Figure 1 shows the results for each interval after treatment. A two-way analysis of variance, with the dose of PCP and the saline or naloxone treatment as the main factors, was conducted for each time point. Both main effects and their interaction were significant ($P < .05$) at 15, 30, and 45 minutes; only the main effect of PCP was significant at 60 minutes. Paired *t*-tests between baseline temperatures and temperatures after treatment reveal that, at all time points, PCP elicited significant hyperthermia at low doses (0.625 to 5.0 mg/kg) and significant hypothermia at high doses (20.0 to 40.0 mg/kg) ($P < .05$). The combination of PCP and naloxone resulted in no significant changes in temperature after 15 minutes and in significant ($P < .05$) hypothermia 30 and 45 minutes after high doses of PCP (40.0 mg/kg and 20.0 and 40.0 mg/kg, respectively). After 60 minutes the effects of PCP plus naloxone did not differ significantly from those of PCP plus saline. Thus naloxone completely antagonized PCP for 15 minutes, after which this effect gradually disappeared.

Reversal of PCP's effects was also demonstrated. After baseline temperatures were determined in eight additional rats, four were administered a hyperthermic dose of PCP (2.5 mg/kg) and four were given a hypothermic dose (20.0 mg/kg). Rectal temperatures were recorded at 15 minutes, and immediately thereafter all the rats were administered naloxone (1 mg/kg). Temperatures were measured again after 15 minutes. The effects of PCP administered alone at doses of 2.5 and 20.0 mg/kg are greater after 30 minutes than after 15 minutes (Fig. 1). In the present instance, however, whereas the hyperthermic and hypothermic effects were readily apparent after 15 minutes, the subsequent administration of naloxone reversed these effects in another 15 minutes. Mean changes in temperature from baseline 15 minutes after the naloxone injections were $0.03° \pm 0.08°C$ for the rats that received 2.5 mg of PCP per kilogram and $-0.15° \pm 0.13°C$ for the rats that received 20.0 mg of PCP per kilogram.

In previous investigations interactions of naloxone with PCP have been absent or negligible (*1*, *6*). The present data, in contrast, indicate that PCP's effects on at least one physiological system are sensitive to naloxone. The gradual waning of the naloxone antagonism after 15 minutes and the reversal of PCP's effects make it very unlikely that the present interaction is in any way attributable to alterations in PCP's pharmacokinetics. It is of interest that morphine also elicits dose-dependent hyperthermia and hypothermia in rats (*7*) and that, as with PCP, both effects can be antagonized by naloxone. Indeed, the sensitivity of PCP to antagonism by naloxone appears similar to the reported sensitivity of morphine to naloxone antagonism (*8*). Antagonism at sigma receptors requires higher doses of naloxone than antagonism at mu receptors (*2*), and naloxone does not appear to antagonize temperature responses elicited by *N*-allylnormetazocine (*9*). Thus, it is likely that PCP and naloxone interact at mu opiate receptors in brain areas controlling thermoregulatory processes.

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Characterization of Estrogen-Concentrating Hypothalamic Neurons by Their Axonal Projections

Abstract. *A method combining steroid autoradiography and fluorescent dye retrograde neuroanatomical tracing has been devised. This method makes it possible to demonstrate that some estrogen-concentrating cells in the ventrolateral subdivision of the ventromedial nucleus of the rat hypothalamus are neurons that send axons to the dorsal midbrain. Other cells only concentrate estrogen or only project to the midbrain. Estrogen-concentrating neurons in the ventromedial hypothalamus that project to the dorsal midbrain are likely to transmit hormone-influenced signals that regulate circuits for reproductive or other behaviors or autonomic functions.*

Gonadal steroid hormones exert powerful effects on the brain, regulating sexual behaviors and gonadotropin release, probably via cells binding gonadal steroid hormones. Autoradiography has made it possible to demonstrate these hormones in the hypothalamus and limbic system of all the vertebrates examined (*1*) including the rat (*2*).

