

# Sex Differences in Androgen Receptors of the Human Mamillary Bodies Are Related to Endocrine Status Rather Than to Sexual Orientation or Transsexuality

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## ABSTRACT

In a previous study we found androgen receptor (AR) sex differences in several regions throughout the human hypothalamus. Generally, men had stronger nuclear AR immunoreactivity (AR-ir) than women. The strongest nuclear labeling was found in the caudal hypothalamus in the mamillary body complex (MBC), which is known to be involved in aspects of cognition and sexual behavior. The present study was carried out to investigate whether the sex difference in AR-ir of the MBC is related to sexual orientation or gender identity (*i.e.* the feeling of being male or female) or to circulating levels of androgens, as nuclear AR-ir is known to be up-regulated by androgens. Therefore, we studied the MBC in postmortem brain material from the following groups: young heterosexual men, young homosexual men, aged heterosexual castrated and noncastrated men, castrated and noncastrated transsexuals, young heterosexual women, and a young virilized woman. Nuclear AR-ir did not differ significantly between heterosexual and homosexual men, but was significantly

stronger than that in women. A female-like pattern of AR-ir (*i.e.* no to weak nuclear staining) was observed in 26- to 53-yr-old castrated male-to-female transsexuals and in old castrated and noncastrated men, 67–87 yr of age. In analogy with animal studies showing strong activational effects of androgens on nuclear AR-ir, the present data suggest that nuclear AR-ir in the human MBC is dependent on the presence or absence of circulating levels of androgen. The group data were, moreover, supported by the fact that a male-like AR-ir (*i.e.* intense nuclear AR-ir) was found in a 36-yr-old bisexual noncastrated male-to-female transsexual and in a heterosexual virilized woman, 46 yr of age, with high levels of circulating testosterone. In conclusion, the sexually dimorphic AR-ir in the MBC seemed to be clearly related to circulating levels of androgens and not to sexual orientation or gender identity. The functional implications of these alterations are discussed in relation to reproduction, cognition, and neuroprotection. (*J Clin Endocrinol Metab* 86: 818–827, 2001)

**I**N ANALOGY WITH the nonhuman vertebrate brain (1, 2), it is thought that in the human also the interaction between sex hormones and their receptors may play an important role in brain development (organizing effects) and may in adulthood alter brain function (activating effects), and that these two mechanisms lead to sex differences in behavior in adult life. Structural and functional sex differences in the brain may be related to reproduction, sexual orientation, gender identity (*i.e.* the feeling of being male or female), cognition, and disease (3, 4). In a number of areas of the human hypothalamus, structural and functional differences between the sexes and between homosexual and heterosexual men have been described (5–7). In addition, our group has found that the central part of the bed nucleus of the stria terminalis (BSTc) is sexually dimorphic, *i.e.* smaller in women, with a female volume and neuron number in male-to-female transsexuals (4, 8).

It has been shown that various areas of the preoptic area (POA) (9, 10), BST (11) and suprachiasmatic nucleus (12, 13) are larger in men than in women, whereas the opposite was found for the anterior commissure (14). Moreover, hypothalamic differences in relation to sexual orientation have been observed. The suprachiasmatic nucleus (15) and the anterior commissure (16) are larger in homosexual than in heterosexual men, whereas the interstitial nucleus of the anterior hypothalamus-3 is smaller in homosexual than in heterosexual subjects (17). These data together with the abundant information showing that sexual orientation and gender identity do not vary with adult endocrine changes (18, 19) suggest that any possible clue to understanding the biological basis of sex differences, sexual orientation, or gender identity will require careful analysis of a large number of brain areas.

Recently we found that in a number of hypothalamic areas men showed stronger androgen receptor (AR) immunoreactivity (AR-ir) than women. Interestingly, in the anterior hypothalamus only moderate sex differences were found, whereas a conspicuous sex difference occurred in the posterior hypothalamus, *i.e.* the medial mamillary nucleus (MMN) and lateromamillary nucleus (LMN) of the mamillary body (MB) complex (MBC) (20). Such sexual dimorphisms may be related to gender differences in certain aspects of reproduction or sexual behavior, as various studies

Received February 8, 2000. Revision received August 29, 2000. Revision received October 9, 2000. Accepted October 16, 2000.

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\* Sabbatical stay in The Netherlands partially supported by a grant from CONACyT (Grant 971017).

† Recipient of a grant from the NWO (Grant 903-47004).

showed that lesions in the rat MB produced a complete loss of sexual activity (21). Moreover, electrical stimulation of this area in squirrel monkeys induces penile erection (22, 23). In addition, Lisk (24) reported in 1967 that implanting testosterone into the MBC of male castrated rats restored sexual excitability in the presence of receptive females (see also Refs. 25 and 26). These data suggest that this area participates in the control of male sexual motivation. In contrast, in the female rat, Galindo-Estaun (27) showed that lesioning the MB did not alter the estrous cycle or alter sexual or maternal behavior.

The present study was carried out to investigate whether the sex difference in nuclear AR-ir of the human MBC is related to sexual orientation, gender identity, or endocrine status. In many species, castration strongly reduces or even eliminates nuclear AR-ir, whereas testosterone, but not estrogen, injection restores such strong nuclear AR-ir (28–30). As nuclear AR-ir is up-regulated by androgens (28, 31, 32), we would expect decreased AR-ir in castrated/aged men. Therefore, we studied AR-ir in the MBC in groups of subjects with different testosterone levels (33, 34), *i.e.* young heterosexual men/young homosexual men, young heterosexual women, aged heterosexual castrated and noncastrated men, and castrated and noncastrated transsexuals.

