Effects of Lesions of the Sexually Dimorphic Nucleus on Sexual Behavior of Testosterone-Treated Female Wistar Rats

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TURKENBURG, J. L., D. F. SWAAB, E. ENDERT, A. L. LOUWERSE AND N. E. VAN DE POLL. *Effects of lesions of the sexually dimorphic nucleus on sexual behavior of testosterone-treated female Wistar rats.* BRAIN RES BULL 21(2) 215-224, 1988.—Discrete bilateral lesions were placed into the sexually dimorphic nucleus (SDN) of the medial preoptic area (MPOA) of ovariectomized female Wistar rats, chronically treated with testosterone (T). Effects of these lesions upon masculine and feminine sexual behavior were studied by comparing the results of pre- and postoperative tests, using sham-operated and unoperated females as controls. Bilaterally-lesioned and, to a lesser extent, unilaterally-lesioned females, showed a marked and significant reduction of masculine sexual behavior (i.e., mounting), especially in the first postoperative tests. Feminine sexual responses, i.e., receptive and proceptive behavior, although slightly lower in bilaterally-lesioned females, did not change significantly. Sexual partner preference, operationalized as the choice between a receptive female and a sexually active male, remained unaffected by the lesions. Plasma levels of testosterone were similar in the various groups. It is concluded that the SDN may be functionally implicated in the control of masculine sexual behavior in T-treated females.

SDN lesions	Masculine sexual behavior	Feminine sexual behavior	Partner-preference	Female rats
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MANY different studies have implicated the medial preoptic area (MPOA) as a brain structure of central importance for the expression of sexual behavior. Lesions within this region markedly impair or abolish masculine sexual behavior in both male (8, 21, 28, 54) and female (25, 40, 48) rats. Comparable results with respect to masculine sexual behavior in males have been reported in other species, i.e., in the dog, cat and rhesus monkey (26, 27, 49). Lesions in the MPOA appear to facilitate feminine sexual responses, almost invariably operationalized as the occurrence of lordosis, in both female (37, 40, 43) and male rats (29,54). Electrical stimulation of the MPOA facilitated male rat's sexual behavior (38, 39, 56). Various studies indicated, moreover, that the MPOA may be an important target area for the activational effects of gonadal hormones on sexual behavior. Androgen and estrogen binding target cells have been identified within this area (47,50), implants of testosterone partially restoring sexual behavior in castrated male rats (7,12). The involvement of the MPOA in hormonal activation of masculine sexual behavior was also indicated by a study on electrical self-stimulation with electrodes in the MPOA. It was shown that

self-stimulation rates diminished following castration and were restored again after subsequent testosterone administration. The alterations in self-stimulation rates showed a close parallel with changes in masculine sexual responses in the same animal after castration and hormone treatment (53).

Within the MPOA the sexually dimorphic nucleus (SDN) has been identified (23,24). Analogous areas now have been described in several other species (9, 30, 52), including man (51). Sex hormones in development play a crucial role in the sexual differentiation of this nucleus in a similar way as for sexually dimorphic behaviors (31-33). Using sex hormone antagonists, Döhler found that the differentiation of the SDN is primarily under estrogenic and not under androgenic control (18). On basis of these results and those of earlier studies on sexual behavior (42) it was indirectly concluded by this author that the SDN would not be directly involved in the control of feminine sexual behavior and patterns of gonadotrophin release.

Only very few experiments until now examined functional aspects of the SDN. In male rats, lesions of the SDN, in contrast to lesions of the dorsal MPOA, were reported not

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to affect masculine sexual behavior (3). However, lesions of the SDN in male rats led to a significant decrement of plasma levels of FSH, LH and prolactin, suggesting the involvement of the SDN in the regulation of pituitary secretion of these hormones (45). In male gerbils, moreover, this nucleus appeared to be essential in the control of masculine sexual behavior as well as in scentmarking behavior (10). An indication that the SDN might be involved in male mating behavior in rats comes from a study which related individual variation of the volume of the SDN to masculine sexual activity in males (2). Prenatal stress reduced the volume of the SDN as well as behavior in this study. A significant positive correlation of masculine sexual behavior and SDN volume was noted in both stressed and unstressed males.

