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The Development of Phenotypic Plasticity: Where Biology and Psychology Meet

"The mechanisms that underlie much of behavioral evolution may reside in the processes studied by developmental psychobiologists . . . " Michel & Moore, 1995, p. 178.

The counterpart in biology of developmental psychobiology is phenotypic plasticity, or the process by which the internal milieu and the environment induce different phenotypes from a given genotype (Sarkar, 1999; Sarkar & Fuller, 2003). After the rediscovery of Mendel's work in the early 1900s, some of the first geneticists were occupied with the problem of how animals and plants that reproduced by cloning or were of fixed genotypes could develop very different morphologies in different environments. As genetics developed, however, this question dropped to the wayside in the United States despite the best efforts of T. Dobzhansky and his student, Richard C. Lewontin (2000). Recently, phenotypic plasticity has been rediscovered in a variety of biological disciplines, from molecular genetics to evolutionary biology. But in developmental psychobiology, there has long been an interest in how behavior emerges (Gottlieb, 1992), and the approaches and concepts of this rich field have much to contribute to this newly emerging field in the biological sciences.

Much of my work has addressed fundamental precepts in behavioral neuroendocrinology, focusing in particular on the evolution of reproductive behaviors and the neural mechanisms that control them (Crews, 2002). To this end, I have capitalized on experiments of nature, or naturally occurring species. But as a student of Danny Lehrman and Jay Rosenblatt at the Institute of Animal Behavior, I was familiar with the principles of developmental psychobiology, and the last decade has found me coming full circle back to these roots, largely for the reasons captured in the quote of George Michel and Celia Moore (1995) at the beginning of this article.

It is in the arena of sex and sexuality that the biological concepts of phenotypic plasticity and the psychobiological principles of development come together. To illustrate this, I will begin by considering the distinction between sex and sexuality and how the study of the former informs, but does not explain, the latter. In so doing, I will describe an animal model system my laboratory has studied for the last decade, one that obviates some of the problems that are inherent in conventional animal models. This work focuses on what constitutes experience and how it influences the development of adult sexuality. Here, I consider the temporal nature and source of experience and how this might affect brain areas that are involved in the display of aggressive and sexual behaviors. I also will present some data on how changes in the brain can constrain future behavior before ending with some comments about transgenerational effects.

SEX VERSUS SEXUALITY

The development of adult sexuality is a case study in phenotypic plasticity, and one where developmental psychobiology has made important contributions. Beginning as a single cell, a unique individual emerges. This is the essence of distinction between sex and sexuality (Crews, 1998, 1999). That is, sex refers to discontinuous and discrete traits such as the genetic complement and/or gonad type that categorize individuals whereas sexuality goes beyond the components of sex and represents the continuously variable suite of traits that emerge during the organism's lifetime and make each individual unique. Because theorists and researchers have tended to emphasize population characteristics, we have not progressed very far in understanding the origins of an individual's sexuality. It is time to turn our attention to some of the sources of this individual variation.

Received 4 January 2002; Accepted 22 January 2003
Correspondence to: D. Crews
Contract grant sponsor: NIMH
Contract grant number: 57874
Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/dev.10115

The first phase in this process I have termed *Primary Organization*. This refers to the genetic and hormonal determination of the primary and secondary sex structures and includes the sexual differentiation process that follows gonad determination of the morphological, physiological, behavioral, and neural traits that are the elements of sexuality.

But the factors that determine gonadal sex do not dictate sexuality. Sexuality results from heritable genetic variation and its sequelae as well as nongenomic factors. These include, but are not limited to, (a) sex steroid hormones, (b) the behavioral and physiological condition of the mother, (c) the environments encountered throughout life, and (d) age and sociosexual experiences. This process I have called *Secondary Organization*.

So sexuality emerges as the organism accumulates experiences throughout the life cycle and also as it undergoes the morphological and physiological transformation that establishes the individual as a reproductive adult. Thus, there are two sources of stimuli that comprise experience: those arising from within the animal and those from without. Of course, there also is the dynamic relationship between these two developmental trajectories in which each influences the other.

The fields of behavioral endocrinology and behavioral neuroscience have provided us with an ample tool kit with which to study sexuality. However, there is a tendency to view development of brain and behavior through the lens of the activation/organization hypothesis. In its simplest form, this assumes that sex hormones, generally early in life, organize the neural substrates of behavior that are in turn activated in adulthood by changes in the nature and pattern of secretion of sex steroid hormones. This, of course, glosses over the many subtleties that have been uncovered, but I believe that it can be argued that this focus on sex differences has hindered study of the development of sexuality.

Another problem arises from the animal model systems that we tend to use. Many of the guiding concepts in developmental psychobiology were developed on mammals and birds, many of them highly domesticated. Such preparations have been very useful in studies of Primary Organization, or sex determination and sexual differentiation. But are these animal models suitable for dissecting the genetic from the environmental components of sexuality, that is, the component processes of Secondary Organization?