## Subjects and Methods

### Subjects

In the present study we included the area of the MBC from the posterior hypothalamus of the following 47 patients: 1) young heterosexual men ( $n = 9$ ), 2) young homosexual men ( $n = 10$ ), 3) old heterosexual castrated men ( $n = 5$ ), 4) castrated male-to-female transsexuals ( $n = 6$ ), 5) old heterosexual intact men ( $n = 5$ ), 6) young heterosexual women ( $n = 8$ ), 7) a 36-yr-old noncastrated male-to-female transsexual, 8) a nontreated 84-yr-old male subject with strong cross-gender identity feelings, 9) a 51-yr-old female-to-male transsexual, and 10) a 46-yr-old woman with high levels of androgens.

Brains were obtained by autopsy (for clinicopathological information and ages, see Table 3). Unless stated otherwise, patients had no primary neurological or psychiatric diseases. The sexual orientation of the subjects was presumed to be heterosexual (15) unless stated otherwise, whereas the sexual orientation of the homosexual group was documented in the clinical records (15). All homosexual patients died of acquired immunodeficiency syndrome or related diseases. The patient data have previously been reported (15). General pathology and neuropathology were performed either at the Free University of Amsterdam (Dr. W. Kamphorst, Prof. F. C. Stam or Prof. P. van der Valk) or at the Academic Medical Center of the University of Amsterdam (Dr. D. Troost). The subjects had no primary endocrine illnesses, except for those who had undergone orchidectomy, had been given hormonal treatment, or had had abnormal hormone fluctuations that are mentioned in Table 2. The pathologically high levels of androgens in a 46-yr-old woman, [androstenedione, 48.0 ng/mL (normal values for women, 0.4–3.5 ng/mL); testosterone, 26.82 nmol/L (normal values for women, 1.04–3.30 nmol/L)] were due to an adrenal cortex carcinoma.

### Histology and immunohistochemistry

After autopsy, the hypothalamus was fixed for about 1 month in 4% formaldehyde at room temperature, dehydrated, and embedded in paraffin. Serial 6- $\mu$ m frontal sections were cut on a Leitz microtome (Rockleigh, NJ).

The immunohistochemical protocol followed for the AR staining has been previously described in detail (20). Briefly, this protocol consisted of mounting paraffin-embedded sections of the posterior hypothalamus onto SuperFrost Plus (Menzel, Darmstadt, Germany) slides. The sections were deparaffinized and rehydrated in a series of ethanol concentrations. To retrieve antigenicity, sections were microwaved (10 min at 700 watts) in 0.1

mol/L citric acid monohydrate buffer (pH 6.0) (35, 36), after which they were rinsed with TBS buffer (0.05 mol/L Tris-0.9% NaCl, pH 7.6). To decrease background, the slides were preincubated for 1 h with TBS-milk [5% milk-TBS solution with commercially available powdered milk (ELK, Campina Melkunie, Eindhoven, The Netherlands)] before incubation with the primary antibody PG21 (donated by Drs. Gail Prins and Geoffrey Greene; 1:1000) for 1 h at room temperature and subsequently kept overnight at 4 C. After rinsing in TBS-milk buffer, sections were incubated for 1 h with a goat antirabbit biotinylated second antibody (1:200), followed by another hour of incubation in the avidin-biotin complex (1:800). The subsequent signal amplification method consisted of an incubation in biotinylated tyramine (1:1000) and 0.01% peroxide (Merck & Co., Darmstadt, Germany) for 20 min (37). Thereafter, sections were rinsed with TBS, and the avidin-biotin complex procedure was repeated. After rinsing in 0.05 mol/L Tris-HCl (pH 7.6), slides were developed by incubation for 10 min in 0.05 mol/L Tris-HCl containing 0.05% 3,3'-diaminobenzidine (Sigma, St. Louis, MO), 0.01% hydrogen peroxide, and 0.3% nickel ammonium sulfate. Developed sections were dehydrated in alcohol, cleared with xylene, and coverslipped with Entellan (Merck & Co.).

### Analysis of AR staining intensity

The sections were rated for staining intensity by three independent investigators blind to the details of the patients. The few differences in rating were concurred by settlement (20). The category assigned to the MMN and LMN corresponded to the predominant cell type within that area according to the following scale: 0 = no staining, 1 = staining diffuse and transparent, and 2 = intense staining with individual granules of the reaction product distinguishable. The staining range was established for both the cytoplasm and the nucleus. The estimates were made at three different microscopic magnifications:  $\times 2.5$ ,  $\times 10$ , and  $\times 40$  objectives (20). The identification of MMN and LMN was made with the aid of maps of coronal sections of the human brain published by Mai *et al.* (38) and using alternating thionine-stained sections for orientation.

### Statistics

The assigned categories of AR-ir in the MMN and LMN were compared using the Kruskal-Wallis ANOVA, followed by the Mann-Whitney U test. The fixation time and postmortem delay were analyzed by the Kruskal-Wallis test. Differences were considered statistically significant at  $P < 0.05$  (two-tailed).

