BRES 25853

Lesions of the suprachiasmatic nucleus do not disturb sexual orientation of the adult male rat

F.P.M. Kruijver^a, F.H. de Jonge^b, W.T. van den Broek^a, T. van der Woude^a, E. Endert^c,
D.F. Swaab^{a,*}

^a Netherlands Institute for Brain Research, Graduate School Neurosciences Amsterdam, Meibergdreef 33, 1105 AZ Amsterdam-Zuidoost, The Netherlands, ^b Agricultural University, Department of Animal Husbandry, P.O. Box 338, 6700 AH Wageningen, The Netherlands, ^c Laboratory of Endocrinology, Faculty of Medicine, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam-Zuidoost, The Netherlands

(Accepted 6 July 1993)

Key words: Suprachiasmatic nucleus; Sexual orientation; Partner preference test; Bilateral SCN lesion; Testosterone

An enlarged suprachiasmatic nucleus (SCN) has been found earlier in a group of homosexual men, as compared to heterosexual controls. In order to assess a possible relationship between the SCN and sexual orientation, the present study was undertaken to investigate whether the rat SCN might play a role with respect to the expression of sexual orientation. Sexual orientation was measured in partner preference tests as the percentage of time spent in the vicinity of sexually active male and female incentives, that were separated from the experimental animal by a wire mesh. The results show that established patterns of sexual orientation towards female incentives by the adult male rat are not influenced by SCN-lesions. Disturbances in circadian rhythmicity of activity in SCN-lesioned animals did not affect this conclusion.

The suprachiasmatic nucleus (SCN) is located just above the optic chiasm (Fig. 1a) and is known to function as the principle component of the biological clock, generating and coordinating hormonal, physiological and behavioral circadian rhythms, e.g., refs. 16,18,19,23. Studies have additionally implicated the SCN area in the regulation of reproduction^{6,13,22,25,30,31}. In an investigation of Swaab and Hofman²⁹, the SCN of ten male homosexuals who died of AIDS (acquired immune deficiency syndrome) was found to be 1.7 times larger in volume and to contain 2.1 times as many cells than heterosexual controls. There are three lines of reasoning that might relate a larger SCN to homosexuality: according to the first, the SCN could have a direct regulatory activity in relation to the development or expression of sexual orientation. According to the second, sexual orientation might influence the size of the SCN. According to the third, an enlarged SCN could have developed due to factors that are associated with, or contribute to the development or expression of sexual orientation. Animal studies may provide empirical evidence favouring one line of rea-

soning over the other and we therefore studied in the adult male rat, whether the SCN might regulate the expression of sexual orientation.

Sexual orientation is generally investigated in partner preference tests in which the rat is given the choice to approach and stay in the vicinity of sexually active male or female incentives, e.g. refs. 7, 17. In such tests, male rats prefer to spend time near the estrous female rather than near the male^{1,8,10,14,17} and evidence indicates that this orientation is indeed sexually motivated. For instance, orientation towards a female is enhanced after sexual experience or after the male has been exposed to copulating rats prior to testing (F.H. de Jonge et al., unpublished observations), orientation towards a female fluctuates with the estrous cycle of the female incentive and parallel with changes in sexual activity, orientation towards a female declines after castration and is reinstated after testosterone supplementation¹⁰, which underlines the importance of testosterone in sexually motivated behavior. Whether preferential orientation towards a male rat is sexually motivated, is only shown in rats that are manipulated during early

* Corresponding author. Fax: (31) (20) 6961006.

Fig. 1. Thionin stained, frontal 6 μ m paraffin sections of a SHAM-lesioned animal (a) and a SCN-X animal (b). OC, optic chiasm; SON, supraoptic nucleus; V III, third ventricle; Intact SCN (a) and lesion site (b). The incisor bar was placed at 2.8 mm and the electrodes were lowered under an angle of 10° relative to the perpendicular, using the following coordinates: 1.5 mm posterior and 0.9 mm lateral relative to the bregma and 9.00 mm ventral relative to the dura. Bar represents 0.5 mm.

development. For instance, neonatally castrated male rats or male rats neonatally treated with the aromatization inhibitor ATD are known to orient towards males and actually engage in sexual contacts with males, when given the opportunity^{5,11}.

