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Developmental Psychobiology

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Table of Contents

DEVELOPMENTAL PSYCHOBIOLOGY

Developmental Determinants of the Neuroendocrine Response to Stress

Hormones in Infancy

Conclusions

Bibliography

DEVELOPMENTAL PSYCHOBIOLOGY

Over the last few decades biological scientists have pursued problems that overlap the boundaries of several traditional disciplines. When this interdisciplinary effort has been particularly fruitful, these interface areas themselves have become formal disciplines; for example, biophysics, neurophysiology, neuroendocrinology, psychosomatic medicine, etc. The rapid growth in research activities at the interface of psychology and biology has necessitated the formalization of a new area—psychobiology. Psychobiology is a very extensive area that includes almost all aspects of the influence of biological systems on behavior and the influence of behavior on biological systems. However, within this classification of psychobiology a number of clear subdivisions are represented, such as brain and behavior, chemical modulations of behavior, and developmental psychobiology.

This paper is concerned with some aspects of developmental psychobiology; however, developmental psychobiology is a broad area that encompasses a number of diverse approaches to the problems of determining how events that occur during critical periods in ontogenesis influence subsequent physiological and behavioral functions. Developmental psychobiologists are interested in the effects of various alterations of environmental and physiological variables in infancy on subsequent physiological function and behavior, both during development and in

adulthood. An attempt at a broad review of this field would be a task of enormous proportions, eventually leading to several volumes of the magnitude of this *Handbook.* Thus, for the purposes of this chapter, we will limit our discussion to basically two phenomena that we hope will serve as examples of the field of developmental psychobiology.

The first of these is the effects of a variety of early experimental interventions, predominantly interventions of a psychological nature upon subsequent hormonal function, in particular the pituitary-adrenal system. The second will be a discussion of the effects of alterations in neonatal-hormonal environments on sexual differentiation, with particular references to sexually dimorphic behavior such as sex, aggressions, and emotions. I have taken this course chiefly because of the extensive body of information that is available and second, of course, because of my own involvement in these particular areas of investigation.

Developmental Determinants of the Neuroendocrine Response to Stress

Perhaps the most labile and responsive of all hormonal systems is that associated with the hypothalamo-hypophyseal-adrenal system that results in the ultimate secretion of corticoids from the adrenal cortex. The numerous stimuli that can activate this system are so diverse as to have resulted in the concept by Hans Selye of nonspecific stress. The range of environmental

events that lead to the ultimate release of ACTH from the pituitary and corticoids from the adrenal vary from seemingly innocuous situations, such as placing an organism in a strange environment, to the most severe traumatic tissue damage. The essentials of the system's operation in response to stress are as follows. Information concerning the stress (coming either from external sources or from internal sources such as a change in body temperature or in the blood's composition) is received and integrated by the central nervous system (CNS) and is presumably delivered to the hypothalamus, the basal area of the brain. The hypothalamus secretes a substance called the cortiocotropin-releasing factor (CRF) that stimulates the pituitary to secrete the hormone ACTH. This in turn stimulates the cortex of the adrenal gland to increase its synthesis and secretion of hormones, particularly the glucocorticoids. In man the predominant glucocorticoid is hydrocortisone; in many lower animals, such as the rat, it is corticosterone.

The entire mechanism is controlled by a feedback system. When the glucocorticoid level in the circulating blood is elevated, the CNS, receiving the message, inhibits the process that leads to secretion of the stimulating hormone ACTH. Two experimental demonstrations have clearly verified the existence of this feedback process. If the adrenal gland is removed, the pituitary secretes abnormally high amounts of ACTH, presumably because the absence of the adrenal hormone frees it from restriction of this secretion. On the other hand, if crystals of glucocorticoid are implanted in the

hypothalamus, the animal's secretion of ACTH stops almost completely, just as if the adrenal cortex were releasing large quantities of the glucocorticoid. However, one of the characteristics of this system is that there are wide individual differences among organisms in response to any given stimulus. This degree of variance could indicate either that a particular stimulus has a different meaning to different organisms or that the same stimulus is responded to differentially as a function of previous events.

This section of the paper represents an attempt to specify some of the factors that contribute to the origin of individual differences in the steroid response to stress. Although there will be no attempt to elaborate a theory to account for such individual differences in stress response, there is the suggestion that such differences originate in the CNS, and that the factors we will cite have their primary action on the organization of the CNS, mediating neuroendocrinological regulation of ACTH release and subsequent steroidogenesis. The steroid response, therefore, is a reflection of the interaction of an organism with its environment. Implicit in this interaction is a perception of the environmental stimuli and integration of the perception to produce a peripheral endocrine response. For such an integrative role the CNS must be the logical candidate. Although central nervous mechanisms governing individual differences in response to stress have not yet been specifically studied, it is difficult to conceive of a peripheral system with the integrative capacity to account for such differences.

Yates and Urquhart have postulated the concept of a centrally located hormonostat that regulates peripheral hormone levels. This concept suggests that there is a regulating mechanism, presumably located in the hypothalamus, that differentially responds to sensory input and peripheral hormonal concentrations to maintain a steady-state, equilibrium concentration for a given stimulus. Differences in the set of the hormonostat should, therefore, produce different peripheral responses to a given stimulus.

