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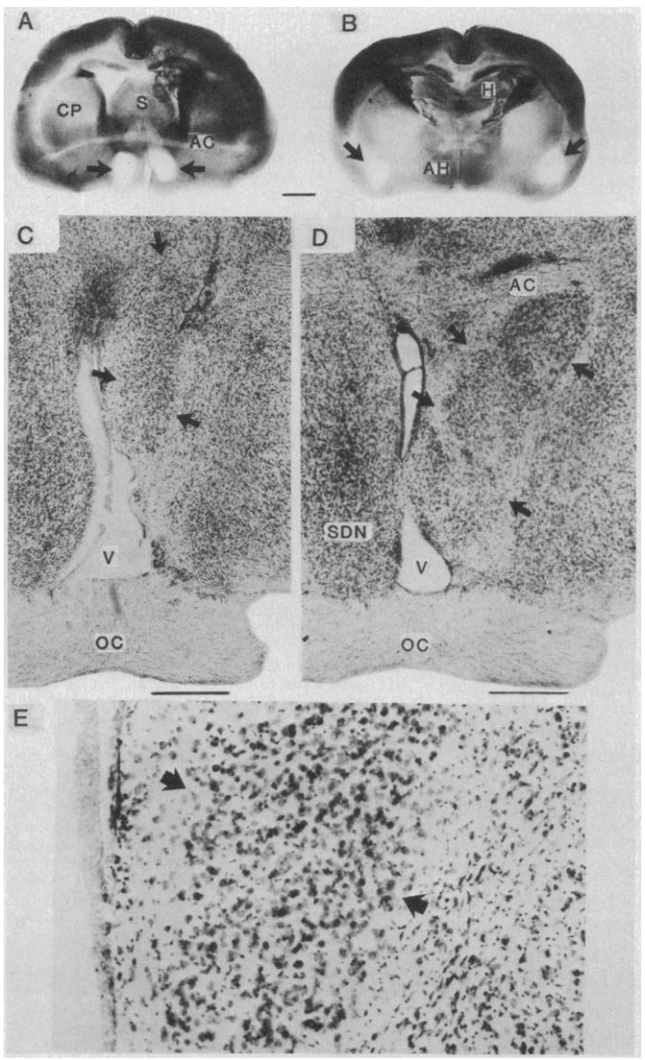
Enhancement of Sexual Behavior in Female Rats by Neonatal Transplantation of Brain Tissue from Males

Abstract. *Transplantation of preoptic tissue from male rat neonates into the preoptic area of female littermates increased masculine and feminine sexual behavior in the recipients during adulthood. This suggests that functional connections develop between the transplanted neural tissue and the host brain. A new intraparenchymal brain transplantation technique was used to achieve these results.*

The mammalian brain is inherently female or bipotential, with sexual differentiation of its reproductive functions largely determined by testicular hormones secreted during perinatal development in the male. One result of this process is the permanent masculinization of reproductive behavior (*1*). In specific neurons testosterone is aromatized to estrogen, which can then act perinatally to masculinize brain function, probably through an interaction with estrogen receptors in the neurons of the medial preoptic area (MPA) (*2*). In the adult rat the MPA is critical for the expression of male sexual behavior (*3*). During the first five postnatal days, however, MPA neurons are relatively undifferentiated, contain few organelles, and make few synaptic connections (*4*). Indeed, the major period of cytoplasmic differentiation and synaptic formation in the rat MPA appears to occur after the critical period for sexual differentiation (*4*).

Fetal and neonatal brain tissue has been successfully transplanted into neonatal (*5*) and adult (*6–8*) rats. Immature neurons can differentiate after transplantation and develop connections with one another as well as with the recipient's brain. We now report that, through the



