Male-to-Female Transsexuals Have Female Neuron Numbers in a Limbic Nucleus

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ABSTRACT

Transsexuals experience themselves as being of the opposite sex, despite having the biological characteristics of one sex. A crucial question resulting from a previous brain study in male-to-female transsexuals was whether the reported difference according to gender identity in the central part of the bed nucleus of the stria terminalis (BSTc) was based on a neuronal difference in the BSTc itself or just a reflection of a difference in vasoactive intestinal polypeptide innervation from the amygdala, which was used as a marker. Therefore, we determined in 42 subjects the number of somatostatin-expressing neurons in the BSTc in relation to sex, sexual orientation, gender identity, and past or present hormonal status. Regardless of sexual orientation, men had almost twice as many somatostatin neurons as women ($P < 0.006$). The number of neurons in the BSTc of male-to-female transsexuals was similar to that of the females ($P = 0.83$). In contrast, the neuron number of a female-to-male transsexual was found to be in the male range. Hormone treatment or sex hormone level variations in adulthood did not seem to have influenced BSTc neuron numbers. The present findings of somatostatin neuronal sex differences in the BSTc and its sex reversal in the transsexual brain clearly support the paradigm that in transsexuals sexual differentiation of the brain and genitals may go into opposite directions and point to a neurobiological basis of gender identity disorder. (*J Clin Endocrinol Metab* **85:** 2034–2041, 2000)

ANIMAL experiments and observations in human brains have convincingly shown that sexual differentiation not only concerns the genitalia but also the brain (1, 2). The strongly connected and sexually differentiated hypothalamus, septum, bed nucleus of the stria terminalis (BST), and amygdala are implicated in sexually dimorphic patterns of reproductive and nonreproductive behaviors (2–18).

Gender identity (*i.e.* the feeling to be male or to be female) is an important trait of a subject. Transsexuals experience themselves as being of the opposite sex, despite having the biological characteristics of one sex (19–21). In line with the hypothesis that in transsexuals sexual differentiation of the brain contrasts with that of the genetic and physical characteristics of sex, our group has recently found that the size of the central subdivision of the BST (BSTc) was within the female range in genetically male-to-female transsexuals (22). In that study the, BSTc was defined on the basis of its vasoactive intestinal polypeptide innervation, which is probably mainly derived from the amygdala (23). A crucial question resulting from that study was, therefore, whether the difference according to gender in the BSTc is based on a neuronal difference in the BSTc itself or rather a reflection of a difference in innervation from the amygdala. To see whether the BSTc itself has a neuronal organization that is opposite to that of the genetic and genitalial characteristics

of transsexuals, we determined the number of somatostatin (SOM)-expressing neurons in the BSTc, which is the major neuronal population in this structure (23).

Materials and Methods

Patients

In the present study, 42 brains of patients were analyzed (for an overview see Table 1). The brains of 34 reference subjects (9 presumed heterosexual males, 9 homosexual males, 10 presumed heterosexual females, and 6 male-to-female transsexuals) ranging from 20–53 yr of age, together with six brains (three males and three females) of patients with sex hormone disorders were obtained at autopsy, after the required permissions had been obtained. Twenty-six of the reference subjects were the same as used in the earlier study of Zhou *et al.* (22), whereas eight new patients (five females, two males, and one homosexual man) were included because not enough sections were left for the present study. A Turner syndrome patient (S6) and a castrated (orchiectomized) male patient (S5) were included in the sex hormone disorder group [n = 6; see the legend to Fig. 1; S1, S2, S3, and M2 were also used in the study of Zhou *et al.* (22)]. A nontreated individual with strong cross-gender identity feelings (S7), which were already present since his earliest childhood, was also analyzed. In addition, we had the exceptional opportunity to be able to study the first collected brain ever of a female-to-male transsexual (FMT). The brains were matched for age, postmortem time, and duration of formalin fixation. Neuropathology of all subjects was systematically performed by Dr. W. Kamphorst (Free University, Amsterdam, The Netherlands), Dr. D. Troost (Academic Medical Centre of the University of Amsterdam, Amsterdam, The Netherlands), or Prof. F. C. Stam (Netherlands Brain Bank, Amsterdam, The Netherlands). Subjects had no primary neurological or psychiatric diseases, unless stated otherwise.

