for other species, for example, the farm animal species. From an applied perspective, it is clearly very important to do further research in this area to clarify the situation, since cortisol levels so often are used as one measure of the level of stress experienced by animals.

An interesting idea is that part of the variation in physiological and behavioral variables seen in stressful situations can be explained by the animal reacting with a different evaluation of the situation (e.g., the noise no longer predicts a predator and is therefore not dangerous) rather than by using the response system (e.g., using oxytocin to lower the stress response). There is very little data either to support or refute this idea, but it is testable and several alternatives have been given in the text.

Stress responses can vary, but certain general principles seem to be visible. In many species, coping strategies, in which the reaction to stress in one context predicts the reaction to stress in another context, can be seen. For stability over time but within context, the evidence is even stronger. There is extensive animal literature suggesting that certain traits (e.g., level of fearfulness) are consistent over time. A very influential theory of coping strategies is the proactive/reactive model. The model has been tested in a number of species with mixed results. On the whole the model seems to be very successful in predicting the behavior of mice and rats over a wide range of different situations but has had less success with domesticated species. Since the model makes strong and important predictions on the ideal composition of a group of animals, it is important to understand what is needed and, if it is at all possible, to implement it on species used by humans, such as farm animals.

One way of coping with stress is through social support. In a number of studies and in a number of species, the effect of social support has been shown to be strong and reliable. Still, we have very little basic knowledge about it and, in fact, know very little about factors that may strengthen or weaken it. Therefore, we can do little to increase the effectiveness of it. Future research in this area is important, both for applications on humans and in farm animals.

Just as a coping system confers a benefit to the individual using it, each coping system also involves a cost. Many times, cost is in the form of energy, something likely to be more

important in nature than in well-fed domestic animals, or in western society. Understanding the costs associated with each coping system will help us to understand not only the level of stress an animal suffers but also the cost they must be to withstand it.

REFERENCES

Alonso, S.J., R. Arevalo, D. Afonso, and M. Rodríguez. 1991. Effects of maternal stress during pregnancy on forced swimming test behavior of the offspring. *Physiol. Behav.* **50**:511–517.

Anderson, D.K., R.W. Rhees, and D.E. Fleming. 1985. Effects of prenatal stress on differentiation of the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the rat brain. *Brain Res.* **332**: 113–118.

Beauchamp, A.J., J.P. Gluck, and M.H. Lewis. 1991. Associative processes in differentially reared rhesus monkeys (*Macaca mulatta*): Blocking. *Dev. Psychobiol.* **24**:175–189.

Bilko, A., V. Altbacker, and R. Hudson. 1994. Transmission of food preference in the rabbit: The means of information transfer. *Physiol. Behav.* **56**:907–912.

Broom, D.M., and K.G. Johnson. 1993. Stress and Animal Welfare, pp.135–136. Dordrecht: Kluwer.

Caldji, C., D. Liu, S. Sharma, J. Diorio, D. Francis, M.J. Meaney, and P.M. Plotsky. 2001. Development of individual differences in behavioral and endocrine responses to stress: Role of the postnatal environment. In: Handbook of Physiology: Coping with the Environment, ed. B.S. McEwen, pp. 271–292. New York: Oxford Univ. Press.

Clancy, B., R.B. Darlington, and B.L. Finlay. 2001. Translating developmental time across mammalian species. *Neuroscience*, in press.

Clarke, A.S., D.J. Wittwer, D.H. Abbott, and M.L. Schneider. 1994. Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. *Dev. Psychobiol.* **27**:257–269.

Cohen, S., W.J. Doyle, D.P. Skoner, B.S. Rabin, and J.M. Gwaltney, Jr. 1997. Social ties and susceptibility to the common cold. *J. Am. Med. Assn.* **277**:1940–1944.

Coplan, J.D., M.W. Andrews, L.A. Rosenblum, M.J. Owens, S. Friedman, J.M. Gorman, and C.B. Nemeroff. 1996. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: Implications for the pathophysiology of mood and anxiety disorders. *Proc. Natl. Acad. Sci. USA* **93**:1619–1623.

