Puberty: A Finishing School for Male Social Behavior

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ABSTRACT: The classical view of steroid-dependent organization of brain and behavior holds that gonadal steroid hormones, acting during an early critical period of development, cause permanent structural changes in neural circuits that determine behavioral responses to hormones in adulthood. This classical view has been modified to incorporate evidence that organizational effects of steroids can occur outside of the established perinatal critical period and that multiple critical periods may exist during development. Experiments in this laboratory indicate that steroid-dependent organization of neural circuits underlying male social behaviors occurs during puberty. This work shows that adult-typical reproductive and flank marking behaviors cannot be activated by gonadal steroids in male Syrian hamsters prior to puberty, suggesting that developmentally timed processes during puberty render the nervous system responsive to activating effects of gonadal steroids in adulthood. Additional experiments demonstrate that the presence or absence of gonadal hormones during puberty is a major factor in the ability of steroids to activate reproductive and flank marking behavior in adult male hamsters and in androgen receptor expression within the neural circuit underlying these behaviors. Thus, gonadal hormones during puberty appear to exert long-lasting changes in neural circuits that are responsible for the programming of activational responses to steroids later in adulthood. A two-stage model for maturation of male social behaviors is proposed: a perinatal critical period for sexual differentiation of neural circuits, followed by the pubertal period, during which gonadal steroids further organize the circuits to enhance behavioral responsiveness to hormones in adulthood. Whether puberty is a critical period for the proposed second wave of steroid-dependent organization of behavioral circuits remains to be determined.

KEYWORDS: puberty; organization; male social behavior; gonadal steroids

INTRODUCTION

The central thesis of this paper is that puberty is not only the time during which reproductive maturation occurs, but it is also a period of development of the nervous system that is dissociable from gonadal maturation. Pubertal maturation of the brain

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Ann. N.Y. Acad. Sci. 1007: 189–198 (2003).
doi: 10.1196/annals.1286.019

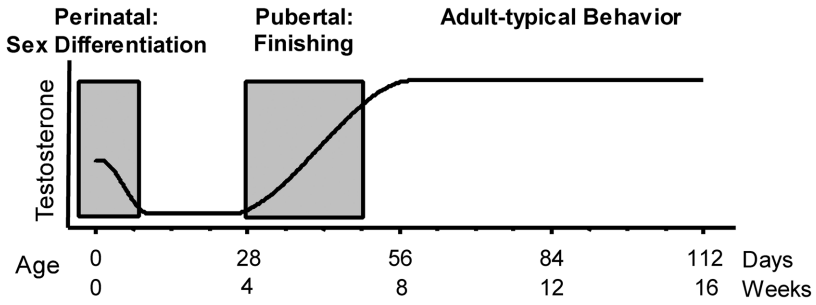

FIGURE 1. Two-stage model for the development of male social behavior. During the perinatal period, exposure to steroid hormones results in sexual differentiation. During the pubertal period, exposure to steroid hormones produces the final maturational changes necessary for adult-typical expression of social behavior.

and reproductive system normally occur around the same time in an individual, and these processes are interactive. Reproductive maturation is initiated through activation of the forebrain gonadotropin-releasing hormone (GnRH) neuronal system, and increased neurosecretory activity of GnRH neurons results in increased production of gonadal steroid hormones. Gonadal steroids, in turn, regulate physiological functions and facilitate the expression of many social behaviors. These actions of steroid hormones are typically described as activational effects. There is increasing evidence that steroid hormones also exert long-lasting organizational effects on neural circuits during puberty.[1,2] In this paper, we develop the idea that developmentally timed events, occurring during pubertal maturation of the brain, render neural circuits sensitive to steroid-dependent organizational change at this stage of development.

The concept of steroid-dependent organization of the nervous system is discussed first and the criteria historically used to establish that organizational effects have occurred are outlined. Next, we review experiments from this laboratory demonstrating that full hormonal activation of male social behaviors in adulthood is dependent in part on long-lasting structural modifications in behavioral circuits organized by steroid hormones during puberty. A two-stage model is proposed for the maturation of adult male social behaviors (FIG. 1). The model incorporates sexual differentiation of neural circuits during a perinatal critical period, followed by further steroid-dependent organization of these circuits during puberty that enhances behavioral responsiveness to hormones in adulthood.