MECHANISMS OF SEX DETERMINATION

Mammals and birds have sex chromosomes. In other words, genetic sex and gonadal sex are inextricably linked. This genetic difference facilitates the study of sex differences, and indeed, one of the great success stories in genetics of the last century was the discovery of a gene on the short arm of the Y chromosome that dictated whether the primordial gonadal ridge would develop into an ovary or a testis.

However, the very nature of genotypic sex determination (GSD) makes it difficult to distinguish epigenetic from genetic contributions to sexuality. Consider, for example, aggressive and sexual behaviors displayed by both sexes, but at different frequencies. To what extent are differences observed between adult males and females due to their differences in sex chromosomes, differences in the nature and pattern of hormone secretion, or differences in nongenomic yet heritable factors such as maternal influences, or even sex-typical experiences?

Thus, in animals with sex chromosomes, the genetic element typically cannot be separated from the epigenetic element. This is particularly important as the sex chromosomes may be involved in the sexual differentiation of the brain as well as the gonads (Arnold, 2002).

Ideally, studies of the development of sexuality would utilize animal models that exhibit sex-typical differences in the traits of interest, yet not have the complications arising from sex-specific chromosomes and maternal care, i.e., a species that can illustrate how different environments can elicit different phenotypes from a particular genotype without the confound of sex-limited genes or parental care giving. Do such organisms exist in nature?

Fortunately, not all vertebrates have sex chromosomes. Indeed, there are many vertebrate species in which sex is determined not by sex chromosomes but by the environment (Crews, 1993, 2002). One form of environmental sex determination (ESD) is temperature-dependent sex determination (TSD). In TSD, gonadal sex is plastic initially, but becomes fixed by the temperature of the incubating egg during the midtrimester of development (Crews, Coomber, Baldwin, Azad, & lez-Lima, 1996). My laboratory has been studying TSD both in terms of its causal mechanisms and its functional outcomes.

Research with the red-eared slider turtle has focused on how the physical stimulus of temperature is transduced into a physiological and ultimately a molecular signal that determines the type of gonad that will be formed (Crews, 1996; Crews, Fleming, Willingham, Baldwin, & Skipper, 2001). Indeed, environmental sex determining mechanisms, including TSD, are believed to be the evolutionary precursors to genotypic sex-determining mechanisms. A second point is that in such animals the trigger for sex determination is external to the animal rather than internal, as is the case in genotypic sex determination.

Animals with environmental sex determination clearly show how the genome is capable of producing males or females, and that the ovary-determining cascade and the testis-determining cascade normally are mutually

exclusive. Our own experimental work and that of others reveal that of the many genes that are active during sex determination, the ten that have been characterized in mammals, birds, and reptiles indicate similarity not only in their sequences but also in their patterns of expression in a way that reflects these same phylogenetic relationships.

EFFECTS OF EXPERIENCE EARLY IN LIFE ON BRAIN AND BEHAVIOR

We have taken advantage of the TSD system to investigate how events early in life can shape adult sexuality independent of the gonad and its products. In this work, we have used another TSD animal, the leopard gecko. In the leopard gecko, high and low incubation temperatures produce only or mostly females while intermediate incubation temperatures produce different sex ratios. That is, 26 and 34°C are female-producing incubation temperatures, 30°C produces a female-biased sex ratio, and 32.5°C produces a male-biased sex ratio (Figure 1).

Although incubation temperature and gonadal sex are linked in TSD, this association is not as fixed as it is in species with GSD. Rather, the effect of incubation temperature and gonadal sex can be dissociated. Thus, the effect of gonadal sex can be determined by comparing males and females from the same incubation temperature, and the effects of embryonic temperature can be determined by comparing males or females from the different temperatures. In other words, when sex differences are the focus, we compare males and females from within a particular incubation temperature, and when the development of sexuality is the focus, we compare males (or females) from the different incubation temperatures.

The results of a variety of experiments indicate that incubation temperature does more than establish the gonadal sex of the individual. That is, the temperature experienced during embryogeny accounts for much of the within-sex variation observed in the morphology, growth, endocrine physiology, and aggressive and sexual behavior of the adult (Crews, 1999; Crews, Sakata, & Rhen, 1998; Rhen & Crews, 1999, 2000, 2001; Rhen, Ross, & Crews 1999).

For example, males in general grow more rapidly and are larger than females. That is, males from the intermediate incubation temperatures are larger than females from the same temperatures. However, females from the male-biased incubation temperature grow as rapidly and become as large as males from female-biased incubation temperatures. The temperature experienced during embryonic development also determines the relative concentrations of sex hormones that the individuals exhibit as adults. In general, there is a log-unit difference in the androgen-to-estrogen (A/E) ratio between males and females. However, the endocrine physiology of the adult varies dramatically with the temperature experienced during incubation. In both sexes, the A/E ratio is highest in animals from the male-biased incubation temperatures (Figure 2).