Coplan, R.J., K.H. Rubin, N.A. Fox, S.D. Calkins, and S.L. Stewart. 1994. Being alone, playing alone, and acting alone: Distinguishing among reticence and passive and active solitude in young children. *Child Dev.* **65(1)**:129–137.

Coplan, J.D., E.L.P. Smith, R.C. Trost, B.A. Scharf, M. Altemus, L. Bjornson, M.J. Owens, J.M. Gorman, C.B. Nemeroff, and L.A. Rosenblum. 2000. Growth hormone response to clonidine in adversely reared young adult primates: Relationship to serial cerebrospinal fluid corticotropin-releasing factor concentrations. *Psychiatry Res.* 95:93–102.

Coplan, J.D., R.C. Trost, M.J. Owens, T.B. Cooper, J.M. Gorman, C.B. Nemeroff, and L.A. Rosenblum. 1998. Cerebrospinal fluid concentrations of somatostatin and biogenic amines in grown primates reared by mothers exposed to manipulated foraging conditions. *Arch. Gen. Psychiatry* **55**:473–477.

Cordon-Cardo, C., J.P. O'Brian, D. Casals, G.L. Rittman, J.L. Biedler, M.R. Melmed, and J.R. Bertino. 1989. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier siyes. *Proc. Natl. Acad. Sci. USA* **86**:695–698.

Dahlöf, L.G., E. Hård, and K. Larsson. 1977. Influence of maternal stress on offspring sexual behavior. *Animal Behav.* **25**:958–963.

De Kloet, E.R., G. Wallach, and B.S. McEwen. 1975. Differences in corticosterone and dexamethasone binding to rat brain and pituitary. *Endocrinology* **96**:598–609.

Duncan, I.J.H., and J.H. Filshie. 1979. The use of radiotelemetry devices to measure temperature and heart rate in domestic fowl. In: A handbook on biotelemetry and radio tracking, ed. C.J. Amlaner and D.W. Macdonald, pp. 579–588. Oxford: Pergamon.

Erhard, H.W., and M. Mendl. 1999. Tonic immobility and emergence time in pigs: More evidence for behavioral strategies. *Appl. Animal Behav. Sci.* **61**:227–237.

Finlay, B.L., and R.B. Darlington. 1995. Linked regularities in the development and evolution of mammalian brains. *Science* **268**:1578–1584.

Forkman, B., I.L. Furuhaug, and P. Jensen. 1995. Personality, coping patterns, and aggression in piglets. *Appl. Animal Behav. Sci.* **45(1–2)**:31–42.

Fraser, A.M., and D.M. Broom. 1990. Farm Animal Behavior and Welfare. London: Bailliere Tindall.

Ginsberg, S.D., P.R. Hof, W.T. McKinney, and J.H. Morrison. 1993. The noradrenergic innervation density of the monkey paraventricular nucleus is not altered by early social deprivation. *Neurosci. Lett.* **158**:130–134.

Gluck, J.P., and G.P. Sackett. 1976. Extinction deficits in socially isolated rhesus monkeys (*Macacca mulatta*). *Dev. Psychol.* **12**:173–174.

Goddard, M.E., and R.G. Beilharz. 1986. Early prediction of adult behavior in potential guide dogs. *Appl. Animal Behav. Sci.* **15**:247–260.

Godfrey, K.M., and D.J.P. Barker. 2000. Fetal nutrition and adult disease. *Am. J. Clin. Nutr.* **71**: 1344–1352.

Gosling, S.D., and O.P. John. 1999. Personality dimensions in non-human animals. *Curr. Dir. Psychol. Sci.* **8**:69–75.

Hansson, L. 1996. Regional differences in behavior in bank voles (*Clethrionomys glareolus*): Dyadic encounters. *Behav. Ecol. Sociobiol.* **39**:331–334.

Harlow, H.F., M.K. Harlow, and S.J. Suomi. 1971. From thought to therapy: Lessons from a primate laboratory. *Am. Sci.* **59**:538–549.