Histology

Brains were weighed, generally followed by 37 days of fixation in 4% formaldehyde at room temperature. The hypothalamic area was subsequently dissected, dehydrated, and embedded in paraffin. Serial 6-μm

TABLE 1. Brain material

NBB patient number	Age (yr)	Brain weight (g)	Postmortem delay (h)	Fixation time (days)	Clinicopathological diagnosis
Reference men (n = 9)					
86042	28	1450	24	46	Guillain-Barré syndrome
84015	29	1400	13	41	Congenital heart disease; cardiac failure
94040	20	1490	8	82	B-cell lymphoma; viral pneumonia, hemorrhage, heart failure
89042	30	1340	30	26	AIDS; disseminated non-Hodgkin's lymphoma
84023	37	1370	39	35	Bronchopneumonia
88011	41	1500	21	33	Suicide
92011	47	1520	<89	77	Pneumococcen sepsis
95102	53	1383	10	33	Aorta dissection
86048	30	1430	8	35	AIDS, pneumocystic carinii pneumonia, lung tuberculosis, toxoplasmosis, heroin addiction
Reference women (n = 10)					
85027	29	1150	13	60	Corrected Fallots' teratology; cardiac failure, hepatic coma
85041	28	ND	5	44	Cardiogene shock
84025	23	1300	<10	35	Acute myeloid leukemia
86032	33	1035	<41	20	Adenocarcinoma with metastasis
92037	32	1280	30	45	Bronchopneumonia
88096	34	1400	<12	31	AIDS; disseminated histoplasmosis
84002	36	1420	86	51	Multiple fractures; rupture of thoracic aorta
80002	46	1300	3	ND	Ovarium carcinoma
89104	49	1260	<41	32	Septic shock; lung carcinoma
86039	53	1410	34	17	Myelocytic leukemia; blastomatosis
Homosexuals (n = 9)					
89031	25	1530	23	28	AIDS; pneumonia
88009	30	1480	5	27	AIDS; cytomegalic infections
87015	30	1640	24	26	AIDS; Pneumocystic carinii pneumonia
87080	39	1320	24	28	AIDS; progressive multifocal leukoencephalopathy
88121	42	1340	19	30	AIDS; cytomegalic meningoencephalitis
86023	43	1260	2	100	AIDS; disseminated Kaposi's sarcoma and pneumonia
88087	41	1240	12	34	AIDS; bronchopneumonia, cytomegalic infections and toxoplasmosis
86046	32	1440	49	11	AIDS: pneumocystic carinii pneumonia
89024	21	1430	<49	25	AIDS; mycobacterial infections, pneumonia, cerebrovascular accident
Male-to-female transsexuals (n = 6)					
84020 (T1)	50	1380	ND	30	Suicide
84037 (T2)	44	1450	ND	34	Cardiovascular death
88064 (T3)	43	1540	ND	ND	Sarcoma
93042 (T4)	36	1145	21	31	AIDS, pneumonia, pericarditis, cytomegaly in brain
93070 (T5)	53	1500	96	34	Acute fatty liver due to alcohol
95018 (T6)	48	1198	24	36	Cardiovascular death, cardiac arrest
Sex hormone disorder cases (n = 6)					
83004 ♀ (S1)	46	1260	ND	34	Adrenocortical carcinoma; postoperative hemorrhage
89103 ♂ (S3)	67	1290	ND	28	Pancreaticocarcinoma; prostate carcinoma; orchidectomy
91044 ♀ (S6)	25	1200	13	103	Turner syndrome (XO); related cardiovascular problems; decompensatio cordis
94090 ♂ (S5)	86	1663	3	93	Lung and prostate carcinoma; orchidectomy; septic shock
89077 ♀ (M2)	73	1090	<48	33	AIDS; pneumonia; epilepsy
91005 ♂ (S2)	31	1377	34	35	Feminizing adrenocortex carcinoma
Nontreated male with cross-gender identity feelings (n = 1)					
96088 ♂ (S7)	84	1433	41	38	Lung carcinoma
FMT (n = 1)					
98138	51		4	32	Cachexia

ND, Not determined.

frontal sections were cut on a Leitz microtome, mounted on SuperFrost/Plus (Menzel-Gläser, Braunschweig, Germany; Art. No. 041300) slides, and subsequently dried overnight on a hot plate at 58 C.

Immunocytochemistry

Sections were hydrated and rinsed in aquadest 2× 5 min and Tris-buffered saline [TBS; 0.05 M Tris, and 0.9% NaCL (pH 7.6)] for 30 min. To enhance antigen retrieval [for a review see Shi *et al.* (24)], sections were put in a plastic jar [filled with a Citrate 0.05 M (pH 4.0) buffer solution] and heated to boiling (120 C) for 10 min at 700 W in a microwave oven (Miele Electronic M696, Darmstadt, Germany). After cooling down for about 10 min, the sections were washed in TBS for 3× 10 min