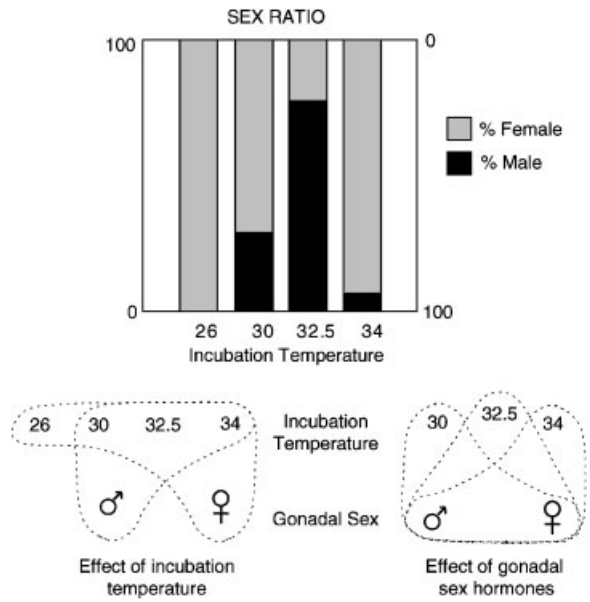

FIGURE 1 Pattern of temperature-dependent sex determination in the leopard gecko and the strategy for uncoupling gonadal (and hence, hormonal) sex and environmental variables in the establishment of individual differences. The middle panel portrays the effect of incubation temperature on sex ratio: Extreme temperatures produce females whereas intermediate temperatures produce different ratios. Since the effects of incubation temperature and gonadal sex co-vary, any difference between individuals could be due to the incubation temperature of the egg, the gonadal sex of the individual, or both factors combined. To assess the contribution of each, they must be dissociated. Studying same-sex animals that differ only in the incubation temperature experienced reveals the effects of temperature (left) whereas comparing males and females from the same incubation temperature reveals the effects of gonadal sex (right). Dotted lines group comparisons made in each condition.

Incubation temperature also has a major influence on the nature and frequency of the behavior displayed by the adult leopard gecko. In general, females usually respond aggressively only if attacked whereas males will immediately posture and then attack male intruders, but rarely female intruders. Females from a male-biased incubation temperature are significantly more aggressive toward males than are females from a low or a female-biased incubation temperature (Figure 2). These same females also show the male-typical pattern of offensive aggression. Similar effects of incubation temperature on aggressive behavior have been documented in males.

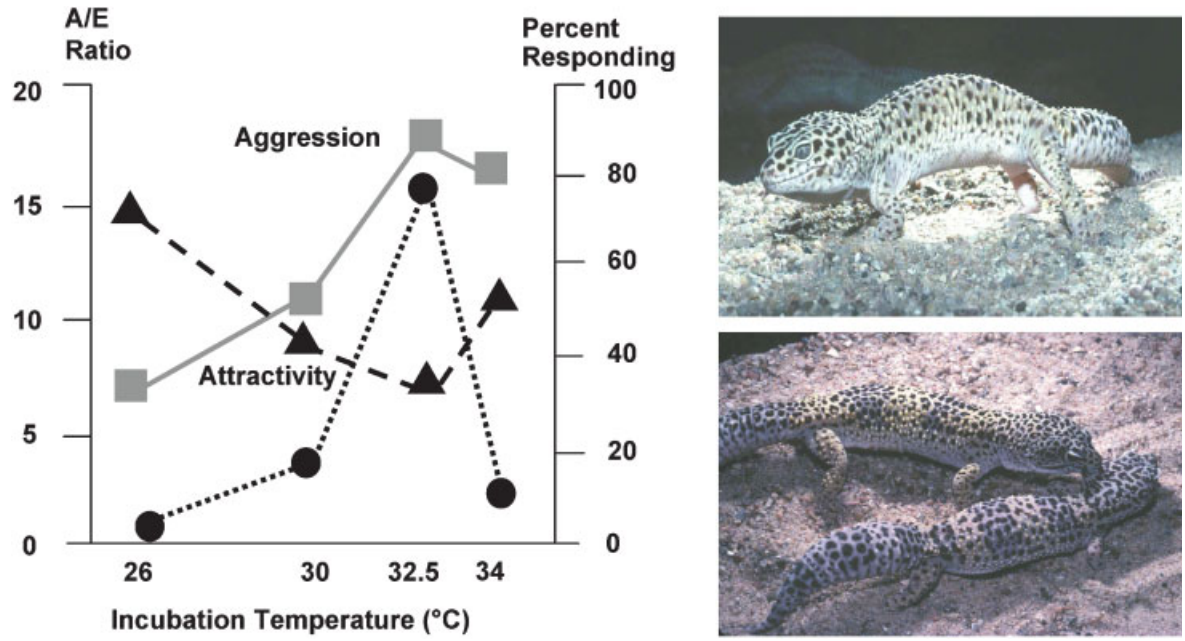

FIGURE 2 Incubation temperature determines circulating hormone concentrations (androgen-to-estrogen, A/E, ratio) and the level of aggressive behavior exhibited by females and their attractivity to males. The right panel depicts high posture aggressive display (upper picture) while the lower panel depicts the male taking a neck grip of an attractive female exhibiting a tail lift, an indication of receptivity.

