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Influence of psychological variables on the activity of the hypothalamic–pituitary–adrenal axis

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Abstract

Psychobiology is the discipline that attempts to integrate the impact of environmental and psychological variables on biological systems. This paper focuses on the psychobiology of the hypothalamic–pituitary–adrenal (HPA) axis and illustrates several processes that influence the response of the HPA axis. The interaction of the developing rodent or primate with their primary care giver has permanent long-term effects on the HPA axis. Manipulations that alter maternal behavior during critical periods of development permanently modify the HPA axis. The HPA axis can be programmed to be hypo-responsive or hyper-responsive as a function of time and length of maternal separation. In the adult organism, the HPA response to stress is highly dependent on specific psychological factors such as control, predictability, and feedback. In primates, social variables have been shown to diminish or exacerbate the HPA stress response. During the post-natal period of development, the mother appears to actively inhibit the pups' HPA axis. Different aspects of maternal behavior regulate different components of the HPA system. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

It is a privilege to participate in this celebration in honor of Professor David de Wied. He was one of a generation of scientists who emerged after the Second World War and whose insights, imagination and visions were to forever alter the face of biology. During that incredible period, new disciplines were born, new relationships between the brain and the peripheral organ systems and the brain and behavior were established. What makes these accomplishments more amazing was that these discoveries were made despite a limited technology. In the ensuing years, we have experienced advances in technology that were beyond the wildest dreams of these post-war biologists. With these new technologies, we are now able to provide exquisite details and to establish mechanisms for the many of the theories postulated by these pioneers in the field. I doubt that many of our students and younger colleagues are familiar with many of the names that shaped our discipline. Such names as Geoffrey Harris, Claude Fortier, Frank Beach, Luciano Martini, George Sayers, Charles Sawyer, Elemer Endroczi, George Solomon and of course David de Wied are notable. Their ideas have become so ingrained in our thought that they have become part of the body of knowledge and their origins long forgotten. To Professor de Wied, we are indebted for the concept of the Neuropeptide and that such compounds profoundly influenced behavior. Although we are chronologically of the same generation and we share a common interest in those systems that are related to stress physiology, our research represents a mirror image of the same problem. Whereas de Wied directed his research efforts towards understanding of how peptide hormones influence the brain and behavior, the persistent theme of my research has been on how behavior and environmental factors influence the patterns of secretion of the so-called stress hormones.

Perhaps, the most recognizable name associated with stress is Selye (1956), who was the first to bring attention to the role of adrenocortical hormones as major component of the physiological response to stress. However, for the most part, the stimuli that were used to challenge the organism were extremely invasive. There were other investigators (Mason, 1968) who suggested that psychological processes played a significant role in the regulation of adrenal secretion of glucocorticoids. The purpose of this

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paper is to discuss the origins of the area of research that is best described as Psychobiology, which can be defined as the study of the environmental and behavioral influences on biological systems. Although I will focus on the psychobiology of the hypothalamic–pituitary–adrenal (HPA) axis, numerous other physiological processes have been studied that fit the definition of psychobiology. One example is the influence of psychological factors on immune function (Psychoneuroimmunology).

There are three sets of studies that will be discussed that are germane to the origins of the psychobiology of the HPA axis. We shall examine the role of early experience in shaping the endocrine responses to stress. How behaviors can modify these responses (coping), and the role of the mother–infant relationship on the development of the neuroendocrine regulation of the HPA axis.

2. Early experience

I have been asked with some frequency what the rationale was for conducting the first experiment on what is now termed early handling. There were two considerations that entered into the motivation to conduct the original study (Levine et al., 1956). This experiment was initiated while I was a post-doctoral fellow at the Institute for Psychosomatic and Psychiatric Research at the Michael Reese Hospital, Chicago. This institute was a pioneer in biological psychiatry and its director Roy Grinker was a Freud-trained psychoanalyst. Freud was one of the early theorists to place an emphasis on the experiences on the infant organism and their profound effects on adult behavior. He also maintained that early traumatic experiences produced long-lasting behavioral effects and contributed to the expression of psychopathology. Our goal was to attempt to provide an animal model of early traumatic events. Based on the available literature at the time, we decided to subject pre-weaning rats to a brief (3 min) exposure to a mild electric shock from post-natal days 1–20. The control groups consisted of pups that were removed from the mother in a manner identical to the shocked group, placed on the apparatus for the exact amount of time but not shocked. We also included a totally non-handled group. The rationale for this group was based on the fact that the non-shocked control pups were experiencing some disruption of the pup–dam relationship and without an appropriate control, this aspect of the early experience could not be properly evaluated. As adults, these animals were required to learn a conditioned avoidance response in a shuttle box. Contrary to every expectation, the early handled and shocked animals differed in subtle ways. However, the non-handled animals not only exhibited extremely poor performance on the learning task but also were overtly more emotionally disturbed. A series of subsequent experiments all yielded similar results. On all the known presumed measures of emotionality, it was the non-handled rats that showed exaggerated emotional responses under a variety of conditions. The first indication that early handling also influenced the HPA system was a study (Levine, 1957) that exposed early handled and non-handled adults to an injection of glucose and examined adrenal weight 24 h after the injection. Although basal adrenal weights did not differentiate between the two early experience groups, adrenal weight were significantly increased only in the non-handled animals that had received the 20% glucose injection. These results were surprising since adrenal hypertrophy following stress usually takes considerably more time to develop than the 24 h that elapsed between the injection and removal of the adrenal.

