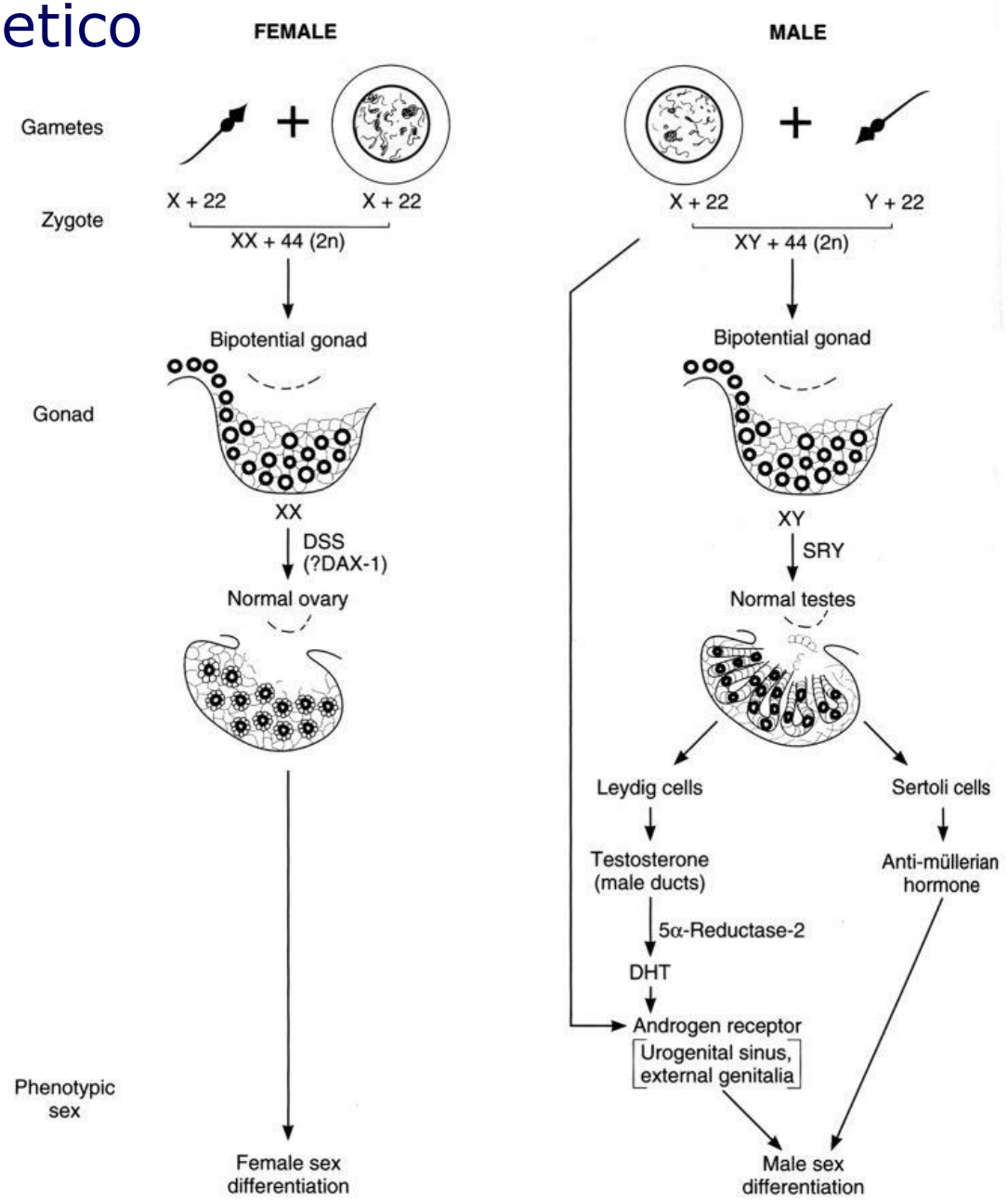
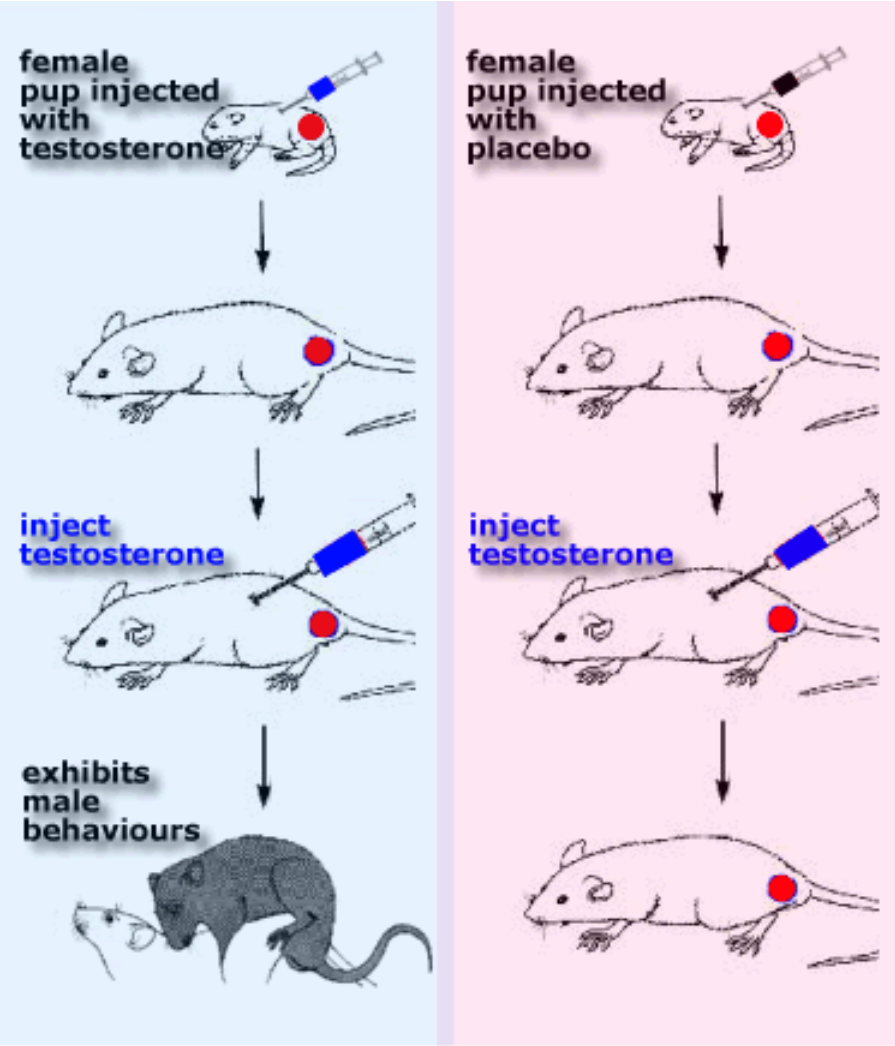
Il ruolo della alfafetoproteina