and preincubated in TBS (pH 7.6) containing 5% nonfat dry milk (Elk, Campina bv., Eindhoven, The Netherlands) to reduce background staining. Subsequently, a circle was drawn around the sections with a Dakopen (Glostrup, Denmark; Code No. S 2002) to prevent the antibody from diffusing. The sections were: 1) incubated with 300-μL rabbit antisomatostatin [SOMAAR, 8/2/89; dilution 1:500; for details and specificity see Van de Nes *et al.* (25)] in 0.5% Triton X-100 (Sigma, Steinheim, Germany), 0.25% gelatin, and 5% nonfat dry milk TBS solution [supermix-milk (pH 7.6)] overnight at 4 C; 2) washed in TBS-milk 3× 10 min, followed by a second incubation with goat antirabbit IgG antiserum (Betsie, NIBR, Amsterdam, The Netherlands; dilution 1:100) in supermix for 60 min; 3) washed in TBS-milk 3× 10 min; 4) incubated with rabbit
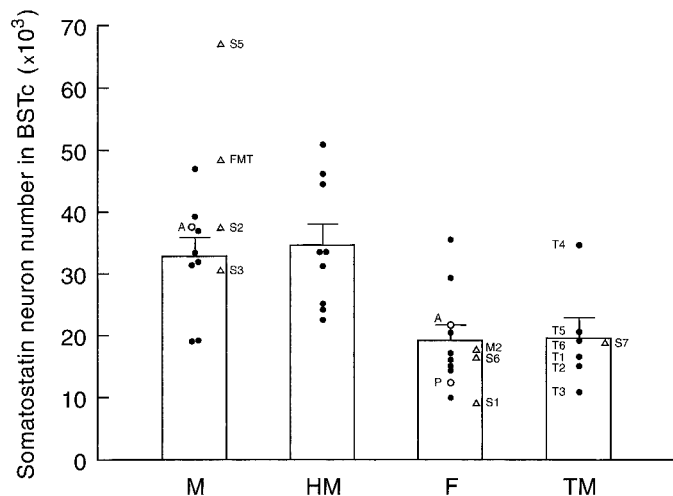
FIG. 1. BSTc neuron numbers. Distribution of the BSTc neuron numbers among the different groups according to sex, sexual orientation, and gender identity. M, Heterosexual male reference group; HM, homosexual male group; F, female group; TM, male-to-female transsexuals. The sex hormone disorder patients S1, S2, S3, S5, S6, and M2 indicate that changes in sex hormone levels in adulthood do not change the neuron numbers of the BSTc. The difference between the M and the TM group ($P < 0.04$) is also statistically significant according to the sequential Bonferonni method if S2, S3, and S5 are included in the M group or if S7 is included in the TM group ($P \leq 0.01$). Note that the number of neurons of the FMT is fully within the male range. Whether the transsexuals were male oriented (T1, T6), female oriented (T2, T3, T5), or both (T4) did not have any relationship with the neuron number of the BSTc. The same holds true for heterosexual and homosexual men. This shows that the BSTc number of somatostatin neurons is not related to sexual orientation. A, AIDS patient. The BSTc number of neurons in the heterosexual man and woman with AIDS remained well within the corresponding reference group (see Fig. 1), so AIDS did not seem to affect the somatostatin neuron numbers in the BSTc. P, Postmenopausal woman. S1 (♀ 46 yr of age): adrenal cortex tumor for more than 1 yr, causing high cortisol, androstendione, and testosterone levels. S2 (♂ 31 yr of age): feminizing adrenal tumor that induced high blood levels of oestrogens. S3 (♂ 67 yr of age): prostate carcinoma; orchiectomy 3 months before death. S5 (♂ 86 yr of age): prostate carcinoma; prostatectomy; orchiectomy, and antiandrogen treatment for the last 2 yr. S6 (♀ 25 yr of age): Turner syndrome (45,X0; ovarian hypoplasia). M2 (♀ 73 yr of age): postmenopausal status.

peroxidase-antiperoxidase (dilution 1:1000 in supermix) for 30 min; 5) rinsed 3× 10 min in 0.05 M Tris-HCL (Merck, Darmstadt, Germany; pH 7.6); 6) incubated in 0.05 mg/mL 3,3'-diaminobenzidine (Sigma), 0.25% nickel ammonium sulphate (BDH, Poole, UK) in 0.05 M Tris-HCL (pH 7.6) containing 0.01% H_2O_2 (Merck) for 15 min; 7) washed in aquadest for 10 min; 8) dehydrated in ethanol; and 9) mounted in Entallan.

Morphometry

Every 50th section stained for SOM along the rostro-caudal axis of the BSTc on one side of the brain (22) was used for analysis with the help of a specially developed program on an IBAS (Kontron Electronik, Munich, Germany) image analysis system. The image analysis system was connected to a scanning stage control box (MCU, Carl Zeiss, Oberkochem, Germany) and had a Sony B/W CCD-camera for image acquisition. Both the scanning stage and the camera were mounted on a microscope (Carl Zeiss) equipped with planapo objectives. To provide optimal contrast and homogenous illumination of the section the voltage of the light source was set maximally. The light was reduced by neutral gray filters (0.03/0.12/0.5/Schott; Mainz, Germany) to improve light contrast. For each section, the analysis consisted of the following steps:
By using the plan ×2.5 objective of the microscope, a low magnification image covering the BSTc area was obtained and loaded into the IBAS image memory.