Large numbers of estradiol-concentrating cells have been demonstrated in the ventrolateral subdivision of the ventromedial nucleus of the rat through the use of steroid autoradiographic methods (*2*). The horseradish peroxidase retrograde tracing method revealed that large numbers of neurons in the ventromedial nucleus, particularly in the anterior and ventrolateral subdivisions, sent their axonal projections to the dorsal midbrain (*3*). Cellular estradiol concentration in an area known to project to the dorsal midbrain prompted us to ask whether any estradiol-concentrating cells in the ventromedial nucleus are neurons that project to the midbrain.

Primuline, true blue, and granular blue are fluorescent dyes which are transported retrogradely through axons to the neuronal soma (*4*). Arnold (*5*) has studied the efferent projections of certain dihydrotestosterone concentrating neurons in the bird telencephalon by a combined method with primuline in unfixed tissue. We have used these retrogradely transported fluorescent dyes combined with steroid autoradiographic procedures to demonstrate the efferent projections of estradiol-concentrating neurons in the ventromedial nucleus of the rat.

Seven ovariectomized 2-month-old Long-Evans (Charles River Laboratories) female rats (body weight, 150 to 200 g) received dorsal midbrain injections of the fluorescent dye primuline (ICN Pharmaceuticals) (*6*). After allow-

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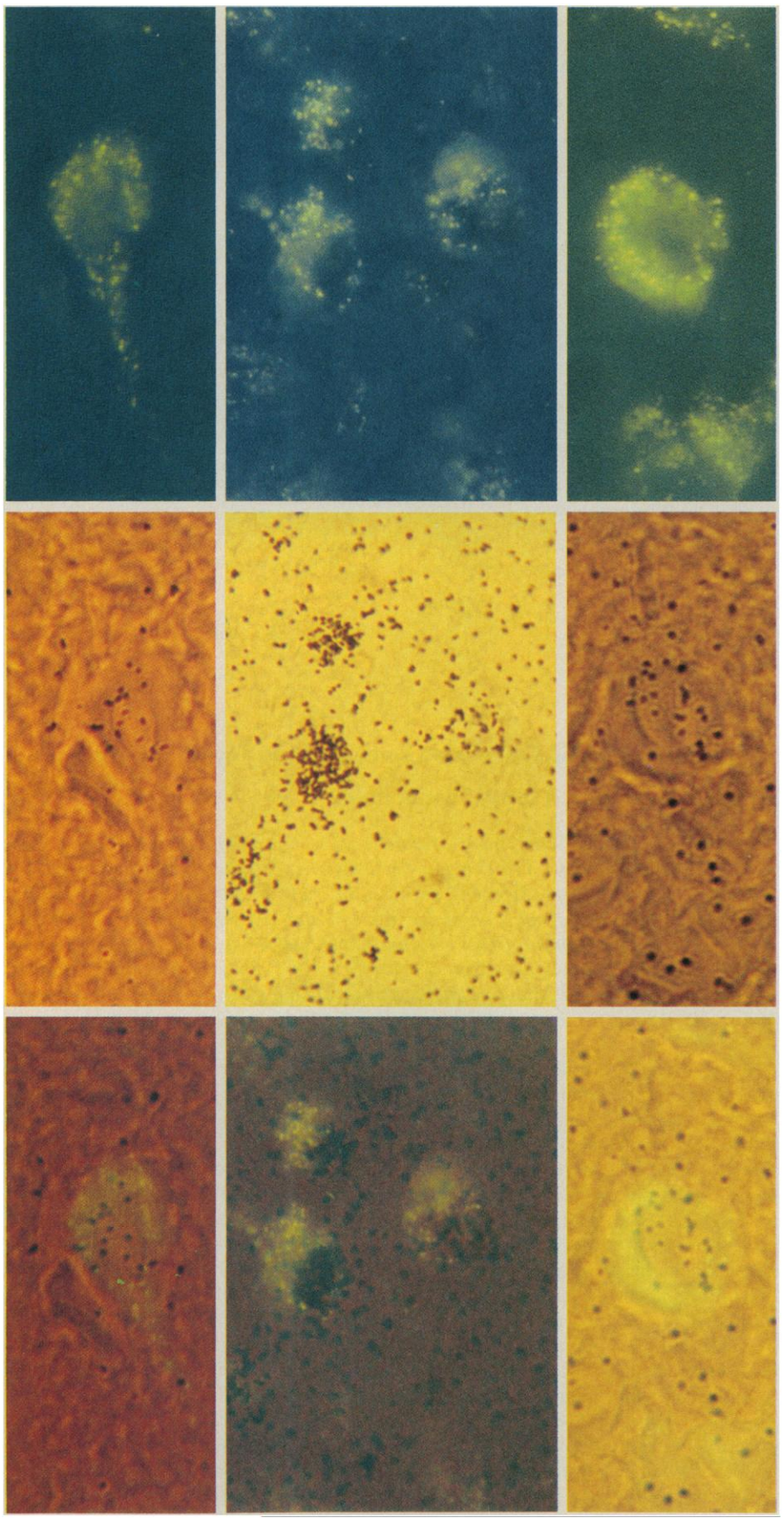