Harvey, P.W., and P.F.D. Chevins. 1985. Crowding pregnant mice affects attack and threat behavior of male offspring. *Horm. Behav.* **19**:86–97.

Heim, C., M.J. Owens, P.M. Plotsky, C.B. Nemeroff. 1997. The role of early adverse life events in the etiology of depression and posttraumatic stress disorder: Focus on corticotropin-releasing factor. *Ann. NY Acad. Sci.* **821**:194–207.

Herrenkohl, L.R. 1979. Prenatal stress reduces fertility and fecundity in female offspring. *Science* **206**:1097–1099.

Higley, J.D., S.J. Suomi, and M. Linnoila. 1992. A longitudinal assessment of CSF monoamine metabolite and plasma cortisol concentrations in young rhesus monkeys. *Biol. Psychiatry* **32**:127–145.

Hopster, H., J.T.N. Van der Werf, and H.J. Blokhuis. 1998. Short- and long-term consistency of behavioral and physiological stress responses in dairy cows during a novel environment test. Coping strategies in dairy cows. Ph.D. diss., pp 67–84. Wageningen University, Wageningen, The Netherlands

Kagan, J., J.S. Reznick, and N. Snidman. 1988. Temperamental influences on reactions to unfamiliarity and challenge. *Adv. Exp. Med. Biol.* **245**:319–339.

Kage, A., A. Fenner, B. Weber, and M. Schoneshofer. 1982. Diurnal and ultradian variations of plasma concentrations of eleven adrenal steroid hormones in human males. *Klin. Wochenschr.* **60**:659–666.

Karssen, A.M., O.C. Meijer, A.G. De Boer, I.C.J. Van der Sandt, E.C.M. De Lange, and E.R. De Kloet. 2000. The multidrug resistance 1a p-glycoprotein affects penetration of glucocorticoids, except corticosterone, in the brain. Abstract 152.17. 30th annual meeting of the Soc. for Neuroscience, New Orleans.

Kay, G., N. Tarcic, T. Polytrev, and M. Weinstock. 1998. Prenatal stress depresses immune function in rats. *Physiol. Behav.* **63**:397–402.

Kinsley, C.H., and B. Svare. 1986. Prenatal stress reduces intermale aggression in mice. *Physiol. Behav.* **36**:783–786.

Kirschbaum, C., T. Klauer, S.H. Filipp, and D.H. Hellhammer. 1995. Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosom. Med.* **57(1)**:23–31.

Klein, S.L., and D.R. Rager. 1995. Prenatal stress alters immune function in the offspring of rats. *Dev. Psychobiol.* **28**:321–336.

Koehl, M., M. Darnaudery, J. Dulluc, O. Van Reeth, M. Le Moal, and S. Maccari. 1999. Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal-axis and hippocampal corticosteroid receptors in adult rats of both gender. *J. Neurobiol.* **40**:302–315.

Künzl, C., and N. Sachser. 1999. The behavioral endocrinology of domestication: a comparison between the domestic guinea pig (*Cavia aperea* f. *porcellus*) and its wild ancestor, the cavy (*Cavia aperea*). *Horm. Behav.* **35**:28–37.

Ladd, C.O., R.L. Huot, K.V. Thrivikraman, C.B. Nemeroff, M.J. Meaney, and P.M. Plotsky. 2000. Long-term behavioral and neuroendocrine adaptations to adverse early experience. In: Progress in Brain Research: The Biological Basis for Mind Body Interactions, vol. 122, ed. E.A. Mayer and C.B. Saper, pp. 81–103. Amsterdam: Elsevier.

Levine, S., J. Weinberg, and L.P. Brett. 1979. Inhibition of pituitary-adrenal activity as a consequence of consummatory behavior. *Psychoneuroendocrinology* **4**:275–286.

Lewis, M.H., J.P. Gluck, A.J. Beauchamp, M.F. Keresztury, and R.B. Mailman. 1990. Long-term effects of early social isolation in *Macaca mulatta*: Changes in dopamine receptor function following apomorphine challenge. *Brain Res.* **513**:67–73.