In this image the BSTc was outlined manually on the basis of the distribution of the SOM immunoreactivity in neurons and fibers (see Fig. 3). Subsequently, the image analyzer covered the outlined area with a grid of rectangular fields, each with the size of the area displayed by the camera when the ×40 objective was installed.
By a random systematic sampling procedure, 50% of the fields (which were for at least 80% covered by the outlined area) were selected for analysis. Taking into account the aberration of the optical axis between the ×2.5 and the ×40 objective, the pixel positions of the selected rectangular fields in the 2.5 image were converted into scanning stage coordinates to position the corresponding areas of the BSTc in front of the camera when using the ×40 objective.
After the ×40 objective was installed, the image analyzer moved the scanning stage automatically to the coordinates of the selected fields. In each field, SOM-positive neurons containing a nucleolus were counted manually, taking into account the exclusion lines according to Gundersen (26). Neurons with double nucleoli were never seen. The spectrum of neuronal sizes was equally distributed among the different groups.
The total volume of the BSTc was calculated by rostro-caudal integration of the outlined areas, taking into account the distance between the measured sections. The neuronal density was calculated on the basis of the nucleolus counts in the sample volume. An estimation of the total number of SOM neurons was obtained by multiplying the total volume with the mean neuronal density. The finding that the mean BSTc volumes of the various groups are almost twice as large as those found in the study of Zhou *et al.* (22) can be explained by the fact that in the present study another peptidergic system (SOM instead of vasoactive intestinal polypeptide) was used as a marker and also an antigen retrieval technique (*i.e.* microwave tissue pretreatment), which makes the staining more sensitive (24, 27).

Statistics