ing 2 days for retrograde transport, the animals were given an intraperitoneal injection of a nuclear saturating dose of 2,4,6,7-[^{3}H]estradiol (New England Nuclear; specific activity, 90 to 111 Ci/mmole), that is, 0.8 μg [^{3}H]estradiol per 250 g. The animals were killed 2 hours after the isotope was injected, when the specific nuclear binding was high (*7*) and the nonspecific low-affinity binding was reduced. The animals were perfused (*8*) and the brains were quickly removed from the skull, blocked, and frozen onto cryostat chucks with liquid nitrogen. Sections (6, 12, or 24 μm) were cut with a cryostat (−20°C) and picked up under safelight conditions on dry emulsion-coated (Kodak NTB-3) slides. The autoradiograms were stored in the dark in dessication boxes at 4°C for 2 to 12 months. After exposure, the autoradiograms were developed, fixed, dehydrated, and covered with Entellan (Merck) (*2, 9*). In addition to the autoradiographic controls for negative and positive chemography, two brains not injected with primuline were prepared as controls for autofluorescence. Neither chemography nor autofluorescence complicated the results. The autoradiograms were examined through a Zeiss microscope with standard bright-field light for the presence of silver grains, and with ultraviolet light (Zeiss UGl filter, 360 nm, epifluorescent system) for the fluorescence of the primuline (*10*).

Five additional animals were prepared with the fluorescent dyes true blue or granular blue (Makromolikulare Chemie) (*11*). All other aspects of the combined procedure were as described.

Criterion for an estradiol-labeled cell was the presence of silver grains over the nucleus equal to or in excess of five times the number of silver grains over an adjacent cell-sized area of neuropil. This criterion has been usual for our previous experiments (*9*). Criterion for retrogradely primuline-labeled neurons was a neuron in which the brilliant white-yellow granules, even-sized and nearly

Fig. 1. Photomicrographs of estradiol-concentrating primuline-labeled neurons in the ventrolateral subdivision of the ventromedial nucleus of the hypothalamus. All photomicrographs were taken with a ×100 oil objective. (Top row) Seven primuline-labeled neurons. White-yellow primuline granules in the cytoplasm, surrounding the nucleus and extending into a process of one cell. The neuronal nuclei are clear of primuline granules. These photomicrographs were taken with UV light (360-nm Zeiss UGl filter) to excite the primuline to white-yellow fluorescence. (Middle row) The same fields as in the top row, showing accumulations of silver grains representing [^{3}H]estradiol concentrated in the nuclei of five cells. Since no counterstain was used, the cells below the grains can be seen only faintly (the photomicrographs were taken with a considerable depth of field). These photomicrographs were taken with standard light. (Bottom row) The same fields were taken with both UV and standard light to show the white-yellow granules of primuline in the cytoplasm and silver grains over the nuclei of five neurons.

spherical, were found throughout the somal cytoplasm, frequently extending into a process of the neuron. The nucleus was clear of these granules. Criterion for a neuron labeled with true blue or granular blue was a brilliant blue cloud of dye over the cell's soma, under which were seen white granules in the cytoplasm, surrounding the clear nucleus, and often extending into a process of the cell.

By this combined method, some cells in the ventrolateral subdivision of the ventromedial nucleus (VL-VM) that concentrate estradiol were discovered to be neurons that projected to the dorsal midbrain. These had silver grains located over the nucleus and primuline granules in the somal cytoplasm surrounding the nucleus (Fig. 1). The granules sometimes extended into a process of the neuron. Estradiol-concentrating neurons that projected to the dorsal midbrain could also be demonstrated with granular blue or true blue used as the retrograde marker.