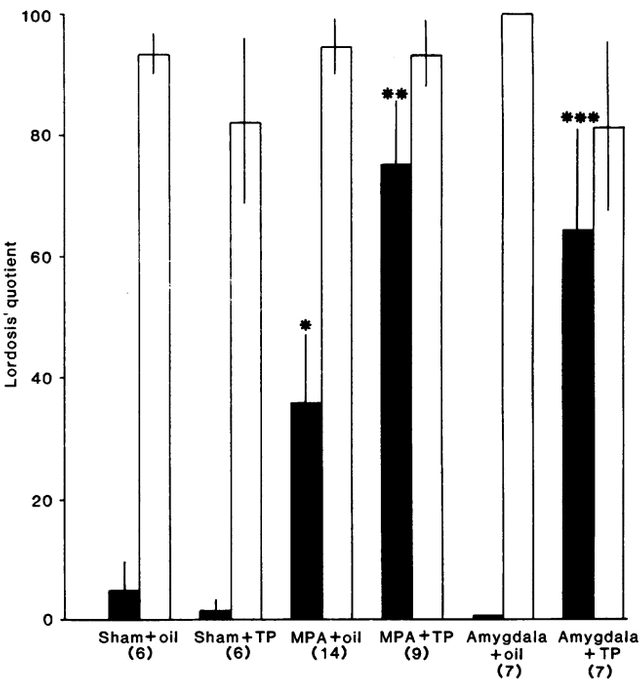


Fig. 1 (left). (A and B) Representative coronal slices from neonatal male donors of MPA (A) or anterior amygdala (B) tissue. Arrows indicate brain areas that were punched out bilaterally and transplanted into neonatal female recipients. Scale bar, 1 mm. (C and D) Photomicrographs of representative sections made through the MPA of two transplant recipients implanted neonatally with male MPA (C) or amygdala (D) tissue. Arrows indicate the location of the transplants. Scale bars, 0.5 mm. (E) High-power photomicrograph of the MPA of the female brain in (C), showing the transplanted male MPA tissue stained by Thionine. Scale bar, 0.1 mm. Abbreviations: *AC*, anterior commissure; *AH*, anterior hypothalamus; *CP*, caudate nucleus and putamen; *H*, hippocampus; *OC*, optic chiasm; *S*, septum; *SDN*, sexually dimorphic nucleus of the MPA; *V*, third ventricle. Fig. 2 (right). Effects of transplanting brain tissue from neonatal males into females on adult lordotic behavior after minimal estrogen priming (filled bars) or estrogen and progesterone priming (open bars). Values are means ± standard errors; numbers in parentheses indicate the number of animals per group. The single asterisk indicates a significant difference ($P < .05$) from other estrogen-only groups treated with oil neonatally; the double asterisk indicates a significant difference ($P < .01$) from all other estrogen-only groups except the one receiving amygdala and TP; and the triple asterisk indicates a significant difference ($P < .01$) from all other estrogen-only groups except those receiving MPA plus oil and MPA plus TP.

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use of a new and accurate intraparenchymal transplantation technique, MPA tissue from male neonates can be successfully transplanted into the MPA of female neonates. Furthermore, the transplanted tissue appears to develop functional connections with the host brain, as evidenced by significantly higher levels of male and female sexual behavior displayed by the female recipients as adults.

The MPA of 1-day-old male Sprague-Dawley rats was bilaterally punched out of the appropriate coronal brain slice with a metal cannula and stereotaxically implanted bilaterally into the MPA of female littermates (*9*). Amygdala, caudate nucleus, or sham (insertion of the empty cannula) transplants were placed in the MPA of other neonatal females. Immediately after surgery, the recipients were given subcutaneous injections of testosterone propionate (TP) (8 μg) or oil vehicle (0.05 ml) (*10*). They were weaned at 21 days of age and ovariectomized at 90 to 100 days of age. Most of the ovaries from females that had been given oil were normal, whereas ovaries from TP-treated females were polyfollicular. Two weeks after ovariectomy, the recipients were tested for female sexual behavior (*11*), and then, 2 weeks later, for masculine behavior (*12*). Finally, the brains were removed, processed histologically (*13*), and stained with Thionine to verify transplant survival and location. Transplant survival rates were excellent: 89 percent of MPA ($N = 54$), 100 percent of amygdala ($N = 32$), and 88 percent of caudate nucleus ($N = 8$) transplants survived the 6-month study, as indicated by the normal appearance of Thionine-stained neurons in the grafts (Fig. 1). Most of the transplants had a volume similar to their neonatal volume (0.2 mm^3).