Research on the possible involvement of the SDN in the female rat's sexual behavior has not been undertaken so far. Female rats, ovariectomized and chronically treated with testosterone, show frequent masculine sexual responses (mounting) in the presence of a receptive female but also feminine sexual responses (receptive and proceptive behavior) if approached by a sexually active male (14, 16, 55). Repeated confrontations with receptive females have been shown not only to markedly increase mounting rates but also to lead to a clear and significant sexual preference for receptive females, in contrast to the normally present preference for males (14). In the present study effects of discrete lesions in the SDN upon various aspects of behavior in chronically testosterone-treated female rats were studied.

METHOD

Animals

Thirty-six virgin female Wistar rats, aged 3 months and weighing 250–300 g, were obtained from the central animal supply house T.N.O. Zeist (The Netherlands). At arrival, the animals were ovariectomized and given a subcutaneous implant under fentanyl anesthesia (Hypnorm® 0.05 ml; 10 mg Flumison/ml, IM). The implant consisted of a 2.5 cm long silastic tube (Dow Corning Medical Products 0.62 in o.d. 0.095 in i.d.) packed with testosterone (Steraloids Wilton). The animals were maintained in groups of 6 per cage under a 12/12 hr reversed dark-light cycle (lights off: 1.30 a.m.–1.30 p.m.) with food and water available ad lib. Behavior testing started after a 3-week period for recovery from ovariectomy and adaptation to the housing conditions.

Procedure

Behavioral tests were carried out before and after brain surgery. Starting 2 weeks after ovariectomy and peripheral testosterone implantation, 2 pretests for masculine sexual behavior were run. These tests, at which sexual activity is commonly relatively low (13,14), served to establish reliable and stable levels of mounting and were not used for analysis of the lesion results. Subsequently, preoperatively as well as postoperatively, 4 tests for masculine sexual behaviour were conducted at regular intervals of 3–4 days. The first and third of these tests were followed by a test for receptive behavior, which resulted in 2 pre-, and 2 postoperative tests for receptivity. Finally, in a subsequent week, all animals were tested for proceptive behavior (1 test pre-, and 1 test postoperatively).

Partner preference was tested in the last preoperative week and as the first postoperative test, after 2 weeks of recovery, thus minimizing the interval and interfering test

experience in between these 2 tests. The rationale for minimizing this interval is that it has been well established that sexual experience interacts with partner preference. Testosterone-treated females frequently tested for masculine sexual behavior with receptive females appear to develop consistent preference for females instead of males (14).

Before brain surgery the animals were subdivided into three groups: a lesion-group (n=23), a sham-operated group (n=7) and a control-group (n=6), that was not stereotactically operated. Care was taken to balance the groups with respect to the results of the preoperative tests for masculine sexual activity and receptivity. The three groups appeared to be equally divided over the group-cages which remained unchanged throughout experimentation. After surgery, the animals were given two weeks to recover.

Behavioral Testing

Tests for *masculine sexual behavior* were conducted in semicircular (r=25 cm) cages illuminated by dimmed red light during the last 4 hours of the dark period. The floor of the cages was covered with sawdust. The animals were tested for masculine sexual activity with stimulus Wistar females, brought into behavioral estrous by estradiol benzoate (50 µg) and progesterone (1 mg) administration (IM), 48 and 17 hours before testing. Experimental females were allowed to adapt to the test environment for 5 minutes before a stimulus female was brought into the test cage. During the following 10 minutes latency to the first mount and frequency of mounts were scored. Mounts were only counted as such when accompanied by pelvic thrusting. Intromission patterns were included in the total mount score.

Tests for *receptive behavior* (lordosis) were conducted in the same observation cages as described above. Sexually highly active males approximately 8 months old and weighing 400–500 g were used to elicit feminine sexual responses. These males, of proven sexual vigor, were allowed to adapt to the test environment for 5 minutes. Thereupon the experimental female was brought into the test cage and the stimulus male was allowed to mount 6 times. Only male mounts involving pelvic thrusting were counted as such and testing did not finish before the criterion of 6 mounts was reached. Lordosis behavior, as a response to mounts of the males, was scored as “no response” (0), “L1” when there was a slight lordosis and head lifting and “L2” when there was full arching of the back and sharp tilting of the head. The lordosis rating as a measure for receptivity was calculated as follows: $L.R. = (nL1 + (2 \cdot nL2) / 12 \cdot 100\%$. The differentiation of lordosis into “L1” and “L2” and the subsequent calculation of the lordosis rating was used in earlier work and provides a more precise quantification of receptive responding (16).