## Results

### Staining specificity

Negative controls, *i.e.* without the first antibody, and positive control sections, *i.e.* tissue of mouse testes and human anterior

**TABLE 1.** Androgen receptor intensity staining in the medial mamillary nucleus (MMN) and the lateromamillary nucleus (LMN) of the mamillary body of heterosexual young men, heterosexual young women, homosexual young men, and castrated transsexual men

	N	LMN		MMN	
		c	n	c	n
Heterosexual men	9	1	2	1	2
Heterosexual women	8	1	1	1	0
Homosexual men	10	1	2	1	1
Transsexual men	6	1	0	1	0

The data of these 33 subjects that were used for the statistical analysis (see *Results* and Table 3) are summarized here with the median values for nuclear and cytoplasmic AR-ir. The table shows median values. The AR-ir intensity was assigned according to the following scale: 0 = no staining; 1 = staining diffuse and transparent, and 2 = intense staining with individual granules of the reaction product still distinguishable. c, Cytoplasmic; n, nuclear. The Kruskal-Wallis and Mann-Whitney U tests for nuclear staining indicated statistically significant differences among the groups (see *Results*). The cytoplasmic staining in both brain areas studied was relatively weak for the four groups and did not differ statistically.

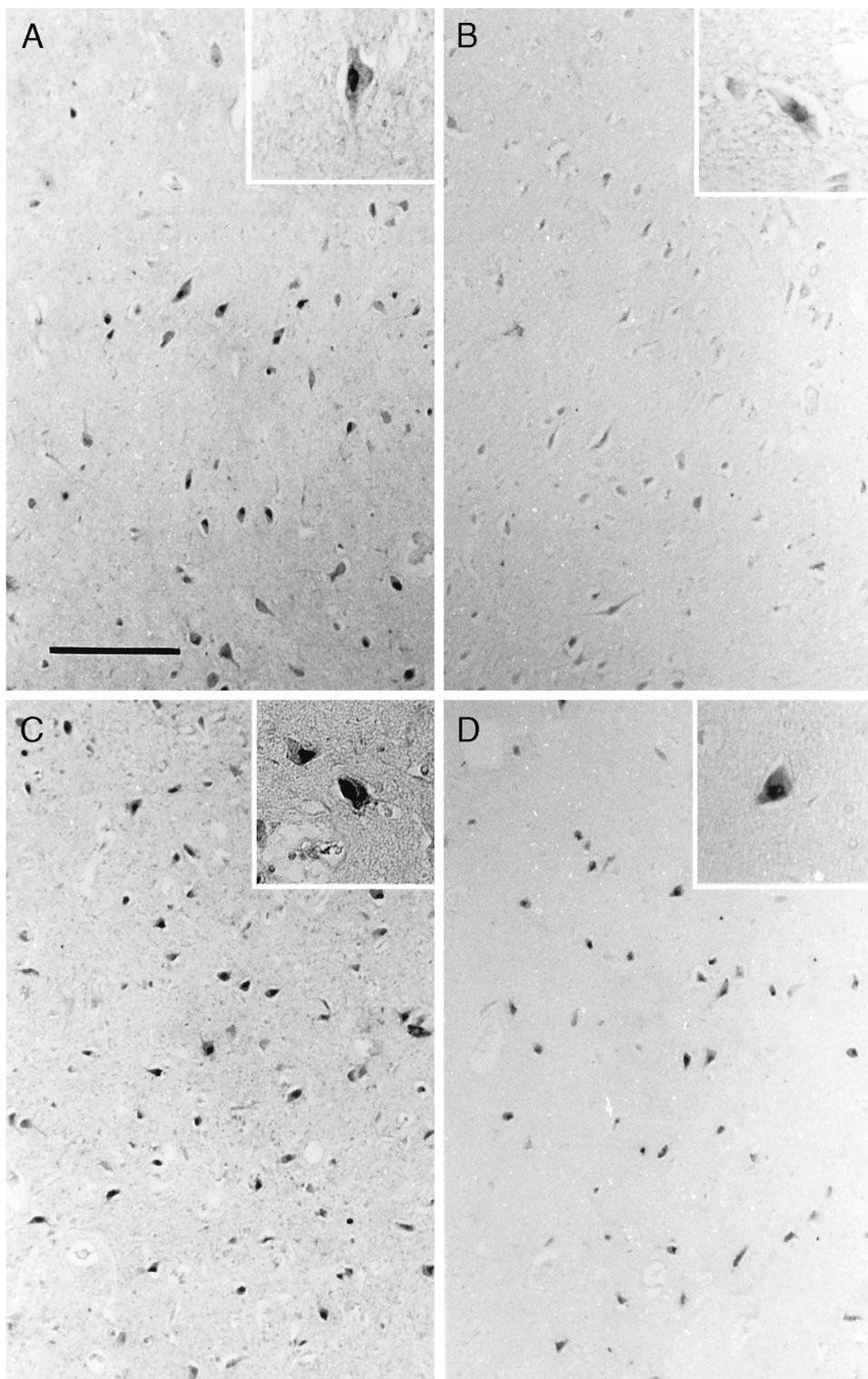


FIG. 1. Photomicrographs showing AR-ir in neurons of the MMN of the mamillary body of a heterosexual man (A), a heterosexual woman (B), a homosexual man (C), and a woman with high levels of androgens (D). Note that in the mamillary body there is a clear sex difference in AR-ir (see A and B), whereas there is no difference in the intensity of AR staining between the representative heterosexual man (A), the homosexual man (C), and the virilized (androgenized) woman (D). Scale bar, 150  $\mu$ m.

pituitary, were run parallel with the hypothalamic sections. Literature data have shown the specificity of the anti-AR antibody for brain immunohistochemical studies (29–31, 39, 40). The specificity data we have added (20) are briefly described below (data not shown). Omitting the AR antibody PG21 totally prevented staining. Paraffin-embedded sections of formalin-fixed mouse and human testes showed clear AR staining in the

peritubular cells as previously reported (41, 42). In the human anterior pituitary isolated groups of cells with moderate to strong AR-ir were observed, as has also been reported in the Brazilian opossum (43) and rat (42). An adsorption test, including an immunoblotting analysis for PG21 with a peptide that consisted of the first 20 amino acids of the peptide that is recognized by PG21, showed the expected concentration gra-

dient on nitrocellulose paper [the technique has been described by Van der Sluis *et al.* (44)]. After adsorption of the PG21 antibody with its corresponding peptide, nuclear and cytoplasmic stainings were completely eliminated (20).