Courtship is a male-typical behavior. In a sexual encounter, the male will slowly approach the female, touching the substrate with his tongue or licking the air. Attractiveness is a female-typical trait and is measured by the intensity of a sexually active male's courtship behavior toward her. Females from a male-biased incubation temperature are less attractive than are females from lower incubation temperatures. Interestingly, attractivity in females from the high (34°C) incubation temperature is greater than that of females from male-biased incubation temperatures and not different statistically from that of low-temperature (26°C) females (Figure 2).

Aggressive and sexual behaviors in the leopard gecko are modulated by sex hormones, and incubation temperature also influences the ability of exogenous sex hormones to maintain or restore sexual and aggressive behaviors in both sexes. For example, we find that depending upon their incubation temperature, adult males respond differently to hormone replacement therapy following castration and, as one would predict, incubation temperature modulates patterns of brain metabolism (see below). Similar results have been found in females. This suggests that incubation temperature influences how the individual responds to steroid hormones in adulthood.

Because the nature and pattern of growth, hormone secretion, and behavior ultimately are expressions of brain activity, it stands to reason that neural phenotypes also must exist, and these might be sensitive to incubation temperature. How might incubation temperature during embryogenesis affect the brain of adult animals?

Given the wealth of evidence that in animals with genotypic sex determination certain limbic nuclei such as the preoptic area (POA) and the ventromedial hypothalamus (VMH) are sexually dimorphic, we expected to find similar sex differences in the leopard gecko. Surprisingly, there are no statistically significant sexual dimorphisms in the POA and VMH between males and females at those incubation temperatures that produce both sexes (Coomber, Crews & Gonzalez-Lima, 1997). There are, however, consistent differences across incubation temperatures. For example, the volume of the POA is larger in both males and females from the male-biased incubation temperature compared to animals from the female-biased incubation temperature. The opposite pattern is found for the VMH. That is, the volume of the VMH is larger in females from low incubation temperatures compared to females from the male-biased incubation temperature. These data suggest that incubation temperature of the embryo may directly organize the development of brain nuclei independent of gonadal sex in a manner similar to that for body growth.

Neural Networks and Behavior

We have learned a great deal from studies in which brain areas have been destroyed or chemically or elec-

trically stimulated, but there are limitations to what they can tell us about behavior–brain relationships. While there is no disagreement that complex behaviors must reflect complex patterns of neural activity involving many areas of the brain, how to visualize such coordinated patterns of brain activity has been a challenge. One approach has been functional brain mapping using methods that include, but are not restricted to, immediate early gene expression, 2-deoxyglucose (2-DG) uptake, and cytochrome oxidase (COX) histochemistry, that correlate patterns of behavioral expression with patterns of activity in various brain nuclei. COX is particularly interesting for studies of the effects of experience on the brain, as it is a rate-limiting enzyme in oxidative phosphorylation, the major pathway in brain metabolism. Thus, COX activity in a brain area is a measure of the metabolic capacity of that brain region and reflects the metabolic history of an area. COX histochemistry is not like 2-DG autoradiography or c-fos immunocytochemistry, which provide information on evoked or immediate activity; instead, COX reflects long-term changes in brain activity.

Sexual behavior is amenable to the study of behavior–brain relationships. A large number of experiments have identified the nuclei of the limbic forebrain to be critically involved in the display of sexual behavior. Further, a variety of methods have revealed them to be interconnected, contain sex steroid hormone receptors, and sexually dimorphic in their volume and synaptic organization as a consequence of the nature and frequency of sex steroid hormones secreted perinatally. Importantly, these properties appear to be evolutionarily conserved. In both mammals and reptiles, for example, metabolic activity in limbic areas reflects the capacity to display sociosexual behaviors and, in turn, that differences in metabolic activity in these areas reflect individual differences in the propensity to display social behaviors (reviewed in Crews, 1992; Sakata, Gupta, & Crews, 2001).