Tests for *proceptive behavior* were conducted in large observation-cages (80×50 cm), the floor being covered with sawdust. A sexually active stimulus male and an experimental female were tested for 10 minutes. All tests were recorded on video tape and scored using a key-board scoring system (57), which allowed for quantification of frequencies of mounts, intromissions and ejaculations (for the males) and frequencies of lordosis and proceptive elements of behavior (“present,” “hopping” and “darting”) of the female (16,17).

Tests for *sexual orientation* (partner-preference) were conducted in semiopen field cages consisting of an open field arena (80×80×35 cm) with two small boxes (15×15×12 cm) positioned opposite to each other. In these boxes stimulus

FIG. 1. Photomicrographs of sections through the brain showing a representative bilateral lesion of the SDN (right) of an experimental female and a comparable section (left) of a control female. The SDN of the control female is visible as a dark area at both sides of the third ventricle. Coronal sections were cut at $6\ \mu$ and stained with thionin.

animals could be placed (15), a metal gauze partition separating these animals from the experimental animal. Both animals could see and smell each other without physical contact. Small areas ($25 \times 25\text{ cm}$) in front of the stimulus compartments were balanced upon microswitches which activated electronic counters to record frequencies and duration of visits of the experimental animals to the stimulus animals. In the present experiment the 2 stimulus animals were always a sexually active male and an ovariectomized Wistar female, brought into estrous by the same treatment as specified for stimulus females (see above). The relative position of these incentives was varied between subjects to correct for possible position effects. Prior to testing, experimental females were allowed to adapt to the semiopen field during 10 min on 2 consecutive days. Tests lasted 15 min and were started by placing a female in the middle of the semiopen field. After each test the apparatus was thoroughly cleaned.

Surgery

The animals were placed in a Kopf stereotact and oper-

ated under Fentanyl anesthesia (Hypnorm® 0.05 ml/100 g, 10 mg Flumison/ml, IM). The incisor bar was placed at -3.0 mm , the vertical angle was at 1° . Bilateral lesions were produced using thermocouple-electrodes (Radionics T.C) with a tip-diameter of $0.30\text{--}0.34\text{ mm}$. The electrode was insulated except for 0.5 mm at the tip. Lesions were made by a radiofrequency alternating current apparatus (Radionics Inc., model RFG-4A). The electrodes were, one after the other, placed in the MPOA using the following coordinates: 0.4 mm posterior to bregma, 1.8 mm lateral and 7.9 mm below the dura. To produce a discrete lesion of the sexually dimorphic nucleus tissue temperature at the tip was maintained at 63°C for 60 sec, after which the electrode was held in position for another 60 sec. Sham procedures were identical except that no current was passed. During this operation the testosterone implant of each animal was renewed. The control animals also received replacement of the implant under the same anesthesia as described for lesioned and sham-operated animals but the skull was not opened. After two days of postoperative isolation all animals were placed in their original cages.

FIG. 2. Schematic reconstruction of the size and locus of lesions of the SDN in a coronal section of the König and Klippel atlas of the rat brain (Fig. 21b). All lesions fall within the hatched area. The smaller dotted area presents the size of representative lesions. The SDN is indicated by the two black spots near the border of the third ventricle.

Autopsy Procedures

Upon completion of all behavioral tests the animals were injected with pentobarbital (Nembutal 0.5 ml/rat IP). One cc blood was taken by heartpuncture and collected in chilled, heparinized plastic tubes to determine plasma testosterone and check for the functional activity of the subcutaneous implant. After centrifugation (1250 g, 15 min, +10°C), 0.5 ml plasma samples were stored at -80°C until assayed for testosterone according to the method described by Pratt *et al.* (44), using a coat-a-count kit from Diagnostic Products Corporation, Los Angeles, CA. The lower limit of detection for plasma testosterone was 0.1 ng/ml.

All animals were then perfused intracardially with saline containing 0.04% NaNO₂ followed by 500 ml fixative (1% paraformaldehyde, 2.5% glutaraldehyde, 2.5% cacodylate-buffer, pH=7.6). The brains were removed and stored in fixative at 4°C for one day. Subsequently the brain was dehydrated and embedded in paraffin. Serial 6 µm frontal sections were cut, mounted on chrome-alum-coated slides and stained with thionin. The location and approximate extent of the lesions was determined and drawn according to König and Klippel (36).