Regardless of the postulated central mechanisms to account for such individual differences, there is now clear evidence that major events that occur during sensitive periods of ontogenesis do indeed determine the manner in which organisms respond to a number of environmental events in adulthood. The major determinant of the organism's responsiveness with regard to pituitary-adrenal activity in adulthood appears to be the mother-infant interaction. Subsumed under this major class of environmental determinants are those studies which involve extra-stimulation of the developing organism, which have been called either handling or manipulation. I believe that the major influences of extra-stimulation result from an alteration of mother-infant interactions as a consequence of treating the young.

Maternal Influences

Extensive clinical and experimental data have indicated that manipulation of mother- infant interaction leads to profound and permanent changes in the subsequent behavior of the offspring. In spite of the large amount of evidence that exists relating maternal variables to behavior, there was, until recently, remarkably little information concerning the possible relationship of maternal factors to subsequent physiological function of the offspring. During the past several years a number of laboratories have been concerned with attempting to specify those factors in the life history of the organism which affect the activity of the hypothalamo-hypophyseal- adrenal system.

In a series of studies that were designed to investigate the effects of infantile experience on the development and maturation of the neuroendocrine regulation of ACTH and adrenocortical activity, it was found that handling infant rats as early as three days of age resulted in an increase in plasma corticosterone in the handled neonate following stress, whereas no such increase was observed in the nonhandled controls. The fact that nonhandled neonates do not respond to stress as early as three days of age is consistent with the general body of information that indicates that the neonate is generally unresponsive. However, the fact that the handled animals responded so very early in development, without what appears to be the appropriate neural and anatomical maturation for this response to occur, indicated that one of the possible mediators for the observed effects of

infantile handling on the pituitary-adrenal system in the newborn was an alteration in the maternal- infant relationships, either in terms of changing maternal behavior or perhaps of modifying the physiological interactions between mother and young. Support for this hypothesis came from preliminary observations in our own laboratory where it was observed in rats that mothers of handled infants had significantly higher plasma corticosteroids than did mothers of nonhandled infants at three days. Further, a study by Young indicated that lactating females showed a distinct preference on retrieval tests for warm, as opposed to cold, pups. These observations led to a series of investigations that studied the effects of various aspects of mother-infant environment on subsequent adrenocortical activity of the offspring.

Levine and Treiman studied, in mice, the role of both genetic and maternal environmental determinants on adult adrenocortical function. They measured circulating plasma corticoids following a brief exposure to electric shock and reported striking differences between four inbred strains of mice. The observed differences were quantitative in terms of the amount of circulating steroids following stress, and qualitative in terms of the different time course of the stress response between different inbred strains. Thus, the $DBA/_2$ strain showed the maximum elevation of corticosteroids approximately fifteen minutes after the base exposure to electric shock, but by the end of sixty minutes had begun to return to the normal basal levels. In

contrast, the C_{57} $BL/_{10}$ strain was still significantly elevated by the end of sixty minutes. Subsequently the two strains, C_{57} $BL/_{10}$ and $DBA/_{2}$, which had been shown to differ in the steroid response to electric shock, were crossed in all four possible combinations to provide a two by two diallel cross, allowing genetic and maternal effects to be assessed. The offspring were tested under two conditions as adults, control and electric shock. The control animals were removed from their cages and rapidly sampled for corticosterone. Shocked animals were placed in the shock compartment and given an electric shock to the feet for one minute. They were then placed in a holding cage and decapitated at one of three time intervals, following the termination of shock —one minute, fifteen minutes, and sixty minutes. The data indicated that the maternal environment is a clear and important modifier of the quantitative and qualitative aspects of steroid response to electric shock. Thus, C_{57} $BL/_{10}$ and C X D crosses, that is, hybrid mice whose mothers came from the C_{57} $BL/_{10}$ strain, showed identical patterns of steroidogenesis following stress. In contrast, the $DBA/_{2}$ and the D X C crosses, whose mothers were of the $DBA/_{2}$ strain, also showed identical patterns of elevations of plasma corticosteroids. Thus, the steroid response of the hybrid animals was dependent upon the mother of the hybrid. The hybrid animals with the C mothers were identical to their parent strain, C_{57} $BL/_{10}$ and in contrast, the hybrid offspring with D mothers were identical to their maternal strain.

Perhaps an even more dramatic series of studies demonstrating the role

of maternal influences on the adult patterns of corticosteroid response to stress are those reported by Denenberg and coworkers.'' Denenberg and coworkers have demonstrated that C_{57} $BL/_{10}$ mice when fostered to lactating rat mothers were markedly less aggressive, less active in an open field, and preferred a rat to a mouse in a two-choice, social-preference test. Further experiments established that one of the principal variables involved in the behavior was the rat mother.

These investigators further reported that when Swiss albino mice were fostered to rat mothers to study the relationship between open-field activity and corticosteroid response following exposure to the open field, it was found that rat-reared mice gave a significantly lower corticosterone response to the novel stimuli of the open field than mouse-reared mice, or rat-reared rats. The mouse reared by the rat mother showed a marked modification of its plasma corticosterone response following exposure to novelty. The rat mother could be influencing the mouse offspring either through her behavioral interactions with the pups between birth and weaning or through biochemical factors present in her milk.

In a recent experiment, in order to bypass the problems of rat milk influencing the mouse offspring, Denenberg and coworkers reared mouse offspring with nonlactating adult female rats—"aunts"—together with pregnant female mice in the expectation that the rat would engage in the

usual caretaking activities while the mouse would supply the milk for the young.