Why did we bother to investigate any aspect of adrenal function in these animals? Science, like most other endeavors, is influenced by the culture of the time. When these studies were conducted, it was believed that some compound existed that was involved in regulating the expression of emotions. One of the logical candidates for this compound was the adrenocortical hormones. The most influential and pervasive thinking concerning stress physiology at the time was Selye's formulation of the General Adaptation Syndrome (GAS), which gave a central role to the adrenal hormones as one of the predominant responses to stress. Given that the response to stress frequently involved affective events, it was a reasonable assumption that if animals or man showed exaggerated emotional behavior, one of the causative factors would be that the adrenal was overactive and releasing excessive amounts of glucocorticoids. Another important influence on the thinking of the time was the knowledge that steroid hormones emanating from the gonads played a regulatory role on sexual behavior. If one class of steroid hormones could regulate specific behaviors related to their putative function, it seemed logical to deduce that other steroid hormones could perhaps serve a similar function. The assumption that the adrenocortical hormones regulate emotional behaviors did not survive experimental scrutiny, although in recent years, there has been more compelling data that some of the hormones of the HPA axis, i.e. corticotropin-releasing hormone (CRH), may play an pivotal role in fear and anxiety (Nemeroff, 1996).

These initial studies did strongly suggest that early experiences have a long-term influence on the activity of the adrenal and the mechanisms that regulate this organ system. Research in this area has continued for four decades and although many other biological processes have been investigated, the original findings have held up remarkably well. It was not until almost 10 years had passed that the effects of early experience on what we now know as the HPA axis was revisited. The impetus was the development of a reliable biochemical assay for examining the levels of circulating corticosterone. This flourometric assay made it possible to examine the dynamics of the release of corticosterone and to obtain repeated measures on the same animal. In the years following the introduction of the flourometric assay, numerous studies emanating from many laboratories studied adrenocortical activity in animals subjected to different early experience using a multitude of different paradigms. In our laboratory, we published numerous studies on the effects of early handling in rats and mice. The effects of early handling on the dynamics of the adrenal response to stress have proved to be an extremely robust phenomenon. The initial experiments (Levine et al., 1967) demonstrated that the corticosterone response following exposure to an open field was significantly reduced in early handled animals. These findings have been robust and reproduced under a variety of different conditions (Meaney et al., 1993). Thus, the adrenocortical response to novelty, restraint, shock, conditioned taste aversion, etc. have all been reported to be significantly reduced in early handled animals. Further, early handled animals appear to have a more efficient negative feedback regulation. Early handling also modifies the response to neonatal malnutrition (Wiener and Levine, 1978) and fetal alcohol syndrome (Wienberg et al., 1995).

It has always been assumed that the effects of early handling were to modify those aspects of the central nervous system that regulated the HPA axis. There were numerous reasons for this assumption. The evidence indicated that there were no differences between handled and non-handled animals in basal levels of corticosterone and adrenocorticotropin (ACTH). No differences were observed between the different early experience groups on adrenal sensitivity to ACTH, pituitary response to exogenous CRH or clearance of corticosterone and ACTH. Adult early handled and non-handled rats have similar levels of corticosterone-binding globulin. In so far as none of the indices of the peripheral aspects of the HPA axis differ as a consequence of early handling, the origins of the differences on the response to stress must be a function of some change in programming of the central regulatory mechanisms. At least two components of the central regulatory mechanisms have been reported to differ between the early experience groups. Plotsky and Meaney (1993) reported that resting levels of CRH mRNA in the paraventricular nucleus (PVN) of the hypothalamus were significantly higher in non-handled compared to handled adult animals. Median eminence levels of CRH and arginine vasopressin (AVP) are also higher in non-handled rats. These difference in CRH gene expression and protein levels are apparent under resting conditions although there are no differences in circulating corticosterone levels. More recently, we have shown increased CRH gene expression following restraint is much more rapid in non-handled rats (Gordon and Levine, 1999). It has been well-established that there are at least two types of glucocorticoid receptors, the so-called mineralocorticoid receptor (MR), and the glucocorticoid receptor (GR). Early handling markedly changes the GR receptor density in the hippocampus of adult rats. In general, early handled animals have increased hippocampal GR sites and also show an increased gene expression for the GR. The MRs do not appear to change with early handling. Bhatnagar et al. (1996) suggested that "it seems that the increase in GR sites is a critical feature of neonatal handling on HPA function. This increased receptor density appears to increase the sensitivity of the hippocampus to circulating glucocorticoids, enhancing the efficacy of negative feedback inhibition on HPA activity, and serving to reduce post-stress secretion of ACTH and corticosterone in handled animals."