Analysis of female sexual behavior revealed a highly significant ($P < .0001$) effect of neonatal treatment following minimal estrogen priming (Fig. 2). Lordotic responsiveness was markedly enhanced in animals that had received MPA or amygdala tissue in combination with TP. Females that received MPA tissue and oil (but not amygdala and oil) also showed a significant, if less dramatic, increase in receptivity after minimal estrogen priming. In contrast to the enhanced lordotic responsiveness observed in transplant recipients after estrogen treatment alone, progesterone-facilitated lordotic behavior was apparently not affected by transplantation or TP treatment.

The transplantation of MPA tissue to female neonates also dramatically enhanced male sexual behavior (Fig. 3). A two-way analysis of variance for repeated measures revealed a highly significant group effect ($P < .0005$) as well as a significant measures effect ($P < .0005$) in a four-test sequence for copulatory activity. Group-effect P values for each successive test were all $<.001$. Females with bilateral MPA transplants showed substantially more mounts and intromission responses in any single test than females receiving caudate nucleus, sham, or unilateral MPA transplants (Fig. 3A). Bilateral implants of male MPA tissue are apparently required to enhance male behavior in females, since unilateral MPA transplants were ineffective.

Masculine copulatory behavior in MPA-implanted females increased even if these animals had not been treated with TP. Indeed, there were no differences between the group given MPA plus oil and the group given MPA plus TP (except on the first test day), with both groups showing the same degree of behavioral enhancement when the number of mounts and intromissions was averaged over the test sequence (Fig. 3B) (*14*). Four of the five MPA plus oil animals showing the highest levels of male behavior also displayed very high levels of female behavior (lordosis quotient ≥70) after minimal estrogen priming, indicating an MPA transplant–mediated enhancement of male and female behavior in the same animal (*15*).

Male sexual behavior in amygdala-implanted females given oil or TP, although elevated, did not differ significantly from that in the control groups during any of the tests (Fig. 3A) or overall (Fig. 3B). The apparent ability of some amygdala transplants to enhance copulatory behavior may not be surprising, since labeled steroids are concentrated in this tissue in the neonate (*2*) and since the amygdala appears to facilitate copulatory behavior in the adult male (*16*).

We conclude that brain tissue from neonatal male rats survives transplantation into the MPA of female neonates for at least 6 months and that male MPA tissue can develop functional connections that dramatically affect sexual behavior in the female recipients as adults. To our knowledge, this is the first time that mammalian intraparenchymal transplants have been reported to produce behavioral changes in recipient animals

