21

Animal Models of Experiential Effects on Neural Metabolism: Plasticity in Limbic Circuits

Jon T. Sakata, Ajay Gupta, and David Crews

CONTENTS

I. Introduction

Sexual differentiation can be defined as the process by which the capacity to display homotypical or heterotypical phenotypes is altered. For example, the brain of a male rodent is both masculinized and defeminized during the process of sexual differentiation, and the end product is an individual who is predisposed to behave like a male. Pioneering studies have highlighted both hormonal and nonhormonal factors early in life that can affect the differentiation of the organism, and there has been a wealth of studies characterizing these neural effects. Intrasexual differences in behavior are also influenced by factors early in life, but the neural bases of these individual

0-8493-0962-X/02/$0.00+$1.50

differences are relatively poorly understood. Historically, the emphasis has been on critical periods during development; in other words, a restricted time period of biological plasticity that molds the differentiation of the organism. However, there has been an increasing awareness of the plasticity of the adult brain and a deeper understanding of immediate factors that can influence both the brain and behavior of adult individuals. For example, photoperiod and temperature are two abiotic factors that profoundly affect the capacity to display sexual behaviors, while the social environment and food abundance are two biotic factors that affect the display of sociosexual behaviors.[1] Sociosexual experience (e.g., copulation, maternal behavior) and social status also alter the reproductive system, the brain, and future behavior.[2]

The most studied aspects of the nervous system are neuromorphology (e.g., brain nucleus size, neuron number and size, dendritic arborization) and protein and gene expression (e.g., sex steroid hormone and neurotransmitter receptors). Each has contributed to a deeper understanding of the organization of the nervous system and of the mechanism by which exogenous and endogenous factors affect sexual differentiation. However, a neural phenotype that is underexamined is neural metabolism. The importance of studying neural metabolism lies in the fact that the activity of neurons mediates behavior. The production and utilization of adenosine triphosphate (ATP) is pivotal in the regulation of brain activity. In this respect, ATP production constrains the activity of neurons and subsequently constrains the expression of behavior.

The enzyme cytochrome oxidase (CO) is a rate-limiting enzyme in oxidative phosphorylation,[3] the major pathway in brain metabolism.[4] Consequently, the amount of CO is critical in determining the amount of activity a brain area can sustain. Phrased differently, CO activity in a brain area reflects the metabolic capacity of that region. Furthermore, CO activity is a marker of the metabolic history of an area; manipulations that decrease synaptic activity also decrease CO activity in efferent brain areas.[5-7] In this respect, CO histochemistry can be used to trace functional pathways activated during a series of experiences. However, information on CO activity is very different from information based on other markers of activity such as 2-deoxyglucose and the immediate early gene (IEG), c-Fos; 2-deoxyglucose consumption and c-Fos expression provide information on evoked or immediate activity, while CO activity tends to reflect long-term changes in brain activity.[8] For example, 2-deoxyglucose consumption during training or learning tasks reflects the neural metabolic changes that occur during learning, while changes in CO activity after learning reflect long-term changes in neural metabolism as a consequence of learning.

Cytochrome oxidase is an enzyme located in the inner mitochondrial membrane. It is a holoenzyme composed of 13 proteins; 10 are encoded in the nuclear genome and 3 are encoded in the mitochondrial genome, and the major catalytic subunits are encoded in the mitochondrial genome.[9,10] Cytochrome oxidase activity is regulated primarily by the abundance of the holoenzyme in the mitochondria.[11] Therefore, CO activity is governed by a

complex interaction between intracellular factors that affect both nuclear and mitochondrial gene expression. Transcription factors such as IEGs[12,13] and sex steriod hormone receptors[14] can consequently alter the expression of CO. In this respect, CO activity can be perceived as a long-term consequence of IEG expression and/or sex steroid hormone stimulation. In summary, we feel that changes in CO activity reflect not only neural plasticity but also a mechanism underlying long-term changes in phenotype.

This chapter is a summary of recent experiments investigating the effects of early and late factors on neural metabolism and the display of sociosexual behaviors. Our primary objective is to argue for the importance of understanding the link between neural metabolism and behavior. We highlight the importance of studying brain metabolism as a measure of neural plasticity. We utilize both mammalian and nonmammalian animal systems in an attempt to identify evolutionarily conserved effects; the importance of discerning such evolutionarily conserved phenotypes is that the more conserved the phenotype, the more likely it is to appear in other species such as humans. We focus first on how factors early in life affect adult neural metabolism, and later emphasize the role of recent experiences on brain metabolism. Finally, we expound on how early and late experiences may interact to affect both neural and behavioral plasticity.

II. Effects of Early Factors on Neural Metabolism and Behavior

The pioneering work on sexual differentiation highlighted the role of the hormonal environment of the developing embryo in shaping adult phenotype. As this work has shown, androgens generally have a masculinizing and/or defeminizing effect on both the brain and behavior. Only recently have the effects of early factors, such as sex steroid environment on brain metabolism been studied. Here we focus on two model systems, the leopard gecko (*Eublepharis macularius*) and the Mongolian gerbil (*Meriones unguiculatus*), to elucidate the effects of early factors on brain organization and the functional significance of these changes.

