The Testosterone Two-Step Is Really a Minuet

Dwayne K. Hamson,[1] Cynthia L. Jordan,[1] and S. Marc Breedlove[1,*]
[1]Neuroscience Program and Department of Psychology, Michigan State University, East Lansing, MI 48824, USA
*Correspondence: breedsm@msu.edu
DOI 10.1016/j.neuron.2010.04.025

Newly developed genetic models indicate that estrogen receptors (ERs) alone mediate prenatal masculinization of the mouse brain to organize reproductive and territorial behaviors, while postnatal activation of androgen receptors (ARs) potentiates specific components of those behaviors. These results and others offer a model of how AR and ER pathways interact to fully masculinize the brain and behavior of male mice.

The courtship behavior of animals has often been likened to a dance, each partner alternating with the other to produce a seamless and pleasing whole. Indeed, dancing has proven to be an important prelude to a future union for many human pairs. In this issue of *Neuron*, we learn that even within a sex an elaborate, highly coordinated dance between forces is required to produce a unified male brain, as Juntti et al. (2010) offer exciting new models for parsing out two fundamentally different contributions to the masculinization of mouse behavior. They also report that these two pathways do not dance in parallel, but rather interact, each regulating the other, to fully masculinize the brain. Not only do these new models provide us with new insights into the sexual differentiation of the mammalian brain, but they also illustrate how these methods can greatly expand our understanding of how males and females come to behave differently.

Over the past 50 years, mammalian models have established that the same testicular androgens, including testosterone (T), that act upon androgen receptors (ARs) to produce a masculine phenotype in the body also masculinize the developing brain, organizing it in a male-like fashion to promote masculine behaviors in adulthood (Phoenix et al., 1959). The relative scarcity of T in females permits the body and brain to develop feminine configurations and therefore feminine behaviors. We have also long known that in some mammals, including rats and mice, T in the brain is converted via aromatase into estrogens, which then activate one or both estrogen receptors (ERα and ERβ) to masculinize many, but not all, aspects of the brain (Feder and Whalen, 1965; Naftolin and MacLusky, 1984). So it has long been clear that both ARs and ERs must be stimulated to fully masculinize the rodent brain (Baum and Vreeburg, 1973; Zuloaga et al., 2008).

What has been difficult to determine is how this activity of both ARs and ERs is normally coordinated to masculinize the brain. Most studies have relied on two traditional methods of surgeries to remove endogenous sources of hormone, and/or pharmacological manipulations with exogenous hormones. While those methods are sufficient to implicate both AR and ER in masculinization of the brain, they have limitations. For example, any surgical or pharmacological manipulation of ARs inevitably affects the body phenotype: blocking AR in developing males results in feminine external genitalia, while stimulating AR in developing females masculinizes genitalia. Thus, when these manipulations affect adult behavior, it is not easy to determine whether ARs affected behavior by directly altering the brain or by altering the periphery, an especially plausible mechanism when studying sexual behaviors. Pharmacological manipulations of ERs do not suffer from this complication, as they tend to have little or no effect on genitalia, but all exogenous hormone treatments tend to be crude, because it is difficult to avoid supraphysiological levels of the hormone, especially when treating small, developing rodents. Thus, if you inject a newborn female with estrogen and later see masculine behavior, even fully masculine behavior, that provides evidence that ERs can organize the brain, but does not rule out a complementary role for AR. Supraphysiological stimulation of ERs might overwhelm any contribution AR might have made to the behavior.

Thus, it is refreshing to have new tools to address these questions with the advent of several genetically modified lines of mice exploited by Juntti et al. (2010). Their observations in mice carrying a reporter construct, where the AR promoter drives β-galactosidase (βGal) expression, indicate that AR is first expressed in the mouse brain in just two regions, in the vicinity of the arcuate nucleus (ARC) and ventromedial hypothalamus (VMH), while AR expression in other brain regions, including the bed nucleus of the stria terminalis (BNST), the preoptic area (POA), and the medial amygdala, does not begin until postnatal day 4 (P4), with full expression resembling the adult brain by P7, well after the sensitive period for masculinizing many behaviors. Presumably, any prenatal manipulations of AR that affect behavior must do so through actions on those two brain regions (ARC or VMH) or through the periphery (where AR arises well before birth). By elimination, prenatal masculinization of the mouse brain seems to be primarily mediated by ERs rather than AR, confirming reports that ERs are doing the heavy lifting for organizing the male mouse brain (Rissman, 2008; Wu et al., 2009). On the other hand, widespread appearance of AR in the brain at P7 is well before puberty, which is emerging as another important phase in sexual differentiation of the brain (Ahmed et al., 2008), so they could respond to the rise of testicular secretions at that stage by regulating, for example, glial cell genesis and/or survival.