In one experiment, mice tended by rat aunts had a significantly lower corticosterone response to novel stimuli of being isolated for thirty minutes. In addition, the aunt-reared group was less active in the open field than the control group. In a second experiment, testing was approximately six months after weaning. Again, significant corticosterone differences were obtained with a group tended by aunts, yielding a lower value than controls. As in the first experiment, the aunt-reared group again had lower activity.

From these experiments it can be concluded that changes in adrenocortical activity and open-field behavior are brought about through behavioral mechanisms involved in the interaction between mothers and young rather than through biochemical differences in the milk of the rat and mouse mothers. These differences also appear to be permanent and profound and persist well into adulthood.

There are several further studies that also clearly indicate the maternal influence on subsequent pituitary-adrenal activity in the offspring. Denenberg and Whimbey reported that in rats the offspring of mothers that had been handled in infancy were heavier at weaning than young rats of mothers that had not been handled. Further, the experience of the mother during her

infancy resulted in different open-field behavior of the pups when they reached adulthood. It has been reported that the offspring of mothers that had been handled in infancy show a reduced plasma steroid response to novel stimuli when compared to weaning rats of nonhandled mothers, although both groups of offspring themselves received no experimental intervention. Further, if the offspring are handled, the differences resulting from maternal differences are abolished. This can be interpreted in either of two ways: first, direct stimulation of the pups is so profound that it overrides the maternal influence or, second, handling the infant alters the maternal responses, and disturbance of the mother as a function of infantile handling tends to counteract the influence of the experience of the mother during her infancy. The work reported in this paper tends to favor the second of these interpretations.

Thus far we have discussed a variety of experiments that have resulted in a reduction of the plasma corticosterone response to stress in the offspring of both rats and mice, and mice reared with rats. Maternal influences, or the lack of maternal influences, can also lead to a significant increase in plasma corticosterone response to a variety of environmental stimuli.

Exposing a lactating female to a stressful experience results in a modification of the stress response in the offspring. Lactating females were subjected to ether exposure at three, six, or nine days postpartum. An

additional group of mothers was subjected to electric shock at three days following birth. The offspring of these females were not disturbed during this period. The data indicate that the offspring of females stressed while nursing showed a significantly greater elevation of plasma corticoids following exposure to novel stimuli when compared to nontreated controls. The absence of a mother during nursing in the rat also leads to a significant augmentation of the response to stress in maternally deprived rat infants.

A technique has been developed for hand-rearing newborn rats. Thus rats can be separated immediately after birth and reared successfully through weaning by the use of the specific set of techniques, which have been described in detail by Thoman and Arnold. This technique involves rearing the animals in an incubator in which there is a warm, moist, pulsating tube that serves as a surrogate to provide warmth and stimulate defecation in the newborn rat. The animals are tube fed at four-hour intervals until they are capable of eating solid food. A group of these hand- reared animals was tested in adulthood for their adrenocortical response to ether. Hand- reared animals showed a significant elevation in basal levels and a significantly greater increase in plasma corticosterone concentrations following stress than did mother-reared animals. Although these data tend to implicate a maternal factor, it should be noted that this procedure is a complicated one involving a large amount of stimulation in the process of hand feeding as well as different dietary conditions, in addition to the many other conditions that differ from

normal rearing. Although the evidence appears to indicate that the absence of a mother results in altered pituitary-adrenal activity in adulthood, it would be difficult to specify that only removal from the mother leads to the differences observed in these experiments.

It is surprising that while there is a large and extensive literature that demonstrates the very profound influences of maternal deprivation in certain primates, there has been an almost total absence of an examination of the physiological influences of these procedures on the adult monkey. One can only hope that, with the availability of better techniques for measuring circulating cortisol in the primate, these studies will soon be accomplished.

Data presented above indicate that the nature of the mother-infant environment is an important determinant of subsequent neuroendocrine regulation of ACTH. The data presented along with the extensive literature on maternal variables affecting behavior emphasize the total organismic effect of those events occurring during critical periods in development. In the mammal this critical period is intimately shared with the mother and it is not surprising, therefore, that maternal variables should affect many aspects of the total system's function.

Infantile Stimulation and Stress

Early research on the influence of infantile stimulation on behavior

dealt with rats treated immediately postnatally either by simply being picked up once daily and placed in a different environment or by being given a brief electric shock once daily from the period immediately following birth until weaning at twenty-one days of age. In adulthood, when placed in a novel environment, these animals explored more freely and defecated less and when placed in avoidance-conditioning situations, they appeared to show "more adaptive behavior" by learning the avoidance conditioning significantly more rapidly. These results also indicated no difference in the later effects between a seemingly innocuous treatment such as simply manipulating the animal or more severe procedures such as shock and shaking. It was therefore concluded that the effects of infantile stimulation were a direct consequence of "stressing" the infant organism and that such stressful infantile experiences resulted in the animal's developing a capacity for being more adaptive. Implicit in this conclusion is that infantile stimulation provides a form of emotional immunization. That is, exposing an animal to stress early in ontogenesis modifies its subsequent stress response such that the emotional response is of a lesser degree, and the animal does perform in a more optimal fashion. The physiological effects of infantile stimulation tended to support this conclusion. Levine and Otis demonstrated that animals that had been stimulated in infancy survived longer under a severe chronic stress of total food and water deprivation.

