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Structural and Functional Sex Differences in the Human Hypothalamus

Dick F. Swaab,*[,1] Wilson C. J. Chung,*[,†] Frank P. M. Kruijver,* Michel A. Hofman,* and Tatjana A. Ishunina*[,‡]

**Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands; †Department of Psychology, University of Massachusetts, Amherst, Massachusetts; and ‡Department of Histology and Embryology, Kursk State Medical University, Kursk, Russia*

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Sex differences in the brain may be the basis not only for sex differences in reproduction, gender identity (the feeling of being male or female), and sexual orientation (heterosexuality vs homosexuality), but also for the sex difference in prevalence of psychiatric and neurological diseases (Swaab and Hofman, 1995). In this brief article we discuss a few examples of structural and functional sex differences in the human brain. © 2001 Academic Press

STRUCTURAL SEX DIFFERENCES

Structural sex differences have been reported in a number of human hypothalamic nuclei (Fig. 1) but the data are still controversial. The sexually dimorphic nucleus of the preoptic area (SDN–POA) that was first described in the rat by Gorski *et al.* (1978) is 3 to 8 times larger in male rats than in female rats and is so evident that it can even be observed with the naked eye in Nissl-stained sections. We have found a sexually dimorphic nucleus in the preoptic area of the human hypothalamus (Swaab and Fliers, 1985; Swaab and Hofman, 1988; Hofman and Swaab, 1989; Fig. 1) that we presume to be homologous to the SDN–POA in the rat as judged from its sex difference in young adults in size and cell number, localization, cytoarchitecture, and neurotransmitter/neuromodulator content. Immunocytochemical studies support such a homology between the SDN–POA in rat and human on the basis of the presence of thyrotropin-releasing hormone, cholecystokinin, galanin, and glutamic acid decarboxylase (for a review see Swaab, 1997). Allen *et al.* (1989) gave this nucleus another name: interstitial nucleus of the anterior hypothalamus 1 (INAH-1).

Morphometric analysis of the human SDN–POA revealed that the volume is more than twice as large in young-adult men as it is in women and contains about twice as many cells in men (Swaab and Fliers, 1985). The magnitude of the SDN–POA sex difference does not remain constant throughout adulthood, but fluctuates with age (Fig. 2). We extended the original observations to a group of 38 females and 42 males (Swaab and Hofman, 1988; Hofman and Swaab, 1989), replicating the sex difference in the young-adult group. However, Allen *et al.* (1989), LeVay (1991), and Byne *et al.* (2000) could not confirm the sex difference in the SDN–POA/INAH-1 (see below). The negative findings of Allen *et al.* (1989) can be explained by the age selection in that study (see Swaab *et al.*, 1992), while LeVay (1991) and Byne *et al.* (2000) used relatively small samples. In addition, there are technical differences between the studies, such as section thickness, that may explain the discrepancies. Recently, by immunocytochemistry, we found stronger androgen receptor and estrogen receptor-(ER)α and -β staining in the SDN–POA of men as compared to those of women, in both intensity and number of neurons stained (Fernández-Guasti *et al.*, 2000; Kruijver *et al.*, 2001, submitted), supporting the presence of a sex difference in the SDN–POA.

Allen *et al.* (1989) described two other cell groups (INAH-2 and -3; Fig. 1) in the preoptic-anterior hypothalamic area of humans that were larger in the male

[1] To whom correspondence and reprint requests should be addressed. Fax: (+31)206961006. E-mail: w.verweij@nih.knaw.nl.