In the present study we tried to reveal whether the SCN might be causally implicated in sexual orientation by lesioning the SCN in male rats after having previously established their patterns of sexual orientation in four tests and retesting them 2 weeks after the operation, exactly according to the schedule of preoperative testing.

Forty adult, 7 weeks old, sexually naive, male albino Wistar rats (weighing ± 210 – 230 g) were obtained from the central animal supply house of T.N.O (Zeist, The Netherlands). The animals were maintained at constant room temperature ($20^\circ \pm 1^\circ\text{C}$; 50% damp atmosphere) in groups of four per cage with food and water available ad libitum, under a 12 h/12 h reversed dark/light cycle. Behavioral testing started at the age of 8 weeks. Tests were run 36 h apart and were therefore run alternately during the dark period and during the light period, in order to control for possible disturbances in circadian rhythmicity of behavior in the SCN-lesioned group. The tests were always started between 4–7 h after lights off (dark) or after lights on (light). Half of the males received their first test during the dark period, the other half during the light period.

Tests for sexual orientation took place in a 78×30 cm test apparatus containing one male and one female incentive in stimulus cages of 8×30 cm at the sides opposite of each other. The incentives were taken from the general lab stock and were sexually active males or ovariectomised females, artificially brought into estrous by a 0.5 cm long silastic tube (0.8 mm i.d., 1.4 mm o.d.) filled with estradiol (Diosynth, Oss, The Netherlands) and subcutaneously implanted 2 weeks before behavioral testing. The experimental animal was separated from the incentives by a wire mesh so that he could clearly see and smell them. The male was free to shuttle for 30 min in the remaining 50×30 cm area which was divided in three areas of equal size. Frequency of visits and time spent in the male, female or neutral area were recorded in between. The apparatus was thoroughly cleaned with water in between the tests. Male and female incentives were always placed in the same stimulus compartment. In order to prevent position preferences, the testing apparatus was rotated 90° after each test.

During brain surgery, bilateral lesions (SCN-X, $n = 24$) were produced²⁰ using thermocouple electrodes (Radionics T.C.) with an uninsulated tip diameter of 0.25×0.25 mm, connected to a radiofrequency alter-

nating current apparatus (Radionics model RFG-4A). Sham procedures (SHAM, $n = 16$) were identical except that no stimulus current was passed.

After completion of behavioral testing all animals were injected with pentobarbital (Nembutal 0.5 ml/rat i.p.). One ml blood was taken by hartpuncture¹² (between 09.00 h and 12.30 h) to determine total basal plasma testosterone levels by radioimmunoassay²¹, since SCN area lesions have been reported to disturb patterns of gonadotropin release^{13,31}. Autopsy procedures were performed by perfusion intracardially with saline followed by 500 ml fixative (4% paraformaldehyde in 0.1 M PBS (phosphate buffer, pH = 7.6). The brains were removed and stored in fixative for 1 day at 4°C and subsequently dehydrated and embedded in paraffin. Serial $6\text{-}\mu\text{m}$ frontal sections were cut according to the coronal plane of Paxinos and Watson²⁰ and every 10th section was mounted on chrome-alum-coated slides and stained with thionin. The location of the lesions (see Fig. 1b) was subsequently determined by two independent investigators. In cases of uncertainty adjacent sections were immunocytochemically stained for vasopressin (VP) according to previously described staining procedures²⁸. Only those animals that did not show VP-immunoreactive SCN-cells were included in the bilaterally SCN-lesioned group. After histological analysis, three different groups of the SCN-X animals ($n = 24$) could be distinguished: (1) SCN intact ($n = 6$); (2) SCN partially lesioned ($n = 12$); (3) SCN completely (= 100%) bilateral lesioned ($n = 6$; SCN-X-group). All SHAM animals had an intact SCN ($n = 16$). In all cases, the lesions inflicted damage to the middle section of the optic chiasm (see Fig. 1b). The behavior of the six completely bilateral lesioned animals (SCN-X-group) was further compared to that of SHAM-lesioned controls ($n = 16$). Behavioral data were analysed by multivariate tests for analysis of variance with repeated measures (ANOVA, 25). Prior tests for homogeneity of variance indicated that ANOVA could be adequately run on this set of data (Bartlett-Box, $P > 0.05$ as a criterium). The behavior of the SHAM-group for sexual orientation during repeated testing was first analysed and involved the variables 'time spent in the female area', 'time spent in the male area' and 'time spent in the neutral area'. This ANOVA included the factors repeated testing (eight levels: four preoperative and four postoperative tests), tests during the dark or during the light period (two levels) and whether the test series began during the light period or during the dark period (two levels). A significant main effect on the factor repeated testing was found for all three variables ($F_{3,36} > 5.29$, $P < 0.005$). Fig. 2 shows the percentage of time spent in different areas during