#### Staining pattern

ANOVAs for fixation time and postmortem delay among the heterosexual men, heterosexual women, homosexual men, and castrated transsexual men did not exhibit statistically significant differences ( $P > 0.1$  and  $P > 0.9$ , respectively).

The immunohistochemical staining for AR in the MMN and LMN revealed cells with nuclear or cytoplasmic labeling or with both types of staining (Tables 1 and 3 and Figs. 1-3).

The heterosexual men showed strong nuclear AR-ir in both brain regions (Tables 1 and 3 and Fig. 1A). In contrast, the women revealed much less intense labeling in the nucleus of neurons of the LMN and MMN (Tables 1 and 3 and Fig. 2B). This sex difference was statistically significant for nuclear staining in both areas ( $P < 0.05$ ). The homosexual men showed a similar staining to that of the heterosexual men for both areas ( $P > 0.2$ ) with a more moderate staining in the MMN (Tables 1 and 3 and Fig. 1C). Women differed significantly from homosexual men in nuclear AR-ir in both the MMN and LMN ( $P < 0.05$ ). The castrated male-to-female transsexual group had a lack of nuclear staining in both brain areas, but had cytoplasmic labeling in the LMN and MNN (Tables 1 and 3 and Fig. 2B). This group was statistically different in the LMN from the heterosexual and homosexual men group ( $P < 0.05$ ) and similar to that in women ( $P > 0.5$ ). The castrated male-to-female transsexual group had significantly less nuclear AR-ir in the MMN than the heterosexual male group ( $P < 0.05$ ). This difference showed only a trend when compared with homosexual men ( $P = 0.10$ ). When

compared with women, the castrated male-to-female transsexual group did clearly not differ from women in the MMN ( $P > 0.7$ ).

In the 36-yr-old noncastrated male-to-female transsexual, strong nuclear and cytoplasmic staining was observed in both areas (Fig. 2A). Similarly, the 46-yr-old woman with high levels of androgens revealed strong nuclear labeling and weak to intermediate cytoplasmic labeling in the LMN and MMN (Fig. 1D). The female-to-male transsexual who did not receive androgen replacement therapy during the last 3 yr before death (Table 2) showed less intense nuclear and weak to intermediate cytoplasmic staining in both the LMN and MMN.

The results of staining in the posterior hypothalamus of old patients are illustrated in Fig. 3 (A and B). In old castrated heterosexual men almost no nuclear and weak cytoplasmic AR-ir were found (Table 3 and Fig. 3B). Thus, in this group of five, two subjects had very weak nuclear and cytoplasmic AR-ir in both areas, whereas three of five had no nuclear but weak cytoplasmic AR-ir (Table 3 and Fig. 3B). A similar trend of weak AR staining with more, but less intense, nuclear AR-ir was observed in five old intact men (Table 3 and Fig. 3A). In this group four of five of the individuals had very weak nuclear as well as cytoplasmic AR-ir (Table 3). Between the castrated old men and the intact old men no statistical significant differences were found.

#### Discussion

The present study confirms the clear sex differences in nuclear AR-ir expression in neurons of the MBC (20) and shows, for the first time, that this sex difference is related to circulating levels of testosterone rather than to sexual orientation or gender identity.

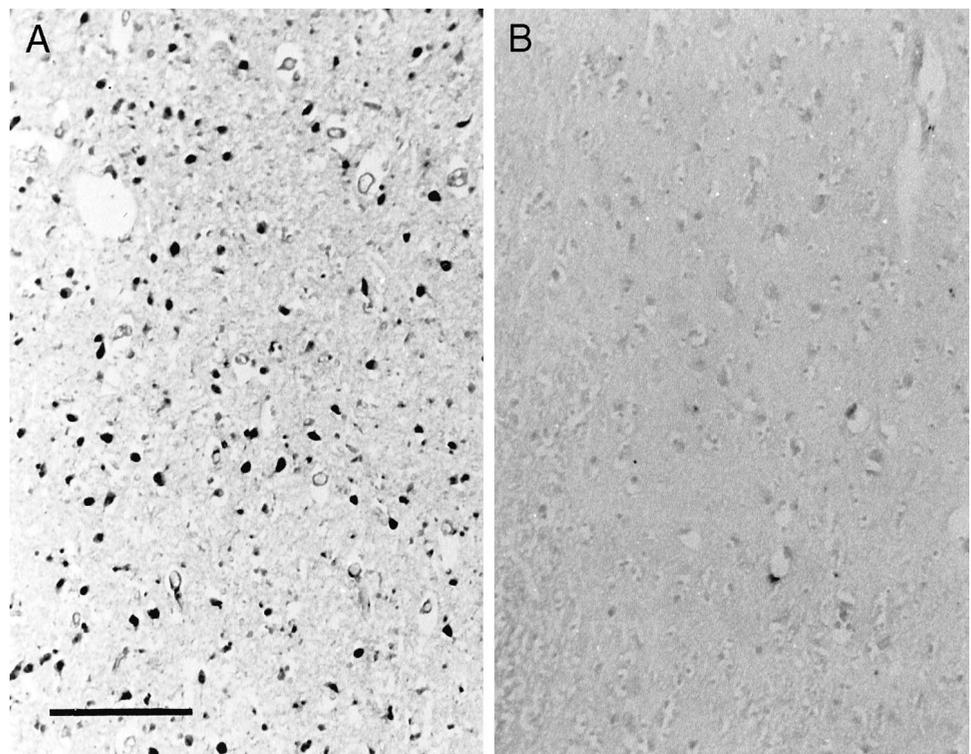


FIG. 2. Illustration of the staining intensity of nuclear AR-ir in neurons of a noncastrated 36-yr-old male-to-female transsexual (A) compared with the lack of such staining in a 26-yr-old castrated male-to-female transsexual. Scale bar, 150  $\mu\text{m}$ .

