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The organizational–activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues

Arthur P. Arnold *

Department of Physiological Science, University of California, Los Angeles, USA
Laboratory of Neuroendocrinology of the Brain Research Institute, University of California, Los Angeles, USA

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ABSTRACT

The 1959 publication of the paper by Phoenix et al. was a major turning point in the study of sexual differentiation of the brain. That study showed that sex differences in behavior, and by extension in the brain, were permanently sexually differentiated by testosterone, a testicular secretion, during an early critical period of development. The study placed the brain together in a class with other major sexually dimorphic tissues (external genitalia and genital tracts), and proposed an integrated hormonal theory of sexual differentiation for all of these non-gonadal tissues. Since 1959, the organizational–activational theory has been amended but survives as a central concept that explains many sex differences in phenotype, in diverse tissues and at all levels of analysis from the molecular to the behavioral. In the last two decades, however, sex differences have been found that are not explained by such gonadal hormonal effects, but rather because of the primary action of genes encoded on the sex chromosomes. To integrate the classic organizational and activational effects with the more recently discovered sex chromosome effects, we propose a unified theory of sexual differentiation that applies to all mammalian tissues.

Ever since 1959

The 1959 publication of the paper by Charles H. Phoenix, Robert W. Goy, Arnold A. Gerall, and William C. Young is appropriately perceived as a major turning point in the study of sex differences in the brain. These authors provided a conceptual framework that has been repeatedly tested and improved since 1959, but has not been substantially undermined by experimental findings in the intervening half century. That's remarkable. The methods used by Phoenix et al. continue to be emulated today in any comprehensive study of sex differences in the brain and behavior, or in non-brain phenotypes (Becker et al., 2005). The framework has been expanded to explain a large majority of sex differences in phenotype of all non-gonadal tissues (e.g., Beatty, 1984; Greenspan et al., 2007). In addition, it has been applied progressively more broadly to new levels of analysis (cellular, molecular, genetic) of sex differences as they became possible in the last 50 years. Along the way, a few amendments were made to the framework, which have served to enhance it. We begin by discussing what Phoenix et al. found and what they concluded, and then discuss some of the "footnotes" that have been added to the framework based on subsequent research. We then discuss sex differences that are not explained by the organizational–activational framework, and merge those findings with the organizational–activational concept to suggest a unified theory of sexual differentiation of all tissues in mammals.

The conceptual framework of Phoenix, Goy, Gerall, and Young: organization and activation

Phoenix et al. injected pregnant guinea pigs with testosterone propionate, and then studied the mating behavior of the offspring when they were adult. They were interested in the behavioral capacity of the animals, defined by whether the experimentally manipulated guinea pigs would behave like a male or female. If the animal showed lordosis behavior, they concluded that it had the capacity to show behavior typical of females. If the animal mounted a receptive female guinea pig, they concluded that it had the capacity to show behavior typical of males. It was important to test the animals under conditions that normally lead to high frequencies of the behaviors. Thus, to test for lordosis, the animals were gonadectomized before puberty and as adults injected with estradiol benzoate followed by progesterone, and then stimulated manually ("fingered") in a manner that reliably elicits lordosis in control females. The hormones injected were thought to mimic the hormones that bring about the female guinea pig's behavioral heat, and the manual stimulation mimicked the tactile stimuli normally provided by the copulating male. In contrast, to test

* Department of Physiological Science, UCLA, 621 Charles Young Drive South, Los Angeles CA 90095-1606, USA. Fax: +1 310 825 8081.
E-mail address: arnold@ucla.edu.

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for masculine mounting, the animals were gonadectomized before puberty and then injected with testosterone propionate as adults and exposed to a receptive female.

The major findings of the paper were as follows.