The possibility that these limbic nuclei might form an integrated neuronal network with overlapping functions that subserve all sex steroid hormone-modulated social and reproductive behaviors has been considered previously, most recently by Newman (1999). Thus, the concept that social and reproductive behaviors may "emerge from the activity of a unitary neuroanatomical framework" in the brain (pp. 252–253), and not simply the product of activity of a single brain area(s), complements more traditional approaches of mapping different behavioral functions on subnuclei in these brain areas. Further, Newman suggested a graphical representation of the data generated in studies of metabolic activity that would allow one to see how nuclei in this network may differ in different behavioral states. It is my opinion that her proposal represents a marked improvement on the traditional tabular form of data presentation that makes it difficult to detect the relationships among the various nuclei measured. While Newman proposed hypothetical representations, I have recently applied and expanded this method. Figure 3 illustrates this method by graphing published data on the effects of sexual experience on metabolic capacity in the "social behavior network" in the male rat (Sakata, Gonzalez-Lima, Gupta, & Crews, 2002b). In this study, we determined that sexual experience elevates COX activity in specific brain nuclei and, further, that these changes in metabolic activity are related to the amount of sexual experience of the individual (Sakata, Gonzalez-Lima, Gupta, & Crews, 2002b).

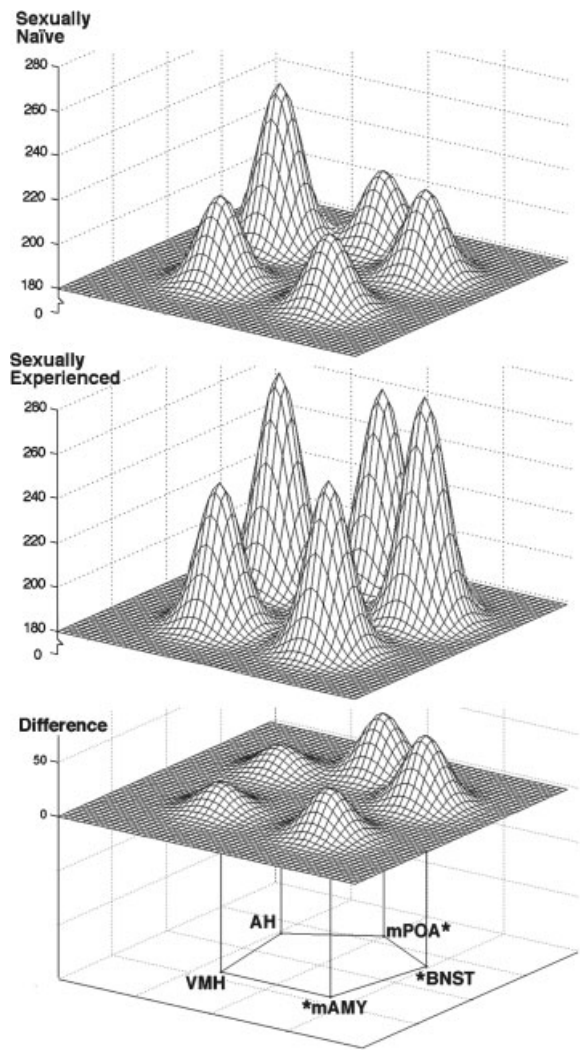

FIGURE 3 Sexual experience changes the social behavior network in male rats. Note that metabolic capacity in sexually experienced males is significantly greater in the medial preoptic area (mPOA), bed nucleus of the stria terminalis (BNST), and the medial amygdala (mAMY), but metabolic activity in the anterior hypothalamus (AH) and the ventromedial nucleus of the hypothalamus (VMH) is not significantly different in sexually experienced and naïve male rats.

Comparing one group of rats that were given daily sex tests for 16 days to a control group that was never exposed to females (naïve), we found that the AH and VMN exhibit little change in COX abundance while the medial preoptic area (mPOA), medial amygdala (mAMY), and bed nucleus of the stria terminalis (BNST) show significant increases in COX abundance. The amount of change also differs among brain areas, with relative abundance of COX in the mAMY being significantly less than that found in the mPOA and the BNST. This effect of experience on the neural landscape is seen in the bottom panel of Figure 3.

We also have been interested in how experience might affect the neural substrates of aggressive and sexual behavior in geckos. In particular, we have been interested in mapping which brain areas mediate experience-dependent changes in behavior. Relative to females, males on average have greater COX activity in the POA. The complement also is true; i.e., relative to males, females on average have greater COX activity in the VMH. However, incubation temperature is an important determinant in both sexes, and these differences correlate well with behaviors exhibited by animals from different incubation temperatures. For example, males from the male-biased incubation temperature are more aggressive and have greater COX activity in the anterior hypothalamus, septum, and the nucleus sphericus (homolog of the medial amygdala) compared to males from the female-biased incubation temperature. As might be expected, females from the female-biased incubation temperature have greater COX activity in the VMH compared to females from the male-biased incubation temperature.

As mentioned previously, there is a significant increase in aggression in females from higher incubation temperatures. This is reflected in COX activity in brain nuclei associated with aggressive behavior. In reptiles, the nucleus sphericus and external amygdala are homologous to the medial and basolateral amygdala of mammals, respectively; as in mammals, both areas are involved in the control of aggression. Analysis of females from different incubation temperatures reveals that COX activity increases in both nuclei as a function of incubation temperature in a manner that parallels the differences in aggression among females from different incubation temperatures (Figure 4).