TIME SPENT IN ARENA

Fig. 2. Percentage of time spent in the female, the male, or the neutral area during repeated testing in the sham operated group. The arrow indicates time of surgery. On each test, half of the animals was tested during the dark phase of the cycle, the other half during the light phase (see methods). Data during the dark and light period are pooled in this graph (ANOVA, repeated testing, $P < 0.005$). The order of test sequence is indicated by the numbers 1 → 8.

repeated testing. In this figure data from tests during the light and tests during the dark period are pooled. As shown in Fig. 2, stable levels of preference behavior are observed only from the third test onwards. Therefore tests 3 and 4 ('preoperative tests') and tests 5 and 6 ('postoperative tests') were further selected for analysis of sexual orientation of SHAM-treated and SCN-X-rats. A subsequently run ANOVA on test 3–6 only, indeed revealed no statistically significant effects on the factor repeated testing ($F_{1,12} < 1.99$, $P > 0.18$), except for the time spent near the male, which slightly increased during testing ($F_{1,12} = 4.87$, $P < 0.05$, see Fig. 2). Sexual orientation was expressed as time spent near the female, calculated as a percentage of the total time spent near the female + male incentives. An ANOVA including the factors preoperative versus postoperative tests (two levels), dark versus light period (two levels) and SHAM versus SCN-X (two levels), revealed no statistical significant main or interaction effects on the factor SHAM versus SCN-X ($F_{1,17} < 1.23$, $P > 0.28$), indicating that sexual orientation of SCN-lesioned animals did not differ from that of controls. A trend

towards significance was found on the interaction effect between the factors dark versus light by preoperative versus postoperative testing ($F_{1,17} = 3.31$, $P = 0.087$), indicating that a greater sexual orientation towards females during the light period tend to disappear during repeated testing both in the SHAM- and in the SCN-X-group. This was possibly due to the effect of repeated testing.

Similar results were obtained when analysing the frequency of total visits to male and female incentives. An ANOVA on 'frequency of visits', including the same factors as described above, revealed a significant interaction effect on the factor dark versus light by preoperative versus postoperative testing ($F_{1,20} = 4.51$, $P < 0.05$). A posteriori contrasts indeed indicated that males visited the different incentives more often in the dark than in the light period during preoperative tests ($F_{1,21} = 28.93$, $P < 0.0001$), but not during postoperative tests ($F_{1,21} = 2.88$, $P < 0.105$). The latter, however, was independent of whether males were SCN- or SHAM-lesioned. The conclusion that SCN-lesions do not influence preoperative patterns of sexual orientation holds therefore true independent of whether or not SCN-lesioned animals showed loss of circadian rhythmicity in activity. Analysis of individual patterns of sexual orientation indicated that all rats from both the SHAM- ($n = 16$) and the total SCN-X-group ($n = 24$; including the intact $n = 6$ and mislocated group $n = 12$) showed a preference for an estrous female over a male, on all preoperative as well as postoperative tests. In harmony with these behavioral data are the analysis of total plasma testosterone levels which revealed no significant differences between the SHAM- and the SCN-X-group (5.1 ± 1.3 ng/ml versus 4.3 ± 1.3 ng/ml, T -test, $P = 0.773$; data are mean scores $\pm$ SD.), indicating that after complete destruction of the SCN, sexual orientation was not influenced by possible significant alterations of basal plasma testosterone levels. This observation in male rats of stable levels of total plasma testosterone after SCN-lesions, together with the observation in female rats of Gray et al.¹³ and Stobie et al.²⁷, that *tonic* luteinizing hormone (LH) release is not affected by SCN-lesions, suggest that in rats the SCN is not responsible for the constant tonic rhythm of gonadotropin release and therefore basal plasma levels of sex hormones, such as testosterone as presented in the present study.