Statistics

The data were subjected to analysis of variance with operation (Factor OP, three levels: bilateral, unilateral lesions and control, see the Results section), and test (Factor TE, two levels: pre- versus postoperative test, repeated measure), as factors. If animals were tested more than once pre- and postoperatively (mounting: 4 tests, receptivity: 2 tests), then these results were included as a second, repeatedly measured variable: factor RE. In order to improve homogeneity of variances, a square root (on frequency

measures) or log (x+1) (on latencies) transformation was applied. No analysis was performed if the $F_{\max}$ -ratio exceeded a value of $p < 0.05$ (59).

RESULTS

Histological Analysis of the Lesions and Experimental Groups

All lesioned animals (n=23) showed small, bilateral lesions of similar size in the MPOA (see Figs. 1 and 2). The lesions were located midway between the anterior commissure and the optic chiasm and varied only slightly in anterior-posterior localization. The lesions never damaged the suprachiasmatic nucleus. Most of the animals showed damage to the ependym of the third ventricle and junctions between the left and right wall of the ventricle to a greater or lesser extent. However, hydrocephalus was never observed.

Two lesion groups were distinguished following histological analysis: 1) Eight animals demonstrating bilateral damage of the major part of the SDN (n=3) or total disappearance of the SDN (n=5). A reconstruction of these lesions is presented in Fig. 2. 2) Thirteen animals were found to have unilateral lesions of the SDN, most of the contralateral lesions being located posterior to the SDN within the MPOA (n=6), some slightly invading the anterior hypothalamus (n=5). Two of the unilaterally SDN-lesioned females had the contralateral lesion positioned too laterally, at the border of the lateral aspect of the preoptic area. Only two animals showed bilateral lesions that did not damage the SDN at all. In the first animal symmetrical lesions of equal size as those of the earlier mentioned groups were localized within the central part of the anterior hypothalamus. In the second animal only one lesion could be well localized, just laterally to the MPOA. The second lesion most probably fell within the third ventricle. Behavioral data of these animals were not used for statistical analysis and will be dealt with in a separate paragraph, together with a further reflection on the unilaterally lesioned group.

Behavioral results of the control- and sham-operated females were statistically compared with Analysis of Variance along the same procedure as used for comparison of the different experimental groups (see the Statistics section). No statistically significant differences in any aspect of observed behaviors were noted if the significance of the interaction OP×TE was taken as a criterion (all p -values >0.539). Both groups were therefore combined into one group (n=13), referred to as controls hereafter. Thus, the results were evaluated and presented on basis of the comparison of 1) a bilateral-lesioned group (n=8), 2) an unilateral-lesioned group (n=13) and 3) a control group (n=13). Mean (and S.E.M.) of testosterone levels for these three groups were: 1.8 (0.1), 1.8 (0.2) and 2.0 (0.3) ng/ml. No differences were noted in testosterone levels when the two lesion groups and the controls were compared.

Masculine Sexual Behavior

The results of the tests for masculine sexual behavior are presented in Figs. 3 and 4. In consequence of the experimental groups being assigned to the various treatments on basis of scores of preoperative testing, analysis of the 4 preoperative tests confirmed that mount frequencies of the three groups were not different if analysed over these four preoperative sessions [OP: $F(2,31)=0.38$, $p=0.690$]. Mount frequencies of all three groups increased in the course of the four tests [RE: $F(3,93)=9.59$, $p<0.001$]. No significant interactions were noted.

FIG. 3. Masculine sexual behavior: mean mount frequencies of bilaterally and unilaterally SDN-lesioned females and controls on 4 pre- and 4 postoperative tests of 10 minutes. Both lesioned groups differ from controls on the postoperative tests (for statistics see the Results section).

Analysis of Variance of the pre- and postoperative mount frequencies indicated that the three groups reacted highly different upon operation [OP $\times$ TE: $F(2,31)=5.15$, $p=0.012$]. The absence of significant interactions involving the factor retesting (RE) indicates that group differences are relatively stable characteristics [OP $\times$ TE $\times$ RE: $F(6,93)=1.35$, $p=0.242$; OP $\times$ RE: $F(6,93)=0.76$, $p=0.602$]. Postoperatively, differences between the three groups were highly significant [OP: $F(2,31)=8.84$, $p=0.001$]. Mount frequencies increased over the four tests [RE: $F(3,93)=4.77$, $p=0.004$] and the interaction with the factor operation did not reach significance [OP $\times$ RE: $F(6,93)=1.70$, $p=0.130$].

