1 (Suppl. 1), S17 (1981); P. Bustany, J. F. Henry, F. Soussaline, D. Comar, in *Functional Radionuclide Imaging of the Brain*, P. G. Magistretti, Ed. (Raven, New York, 1983), p. 319–326.
4. A portion of these studies has been reported in preliminary form by C. A. Marotta and E. M. Sajdel-Sulkowska [*Trans. Am. Soc. Neurochem.* **15**, 14 (1984)].
5. C. Niedergang, H. Okazaki, M. E. Ittel, D. Munoz, F. Petek, P. Mandel, *Biochim. Biophys. Acta* **358**, 91 (1974); H. Okazaki, M. E. Ittel, C. Niedergang, P. Mandel, *ibid.* **391**, 84 (1975).
6. J. S. Roth, *J. Biol. Chem.* **231**, 1097 (1958); K. Shortman, *Biochim. Biophys. Acta* **51**, 37 (1961); J. S. Roth, *ibid.* **61**, 903 (1962); P. Rosso and M. Winick, *J. Nutr.* **105**, 1104 (1975).
7. N. Kraft and K. Shortman, *Biochim. Biophys. Acta* **217**, 164 (1970); D. K. Liu, G. H. Williams, P. J. Fritz, *Biochem. J.* **148**, 67 (1975).
8. H. Koenig, D. Gaines, T. McDonald, R. Gray, J. Scott, *J. Neurochem.* **11**, 729 (1964).
9. L. Franzoni and C. A. Garcia Argiz, *Acta Physiol. Lat. Am.* **28**, 185 (1978).
10. M. E. Ittel and P. Mandel, *J. Neurochem.* **33**, 521 (1979).
11. Y. Takahashi, K. Mase, Y. Suzuki, *ibid.* **17**, 1433 (1970); P. Blackburn, G. Wilson, S. Moore, *J. Biol. Chem.* **252**, 5904 (1977).
12. J. S. Roth, *Biochim. Biophys. Acta* **21**, 34 (1956).
13. Y. Takahashi, K. Mase, H. Sugano, *ibid.* **119**, 627 (1966); G. R. Lawford, P. Langford, H. Schachter, *J. Biol. Chem.* **241**, 1835 (1966); G. Scheele and P. Blackburn, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4898 (1979).
14. R. D. Terry and P. Davies (Albert Einstein College of Medicine) provided all AD specimens and controls C63a and C86 in addition to making the neuropathologic evaluations; H. J. Manz (Georgetown Medical School) contributed samples and data for C63b and C76.
15. Total cellular RNA was prepared by method IV described in E. Sajdel-Sulkowska, J. F. Coughlin, C. A. Marotta, *J. Neurochem.* **40**, 670 (1983).
16. D. M. A. Mann, D. Neary, P. O. Yates, J. Lincoln, J. S. Snowden, P. Stanworth, *J. Neurol. Neurosurg. Psychiatry* **44**, 97 (1981).
17. Poly(A)$^+$ RNA was prepared by the one-step oligo(dT)-cellulose column chromatography (*15*). The RNA yields obtained after precipitation with ethanol (measured by optical absorbance at A_{260}) are indicated in the text and in Fig. 1B. The RNA yields of column eluates, prior to precipitation, are as follows: controls, 3.9 ± 0.8 μg/g; AD, 1.6 ± 0.3 μg/g. The latter value represented a significant relative reduction [59.7 percent decrease, $t(10) = 2.78$, $P < 0.05$].
18. Acid ribonuclease was assayed as in Fig. 2 except that incubations were carried out in 0.02*M* citrate-phosphate buffer at *p*H 6.0.
19. C. Gagnon, G. Lalonde, G. de Lamirande, *Biochim. Biophys. Acta* **353**, 323 (1974).
20. R. C. Imrie and W. C. Hutchinson, *ibid.* **108**, 106 (1965); R. L. Greif and E. F. Eich, *ibid.* **286**, 350 (1972).
21. We thank Drs. Alfred Pope, Ralph Nixon, and Adam Sulkowski for comments concerning this manuscript. Supported by NIH grants AG 02126 and RCDA AG 00084 and a MacArthur Foundation Award to C.A.M.
* Address correspondence to McLean Hospital.