The results show that SCN-lesions do not change preoperative patterns of sexual orientation in the adult male rat. This conclusion is corroborated by the stable total plasma testosterone levels after complete destruction of the SCN. These results therefore seem to exclude the possibility that the SCN has a direct regu-

latory activity with respect to the *expression* of sexual orientation in the male rat with an already established female directed orientation, but obviously leaves the possibility open that the SCN may play a role with respect to the *development* of sexual orientation. A relevant question, regarding the present study, is if there are non-cognitive aspects in human sexual orientation which may be adequately studied in a non-primate model on sexual orientation. Variations in sexual differentiation due to varied pre- or neonatal hormonal exposure²⁴ is mentioned as one of such non-cognitive factors influencing both animal^{1,5,7,17} and human sexual orientation⁹.

Although the findings of subsequently Swaab and Hofman²⁹, LeVay¹⁵ and Allen and Gorski² within the human brain and the twin studies of Bailey and Pillard^{3,4} clearly suggest a neurobiological basis for sexual orientation, it is unknown at present whether or not the spectrum of human heterosexual, homosexual or bisexual orientation can be attributed to a variation in the differentiation of (sexually dimorphic) structures in the human brain.

The authors wish to thank Mr. B. Fisser, Mr. R. Joosten and Ms. A.L. Louwerse for their technical assistance and Mr. G. van der Meulen for his assistance with the photography. Special thanks to Mr. M.J. Wakefield and Mr. M.A. Hofman for their helpful discussions and valuable comments on the manuscript.