Differences among the groups were statistically evaluated by the nonparametric Kruskal-Wallis multiple comparison test. Differences between the groups were analyzed two-tailed using the Mann-Whitney *U* test with a 5% experiment wise error rate (sequential Bonferroni method). Throughout this study values are expressed as mean ± SEM. A significance level of 5% was used in all statistical tests.

Results

Differences among the groups were statistically significant by the nonparametric Kruskal-Wallis multiple comparison test ($P = 0.002$ for SOM neuron number). No statistical group differences were found for age ($P = 0.090$), brain weight ($P = 0.125$), postmortem time ($P = 0.738$), fixation time ($P = 0.065$), or storage time ($P = 0.308$). To further test whether the differences in the BSTc between the groups were affected by possible confounding factors, such as paraffin-embedded storage time of sections, fixation time, postmortem time, or brain weight, an analysis of covariance was carried out. These factors seemed to have no significant effect on the BSTc SOM neuron numbers ($P > 0.10$).
The number of SOM neurons in the BSTc of heterosexual men ($32.9 \pm 3.0 \times 10^3$) was 71% higher than that in heterosexual women ($19.2 \pm 2.5 \times 10^3$) ($P < 0.006$), whereas the number of neurons in heterosexual and homosexual men ($34.6 \pm 3.4 \times 10^3$) was similar ($P = 0.83$). The BSTc number of neurons was 81% higher in homosexual men than in heterosexual women ($P < 0.004$). The number of neurons in the BSTc of male-to-female transsexuals was similar to that of females ($19.6 \pm 3.3 \times 10^3$) ($P = 0.83$) (see also Figs. 1 and 2). In addition, the neuron number of the FMT was clearly in the male range (see Fig. 1). The number of neurons in transsexuals was 40% lower than that found in the heterosexual reference males ($P < 0.04$; see the legend to Fig. 1) and 44%
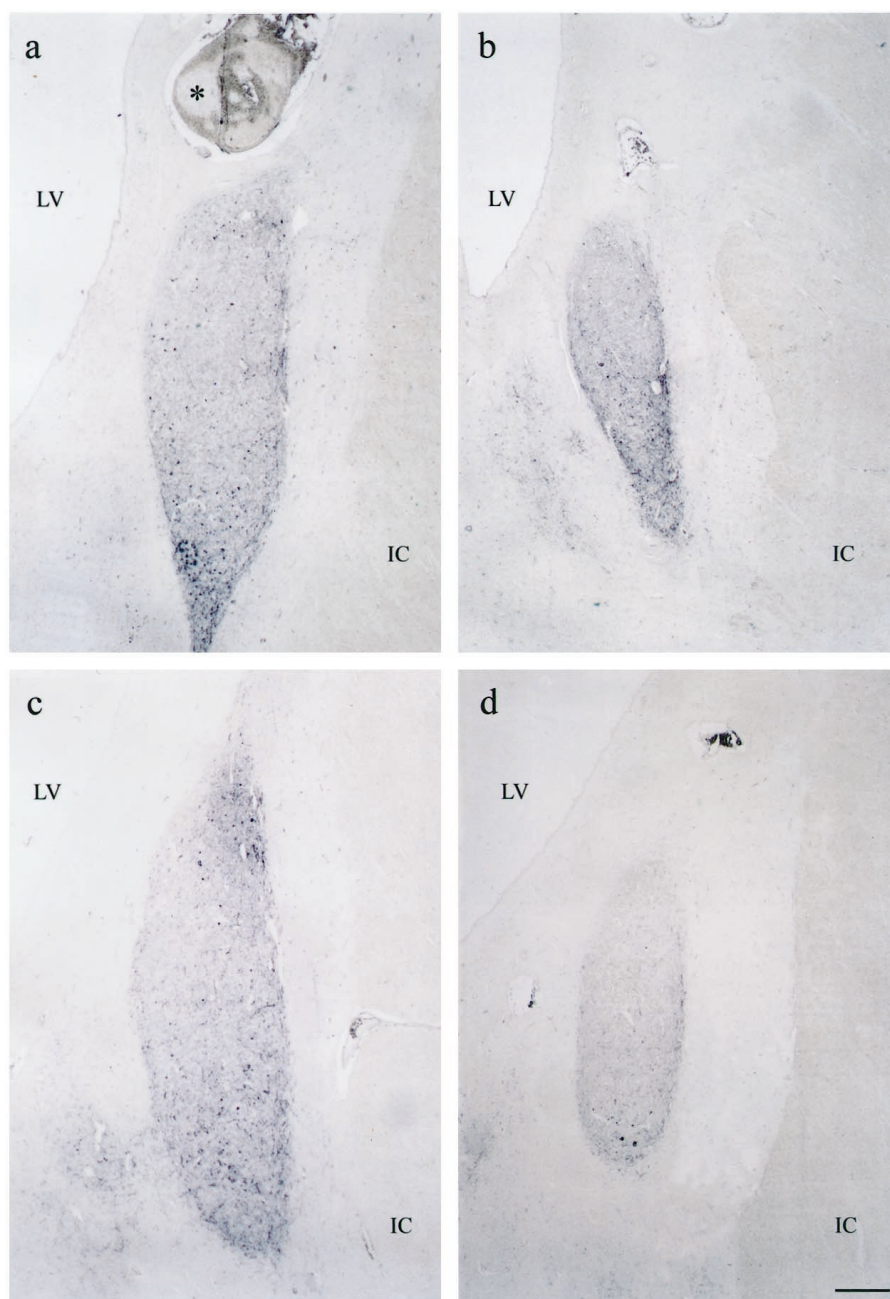
FIG. 2. Representative immunocyto-chemical stainings of the somatostatin neurons and fibers in the BSTc of a reference man (a), reference woman (b), homosexual man (c), and male-to-female transsexual (d). Note the sex difference regardless of sexual orientation. The male-to-female transsexual has a BSTc in the female range. *, Blood vessel. *Bar* represents 0.35 mm.