Other studies indicated that adrenal hypertrophy, which occurs under

conditions of chronic stress, was significantly greater in nonmanipulated animals. It is important to note, however, that these experiments dealt with animals placed in relatively chronic situations leading to a prolonged stress response and that these sustained responses can and often do lead to pathological changes in the organism. Experiments based on more acute stressful conditions, both in the infant and the adult organism, tended to indicate that quite contrary to the original hypothesis, the stimulated animal was indeed more responsive to stress under some conditions. This has been demonstrated in an investigation of the effects of infantile stimulation on the acute response to a brief electric shock. Adult rats subjected to a brief electric shock were sampled at various periods of time following exposure to the stimuli, and their plasma corticoids were determined. The manipulated animals showed a much more significant rise and, for the period measured, showed a more sustained increase in adrenal steroids. Further studies have demonstrated, however, that although the stimulated animals do show a more rapid elevation of plasma corticoids, thus indicating a more immediate secretion of ACTH, they do tend to return to base levels significantly sooner than do nonmanipulated animals. It is clear from these data that the simple proposition that infantile stimulation reduces physiological and emotional responsiveness to stress was in error and, in fact, the animals manipulated in infancy appear to be more sensitive to their environment. On the basis of this experiment, an alternate hypothesis was developed that postulates that one

of the major consequences of early stimulation may be to endow the organism with the capacity to make finer discriminations concerning the relevant aspects in the environment. The animal then is able to make responses more appropriate to demands of the environment, including appropriate responses to stress. Perhaps this is the real meaning of adaptiveness, the ability to make the appropriate discrimination in a particular situation and respond according to the demands of that situation. A more generalized response pattern, based on gross discriminations or, in fact, a lack of discrimination, appears to be characteristic of the nonmanipulated animal and leads to responses that are often inappropriate to the situation. Such responses may be viewed as maladaptive. Having thus postulated that the manipulated animals possess the capacity for more appropriate discrimination, we should then be able to predict that their responses to novel situations, where the environmental changes are not so drastic, would be much less than that of nonmanipulated animals.

In an experiment conducted by Levine et al. rats were handled for the first twenty days in infancy and compared to nonhandled controls. In adulthood these animals were subdivided and tested in open field for one, two, three, or four days. Activity and defecation in the open field were recorded and, in addition, following the termination of testing, the animals were killed either immediately, five minutes afterward or fifteen minutes afterward, and plasma corticosterone was determined. Animals handled in

infancy were more active in the open field on the last three test days, defecated less on all of the test days, and had a significantly lower corticosterone response on all four of the days. These data allow one to draw the conclusion that stimulation in infancy results in an animal that is less responsive to novel stimuli as measured both at the behavioral and physiological level. Thus, to reiterate, in situations where distinctly noxious stimuli are involved, the handled animal seems to be more active in terms of its pituitary-adrenal system. However, where the test situation does not involve intense noxious stimulation, the steroid response of the handled animal is of a smaller magnitude. The nonhandled animal appears to discriminate less and reacts with a large corticosterone response regardless of the specific aspects of the test situation.

Development and Early Experience

Thus far we have been concerned predominantly with the long-term effects of variations in the environment of the neonate. The psychobiologists, however, have also investigated the developmental consequences of early environment. The results of these studies have found that the rate of development is also dependent upon variations in the infantile environment. It has been observed that stimulated organisms are heavier at weaning and maintain these weight differences through adulthood. The normal time of eye opening in nonstimulated animals is approximately fifteen to sixteen days.

Stimulated rats open their eyes at about thirteen to fourteen days, and eye opening has been observed as early as twelve days of age. Following these observations of differences in the development of gross morphological characteristics, there has been a series of investigations into the effects of early stimulation on other aspects of development. In the first of these studies, the maturation of the hypothalamo-hypophyseal-adrenal system was studied. Prior to the advent of appropriate measures of circulating plasma corticosterone, one response of the rat adrenal to ACTH that was used as an indicant of adrenal function was the depletion of ascorbic acid present in the adrenal. It had been demonstrated that infant rats do not respond to environmental stress with depletion of adrenal ascorbic acid until about sixteen days of age. This was true in nontreated animals. However, animals handled in infancy showed a significant adrenal, ascorbic-acid response as early as twelve days of age.

Since the maturation of the neuroendocrine regulation of ACTH is indicative of one aspect of neural development, which appeared to be accelerated as a consequence of infantile stimulation, it seemed reasonable that other aspects of neural development would also show similar acceleration. Thus myelination in the CNS has been shown to occur earlier in animals stimulated neonatally. Meier demonstrated an earlier onset in adult EEG patterns in Siamese kittens that had been handled. These findings would indicate that neural maturation is accelerated as a consequence of infantile

stimulation. Altman et al. have investigated whether handling during infancy has any effect upon the development of the brain, with particular attention to the rate and kinetics of cell proliferation and other quantifiable aspects of brain development. Rats were handled daily from day two today eleven after birth. These animals and unhandled controls were injected on day eleven with radio- actively labeled precursor of DNA and killed either six hours, three, or thirty days later. In a subsequent experiment, uninjected rats were permitted to survive until eleven, fourteen, forty-one, or one hundred and one days of age. Brain-weight measurements were taken in all animals, and the brains were compared for histology and autoradiography, and evaluated. These investigators found the following differences between the brains of handled and nonhandled animals: (1) the brains of the handled animals were consistently lighter than controls at eleven and fourteen days of age and these differences were not associated with differences in body weight; (2) planimetric measurements of sampled regions show that the brain-weight differences were correlated with areal size differences, and (3) autoradiographic cell counting indicated that cell proliferation and the formation of new micro-neurons were higher following injection of the radioactive precursor in the handled animals than in nonhandled rats.