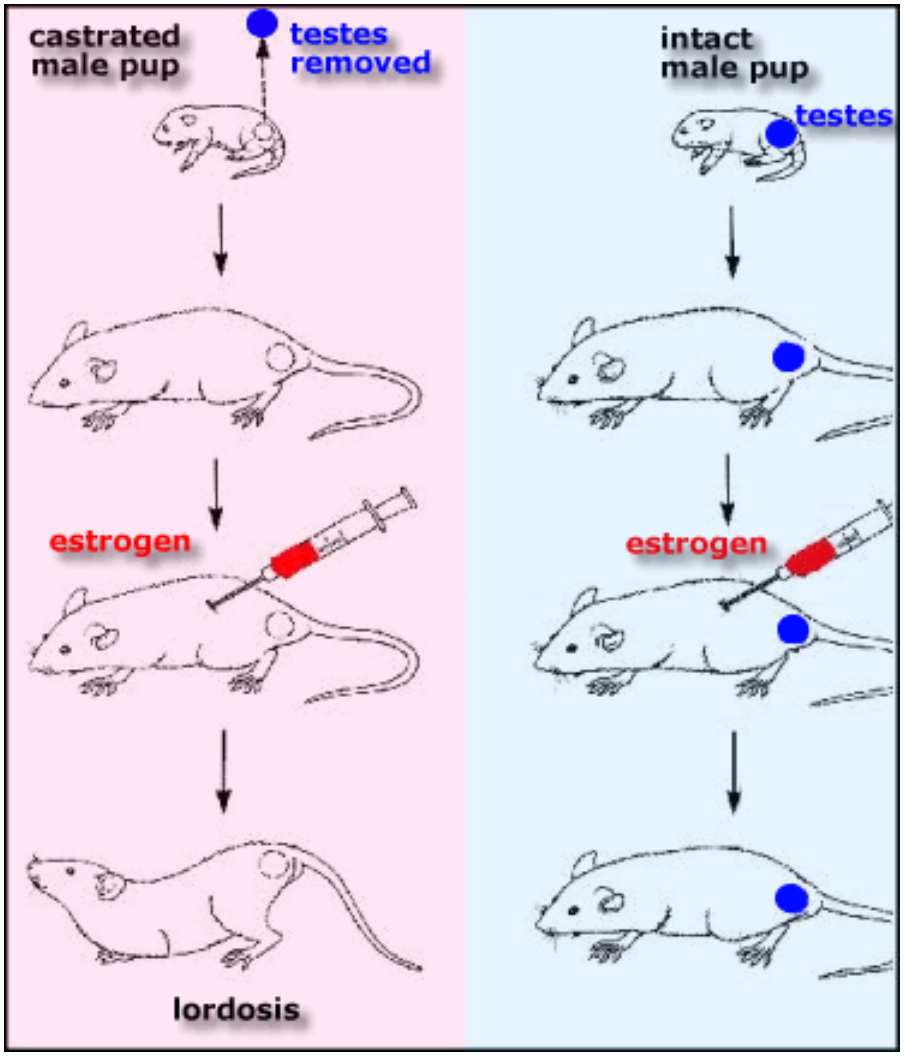
Sesso gametico



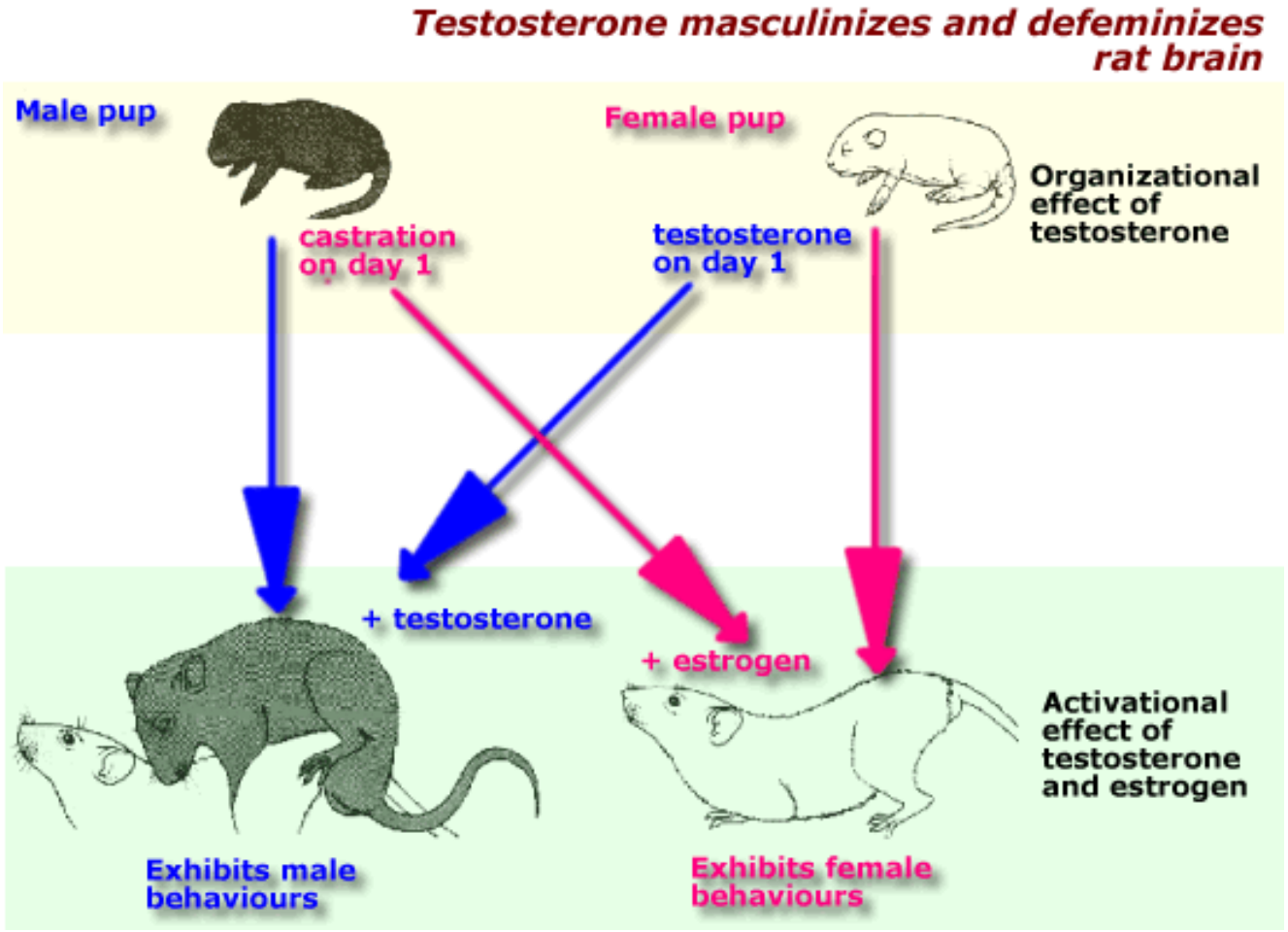
Mascolinizzazione



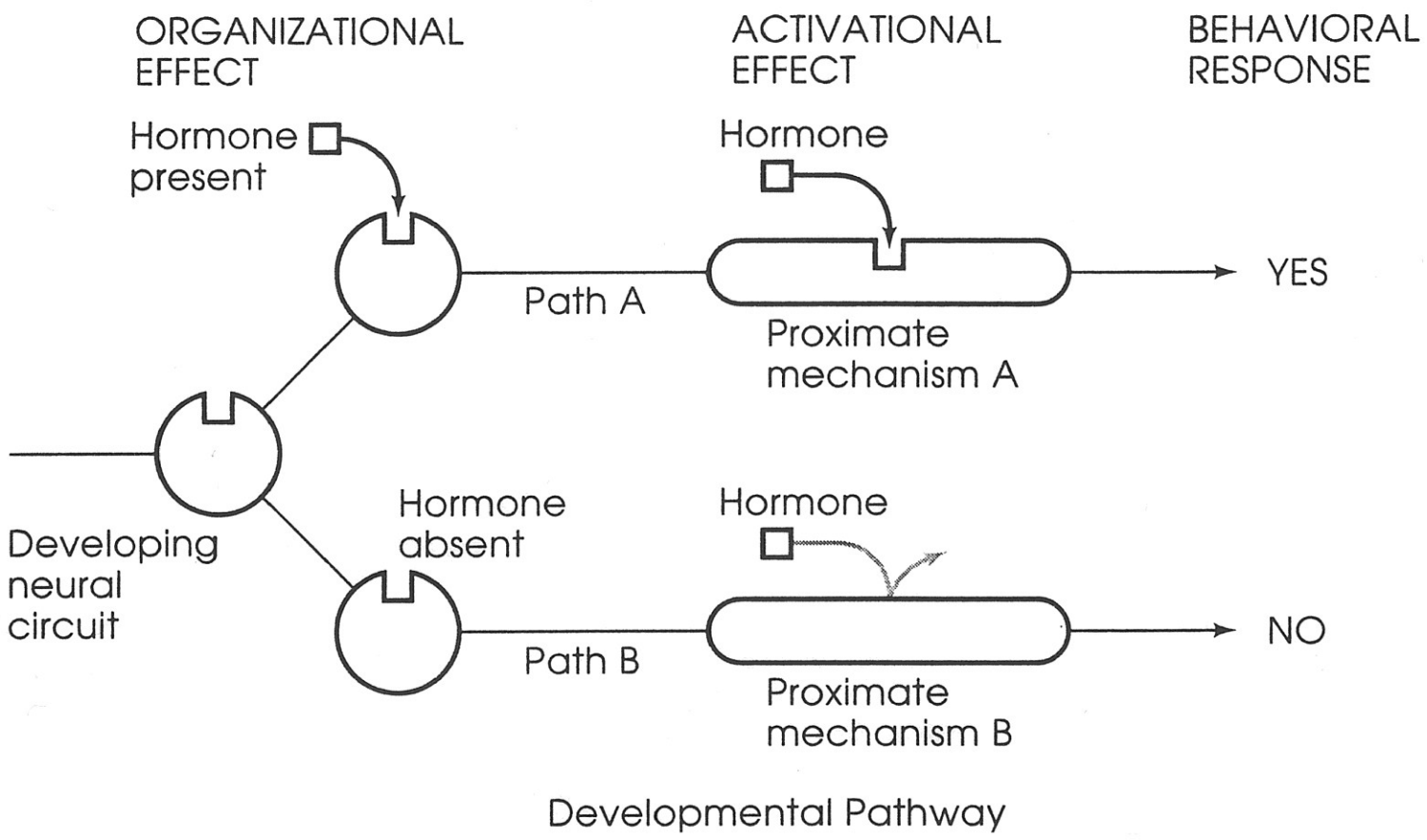
Defemminizzazione



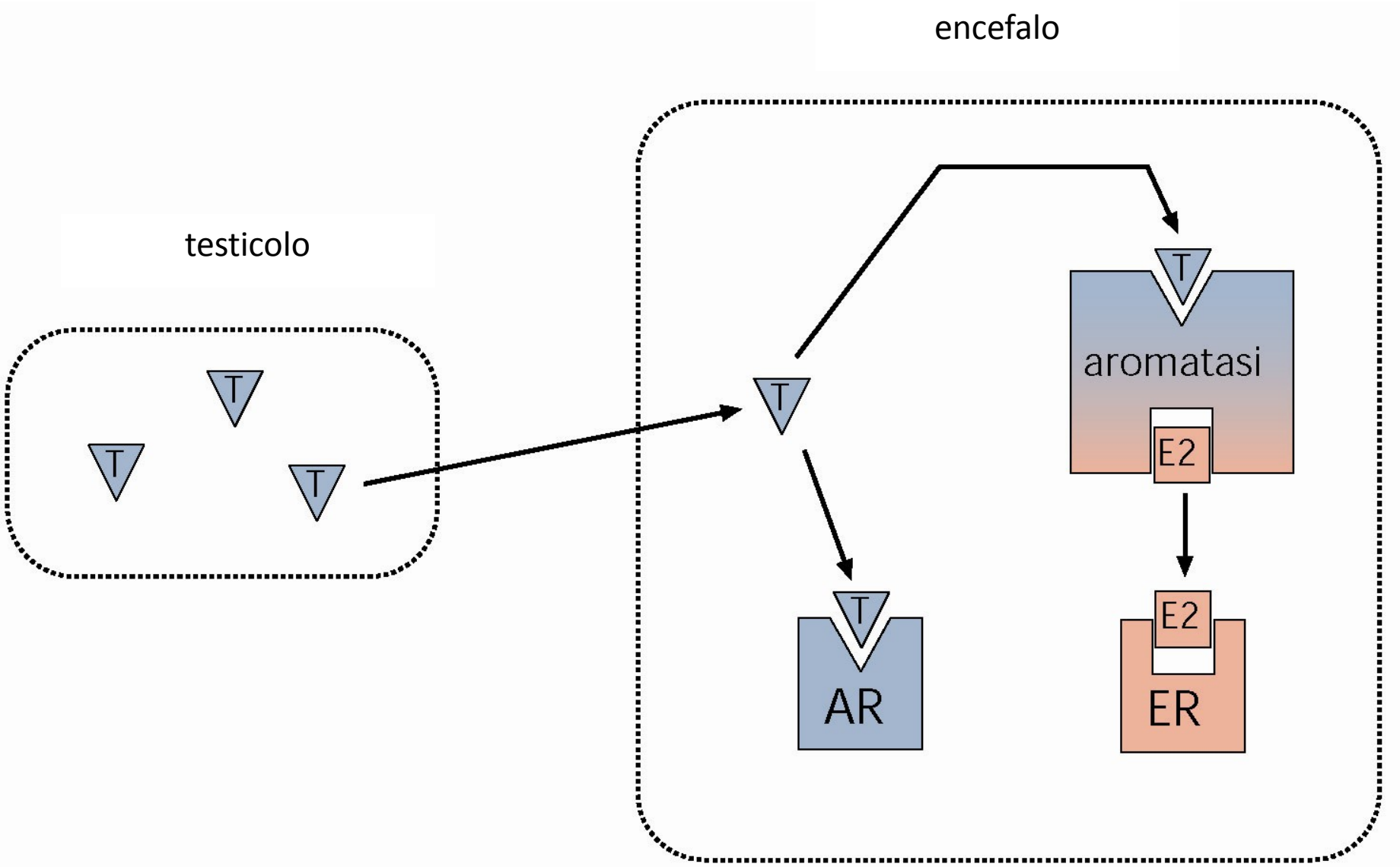
Schema differenziamento sessuale



Organizzazione e attivazione: il sesso neutro



La doppia azione di T



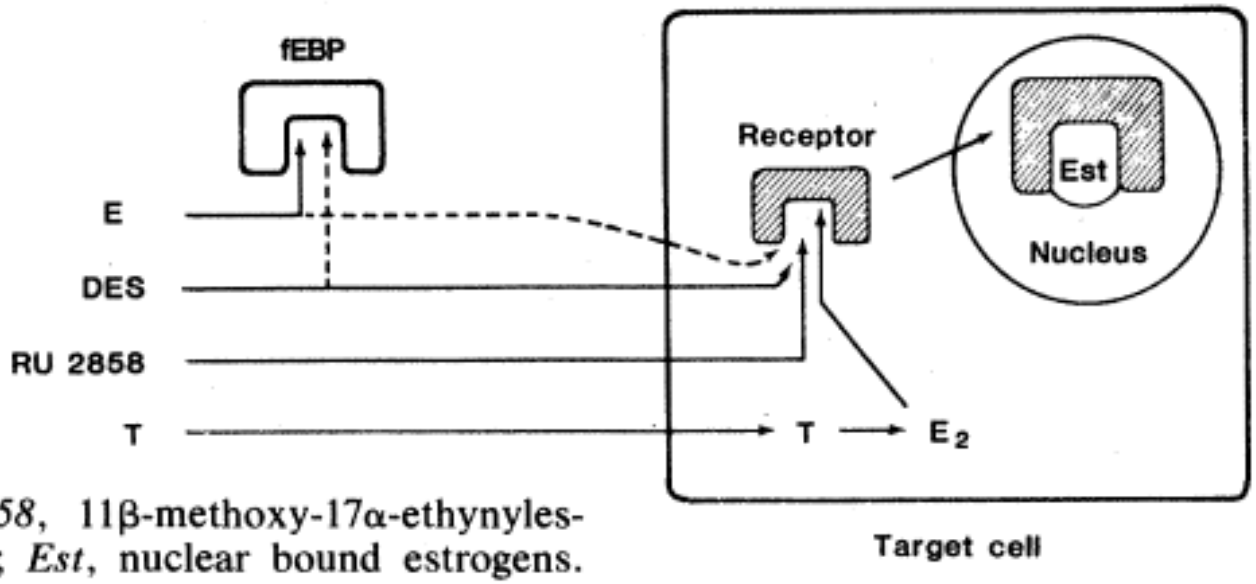


Fig. 3. Schematic diagram of the protective role of fetoneonatal estrogen binding protein (*fEBP*) in neonatal rats, and the ability of synthetic estrogens and testosterone to bypass this mechanism. Abbreviations: E_2, estradiol; *DES*, diethylstilbestrol; *Ru2858*, 11β-methoxy-17α-ethynylestradiol; *T*, testosterone; *Est*, nuclear bound estrogens. [From (*68*); courtesy of *Brain Research*]

"….these two clearly opposing views on the function of AFP in brain sexual differentiation have not been experimentally tested owing to the absence of a suitable animal model…"