**TABLE 2.** Patient data

Male to female transsexuals (n = 7 with 6 orchiectomized subjects)			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
Transsexual (84020)	50	42	44
Hormone treatment Age 42: stilbestrol (5 mg 1 dd); after 2 months to 5 mg 2 dd; age 44: CPA (50 mg 2 dd; treatment lasted 4 yr; stopped 2 yr before death); ethinyloestradiol (50 µg 2 dd; treatment lasted 8 yr until death)			
Cause of death: suicide			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
Transsexual (88064)	43	36	39
Hormone treatment Age 36: received standard CPA treatment (50 mg 2 dd) until 2 yr before death; at age 39 received standard ethinyl estradiol treatment (50 µg 2 dd) that stopped 3 months before death			
Cause of death: sarcoma, right side temporal			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
Transsexual (93042)	36	NA	no orchiectomy, testes atrophy
Hormone treatment CPA (50 mg 1 dd) at least the last 10 months before death; the patient did receive estradiol in combination with hydroxyprogesterone in therapeutic dosages. Exact period of treatment is not known but based on the significant testes atrophy she was probably treated for about 5 yr or more.			
Cause of death: AIDS, pneumonia, pericarditis, cytomegaly in brain			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
Transsexual (93070)	53	40	50
Hormone treatment Age 40: stilbestrol treatment (stopped after 1 yr); at age 43–47: premarin (0.625 mg dd); at age 47–50: Premarin (3.75 mg dd); at age 50–53: Premarin (2.5 mg 3 dd); CPA (50 mg 1 dd); topical estrogen cream (estrogen treatment stopped 3 months before death)			
Cause of death: acute fatty liver due to alcohol abuse			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
Transsexual (95018)	48	35	36
Hormone treatment Age 35: spironolactone (100 mg 2 dd); CPA (50 mg 2 dd); ethinyl estradiol (50 µg 2 dd); at age 36–40: CPA (50 mg 2 dd); ethinyl estradiol (50 µg 2 dd); at age 40–48: aldosterone (100 mg 1 dd); ethinyl estradiol (50 µg 1 dd; treatment lasted until death)			
Cause of death: Cardiovascular death			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
Transsexual (98334)	26	23	24
Hormone treatment Age 23: received standard CPA treatment (50 mg 2 dd) and ethinyl estradiol (50 µg 2 dd) treatment until death			
Cause of death: Suicide			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
Transsexual (98141)	74	64	64
Hormone treatment Age 64: received standard CPA treatment (50 mg 2 dd) and ethinyl estradiol (50 µg 2 dd) treatment; at age 67: received estraderm (100 mg 1 dd); at age 74 received spironolactone (50 mg 1 dd) and estraderm (100 mg 1 dd)			
Cause of death: coma after appendicitis, pneumonia, lung embolism, and occipital cerebral infarction			
Nontreated male with cross-gender identity feelings (n = 1)			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
(96088)	84	—	no orchiectomy or sex reassignment therapy
Hormone treatment Male patient with strong cross-gender identity feelings who did not receive sex hormone replacement therapy			
Cause of death: lung carcinoma			
Female to male transsexual (n = 1)			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-ovariectomy
(98138)	51	27	28
Hormone treatment At age 27 testosterone, Sustanon (250 mg), twice a month injections; at age 30 testosterone undecanoate (40 mg 3 dd); at age 34 testosterone undecanoate (40 mg 2 dd); at age 36 testosterone undecanoate (40 mg 4 dd); at age 44 testosterone, Sustanon (250 mg) twice a month injections; at age 47–48 testosterone, Sustanon (250 mg) every 3 weeks From age 48 until death (51) no testosterone replacement therapy			
Cause of death: cachexia			
Castrated males (n = 5)			
Patient no. (NBB)	Age (yr)		Age of hormonal treatment/-orchiectomy
(94090)	86	85	85
Hormone treatment Male patient with prostate cancer; orchiectomy 20 months before death; patient received an additional antiandrogen therapy, Androcur (50 mg 4 dd) during the first 14 months, 50 mg 2 dd during the last 6 months)			