Thus, in contrast to the aforementioned studies that have indicated more rapid neural development as a consequence of handling, the weight and areal measurements indicated that the brains of the handled animals were

retarded in development. The autoradiographic cell counts also appeared to indicate some aspects of retarded brain development in the handled animals. In the nonhandled animals cell proliferation declined by the latter half of the second week. The cell proliferation was still brisk during this period in the handled animals, which would indicate further a delay in brain maturation. Altman interprets his data as follows:

> How can this effect, the decelerated maturation of the brain, be related to the adaptive superiority displayed by handled animals in adulthood? We have postulated elsewhere that the postnatal origin of the modulatory micro-neurons in some brain structures may represent an opportunity for the exertion of input-regulated (behavioral) control over the finer aspects of the interconnection of neurons. This would imply that delay in the proliferation and migration of the precursors of micro-neurons extends the time available for the exertion of environmental modulatory influences on the organization of the brain. This hypothesis is akin to the concepts of "fetalization"718 and "infantilization" postulated by writers on anthropogenesis, which denote the retention of fetal properties after birth and excessively prolonged postnatal development, respectively, as biological characteristics of man. These evolutionary adaptations are conceived to provide longer opportunity for the exertion of environmental influences on the organization of the control mechanisms of behavior. The specific hypothesis that we are presenting here is that infantilization, which is a property shared by all altricial species, is not entirely genetically determined but is subject, during a critical period of development, to environmental influences, such as some factor (stress?) inherent in the handling procedure. [p-19]

Although it is difficult at this time to reconcile those studies which report an acceleration in the maturation of certain neural systems and the study by Altman, which shows a retardation of some other neural systems, it

is clear that there are definitive environmental influences on the maturation of the structure and function of the brain. These alterations in neural development and function are observed throughout the life history of the organism in alterations of behavioral and physiological processes.

Hormones in Infancy

Thus far in this paper we have dealt primarily with the influence of various environmental conditions upon the subsequent neuroendocrine activity of the adult animal. However, this section of the paper deals with another experimental approach used by the developmental psychobiologist, namely, altering physiological processes during infancy and studying the influence upon subsequent development and behavior. The best example of this type of investigation comes from a now extensive series of studies that have investigated the influence of the presence or absence of specific gonadal hormones during critical periods in development on adult, sexually dimorphic behavior.

Under normal circumstances, the adult female rat becomes sexually receptive during a period in the estrous cycle when there exists the appropriate hormonal balance between estrogen and progesterone that results in ovulation. Sexual receptivity of the female rat is manifested by the presence of a lordosis response. If the female is deprived of the appropriate

circulating hormones by ovariectomy, sexual receptivity is immediately abolished. However, when the appropriate hormones are replaced either in the form of chronic high doses of estrogen or small doses of estrogen followed by progesterone, sexual receptivity appears within a very short time following progesterone administration. Sexual behavior of the male involves a much more complex pattern of mounts with intromissions and ejaculation. In contrast to the cyclic pattern of receptivity exhibited by the female, the adult male is acyclic in his sexual behavior and will under normal conditions copulate as long as there is an appropriate stimulus object. Again in contrast to the female, when the male is castrated there ensues a period of time during which the male is sexually active even in the absence of circulating androgens. However, eventually the male will cease normal sexual activity. But, following androgen replacement, it will resume behavior that is indistinguishable from that of the normal intact male. However, no amount of estrogen and progesterone has yet proved capable of reliably eliciting in an adult castrate male patterns of sexual behavior that are typical of the normal female. Adult castrate females administered testosterone propionate (TP) will exhibit mounting and mounts with behavioral patterns that closely resemble intromission.

It has been suggested' that gonadal hormones act on the central nervous system in different ways at two different stages of development. First, during fetal or neonatal life, sex hormones organize the sexually undifferentiated

brain with regard to patterns of gonadotrophin secretion and sexual behavior. Specifically, this hypothesis states that androgens, acting on the central nervous system during critical periods in development, are responsible for the programming of male patterns of gonadotrophin secretion and sex behavior in much the same way that they determine the development of anatomical sexual characteristics. Second, during adult life gonadal hormones activate the sexually differentiated brain and elicit the responses that were programmed earlier. Third, one of the components of the process of sexual differentiation is to render the tissues that are responsive to gonadal hormones differentially sensitive in the male and the female.

The evidence regarding normal patterns of sexual behavior and their dependency upon circulating hormones is consistent with the hypothesis that there are differences between the male and the female central nervous system with regard to patterns of hormone secretion and behavior. First, whereas the female is cyclic in her sexual activity, the male tends to be acyclic. Second, the female pattern of sexual receptivity is easily elicited with the appropriate regime of estrogen and progesterone replacement following castration, whereas in the male these patterns appear to be completely suppressed and cannot be elicited with doses of estrogen and progesterone that are a thousand-fold higher than those required in the female. Thus, one of the primary aspects of sexual differentiation in the rat appears to be the suppression of the capacity of the normal male to respond to estrogen and

progesterone.

Although the designations male and female have been used in a seemingly specific way with regard to sexual behavior, there are many experiments that cast doubt upon the validity of making behavioral distinctions between male and female rats. While normal male rats almost never exhibit any of the kinds of behavior that female rats show during estrus, lordosis cannot be called a genetically determined “female” response, since it will be shown that males castrated at birth will exhibit this behavior when administered estrogen and progesterone. As has been mentioned, normal female rats will often perform behavioral mounts and intromission patterns identical in form to those exhibited by male rats, although the quantity and temporal patterning of these responses is different. Behavioral ejaculation has been reported in genetic females.