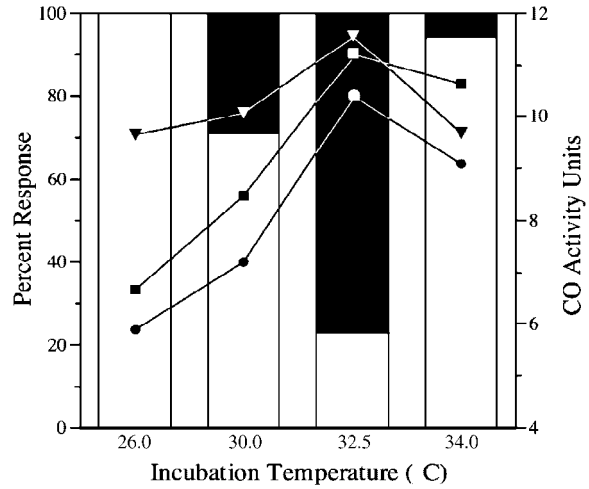

FIGURE 4 Level of aggression (circles) in female leopard geckos from different incubation temperatures compared to mean cytochrome oxidase (COX) activity units in the external amygdala (AME, squares) and nucleus sphericus (NS, inverted triangles) of females from the same incubation temperatures. Histogram reflects sex ratio at birth at different incubation temperatures. Open bars, females at hatching.

EFFECTS OF EXPERIENCE LATER IN LIFE ON BRAIN AND BEHAVIOR

Experience during adulthood also can influence brain neurochemistry and behavior in leopard geckos. It is well known that, relative to sexually naïve males, sexually experienced male rats and cats initiate copulation sooner, tend to be more aggressive, continue to copulate longer after castration, and respond more rapidly to androgen replacement. In mammals, it also is common to find that experienced males exhibit greater changes in sex steroid hormone concentrations and immediate early gene expression when presented with cues that predict the introduction of a female. Altogether, it appears that sexually experienced males are more primed for sexual behavior.

Similar effects of sexual experience are found in the leopard gecko. For example, sexually experienced male geckos begin to mark sooner, are less likely to flee from a territorial male, and have higher circulating concentrations of testosterone than naïve males (Crews et al., 1998; Sakata, Gupta, Chuang, & Crews, 2002c). In general, we find that sociosexual experience also increases metabolic capacity in certain nuclei, but reduces it in others; there also are nuclei where there is no discernable effect.

However, this effect of sexual experience on both volume and metabolic capacity of brain nuclei is dependent upon incubation temperature (Figure 5). Again, the effects can vary from brain area to brain area. For example, COX activity in the POA increases with sexual experience in low-temperature females, but not in females from the male-biased incubation temperature whereas COX activity in the VMH increases in females from the male-biased incubation temperature, but not in low-temperature females.

Another wrinkle comes in when we consider age. We have looked at this question by incubating eggs at different

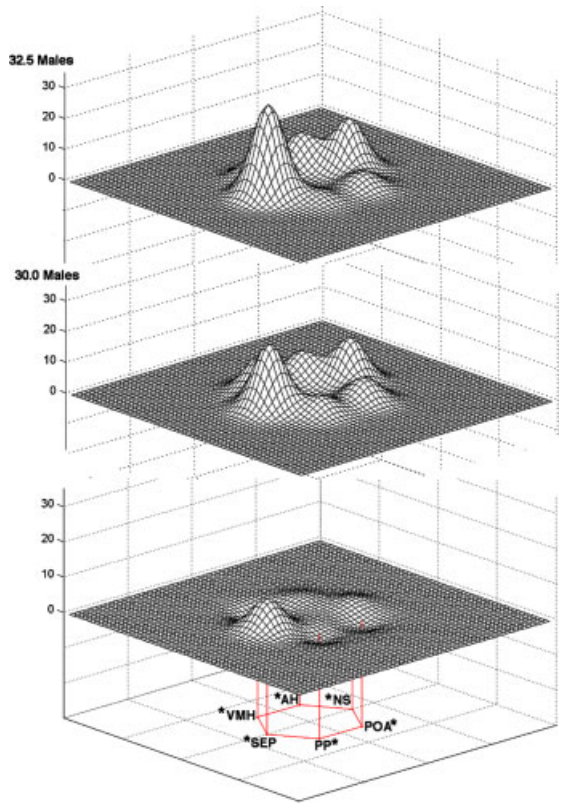

FIGURE 5 Cytochrome oxidase (COX) activity in the interconnected limbic nuclei of adult male geckos from 32.5°C incubation temperature (top panel) and from 30°C incubation temperature (middle panel). Bottom panel indicates the difference between the neural landscapes, revealing the effect of temperature during incubation on metabolic activity in the adult brain. All these nuclei either accumulate sex steroid hormones or are involved in the expression of social behaviors. Positive peaks indicate nuclei in which change was more positive in males from a 32.5°C incubation temperature whereas negative peaks indicate nuclei in which change was more positive in males from a 30°C incubation temperature. AH = anterior hypothalamus; VMH = ventromedial hypothalamus; SEP = septum; PP = periventricular nucleus of the preoptic area; POA = preoptic area; NS = nucleus sphericus.