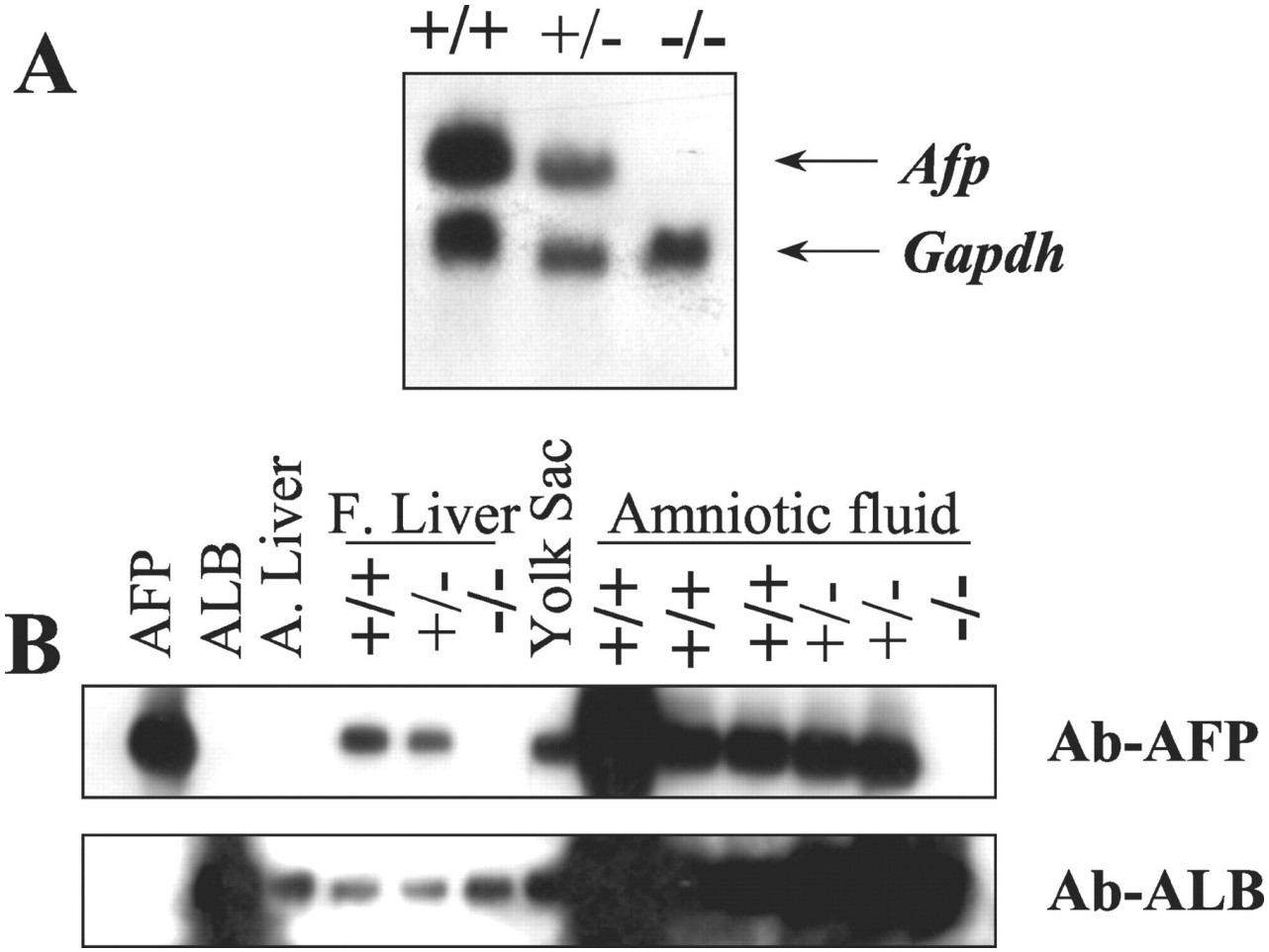
Bakker et al. Nature Neuroscience 9, 220 - 226 (2006)

PNAS October 1, 2002 vol. 99 no. 20 12865-12870

Alpha-fetoprotein, the major fetal serum protein, is not essential for embryonic development but is required for female fertility

Philippe Gabant*, Lesley Forrester†,‡, Jennifer Nichols†, Thierry Van Reeth*, Christelle De Mees*, Bernard Pajack§, Alistair Watt†, Johan Smitz¶, Henri Alexandre||, Claude Szpirer*,**, and Josiane Szpirer*

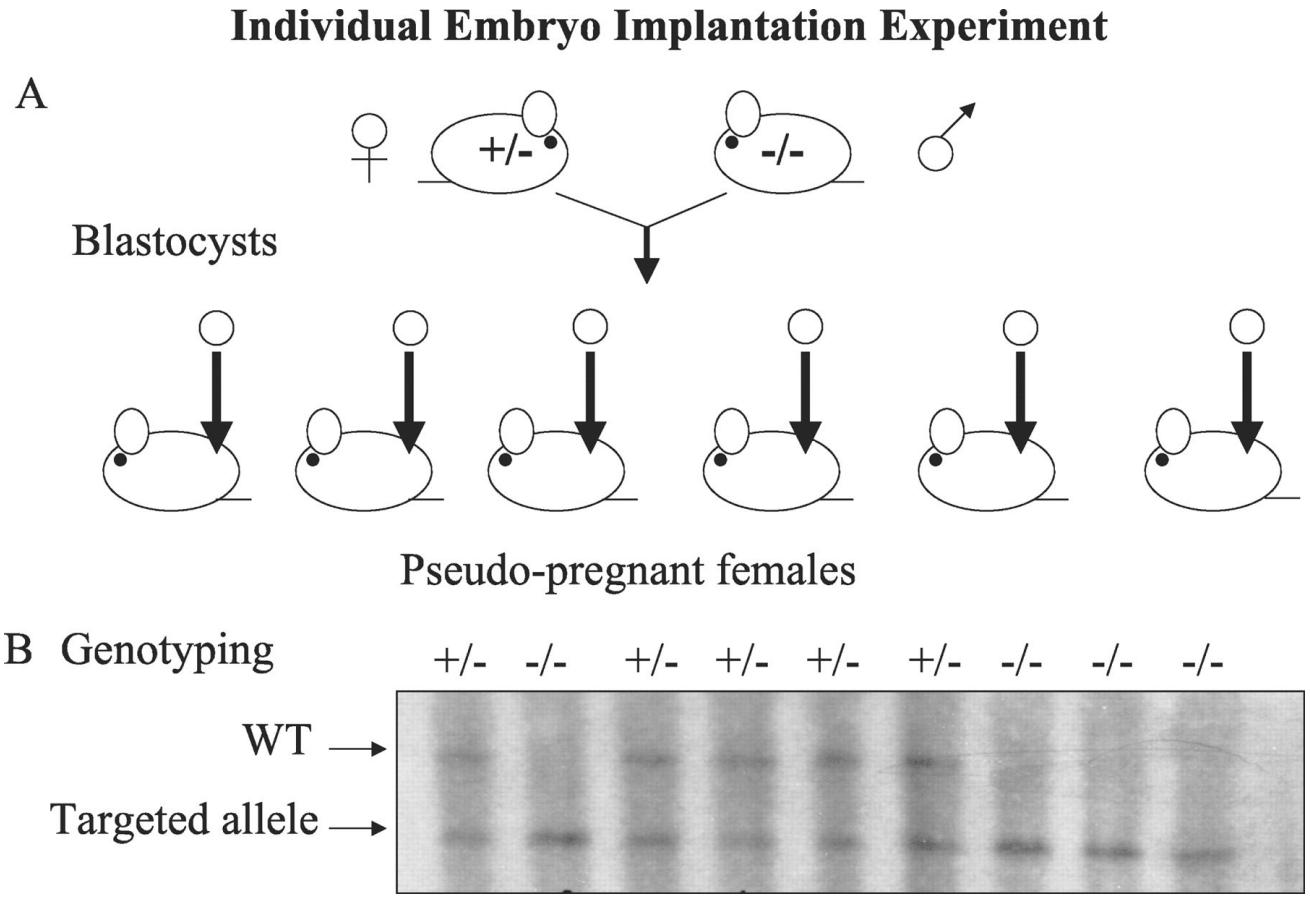
(A) Northern blot analysis of intercross litters



(A) Northern blot analysis of intercross litters. Total RNA from 16.5 E embryos (genotyped by Southern blot) was analyzed by Northern blot. The mouse Afp probe detects a 2.2-kb transcript. A human GAPDH probe was used as loading control. (B) Western blot analysis with protein extracts from different tissues. Pure AFP, albumin (ALB), and adult liver (A. liver) were used as controls. Extracts were from wild-type (+/+), heterozygous (+/−), and Afptm1Ibmm/tm1Ibmm mutant (−/−) mice. Protein from fetal liver (F. Liver), yolk sac, and amniotic fluid were tested with a serum raised against AFP (Ab-AFP). The same blot was tested with a serum raised against albumin (Ab-ALB).