A. Leopard Geckos

As in many reptilian species, the gonadal sex of leopard geckos is determined not by sex chromosomes but by the temperature experienced by the egg during embryogenesis. In this species, individuals hatched from eggs incubated at low (e.g., 26°C) or high (e.g., 34°C) incubation temperatures are almost always females. Sex ratios at intermediate incubation temperatures are more mixed. For example, at 30°C less than a third of the individuals are male, while at 32.5°C about two-thirds are male. Interestingly, incubation

temperature not only determines gonadal sex but also influences the sexual differentiation of the individual.[15]

Past experiments with the leopard gecko have studied phenotypic differences among males and females from four incubation temperatures: 26 (low), 30 (female-biased), 32.5 (male-biased), and 34°C (high). While sex differences in sociosexual behaviors as well as reproductive physiology and neural phenotype have been examined,[15] in this chapter we focus on intrasexual differences based on incubation temperature. The effects of incubation temperature on adult male phenotype can only be readily examined at the female-biased and male-biased incubation temperatures, while the effect of incubation temperature among females can be studied at all four temperatures. Males from the male-biased incubation temperature are more aggressive than males from the female-biased incubation temperature, whereas females from the male-biased and high incubation temperatures are more aggressive than females from the low incubation temperature.[16] Males from the male-biased incubation temperature display more territorial marking behaviors after gonadectomy and androgen replacement relative to identically treated males from the female-biased incubation temperature.[17] Females from the male-biased incubation temperature are more likely to exhibit male-typical courtship behavior in response to gonadectomy and androgen treatment than identically treated females from the low incubation temperature.[18] On the other hand, males from the female-biased incubation temperature show more homotypical courtship behavior following gonadectomy and androgen replacement than identically treated males from the male-biased incubation temperature.[17]

By comparing differences in brain metabolic capacity between same-sex geckos from different incubation temperatures, we can begin to understand the link between brain metabolism and propensities to display aggressive or courtship behavior. For example, relative to males from the female-biased incubation temperature, males from the male-biased incubation temperature have elevated metabolic capacity in the anterior, dorsolateral, and periventricular hypothalamic areas, nucleus sphericus (NS), and septum.[19] Conversely, males from the female-biased incubation temperature have elevated metabolic capacity in the preoptic area (POA), ventromedial hypothalamus (VMH), and dorsal ventricular ridge (DVR).[19] Among females, females from the male-biased incubation temperature have elevated CO activity in areas such as the POA, anterior and dorsolateral hypothalamus, NS, DVR, and septum relative to females from the low incubation temperature.[19] Across both sexes, individuals that are more territorial or aggressive (i.e., males and females from the male-biased incubation temperature) have elevated metabolic capacity in the anterior and dorsolateral hypothalamus, NS, and septum relative to their same-sex counterparts. Interestingly, the anterior hypothalamus and septum have been implicated in the regulation of aggressive behaviors in a number of species.[20,21] Individuals predisposed to display courtship behavior in response to androgen treatment (i.e., males from the female-biased incubation temperature and females from the male-biased temperature) have elevated CO activity in the POA and DVR. Both areas concentrate

androgens in lizard species studied to date,[22,23] and the POA is an evolutionarily conserved brain area modulating the expression of male-typical courtship and copulatory behavior.

Based on these differences, we propose that differences in metabolic capacity not only reflect differences in the metabolic history of the brains of these animals but may also reflect differences in the propensity to display specific behaviors. In other words, CO activity may, in part, reflect neural priming.

B. Mongolian Gerbils

The intrauterine position of the rodent embryo affects the amount of exposure to prenatal androgens. For example, embryos situated between two male fetuses (2M embryos) are exposed to more androgens than embryos situated between two female fetuses (2F embryos).[24] This difference in androgen exposure causes differences in reproduction. Relative to 2M female gerbils, 2F females experience vaginal opening and reproduce at an earlier age and produce more litters.[25,26] Furthermore, late-maturing female gerbils tend to attack strange males introduced into their home cage more than their early maturing sisters.[27] Regarding neural differences between 2F and 2M female gerbils, 2M individuals have elevated CO reactivity in the medial and posterior anterior hypothalamic area (AH).[28] Because the medial AH as defined in this study overlapped with the ventrocaudal portion of the medial sexually dimorphic nucleus of the POA, this increase in 2M females may reflect the general masculinity of 2M females. The increase in CO activity in the posterior AH may also reflect this masculinization of the nervous system. The posterior AH contains many cells that secrete gonadotropin-releasing hormone (GnRH), which mediates the release of luteinizing hormone (LH) from the pituitary. Females generally have a pulsatile secretion of LH, whereas LH release in males is more tonic and constant. If the release of LH is masculinized in 2M females, the more persistent release of GnRH may be reflected as an increase in CO activity in the posterior AH. The functional significance of these differences will become clearer as more manipulations on brain functions as well as more functional mapping of circuits are completed.