TABLE 2. Patient data

Cause of death: septic shock with lung and prostate carcinoma			
Patient no. (NBB) (94109)	Age (yr) 82	—	Age of hormonal treatment/-orchiectomy 82
Hormone treatment Male patient with prostate cancer; orchiectomy 20 days before death; patient did not receive additional antiandrogen therapy			
Cause of death: respiratory insufficiency, prostate carcinoma, renal insufficiency			
Patient no. (NBB) (95062)	Age (yr) 80	—	Age of hormonal treatment/-orchiectomy 75
Hormone treatment Male patient with prostate cancer; orchiectomy 5 yr before death; patient did not receive additional antiandrogen therapy			
Cause of death: renal insufficiency with metabolic			
Patient no. (NBB) (97157)	Age (yr) 69	67	Age of hormonal treatment/-orchiectomy 67
Hormone treatment Male patient with prostate cancer; orchiectomy 3 yr before death; patient received anadron (150 mg 1 dd) during the last 3 yr before death.			
Cause of death: prostate cancer with metastases			
Patient no. (NBB) (89103)	Age (yr) 67	—	Age of hormonal treatment/-orchiectomy 67
Hormone treatment Male patient with prostate cancer; orchiectomy 3 months before death; patient did not receive additional antiandrogen therapy			
Cause of death: carcinoma of pancreas with multiple metastases; cachexia			
Virilized syndrome			
Patient no. (NBB) (83004)	Age (yr) 46	—	Age of hormonal treatment/-orchiectomy 44
Hormone treatment Female patient with a virilizing adrenocortical carcinoma for >1 yr that produced high levels of cortisol, androstendione, and testosterone levels; latest androstendione serum level before death was 48.0 ng/mL (normal range for women 0.4–3.5 ng/mL); the latest serum testosterone level before death, 26.82 nm/L (normal range for women is 1.04–3.30 nm/L).			
Cause of death: adrenocorticocarcinoma; postoperative hemorrhage			

NBB, patient number of The Netherlands Brain Bank; T, male-to-female transsexual; CPA, cyproterone acetate; NA, not available; AIDS, acquired immune deficiency syndrome.

Heterosexual and homosexual men had the most intense nuclear AR-ir in the LMN and MMN which were statistically not different from each other but showed significantly more AR-ir than women. A similar male-like staining intensity was also found in a 36-yr-old bisexual noncastrated male-to-female transsexual and in a 46-yr-old heterosexual virilized woman with high circulating levels of testosterone. In all cases studied, a close relationship was found between the endocrine status and the intensity of AR staining. High levels of testosterone went together with high nuclear AR-ir and low levels of testosterone with weak or no nuclear AR-ir. The fact that homosexual men showed more variability in their nuclear AR-ir profiles compared with heterosexual men, whereas in the MMN the AR-ir did not differ statistically from the transsexual group might be due to their acquired immunodeficiency syndrome status, as some of these patients have subnormal testosterone levels (45–47). The strong decrease in nuclear MBC AR-ir in five old male heterosexual intact subjects also fit the idea of an androgen-dependent nuclear expression of the AR, as decreased circulating levels of androgens occur with aging (48, 49). The weak AR-ir in an 84-yr-old man, who was gynecophilic and who had well documented strong cross-gender identity feelings but never received hormonal treatment or sex reassignment therapy, fits with his age. The fact that the 51-yr-old female-to-male transsexual who did not receive testosterone replacement

during the last 3 yr before death still had a female-like pattern of AR-ir is also fully in agreement with the assumption that circulating testosterone is crucial for nuclear AR-ir.

From our data there appeared no relationship between AR-ir and sexual orientation or gender identity. Regardless of sexual orientation or gender identity, a female pattern of AR-ir, *i.e.* low nuclear staining in the LMN and MMN, was observed in women, castrated male-to-female transsexuals, a female-to-male transsexual, and old men.

Animal studies show that castration induces a shift from strong nuclear to weak cytoplasmic AR-ir in hypothalamic neurons, which can be reversed by treatment with androgens (29, 30). Regarding this point it seems of particular interest to note that ongoing testosterone treatment in female-to-male transsexuals was accompanied by up-regulation of AR in peripheral ectocervix tissue, resulting in increased nuclear AR-ir (32). In addition to the specificity tests (Refs. 29, 36, 39, and 43 and our additional specificity data), the analogy between data on ARs in animals and humans under different levels of testosterone (33, 34) now also seems to provide biological evidence that in neurons of the human brain, PG21 indeed recognizes ARs. Our data from postmortem tissue are in agreement with the experimental data reported by Wood and Newman (29, 30), as we also show that gonadectomy in transsexuals (34) goes together with cytoplasmic AR-ir, in contrast with the mainly nuclear AR-ir pattern in the male

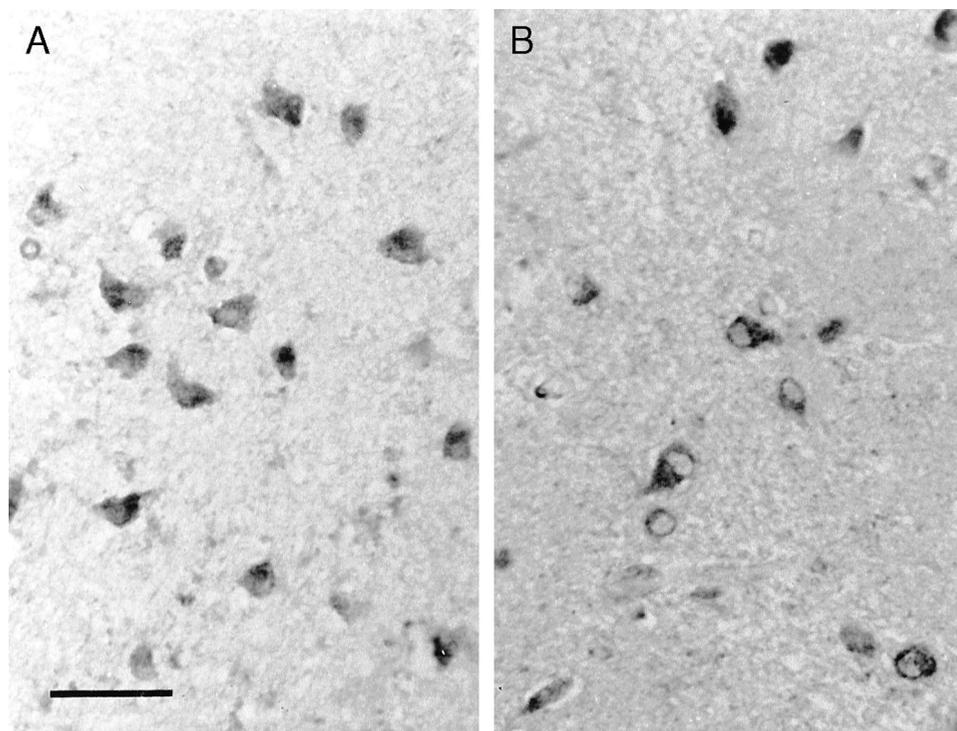


FIG. 3. Illustration of the almost complete lack of nuclear AR-ir in a noncastrated old man (A) and in a castrated old man (B). Scale bar, 50  $\mu$ m.