ORGANIZATION OF THE BRAIN AND BEHAVIOR BY STEROID HORMONES

The concept of the organization of behavior and its underlying neural circuits originated with the demonstration that perinatal manipulation of gonadal steroids affected the propensity to display masculine or feminine sexual behavior in response to hormone treatment in adulthood.[3] The classical definition of organizational effects of steroid hormones includes (1) permanent or long-lasting effects of perinatal

steroid hormone manipulation on behavior and structural features of underlying neural circuits; (2) the programming of behavioral responses to steroid hormones in adulthood; and (3) the existence of a perinatal critical period during which sensitivity to the organizational effects of steroid hormones is highest.

Several revisions to the organizational hypothesis have occurred over the past 40 years. Scott and colleagues laid the theoretical groundwork for the existence of multiple critical periods during development and suggested that organizational change in earlier critical periods may determine the capacity and direction of organizational change in successive critical periods.[4] Arnold and Breedlove[5] pointed out that steroid hormones can exert long-lasting structural changes in the nervous system well beyond the perinatal critical period. Finally, demonstrations that manipulations of gonadal steroids during puberty irreversibly alter the emergence of sex differences in nonreproductive adult social interactions indicated that puberty is a sensitive period for further steroid-dependent organization of behavioral circuits.[1] Data from this laboratory reviewed below support the idea that puberty is a sensitive period for hormonal organization of neural circuits underlying male reproductive and communicative behaviors.

ACTIVATION AND ORGANIZATION OF BEHAVIOR DURING PUBERTY

One of the first indications that organization of neural circuits underlying male reproductive behavior occurs during puberty came from observations that in a number of species doses of testosterone that reliably activate copulatory behavior in adult males do not activate behavior to the same extent in prepubertal males.[6–8] In a series of studies, we compared the activation of reproductive behavior in sexually naive prepubertal and adult male Syrian hamsters that were castrated and treated for one week with one of three doses of either testosterone, dihydrotestosterone, or estradiol benzoate via subcutaneous pellet implants.[8–10] Hormone treatment increased anogenital investigation of the female by both prepubertal and adult males, indicating

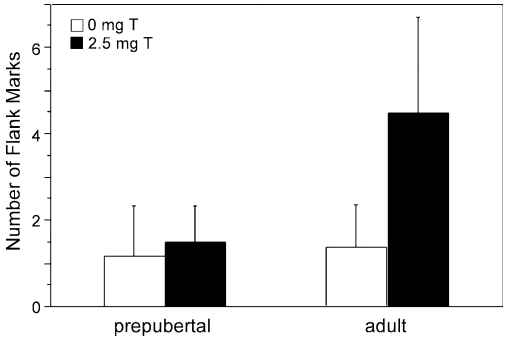

FIGURE 2. Number of mounts displayed by prepubertal and adult males during an interaction with an estrous female. All behavior tests occurred one week after castration and treatment with either TP (2.5 mg), DHT (0.5 mg), or EB (0.05 mg). Data are expressed as mean ± SEM.

similar activational effects of gonadal steroids on this component of reproductive behavior before and after puberty. In contrast, mounts (FIG. 2), intromissions, and ejaculations were activated by hormone treatment only in adults. These data suggest that developmental events occurring during puberty render the nervous system responsive to the activating effects of gonadal steroids on these components of reproductive behavior.

Another social behavior regulated by testosterone in Syrian hamsters is flank marking.[11,12] Males and females rub pigmented sebaceous glands located on their dorsal flank region against objects in the environment as an important form of communication about reproductive and social status.[13] Recent data from this laboratory suggest that testosterone does not facilitate flank marking during male social interactions until after pubertal development. Prepubertal and adult males were gonadectomized and treated with 0 or 2.5 mg of testosterone. After one week of treatment with testosterone, the flank marking behavior of prepubertal and adult males was observed during a social interaction with an unfamiliar male in a resident-intruder test. In adults, testosterone increased flank marking during the social interaction but, in juveniles, testosterone did not increase flank marking. These data suggest that, similar to neural circuits underlying reproductive behavior, circuits underlying flank marking are not responsive to the activating effects of testosterone until *after* pubertal maturation (FIG. 3).