III. Effects of Late Factors on Neural Metabolism and Behavior

With respect to male-typical sexual behavior, one factor that dramatically influences the display of copulatory behavior as well as neural phenotype is sexual experience. For example, relative to sexually naïve males, sexually experienced male rats are quicker to initiate copulation,[29] continue to copulate longer after castration,[30,31] and begin to copulate sooner after androgen replacement.[31] Experienced males also exhibit greater changes in sex steroid

hormone concentrations[32,33] and immediate early gene expression[34,35] when presented with cues that predict the introduction of a female. Ejaculation also leads to greater increases in Fos-immunoreactive neurons in the medial POA (mPOA) in sexually experienced males relative to males copulating for the first time,[36] and experienced males are less detrimentally affected by pharmacological manipulations[36,37] and novel environments.[38] Furthermore, sexually experienced male rats are less aversely affected by lesions within the vomeronasal limbic system.[39-42] Altogether, it appears that sexually experienced males are more primed for sexual behavior.

We have been interested in how sociosexual experience affects neural metabolism. In particular, we have been interested in mapping which brain areas mediate experience-dependent changes in behavior. Moreover, by comparing the neural and behavioral effects of sociosexual experience in both mammalian and nonmammalian species, we can search for evolutionarily conserved neural areas modulating these changes. We have been most interested in the effects of sexual experience on robustness to castration and on sensitivity to androgen replacement.

A. Rats

Because CO activity constrains the production of ATP and, consequently, the activity of a brain area, we postulated that expression of sexual behavior is constrained by metabolic capacity in key limbic areas such as the mPOA, bed nucleus of the stria terminalis (BNST), and medial amygdala (MeA). For example, we hypothesized that the reduction in the expression of copulatory behavior following castration is caused, in part, by a reduction in metabolic capacity in key brain areas (see below). Additionally, we hypothesized that the reinstatement of copulatory behavior by androgen replacement therapy is due, in part, to an increase in neural metabolism in limbic circuits. Phrased differently, we propose that one of the actions of testicular steroid hormones is to maintain the neural metabolism in pertinent circuits necessary to display male-typical copulatory behavior. Indeed, there may be a neural metabolic threshold below which copulatory behavior cannot be expressed. This would be a neural corollary to the androgen threshold hypothesis proposed by Frank A. Beach (Figure 21.1). Relatedly, sexually experienced males may be more robust to castration than naïve males because it takes longer for neural metabolic capacity to fall below this threshold. It is possible that (1) sexually experienced males have elevated CO activity in areas like the mPOA, BNST, and MeA prior to castration and/or that (2) castration leads to slower decrements in metabolic capacity in sexually experienced males. Both possibilities would translate into an increased latency for metabolic capacity to fall below this neural threshold in experienced males.

We first investigated the effects of different amounts of sociosexual experience on neural metabolism in intact male rats.[43] Males were either kept sexually naïve (NAÏVE males), given three opportunities to copulate with a female (3F

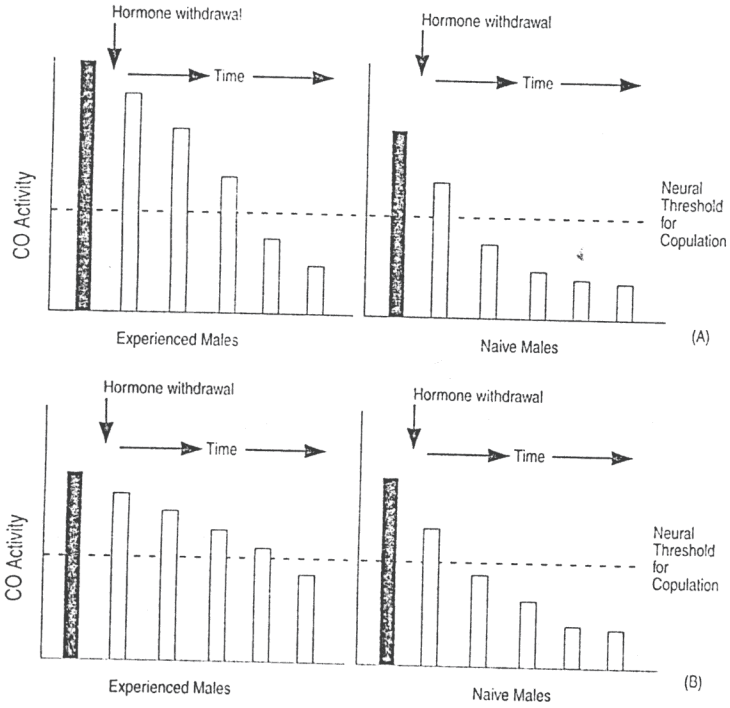

FIGURE 21.1
The hypothesized relationship between CO activity in limbic brain areas, the display of copulatory behavior, and castration. Castration leads to a decrement in metabolic capacity, and when metabolic capacity falls below the neural threshold for copulation, sexual behavior is not exhibited. (A) Schematic of the hypothesis that sexually experienced males are more robust to castration because they have elevated metabolic capacity in limbic brain areas prior to castration. (B) Schematic of the hypothesis that sexually experienced males are more robust to castration because the rate of decline in neural CO activity following castration is slower than in naïve males. Both translate into a longer period of time before metabolic capacity falls below the neural threshold for copulation. It should be noted that the two models are not mutually exclusive.