Gabant P et al. PNAS 2002;99:12865-12870

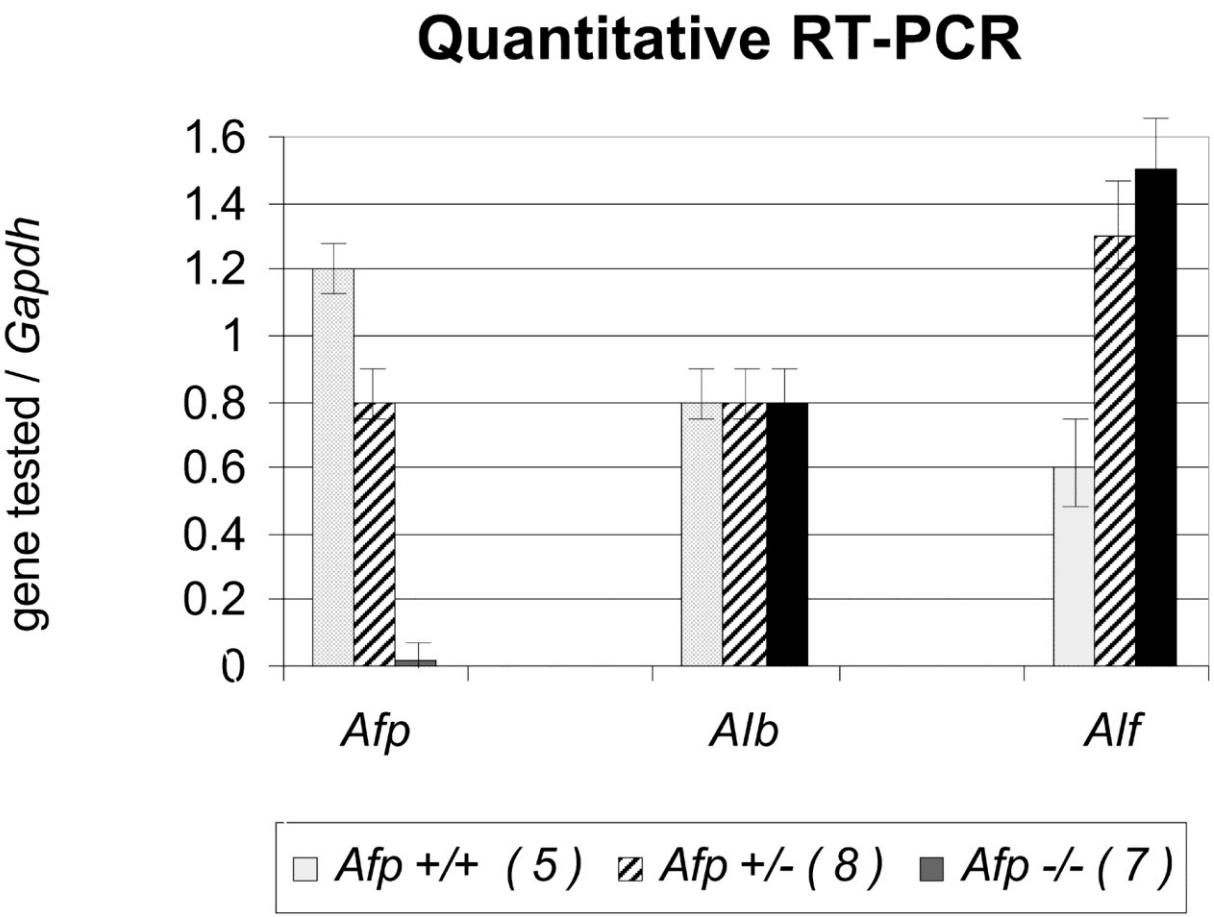
Single embryo implantations



Single embryo implantations. (A) Heterozygous Afptm1Ibmm/+ females and homozygous Afptm1Ibmm/tm1Ibmm mutant males were mated. Blastocysts were collected and implanted individually into pseudopregnant females. (B) Southern blot analysis of mice obtained from different females.

Gabant P et al. PNAS 2002;99:12865-12870

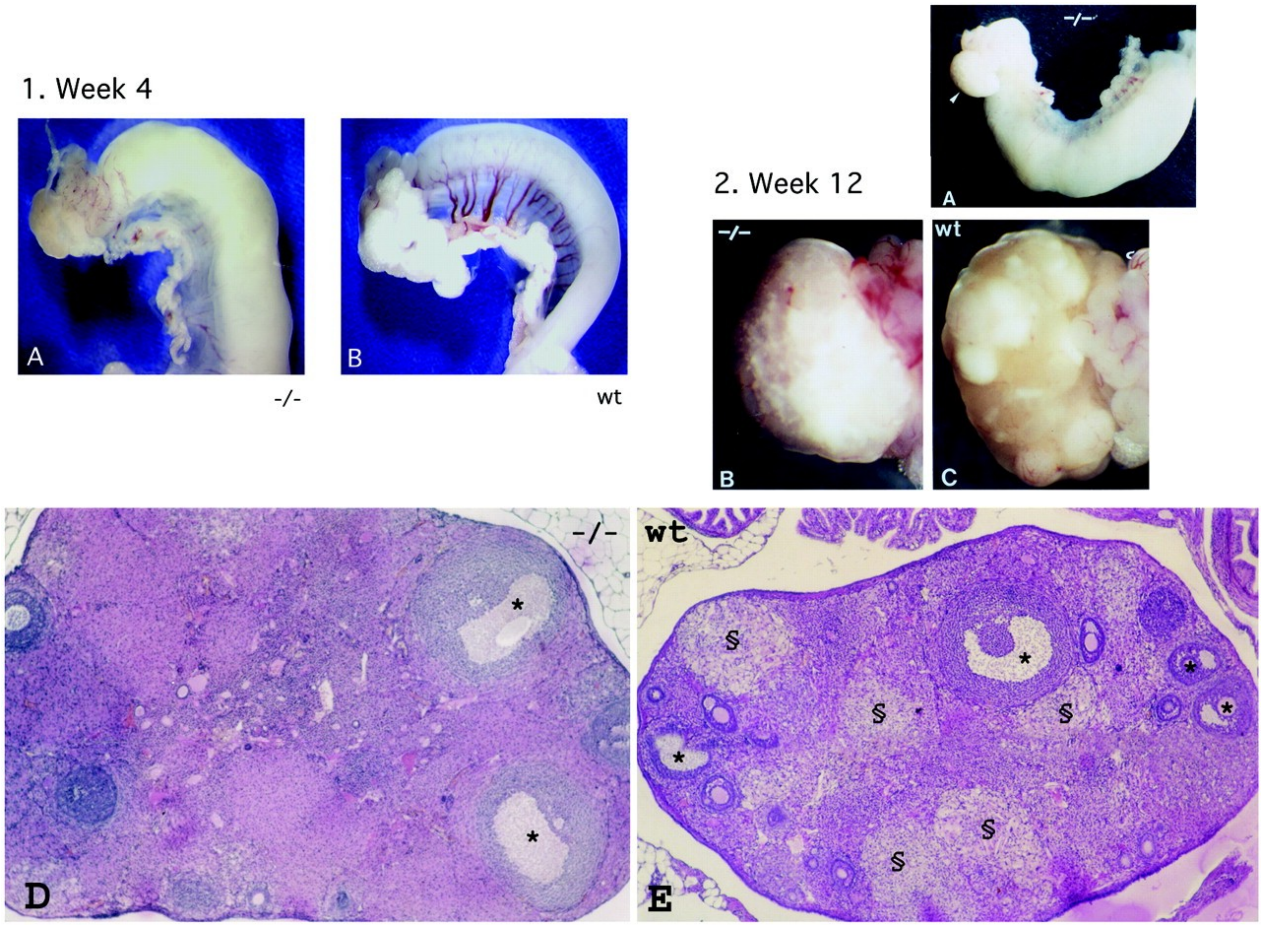
Quantitative RT-PCR on the different genes of the albumin family



Quantitative RT-PCR on the different genes of the albumin family. Embryos (15.5 E) from intercrosses matings were dissected and genotyped, and total RNA from their livers was extracted. The concentration of Gapdh transcript was measured for each sample tested, and the ratio of the tested transcript [Afp, albumin (Alb), alpha-albumin (Alf)] on the Gapdh transcript was calculated. The relative amounts the three mRNAs tested is given for wild-type embryos (Afp+/+), heterozygous (Afp+/−), or homozygous (Afp−/−). The number of each sample tested for each genotype is indicated in parentheses.