1. *Fetal masculinization*. Females treated prenatally with testosterone showed less lordosis and more mounting as adults than control females. The authors concluded that exposure to testosterone during fetal life makes female guinea pig behavior more like that of males. The implication (almost tacit in their 1959 article) was that the male is normally masculinized by testosterone secreted by his testes during fetal life. (Phoenix et al. did not use the term "defeminization" presumably because they viewed masculinization as involving both an increase in masculine behavior and decrease in feminine behavior; see below for further discussion).
2. *Permanence*. The prenatal effects were permanent, since they were observed months after the end of the fetal testosterone treatment. The activational effects of gonadal hormones were seen as acute and reversible, not permanent.
3. *Organizational*. The effects of prenatal testosterone were interpreted to have changed the response to gonadal hormones that activate behaviors in adulthood. "The data are uniform in demonstrating that an androgen administered prenatally has an organizing action on the tissues mediating mating behavior in the sense of producing a responsiveness to exogenous hormone which differs from that of normal adult females" (page 369).
4. *Dichotomy*. The authors dichotomized the hormonal effects: organizational ("differentiating") vs. activational. During the prenatal period, testosterone acted to organize tissues so that they respond differently to gonadal hormones in adulthood. In adulthood, the hormones activate tissues organized prenatally. "The embryonic and fetal periods are periods of organization or "differentiation" in the direction of masculinization or feminization. Adulthood, when gonadal hormones are being secreted, is a period of activation; neural tissues are the target organs and mating behavior is brought to expression. Like the genital tracts, the neural tissues mediating mating behavior respond to androgens or to estrogens depending on the sex of the individual, but again the specificity is not complete" (pages 379–380).
5. *Hormonal effects on the brain*. The authors favored the idea that the brain, like the genital tracts, was permanently masculinized (differentiated) by testosterone. Although their wording carefully leaves open the site of testosterone action ("on the tissues mediating mating behavior", page 369), the authors clearly favored the view that testosterone or its metabolites acts on the CNS. "We are assuming that testosterone or some metabolite acts on those central nervous tissues in which patterns of sexual behavior are organized" (page 381). (The identification of estradiol as an important metabolite in the brain was an important footnote added later (MacLusky and Naftolin, 1981).
6. *Critical period*. The authors presented evidence for a critical period for testosterone's action on the brain. Treating females with testosterone postnatally, or in adulthood, did not change their responsiveness to hormones in the long term.
7. *Diverse actions of testosterone*. Although prenatal exposure to testosterone also caused masculinization of the external genitals of females, the effects on mating behavior were dissociated from those on the genitalia because they were not always correlated. This dissociation was not discussed at length by Phoenix et al., but implies that the behavioral effects are not the result of the actions of testosterone on the genitalia, an issue that recurred in later discussions (Beach, 1971).

A critical emphasis of the Phoenix et al. paper was that they were applying and extending a conceptual framework, already developed by Lillie (1916; 1939), Jost (1947; Jost et al., 1973) and others, based on the study of sexual differentiation of the external genitalia and genital tracts. "Attention is directed to the parallel nature of the relationship, on the one hand, between androgens and the differentiation of the genital tracts, and on the other, between androgens and the organization of the neural tissues destined to mediate mating behavior in the adult" (page 369). Specifically, Phoenix et al. argued that the fetal actions of hormones permanently change the substrate (probably neural) on which gonadal hormones act in adulthood, just as they do in the genitalia and genital tracts. By explaining behavioral and genital sexual differentiation in much the same way, the authors provided a heuristically pleasing single framework for explaining all non-gonadal sexual differentiation. The comparison was an invitation to the reader to apply to behavior a host of experimental findings in the period 1916–1959 that indicated that gonadal hormones cause permanent sex differences in tissue differentiation and growth, even though the Phoenix et al. experiments themselves did not measure morphological differentiation and growth. "…When what has been learned from the present investigation is related to what has long been known with respect to the action of androgens in the genital tracts, a concept much broader than that suggested by the older studies emerges" (page 379).

Yet, Phoenix et al. realized that both sexes have significant capacity to show behavior normally seen mostly in the other sex. Thus, behavioral sexual differentiation is incomplete, and the two sexes are each somewhat bisexual. "We suggest… that in the adult this bisexuality is unequal in the neural tissues as it is in the case of the genital tissues. The capacity exists for giving behavioral responses of the opposite sex, but it is variable and, in most mammals that have been studied and in many lower vertebrates as well, it is elicited only with difficulty…" (page 380).

Conceptual frameworks determine experimental designs

The Phoenix et al. theory has dominantly influenced how experiments have been performed ever since. For example, in the brain sexual differentiation literature, the effects of hormones are not actually measured equally at all life stages. Rather, there has been the tendency to investigate adult hormone and fetal/neonatal hormone effects intensively because those are the focus of the organizational–activational theory. Most investigators now think of adulthood as an extended period in which hormones act on a relatively unchanging neural substrate. Admittedly there are the slow changes related to aging, and some experiences might cause longer lasting changes to the adult neural substrate. But as a rule, if the effects of gonadal hormones are to be tested in adult animals (via manipulations of hormone levels or receptors or synthetic enzymes), the age of gonadal hormone manipulation of adults is not thought to be critical. On the other hand, if the investigator believes that adult hormone levels do not explain a specific sex difference, then the most common manipulation is to administer testosterone to fetal or neonatal females, or to reduce testosterone action in fetal or neonatal males, based on the organizational hypothesis. This focus on two times of life has left some important questions relatively unanswered. For example, does the surge of gonadal hormone secretion at puberty have long-lasting effects similar to the perinatal organizational effects? Recent experiments support that idea (Sisk and Zehr, 2005; Sisk, this volume). Indeed, one might now ask if the pubertal period can be considered a second wave of differentiation of the "tissues that mediate" sexually dimorphic behaviors in adulthood (Ahmed et al., 2008).

The experimental design of Phoenix et al. set a standard for succeeding generations: to measure the permanent effects of gonadal hormones that act during the fetal/neonatal period, compare groups that differ in the levels of fetal hormones but keep the levels of hormones equal across groups at the time of behavioral testing. By