males), or given sixteen daily opportunities to copulate with a female (16F males). In general, metabolic capacity in limbic nuclei was not significantly different between NAÏVE and 3F males. However, dramatic increases in CO activity were found in the mPOA, BNST, MeA, and VMH in 16F males relative to NAÏVE and 3F males. Other areas that showed increases in metabolic capacity with extensive sociosexual experience were the habenula, zona incerta, and arcuate nucleus. Cortical amygdalar nuclei, lateral POA, AH, lateral hypothalamus, paraventricular hypothalamic nucleus, and dorsal thalamic nuclei did not show metabolic changes with sociosexual experience.

There appears to be a relationship between the amount of change in brain metabolism, documented patterns of c-Fos induction during copulation, and documented distributions of androgen receptor (AR) mRNA abundance in male rats. Areas that show c-Fos induction during copulation in addition to high AR mRNA expression[44-46] tend to show increases in CO activity with extensive sociosexual experience. However, areas that have neither c-Fos induction during copulation nor high AR mRNA expression tend not to show increases in CO activity with experience. It is less clear whether high levels of AR mRNA expression or c-Fos expression are, by themselves, sufficient to induce metabolic changes. Though estrogen is also important for the display of copulatory behaviors (reviewed in reference 47) and neural plasticity (reviewed in references 48 and 49), the abundance of estrogen receptor alpha (ERα) mRNA[44] does not correlate as well with changes in CO activity. Therefore, we propose that androgenic stimulation, more than estrogenic stimulation, contributes to this metabolic plasticity with sociosexual experience. The idea that AR mRNA expression corresponds more readily with neural plasticity in metabolism than ERα mRNA expression is consistent with the observation that more c-Fos is induced by copulation in AR-expressing neurons than in ER-expressing neurons.[50]

In summary, evidence exists that changes in metabolic capacity may underlie experience-dependent changes in robustness to castration. Though the effects of castration are unknown in rats, we predict that, as in the leopard gecko (see below), androgen deprivation will lead to a decrement in CO activity in limbic brain areas. We also predict that androgen replacement will increase metabolic capacity in these brain areas. Furthermore, because sexually experienced male rats display copulatory behavior sooner after androgen replacement therapy than naïve males,[31] we predict that CO activity rises to a level that supports copulatory behavior sooner in experienced males.

B. Leopard Geckos

In a study investigating the neural effects of sociosexual experience in adult male geckos from the male-biased incubation temperature, it was found that males housed with intact, cycling females for one to two years had elevated metabolic capacity in the VMH, AH, lateral hypothalamus (LHA), dorsolateral hypothalamus (DLH), and dorsal lateral nucleus of the thalamus relative to age-matched males housed in isolation.[51] However, isolate, or sociosexually naïve males had elevated metabolic capacity in the DVR, SEP, periventricular hypothalamus, and striatum. Though serum testosterone concentrations were also elevated in sociosexually experienced males, this hormonal difference does not account for the observed neural difference.[52]

The effect of sociosexual experience on brain metabolism varies dramatically between male leopard geckos and male rats. While the AH, LHA, DLH, and dorsal lateral thalamus showed increases with sociosexual experience in male geckos, none of these areas showed changes with repeated copulatory

experiences in male rats. Furthermore, sociosexual experience leads to decrement in CO activity in some limbic brain areas, whereas in rats only increases were observed. In fact, the only brain area that showed increases in metabolic capacity across both species is the VMH. This is interesting because the VMH is primarily regarded as an area modulating female-typical receptive behaviors. However, copulation induces c-Fos in the VMH in male rats,[45] suggesting that it may modulate the expression of copulatory behavior. Furthermore, the VMH is replete with androgen receptors,[53] and antagonism of androgen receptors in the VMH significantly inhibits copulatory behavior.[54] Therefore, this commonality in plasticity in the VMH may translate into similar behavioral changes accompanying sexual experience.

In order to assess the functional significance of the metabolic changes in geckos, we subsequently investigated the behavioral difference between naïve, isolated males and sociosexually experienced males.[55] Because sexually naïve rats are more detrimentally affected by exposure to novel environments than sexually experienced male rats,[38] we first examined the differences in reactivity to novel environments. We found that sexually experienced male geckos are less likely to flee in a novel test environment than naïve males. This suggests that the effect of sexual experience on reactivity to novel environments is evolutionarily conserved. Furthermore, we found that sexually experienced males exhibit more territorial marking behavior in a novel test chamber than naïve males. The increased propensity to mark may be due to the fact that metabolic capacity is elevated in the AH in sexually experienced males. Interestingly, as mentioned previously, males from the male-biased incubation temperature show more territorial marking behavior after gonadectomy and testosterone replacement than identically treated males from the female-biased incubation temperature. Furthermore, males from the male-biased incubation temperature also have elevated metabolic capacity in the AH relative to males from the female-biased incubation temperature. Taken together, this supports the notion that elevated metabolic capacity in the AH translates into an increased priming to display territorial marking behaviors. Alternatively, because the VMH has also been found to modulate the expression of agonistic behavior in male mice,[56] it is possible that the elevated metabolic capacity in the VMH underlies this phenotypic difference.