lower than that found in the homosexual males ($P < 0.02$). Including patients S2, S3, and S5 in the male group and S1, S6, and M2 in the female group or S7 in the transsexual group to increase the number of their respective gender groups enhanced the level of significance among the groups ($P < 0.001$ for SOM neuron number). There seemed to be no clear difference in the BSTc number of neurons between early onset (T2, T5, T6) and late-onset transsexuals (T1, T3), indicating that their smaller number of neurons is related to the gender identity *per se* rather than to the age at which it became apparent. No indication was found for a relationship between cause of death and BSTc neuron numbers. Analysis of the BSTc volumes showed a similar pattern of differences among the groups with heterosexual men having a BSTc

volume of 4.60 ± 0.28 mm^3, similar to that in homosexual men (5.00 ± 0.39 mm^3) ($P = 0.76$). The BSTc volume of females (3.38 ± 0.41 mm^3) and that of transsexuals (3.58 ± 0.19 mm^3) did not differ either ($P = 0.50$). The volumes of all males, regardless of sexual orientation, *vs.* all females or *vs.* all genetic male transsexuals were statistically highly significant ($P \leq 0.01$). The FMT had a BSTc volume in the male range (4.80 mm^3).

Discussion

In the present study, we show regardless of sexual orientation: 1) a sex difference in SOM neuron numbers in the human BSTc, with males having almost twice as many SOM
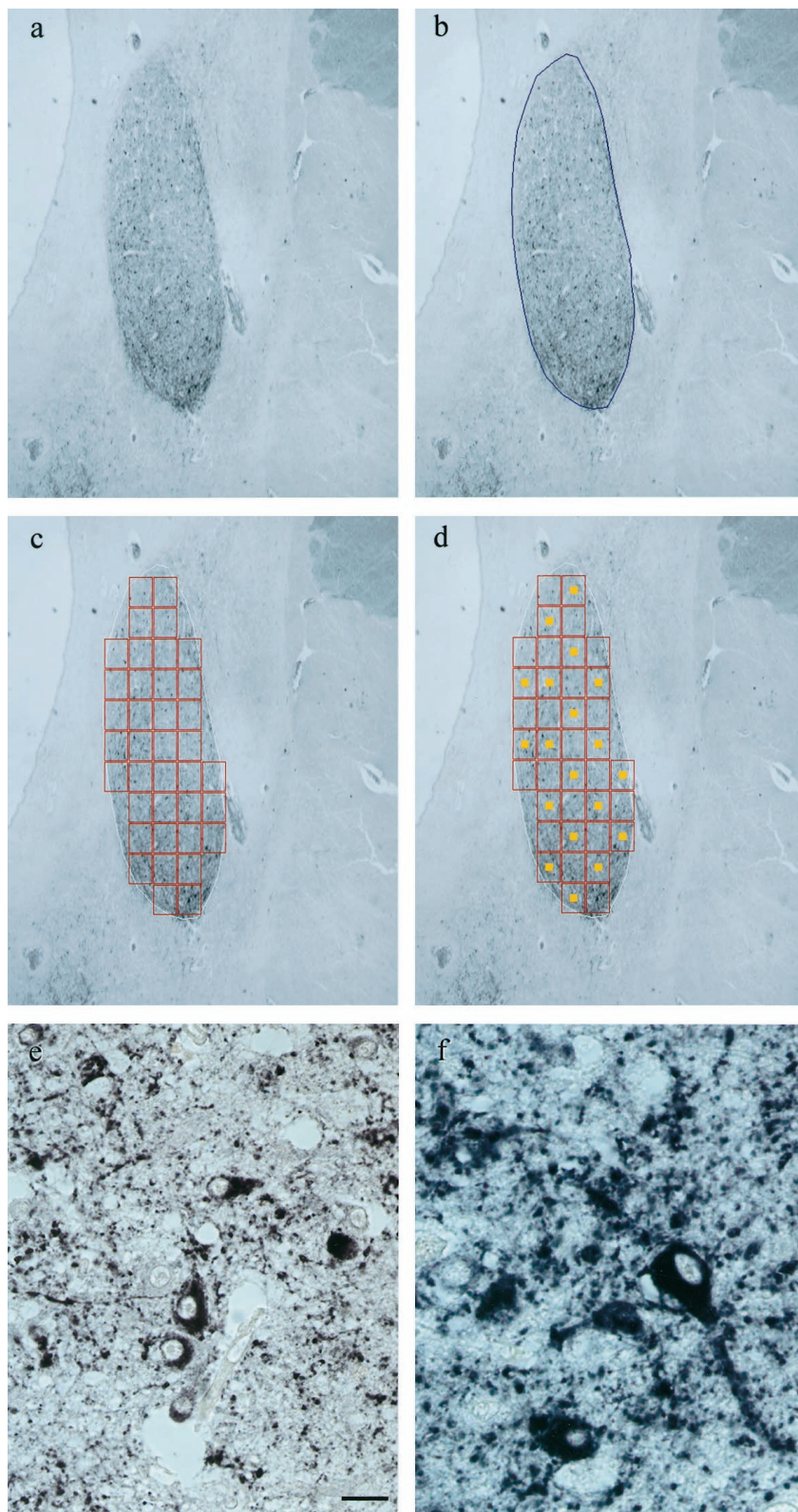
FIG. 3. The image analysis procedure. a, Illustration of a somatostatin immunoreactive BSTc. b, The BSTc is outlined manually. c, Outlined BSTc is divided automatically into rectangular fields. d, Fifty percent of the fields is selected by a random systematic sampling procedure. e, Higher magnification of somatostatin neurons in a field displayed by the camera when the ×40 objective is installed. Only somatostatin-positive neurons with a visible nucleolus were counted (see *Morphometry* in *Materials and Methods*). *Bar* represents 40 μm. f, Example of a clearly visible nucleolus in a somatostatin immunoreactive neuron.

neurons as females; 2) a number of SOM neurons in the BSTc of male-to-female transsexuals in the female range; and 3) an opposite pattern in the BSTc of a female-to-male transsexual with a SOM neuron number in the male range.

Analysis of the total number of SOM neurons of the human BSTc in individual patients with highly different hormone levels does not give any indication that changes in sex hormone levels in adulthood change the neuron numbers. Be-

cause the transsexuals had all been treated with estrogens, at least for some time (see Table 2), the reduced neuron numbers of the BSTc could theoretically be due to the presence of high levels of circulating estrogens. Arguments against this possibility come from the finding that transsexuals T2 and T3 both showed a small BSTc (Fig. 1), despite the fact that T2 stopped taking estrogens about 15 months before her death because of hyperprolactinemia, and T3 no longer received hormone treatment when a sarcoma was found about 3 months before she died. T5 continued to take estrogens until 3 months before death and had even more SOM neurons than T3, whereas T1 and T6 continued to take estrogens until death and even had higher SOM neuron numbers than T2 and T3 (Fig. 1). Furthermore, a 31-yr-old man (S2), who suffered for at least 1 yr from a feminizing adrenal tumor that produced high blood levels of estrogens, still had a BSTc neuron number in the normal male range (the latest highest serum estradiol levels before death varied between 577–779 pmol/L; the normal range is 50–200 pmol/L).