Although there have been several hypotheses proposed to attempt to account for the effectiveness of early handling, one hypothesis that seems to have received the most attention and support from the existing data is the "maternal mediation" hypothesis (Smotherman and Bell, 1980). This hypothesis emerged once again as a consequence of the research on early handling. Intuitively, it was difficult to understand why the seemingly innocuous manipulation of very brief bouts of maternal separation could have such permanent and pervasive influence on behavior and the HPA axis. The first suggestion that the effects of early handling could be maternally mediated was an experiment by Deneberg and Whimby (1963). These investigators reared pups with mothers who had no prior early interventions and compared them to offspring of mothers who had been handled as infants. Elaborate cross-fostering procedures were included in an attempt to tease out post-natal from pre-natal effects. The results indicated that on some measures of emotionality, the experience of the post-natal mother significantly altered the behavioral outcome measure. On other indices of emotionality, the effects were a result of the interaction between pre- and post-natal influences. Levine (1967) demonstrated that maternal early experience could influence the adrenocortical response of the pups. The corticosterone response to novelty of pups reared by handled dams was reduced compared to pups reared by non-handled dams. Handling the pups resulted in a reduction of the response when reared by a non-handled dam. In contrast, handled pups from handled dams did not differ from their non-handled counterparts if the dam had been handled as a pup. There is direct evidence that handling altered maternal behavior (Smotherman et al., 1977). They observed maternal behavior in dams when reunited with pups that were handled or shocked. Several aspects of maternal behavior were intensified when the treated pups were returned to the dams. Clearly, manipulation of the pup altered and increases dam–pup interactions.

For those of us who have had the opportunity to observe the field over an extended period of time, the cyclic quality of science is apparent. Questions that were posed decades ago are revisited often with new approaches, always with new techniques and insights. Recently, Meaney and co-workers (Liu et al., 1997; Francis et al., 1999) have used a naturalistic approach to the question of maternal mediation. They observed maternal behavior in undisturbed females. They report that variations in maternal behavior influence the development of behavioral and endocrine responses to stress in the offspring. In particular, increased licking/grooming and arched back nursing are correlated with a reduction in HPA activity and less fearful behavior in the offspring. They further demonstrated that variations in maternal care serve as the basis for non-genomic behavioral transmission of individual differences in stress reactivity.

In recent years, there has been a resurgence of interest in this area of developmental psychobiology. Specifically new paradigms have been introduced in an attempt to investigate the effects of adverse early experiences on the neurobiology of stress. Thus, several laboratories have begun to explore the consequences of more prolonged periods of maternal separation (Plotsky and Meaney, 1993; Patchev et al., 1997). Although there are only a limited number of published papers using these paradigms, it does appear as though these longer periods of separation can reverse the effects of brief separations (early handling). In some instances, these animals as adults exhibit hyper-reactive HPA activity in contrast to the well-established hypo-reactivity that results from early handling. Although the ultimate outcome of these post-natal manipulations depends upon a number of different variables, there is little question that one major source of individual differences in the neuroendocrine regulation of the HPA axis is based on the environment during development and these effects are pervasive and difficult to reverse.

3. Stress and coping

There are perhaps fewer exercises that have humbled more scholars in this field than the definition of stress. The simplistic explanations evoke the concept of homeostasis and define stress as any threat to homeostasis. This definition may work when dealing with the many invasive stimuli that have been used in stress research. It is not difficult to assume that tissue damage, toxic agents, severe abrupt changes in ambient temperature, or being chased by a lion represent threats to survival that would disturb homeostasis. The difficulty arises when similar increases in the so-called stress hormones, that are indistinguishable from those observed in response to physically invasive stimuli, occur when the organism is confronted with having positive reinforcement withheld, exposure to novelty, sudden changes in noise, taking an examination, public speaking, etc. Further, there is evidence that in animals and man, the anticipation of an event is as potent an activator of HPA axis than the event itself. Phobic patients show the highest elevation of cortisol on the day prior to being exposed to the phobic stimulus (Wiedenfeld et al., 1990). The fact that there are obvious differences between the stimuli that are capable of inducing secretion of the stress hormones was recognized early in the history of stress research. Fortier (1951) proposed a distinction between systemic and neurotropic stimuli and proposed that systemic (adrenaline, cold, histamine) stimuli were under humoral control and neurotropic stimuli (sound, immobilization) were mediated by the central nervous system. Throughout the history of stress research, these types of distinctions have been demonstrated (Dallman, 1979; Feldman et al., 1993). Recent methodological advances have made it possible to examine the neural pathways in the central nervous system in response to different stimuli that induce increased secretion of corticosterone and ACTH. Sawchenko (1991) has described at least five different pathways that converge on the PVN and stimulate the release of CRH. Herman and Cullinan (1997) have presented the most recent version of this stress dichotomy. They state “Stressors involving an immediate physiologic threat (systemic stressors) are relayed directly to the PVN, probably via brainstem catecholaminergic projections. By contrast, stressors requiring interpretation by higher brain structures (processive stressors) appear to be channeled through limbic forebrain structures.” It is perplexing that even though there have been several highly sophisticated attempts to define stress and to elaborate the neurobiology of the HPA axis, the persistent definition of stress is still “anything that induces increased secretion of glucocorticoids.”