After these behavioral tests, we gonadectomized the naïve and experienced male geckos, allowed a week to recover from surgery, then tested for sexual behavior every four days for three months. Though a higher proportion of sociosexually experienced males showed courtship behavior (i.e., body gripped stimulus female) following castration, the difference never reached statistical significance. Analyzed differently, the date at which males reached a criterion for extinction (i.e., five consecutive tests without body gripping the female) also did not differ significantly across groups. Therefore, unlike rats, it does not appear that sexual experience engenders a heightened robustness to castration in the leopard gecko.

This behavioral finding may be explained by comparing the effects of castration and sociosexual experience on brain metabolism. In adult male geckos, castration leads to significant decrements in metabolic capacity in the POA, amygdaloid nuclei, AH, DLH, SEP, and DVR.[57] The only androgen-sensitive brain areas that show metabolic increases with sociosexual experience are the AH and DLH. Metabolism in the VMH, the only brain area to show experience-dependent increases in CO activity in both rats and geckos, does not seem to be significantly affected by androgen concentrations. Moreover, the POA and amygdala, areas important in the expression of copulatory behavior in a variety of vertebrates[47] and that show experience-dependent metabolic increases in the rat, do not show increases with sociosexual experience in male geckos. Indeed, the differential effect of sociosexual experience on the POA and amygdala may explain why experience-dependent increases in robustness to castration are found in male rats but not in male geckos.

If this is true, an interesting hypothesis is that sexually naïve male geckos from different incubation temperatures differ in their robustness to castration. For example, because naïve males from the female-biased incubation temperature have elevated CO activity in the POA than naïve males from the male-biased incubation temperature,[19] sexually naïve males from the female-biased incubation temperature may be more robust to castration than naïve males from the male-biased incubation temperature.

IV. Interactions between Early and Late Factors

In Chapter 26, Wagner et al. expound the masculinizing influence of prenatal progesterone exposure in rats. Our lab has focused on the effects of progesterone in adulthood on male-typical courtship and copulatory behavior. We have demonstrated that progesterone facilitates copulatory behavior in rats[55] as well as in two species of whiptail lizards[59,60] and green anole lizards.[61] Therefore, progesterone stimulation both early and later in life facilitates copulatory behavior. Recent experiments with progesterone receptor knockout (PRKO) mice have focused on how the lack of progesterone stimulation throughout life can affect not only the display of sociosexual behavior in adulthood but also the degree of behavioral plasticity shown in adulthood.

Relative to males with functional progesterone receptors (i.e., wild-type [WT] males), PRKO male mice show significant decrements in copulatory behavior on their first sexual experience. For example, PRKO males show lower mounting frequency (i.e., number of mounts per five minutes)[62] as well as increased mounting latencies[63] on their first test with receptive females. However, after the first test, PRKO males improve their performance such that there is no longer a significant difference between WT and PRKO males. Sexual experience appears to compensate for the persistent lack of progesterone stimulation. Furthermore, stimulation of dopamine receptors,

specifically the D1 subtype, during the first behavioral test also eliminates the difference between WT and PRKO males.[63]

Interestingly, sexual experience has different effects on the robustness to castration in WT and PRKO males.[62] While sexually experienced WT males show the expected increase in robustness to castration relative to naïve WT males, sexually experienced and sexually naïve PRKO males are not different in their postcastration copulatory behavior. At three weeks following castration, very few sexually experienced PRKO males exhibit mounting behavior, while many WT males continue to mount receptive females. Naïve males of both genotypes do not show sexual behavior three weeks after castration. Therefore, the lack of experience-dependent increases in robustness to castration in PRKO males is due not to a heightened capacity of naïve males to copulate following castration, but to a reduced retention of copulatory behavior in sexually experienced males. Strain differences in the retention of copulatory behavior following castration have also been documented,[64,65] and it is possible that these strain differences may be mediated by differences in progesterone stimulation early and/or later in life.

Finally, sensitivity to testosterone administration following castration also differs across genotypes. Wild-type male mice show more copulatory behavior after testosterone replacement than PRKO males.[62] Therefore, it appears that progesterone stimulation early and/or later in life may also modulate sensitivity to testosterone following castration.

Relating these findings to the experiments in rats and leopard geckos, it would be interesting to assess how neural metabolism changes with sexual experience in WT and PRKO males. Because we hypothesize that increases in metabolic capacity in preoptic and amygdalar areas are critical in engendering a heightened robustness to castration, we predict that only in WT males will sexual experience lead to increases in CO activity in these brain areas. Given that male leopard geckos and male rats that are more sensitive to the activational effects of androgen treatment on courtship behavior (i.e., male geckos from the female-biased incubation temperature versus males from the male-biased incubation temperature, or sexually experienced male rats versus sexually naïve males) also have elevated metabolic capacity in the POA, we also predict that WT males are more sensitive to the activational effects of androgens on copulatory behavior because they have elevated CO activity in the POA.