heterosexual and homosexual group, the noncastrated young transsexual subject, and the virilized woman who also had strong nuclear AR-ir. These observations support the concept that the AR occupied by androgens is mainly present in the nucleus, where it can alter gene expression and regulate cell activity, whereas the unoccupied AR is for the most part displaced to the cytoplasm (29, 30). The strikingly complete lack of nuclear AR-ir in the MBC of one male control patient (no. 97101) and in the MMN of two homosexual patients (no. 87084 and 88087) may be due to their antemortem status with a severely compromised immune system that may be accompanied by a strong down-regulation of testosterone levels (45–47). In aged males the decreased levels of testosterone (50) do not seem to have a strong effect on the AR in the MBC, as after castration no significant change in AR distribution was noticed.

#### *Hormones and their receptors in relation to sexual orientation and transsexuality*

The possible role of steroid hormones in the development of sexual orientation has been studied in various animal models (51) and in humans (52). The animal data show that steroid hormones during the neonatal period contribute to the organization of the brain and influence sexual preference (53). In contrast, the exposure to sex steroids during adulthood stimulates sexual behavior, but does not modify sexual orientation in animals or humans (18, 54). In the present study no statistical difference was found in AR-ir between heterosexual and homosexual men. The lack of differences in the AR-ir in the posterior hypothalamus and in the sequence variation in the AR gene (55) between homosexual and heterosexual men suggests that neither the intensity of AR-ir nor a variation in AR structure is related to the expression of

homosexuality. Although possible differences between homosexual and heterosexual men in the AR-ir in other brain areas have not yet been systematically studied, the data obtained to date reinforce the idea that homosexuality does not depend on differences in activational effects of testosterone in adulthood (18).

In the present study we found no clear relationship between MBC AR-ir and gender identity as we did, for instance, find for the size of the BSTc and gender identity (4, 8). Recent studies (56–59) and our observation that the volume and neuron number of the BSTc in male-to-female transsexuals in adulthood is independent of sex hormone levels (4, 8) support the idea that steroids do not act in adulthood but, rather, earlier during development to establish gender identity.

#### *Functional implications*

The results obtained in the present study fully agree with the idea that the AR-ir sex differences in the MBC of the posterior hypothalamus are due to differences in circulating levels of androgens and give additional support to the paradigm that endocrine features during adulthood do not contribute to sexual orientation or gender identity.

Anatomical and functional studies in rats have shown that the MMN and LMN are larger in males than in females (60), a sex difference that is accompanied by an increase in the rate of global protein synthesis (61). In addition, experimental data in animals show a sex-specific involvement of the MB in aspects of sexual behavior such as sexual motivation, penile erection, and sexual activity (21–24, 27). Also in humans, the MBC has been implicated in the regulation of reproduction, possibly by inhibiting the release of gonadotropins, as lesions in the posterior hypothalamus go together with precocious puberty (62). Our findings of gonadal hor-