Further analysis of the postoperative results concentrated upon the comparison of both experimental groups (unilaterally- and bilaterally-lesioned females) with the controls. Bilaterally-lesioned females clearly differed from controls [OP: $F(1,19)=22.98$, $p<0.001$]. Although there was a significant effect of repeated testing, both groups showing increased mount frequencies [RE: $F(3,57)=4.35$, $p=0.008$], there was only a marginal interaction of operation and repeated testing [OP $\times$ RE: $F(3,57)=2.14$, $p=0.105$], which again confirms that the group differences were rather stable over the sessions.

Unilaterally-lesioned females differed from controls [OP: $F(1,24)=7.32$, $p=0.012$]. There was no significant effect of the factor repeated testing or the interaction of this factor with the operation to be noted (both p -values, $p>0.35$), indicating that the lesion effects remained to be seen on all four sessions. The comparison of the bi- and unilaterally-lesioned females is of special interest with respect to the question whether lesions have to be bilaterally to be effective (see the Discussion section). Analysis indicated that when all four postoperative sessions were compared, no significant effect was to be noted [OP: $F(1,19)=2.29$, $p=0.146$]. Both groups showed increased mounting frequencies as testing progressed [RE: $F(3,57)=4.20$, $p=0.009$] with a marginal interaction of these two factors [OP $\times$ RE: $F(3,57)=1.75$, $p=0.168$]. Inspection of Fig. 3 indicates that this interaction might result

from differences between the first and the second two postoperative tests. Indeed, statistically this suggestion was corroborated when analysis was applied to the first two postoperative tests only: OP: $F(1,19)=4.45$, $p=0.048$. This difference was absent in the last two tests (OP: $p>0.493$).

In general, mount latencies reflected and confirmed all group differences already established in the analysis of mount frequencies (Fig. 4). Mount latencies, although highly variable on preoperative Test 1, became quite consistent on the subsequent tests thereafter, and latencies did not reveal any differences between the three groups [OP: $F(2,31)=0.48$, $p=0.620$]. Postoperatively, a significant difference between the three groups was noted [OP: $F(2,31)=4.72$, $p=0.016$]. Bilaterally-lesioned animals had longer mount latencies than controls [OP: $F(1,19)=11.60$, $p=0.003$] Unilaterally-lesioned animals had intermediate latencies which were not significantly different from either controls [OP: $F(1,24)=2.10$, $p=0.160$] or bilaterally-lesioned females [OP: $F(1,19)=2.82$, $p=0.109$].

Feminine Sexual Behavior: Receptivity

Lordosis ratings (means of 2 pre- and 2 postoperative tests) are presented in Fig. 5. Analysis of variance of the three groups on the 2 pre- and 2 postoperative tests did not reveal any group-specific effects. It was further indicated in this material that the results of the 2 tests, preoperatively as well as postoperatively, were highly comparable. However, the postoperative tests, in general, were highly different from the preoperative tests [TE: $F(1,31)=10.16$, $p=0.003$], irrespective of the factor treatment [OP $\times$ TE: $F(2,31)=1.48$, $p=0.243$].

Separate comparisons of the bilaterally- and unilaterally-lesioned females with controls confirmed that there were no significant effects of these lesions on receptive behavior, although a marginal interaction of the factor OP and TE in the comparison of bilaterally-lesioned females suggested that receptivity was not increased in this group after operation. in

FIG. 4. Masculine sexual behavior: mean mount latencies of bilaterally and unilaterally SDN-lesioned females and controls on 4 pre- and 4 postoperative tests of 10 minutes (for statistics see the Results section).

FIG. 5. Feminine sexual behavior: means and S.E.M.'s of receptive responses calculated as lordosis ratings of bilaterally (b), unilaterally (u) SDN-lesioned females and controls (c) of 2 pre- and 2 postoperative tests (for statistics see the Results section).

contrast to controls [OP×TE: $F(1,19)=3.12$, $p=0.093$]. No such effect was seen in unilaterally-lesioned females [OP×TE: $F(1,24)=0.28$, $p=0.600$].