Finally, it would also be interesting to investigate how early factors that affect neural metabolism (i.e., incubation temperature in leopard geckos and intrauterine position in rodents) can constrain the degree of neural and behavioral plasticity following sociosexual experiences. For example, male leopard geckos from the female-biased incubation temperature may show different amounts or types of changes following sociosexual experiences. Phrased differently, early factors may not only shape baseline differences in behavior but also mold individual differences in the phenotypic plasticity.

V. Acknowledgments

This research was supported by NSF DGE-9616181 (JTS), University Preemptive Fellowship (JTS), Howard Hughes Medical Institute Fellowship (AG), Barry M. Goldwater Foundation Scholarship (AG), NIH NS 37755 (F. Gonzalez-Lima), and NIMH 41770 (DC). Special thanks to Dr. F. Gonzalez-Lima for providing training and guidance throughout these experiments and to Sarah Woolley for her comments on the manuscript.

References

1. Wallen, K. and Schneider, J.E. *Reproduction in Context. Social and Environmental Influences on Reproduction*, MIT Press, Cambridge, 2000.
2. Stern, J.M. Multisensory regulation of maternal behavior and masculine sexual behavior: a revised view. *Neurosci. Biobehav. Rev.*, 14, 183, 1990.
3. Wong-Riley, M.T.T. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. *TINS*, 12, 94, 1989.
4. Erecinska, M. and Silver, I.A. ATP and brain function. *J. Cerebr. Blood Flow Metab.*, 9, 2, 1989.
5. Borowsky, I.W. and Collins, R.C. Histochemical changes in enzymes of energy metabolism in the dentate gyrus accompany deafferentation and synaptic reorganization. *Neuroscience*, 33, 253, 1989.
6. Wong-Riley, M. and Caroll, E.W. Effect of impulse blockage on cytochrome oxidase activity in monkey visual system. *Nature*, 307, 262, 1989.
7. Hevner, R.F. and Wong-Riley, M.T.T. Regulation of cytochrome oxidase protein levels by functional activity in the macaque monkey visual system. *J. Neurosci.*, 10, 1331, 1990.
8. Gonzalez-Lima, F. Brain imaging of auditory learning functions in rats: studies with fluorodeoxyglucose autoradiography and cytochrome oxidase histochemistry, in *Advances in Metabolic Mapping Techniques for Brain Imaging of Behavioral and Learning Functions*, Gonzalez-Lima, F., Ed., Kluwer Academic, Dordrecht, 1992, chap. 2.
9. Hevner, R.F. and Wong-Riley, M.T.T. Mitochondrial and nuclear gene expression for cytochrome oxidase subunits are disproportionately regulated by functional activity in neurons. *J. Neurosci.*, 13, 1805, 1993.
10. Wong-Riley, M.T.T., Nie, F., Hevner, R.F., and Liu, S. Brain cytochrome oxidase: functional significance and bigenomic regulation in the CNS, in *Cytochrome Oxidase in Neuronal Metabolism and Alzheimer's Disease*, Gonzalez-Lima, F., Ed., Plenum Press, New York, 1998, chap. 1.
11. Hevner, R.F. and Wong-Riley, M.T.T. Brain cytochrome oxidase: purification, antibody production, and immunohistochemical/histochemical correlations in the CNS. *J. Neurosci.*, 9, 3884, 1989.
12. Curran, T. and Franza, B.R. Fos and Jun: the AP-1 connection. *Cell*, 55, 395, 1988.
13. Morgan, J.I. and Curran, T. Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. *TINS*, 12, 459, 1989.