Lewis, M.H., J.P. Gluck, J.M. Pettito, L.L. Hensley, and H. Ozer. 2000. Early social deprivation in nonhuman primates: Long-term effects on survival and cell-mediated immunity. *Biol. Psychiatry* **47(2)**:119–126.

Lubach, G.R., C.L.Coe, and W.B. Ershler, 1995. Effects of early rearing environment on immune responses of infant rhesus monkeys. *Brain Behav. Immun.* **9(1)**:31–46.

Lyons, D.M., F.L. Martel, S. Levine, N.J. Risch, and A.F. Schatzberg. 1999. Postnatal experiences and genetic effects on squirrel monkey social affinities and emotional distress. *Horm. Behav.* **36(3)**:266–275.

MacLusky, N.J., and F. Naftolin. 1981. Sexual differentiation of the central nervous system. *Science* **211**:1294–1303.

Martin, R.E., G.P. Sackett, V.M. Gunderson, and B.M. Goodlin-Jones. 1988. Auditory evoked heart rate responses in pigtailed macaques (*Macaca nemestrina*) raised in isolation. *Dev. Psychobiol.* **22**:251–260.

Matthews, K., F.S. Hall, L.S. Wilkinson, and T.W. Robbins. 1996. Retarded acquisition and reduced expression of conditioned locomotor activity in adult rats following repeated early maternal separation: Effects of prefeeding, d-amphetamine, dopamine antagonists, and clonidine. *Psychopharmacology* **126(1)**:75–84.

McIntosh, J., H. Anisman, and Z. Merali. 1999. Short and long periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: Gender-dependent effects. *Brain Res. Dev. Brain Res.* **113(1–2)**:97–106.

Meijer, O.C., E.C.M. De Lange, D.D. Breimer, A.G. De Boer, J.O. Workel, and E.R. De Kloet. 1998. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. *Endocrinology* **139**:1789–1793.

Moyer, J.A., L.R. Herrenkohl, and D.M. Jacobowitz. 1978. Stress during pregnancy: Effect on catecholamines in discrete brain regions of offspring as adults. *Brain Res.* **144**:173–178.

Netto, W.J., and D.J.U. Planta. 1997. Behavioral testing for aggression in the domestic dog. *Appl. Animal Behav. Sci.* **52**:243–263.

Newell-Morris, L.L., C.E. Fahrenbruch, and G.P. Sackett. 1989. Prenatal psychological stress, dermatoglyphic asymmetry, and pregnancy outcome in the pigtailed macaque (*Macaca nemestrina*). *Biol. Neonate* **56**:61–75.

Pariante, C.M., R. Dayanandan, A. Heyden, S. Ackerley, A.J. Grierson, G. Nebbia, S. Lovestone, A.M. Makoff, M. Danielsen, B.D. Pearce, A.H. Miller, and R.W. Kerwin. 2000. A new green fluorescent protein-glucocorticoid receptor (GR) chimera to study steroid-independent regulation of the GR in cell lines and in rat cortical and hippocampal neurons. Abstract 152.1. 30th annual meeting of the Soc. for Neuroscience, New Orleans.

Phillips, D.I.W., B.R. Walker, R.M. Reynolds, D.E.H. Flanagan, P.J. Wood, C. Osmond, D.J.P. Barker, and C.B. Whorwood. 2000. Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. *Hypertension* **35**:1301–1306.

Rosenblum, L.A., and M.W. Andrews. 1994. Influences of environmental demand on maternal behavior and infant development. *Acta Paediatr.* **Suppl. 397**:57–63.

Roy, M.P., C. Kirschbaum, and A. Steptoe. 2001. Psychological, cardiovascular, and metabolic correlates of individual differences in cortisol stress recovery in young men. *Psychoneuroendocrinology* **26**:375–391.

Roy, M.P., A. Steptoe, and C. Kirschbaum. 1998. Life events and social support as moderators of individual differences in cardiovascular and cortisol reactivity. *J. Personality & Soc. Psychol.* **75**:1273–1281.