- 1 Adkins-Regan, E., Sex hormones and sexual orientation in animals, *Psychobiology*, 16 (1988) 335–347.
- 2 Allen, L.S. and Gorski, R.A., Sexual orientation and the size of the anterior commissure in the human brain, *Proc. Natl. Acad. Sci. USA*, 89 (1992) 7199–7202.
- 3 Bailey, J.M. and Pillard, R.C., A genetic study of male sexual orientation, *Arch. Gen. Psychiatry*, 48 (1991) 1089–1096.
- 4 Bailey, J.M., Pillard, R.C., Neale, M.C. and Agyei, Y., Heritable factors influence sexual orientation in women, *Arch. Gen. Psychiatry*, 50 (3) (1993) 217–223.
- 5 Brand, T., Kroonen, J., Mos, J. and Slob, A.K., Adult partner preference and sexual behavior of male rats affected by perinatal endocrine manipulations, *Horm. Behav.*, 25(3) (1991) 323–341.
- 6 Brown-Grant, K. and Raisman, G., Abnormalities in reproductive function associated with the destruction of the suprachiasmatic nuclei in female rats, *Proc. R. Soc. Lond. B.*, 198 (1977) 279–296.
- 7 De Jonge, F.H., Muntjewerff, J.W., Louwerse, A.L. and Van de Poll, N.E., Sexual behavior and sexual orientation in the female rat as a function of hormonal treatment during various stages of development, *Horm. Behav.*, 1 (1988) 100–115.
- 8 De Jonge, F.H. and Van de Poll, N.E., Relationships between sexual and aggressive behavior in male and female rats: effects of gonadal hormones, *Progr. Brain Res.*, 61 (1984) 283–302.
- 9 Ehrhardt, A.A., Meyer-Bahlburg, H.F.L., Rosen, L.R., Feldman, J.F., Veridiano, N.P., Zimmerman, I. and Mc Ewen, B.S., Sexual orientation after prenatal exposure to exogenous estrogen, *Arch. Sex Behav.*, 14 (1985) 57–75.
- 10 Eliasson, M. and Meyerson, B., Development and approach behavior in male laboratory rats, *J. Consult. Clin. Psychol.*, 95 (1981) 160–165.
- 11 Fadem, B.H. and Barfield, R., Neonatal hormonal influences on the development of proceptive and receptive feminine sexual behavior in rats, *Horm. Behav.*, 15 (1981) 282–288.
- 12 Goudsmit, E., Fliers, E. and Swaab, D.F., Testosterone supplementation restores vasopressin innervation in the senescent rat brain, *Brain Res.*, 473 (1988) 306–313.
- 13 Gray, G.D., Södersten, P., Tallentire, D. and Davidson, J.M., Effects of lesions in various structures of the suprachiasmatic-preoptic region on LH regulation and sexual behavior in female rats, *Neuroendocrinology*, 25 (1978) 174–191.
- 14 Hetta, J., Sexual motivation in the male rat. A methodological study of sex-specific orientation and the effects of gonadal hormones, *Acta Physiol. Scand.*, (1978) suppl.453.
- 15 LeVay, S., A difference in hypothalamic structure between heterosexual and homosexual men, *Science*, 253, (1991) 1034–1037.
- 16 Meyer, J.H. and Rietveld, W.J., The neurophysiology of the suprachiasmatic pacemaker in rodents, *Physiol. Rev.*, 69 (1989) 671–707.
- 17 Meyerson, B.J., Eliasson, M. and Hetta, J., Sex-specific orientation in female and male rats: development and effects of early endocrine manipulation, *Adv. Biosci.*, 25 (1979) 451–460.
- 18 Moore, R.Y., The organization of the human circadian timing system, *Progr. Brain Res.*, 93 (1992) 101–117.
- 19 Moore-Ede, M.C., Sulzman, F.M. and Fuller, C.A., The clocks that time us. In *Physiology of the Circadian Timing System*, Harvard University Press, Cambridge, MA, (1982).
- 20 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, 2nd edn., 1986, Academic Press, Sydney.
- 21 Pratt, J.J., Wiegman, T., Lapphöhn, R.E. and Woldring, M.G., Estimation of plasma testosterone without extraction and chromatography, *Clin. Chim. Acta*, 59 (1975) 337–346.
- 22 Raisman, G. and Brown-Grant, K., The 'suprachiasmatic syndrome': endocrine and behavioural abnormalities following lesions of the suprachiasmatic nuclei in the female rat, *Proc. R. Soc. Lond. B.*, 198 (1977) 297–314.
- 23 Rusak, B. and Zucker, I., Neural regulation of circadian rhythms, *Physiol. Rev.*, 59 (1979) 449–526.
- 24 Segovia, S. and Guillemin, A., Sexual dimorphism in the vomeronasal pathway and sex differences in reproductive behaviors, *Brain Res. Rev.*, 18 (1993) 51–74.
- 25 Södersten, P., Hansen, S. and Srebro, B., Suprachiasmatic lesions disrupt the daily rhythmicity in the sexual behavior of normal male rats and of male rats treated neonatally with antioestrogen, *J. Endocr.*, 88 (1981) 123–130.
- 26 *SPSSX-X User's guide (3rd edn.)*, SpssX, 1989.
- 27 Stobie, K.M. and Weick, R.F., Effects of lesions of the suprachiasmatic and paraventricular nuclei on the inhibition of pulsatile luteinizing hormone release by exogenous vasoactive intestinal peptide in the ovariectomized rat, *Neuroendocrinology*, 51 (1990) 649–657.
- 28 Swaab, D.F., Fliers, E. and Partman, T.S., The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia, *Brain Res.*, 342 (1985) 37–44.
- 29 Swaab, D.F. and Hofman, M.A., An enlarged suprachiasmatic nucleus in homosexual men, *Brain Res.*, 537 (1990) 141–148.
- 30 Swaab, D.F., Roozendaal, B., Ravid, R., Velis, D.N., Gooren, L. and Williams, R.S., Suprachiasmatic nucleus in aging, Alzheimer's disease, transsexuality and Prader-Willi syndrome, *Progr. Brain Res.*, 72 (1987) 301–311.
- 31 Wiegand, S.J., Terasawa, E., Bridson, W.E. and Gray, R.W., Effects of discrete lesions of preoptic and suprachiasmatic structures in the female rat. Alterations in the feedback regulation of gonadotropin secretion, *Neuroendocrinology*, 31 (1980) 147–157.