Quantitative analysis of estradiol-concentrating cells (N = 3735) in the VL-VM showed that 26 to 36 percent were neurons that projected to the dorsal midbrain (Fig. 2) (*12*).

When the hypothalamic arcuate nucleus was charted, many cells that concentrated estradiol were found, but few projected to the midbrain. In the arcuate, of estradiol-concentrating cells (N = 2952) counted, only 1 to 2 percent were also retrogradely labeled.

In addition, as expected, large numbers of other estradiol-concentrating cells were found in the VM, the region ventral and lateral to it, and in the arcuate nucleus. The pattern and number of these estradiol-concentrating cells are identical to those previously reported in unfixed tissue (*2*). Moreover, many other neurons that projected to the dorsal midbrain were found in the VM, in the adjacent lateral hypothalamus, and in the zona incerta, particularly its rostromedial extent. The pattern of retrogradely labeled neurons seen after the combined method with primuline, true blue, or granular blue as the marker was virtually identical to that previously reported with horseradish peroxidase as the marker (*3*).

These experiments show that some estradiol-concentrating cells in the VL-VM are neurons that send axonal projections to the dorsal midbrain. Fewer than half of those cells also project to the dorsal midbrain. This demonstrates an anatomical heterogeneity even among estradiol-concentrating cells in a small hypothalamic region. Conversely, only a small percentage of all the neurons in the VL-VM that project to the dorsal midbrain concentrate estradiol, suggesting a functional heterogeneity even among cell bodies located in this small region and having common axonal projections. The particular anatomical and chemical roles filled by individual neurons emphasize the need for cytochemical techniques, as we have used, with high spatial resolution.

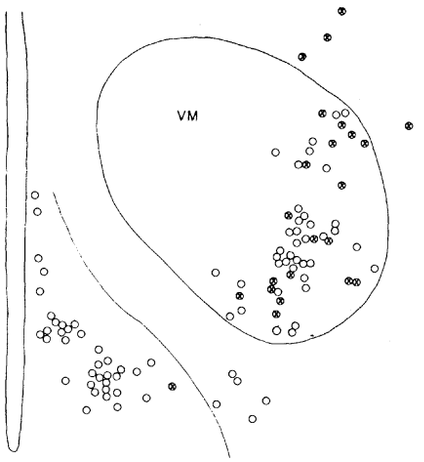

Fig. 2. Diagrammatic representation of one transverse section of one half of the medial basal hypothalamus, showing the third ventricle, arcuate nucleus, and ventromedial nucleus (*VM*), after use of the combined steroid autoradiography–retrograde tracing method. Dorsal is toward the top. All symbols: ○, estradiol-concentrating cells; ⊗, cells that also have primuline in their cytoplasm.

Estradiol-concentrating VM neurons that project to the dorsal midbrain can readily be hypothesized to be involved in the control of estradiol-dependent female sexual behaviors (*13*). The VM is the most sensitive site at which implantation of extremely small amounts of estradiol consistently leads to lordosis (*14*). Lesions there interfere with the induction of lordosis by estrogen (*15*), while electrical stimulation of VM can facilitate this behavior (*16*). Cutting connections between VM and the dorsal midbrain can reduce or eliminate lordosis (*17*).

The dorsal midbrain, specifically the central gray (CG), has a well-documented role in the neural mechanism underlying sexual behavior in the female rats (*13*). Lordosis can be facilitated by stimulating the CG and abolished by lesions placed in the CG (*18*). The CG contains units which respond to somatosensory information relevant for lordosis (*19*), and the CG is the target for the axons of many hypothalamic neurons, some of them estradiol-concentrating. This region probably serves a role in the integration of these types of information, which is then transmitted via axons of CG neurons to brainstem and spinal cord structures that control spinal motor neurons that produce the lordosis reflex (*13*).

Hormone-concentrating neurons in the VM could also play a role in other phenomena such as aggressive behaviors and control of autonomic functions. For analyzing such functions combinations of cytologic techniques will be useful for determining the connections between neurons influenced by steroid hormones and the rest of the central nevous system.