**TABLE 3.** Brain material showing nuclear and cytoplasmic androgen receptor labeling (AR-ir) in the MBC

NBB	Sex	Age	Pmd	Fix	MMN		LMN		Cause of death and clinicopathological information
					N	C	N	C	
Young heterosexual men									
81-009	m	28	23:00	32	2	1	2	2	Medial cerebral artery aneurysm; lung emboli
82-020	m	27	41:00	40	2	1	2	1	Drug addiction; sepsis ( <i>S. aureus</i> ); cerebral edema
84-023	m	37	39	35	2	0	2	0	Bronchopneumonia
88-017	m	31	96:00	24	1	1	2	1	Heart failure due to coronary anomaly (birth defect)
88-035	m	23	65:00	39	1	1	2	2	Cardiac arrest
94-040	m	20	08:00	82	2	1	2	0	Heart failure and acute fibronous hemorrhagic pneumonia
97-075	m	33	18:45	—	1	1	2	1	Brain damage after motor accident
97-083	m	22	16:29	—	2	1	2	2	Hypertrophic cardiomyopathy
97-101	m	43	09:15	—	0	0	0	0	<i>Aspergillus</i> pneumonia
Mean ± SEM		29.3 2.7	35:10 10:19	42.0 9.13					
Young homosexual men									
86-038	m	37	5	—	2	1	2	1	AIDS
86-043	m	42	—	24	2	1	1	1	AIDS, disseminated Karposi sarcoma and generalized mycobacterium avium infections
86-046	m	32	49:00	11	1	1	1	1	AIDS, pneumocystic carinii pneumonia
87-030	m	41	—	40	1	2	2	2	Respiratory insufficiency
87-084	m	40	24:00	28	0	0	1	1	Cerebral (lymfome, fungal infection)
88-087	m	41	12:00	34	0	1	2	2	AIDS, bronchopneumonia, cytomegalic infections and toxoplasmosis
88-121	m	42	19:00	30	2	1	2	1	AIDS, cytomegalic meningoencephalitis
89-031	m	25	23:00	28	2	1	2	1	AIDS, pneumonia
89-084	m	39	—	—	1	1	1	2	AIDS, kaposisarcoma, suicide
89-109	m	32	49:00	11	1	1	2	2	AIDS, HIV encephalopathy
Mean ± SEM		37.1 1.8	25:51 6:59	25.75 3.88					
Young heterosexual women									
80-008	f	35	08:00	26	0	1	1	0	Acute lymphoblastic leukemia
84-002	f	36	86:00	51	0	0	0	0	Multiple fractures; rupture of thoratic aorta
84-026	f	33	41:00	20	0	1	0	1	Anoxia, status after resuscitation after progressive bronchial asthma
85-027	f	29	13:00	60	2	1	2	1	Hepatic coma
85-041	f	28	05:00	44	0	1	2	1	Cardiogenic shock
86-032	f	33	<41:00	20	0	0	0	0	Adenocarcinoma with metastases
92-037	f	32	30:00	45	0	0	0	0	Bronchopneumonia/bronchitis
96-410	f	38	53:00	—	1	0	1	1	Pneumonia, respiratory insufficiency
Mean ± SEM		33.0 1.3	34:38 10:14	38.0 6.52					
Transsexuals									
84-020	mtf	50	—	30	0	1	0	1	Suicide
88-064	mtf	43	—	—	1	2	1	2	Sarcoma, right side temporal
93-042	mtf	36	21:00	31	2	1	2	1	Pneumonia after CMV and pseudomonas aeruinososa infection
93-070	mtf	53	96:00	34	0	0	0	0	Acute fatty liver (due to alcohol abuse)
95-018	mtf	48	24:00	36	2	1	2	1	Cardiac arrest
98-137	mtf	26	—	40	0	0	0	0	Suicide: XTC overdose
98-138	ftm	51	04:15	32	1	2	1	2	Cachexia
98-141	mtf	74	06:35	33	0	1	0	1	Recent multiple cerebral infarction, cardiac failure, pneumonia
Mean ± SEM		47.6 5.3	30:22 18:51	33.7 1.4					
Old heterosexual, castrated men									
89-103	m	67	24:00	28	0/1	0/1	0/1	0/1	Carcinoma of pancreas with multiple metastases; cachexia
94-090	m	86	03:00	93	0/1	0/1	0/1	0/1	Septic shock with lung and prostate carcinoma
94-109	m	82	05:35	32	0	0/1	0	0/1	Respiratory insufficiency; prostate carcinoma; orchiectomy; renal insufficiency
95-062	m	80	04:30	24	0	0/1	0	0/1	Renal insufficiency with metabolic acidosis and hyperkalemia
97-157	m	69	05:55	45	0	0/1	0	0/1	Serious prostate cancer with metastasis
Mean ± SEM		76.8 4.2	8:02 4:31	44.4 14.1					
Old heterosexual men									
80-005	m	70	17:00	—	0/1	0/1	0/1	0/1	Pneumonia
82-005	m	68	05:45	30	0/1	0/1	0/1	0/1	Myocardial infarction; glomerulosclerosis of kidneys
93-019	m	78	—	70	0/1	0/1	0/1	0/1	Cardiopulmonary insufficiency; bronchopneumonia
93-039	m	79	3:00	53	0	0/1	0	0/1	Internal bleeding; decompensatio cordis
97-039	m	87	4:00	45	0/1	0/1	0/1	0/1	Myocardial infarction
Mean ± SEM		76.4 3.8	7:26 3:44	49.5 9.6					
Untreated transsexual									
96-088	m	84	41:00	38	0	0/1	0	0/1	Small cell carcinoma of the lungs with metastasis to the liver
Virilized syndrome									
83-004	f	46	6:00	34	2	1	2	1	Adrenocortical carcinoma; postoperative hemorrhage

m, Male; f, female; mtf, male-to-female transsexual; ftm, female to male transsexual.

mone receptors in neurons of the MBC underline the possibility of its involvement in reproduction.

In addition to reproduction, the MBC plays a crucial role in memory function (63). Mamillary bodies atrophy with age (64) and even more so in Alzheimer's disease (65) and are damaged in alcohol-associated Wernicke-Korsakoff's disease (66). The decline with age in nuclear AR-ir in the male MBC as found in the present study may also be reflected in functional changes. Whether the observed changes in the MBC play a role in the relationship between low levels of sex hormones and impairment in sexual and cognitive functioning (48, 67, 68) or in the increased prevalence of nonfamiliar Alzheimer's disease in the elderly (69, 70) should be further investigated. Protective actions of androgens on neurons (71) and memory loss (72, 73) have been described. It may in this connection also be of interest to investigate the possible neuroprotective effects of androgens in age-related diseases in men, in a similar way as is done for estrogen replacement therapy in postmenopausal women with reported beneficial effects on physical status, mood, cognition, and the prevention of Alzheimer's disease (67, 74, 75), although the latter certainly requires more investigation.

In conclusion, here we show for the first time that the sex differences in nuclear AR-ir in the MBC of the posterior hypothalamus reflect differences in circulating levels of androgens rather than differences in sexual orientation or gender identity. The functional implications of these alterations should be studied in the future.

### Acknowledgments

Brain material was obtained from The Netherlands Brain Bank (Anne Holtrop, Michiel Kooreman, and José Wouda; coordinator Dr. R. Ravid). We thank Bart Fisser for his technical assistance, Dr. F. W. van Leeuwen for the antibody PG21, Dr. S. Kaiser for critically reading the manuscript, G. van der Meulen for photography, and W. Verweij for secretarial help.

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