

# Defending the brain from estrogen

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**Alpha-fetoprotein binds estrogens in the developing brain. A new paper shows that inhibiting estrogen rescues the brain masculinization found in female mice lacking this gene, suggesting that alpha-fetoprotein inhibits estrogen activity in females.**

In some circles, it is popular to blame 'testosterone poisoning' for the aggressive tendencies and indiscriminate sexual interests of men, but evolutionary theorists insist such characteristics are adaptive male reproductive strategies. Thus, in many mammalian species, prenatal exposure to testosterone and other androgens induces the male, in adulthood, to behave in ways that help him compete with other males for mates. But what is good for the gander may not be good for the goose: an aggressive response to males is maladaptive for female reproductive success. Instead, the female must, at some point, be sexually receptive to males if she is to reproduce. So for females, testosterone exposure during development might really constitute poisoning. How do females avoid testosterone exposure, especially in mammalian species with large litters where almost every female fetus shares the uterus with brothers? In this issue, Bakker *et al.*<sup>1</sup> examine knockout mice to demonstrate how a particular protein prevents steroid hormones from masculinizing the brains of developing females.

To understand their study, one must first realize that in rats and mice it is not testosterone itself that masculinizes the developing brain, but rather metabolites of testosterone: estrogens such as estradiol masculinize many brain regions<sup>2</sup>. The fetal testes produce androgenic steroids, such as testosterone, that themselves masculinize many body structures. In the brain, however, testosterone is converted in a single step to estradiol. This conversion is accomplished by the enzyme aromatase, and the developing brain has loads of aromatase on hand for the task<sup>3</sup>. The locally produced estrogen then binds with estrogen receptors (not androgen receptors) to modulate gene expression and sculpt developing brain circuits into a masculine configuration (**Fig. 1a**). For this rea-

son, developing females need to be protected both from estrogens, which can directly masculinize the brain, and from testosterone, which can be converted to estrogens. The most compelling demonstration of how early estrogen exposure can affect females is that a single dose of estrogen given to a newborn female rat will prevent her brain from maintaining ovulatory cycles<sup>4</sup>. What's more, even if such a female is treated in adulthood with the steroid hormones that normally induce sexual receptivity, she will not respond<sup>5</sup>. When mounted by a male, she will not display the lordosis posture (raised head and rump, deflected tail) that permits the male to insert his penis into her vagina. Such a female is called 'defeminized' because she is less likely to display female-typical behaviors such as lordosis. She may also be 'masculinized': that is, more likely to display male-typical behaviors. For example, as adults, such females will be more likely to mount other females. In any case, perinatal exposure of the female brain to estrogen severely disrupts her chances to reproduce.

Fetal females are protected from testosterone itself by the placenta. Because it is rich in aromatase, the placenta readily converts androgens passing through, such as testosterone, into estrogens<sup>6</sup>. This means that little testosterone from mouse brothers *in utero* is likely to reach their sisters. Obviously, though, this mechanism does nothing to shield females from estrogens, which molecule-for-molecule are more effective than testosterone at masculinizing the developing brain. In addition to aromatized metabolites of testosterone from her brothers, the female fetus is also exposed to estrogens from the maternal ovary and possibly her own. What keeps all this circulating estrogen from masculinizing the female brain?

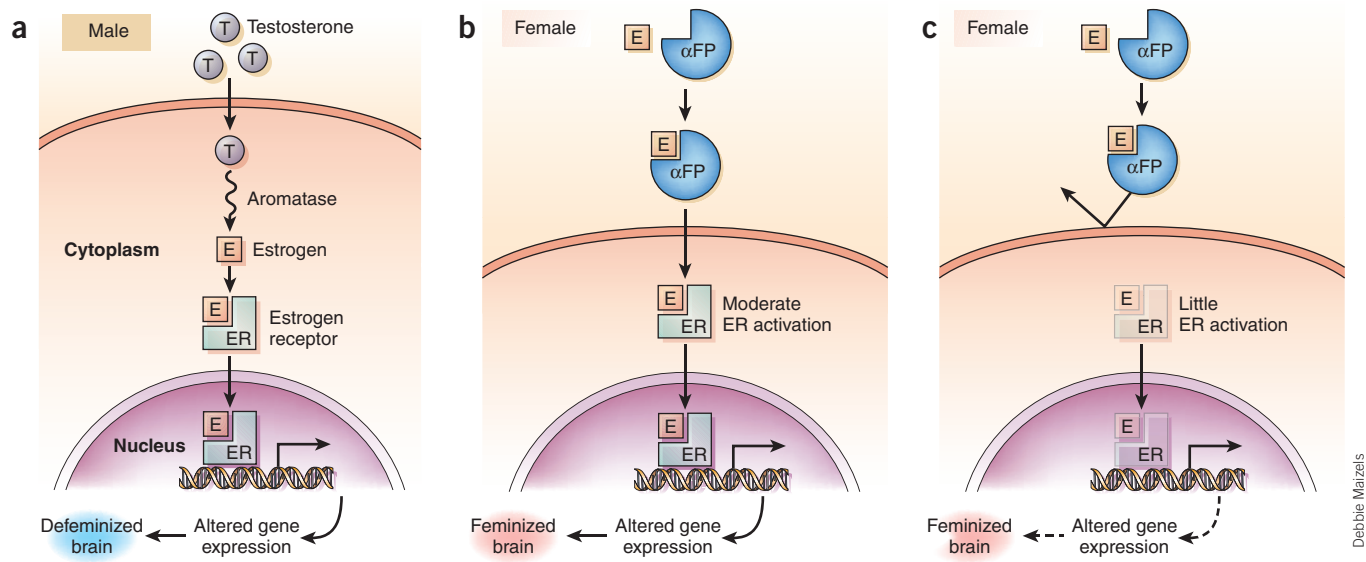
Several decades ago, Bruce McEwen of Rockefeller University proposed that a circulating protein, alpha-fetoprotein (AFP) might do the trick<sup>7</sup>. As the name suggests, this protein is found in the circulation of fetuses, and in rats and mice AFP binds a wide variety of steroid hormones, including estrogens. Conveniently enough, AFP does not bind testosterone very well, so in