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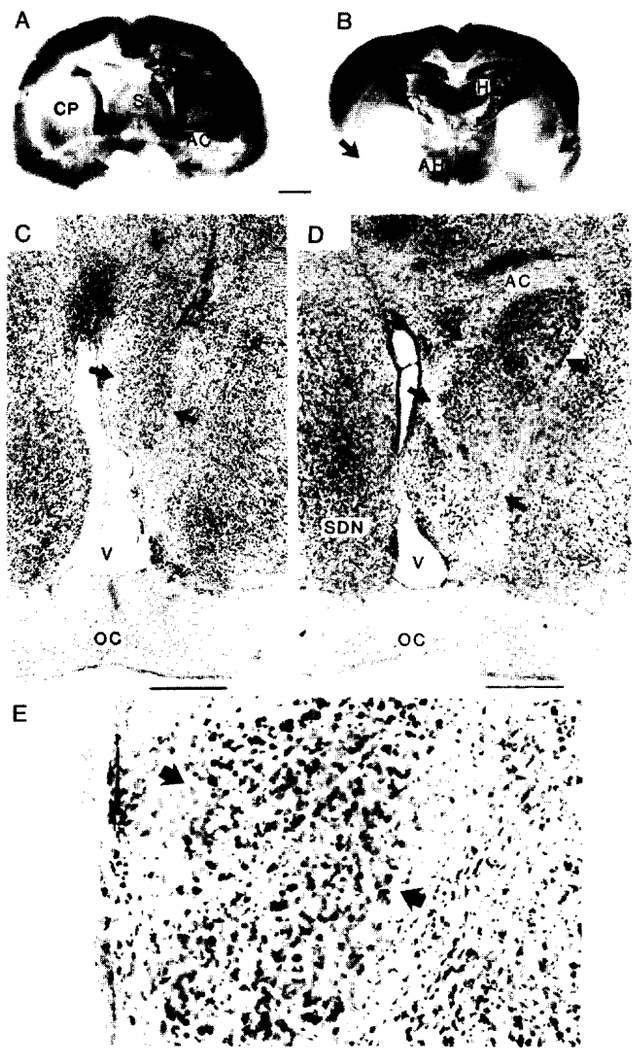
Enhancement of Sexual Behavior in Female Rats by Neonatal Transplantation of Brain Tissue from Males

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



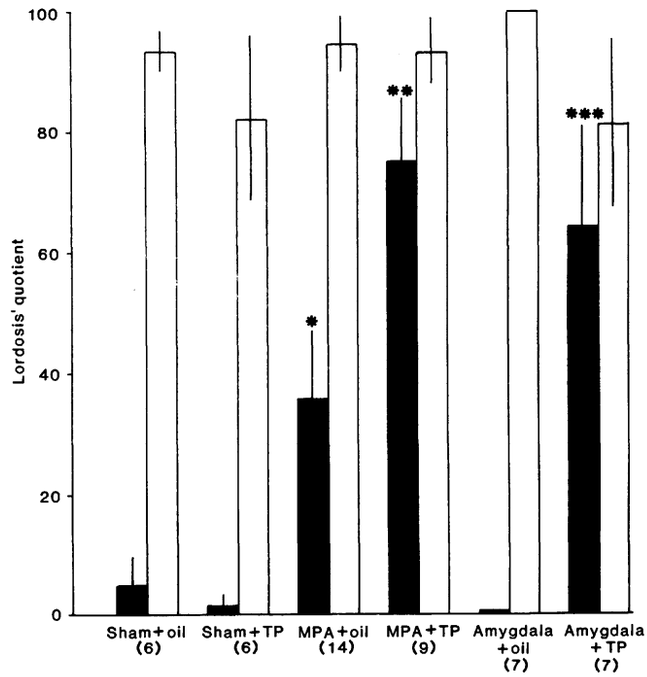


Fig. 1 (left). (A and B) Representative coronal slices from neonatal male donors of MPA (A) or anterior amygdala (B) tissue. Arrows indicate brain areas that were punched out bilaterally and transplanted into neonatal female recipients. Scale bar, 1 mm. (C and D) Photomicrographs of representative sections 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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