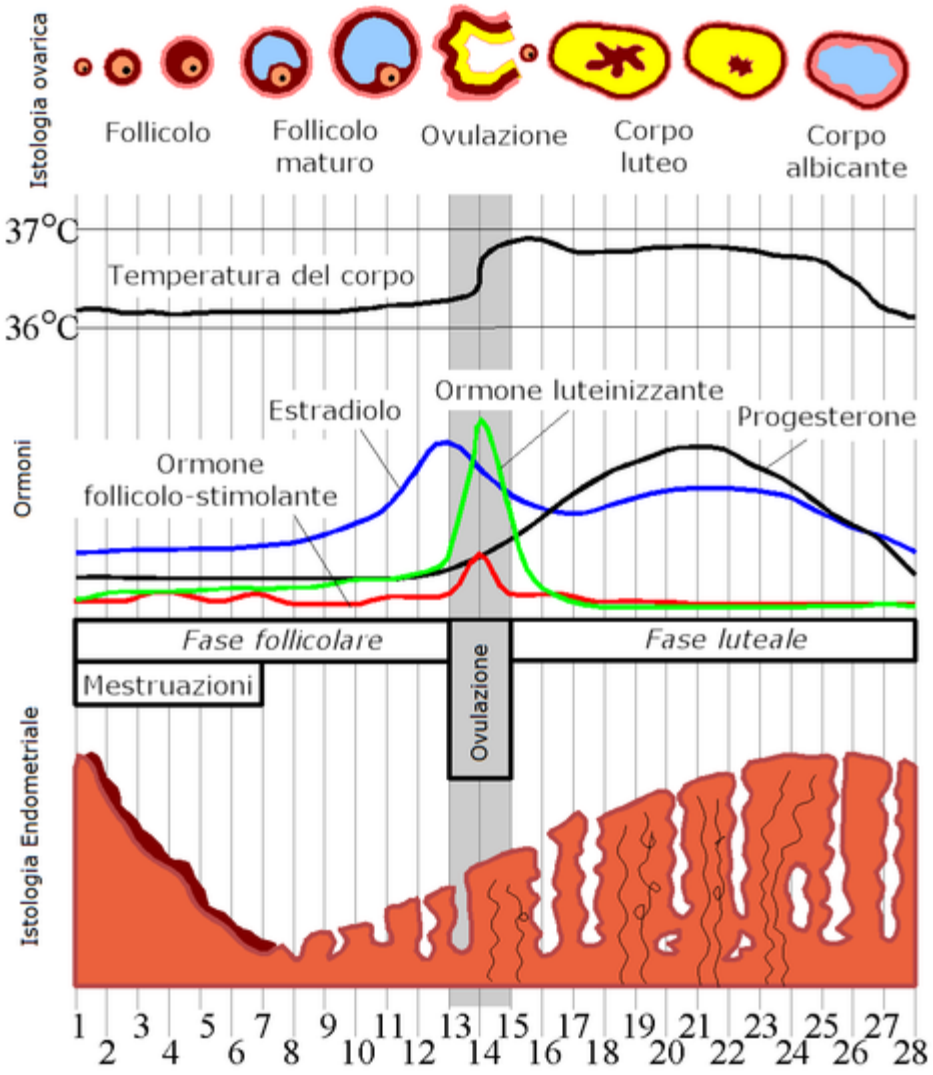
Gabant P et al. PNAS 2002;99:12865-12870

Anatomical and histological analysis of Afp mutant (Afptm1lbmm/tm1lbmm) ovaries and uteri of preburtal (week 4) and adult (week 12) mice



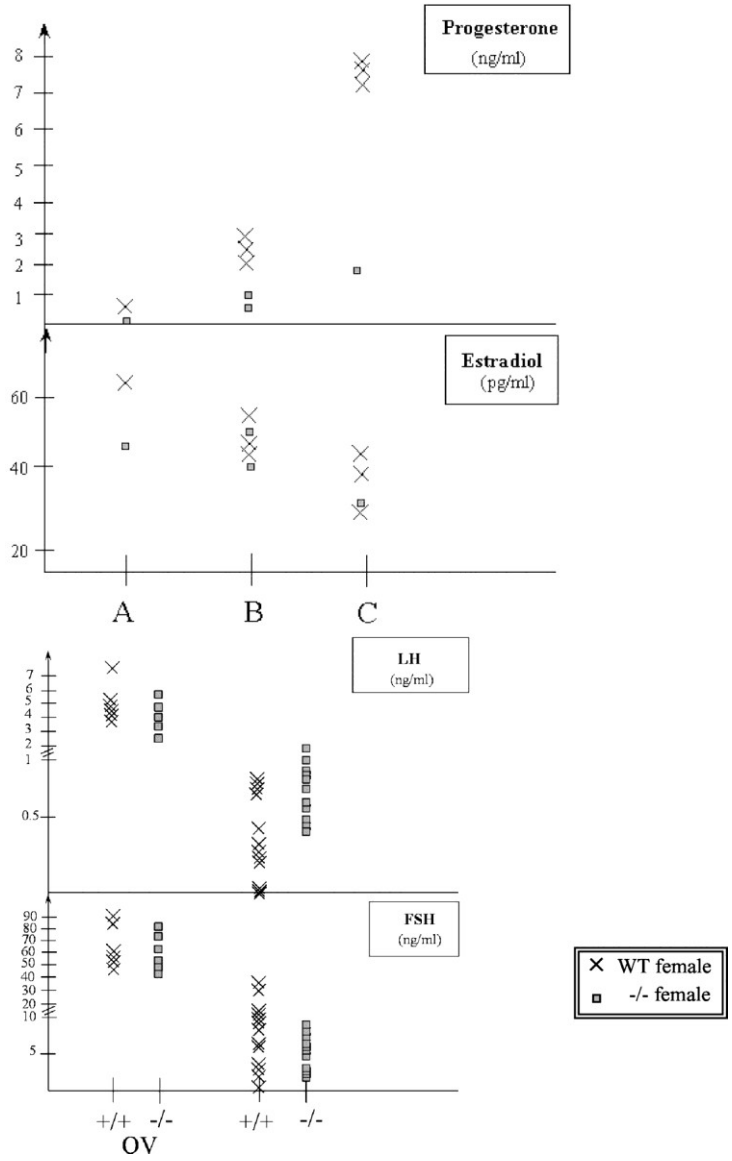
Anatomical and histological analysis of Afp mutant (Afp -/-) ovaries and uteri of preburtal (week 4) and adult (week 12) mice. (1A) Uterine horn and ovary of a 4-week-old Afp -/- female. (1B) Uterine horn and ovary of a wild-type 4-week-old female. (2A) Uterine horn and ovary (arrowhead) of an adult Afp -/- female. (2B) Ovary from a 12-week-old Afp -/- female. (2C) Ovary from a 12-week-old wild-type female. The surface distortions caused by large corpora lutea are not observed in the Afp -/- female. (2D) General histological structure of an Afp -/- ovary (section from a 16-week-old female) showing that mature Graafian follicles (*) are present. (2E) At the same age, wild-type ovaries exhibit large corpora lutea (§), indicative of successful ovulation (these structures were never found in Afp -/- ovaries).

Gabant P et al. PNAS 2002;99:12865-12870



(Valori medi. Durata e valori possono variare tra diverse femmine o diversi cicli.)

Hormonal levels



Hormonal levels. Each point corresponds to a single mouse. (Upper) Results of progesterone and estradiol assays in Afptm1Ibmm/tm1Ibmm mutant females and controls. Assays were performed on serum from different batches of females maintained for at least 6 weeks in three different cages (A, B, C). Note the lack of progesterone in the mutant mice; the difference with the control mice is significant (P = 0.05). (Lower) Results of gonadotropin (LH and FSH) measurements in wild-type and Afptm1Ibmm/tm1Ibmm mutant females ovarectomized (OV: first two series) or not ovarectomized (last two series). The difference in the LH levels is significant (P = 0.01), whereas that in the FSH levels is not (P = 0.16).

Gabant P et al. PNAS 2002;99:12865-12870

Article *Nature Neuroscience* ***9, 220 - 226 (2006)***
Published online: 1 January 2006; | doi:10.1038/nn1624

Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens

Julie Bakker[1], Christelle De Mees[2], Quentin Douhard[1], Jacques Balthazart[1], Philippe Gabant[2], Josiane Szpirer[2] & Claude Szpirer[2]