14. Tsai, M-J. and O'Malley, B.W. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Ann. Rev. Biochem.*, 63, 451, 1994.
15. Crews, D., Sakata, J., and Rhen, T. Developmental effects on intersexual and intrasexual variation in growth and reproduction in a lizard with temperature-dependent sex determination. *J. Comp. Biochem. Physiol. C*, 119, 229, 1998.
16. Flores, D.L., Tousignant, A., and Crews. D. Incubation temperature affects the behavior of adult leopard geckos. *Physiol. Behav.*, 55, 1067, 1994.
17. Rhen, T. and Crews, D. Embryonic temperature and gonadal sex organize male-typical sexual and aggressive behavior in a lizard with temperature-dependent sex determination. *Endocrinology*, 140, 4501, 1999.
18. Flores, D.L. and Crews, D. Effect of hormonal manipulation on sociosexual behavior in adult female leopard geckos (*Eublepharis macularius*), a species with temperature-dependent sex determination. *Horm. Behav.*, 29, 458, 1995.
19. Coomber, P., Crews, D., and Gonzalez-Lima, F. Independent effects of incubation temperature and gonadal sex on the volume and metabolic capacity of brain nuclei in the leopard gecko (*Eublepharis macularius*), a lizard with temperature-dependent sex determination. *J. Comp. Neurol.*, 380, 409, 1997.
20. Ferris, C.F., Gold, L., de Vries, G.J., and Potegal, M. Evidence for a functional and anatomical relationship between the lateral septum and the hypothalamus in the control of flank marking behavior in golden hamsters. *J. Comp. Neurol.*, 293, 476, 1990.
21. Goodson, J.L., Eibach, R., Sakata, J., and Adkins-Regan, E. Effect of septal lesions on male song and aggression in the colonial zebra finch (*Taeniopygia guttata*) and the territorial field sparrow (*Spizella pusilla*). *Behav. Brain. Res.*, 101, 115, 1999.
22. Morrell, J.I., Crews, D., Ballin, A., Morgentaler, A., and Pfaff, D.W. ^{3}H-Estradiol, ^{3}H-testosterone, and ^{3}H-dihydrotestosterone localization in the brain of the lizard, *Anolis carolinensis*: an autoradiographic study. *J. Comp. Neurol.*, 188, 201, 1979.
23. Young, L.J., Lopreato, G.F., Horan, K., and Crews, D. Cloning and *in situ* hybridization analysis of estrogen receptor, progesterone receptor, and androgen receptor expression in the brain of whiptail lizards (*Cnemidophorus uniparens* and *C. inornatus*). *J. Comp. Neurol.*, 247, 288, 1994.
24. Clark, M.M., Crews, D., Galef, B.G., Jr. Concentrations of sex steroid levels of pregnant and fetal Mongolian gerbils. *Physiol. Behav.*, 49, 239, 1991.
25. Clark, M.M., Spencer, C.A., and Galef, B.G., Jr. Improving productivity of breeding colonies of Mongolian gerbils (*Meriones unguiculatus*). *Lab. Anim.*, 20, 313, 1986.
26. Clark, M.M. and Galef, B.G., Jr. Effects of uterine position on rate of sexual development in female Mongolian gerbils. *Physiol. Behav.*, 42, 15, 1988.
27. Clark, M.M., vom Saal, F.S., and Galef, B.G., Jr. Intrauterine positions and testosterone levels of adult male gerbils are correlated. *Physiol. Behav.*, 51, 957, 1992.
28. Jones, D., Gonzalez-Lima, F., Crews, D., Galef, B.G., Jr., and Clark, M.M. Effects of intrauterine position on the metabolic capacity of the hypothalamus of female gerbils. *Physiol. Behav.*, 61, 513, 1997.
29. Dewsbury, D.A. Copulatory behaviour of rats (*Rattus norvegicus*) as a function of prior copulatory experience. *Anim. Behav.*, 17, 217, 1969.
30. Larsson, K. Experiential factors in the development of sexual behaviour, in *Biological Determinants of Sexual Behaviour*, Hutchinson, J.B., Ed., John Wiley & Sons, Chichester, 1978, chap. 3.

31. Retana-Marquez, S. and Velazquez-Moctezuma, J. Cholinergic-androgenic interaction in the regulation of male sexual behavior in rats. *Pharmacol. Biochem. Behav.*, 56, 373, 1997.
32. Kamel, F., Mock, E.J., Wright, W.W., and Frankel, A.I. Alterations in plasma concentrations of testosterone, LH, and prolactin associated with mating in the male rat. *Horm. Behav.*, 6, 277, 1975.
33. Graham, J.M. and Desjardins, C. Classical conditioning: induction of luteinizing hormone and testosterone secretion in anticipation of sexual activity. *Science*, 210, 1039, 1980.
34. Heeb, M.M. and Yahr, P. c-Fos immunoreactivity in the sexually dimorphic area of the hypothalamus and related brain regions of male gerbils after exposure to sex-related stimuli or performance of specific sexual behaviors. *Neuroscience*, 72, 1049, 1996.
35. Kollack-Walker, S. and Newman, S.W. Mating-induced expression of c-Fos in the male Syrian hamster brain: role of experience, pheromones, and ejaculation. *J. Neurobiol.*, 32, 481, 1997.
36. Lumley, L.A. and Hull, E.M. Effects of a D1 antagonist and of sexual experience on copulation-induced Fos-like immunoreactivity in the medial preoptic nucleus. *Brain Res.*, 829, 55, 1999.
37. Guiliani, D. and Ferrari, F. Differential behavioral response to dopamine D2 agonists by sexually naïve, sexually active, and sexually inactive male rats. *Behav. Neurosci.*, 110, 802, 1996.
38. Pfaus, J.G. and Wilkins, M.F. A novel environment disrupts copulation in sexually naïve but not experienced male rats: reversal with naloxone. *Physiol. Behav.*, 57, 1045, 1995.
39. Harris, V.S. and Sachs, B.D. Copulatory behavior in male rats following amygdaloid lesions. *Brain Res.*, 86, 514, 1975.
40. Arendash, G.W. and Gorski, R.A. Effects of discrete lesions of the sexually dimorphic nucleus of the preoptic area or other medial preoptic regions on the sexual behavior of male rats. *Brain Res. Bull.*, 10, 147, 1983.
41. de Jonge, F.H., Louwerse, A.L., Ooms, M.P., Evers, P., Endert, E., and van de Poll, N. Lesions of the SDN-POA inhibit sexual behavior of male Wistar rats. *Brain Res. Bull.*, 23, 483, 1989.
42. Kondo, Y. Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperienced male rats. *Physiol. Behav.*, 51, 939, 1992.
43. Sakata, J.T., Gonzalez-Lima, F., Gupta, A., and Crews, D. Differential changes in brain metabolic capacity in response to sociosexual experience in the male rat. Submitted.
44. Simerly, R.B., Chang, C., Muramatsu, M., and Swanson, L.W. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an *in situ* hybridization study. *J. Comp. Neurol.*, 294, 76, 1990.
45. Greco, B., Edwards, D.A., Michael, R.P., and Clancy, A.N. Androgen receptor immunoreactivity and mating-induced Fos expression in forebrain and midbrain structures in the male rat. *Neuroscience*, 75, 161, 1996.
46. Pfaus, J.G. and Heeb, M.M. Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. *Brain Res. Bull.*, 44, 397, 1997.
47. Meisel, R.L. and Sachs, B.D. The physiology of male sexual behavior, in *The Physiology of Reproduction*, Vol. 2, Knobil, E. and Neill, J.D., Eds., Raven Press, New York, 1994, chap. 1.