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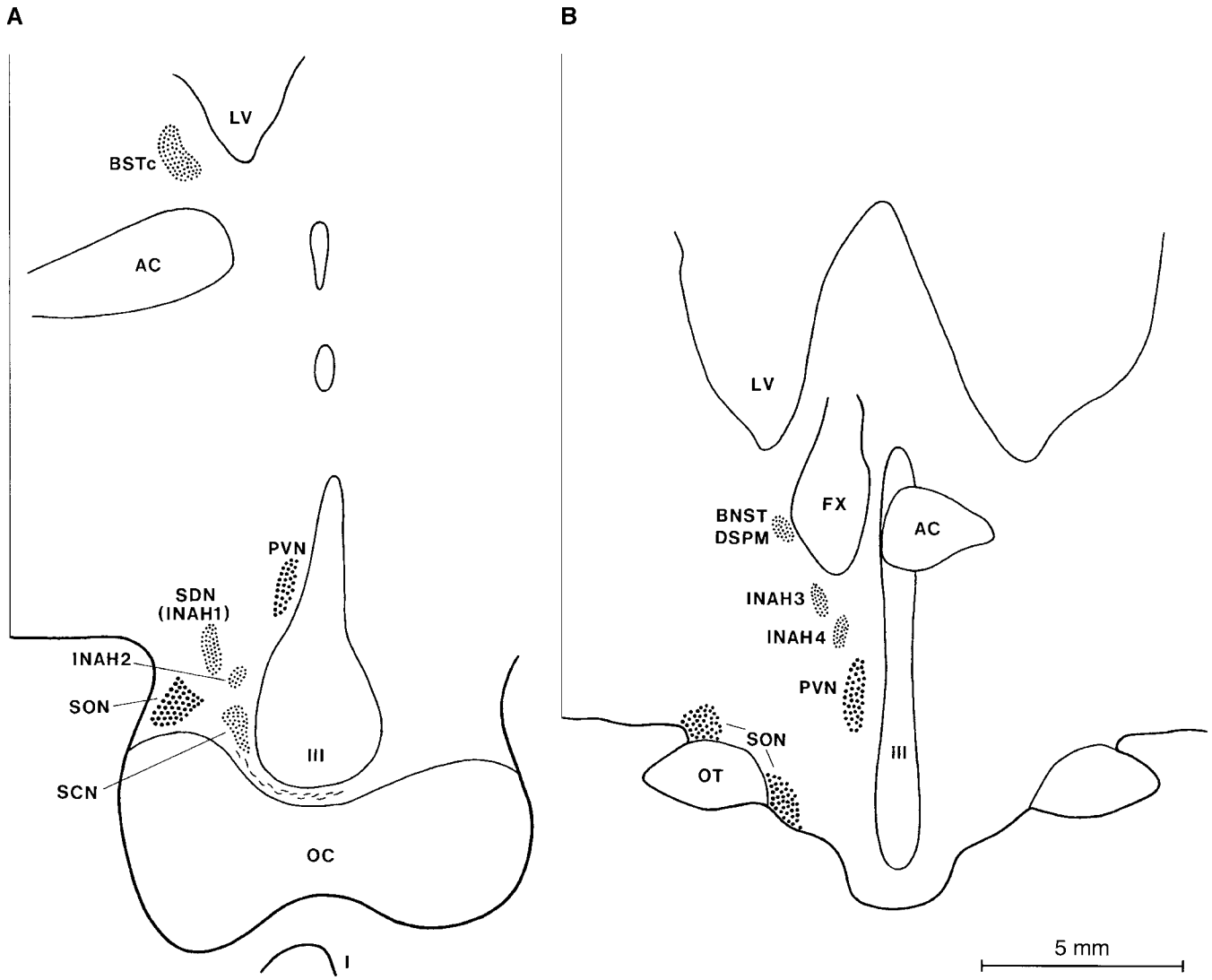

FIG. 1. Topography of the sexually dimorphic structures in the human hypothalamus. A is more rostral than B. III, Third ventricle; AC, anterior commissure; I, infundibulum; BSTc, central nucleus of the bed nucleus of the stria terminalis; FX, fornix; LV, lateral ventricle; OC, optic chiasm; OT, optic tract; SCN, suprachiasmatic nucleus; SDN, sexually dimorphic nucleus of the preoptic area (SDN–POA); PVN, paraventricular nucleus; SON, supraoptic nucleus; BNST-dspm, darkly staining posteriomedial component of the bed nucleus of the stria terminalis; INAH1-4, interstitial nuclei of the anterior hypothalamus 1–4.

brain than in the female brain. It is unclear which nuclei in the rat are homologous to INAH-2 and -3, which is further hampered by the lack of knowledge about their neurotransmitter content. Neither LeVay (1991) nor Byne *et al.* (2000) could confirm the sex difference in INAH-2, but they both did confirm a sex difference in INAH-3.

Another clear sex difference was described by Allen and Gorski (1990) in what they called the "darkly staining posteromedial component of the bed nucleus of the stria terminalis" (BNST-dspm). The volume of the BNST-dspm was 2.5 times larger in males than in females. We found a similar sex difference in the central nucleus of the bed nucleus of the stria terminalis (BSTc; Fig. 1). The BSTc is defined by its dense vasoactive intestinal polypeptide (VIP) innervation, which probably originates from the amygdala and is characterized by its somatostatin fiber plexus and neurons and which is sexually dimorphic. The BSTc in men is 40% larger than in women and men have almost twice