These facts, coupled with the occurrence of mounting behavior in the prepuberal play of both sexes, make it very difficult to make rigorous behavioral distinctions between male and female rats. One way of establishing criteria for the validation of male and female behavior is to use as a basis the behavioral patterns necessary for successful reproduction. Thus, mounts and intromissions and ejaculation patterns could be termed male reproductive behavior, since these are the patterns that animals must exhibit in order for fertilization to take place. This, however, leaves the problem of

finding some new term to describe the mounting that is done by females. Another, and perhaps the most satisfactory way of dealing with this problem, is to describe the behavior involved with no reference to its maleness and femaleness. Thus, a lordosis is a given pattern of behavior without regard to the genetic sex of the animal performing it. In the same way, a mount is a behavior pattern that involves a given sequence of motor acts. Since the experiments we will describe involve many situations in which genetic males and females are treated with homotypical and heterotypical hormones, the definitional problem is best handled by referring to these patterns themselves rather than to the maleness or femaleness of a given behavior.

In 1959, Young and his colleagues reported that administering testosterone to the pregnant guinea pig resulted in the birth of pseudohermaphroditic female offspring that failed to respond normally to gonadal hormones in adulthood. As adults the female offspring of testosterone-treated pregnant guinea pigs did not become receptive to males when treated with estrogen and progesterone. They did display an unusually high frequency of mounting responses when administered with testosterone. These findings were interpreted to indicate that gonadal hormones played a crucial role during development in a differentiation of neural tissues that mediate sexual behavior in the adult organism.

Research on the rat also indicated that if large amounts of TP were

administered to the newborn female rat within the first five days of life mating behavior was abolished. However, in these experiments, mating behavior was defined as the presence or absence of sperm in the vagina when placed with a vigorous sexually active male. In 1962, Harris and Levine injected five-day-old female rats with a single dose of 500 μg of TP. As adults these animals failed to exhibit lordosis when injected with large doses of estrogen and progesterone. Goy, Phoenix, and Young gave 1 mg of TP for seven consecutive days beginning on days one, ten, or twenty after birth. Those female rats receiving injections starting on day one and ten showed markedly decreased female sex behavior when given estrogen and progesterone in adulthood. Thus, it appeared as though the presence of androgen prenatally in the guinea pig and postnatally in the rat was capable of abolishing the normal patterns of receptivity in the female that are elicited by estrogen and progesterone replacement. However, Barraclough and Gorski' reported a behavioral dichotomy between female rats receiving high and low doses of TP neonatally. Whereas females receiving 1.25 mg of TP at five days of age would not exhibit lordosis, even when primed with estrogen and progesterone following castration in adulthood, females receiving 10 μg of TP were consistently sexually receptive when intact and did respond to estrogen and progesterone after castration.

Levine and Mullins administered to independent groups of neonatal females doses of TP ranging from 5 to 1000 μg. These females fell into three

distinct groups with regard to their capacity to exhibit lordosis in adulthood. Animals receiving 5 to 10 μg of TP in infancy showed the pattern of behavior reported by Barraclough and Gorski of continual receptivity on seven consecutive nights. Animals receiving 50 μg of testosterone tended to show receptivity early in the testing procedure, but toward the latter part of the test series showed a marked drop in female sexual receptivity. Finally, animals receiving 100 μg or above showed very low mount-to-lordosis ratios.

It is interesting to note that although the low-dose females were judged to be receptive, their behavior was quite different from that seen in normal estrous females. The darting, hopping, and ear wiggling were almost entirely absent. The passivity of these animals was exhibited by the nature of the lordosis. Untreated female rats will lordose when mounted by the male, but will hop away and often groom as soon as he dismounts. The TP females, however, would frequently freeze with their head near the floor and their hindquarters elevated and hold this position until the male ejaculated. When these females were judged nonreceptive, many just sat with their backs hunched while the male attempted to mount instead of exhibiting the vigorous back kicking seen in untreated nonreceptive females.

In this experiment, all females were ovariectomized and, following a two-week period, were injected with estrogen and progesterone. It is interesting to note that the lordosis to mount ratios observed with estrogen

and progesterone replacement were identical to those observed during the first night of testing when intact. These results appear to be another example of the differential sensitivity of the neural tissues mediating sexual behavior and to indicate that all the circulating hormone is able to replace is that level of behavior which will normally be exhibited when the animal's own endogenous hormones are active. These data do raise many questions, however, concerning the capacity of neonatal androgen to masculinize female rats. Under all doses of androgen the ovaries are atrophied and there is no evidence of ovulation in any of the TP- treated groups. However, the low dose of TP treatment appears to produce an organism that is by no means incapable of exhibiting lordosis, albeit in many of these animals the pattern of lordosis appears to be aberrant. The data concerning neonatal androgen treatment in females tend to be ambiguous. Whereas the patterns of gonadotrophin secretion always appear to be acyclic, the effects of sexual behavior are paradoxical.

In contrast, it does appear that removal of the androgens prior to sexual differentiation uniformly leads to marked behavioral and physiological feminization of the animal. Thus, male rats that have been castrated twenty-four hours after birth appear capable of exhibiting cyclic patterns of gonadotrophin secretion that result in cyclic ovulation.