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Mating and Pregnancy Can Occur in Genetically Hypogonadal Mice with Preoptic Area Brain Grafts

Abstract. *Adult female hypogonadal mice, in whom hypogonadism is secondary to a genetic deficiency in hypothalamic gonadotropin-releasing hormone (GnRH), are infertile. Mating, pregnancy, and delivery of healthy litters were achieved after transplantation of normal fetal preoptic area tissue, a major site of GnRH-containing cell bodies, into the third ventricle of adult female hypogonadal mice. Immunocytochemistry revealed GnRH-containing neurons in the grafts and GnRH-containing processes extending to the lateral median eminence of the host brains.*

Infertility in the hypogonadal mouse is secondary to a genetic deficiency of hypothalamic gonadotropin-releasing hormone (GnRH) (*1*). Affected animals have infantile gonads, undeveloped secondary sexual tissue, and low concentrations of pituitary and plasma gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)]. A similar condition in humans, familial gonadotropin deficiency (*2*), appears to be inherited as an autosomal recessive trait, as is the hypogonadal defect (*1*). GnRH injections stimulate LH and FSH production in affected mice (*3*) and humans (*4*). In normal adult rodents, GnRH-containing cell bodies are present in the preoptic area (POA) of the brain. Several diverse neurochemical deficits related to genetic or surgical lesions have been alleviated by grafts of brain tissue (*5*). We previously reported that grafts of normal fetal POA tissue into the third ventricle in adult male (*6*) or female (*7*) hypogonadal mice corrected many of their endocrine defects. In both sexes significant increases in pituitary and plasma LH and FSH were associated with increased weights of gonadal and accessory sex organs. However, after vaginal opening, the cells in daily vaginal smears were cornified, indicating constant secretion of estrogen rather than the characteristic 4- to 6-day ovulatory cycle of normal mice. Since no corpora lutea were seen in the ovaries, we had no evidence that the females were capable of ovulation.

We now report that ten adult hypogonadal females with POA grafts (*8*) mated when paired overnight with a normal male. Seven of these females became pregnant and six delivered healthy litters. Histological and immunocytochemical analyses of the brains of these females confirmed that all had received GnRH-containing grafts in the third ventricle.

In view of the known sexual dimorphism of the POA region (*9*), we sought to determine whether the sex of the donor was important in the success of the POA transplant. Therefore, five of the hypogonadal females received grafts of POA tissue from female fetuses and five received grafts from male fetuses. Vaginal opening, induced by estrogen secretion from the stimulated ovaries, occurred 16 to 40 days after transplantation, and the females entered constant vaginal estrus.

Ten weeks after transplantation the hypogonadal females were each paired with a normal male overnight. In the morning nine of the females were found to have vaginal plugs, a sign of successful copulation. The remaining female was paired with one of the proven males a second night, and she too mated. The females were weighed every 3 to 4 days to check for pregnancy; seven showed weight gains. Six females delivered live litters of four to seven pups each. One female died 11 days after mating, but an autopsy showed that she held three embryos.

When the pups were 1 month old, blood was collected under ether anesthesia from the nine surviving dams. The plasma was stored at −20°C for later radioimmunoassay of LH and FSH (*10*). The dams' brains were then prepared for immunocytochemical identification of GnRH. Four animals were perfused (*11*) for subsequent sectioning of brain tissue with a Vibratome. The remaining animals were decapitated and their brains were processed for paraffin sectioning (*12*); pituitaries were frozen for subsequent radioimmunoassay of LH and FSH. The ovaries and uteri of all animals were removed and weighed.

Table 1 gives mean concentrations of pituitary and plasma LH and FSH in hypogonadal females with and without POA grafts and in normal homozygous mice of the same strain. Gonadotropin concentrations in hypogonadal females with grafts were in the normal range. Similarly, ovarian and uterine weights in hypogonadal females with POA grafts (12.4 ± 1.6 and 136.5 ± 15.1 mg, respectively; means ± standard errors) were comparable to those of the normal adults (12.5 ± 1.0 and 161.0 ± 31.3 mg) and vastly greater than in the untreated hypogonadal adults (2.8 ± 0.6 and 12.2 ± 1.6 mg).