Our results might theoretically also be explained by a lack of androgens in the transsexual group because all subjects, except for T4, had been orchiectomized. We, therefore, studied two nontranssexual men (S3 and S5) who had been orchiectomized because of prostate cancer 3 months and 2 yr before death, respectively, and found that the BSTc neuron number of S3 was close to the mean of the male group and that the BSTc number of neurons of S5 was even the highest observed (Fig. 1), indicating that orchiectomy did not cause any decrease in SOM neuron numbers. Not only were five of the transsexuals orchiectomized, they all used the antiandrogen cyproterone acetate (CPA). However, an effect of CPA reducing the number of SOM neurons of the BSTc is highly unlikely because S5 had taken CPA during the last 2 yr of his life and his BSTc neuron number was at the upper end of the male range, whereas T6 had not taken CPA for the past 10 yr, and T3 took no CPA during the last 2 yr before her death, and they still had relatively low numbers of SOM neurons.

The BSTc SOM neuron numbers of two postmenopausal women [73- (M2) and 53-yr-old (P)] and of a 25-yr-old woman with Turner syndrome (S6: complete 45,X0, with ovarian hypoplasia) were completely within the normal female range (Fig. 1). If high estrogen levels would have a reducing effect on BSTc neuron numbers, the opposite effect (high neuron numbers) would be expected in the postmenopausal women and the Turner syndrome patient due to their low endogenous sex hormone level status. However, this was not the case. Noteworthy is that according to the available clinical data the two postmenopausal women did not receive any estrogen replacement therapy either. Although the Turner syndrome patient had been receiving hormone replacement therapy since she was 16 yr of age, her neuron numbers were even higher than P, whereas she had almost the same BSTc neuron number as M2 who did not receive such a therapy. Again, this argues against the probability of an estrogen-induced reduction effect on the number of SOM neurons. Finally, the BSTc neuron number of a 46-yr-old woman who had suffered for at least 1 yr from a virilizing tumor of the adrenal cortex (that produced very high blood levels of androstendione and testosterone) was also clearly within the lower spectrum of that of other women (Fig. 1; S1:

latest androstendione serum level before death was 48.0 ng/mL; the normal range for women is 0.4–3.5 ng/mL; the latest serum testosterone level before death was 26.82 nm/L; the normal range for women is 1.04–3.30 nm/L). Thus, an increasing effect of testosterone on the BSTc neurons does not seem likely to be the case either. Furthermore, it should be noted that the FMT stopped taking testosterone 3 yr before death while having a BSTc neuron number clearly within the male range.

In conclusion, estrogen treatment, orchiectomy, CPA treatment, or hormonal changes in adulthood did not show any clear relationship with the BSTc SOM neuron number. In addition, we had the unique opportunity to study the brain of an 84-yr-old man (S7) who also had very strong cross-gender identity feelings but was never orchiectomized, sex re-assigned, or treated with CPA or estrogens. Interestingly, this man had also a low BSTc SOM neuron number that was fully in the female range (see Fig. 1, S7). This case provides an additional argument against the view that orchiectomy, CPA, or adult estrogen treatment of the transsexuals would be responsible for the reduced somatostatinergic neuron numbers. Moreover, studies that investigated the effects of estrogen treatment on hypothalamic SOM neurons in (castrated) rats are also not in support of such an effect. Estrogen treatment does not reduce the amount of SOM messenger RNA (mRNA) in neurons but even enhances its neuronal expression (28). Moreover, another animal study indicates that, although changes occur in the hypothalamic neuronal expression of SOM mRNA due to castration or testosterone treatment of male rats, no differences in hypothalamic SOM neuron numbers are induced at all by either of such treatments (29). This observation is also in agreement with the control SOM neuron numbers of the castrated male patients (S3, S5) and testosterone-exposed (S1) female patient. Together, all these data clearly indicate that sex hormone-mediated reduction (or enhancement) effects on transsexual BSTc neurons in adulthood are extremely unlikely to be the underlying mechanism of the observed somatostatinergic BSTc differences.

In short, our findings seem to support the hypothesis that the somatostatinergic sex differences, the female number of SOM neurons in the BSTc of the male-to-female transsexual brain and the male number of SOM neurons in the BSTc of the FMT are not the result of changes of sex hormone levels in adulthood. Instead, the neuronal differences are likely to have been established earlier during development [see also Zhou *et al.* (22), and for functional differences see Cohen-Kettenis *et al.* (30)]. In line with this reasoning are the developmental data on the rat BST showing that adult volumes and neuron numbers of BST subdivisions are orchestrated by androgen exposure during early brain development (31, 32). Such a mechanism is also in agreement with data of Breedlove (33, 34) showing that perinatal androgens but not adult variations in androgen exposure induce differences in the total neuron number of the rat spinal nucleus bulbocavernosus. Apart from such well known irreversible "organizing" effects of sex hormones on the developing brain, the possibility of a direct action of genetic factors on sexual differentiation of the brain should not be ruled out (35).