Ruis, M.A., J.H.A. te Brake, B. Buwalda, S.F. De Boer, P. Meerlo, S.M. Korte, H.J. Blokhuis, and J.M. Koolhaas. 1999. Housing familiar male wildtype rats together reduces the long-term adverse behavioral and physiological effects of social defeat. *Psychoneuroendocrinology* **24(3)**:285–300.

Ruis, M.A.W., J.H.A. te Brake, J.A. van der Burgwal, I.C. de Jong, H.J. Blokhuis, and J.M. Koolhaas. 2000. Personalities in female domesticated pigs: Behavioral and physiological indications. *Appl. Animal Behav. Sci.* **66**:31–49.

Sachser, N., D. Hierzel, and M. Dürschlag. 1998. Social relationships and the management of stress. *Psychoneuroendocrinology* **23**:891–904.

Sachser, N., and S. Kaiser. 1996. Prenatal social stress masculinizes the females' behavior in guinea pigs. *Physiol. Behav.* **60**:589–594.

Sackett, G.P. 1981. A nonhuman primate model for studying causes and effects of poor pregnancy outcomes. In: Preterm Birth and Psychological Development, ed. S. Friedman and M. Sigman, pp. 41–63. New York: Academic.

Sanchez, M.M., E.F. Hearn, D. Do, J.K. Rilling, and J.G. Herndon. 1998. Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys. *Brain Res.* **812**:38–49.

Schmidt, L.A., N.A. Fox, J. Schulkin, and P.W. Gold. 1999. Behavioral and psychophysiological correlates of self-presentation in temperamentally shy children. *Dev. Psychobiol.* **35(2)**:119–135.

Schneider, M.L. 1992a. Delayed object permanence development in prenatally stressed rhesus monkeys infants (*Macaca mulatta*). *Occ. Therapy J. Res.* **12**:96–110.

Schneider, M.L. 1992b. Prenatal stress exposure alters postnatal behavioral expression under conditions of novelty challenge in rhesus monkey infants. *Dev. Psychobiol.* **25**:529–540.

Schneider, M.L. 1992c. The effect of mild stress during pregnancy on birth weight and neuromotor maturation in rhesus monkey infants (*Macaca mulatta*). *Infant Behav. Dev.* **15**:389–403.

Schneider, M.L., A.S. Clarke, G.W. Kraemer, E.C. Roughton, G.R. Lubach, S. Rimm-Kaufman, D. Schmidt, and M. Ebert. 1998. Prenatal stress alters brain biogenic amine levels in primates. *Dev. & Psychopathol.* **10**:427–440

Scott, J.P., and J.L. Fuller. 1965. Genetics and the Social Behavior of the Dog. Chicago: Univ. of Chicago Press.

Van Kalken, C.K., H.J. Broxterman, H.M. Pinedo, N. Feller, H. Dekker, J. Lankelma, and G. Giaccone. 1993. Cortisol is transported by the multidrug resistance gene product P-glycoprotein. *Br. J. Cancer* **67**:284–289.

Visser, E.K., C.G. van Reenen, H. Hopster, M.B.H. Schilder, J.H. Knaap, A. Barneveld, and H.J. Blokhuis. 2001. Quantifying aspects of young horses' temperament: Consistency of behavioral variables. *Appl. Animal Behav. Sci.* **74**:241–258.

Ward, I. 1972. Prenatal stress feminizes and demasculinizes the behavior of males. *Science* **175**:82–84.

Weinstock, M. 1997. Does prenatal stress impair coping and regulation of hypothalamic-pituitary-adrenal axis? *Neurosci. Biobehav. Rev.* **21(1)**:1–10.

Weinstock, M., T. Polytrev, D. Schorer-Apfelbaum, D. Men, and R. McCarty. 1998. Effect of prenatal stress on plasma corticosterone and catecholamines in response to footshock in rats. *Physiol. Behav.* **64(4)**:439–444.

Wigger, A., and I.D. Neumann. 1999. Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats. *Physiol. Behav.* **66(2)**:293–302.