The developmental processes during puberty that permit activating effects of steroid hormones on reproductive and flank marking behaviors in adulthood are still unknown. Recent work in this laboratory provides evidence that these processes include organizational change in neural circuits underlying behavior and, further, that gonadal hormones are the agents for organization. Two experiments investigated the effects of the presence of gonadal hormones during puberty on reproductive and flank marking behavior. In both studies, groups of male hamsters were gonadectomized either before or after puberty. Thus, the testes and gonadal hormones were not present during puberty in males castrated before puberty (NoTduringP), whereas gonadal hormones were present during puberty in males castrated afterward

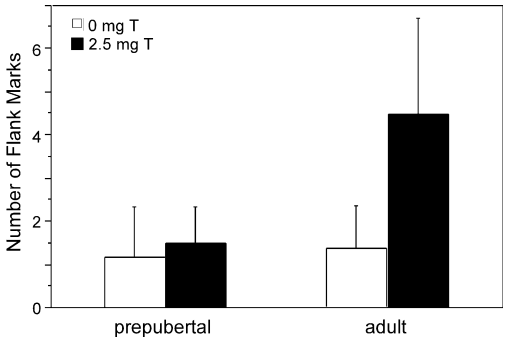

FIGURE 3. Number of flank marks displayed by prepubertal and adult males in their home cage during an interaction with an unfamiliar age- and weight-matched male intruder. All behavior tests occurred one week after castration and treatment with either T (2.5 mg) or vehicle (T, 0 mg). Data are expressed as mean ± SEM.

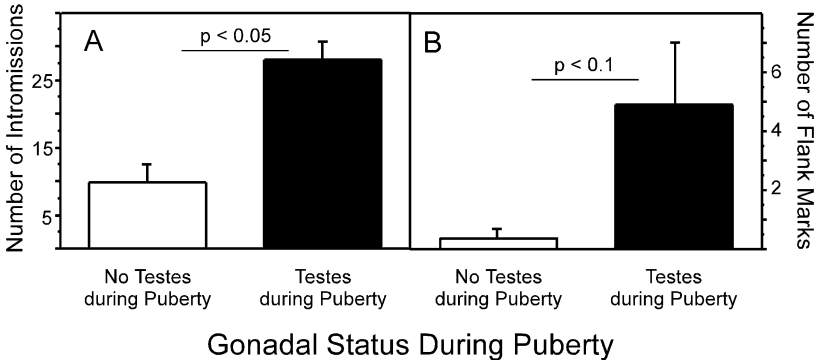

FIGURE 4. Number of intromissions during a reproductive test with a female (**A**) and number of flank marks during a resident-intruder test with an intact stimulus male (**B**) displayed by experimental males gonadectomized before (No Testes during Puberty) or after puberty (Testes during Puberty). Testosterone (2.5–3.0 mg) was administered 6 weeks after gonadectomy, and behavior tests occurred one week following the onset of testosterone treatment (7 weeks after gonadectomy). Each subject was tested in only one behavioral paradigm. Data are expressed as mean ± SEM.

(TduringP). Six weeks after prepubertal or postpubertal gonadectomy, when all males were chronologically adults, males were treated with testosterone and tested one week later either for reproductive behavior with a receptive female or for flank marking behavior during interactions with a male in a resident-intruder paradigm. NoTduringP males displayed fewer intromissions (FIG. 4A) and ejaculations than TduringP males, and these differences persisted even after 17 days of testosterone treatment. Similarly, NoTduringP males gonadectomized before puberty flank marked less frequently than TduringP males (FIG. 4B). Since behavioral differences between NoTduringP and TduringP males were present more than 7 weeks after gonadectomy, exposure to gonadal hormones during puberty appear to exert long-lasting changes in steroid-sensitive neural circuits underlying reproductive and flank marking behaviors. Furthermore, these changes induced by the presence of gonadal hormones during puberty alter the ability of testosterone to activate reproductive and flank marking behavior in adulthood. Thus, these outcomes of the presence of gonadal hormones during puberty fulfill two of the criteria for classical organizational effects: *long-lasting changes* that *program activational responses* to steroids in adulthood.

ACTIVATION AND ORGANIZATION OF BEHAVIORAL NEURAL CIRCUITS DURING PUBERTY

Pubertal development of sexual and flank marking behavior is paralleled by developmental changes within the neural circuits underlying social behaviors. Some differences in neural circuits before and after puberty are directly related to pubertal changes in circulating hormones, representing activational effects of steroid hormones on brain structure. For example, androgen and estrogen treatment of prepu-