We are aware of the fact that our data are based on post-mortem brain material derived from a heterogeneous patient population of which each individual's clinical status might

TABLE 2. Clinicopathological data of subjects with gender identity disorder

Patient no. (NBB)	Age (yr)	Age of hormonal treatment/orchiectomy	Hormone treatment	Cause of death
Male-to-female transsexuals (n = 7)				
T1 (84020)	50	42/44	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)	Suicide
T2 (84037)	44	35/37	Age 35: stilbestrol 5 mg 3 dd; after 2 months to 5 mg 2 dd; CPA 50 mg 1 dd; 1977: CPA 50 mg 2 dd; stilbestrol 5 mg 1–2 dd (generally this lasted 7 yr until death; stilbestrol stopped about 15 months before death)	Cardiovascular death
T3 (88064)	43	36/39	Age 36: received standard CPA treatment (50 mg 2 dd) until 2 yr before death; At age 39 received standard ethinylestradiol treatment (50 µg 2 dd) that stopped 3 months before death	Sarcoma, right-side temporal
T4 (93042)	36	NA/no orchiectomy, testes atrophy	CPA 50 mg 1 dd at least the last 10 months before death; the patient received estradiol in combination with hydroxyprogesterone in therapeutical dosages. Exact period of treatment is not known but based on the significant testes atrophy she was most probably treated for a period of about 5 yr or more.	AIDS, pneumonia, pericarditis, cytomegaly in brain
T5 (93070)	53	40/50	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)	Acute fatty liver due to alcohol abuse
T6 (95018)	48	35/36	Age 35: spironolactone 100 mg 2 dd; CPA 50 mg 2 dd; ethinyloestradiol 50 µg 2 dd; at age 36–40: CPA 50 mg 2 dd; ethinyloestradiol 50 µg 2 dd; at age 40–48; aldoctone 100 mg 1 dd; ethinyloestradiol 50 µg 1 dd (treatment lasted until death)	Cardiovascular death
S7 (96088)	84	No orchiectomy or sex reassignment therapy	Patient did not receive sex hormone replacement therapy	Lung carcinoma
FMT (n = 1)				
FMT (98138)	51	27/28	Age 27: testosterone sustanon 250 mg, twice a month injections; at age 30 testosterone undecanoaat 40 mg 3 dd. At age 34 testosterone undecanoaat 40 mg 2 dd; At age 36 testosterone undecanoaat 40 mg 4 dd; At age 44 testosterone sustanon 250, twice a month injections; At age 47 to 48: testosterone sustanon 250, every 3 weeks; from the age 48 until the age of death (51), no testosterone replacement therapy anymore	Cachexia

NBB, Patient number of the Netherlands Brain Bank; CPA, cyproterone acetate; NA, not available; AIDS, acquired immune deficiency syndrome.

have had an impact on the brain. However, despite that we were still able to find striking sexual dimorphic differences (that become even more significant if patients S1, S2, S3, S5, S6, S7, and M2 are included in their respective gender groups; see statistics and the legend to Fig. 1). An exciting additional new finding came from the FMT who revealed a "masculine" BSTc, which is completely in line with the sexual brain paradigm (7, 22, 30, 36–40).

Although our collection of male-to-female transsexual brains is small, it offers new opportunities to explore neurobiological correlates of transsexualism, as has previously been done in relation to sexual orientation (4–6). The development of high resolution imaging techniques may allow *in vivo* volume measurements of particular brain areas in much larger groups of transsexuals, which could extend our findings in the distant future. Although brain imaging proved to be useful in visualizing [*e.g.* septo-hypothalamic brain injuries leading to hypersexuality or altered sexual preference (9, 10)], precise neuroanatomical delineation of small brain structures such as the BSTc or neuronal counts are, at present, not possible using such techniques.

Taking into account the aforementioned limitations of our studies, the present study of SOM neurons in the human BSTc provides unequivocal new data supporting the view that transsexualism may reflect a form of brain hermaphroditism such that this limbic nucleus *itself* is structurally sexually differentiated opposite to the transsexual's genetic and genital sex. It is conceivable that this dichotomy is just the tip of the iceberg and holds also true for many other sexually dimorphic brain areas.

Because the sexually differentiated brain in general (41) may be the basis of sex differences in the prevalence of many neurobiological diseases and disorders (7), more studies are needed to further unravel the potential determinants of the sexual dimorphic brain and its related clinical disorders.

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