males it would leave testosterone free to enter the brain and be locally aromatized to estrogen to masculinize and defeminize the male's brain. In females, though, the AFP binding of estrogens could keep them out of the brain, circulating long enough to be metabolized into inactive steroids. This vision of brain sexual differentiation comfortably explained most reports in the literature, and seemed to resolve the question of how estrogens could masculinize the male brain but not the female brain.

As is usual with a new hypothesis, findings soon arose that were inconsistent with the idea that AFP acts by keeping estrogen out of the brain. For one thing, several studies suggested that removing the ovaries from newborn rats made their behavior less feminine<sup>8–12</sup>, not more feminine as one would predict if ovarian estrogens masculinize the brain. These findings are somewhat inconsistent across studies, indicating that the feminizing effect of early estrogens, if real, is a subtle one. Because we know that a lot of estrogen will defeminize the brain, the question is whether a very modest dose of estrogen is needed to complete feminine development.

Such findings led Dominique Toran-Allerand of Columbia University to suggest that perhaps the role of AFP was to bring estrogen into the brain<sup>13</sup>, a suggestion strengthened by her immunocytochemical detection of AFP inside brain neurons<sup>14</sup>. What's more, the AFP does not seem to be made in the brain, so it is being transported into neurons from the outside. Because AFP binds estrogens, perhaps it is escorting estrogen into the brain to favor feminine behavioral development (**Fig. 1b**). Presumably this effect of AFP-escorted estrogen would result in very selective and specific estrogen delivery to particular sets of neurons equipped to internalize the AFP-steroid complex. It is easy to imagine that selective estrogen delivery to a specific neural circuit might contribute to feminine brain development. Because it was difficult to identify which particular neurons are benefiting from this estrogen stimulation, it was difficult to disprove definitively either the hypothesis that

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**Figure 1** Sexual differentiation of the brain. (a) In male rodents, testosterone reaches the fetal brain and is aromatized there into estrogens (E), which bind to estrogen receptors (ER) to promote gene expression that masculinizes some neural circuits and defeminizes others. There remained the question of whether optimal development of the female brain is best served by delivery of some estrogen to specific neural circuits or by protecting the brain from estrogen as much as possible. These competing hypotheses suggested different roles for alpha-fetoprotein (AFP), which binds estrogens. (b) AFP might deliver estrogen to specific neural elements to promote feminization of those circuits. (c) Alternatively, AFP might serve to keep estrogens out of the brain of fetal females. Females lacking the *Afp* gene were found to be defeminized and masculinized, as both hypotheses would predict. However, blockade of estrogen synthesis in the *Afp*<sup>-/-</sup> females restored feminine behavior, which is compatible only with the hypothesis in c.

AFP keeps estrogen out of the brain (Fig. 1c) or that it selectively brings estrogen into the brain.

For example, in the mice studied by Bakker *et al.*<sup>1</sup> that have the gene for AFP knocked out (*Afp*<sup>-/-</sup> mice), both hypotheses would predict that the males would be more or less normal but that the females would be defeminized and masculinized. That is what was found: *Afp*<sup>-/-</sup> females treated as adults with hormones that normally induce sexual receptivity show very little lordosis response to mounting males (they are defeminized) and are more likely to mount other females (they are masculinized)<sup>1</sup>. By themselves, these results cannot tell us whether the absence of AFP defeminized and masculinized the brain by allowing estrogen in or by not bringing estrogen to the select neuronal circuit. However, treating the developing *Afp*<sup>-/-</sup> females with a drug that systemically blocks estrogen synthesis dramatically rescued the feminine phenotype. In other words, by reducing the amount of estrogen to which the fetuses were exposed, the investigators allowed *Afp*<sup>-/-</sup> females to display sexual receptivity again. Blocking perinatal estrogen synthesis also restored female levels of tyrosine hydroxylase-expressing neurons in the hypothalamus of *Afp*<sup>-/-</sup> females. Of

the two competing hypotheses, these outcomes are consistent only with the notion that AFP functions by excluding estrogen from the female's brain.

What's next? This finding does not explain why neonatal ovariectomy sometimes interferes with feminine development. Perhaps there is a mechanism for selectively delivering a modicum of estrogen to a developing feminine circuit, but AFP just does not happen to be that mechanism. Likewise, we are still left with the question of why AFP seems to be internalized into neurons in the developing brain. Perhaps AFP is escorting some other player into neural circuits. It is clear that such putative escorting of 'factor X' is not required for females to display lordosis because *Afp*<sup>-/-</sup> mice, when protected from estrogens perinatally, were sexually receptive as adults, but it may well be involved in some other brain function. Perhaps a closer look at the neurons that contain AFP will suggest a function for the protein in these cells. If so, it could be a function common to both sexes, as males also produce AFP. It will be interesting to see whether these *Afp*<sup>-/-</sup> mice have other, unanticipated differences in behavior compared to wild-type mice. In the meantime, we now know that AFP is crucial indeed in sexual differentiation of

mouse behavior. The protein is not a chaperone, ushering estrogen into the female brain, but more like a matron, firmly excluding estrogen from the developing brain so that females can feminize themselves, untainted by steroidal interference.

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