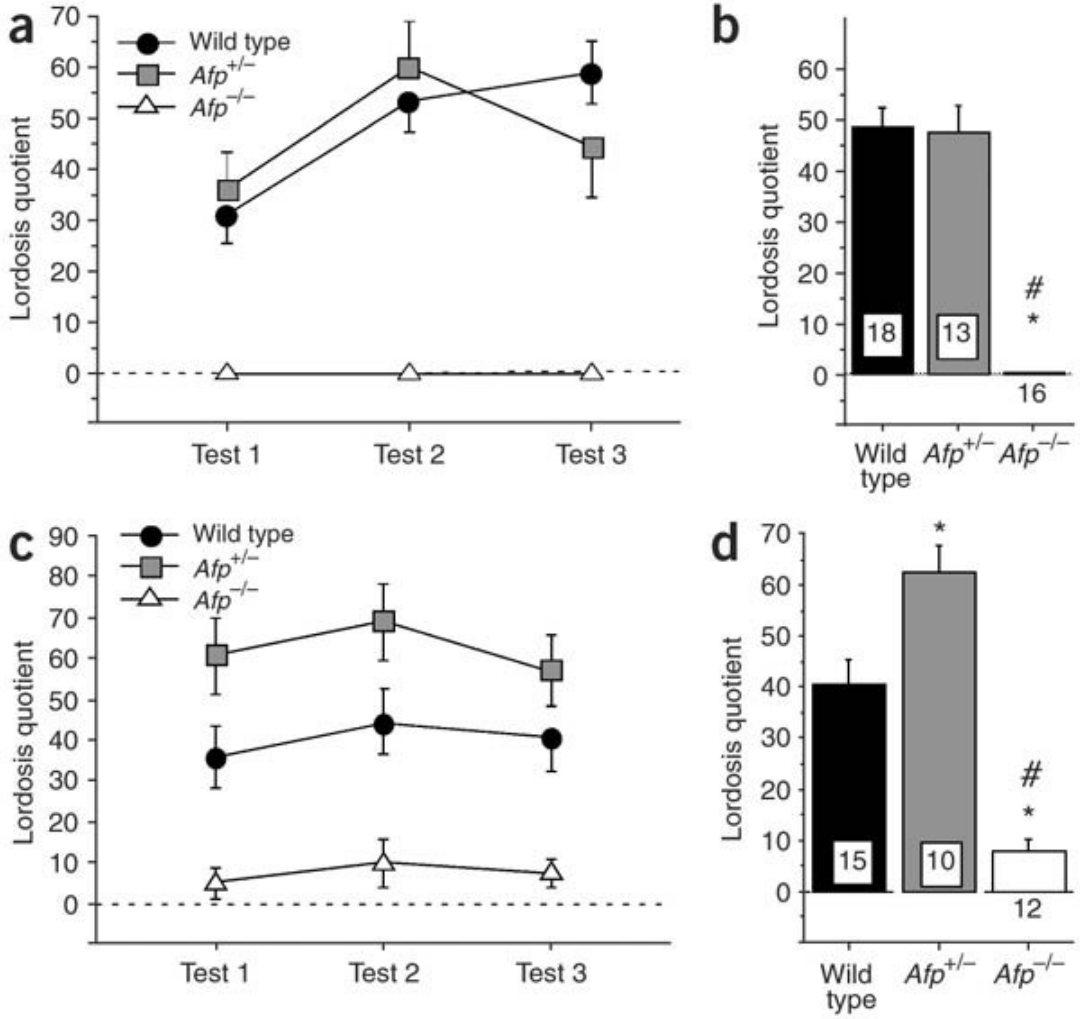


Figure 1. Complete absence of female sexual behavior in female mice lacking AFP.
(**a**,**b**) Lordosis quotients in three consecutive tests of *Afp1*$^{-/-}$ females of the CD1 strain (**a**) and their average (**b**). (**c**,**d**) Lordosis quotients in three consecutive tests of *Afp1*$^{-/-}$ females of the C57Bl6/j strain (**c**) and their average (**d**). Results of *post-hoc* comparisons by the Fisher PLSD test are indicated as follows: * $P < 0.05$ compared to the wild type; $\#P > 0.05$ compared to *Afp*$^{+/-}$. The numbers of subjects are indicated in the bars in **b** and **d**. Data are expressed as mean s.e.m.

Bakker et al. Nature Neuroscience 9, 220 - 226 (2006)

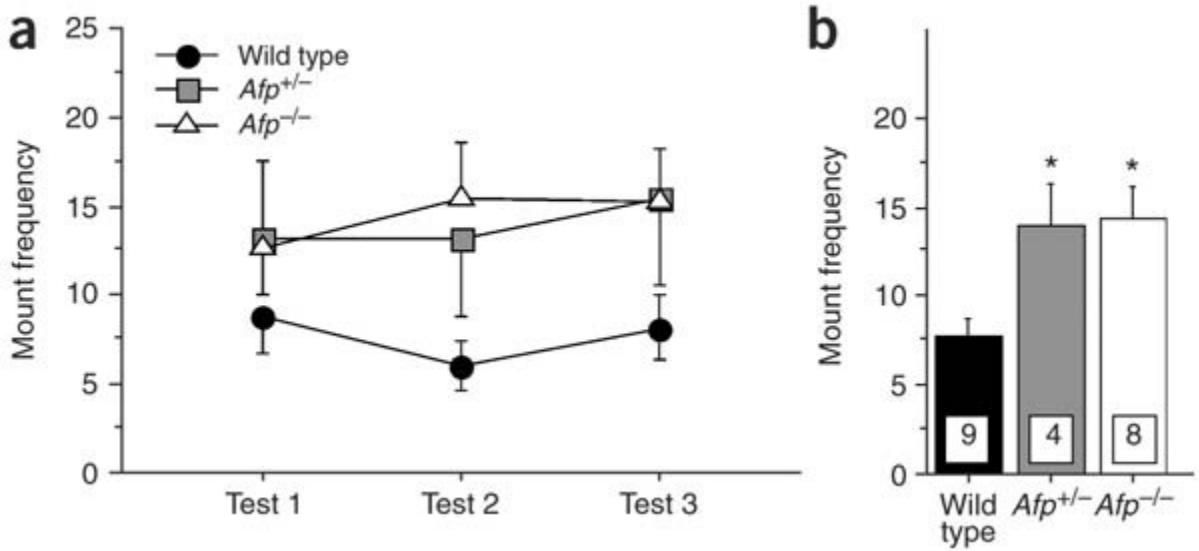


Figure 2. Increased male-typical sexual behavior in female mice lacking AFP.
(**a**,**b**) Frequencies of mounts plus pelvic thrusting in three consecutive tests (**a**) and their average (**b**). Results of *post-hoc* comparisons by the Fisher PLSD test are indicated as follows: * $P < 0.05$ compared to the wild type. The numbers of subjects are indicated in the bars in **a** and **b**. Data are expressed as mean s.e.m.

Bakker et al. Nature Neuroscience 9, 220 - 226 (2006)

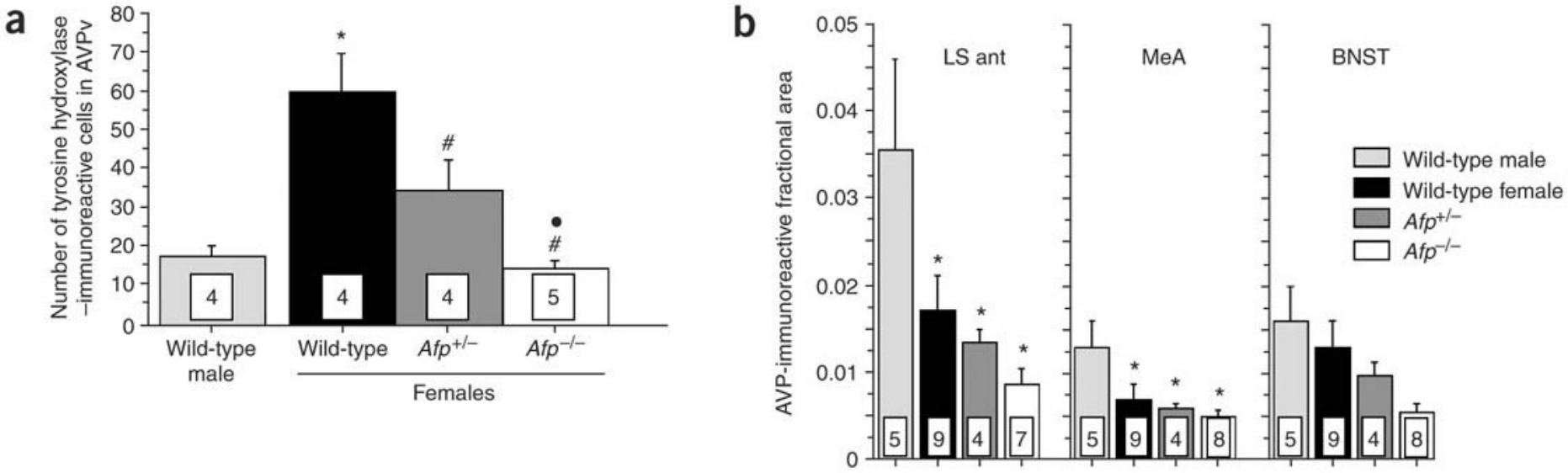


Figure 3. Neurochemical changes in female mice lacking AFP.
(**a**) Numbers of tyrosine hydroxylase–immunoreactive neurons in the AVPv of *Afp*$^{-/-}$ females. (**b**) Brain AVP expression assessed by the fractional areas covered by AVP-immunoreactive structures in the lateral septum anterior (LS ant), medial amygdala (MeA) and bed nucleus of the stria terminalis (BNST). Results of *post-hoc* comparisons by the Fisher PLSD test are indicated as follows: * $P < 0.05$ compared to wild-type males; #$P < 0.05$ compared to wild-type females; $P < 0.05$ compared to *Afp*$^{+/-}$ females. The numbers of subjects are indicated in the bars. Data are expressed as mean s.e.m.