temperatures, raising hatchlings in isolation before transferring them to breeding cages on their first birthday or several years later. In gecko life, this corresponds to an 18-year-old human and a 36-year-old. In this way, we are able to assess the relative effects of age independent of social and sexual experience and, further, to determine if embryonic experience could affect the response.

As mentioned earlier, sexual experience can increase the volume of the POA in females whereas age can decrease the volume of the POA (Crews, Coomber, & Gonzalez-Lima, 1997). This points to an important principle that often is not taken into account in psychobiology or in phenotypic plasticity studies: namely, that organisms age as they gain experience, but do not necessarily gain experience as they age. The only other example that I have found that controlled for experience independent of age was a study of Joan Witkin (1992) demonstrating that aging in rats is associated with a decline in the density of synaptic input to GnRH neurons in the POA, but that reproductive experience will counter this trend and maintain synaptic input in old females at the levels of young adults.

Finally, we have discovered that sexual experience can reorganize the functional associations between brain nuclei. Sakata, Coomber, Gonzalez-Lima, and Crews (2000) developed methods to analyze correlations between COX levels in different brain nuclei and combine these results with knowledge of neuroanatomical pathways. Such analysis shows that among some nuclei, sexual experience has no influence on the strength of neural connections, but among other nuclei the functional associations are altered completely.

Taken together, such results indicate that (a) the volume and metabolic capacity of specific brain regions are dynamic in adulthood, changing as individuals age and gain sociosexual experience, (b) the size and activity of brain areas can be independent, and (c) the embryonic environment influences the nature and degree of these changes.

HOW CHANGES IN THE BRAIN AFFECT FUTURE BEHAVIOR

Do changes in brain metabolism constrain the likelihood of future behaviors? Because COX activity constrains neural activity, and because neural activity in specific brain circuits constrains the expression of behavior, we have proposed that changes in COX activity reflect changes in the capacity to display particular behaviors (Sakata et al., 2001). In the context of sexual behavior, we propose that there is a threshold amount of COX activity in areas such as the mPOA that is necessary for the display of copulatory behavior. This is a metabolic equivalent of the androgen threshold hypothesis put forward by Frank Beach many years ago.

We have suggested further that one of the actions of testicular steroid hormones is to maintain the levels of metabolism in neural circuits critical to the display of male-typical copulatory behavior (Sakata et al., 2001). Indeed, there may be a neural metabolic threshold below which copulatory behavior cannot be expressed. In geckos, there is a decline in COX in relevant brain areas following castration. That is, a reduction in COX activity may translate into an inability to maintain sufficient neural activity to express copulatory behavior.

So, the oft-heard statement that sexual experience may take the place of hormones may have a metabolic basis. In other words, sexually experienced males are more robust to castration than naïve males because it takes longer for neural metabolic capacity to fall below this threshold.

Sakata and colleagues (2002a) tested this idea using whiptail lizards by determining the metabolic capacity in different brain areas of adult males that had been housed recently with females versus those that had been housed in isolation. All animals were given the same amount of sexual experience prior to the experimental manipulation. They found that males with recent sexual experience had elevated COX activity in areas such as the mPOA. In a similar experiment, castrated males that had been housed with females continued to display high levels of sexual behavior compared to those males that had not been housed with females.

TRANSGENERATIONAL EFFECTS

Finally, experiences both early and late in life can be transmitted across generations. In various litter-bearing mammals, for example, the sex of an individual's neighbors in the uterus will influence its physiology and behavior in adulthood (vom Saal, Clark, Galef, Drickamer, & Vandenbergh, 1999). That is, the endocrine microenvironment during the embryonic environment modifies the physiology, behavior, and neurochemistry of the individual. Thus, a female fetus located between two males (2M female) is exposed to higher levels of androgen produced by the neighboring males compared to a female fetus located between two females (2F female). Largely through the work of Mertice Clark and Jeff Galef on gerbils, we have learned that as adults these 2M females have lower estrogen and higher testosterone levels, have a masculinized phenotype, are less attractive to males and more aggressive to females, and produce litters with significantly more male-biased sex ratios relative to 2F females. Not only does the fetus's intrauterine position influence its sensitivity to exogenous hormones in adulthood, the metabolic activity of brain nuclei also varies according to uterine position. In gerbils, the sexually dimorphic area of the preoptic area (SDA-POA) is responsible for copulatory behavior in males, and, as females differ in their sexual behavior according to intrauterine position, the SDA-POA is likely to be involved in their behavior as well. COX histochemistry reveals long-term changes in the metabolic capacity in the SDA-POA, with 2M females having greater activity compared to 2F females (Jones, Gonzalez-Lima, Crews, Galef, & Clark, 1997). There also is a difference in COX activity in the posterior anterior hypothalamus, an area replete with neurons containing GnRH, which may partly explain the physiological differences between 2M and 2F females.