3.1. Control and expectancies

Regardless of one’s definition of stress, the evidence unambiguously supports the hypothesis that there are psychological factors important in determining the endocrine responses to stress. This field is indebted to Weiss (1972) for his innovative research on coping. What was demonstrated in these studies was that a physiological response (stomach ulceration), in response to a well-defined stimulus (electric shock), can be modified: If the animal is permitted to exert *control* which regulates in some manner the duration and/or intensity of the shock, is presented with information concerning the onset or offset of the shock, *predictability*, or is given information concerning the efficacy of the response, *feedback*. Weiss further postulated that the amount of stress an animal actually experiences when exposed to noxious stimuli depends on two variables: the number of coping attempts (responses) and the amount of relevant feedback these responses produce.

Some of these psychological principles appear to be directly applicable to the regulation of the HPA axis. Evidence that control is a potent modulator of HPA activity is found in studies using a variety of species. Coover et al. (1973) examined corticosterone levels during active avoidance in rats. Plasma samples were obtained following the first training session during which time the rats received shock on the majority of the trials, following the seventh training session when the animals has achieved asymptotic performance and 10 days later. There was a decline in plasma corticosterone levels from the first to the seventh training session, which was attributed to the absence of shock. However, as training continued, there was a further decline in corticosterone levels although performance of the avoidance task did not differ from the early training. This decline was interpreted as evidence for the effects of control and predictability on the response of the HPA axis. Other studies (Weinberg and Levine, 1977) reported similar findings. Davis et al. (1977) found similar declines in adrenocortical activity using a lever press escape paradigm that permitted escape but not avoidance of the aversive stimulus. Thus, the avoidance component of the shuttle box task appears to be less important than the ability to make an active response (control) that terminates the noxious event. Dess et al. (1983) examined the issues of control and predictability in dogs. The results revealed that control reduced the cortisol response to shock, whereas, predictability in the absence of control has no discernable immediate effect. Increases in cortisol induced by shock were elevated whether or not the shock was predictable. Hanson et al. (1976) presented a clear demonstration that monkey's control could reduce the cortisol response to loud aversive noise levels. These are but a few of the many examples of importance of control in modulating the glucocorticoid and presumably the neural components of the HPA axis.

The influence of predictability on HPA activity is more problematic. Davis and Levine (1982) and Dess et al. (1983) failed to show that predictability, in the absence of control, exerted any effect on the HPA axis. However, these investigators pointed out the interaction between control and predictability. Thus, whereas prediction may occur with or without control, the converse is not necessarily true. The very act of making a stimulus (or its offset) response contingent dictates that the stimulus will also be predictable. It is not surprising, therefore, that the effects of predictability are most profound when control is simultaneously available.

As we have documented, the absence of control results in an exacerbated adrenocortical response. There is further evidence that the loss of control can induce increased HPA activity. In the study by Coover et al. (1972), a procedure was introduced that prevented the animal from making the now well-learned avoidance response. During this "forced extinction" period, a locked door was placed between the compartments. Although the conditioned stimulus (CS) was presented and shock was omitted, corticosterone levels were again elevated compared to the plasma levels of corticosterone during avoidance conditioning. These data were seen to indicate that preventing the rat from making its response contingent avoidance response represented a loss of control. Another example of the effects of loss of control is the rise in corticosterone following extinction of an appetative response. Rats trained to press a lever for water or food on a continuous reinforcement schedule show an elevation of corticosterone levels as a consequence of reinforcement being withdrawn during extinction. These data were interpreted as suggesting that "frustration," defined as the absence of reinforcement occurring in a context where reinforcement is expected, does result in activation of the HPA axis. Frustration however, can also be viewed as loss of control. This notion is supported by a study by Davis et al. (1976). Extinction of an instrumental response can be achieved in several ways. The traditional procedure is to permit the animal to respond and omit the reward. Another extinction paradigm is to permit the subject to continue to respond and receive reward but to make obtaining the reinforcement no longer response-contingent. Under this extinction procedure, no change in corticosterone levels occurs.

The concept of loss of control implies that there is an accompanying loss of predictability. This would suggest that HPA activation would also be observed under circumstances where predictability of obtaining reward is altered from high to low predictability, and conversely that a shift from low to high predictability should result in a reduction of arousal and therefore a decrease in HPA activity. Goldman et al. (1973) trained rats to bar press for water on either a continuous reinforcement or a variable interval schedule. When the variable interval trained rats were shifted to continuous reinforcement, their corticosterone levels decreased. In contrast, when rat are shifted from a predictable schedule of reward to a more unpredictable schedule, corticosterone levels invariably elevate (Levine et al., 1972). That the HPA axis is bi-directional has been well-established. The presentation of food or water to deprived rats results in a decline in the levels of ACTH and corticosterone (Gray et al., 1978; Romero et al., 1998). Perhaps, even more impressive is that simply presenting cues signaling the occurrence of food and water also produce declines in circulating levels of corsticosterone (Levine and Coover, 1976; Coover et al., 1977).

Is there an overall set of assumptions that can account for the data just presented? Several attempts were made to provide a theoretical basis to account for the activation and inhibition of the HPA axis (Hennessy and Levine, 1979; Levine and Ursin, 1991; Ursin, 1988). The underlying principle that pervades most psychobiological approaches to stress invokes cognitive processes. The primary cognitive operation is one of comparison between the immediate external event and some cognitive representation based on prior experiences. When discrepancies occur between the event and the cognitive representation, arousal is increased. The neurophysiological basis for this concept is derived from Sokolov's (1963) theory of the orienting response. In so far as stress and arousal appear to be analogous concepts (Hennessey and Levine, 1979), those events that serve to increase arousal should activate the HPA axis and those, which would reduce arousal, should result in inhibition. Thus, such notions as uncertainty, expectancies, response outcomes, etc., have all been invoked to deal with the manner in which psychological events can influence the HPA system. What is clear is that psychological processes can exert profound influences on the magnitude and direction of the response characteristics of the HPA axis.