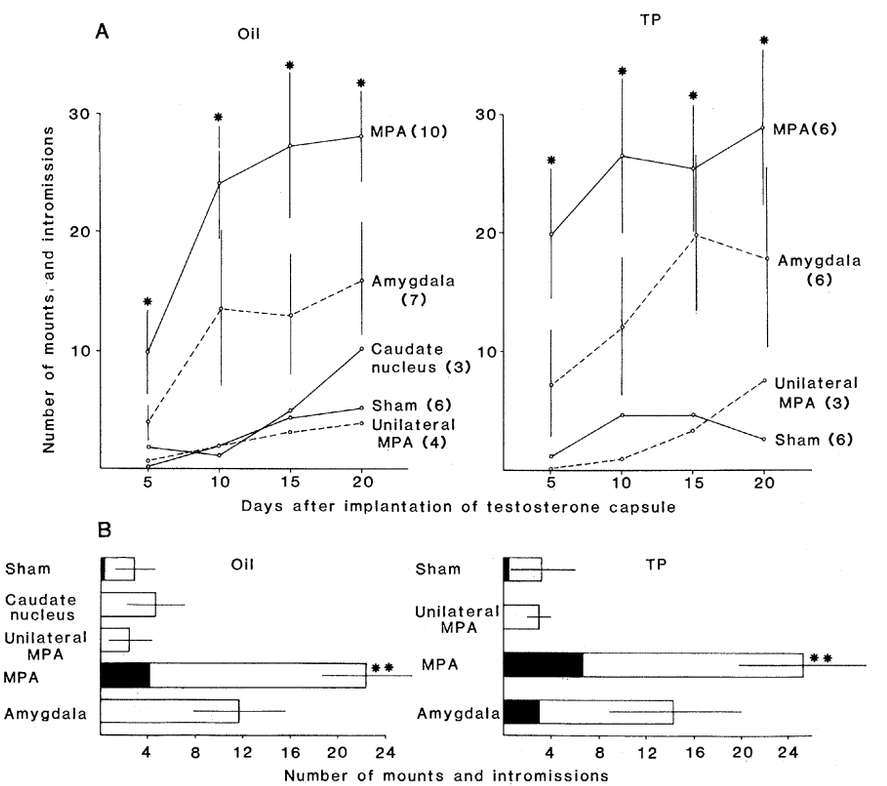

Fig. 3. (A) Masculine sexual behavior shown during four tests by adult female rats given male brain tissue and oil or TP as neonates. Values are means ± standard errors; the number of animals in each group is shown in parentheses. Single asterisks indicate a significant difference ($P < .05$) from all the control groups. (B) Mean number of mounts and intromissions shown by recipient females during the tests for male behavior. The mean number of intromissions for each group is depicted by the filled zone of each bar. Double asterisks indicate a significant difference from the group that received amygdala and oil ($P < .05$) and from all other groups ($P < .01$) except the one that received amygdala and TP.

that are consistent with the normal function of the transplanted tissue. Although histological examination of stained sections indicates that healthy, normal neurons are present in the transplanted tissue (Fig. 1), this does not prove the existence of connectivity. However, we subsequently used a modified Golgi impregnation technique to verify the existence of neuronal connections between the male transplant and the recipient female's brain (*17*). In addition, we recently were able to radioactively label neurons in the male MPA before birth and to transplant large numbers of these neurons into the MPA of neonatal females not previously exposed to radioactivity (*18*).

The enhancement of male sexual behavior in females neonatally implanted with male MPA tissue was observed even if the recipients were not given testosterone concurrently. This suggests that the day-old male MPA already has the potential to behaviorally masculinize the neonatal female brain. Weisz and Ward (*19*) found that testosterone concentrations in males are consistently higher than those in females only on days 4 and 5 before birth, and suggested that this brief prenatal exposure sensitizes the developing male central nervous system to the masculinizing action of testosterone circulating in relatively low amounts (not consistently higher than in females) at later stages of development. Such a testosterone-induced sensitization of male MPA transplants could explain the enhanced male behavior observed in females in the present study. However, proof of the dependence of this behavioral masculinization on transplanted male brain tissue will require similar studies involving the transplantation of female MPA tissue into female littermates.

The increased lordotic responsiveness seen in transplant recipients after minimal estrogen priming (Fig. 2) was an unexpected, although not unreasonable, finding (*20*). Estrogen receptors are present in the MPA and amygdala of male and female rat neonates (*2*). It is possible that, in the process of transplanting these two brain regions neonatally, a sizable number of estrogen receptors were also transferred, resulting in an enhanced MPA estrogen receptor population in the recipient's brain. Stenevi *et al.* (*8*) transplanted fetal hypothalamic tissue into the brains of adult female rats and found that estrogen-concentrating cells had apparently differentiated in the transplanted tissue.

The intraparenchymal transplantation technique is versatile in that, theoretically, any discrete area of brain tissue can be accurately transplanted stereotaxically into any part of a recipient neonate's brain. Since immature rat brain tissue survives transplantation into adults (*7*), it is likely that adults as well as neonates can serve as recipients.

In contrast to techniques in which some portion of the ventricular system is used as the transplantation site (*6, 8, 21, 22*), the present approach allows a direct anatomic relation to be established between the transplant and the recipient's neural tissue, maximizing the chances for development of functional connections and neuropil continuity (*23*). Indeed, no enhancement of male behavior was observed in the present study unless male tissue was bilaterally resident in the recipient female's MPA. Apparently, however, transplant tissue need not be placed intraparenchymally in order to have some functional impact since (i) ventricular transplants of fetal dopamine neurons decrease motor abnormalities caused by lesions of the adult substantia nigra (*21*) and (ii) ventricular transplants of fetal vasopressin neurons into adult Brattleboro rats alleviate their polydipsia and polyurea (*22*).