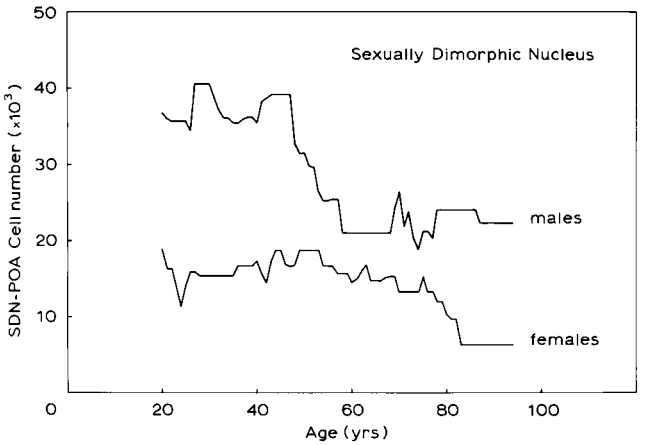

FIG. 2. Age-related changes in the total cell number of the sexually dimorphic nucleus of the preoptic area (SDN–POA) in the human hypothalamus. The general trend in the data is enhanced by using smoothed growth curves. Note that in males, SDN–POA cell number declines steeply between the ages of 50 and 60 years, whereas in females, from the age of about 50 years, a more gradual cell loss is observed, which continues through old age. These growth curves demonstrate that the reduction in cell number in the human SDN–POA in senescence is a nonlinear, sex-dependent process (Hofman and Swaab, 1989).

as many somatostatin neurons as women (Zhou *et al.*, 1995b; Kruijver *et al.*, 2000).

The anterior commissure was found to be 12% larger in females, and the interthalamic adhesion (or massa intermedia), a gray structure that crosses the third ventricle between the two thalami, was present in more females (78%) than males (68%). Among subjects with a massa intermedia, the structure was on average 53% larger in females than in males (Allen and Gorski, 1991). The latter observations point to a greater connectivity between the cerebral hemispheres of women as compared to that of men.

AGE-RELATED STRUCTURAL SEX DIFFERENCES

A number of structural sex differences vary strongly with age. Both the process of neuronal aging and changes in sex hormone levels during aging seem to be instrumental in these changes. In males, a major reduction in SDN–POA cell number was observed between the ages of 50 and 60 years (Fig. 2), which resulted in a period of much less pronounced sex difference in cell numbers. In females of over 70 years of age cell death was found to be prominent, dropping to values which were only 10–15% of the cell number found in early childhood, so that it appears that the sex difference in the SDN–POA increases again in old people (Hofman and Swaab, 1989; Fig. 2).

The VIP-containing subnucleus of the human suprachiasmatic nucleus was found to be twice as large in young men (10 to 30 years) as in young women and contained twice as many VIP cells. From the age of about 40 onward this sex difference was reversed (Swaab *et al.*, 1994; Zhou *et al.*, 1995b). These observations show again how important age is for sexual dimorphisms of the human brain.

In the mediobasal hypothalamus of aged subjects a striking sex difference has been reported in neurofibrillary pathology associated with abnormally phosphorylated tau protein. The pathology in the median eminence and infundibular nucleus is characterized by a dense network of large dystrophic neurites with neurofibrillary tangles that are interspersed among them. The terminal-like processes contact the neurohemal vasculature of the posterior median eminence and the adjacent infundibular nucleus. The Alzheimer pathology in the infundibular nucleus was identified in up to 90% of the older males and in only 8–10% of the females. The vessel-associated neurofibrillary lesions of the mediobasal hypothalamus develop independently of Alzheimer's disease-related neocortical pathology (Schultz *et al.*, 1996). In the arcuate nucleus of postmenopausal women, LHRH neurons and interneurons are strongly activated. We propose to explain the lack of neurofibrillary changes in the mediobasal hypothalamus of females as an illustration of how activated neurons are protected against the development of Alzheimer changes, a principle we paraphrased as "use it or lose it" (Swaab, 1991).

An opposite sex difference in Alzheimer pathology was observed in the nucleus basalis of Meynert (NBM), which is the major source of cholinergic innervation of the neocortex and which is severely affected in Alzheimer's disease. The percentage of NBM neurons containing pretangles with hyperphosphorylated tau was higher in females than in males (Salehi *et al.*, 1998). This sex difference may be related to the higher prevalence of Alzheimer's disease observed in women.