The antiserum used for immunocytochemical demonstration of GnRH visualizes these peptidergic neuron cell bodies in normal mouse brain without requiring treatment of the animal with colchicine, and the reactive cells and fibers in this species have a distribution similar to that in rat brain (*13*). In normal mouse brain many GnRH-containing cell bodies are present in the POA-septal region, and a major projection of reactive fibers is observed in the lateral median eminence. With the antiserum no reactive cells or

Table 1. Concentrations of LH and FSH in pituitaries and plasma from homozygous normal female mice of the hypogonadal strain, homozygous hypogonadal females, and hypogonadal females with POA grafts. Values are means ± standard errors.

Group	*n*	Nanograms per pituitary		*n*	Nanograms per milliliter of plasma	
		LH	FSH		LH	FSH
Normal	6	145.0 ± 36.6	32.7 ± 5.3	6	0.68 ± 0.12	4.3 ± 2.3
Hypogonadal	6	44.7 ± 3.3	6.4 ± 0.8	6	≤0.5	≤2.0
Hypogonadal with graft	4	155.0 ± 22.8	38.3 ± 5.5	9	0.72 ± 0.08	8.3 ± 1.3

fibers are detectable in serial brain sections from homozygous hypogonadal male or female mice when these sections are taken from as far rostral as the olfactory bulbs to the caudal medulla.

The transplanted tissue was located primarily in the third ventricle, as far anterior as the medial POA and as far caudal as the premammillary recess. In a few animals donor tissue was also found along the needle track in the parenchyma of the hypothalamus and thalamus and in the foramen of Monro. In all cases transplanted tissue was present in the infundibular recess and merged with the median eminence (Fig. 1A). The immunocytochemical studies showed GnRH-containing cells and fibers in the transplants and host tissue (Fig. 1B). Only 1 to 16 immunoreactive cells were found per animal, perhaps reflecting nonrandom selection of the paraffin sections (only 20 percent were reacted), incomplete penetration of immunological reagents in the thicker Vibratome sections, or low cellular content of GnRH due to rapid turnover of the hormone in some transplanted cells. However, it is possible that these grafts can function physiologically even with far fewer than the normal complement of GnRH neurons (190 cells were counted in serial 50-μm Vibratome sections of the POA from one normal male mouse).

Most fibers exiting from the graft did so from the lateral or ventral surface. Fibers that exited from the lateral edge of the graft arched through the arcuate nucleus (Fig. 1D), apparently following the curved distribution of tanycytes in this region (*14*), as do many fibers in the normal animal. Several axons could be traced from the graft to the median eminence. As in normal brains, fibers were particularly concentrated at the lateral borders over the tuberoinfundibular sulci. In all the animals fibers extended through the median eminence as far caudal as the premammillary recess. We could not detect any notable differences between mice that became pregnant and those that did not in the pattern of reactive cells and fibers.

In rats, extrahypothalamic projections of GnRH-containing fibers to the central gray matter of the midbrain have been implicated in the mediation of lordosis (*15*). However, such projections were not observed in our successfully mated hypogonadal females and may not be necessary for mating behavior in female mice. In one animal a few fibers exited the most rostral portion of the graft and entered the septum, and in another animal with a substantial portion of the transplant in the premammillary recess, a few GnRH-containing axons were present in the mammillary body. These were the only extrahypothalamic fibers seen.

The sex of the donor apparently did not affect the success of the grafts in supporting reproduction, since four of the seven fertile females received tissue from male fetuses. If grafts from neonates prove viable, it would be desirable to determine whether transplants of POA's from 5-day-old, sexually differentiated mice (*16*) (fetal brains may not yet show such dimorphism) will similarly support ovulation in the hypogonadal female.