Feminine Sexual Behavior: Proceptivity

Both masculine behavior of the stimulus male and feminine sexual behaviors of the experimental females were examined in tests for proceptivity: 1 preoperative and 1 postoperative test. A tendency to decreased masculine sexual activity was noticed in the males confronted with bilaterally SDN-lesioned females. This was reflected most clearly in increased intromission latencies and the lower number of ejaculating males. Statistically, however, no significant differences were found between the males tested against the different groups of females.

The frequencies of proceptive elements of behavior of the females showed a slight decrement in bilaterally- as well as unilaterally-lesioned females, in contrast to a postoperative increment in controls (Fig. 6). Analysis of the behavioral changes in the three groups revealed a marginally significant interaction of the factor treatment and test [OP×TE: $F(2,31)=2.88$, $p=0.071$]. Comparison of the uni- and bilaterally-lesioned females with controls failed to show clearly significant differences between controls on the one hand and both bilaterally-lesioned females [OP×TE: $F(1,24)=2.94$, $p=0.099$], and unilaterally-lesioned females on the other hand [OP×TE: $F(1,19)=3.81$, $p=0.066$], due to the large variance.

Sexual Orientation: Partner-Preference

For the comparison of lesion effects upon preference, the time spent near the stimulus male, divided by the total time spent near stimulus male and female, was calculated. The groups appeared to differ with respect to this measure of preference on the preoperative tests; these differences, however, were not significant (Fig. 7). If the absolute time spent near the male was compared with the female, a marginal difference was noted in the control group only, $t=1.93$, $p<0.08$; t -test for paired data. As the groups obviously could not be matched for this aspect of behavior, this difference presumably occurred by chance.

Analysis of the lesion effects indicated major differences between pre- and postoperative tests [TE: $F(1,31)=14.93$, $p=0.001$], however without group-specific effects [OP×TE: $F(2,31)=2.18$, $p=0.130$]. Inspection of the data indicates that in contrast to the preoperative data, postoperatively all groups preferred females to males, which is confirmed by statistical comparison of the time spent near females vs. time spent near males. The most drastic change occurred in the controls which postoperatively showed a statistically significant preference for females, $t=2.55$, $p=0.025$; t -test for paired data.

FIG. 6. Feminine sexual behavior: means and S.E.M.'s of frequencies of proceptive responses of bilaterally (b) and unilaterally (u) SDN-lesioned females and controls (c) of 1 pre- and 1 postoperative test with sexually active males (for statistics see the Results section).

Further Analysis on Basis of Anatomical Data

Unilaterally SDN-lesioned females, thus, showed significantly lower frequencies of mounting. As all these females had a contralateral lesion which did not invade the SDN, it could be of interest to further analyse this group on basis of the position of this lesion. Inspection of the histological material indicated that 6 animals of the unilaterally SDN-lesioned group had lesions within the MPOA, slightly lateral and posterior to the SDN. In 5 animals this lesion was positioned more posterior and invaded the anterior hypothalamus. A tentative comparison of behavioral changes of these two groups suggested that mount frequencies in animals in which damage was restricted to the MPOA were slightly more affected than those of the other group, but these results could not be statistically corroborated, due to the low number of animals: mean (and S.E.M) of the difference-scores of all 4 postoperative tests minus all 4 preoperative tests of these two groups: 3.5 (4.8), $n=6$ and 16.4 (11), $n=5$. No further conspicuous behavioral differences separated these two groups.

Of the two remaining animals with lesions not damaging the SDN at all, one animal was of special interest. In this animal symmetrical lesions were localized within the central part of the anterior hypothalamus. Analysis of behavior showed that mount frequencies of this animal were not affected: all frequencies of the four pre- and postoperative tests were slightly higher than the means of the controls. Scores for receptivity and proceptivity on the postoperative tests, in contrast, were low (25 and 0) as compared to those of all groups, analysed in this experiment. The behavioral data of the remaining animal did not markedly differ from those of the controls.