Bakker et al. Nature Neuroscience 9, 220 - 226 (2006)

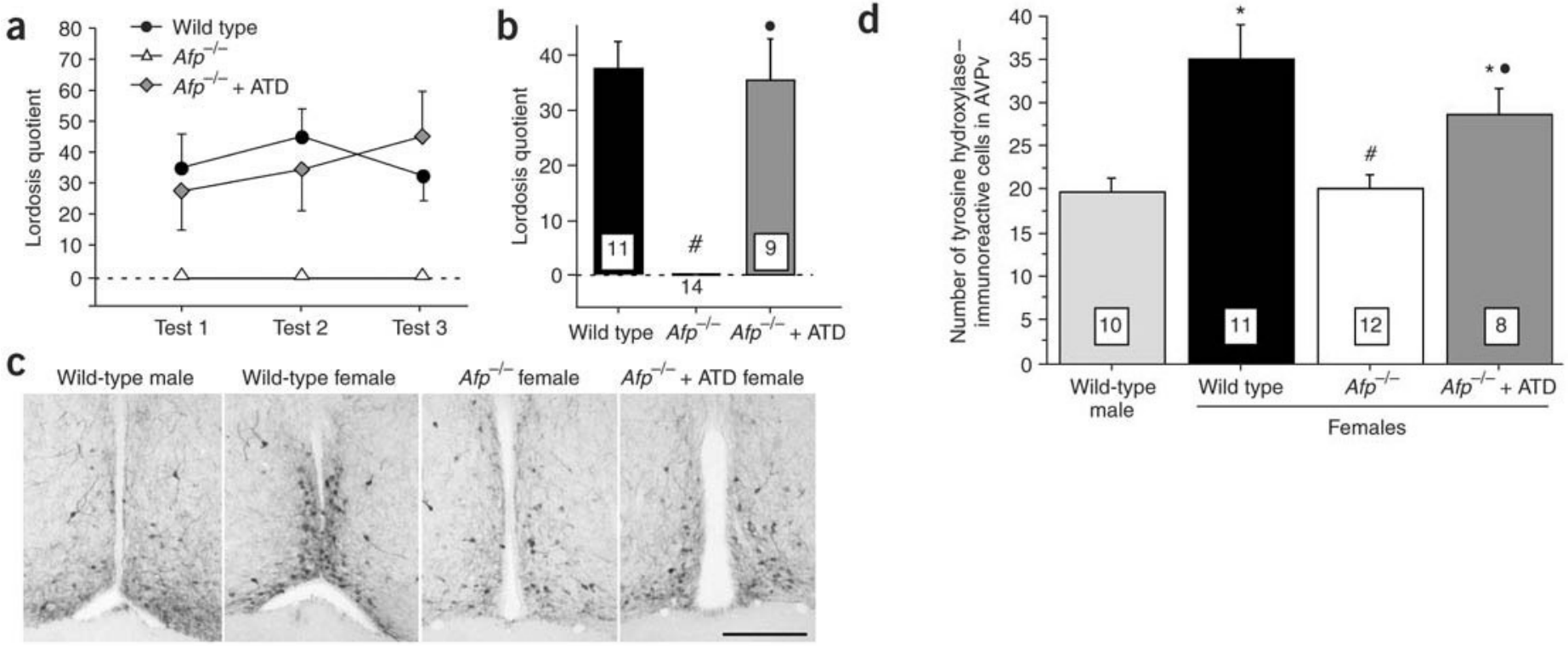


Figure 4. Prenatal treatment with the aromatase inhibitor ATD rescued the female phenotype of *Afp2*$^{-/-}$ females.

(**a**,**b**) Lordosis quotients in three consecutive tests (**a**) and their average (**b**). (**c**) Photomicrographs of sections in AVPv stained by immunohistochemistry for tyrosine hydroxylase in males and females of the three genotypes. Scale bar, 200 m. (**d**) Number of tyrosine hydroxylase–immunoreactive neurons in these sections. Results of *post-hoc* comparisons by the Fisher PLSD test are indicated as follows: * $P <$ 0.05 compared to wild-type males; #$P < 0.05$ compared to wild-type females; $P < 0.05$ compared to *Afp2*$^{-/-}$ females. The numbers of subjects are indicated in the bars. Data are expressed as mean s.e.m.

Bakker et al. Nature Neuroscience 9, 220 - 226 (2006)

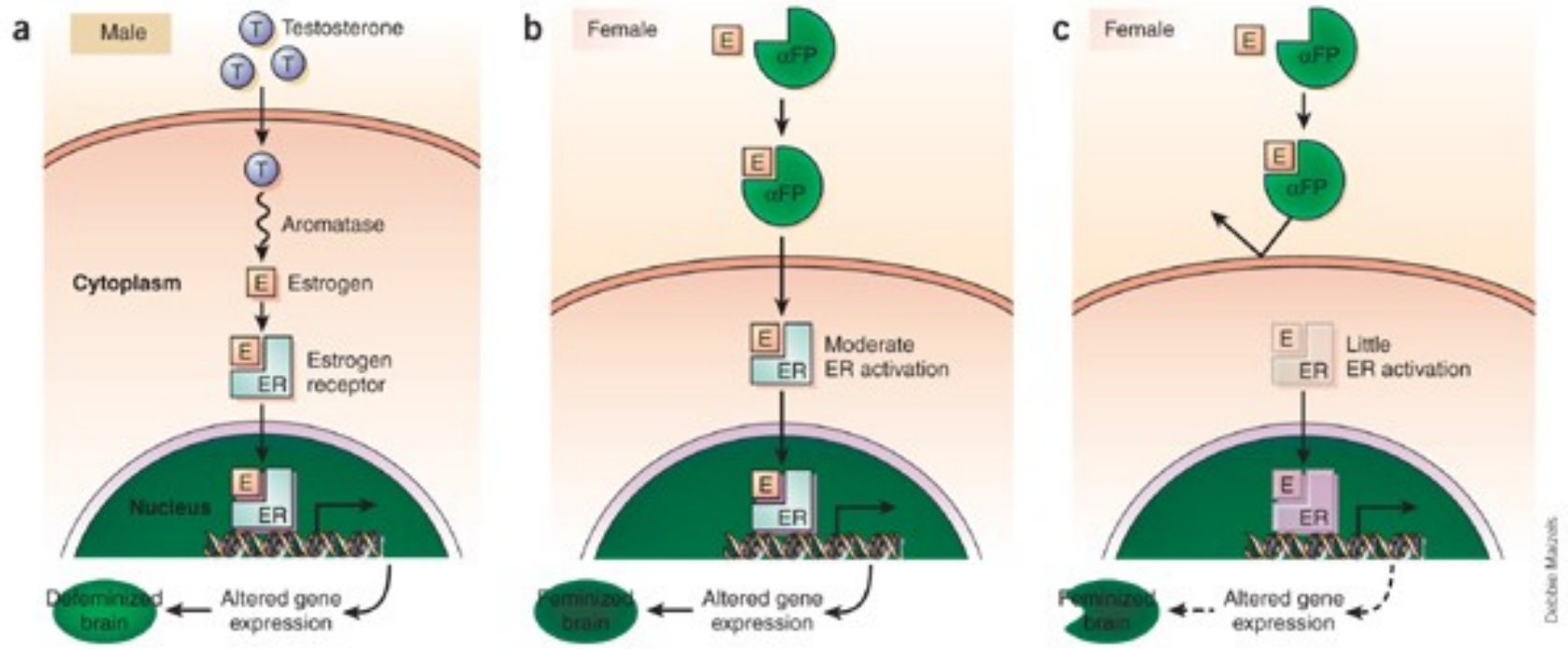


Figure 1. Sexual differentiation of the brain.
(**a**) In male rodents, testosterone reaches the fetal brain and is aromatized there into estrogens (E), which bind to estrogen receptors (ER) to promote gene expression that masculinizes some neural circuits and defeminizes others. There remained the question 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*$^{-/-}$ females restored feminine behavior, which is compatible only with the hypothesis in **c**.

Debbie Maizels