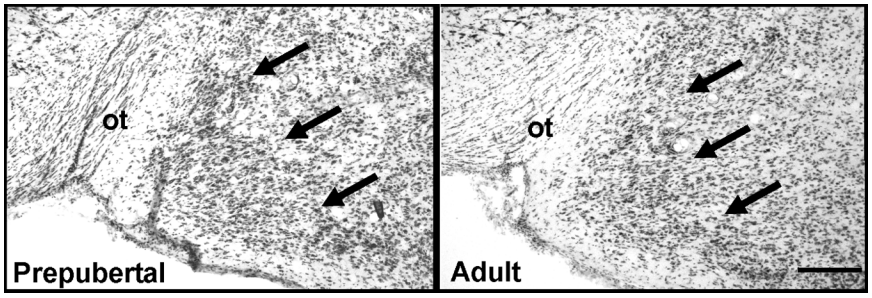

FIGURE 5. Photomicrograph of the anterior portion of the medial amygdala (MeA) in a prepubertal and adult male hamster. *Arrows* outline the outer boundary of the MeA. *Bar*, 200 μm. *Abbreviation*: ot, optic tract.

bertal male hamsters increases hypothalamic aromatase activity and progesterone receptor immunoreactivity, respectively, to levels comparable to those found in adults.[10,14] Therefore, in these respects prepubertal males can be made "adults" simply by experimentally increasing circulating steroids to adult levels.

Other experiments indicate that structural change in the amygdala occurs during puberty as the result of increased steroid secretion. The cross-sectional area of the posterodorsal portion of the medial amygdala (MePD) increases during puberty in male hamsters, and this increase in MePD area is reversible if adult males are housed in short photoperiods to induce gonadal regression and reduced testosterone secretion.[15] Experiments from other laboratories show that in adulthood, MePD size and dendritic branching are elaborated by testosterone and its estrogenic metabolites.[16,17] Thus, structural plasticity in the MePD appears to be related to circulating steroid levels during puberty and in adulthood.

In contrast to the MePD, the cross-sectional area of the medial amygdala (MeA) *decreases* during puberty in gonad-intact male hamsters (FIG. 5), and the MeA area does not revert back to the larger prepubertal size when males are exposed to short days to induce gonadal regression.[15] Whether the pubertal decrease in MeA area is dependent upon exposure to testosterone during puberty has not been determined. However, in adulthood, dendritic branching of MeA neurons is not influenced by circulating testosterone.[16] Collectively, these experiments suggest that pubertal development of the MeA involves regressive events that result in permanent structural organization that is not modifiable by testosterone in adulthood.

Androgen receptor (AR) expression within cell groups forming the forebrain neural circuit underlying male reproductive behavior is influenced by testosterone. Similar to behavioral activation by testosterone, AR regulation by testosterone differs in prepubertal and adult hamsters. However, unlike behavioral responses which are of less magnitude prior to puberty, the AR response is of *greater* magnitude in prepubertal males compared to adults. For example, the cells/unit area (density) of androgen receptor-immunoreactive (AR-ir) cells is higher in preoptic area nuclei of castrated, androgen-treated prepubertal males compared with similarly treated adults (FIG. 6).[8,9] Interestingly, as we found with behavioral activation, AR immunoreactivity in adulthood is influenced by whether gonadal hormones are present

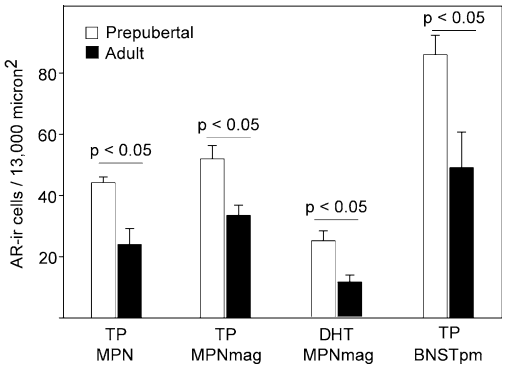

FIGURE 6. Androgen-receptor immunoreactivity (AR-ir cells/unit area) in brain regions controlling male sexual behavior after treatment of gonadectomized prepubertal and adult males with testosterone propionate (TP, 2.5 mg) or dihydrotestosterone (DHT, 0.5 mg) for one week prior to tissue collection. Data are expressed as mean ± SEM. *Abbreviations*: MPN, medial preoptic nucleus; MPNmag, magnocellular medial preoptic nucleus; BNSTpm, posterodorsal division of the bed nucleus of the stria terminalis.

during puberty. In an experiment similar in design to behavioral experiments described above, males were castrated either before (noTduringP) or after (TduringP) puberty, and several weeks later when all males were adults, a single injection of testosterone was administered 4 h prior to sacrifice to translocate AR to the nucleus for immunocytochemical visualization. The density of AR-ir cells in both preoptic area nuclei and bed nucleus of the stria terminalis (BNST) was *higher* in males gonadectomized before puberty (NoTduringP) when compared with males gonadectomized after puberty (TduringP) (FIG. 7).[18] These experiments suggest that prepubertal gonadectomy prevented a testosterone-dependent decrease in the number of AR-expressing cells in these brain areas, which caused AR expression to remain at prepubertal levels. These findings corroborate the observations on the MeA area in providing evidence that pubertal development of the nervous system involves steroid-dependent regressive events resulting in long-lasting structural and functional change in the forebrain neural circuits underlying male social behaviors.