The finding that nine of the ten females in this study mated with the male on the first night of pairing is in contrast to the experience with similarly paired, normally cycling virgin females (n = 317), when only 14 percent mated the first night, another 14 percent for the first time on the second night, and 46 percent on the third night (*17*). Since seven of these mice in constant estrus became pregnant, reflex ovulation may have oc-

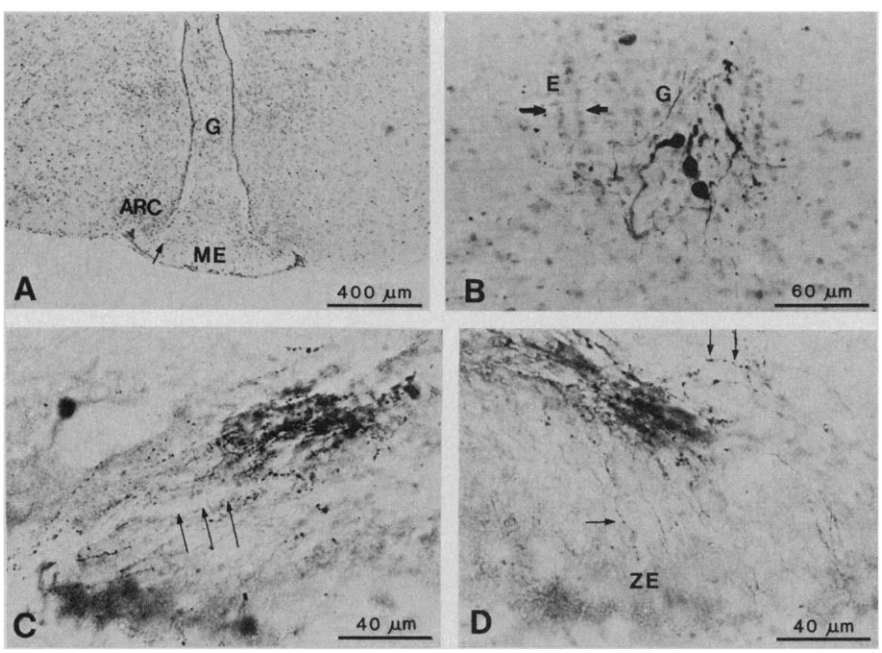

Fig. 1. Fetal POA grafts in the third ventricle of hypogonadal adult mice that produced litters. (A) Low-power micrograph of a 6-μm coronal section stained with cresyl violet, showing a graft (G) filling and expanding the third ventricle. The base of the graft merges (arrow) with the median eminence (ME). ARC, arcuate nucleus. (B to D) Higher power views of 60-μm Vibratome sections reacted with antiserum to GnRH. (B) Cell bodies and fibers containing reactive GnRH in a graft in the third ventricle, which is lined by ependyma (E; left-hand arrow). The ventricular wall of the host is also visible (right-hand arrow), as is a reactive fiber exiting the graft through the ependyma to pass into the host brain. (C) Lateral corner of the median eminence, similar to the area indicated by the arrow in (A), into which the graft is sending numerous fibers containing reactive GnRH. Some of the fibers are associated with a penetrating capillary (arrows); note the row of red blood cells within it. (D) Right-hand corner of a transplant–median eminence junction, showing reactive GnRH-containing fibers exiting the graft to follow the normal arching course through the arcuate nucleus (vertical arrows) or extending ventrally (horizontal arrow) to project to the portal capillary bed in the zona externa (ZE).

curred. This phenomenon has been described in rats, which, like mice, normally ovulate spontaneously and exhibit regular estrous cyclicity, but which, when maintained in conditions of constant light for prolonged periods, enter persistent vaginal estrus (*18*). Such rats may ovulate "reflexively" in response to copulation (*19*). The neural pathway involved in reflex ovulation has not been fully defined, but the response is abolished by pelvic neurotomy (*20*). The female mouse is less affected by conditions of constant light (*21*), since after 120 days in constant light only 12.5 percent of female mice exhibited persistent vaginal cornification compared to 80 percent of rats housed in the same chamber. In fact, despite having more variable cycle lengths than rats, young adult mice do not show prolonged periods of persistent estrus, although with aging (13 to 16 months), such episodes begin to appear (*22*). Thus, although estrous cyclicity in mice is affected by male odor (*17*), there have not, to our knowledge, been any previous reports in mice of reflex ovulation within hours of the stimulus.