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9. The males were decapitated and their brains were quickly removed from the skull and placed in cold isotonic saline for 5 minutes. A coronal slice approximately 0.5 mm thick and sexually dimorphic structurally [R. A. Gorski, J. H. Gordon, J. E. Shryne, A. M. Southam, *Brain Res.* **148**, 333 (1978)] was removed at the level of the MPA. The right MPA was punched out of the brain slice with a metal cannula (inner diameter, 0.7 mm) sharpened at the end and equipped with a stylet of the same length but drawn slightly into the cannula. The cannula containing this tissue was stereotaxically positioned in the right MPA of a female anesthetized on ice and held in a special stereotaxic adapter unit. The stylet was then depressed to extrude the transplant. The left MPA was similarly punched out and transplanted. After surgery the rats were returned to their mothers. Each fresh brain slice was placed on a microprojector for immediate verification of tissue punch accuracy; then the slice was placed in Bouin's fixative for histological processing.
10. In planning this study, we assumed that a small dose of TP (not known to affect adult levels of sexual behavior when given to females neonatally) might be necessary to provide a more suitable hormonal milieu for normal masculine differentiation of neurons in the male tissue transplants. The results indicate, however, that this neonatal TP treatment was not necessary to observe transplant-induced behavioral modifications.
11. Transplant recipients were given estradiol benzoate (2 μg, subcutaneously) daily for 3 days and tested on the 4th day starting 1 hour after the onset of the 10-hour dark period. They were placed in 18 by 18 inch Plexiglas testing arenas containing two or three sexually vigorous male Long-Evans rats. After each mount, the female was scored as to whether or not she had displayed lordosis. Each test consisted of ten mounts, with the number of lordotic responses expressed as the lordosis quotient (number of lordotic responses multiplied by 10). After this initial test, the females were injected with progesterone (0.5 mg, subcutaneously) and tested 4 hours later in the same manner. Between-group differences were determined by analysis of variance and Duncan's multiple range test.
12. Each female was implanted subcutaneously with a 15-mm testosterone-filled Silastic capsule. These capsules maintain copulatory behavior at normal levels for well over 1 month after implantation into castrated rats [D. A. Damassa *et al.*, *Horm. Behav.* **8**, 275 (1977)]. Tests of copulatory activity were conducted 5, 10, 15, and 20 days after capsule implantation. Each test was started 1 hour or less after the onset of the dark period by placing the experimental females alone into a Plexiglas arena for adaptation. A highly receptive stimulus female was then introduced to begin the 30-minute test. The number of mounts and intromissions displayed by experimental females were recorded during this period. No ejaculatory responses were observed. Statistical analyses were the same as those used to evaluate female behavioral performance.
13. The animals were perfused with 10 percent Formalin and their brains were removed and stored in Formalin for at least 3 days. The brains were then sectioned serially (thickness, 50 μm), mounted, and stained.
14. When averaged over the four-test sequence, both the MPA plus oil and MPA plus TP groups also displayed significantly higher numbers of intromissions than any other group (Fig. 3B) except for the amygdala plus TP group.
15. This dual behavioral enhancement was not observed for animals given amygdala or caudate nucleus and oil.
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17. This work is still in progress in collaboration with R. Hammer of the National Institute of Mental Health.
18. Tritiated thymidine injected intravenously into the dam late in gestation is permanently incorporated into the genomic apparatus of fetal MPA neuroblasts still undergoing mitosis at the time of injection [C. D. Jacobson and R. A. Gorski, *J. Comp. Neurol.* **196**, 519 (1981)].
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20. Although TP treatment facilitated (but was not necessary for) this enhancement of female behavior in MPA transplant recipients, it was required for enhancement in amygdala recipients. The mechanism of this synergism is unknown.
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23. It must be emphasized that, until the connectivity of such grafts can be described fully, these behavioral effects must be interpreted cautiously. For example, transplanted male MPA tissue could act as a local source of hormone receptors, testosterone, or a hypothetical masculinizing substance.
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