There are parallels to such effects in nonmammalian species. For example, it has been known for some time that the yolk of birds and other egg-laying vertebrates contains sex steroid hormones. Howard Bern (1990) suggested that these maternal hormones might have effects on the growth and physiology of the offspring. Hubert Schwabl (1993) discovered subsequently that in the canary, the yolk concentrations of testosterone and androstenedione are higher in eggs laid later and that these hatchlings exhibit more rapid growth and higher level of aggressive behavior than chicks from eggs laid earlier in the clutch. These hormones are deposited in layers that reflect the changes in the pattern of hormone secretion in the female. Depending upon how the yolk is utilized during embryogenesis, it is possible that the embryo is being dosed by different amounts and kinds of steroid hormones in a manner reflecting the layering pattern. Thus, when one considers how the physical and biotic environments alter hormonal profiles of the female during this period, it suggests that the yolk represents the physiological distillation of the female's interaction with her past and present environment.

Finally, there is the long history of outstanding work demonstrating that what happens in pregnancy can be magnified during the neonatal period. About 45 years ago Christian and LeMunyan (1959) demonstrated that if pregnant mice were crowded, the physiology and behavior of at least two generations of progeny would be affected. The laboratories of Seymour Levine, Victor Denenberg, and Ingeborg Ward delved into this problem and demonstrated that handling pregnant dams could produce similar effects in their young. The mechanism of action of these stress effects appears to be due to changes in the mother's behavior brought about by the activation of adrenocortical responses of the mother and the consequent effects on fetal endocrine physiology. Michael Meaney (2001) added brilliantly to this story by taking it into the brain and documenting how the amount of licking, per se, a pup receives can modulate the effects of the altered maternal behavior and hence the stress reactivity of the pup later in life. In addition, there is the work of Celia Moore, who discovered that mother rats lick the anogenital region of male pups more than they do female pups and, further, that this difference accentuates the copulatory behavior of the pup when it reached adulthood (Moore, 1995).

It is common in these types of studies for the sex ratio of the litter to be balanced such that there are equal numbers of male and females and to contrast them with single-sex litters. But in nature, sex ratios in the litter vary. To see whether the sex ratio in the litter matters, Stephanie Rees, Alison Fleming, and I set out to determine whether sex

ratio might influence maternal behavior and hence the adult behavior of the dam's offspring. In this study, natural litters were balanced to either equal numbers of males and females, a male-biased sex ratio consisting of 6 males and 2 females, or a female-biased sex ratio consisting of 6 females and 2 males. Observations of mothers with their pups revealed that maternal licking was significantly greater in the male-biased group compared to the other groups, but that the males from the female-biased litter exhibited significantly more mounting behavior than did males from the male-biased litters. This suggests that in the male-biased litters having the higher level of licking, maternal licking might have been distributed equally across the males whereas in the female-biased litters where there was less licking, maternal licking was more focused on the 2 males.

DISCUSSION

I have attempted to show that by focusing on sex differences we ignore the problem of individual variation—the crux of sexuality. Second, I have tried to illustrate how the principles of developmental psychobiology can be extended to unconventional animal models and, further, that the unusual aspects of these model systems enable us to parse the relative contributions of genetic and epigenetic factors that lead to the development of sexuality in ways that are simply not possible with mammals and birds. For example, the evidence presented for the formative effects of early experience (incubation temperature) on brain development reminds one of Gerald Edelman's Selective Theory. Similarly, the results of our studies of the effects of sexual experience on metabolism in the adult brain are reminiscent of Donaldo Hebb's concept of the self-organizing brain. The finding that age and experience can have opposite effects on brain metabolism and behavior points to the importance of separating the effects of age from those attributable to experience.

Finally, I hope that I have been successful in showing how a biologically informed perspective coupled with an approach that integrates different levels of analysis is likely to lead to a fuller understanding of the cumulative nature of experience, depending as it does upon preceding events while setting the stage for future experience. In this way, we not only uncover answers to questions at specific levels of biological organization but also ideally will find that information at one level influences interpretations at other levels.

NOTES

Invited address to the 34th annual meeting of the International Society for Developmental Psychobiology, November 2001, San Diego, CA. This address was supported by a grant from John Wiley & Sons, Inc., Publisher.

I thank Nicholas Sanderson for comments on the manuscript.

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