3.2. Social factors

Thus far, we have discussed several variables that can modify the response to stress, or determine whether or not a response will be elicited. Another important influence on the HPA axis appears to be the presence of familiar social partners and/or salient social relationships. There is now an extensive literature that points to the importance of social relationships in determining an individual's ability to cope with stress. Hamburg and Adams (1967) emphasized the great need for continuity of personal relationships when individuals are involved in crisis situations. In addition, there have been numerous studies that have pointed to social support as a major factor in specific health-related issues (Elliot, 1989). In our laboratory, we have studied the influence of psychosocial variables on the response of the HPA axis to stress. In order to examine the influence of psychosocial factors on physiological processes, it was first necessary to find an appropriate animal model in which social behavior was an integral part of the animal's adaptive processes. Although there are many forms of social organization and social behavior among animals, one of the most striking features of adaptation in all primate species appears to be living and interacting in social groups. Kummer (1971) has stated "Primates seem to have only one unusual asset in coping with their environments: a type of society, which, through constant associations of young and old animals and through a long life duration, exploits their large brains to produce adults of great experience. One may, therefore, expect to find specific primate adaptations in the way primates do things as social groups." For these and other cogent reasons, we chose, as our experimental animal, a small South American primate: the squirrel monkey (*Saimiri sciureus*). Our primary goal when we initiated these studies was to investigate the psychobiology of attachment. If one invokes Bowlby's (1969) definition of attachment, it was apparent that a primate model was more appropriate for studying attachment than the more commonly used rodent models, which failed in many respects to fulfill the criteria for human attachment relationships. Another intriguing feature of the squirrel monkey was the unique adrenocortical system of this primate. Not only does the squirrel monkey have exceptionally high basal levels of cortisol, but it is also highly responsive to stress.

Our initial studies (Mendoza et al., 1978) on the influence of social factors on endocrine regulation occurred within the context of an extensive series of experiments dealing with the psychobiology of mother–infant relationships. In these studies, we were attempting to characterize the effects of disruptions of mother–infant relationships on the activation of the HPA axis. Using a separation paradigm, our initial observations demonstrated unequivocally that separation of mothers and infants resulted in a striking and reproducible elevation of plasma cortisol in both members of the dyad (Levine and Wiener, 1989). The early studies were conducted by isolating the infant in a novel environment. Thus, the infant was exposed to at least two conditions that were capable of activating the HPA axis: novelty and maternal loss. Separation of mother and infant can occur in several different ways. These different separation procedures have been described anthropomorphically as: (1) Infant goes to hospital — The infant is separated from his mother and all other members of its social group and placed in unfamiliar surroundings; (2) Mother goes to hospital — In this case, the mother is absent but all other physical and social aspects of the environment remain in place for the infant. A third separation paradigm consists of removing both mother and infant from their familiar surroundings, separating them but preventing physical contact. Thus, both members of the mother–infant dyad are in proximity to each other, with visual, auditory, and olfactory communication available. The findings of these studies demonstrated that the endocrine response to separation was dependent upon the separation paradigm that was used (Wiener et al., 1987, 1990). Although all infants responded to separation with increases in circulating levels of cortisol, the magnitude of the elevations appeared to be a function of the degree of social support available during the period of separation. Thus, the most severe of these procedures in terms of elevations of plasma levels of cortisol is the first condition, infant goes to hospital. When the mother is removed, leaving the infant in its familiar surroundings (including other mother–infant dyads), the infant emits relatively few vocalizations and a marked reduction in the cortisol response to the absence of the mother. These data could be interpreted as indicating that familiarity with the environment is the important factor in modulating the HPA response to separation. The results of other experiments have demonstrated that the critical variable is the presence of familiar social partners. The infants adjacent to their mothers during separation showed a cortisol response that was reduced in comparison to that of the totally isolated infants but higher than that observed in those infants that were in the presence of their familiar companions. Thus, for the infant squirrel monkey, the availability of social support does appear to 'buffer' the response of the stress-related hormones of the HPA axis. Although most of our studies have used only separations of short duration (maximum 24 h), following separation at weaning (when the infants are about 6–7 months of age), cortisol can remain elevated for at least 14 days. Infants that have been previously separated show higher cortisol than infants who have not experienced prior separation. However, the presence of familiar social companions at the time of weaning markedly reduces the behavioral and endocrinological responses to the loss of the mother