CONCLUSIONS

The experiments substantiate the claim that prepubertal and adult males differ in more than just circulating gonadal steroid hormone levels, because adult levels of hormone administered to prepubertal males fail to elicit certain behavioral responses. These findings further suggest that the prepubertal brain is not merely an adult brain in limbo waiting for steroids to exert activational effects, but rather that the prepubertal brain requires additional development and maturation during puberty before full expression of adult-typical behavioral responses to hormones can occur.

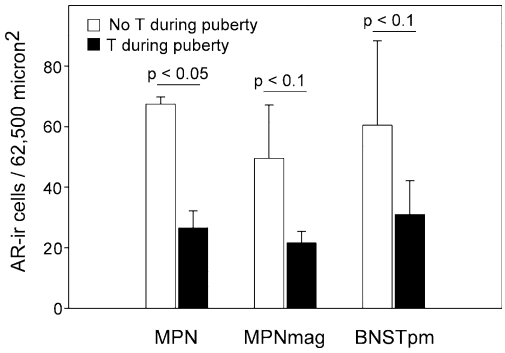

FIGURE 7. Androgen-receptor immunoreactivity (AR-ir cells/unit area) in brain regions controlling male sexual behavior in adult males gonadectomized before puberty (no T during puberty) and in adult males gonadectomized after puberty (T during puberty). All subjects received a subcutaneous injection of testosterone propionate (2.5 mg) 4 h prior to tissue collection. Data are expressed as mean ± SEM. *Abbreviations*: MPN, medial preoptic nucleus; MPNmag, magnocellular medial preoptic nucleus; BNSTpm, posterodorsal division of the bed nucleus of the stria terminalis.

They also provide evidence that pubertal maturation of the brain involves, at least in part, steroid-dependent organizational changes within forebrain behavioral circuits.

These observations have led us to propose a two-stage model for development of male social behavior (FIG. 1). The model holds that maturation of adult-typical behavioral responses to gonadal steroid hormones involves a perinatal critical period of steroid-dependent sexual differentiation of neural circuits underlying behavior, which determines whether male-typical or female-typical behavioral responses to gonadal steroids will occur in adulthood. A second period for steroid-dependent organizational change occurs during puberty when gonadal steroids further organize sexually differentiated neural circuits to enhance male-typical responses to steroid hormones in adulthood.

To date, our experiments provide evidence that effects of steroid hormones during puberty on adult behavior satisfy two of the criteria associated with the classical definition of organizational effects: *long-lasting* changes that *program activational responses* to hormones in adulthood. Arnold and Breedlove[5] effectively argued that steroid hormones can exert organizational effects on the nervous system outside of a critical period, and we contend that steroid hormones do organize neural circuits during puberty. However, Scott[4] made a compelling case for multiple critical periods during behavioral development and even argued that puberty is a likely candidate for a critical period because it is a time of rapid developmental change. While the proposed two-stage model incorporates puberty as a second developmental period during which steroids organize neural circuits and behavior, it does not *require* that puberty be a critical period for organizational change. That is, steroid hormones could theoretically further organize sexually differentiated neural circuits at any time following the initial perinatal critical period. Puberty may not be a critical period per se, but the second stage of organization may normally occur during puberty

because that is when the reproductive neuroendocrine axis is reactivated. However, preliminary evidence from this laboratory suggests that puberty may indeed be a second critical period for steroid-dependent organizational change. First, treatment of prepubertal males with testosterone for up to two weeks (days 14–28 of age) still does not permit the activation of reproductive behavior prior to puberty. This finding suggests that neural circuits are not sensitive to potential organizing effects of testosterone prior to puberty. Second, up to 17 days of testosterone treatment administered to adult males that were castrated prior to puberty still does not permit adult-typical activation of reproductive behavior. This finding suggests that neural circuits are not sensitive to potential organizing effects of testosterone after puberty. Future work will include direct tests of the hypothesis that puberty is a critical period for steroid-dependent organization of behavior and focused investigation of the structural mechanisms by which steroids organize neural circuits during puberty, regardless of whether puberty is a critical period.

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