It is important to determine whether any hypogonadal mice with POA grafts are capable of normal estrous cyclicity. Such capability may depend on the establishment of certain critical neural connections between the host brain and the transplant. Various peptide and nonpeptide transmitters have been implicated in the regulation of GnRH-containing neurons (*23*), but the essential interactions are unknown. Nevertheless, our present understanding of the control of ovulation indicates that reflex ovulation is also a neurally mediated event, although the final pathway is not yet defined. In at least seven hypogonadal females with fetal POA grafts, neuronal connections necessary to permit ovulation were established.

MARIE J. GIBSON
DOROTHY T. KRIEGER
Department of Medicine, Mount Sinai School of Medicine, New York 10029
HARRY M. CHARLTON
Department of Human Anatomy, Oxford University, Oxford, United Kingdom OX1 3QX
EARL A. ZIMMERMAN
ANN-JUDITH SILVERMAN
Departments of Neurology and *Anatomy, College of Physicians and Surgeons, Columbia University, New York 10032*
MARK J. PERLOW
Augustana Hospital and Neurology Service, Veterans Administration, Chicago, Illinois 60680

References and Notes

1. B. M. Cattanach, C. A. Iddon, H. M. Charlton, S. A. Chiappa, G. Fink, *Nature (London)* **269**, 338 (1977).
2. R. W. Ewer, *J. Clin. Endocrinol. Metab.* **28**, 783 (1968).
3. H. M. Charlton *et al.*, *Endocrinology* **113**, 535 (1983).
4. Y. Yoshimoto, K. Moridera, H. Imura, *N. Engl. J. Med.* **292**, 242 (1975); C. H. Mortimer, A. S. McNeilly, R. A. Fisher, M. A. F. Murray, G. M. Besser, *Br. Med. J.* **4**, 617 (1974).
5. D. Gash and J. R. Sladek, Jr., *Peptides* **1**, 11 (1980); M. J. Perlow *et al.*, *Science* **204**, 643 (1979); W. C. Low *et al.*, *Nature (London)* **300**, 260 (1982).
6. D. T. Krieger *et al.*, *Nature (London)* **298**, 468 (1982).
7. M. J. Gibson *et al.*, *Endocrinology* **114**, 1938 (1984).
8. Tissue from the ventral brains of 16- to 18-day fetuses was dissected just posterior to the bifurcation of the anterior cerebral artery. The block of tissue included the POA and was approximately 1.0 mm wide by 0.5 mm deep by 0.7 mm anterior to posterior. Segments from two fetuses were pooled for each graft in a drop of 0.9 percent sterile saline. Tissue was injected stereotaxically (*6*) with a modified 22-gauge needle into the anterior third ventricle of anesthetized mice 2½ months of age.
9. G. Raisman and P. M. Field, *Brain Res.* **54**, 1 (1973); R. A. Gorski, J. H. Gordon, J. E. Shryne, A. M. Southam, *ibid.* **148**, 333 (1978).
10. Radioimmunoassay of LH and FSH was performed with kits for rat FSH and LH (provided by the National Institute of Child Health and Human Development); rLH-RP-2 and rFSH-RP-2 were used as reference standards. Because these reference preparations are more potent than the respective RP-1's previously used, the NICHD advises that with RP2 the values for LH will be 61 times lower than formerly reported, while those for FSH will be 45 times lower.
11. After perfusing four animals intracardially with saline (20 to 30 ml) and Zamboni's fixative (*12*), we removed their brains, cut them into blocks (*6*), and immersed them in Zamboni's overnight. The region from the septal nuclei to the mammillary bodies was then cut into 50- to 60-μm sections on a Vibratome. The sections were washed and incubated sequentially in 0.1*M* sodium periodate (10 minutes), 0.1 percent sodium borohydride (10 minutes), and antiserum to luteinizing hormone–releasing hormone (LR-1, a gift of R. Benoit) diluted 1:10,000 and containing 0.02 percent saponin (48 to 72 hours), a biotinylated goat antibody to rabbit serum (60 minutes), and a complex of avidin, biotin, and horseradish peroxidase (60 minutes) (Vectastain). The horseradish peroxidase was visualized with 3,3′-diaminobenzidine and the hydrogen peroxide was generated with glucose oxidase; this reaction was allowed to continue for 30 to 60 minutes. All reactions were carried out at room temperature except for the exposure to primary antiserum, which took place at 4°C. After their exposure to diaminobenzidine the sections were washed, mounted on glass slides, dehydrated, and covered with cover slips.
12. The brains were cut into blocks (*6*), immersed in Bouin's fixative overnight, dehydrated in graded alcohols, embedded in paraffin, cut serially in the coronal plane, and placed on glass slides. Sections were deparaffinized and every tenth section was stained with cresyl violet; intervening sections were reacted with rabbit antiserum to GnRH diluted 1:10,000 for 48 hours and then exposed to avidin-biotin-peroxidase complex and diaminobenzidine to produce reaction products [(*6*); S. M. Hsu, L. Raine, H. Fanger, *J. Histochem. Cytochem.* **29**, 577 (1981)].
13. J. W. Witkin, C. M. Paden, A. J. Silverman, *Neuroendocrinology* **35**, 429 (1982).
14. K. M. Knigge and A. J. Silverman, in *Handbook of Physiology*, S. R. Geiger, Ed. (Academic Press, New York, 1974), pp. 1–32.
15. Y. Sakuma and D. W. Pfaff, *Nature (London)* **283**, 566 (1980); *Neuroendocrinology* **36**, 218 (1983).
16. C. A. Barraclough and J. H. Leathem, *Proc. Soc. Exp. Biol. Med.* **85**, 673 (1954); R. A. Gorski, *Endocrinology* **82**, 1001 (1968).
17. W. K. Whitten, *J. Endocrinol.* **13**, 399 (1956).
18. L. G. Browman, *J. Exp. Zool.* **75**, 375 (1937); J. W. Everitt, *Endocrinology* **25**, 123 (1939).
19. K. Brown-Grant, J. M. Davidson, F. Greig, *J. Endocrinol.* **57**, 7 (1973); K. Brown-Grant, *Horm. Behav.* **8**, 62 (1977).
20. M. X. Zarrow and J. H. Clark, *J. Endocrinol.* **40**, 343 (1968).
21. W. Jochle, *Ann. N.Y. Acad. Sci.* **117**, 88 (1964); C. S. Campbell, K. D. Ryan, N. B. Schwartz, *Biol. Reprod.* **14**, 292 (1976).
22. J. F. Nelson, L. S. Felicio, P. K. Randall, C. Sims, C. E. Finch, *ibid.* **27**, 327 (1982).
23. G. Fink, H. F. Stanley, A. G. Watts, in *Brain Peptides*, D. T. Krieger, M. J. Brownstein, J. B. Martin, Eds. (Wiley, New York, 1983), p. 413.
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The Goldfish Ear Codes the Axis of Acoustic Particle Motion in Three Dimensions