48. McEwen, B.S., Coirini, H., Westlind-Danielsso, A., Frankfurt, M., Gould, E., Schumacher, M., and Woolley, C. Steroid hormones as mediators of neural plasticity. *J. Steroid Biochem. Mol. Biol.*, 39, 223, 1991.
49. Woolley, C.S. Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. *Horm. Behav.*, 34, 140, 1998.
50. Greco, B., Edwards, D.A., Michael, R.P., and Clancy, A.N. Androgen receptors and estrogen receptors are colocalized in male rat hypothalamic and limbic neurons that express Fos immunoreactivity induced by mating. *Neuroendocrinology*, 67, 18, 1998.
51. Crews, D., Coomber, P., and Gonzalez-Lima, F. Effects of age and sociosexual experience on the morphology and metabolic capacity of brain nuclei in the leopard gecko (*Eublepharis macularius*), a lizard with temperature-dependent sex determination. *Brain Res.*, 758 169, 1997.
52. Sakata, J.T. and Crews, D. Unpublished data, 2000.
53. Roselli, C.E., Handa, R.J., and Resko, J.A. Quantitative distribution of nuclear androgen receptors in microdissected areas of the rat brain. *Neuroendocrinology*, 49, 449, 1989.
54. McGinnis, M.Y., Williams, G.W., and Lumia, A.R. Inhibition of male sex behavior by androgen receptor blockade in preoptic area or hypothalamus, but not amygdala or septum. *Physiol. Behav.*, 60, 783, 1996.
55. Sakata, J.T., Gupta, A., Chuang, C-P., and Crews, D. Unpublished data, 2000.
56. Nyby, J., Matochik, J.A., and Barfield, R.J. Intracranial androgenic and estrogenic stimulation of male-typical behaviors in house mice (*Mus domesticus*). *Horm. Behav.*, 26, 24, 1992.
57. Crews, D., Coomber, P., Baldwin, R., Azad, N., and Gonzalez-Lima, F. Brain organization in a reptile lacking sex chromosomes: effects of gonadectomy and exogenous testosterone. *Horm. Behav.*, 30, 474, 1996.
58. Witt, D.M., Young, L.J., and Crews, D. Progesterone modulation of androgen-dependent sexual behavior in male rats. *Physiol. Behav.*, 57, 307, 1995.
59. Lindzey, J. and Crews, D. Interactions between progesterone and androgens in the stimulation of sex behaviors in male little striped whiptail lizards, *Cnemidophorus inornatus*. *Gen. Comp. Endocrinol.*, 86, 52, 1992.
60. Grassman, M. and Crews, D. Progesterone induction of pseudocopulatory behavior and stimulus-response complementarity in an all-female lizard species. *Horm. Behav.*, 20, 327, 1986.
61. Young, L.J., Greenberg, N., and Crews, D. The effects of progesterone on sexual behavior in male green anole lizards (*Anolis carolinensis*). *Horm. Behav.*, 25, 477, 1991.
62. Phelps, S.M, Lydon, J.P., O'Malley, B.W., and Crews, D. Regulation of male sexual behavior by progesterone receptor, sexual experience, and androgen. *Horm. Behav.*, 34, 292, 1998.
63. Woolley, S.C. and Crews, D. Unpublished data, 2000.
64. McGill, T.E. and Tucker, G.R. Genotype and sex drive in intact and in castrated male mice. *Science*, 145, 514, 1964.
65. McGill, T.E. and Manning, A. Genotype and the retention of the ejaculatory reflex in castrated male mice. *Anim. Behav.*, 24, 507, 1976.