The question that was raised by these data was whether ‘social buffering’ was exclusive to the mother–infant attachment relationship or if social buffering could be observed in the adult monkey. One example of social buffering in adults was observed in the separation experiments. Squirrel monkey mothers living in isolated dyads show an elevation of cortisol when their infant was removed. Mothers maintained in social groups did not show a cortisol response when the infant was removed. To further investigate this issue, adult monkeys in their home cages were presented with unfamiliar objects such as moving toys. Our initial studies were conducted on group-housed animals, and although we frequently observed behavioral avoidance of these objects, we could not detect any changes in cortisol secretion. These data could be interpreted as indicating (1) that these objects were not sufficiently intense to elicit an adrenal response, or (2) that in so far as the monkeys were with familiar social partners social buffering had indeed occurred. In order to solve the problem of stimulus intensity, we chose a more salient stimulus, visual exposure to a live snake (Vogt et al., 1981). This has been shown to provoke strong behavioral reactions in non-human primates, which do not readily habituate. Thus, squirrel monkeys were exposed to a live boa constrictor, which was presented above their cages. The snake was contained in a wire mesh box and no contact with the snake occurred during the period of exposure. The animals lived in social groups consisting of two males and two females and were exposed to the snake for 10 min both while in their social group and after being placed in an individual cage. All monkeys showed increased vigilance, agitated activity and avoidance of the snake in both the group and individual condition. Although the behavioral responses to the snake did not differ according to their housing condition, the snake did not elicit an increase in adrenocortical activity when the monkeys were tested as a group. These results indicate that the presence of social partners does have the capacity to ameliorate the response of the HPA system to potentially threatening stimuli. However, it should be noted that social buffering of the HPA axis occurs only when the monkeys are living with multiple partners. When the animals are housed as pairs, not only do we not see evidence for social buffering, and in some cases the hormonal response to the snake is exacerbated (Coe et al., 1982).

The above experiments provide compelling evidence that social factors can act to modify the neuroendocrine responses to potential threatening stimuli. However, in the snake experiments, it was also possible for the monkeys to distance themselves from the snake by moving to the lower perches and thus, exercising some degree of control in this experimental situation. In a subsequent study, we used a different paradigm that prevented the monkeys from exerting any control. In this study, it was also possible to more clearly delineate both the stress stimulus and the behavioral reactions to the stimulus. To accomplish this, we used a classical conditioning procedure (Stanton et al., 1985). The animals were trained to associate a previously neutral light with an aversive event. Individual monkeys were presented in a test chamber with multiple parings of light (CS) and shock (unconditioned stimulus (UCS)). Subsequently, all animals were tested in their home cage and their behavior and adrenocortical responses were measured following presentation of the flashing light when they were alone, in pairs, or in a group of six monkeys. When the monkeys were alone or in pairs, exposure to the light resulted in a significant elevation of plasma cortisol levels. In contrast, when the light was presented to the animals in the larger social group consisting of six monkeys, no elevations of cortisol were observed. There was a significant increase in behavioral activity in all monkeys regardless of housing conditions, which indicates that the CS was capable of eliciting the behavioral conditioned response in the home cage. Social buffering appears to require some critical number of animals before it is manifested. Hennessy (1986) found no evidence of social buffering when monkeys were removed from their home cages and placed in a novel cage either alone or as a pair. Under these conditions, as in the conditioning and snake experiments, the presence of a single social partner does not result in social buffering.

At least for the infant monkey, disruption of their primary social relationship with the mother results in acute and chronic elevations of plasma cortisol. This response does not appear to habituate nor is it tightly dependent upon age. Both pre- and post-weaned monkeys respond to relatively brief periods of separation and maternal loss. Mendoza and Mason (1997) distinguish between attachment and affiliation. The response to separation in attachment relationships invariably activates the HPA axis, whereas there is little evidence that adult squirrel monkeys respond to separation from their adult companions.

There have been several attempts to integrate the phenomenon of social buffering within the broader framework of stress and coping theory. For the infant, the mother is the primary source of emotional security and serves to reduce the infant’s arousal levels. Thus, the infant’s ability to make contact and maintain proximity to the mother is perhaps the infant’s earliest experience with the use of control to modulate arousal. This explanation cannot account for the effects social partners have on modulating the response of the HPA axis to stress. Why does the presence of other familiar animals serve to inhibit HPA activity in affiliative relationships? There is evidence that in stress-inducing situations, the capacity to make some response, although the response does not alter the characteristics of the stress-inducing event, can diminish the HPA response. Thus, the ability to interact with other monkeys provides another element of control. Another possible hypothesis is that presence of familiar cage mates provides the monkey with a more stable and predictable environment. Predictability has been shown, under certain conditions, to reduce the endocrine responses to stress. That any of these hypotheses can provide a framework for understanding the influence of social factors in modulating the response to stress is debatable. What is not in question is the overwhelming data that the response of the HPA axis is highly influenced by psychological processes.

4. The stress hypo-responsive period

If there is one principle that I have communicated to the many students who have passed through my laboratory, it is that "the neonate plays by different rules than the adult." Often, the assumption is made that the neonate is a miniature adult. When many of the procedures used to study adult organisms are applied to neonates, the results obtained are frequently inaccurate and generate erroneous conclusions. In this section, we will deal with the influence of maternal factors on the development of the endocrine and neural responses to stress.