Abstract. *Auditory and vestibular nerve fibers of the goldfish are strongly directionally sensitive to whole-body acceleration at audio frequencies. The three-dimensional pattern of sensitivity shows that input from a receptor ensemble (hair cells) is essentially equivalent to that expected from a single hair cell having a given three-dimensional orientation of best sensitivity. Fibers from the sacculus, lagena, and utriculus differ with respect to distributions of directional orientation, but are similar in best threshold (less than 1 nanometer, root mean square, at 140 hertz). In combination with other mechanisms for detection of sound pressure, this directionality is a likely basis for directional hearing in fishes, and it could allow the determination of underwater acoustic intensity.*

Otolith organs of the ear respond to accelerations resulting from gravity and movements in space and generally operate on the principle of an accelerometer (*1*). In terrestrial animals, the saccular and utricular organs are part of the vestibular system, and their function is usually restricted to frequencies below 10 Hz (*2*). In fishes, however, the otolith organs may have auditory functions as well and a usable frequency range exceeding 1 kHz in some species (*3, 4*). A general route of sound to the ear in all fishes operates in an essentially "vestibular" fashion: acoustic particle movement engages the animal's body, and the mass-loaded otolithic ears function essentially as inertial devices in sensing this motion (*5*).

The sensing elements are the hair