FUNCTIONAL SEX DIFFERENCES IN THE HYPOTHALAMUS

Although the number of vasopressin neurons in the supraoptic nucleus (SON) did not differ between men

and women, a sex difference was reported in vasopressin plasma levels. Males have higher vasopressin levels than females (Share *et al.*, 1988; Van Londen *et al.*, 1997). This sex difference is explained by the higher activity we found in vasopressin neurons in the SON (Fig. 1) of young males as compared to females using the size of the Golgi apparatus as a measure for neuronal activity. In the course of aging, possibly triggered by the decrease in estrogen levels in postmenopausal women, the neuronal activity in the SON gradually increases in females, while it remains stable in males. The sex difference in neuronal activity in the SON thus disappears after the age of 50 (Ishunina *et al.*, 1999). Consequently, this is an example of a hypothalamic system that shows no structural sex difference but a functional sex difference instead. It is also an example of a sex difference based on the "activating" (or in this case "inhibiting") effect of sex hormones. The activation of neurosecretory vasopressin neurons in postmenopausal women was confirmed by measurement of the cell size as a parameter for neuronal activity in immunocytochemically stained vasopressin neurons. Vasopressin neurons in the SON and paraventricular nucleus (PVN) of the hypothalamus appeared to be larger in young men than in young women. In elderly women (>50 years old) vasopressin cell size considerably exceeded that of young women. In addition, vasopressin cell size correlated positively with age in women, but not in men in both nuclei. Sex differences in the size of the PVN vasopressin neurons were pronounced on the left side and absent on the right, indicating the presence of functional lateralization of this nucleus. These data demonstrate sex differences in the size of the vasopressin neurons, and thus in their function, that are age-dependent and probably also lateralized. No such changes were observed in oxytocin neurons in the PVN (Ishunina and Swaab, 1999). Sex- and age-related differences in the activity of vasopressin neurons in the human SON are probably mediated by differences in estrogen receptor-α and -β expression by these cells. Young women ($\leq$50 years old) show 50 times more ERβ nuclear positive vasopressin neurons than young men and 250 times more than postmenopausal women. On the contrary, ERα is present in a higher proportion of the SON cells in young men and elderly women than in young women. The activation of vasopressin neurons in postmenopausal women is thus probably mediated by a decrease in estrogen receptor-β as a possible mediator of inhibitory effects of estrogens, and an increase in estrogen receptor-α as a possible mediator of stimulatory effects of estrogens in these neurons (Ishunina *et al.*, 2000).

Another example of a sex difference based on the activating effect of sex hormones was found in the mamillary body complex (MBC) that shows much stronger androgen receptor staining in males than in females (Fernández-Guasti *et al.*, 2000). Electrical stimulation of this area in monkeys induces penile erections (MacLean and Ploog, 1962; Poeck and Pilleri, 1965). In a follow-up study we have shown that this sex difference depends fully on the amount of circulating androgens in adulthood, while the sex difference did not seem to be related to sexual orientation or gender identity (Kruijver *et al.*, 2001). Together, these data support the notion that a number of sex differences in the human hypothalamus are related to circulating levels of sex hormones.

REVERSED SEX DIFFERENCE IN TRANSSEXUALITY

A reversed sexual dimorphism is found in the brain of transsexuals. Transsexuals have, often from childhood onward, the strong feeling of having been born the wrong sex. Their desire to resemble the opposite sex is so strong that they are even willing to undergo major surgery and hormone treatments to achieve this end. This gender-identity problem has been proposed to develop as a result of a disturbed interaction between the developing brain and sex hormones. The search for structures that may be directly related to gender identity, i.e., structures whose anatomy is "female" in genetically male transsexuals, has so far led to our studies of the central nucleus of the bed nucleus of the stria terminalis (BSTc). A female-sized nucleus was found in male-to-female transsexuals. The size of the BSTc was not influenced by sex hormones in adulthood and was independent of sexual orientation. Similar results were obtained when the total number of somatostatin neurons was determined in the BSTc. In the BSTc of one female-to-male transsexual a male volume and somatostatin neuron number was found (Zhou *et al.*, 1995b; Kruijver *et al.*, 2000). Although the BSTc may be one of many structures involved in the phenomenon of gender identity, these results do support the hypothesis that gender identity develops as a result of an interaction between the developing brain and sex hormones.

CONCLUSION

There are now quite a number of structural and functional sex differences known in the human brain that may be related not only to reproduction, sexual orientation, and gender identity, but also to the often pronounced sex differences in prevalence of psychiatric and neurological diseases. One of the recent focuses of interest in this respect is the possible beneficial effect of sex hormones on cognition in Alzheimer patients. The immunocytochemical localization of estrogen receptors-α and -β and androgen receptors has shown that there are indeed numerous targets for sex hormones in the adult human brain. Observations in the infundibular nucleus have, however, indicated that in this brain area the hyperactivity resulting from a lack of estrogens during menopause seems to protect females against Alzheimer changes, in contrast to males. It is thus quite possible that estrogen replacement therapy may, in these brain areas, lead to inhibition of neuronal metabolism and thus to the same proportion of Alzheimer changes as are observed in men. Knowledge about the functional sex differences in the brain and the effect of sex hormones on neuronal metabolism may thus provide clues not only for the possible beneficial effects of these hormones (e.g., on cognition or hypertension), but also on possible central side effects of estrogen replacement therapy.

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