Early in the history of developmental endocrinology, it was shown that the HPA axis of the neonate responded in a manner idiosyncratic to that specific period of development. Whereas the adult rat exhibits a robust adrenocortical response to a wide variety of stimuli, the developing pup shortly after birth enter a period during which it become stress non-responsive. This stress non-responsive period was believed to persist for at least 2 weeks. In our laboratory, we had observed that several aspects of the development of the pup seemed to be accelerated as a consequence of early handling. Thus, the opening of the eyes occurred several days earlier than their non-handled counter parts. Levine et al. (1958) therefore reasoned that if one aspect of development could be so affected by these early manipulations, then the maturation of the systems regulating the stress response should also be influenced. The data from our first study unequivocally supported this hypothesis. Whereas adrenal ascorbic acid depletion in response to cold did not occur until day 16 in non-handled pups, pups that had been handled (removed from the nest for 3 min daily) responded as early as post-natal day 12. In retrospect, this was a landmark study. It is not often that we have the privilege of revisiting archival papers and re-evaluating their significance. This study had many implications that were not appreciated at the time. It was one the first demonstrations of the plasticity of those neural mechanisms regulating the stress response. Although neuroendocrinology was in its infancy, based on these results, it was postulated that the effects of early handling was to influence the development of the "hypothalamo–hypophysial system, which appears essential in the reactions to certain types of stress." Other studies (Levine and Lewis, 1959) using adrenal ascorbic acid depletion as the measure of HPA activity also were the first to suggest that the effects of early handling were maternally mediated. In this experiment, it was shown that the accelerated maturation of the HPA axis could be observed if the early manipulation involved simply removing the dam for the home cage without any direct treatment of the pups.

4.1. Maternal deprivation

It is not the purpose of this paper to review the history of the so-called stress non- or hypo-responsive period, but to highlight how a psychobiological approach to the study of HPA development was instrumental in altering many of the prevailing views concerning the ontogeny of the HPA axis. However, one of these studies reported (Levine et al., 1967) deserves mention. This experiment determined that the neonatal adrenal appeared to be relatively insensitive to exogenous ACTH. Previous studies assumed that the major deficit during the stress non-responsive period was the inability of the neonate to secrete ACTH due to the immaturity of the central regulation of the pituitary. This study suggested that the failure to observe adrenal response to stress might in part be a function of adrenal insensitivity.

The importance of maternal factors in the development of the HPA axis is based on a series of studies that are of more recent vintage. The importance of serendipity in science should never be underestimated. Although the very early studies on HPA development suggested that maternal factors may be involved, more convincing evidence of the role of the dam on the regulation of the HPA axis during development was first apparent in a study that was designed to explore a totally different question. Stanton et al. (1988) were testing the ontogeny of food-induced down regulation of the corticosterone, which had been observed in adult rats (see Section 3). In order to insure that pups would actively suckle, they were removed from the dams and maintained on a heating pad for 24 h. The pups were then placed in a novel chamber alone or with an anesthetized dam. Half the pups were fed through indwelling cannulae. The results indicated that the maternally deprived pup responded to the novel chamber with elevations of corticosterone. Although feeding did not affect this response, contact with the dam effectively suppressed the response of the adrenal. The most surprising finding that emerged from this study was that pups tested while presumably in the stress hypo-responsive period (day 12) had elevated basal levels of corticosterone and further increased hormone levels following stress (novelty). We repeated this experiment using much younger pups and regardless of age at the time of deprivation, elevated basal levels and a robust response to stress were found. These data suggested that some aspect of maternal behavior was actively inhibiting the developing HPA axis. Many previous studies of the stress hypo-responsive period have reported deficits in one or several of the elements that comprise the HPA axis. Thus, CRH appeared reduced, CRH mRNA was unresponsive to stress, and pro-opiomelanocortin (POMC) and ACTH levels were reduced. These data were inconsistent with what was observed in maternally deprived pups. Following maternal deprivation, not only were corticosterone levels increased in response to stress but in addition, ACTH levels were also elevated following relatively mild stress (novelty and injection of isotonic saline). In so far as both the adrenal and the pituitary were activated in response to stress in maternally deprived pups, we made the assumption that the HPA axis was yet another example of a biological system that was under maternal regulation.

There are many examples that indicate that the mother regulates physiological processes in the developing organism. Hofer (1978) coined the term "hidden regulators" to describe the role of the maternal figure in regulating many aspects of development. What is implied in the concept of "hidden regulators" is that during development, the biological unit consists of both mother and infant. This relationship is reciprocal in that the infant also can participate in regulating some aspects of the mother's physiology. Among some of the examples of "hidden regulators" are sleep/wake cycles, heart rate, secretion of growth hormone and as we are proposing, HPA activity. In some of the examples, the maternal role is to participate in the activation of a function; in others, the mother serves to actively inhibit the system under regulation (see Levine, 1994 for review). Several abortive attempts were made to uncover what specific maternal behaviors were involved in the inhibition of the HPA axis. In particular, the presence of a non-nutritive dam did not alter the pattern of corticosterone in the pup or preventing the pup's ability to obtain milk but suckle did not modulate the deprivation-induced changes in corticosterone. These data strongly suggested that the critical feature of maternal behavior that affected the HPA axis was feeding. When the pups were fed via an intraural cannulae in the absence of the dam, basal levels of corticosterone were reduced to the levels of the non-deprived pups and the stress response, although not completely suppressed, was markedly reduced (Rosenfeld et al., 1993). It was in a study that examined the role of feeding in the regulation of ACTH that a surprising and perplexing result was obtained. If ACTH and corticosterone were examined simultaneously in fed vs. non-fed deprived pups, the effects of feeding on corticosterone secretion were replicated. However, in all the experimental groups, the stress-induced ACTH response did not occur. Neonatal rats are dependent upon the dam to regulate their eliminative functions. Ano-genital licking by the dam stimulates both urination and defecation. In all the previous studies when the pup was artificially fed, the experimenter applied gentle stroking to the ano-genital area of the pup to induce elimination by the pup. This stroking occurred on only three occasions during the 24-h deprivation period. A systematic investigation of stoking, in the absence of feeding, revealed that different components of the HPA axis were differentially regulated by distinctly different maternal behaviors. Stroking appears to act directly on the mechanisms that regulate ACTH, whereas the adrenal requires feeding to remain down-regulated (Suchecki et al., 1995).