Juntti et al. use other mouse lines with cre-lox technology to disrupt AR only in the nervous system. These are truly new

animals for the field, as they appear to have a fully masculine periphery, including male-typical secretion of testosterone, yet have little or no AR in the brain (Juntti et al., 2010; Raskin et al., 2009). There are several examples of spontaneous mutations that disrupt AR in rats, mice, and humans (Zuloaga et al., 2008), but in those cases, the disruption is global, and therefore the periphery is entirely feminine. Juntti et al.'s new mice, sporting a masculine periphery with an AR-devoid brain, appear to show all the reproductive and territorial behaviors of normal males, again supporting the idea that the mouse brain is primarily masculinized by ER. However, these males without AR in the brain do show deficits, as particular aspects of behavior are reduced in vigor. For example, while the males without brain AR can successfully breed and display all the components of sexual behavior, including mounting and intromissions, those behaviors are less common than in control males (ejaculations are also less likely than in control males, but that behavior was rare in the tests and the difference was not statistically significant). Likewise, although males without AR in the nervous system establish territory by urine marking and also attack other males, they do so less often than control males. Because the periphery of these males seems entirely masculine, and they are secreting ample T, these results indicate that activation of AR somewhere in the nervous system indeed contributes to these behaviors. The observations in the reporter mice indicate that this action of AR in the brain takes place either in the ARC or VMH before birth, or in some other brain regions on P7 or later in development.

This paper also represents a promissory note, as such genetic methods should bring further details in the near future. Already there are mouse lines that express cre-recombinase in particular types of cells, so tissue-specific deletion of AR may someday pinpoint which part(s) of the brain mediate AR's potentiation of reproductive and territorial behaviors. At present, small implants of hormone directly into brain subregions provide some selectivity of steroid treatment, but this approach is limited when applied to small, newborn brains. The prospect of pinpointing a contribution of AR action in astrocytes (Garcia-Segura et al., 2008) versus neurons, or in forebrain versus brainstem, for masculinization of behavior is especially tantalizing as it is far beyond prior methodology. Furthermore, inducible cre lines should make it possible to disrupt AR globally in an adult male mouse. As the animals in Juntti et al. were deprived of AR function in neural cells throughout life, compensatory mechanisms may come into play in development. If so, then deleting AR in adulthood might reveal an even greater contribution of brain AR to masculine behavior. Finally, it seems likely that in the future we can effectively disrupt AR only in adulthood, and only in particular classes of cells, such as astrocytes or hypothalamic neurons.

Taken together, these results suggest that T acts primarily on ERs during early development to organize a male brain, and then also upon ARs postnatally to activate fully masculine behavior. These could have been two independent systems, working in parallel, but the genetic models also indicate that the AR and ER pathways interact with one another. In the mice reporting AR expression, more cells are labeled with βGal in the brains of males than of females at P7. The greater expression in males is not due to activation of AR itself, but appears to be dependent on ER activation. The authors show this first the old-fashioned way, by treating newborn females with exogenous hormone and boosting βGal expression in the brain to male-typical levels with either estrogen or T. Then they use yet another mouse line. Male mice carrying a null mutation for the aromatase gene, and hence unable to synthesize estrogens, express female-typical levels of the βGal reporter. Hence, early activation of ERs appears to upregulate AR expression in the developing male brain (McAbee and DonCarlos, 1999), and as the authors have previously shown (Wu et al., 2009), aromatase as well. As the authors discuss, in the rat brain activation of AR can boost aromatase expression in many brain regions (Roselli et al., 1997), presumably boosting the stimulation of ERs in those regions. Indeed, the reduced masculine behavior in males lacking AR in the brain might be due to reduced aromatase in particular brain regions.

So these two systems are not ships passing through the night, each making its contribution in isolation. Rather, they are acting on different regions, at different times in development, and each is regulating the effectiveness of the other. Thus, what could have been a "testosterone two-step" looks more like a minuet, where AR and ER each regulate and support each other to make a wholly masculine brain (Figure 1). Because of these new genetic models, we are poised to better understand the complementary efforts of these two metabolites of T, acting on two different classes of steroid

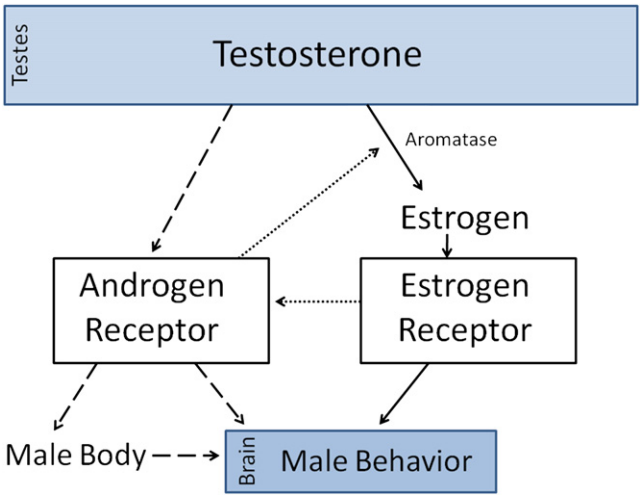

Figure 1. Two Systems Interact to Masculinize the Mammalian Brain
Testicular androgens such as testosterone (T) act on androgen receptors (ARs) to masculinize the body and brain (dashed arrows). However, the enzyme aromatase converts T to estrogens, which then act on estrogen receptors (ERs), and the present paper confirms that this pathway (solid arrows) is primarily responsible for masculinizing both reproductive and territorial behaviors in mice. They also find that AR acting in the brain potentiates those behaviors. The two systems of steroid receptors interact (dotted lines), as AR stimulation has been shown to boost brain aromatase in many previous reports, and the present paper indicates that ER stimulation boosts AR expression in the neonatal brain of mice.

receptors, to masculinize the brain and behavior of mice. Sharpened by natural selection, the elaborate coordination of these two different pathways driving male reproductive success offers as beautiful a pas de deux as was ever conceived by any human choreographer.

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