Grady, Phoenix, and Young castrated male rats on days one, five, ten,

twenty, thirty and ninety after birth and administered estrogen and progesterone to these animals when they were one hundred and twenty to one hundred and fifty days of age. Animals castrated within twenty-four hours after birth displayed a lordosis response that was indistinguishable from that of normal female rats when mounted by sexually active males. Those animals which had been castrated at five days of age showed a marked reduction in lordosis, while those castrated after five days of age failed to display lordosis when mounted. This finding has been replicated by several investigators.' Whalen and Edwards reported further that males that had been castrated in infancy and given 2.5 mg. of TP at the time of castration failed to exhibit the lordosis response following estrogen and progesterone in adulthood. In a similar study, Mullins and Levine demonstrated that doses as low as 10 μg of TP given to the neonatal castrate at ninety-six hours after birth were also capable of suppressing lordosis in the adult male animal when given the appropriate hormonal treatment in adulthood. It should be noted that this dose of androgen given to the female does not abolish the lordosis response and, in fact, results in an animal that is continually responsive to a sexually active male. It appears that even at birth the genetic male is differentially sensitive to androgen. Thus, whereas androgen given to the female does not suppress sexual receptivity, androgen given to the male deprived of its gonads at this time strikingly suppresses sexual receptivity. One possible explanation for this suppression of the lordosis response in

these males is the possibility that these animals have had functioning testes present until shortly after birth. The secretions of the fetal gonads may already have sensitized the neural mechanisms that mediate the lordosis response, so that the injection of TP four days after castration resulted in the observed suppression. Further evidence of the ability of androgen present in infancy to sensitize neural mechanisms to later injections of androgen is given by Morrison and Johnson. A genetically determined sensitivity to neonatal testosterone might also explain the difference observed between the males and females that received the lower doses. Since the females given the lower doses of TP did exhibit lordosis while the females with higher doses did not, it is possible that more than one injection of the smaller amounts would be necessary in order to duplicate the conditions found in the normal male. However, it should be noted that at all doses of androgen the male suppression of the lordosis response is always greater than that observed in the female.

Thus far we have focused primarily on the presence or absence of the estrogen-progesterone-induced lordosis response with little mention of those behavior patterns normally associated with the "male." Although it does appear necessary to bring these patterns of behavior into this discussion, they are confounded by many of the manipulations performed neonatally. As mentioned before, the female appears to have represented in the CNS the capacity to exhibit mounting and mounting with intromissions. Although

Harris and Levine reported that female rats treated with 500 *fig* of TP ninety-six hours after birth showed more frequent and vigorous mounting than control females, this result has not been systematically reproduced. Finally, males castrated in infancy do not develop a normal penile structure, and the cornified papillae of the penis appear to be insensitive to androgen treatments later in life. It thus becomes difficult to discuss intromissions and ejaculations in organisms that have deficient penile structure.

Throughout this paper we have made the assumption that the function of gonadal hormones in infancy is to organize the CNS with regard to neuroendocrine function and patterns of behavior.

It is important to note that the concept of organization of the CNS does not necessarily imply that there are structural changes in the brain as a consequence of these gonadal hormones. In fact, it appears that one of the major influences of testosterone on the developing brain is to alter the thresholds of sensitivity to hormones in adulthood. Males that have been castrated in infancy are now rendered sensitive to minute quantities of estrogen and progesterone and will under these treatments exhibit normal lordosis. In contrast, neonatal androgenization of the female abolishes the behavioral response to progesterone; similarly, neonatal castration makes the male rat subject to progesterone facilitation. Clemons et al. have proposed that the reason female rats that have been treated with androgen do not

exhibit lordosis is because they are now insensitive to progesterone treatment. There have been many studies that have also shown that thresholds of response to estrogen are clearly depressed in androgenized females.

Although the relationship between differentiation of male and female behavior patterns is still unsettled, the organizational effects of early androgen might conform to a “one anlage” model of differentiation in which a single undifferentiated mechanism in the brain of the fetus is influenced by androgen to develop in the male direction. This is analogous to the genital tubercle that differentiates either to a penis or to a clitoris, with intermediate forms but not dual structures as possible outcomes of incomplete differentiation. Alternatively, a “two anlage” model might apply, resembling the process of reproductive-tract differentiation. In this case, although the development of the male primordium usually parallels the suppression of the female primordium, both are actually retained, albeit vestigially, into adulthood, and the possibility exists of simultaneous development of both primordia. In favor of the double anlage hypothesis is the well-established fact that normal adults of both sexes can respond to heterotypical gonadal hormones by showing heterotypical sexual behavior.

Although we have focused, thus far, primarily on reproductive behavior, there have been numerous reports in the literature that have indicated that

there are sex differences in nonsexual behavior.

In recent years, we have seen a growing emphasis on the physiological mechanisms regulating aggressive behavior. Aggressive behavior is generally studied in the laboratory by two principal methods. The first is the observation of spontaneous aggression that occurs frequently in laboratory mice and is usually seen when individuals are exposed to each other following a fairly long period of isolation. Spontaneous aggression is sexually dimorphic, occurring predominantly in males and rarely in females. The second laboratory method utilizes a procedure originated by Ulrich and Azrin called shock-induced aggression, and is used generally in rats. Pairs of animals are placed in a small compartment and a train of electric shocks is delivered, in response to which the animals take a characteristic fighting posture, strike at each other, and usually show a full pattern of fighting behavior. This is also sexually dimorphic, as it is elicited more easily in male than in female rats.