4.2. Central control

One of the most important methodological advances in neuroscience provided by molecular biology has been the technology to examine gene expression and protein level of those peptides involved in the HPA axis. The expression of a class of immediate early genes, in particular c-*fos*, has proved to be a marker for neural activation. Changes in CRH and AVP mRNA are critical to understanding the role of these peptides in the regulation of ACTH and ultimately corticosterone. Studies that examined gene expression of these peptides during development, in general, failed to see any changes in CRH or AVP mRNA during the stress hypo-responsive period. In so far as maternal deprivation rendered the pup stress responsive, it was hypothesized that the central markers of stress would also reflect these changes. Activation of the PVN as measured by c-*fos* was enhanced in the deprived pups. However, levels of CRH mRNA were lower in the pup following the deprivation period and did not change following stress (Smith et al., 1997). It was argued that the failure to increase CRH mRNA in deprived pups was a result of either: (a) a reduced capacity of the neonate to activate CRH, (b) that the stimulus was too mild, or (c) that CRH mRNA was measured 2 h following the initiation of stress and therefore sufficient time had not elapsed for gene expression to be manifested. Stroking reversed all of these changes in the central markers of deprivation (Van Oers et al., 1998). These results supported the hypothesis that the crucial element of the dam's behavior in the regulation of the central mechanisms controlling ACTH was licking/grooming. The importance of these behaviors in determining the outcome of several early experience manipulations was discussed previously.

Over the past decade, the concept of a stress hypo-responsive period has been so modified as to lose its original meaning. Although the adrenal of the neonate is insensitive to ACTH, this appears to be a process that is actively inhibited by aspect of feeding. However, the pituitary and hypothalamus can and do respond to stress, but do so in a manner idiosyncratic to the neonate. One characteristic of the developing HPA axis is that the response is more stimuli-specific. Recently, Dent et al. (2000b) have shown that the expression of CRH mRNA in the neonate is indeed responsive but gene expression in the pup follows a time course that has not been observed in the adult. The impetus for these studies was yet another attempt to determine changes in CRH as a consequence of maternal deprivation. Thus, in addition to examining mRNA, the primary transcript was also studied. The time course of hnRNA has been shown to be more rapid and is similar to that seen for ACTH. Thus, hnRNA and mRNA for CRH were measured following an injection of isotonic saline. The results were counter-intuitive, whereas non-deprived pups showed rapid expression of hnRNA. They also showed a similar rapid activation of CRH mRNA. In contrast, deprived pups showed a much more sluggish pattern of hnRNA and mRNA expression. There is an obvious paradox that requires some resolution. How is it possible for maternal deprivation to result in a pup that responds to relatively mild stress with increased ACTH and corticosterone secretion, shows increase c-*fos* in the hypothalamus but not have an equivalent change in the primary secretagog for ACTH? In adult rats, it has been shown that the pattern of secretagog release changes with chronic stress. Whereas following acute stress, CRH mRNA increases under conditions of chronic stress, there is a change in the relationship between CRH and AVP message and the primary secretagog now appears to be AVP. If CRH and AVP mRNA are measured in the deprived and non-deprived pups, the results indicate that for the neonate, the primary secretagog for ACTH may not be CRH but AVP. Only in pups that show an increase in ACTH do we see an increase in AVP mRNA (Dent et al., 2000a).

5. Concluding remarks

The studies reviewed here have attempted to demonstrate the importance of psychological variables in modulating one of the major stress responsiveness systems, the HPA axis. It should be cautioned that this is by no means an exhaustive review and that it focuses entirely on one biological system. Clearly, many neurotransmitters and other central nervous system structures play a role in regulating the central components of the HPA axis. There is now evidence that many of the variables discussed in this review also affect these other regulatory processes. What is striking is that we have been able to apply very sophisticated behavioral methodologies that have permitted us to understand some of the psychological parameters that are involved. There is, however, little information concerning the cellular and molecular changes that are involved in translating behavioral and environmental events into modifications of basic physiological processes. Psychobiology by definition cannot easily utilize in vitro methodology. One possible approach that may help unravel some of the mechanistic issues is to utilize the techniques of gene manipulation available through transgenic mouse preparations. The current available technologies may eventually permit us to understand which neural pathways are modified either permanently or transiently by psychological variables. What psychobiology has contributed is an impressive body of data that highlights the plasticity of the nervous system at all stages of development.

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