The role of androgens in the regulation of spontaneous aggression was noted as early as 1947 by Beeman. Subsequent studies have all indicated that castration usually inhibits or markedly suppresses this behavior. In contrast to castrated males, which show the full pattern of isolation-induced aggressive behavior following testosterone replacement, females do not show spontaneous or androgen-induced aggression. Normally male mice did not

show spontaneous aggression against females. However, Mugford and Nowell have shown that the tendency for male mice to attack females was increased significantly if the females were given a course of testosterone treatments.

On the basis of previous work, Mugford and Nowell concluded that the change in androgenized female mice was not due directly to some pheromone that is released as a consequence of androgen, but rather that the female releases a pheromone in her urine that normally inhibits attack and that the treatment with androgen appears to suppress this urinary substance, thus changing the female's stimulus properties.

In view of the highly predictable sexual dimorphism in aggressive behavior in mice, it seems natural that the possible "organizational" role of testosterone in differentiation of aggressive behavior should receive considerable attention.

Conner and Levine have demonstrated that neonatally castrated male rats show all the characteristics of the female when tested for shock-induced aggression. Castration at weaning tends to suppress aggressive behavior, but it is fully restored when testosterone is administered in adulthood. However, testosterone replacement in adulthood does not influence the aggressive behavior of neonatal castrates. Female mice given an injection of testosterone on the day of birth and then given androgen in adulthood show aggressive

behavior comparable to that seen in male mice. Furthermore, male mice castrated on the day of birth are less aggressive following androgen-replacement therapy in adulthood than males castrated on the tenth day of life.

Similar results have been obtained by Bronson and Desjardins. These investigators have reported that single injections of testosterone were most effective in facilitating aggressive behavior in adulthood when administered to the female on the day of birth and less effective when given after that time, becoming ineffective some time between the twelfth and twenty-fourth day. Also, neonatal androgen was effective in enhancing adult aggressiveness only if it was again administered before testing. The implication of both of these studies is that early androgen treatment sensitizes appropriate neural elements to androgen encountered in adulthood. The same conclusions can be reached on the basis of studies using shock-induced aggression in rats.

More recently Edwards and Herndon have shown that neonatal estrogen treatments to female mice mimic the effects of neonatal androgen, in terms of facilitating the differentiation of androgen sensitive mechanisms for adult aggressive behavior. Thus, ninety percent of the pairs of females given neonatal estrogen fought when treated with androgen in adulthood, compared to twenty-five percent of control females and one hundred percent of testosterone-treated females.

Further evidence of the control of sexually dimorphic behavior by neonatal hormone treatments comes from experiments on the learning of an avoidance response in the rat. The procedure consists usually of presenting a rat with a conditioned signal—a tone, buzzer, light, etc.—followed closely by electric shock. The animal can usually cross a barrier to another compartment to either escape further shock or avoid it by responding to the signal prior to the onset of the shock. It has been reported that normal females tend to learn the active avoidance response more rapidly than do normal males. The Beattys found that castrating male rats in adulthood did not influence avoidance conditioning. Testosterone injections to females in infancy, when combined with testosterone treatment in adulthood, produced rats whose avoidance behavior was masculinized in that they showed the same deficit in avoidance learning shown by males.

Further evidence of modification of sexually dimorphic behaviors comes from Pfaff and Zigmond who studied yet another behavior that usually shows sex difference, namely, timidity or emotionality as exhibited by activity and defecation in an open field (a circular arena usually brightly lit). Commonly, females tend to be more active in the open field and to emerge from the home cage faster than males. Neonatally castrated male rats tend to behave more like females and neonatally androgenized females more like males in both the open- field test and tests of emergence. Similar findings have been reported by Gray and Levine and by Swanson.

In the question surrounding the problem of the cellular mechanisms of the differentiating action of androgen on the brain, certainly the first step is to characterize the physiological or biochemical differences between the brains of males and females, and this can hardly be said to have been accomplished. A promising development has emerged from the ultrastructure studies by Raisman and Field on projections from the amygdala to the hypothalamus. They have found that the ratio of the number of preoptic region synapses ending on dendritic spines to those ending on dendritic shafts was significantly lower in male than in female rats.

Several laboratories have sought changes in RNA or protein synthesis in relation to androgenization. Shimada and Gorbman found evidence for synthesis of new species of RNA in the rat forebrain. Clayton, Kogura, and Kraemer report that neonatal testosterone significantly affects synthesis of labeled RNA from tritiated uridine in the amygdala and preoptic areas of the female brain. Using a different autoradiographic technique, MacKinnon has found changes in protein synthesis in roughly the same two regions of the mouse brain in relation to puberty and the estrous cycle. No effects of neonatal androgen on brain uptake of testosterone could be demonstrated in the female rat. Several investigators have reported lower retention of H-estradiol in brain tissue from androgen-sterilized females, but these data do not seem to be consistent. At any rate, any differences in the uptake of hormones that may exist between males, females, and neonatally

manipulated animals would seem to be rather small.

Conclusions

In this paper we have attempted to do two things: first, to communicate a body of information illustrative of the very profound effects that alterations in the neonatal environment, whether they be endogenous or exogenous, have upon the subsequent function of the developing organism; second, to define an area called developmental psychobiology and to illustrate how the developmental psychobiologist proceeds to understand the nature of his universe. Developmental psychobiology is in its infancy. This is indeed a truism as it can be said of many of the areas of investigation that attempt to view the organism as a totality and to step across the traditional limitations of defined disciplines. I believe that one can only achieve a full understanding of the organism's function by viewing the organism ontogenetically.

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