DISCUSSION

Discrete lesions in the sexually dimorphic nucleus of the medial preoptic area decreased masculine sexual behavior in ovariectomized testosterone-treated female rats, without influencing feminine sexual behavior or sexual orientation. A subdivision of the lesioned group into unilaterally- and

FIG. 7. Partner-preference: time spent near males as a percentage of the total time spent near males and females of bilaterally (b) and unilaterally (u) SDN-lesioned females and controls (c) on 1 pre- and 1 postoperative test (for statistics see the Results section).

bilaterally-lesioned animals was based upon the accuracy of the lesion. Both groups had markedly lower mount frequencies than controls which showed levels of mounting in the postoperative tests which were further increased as compared to the preoperative tests. Bilaterally SDN-lesioned animals differed from the unilaterally lesioned animals, in that significantly lower mount frequencies and longer mount latencies were noted in the first postoperative tests. Some recovery of the mounting frequencies of the bilateral group took place which led to the absence of differences between the two lesioned groups in the last postoperative test, five weeks after lesioning. It is important to note, however, that bilateral lesions resulting in total destruction of the SDN did not by any means abolish mounting in females receiving exogenous testosterone.

The control group showed high and stable levels of mounting in the four postoperative tests which were clearly higher than those of the last preoperative tests. An increase in mounting frequencies in (testosterone-treated) females with repeated testing has been found in many of our experiments (13,14). It is, however, difficult to find an explanation for the increase noticed in the present postoperative tests which might result from further increased experience within the social setting of the home-cage or from a process which progresses, even in the absence of testing (provided that testosterone remains present within the animal). The markedly lower mounting frequencies in females with small and selective SDN lesions and the fact that even unilaterally SDN-lesioned females differed significantly from controls, underlines the relative importance of this structure as part of the MPOA for masculine sexual behavior in the female rat. Functional aspects of the SDN proper are only investigated in a very few experiments exclusively concentrating on male subjects. Lesions located in the SDN of male rats did not affect masculine sexual behavior in contrast to comparable lesions in the dorsal aspect of the MPOA (3). It is not clear from this study, however, whether these lesions damaged the SDN bilaterally, which may be a critical aspect (vide supra). In male gerbils, however, lesions bilaterally destroying 25% to 100% of the sexually dimorphic area disrupted copulation to a significant extent (10). The specific significance of the SDN for the expression of masculine sexual

behavior in male rats is implicated, moreover, by studies relating the development and volume of this area to sexual performance. It was shown that masculine sexual activity is positively correlated to the volume of the SDN (2,63).

Former experiments on the MPOA almost invariably showed a decrement or abolition of sexual behavior in males (8, 21, 22, 28, 54). Implants of testosterone or estradiol resulted in an activation of masculine sexual behavior (7,12) and aggression (6), lesions disrupting this behavior (1) in male rats. Little attention was paid to the specific localization of the most relevant part of the MPOA as lesions generally were extensive and effects were only noted if the MPOA was bilaterally damaged to a significant extent (34). One anatomically well defined aspect of the MPOA is the localization of androgen and estrogen receptors, both in males and females (47,50). Autoradiographic localization of these hormone receptors in the male gerbil brain indicated the presence of a concentration within the SDN which appeared to play a role in sexual and scentmarking behavior (11,60). Lesions differentially disrupted these aspects of the male gerbil's behavior (10).

Scarce data thus far indicated that in females as in males, testosterone-activated masculine sexual behavior could be affected by lesions although the localization of the most vital part of the medial preoptic-anterior hypothalamic area (MPOA-AHA) remained uncertain (25, 40, 48). Lesions in the dorsal aspect of the MPOA were shown to be effective for attenuating masculine sexual behavior whereas lesions in the ventral aspects were not (40). The MPOA, in contrast to the AHA, was indicated as the most relevant area (48), whereas also a more general dispersement of neurons important for this behavior was suggested (25). The present results are not necessarily at odds with these earlier results, as it is indicated now that neurons may show a critical concentration in or around the SDN which is situated in the caudal part of the MPOA, and some of the lesions in the studies mentioned above may have afflicted damage to this area. Analysis of the data of unilaterally-lesioned females on basis of the position of the contralateral lesion, together with the absence of an effect in the animal with bilateral lesions within the anterior hypothalamus further substantiate the relevance of the importance of the MPOA.

Mount frequencies of the bilaterally lesioned females partly recovered, although differences between this group and controls remained at the final tests of the experiment. This is consistent with similar effects of small lesions in the MPOA in male rats, reported earlier (22). Bilaterality of inflicted damage was shown to be vital in males where lesions were placed on either side of the brain in two stages, and bilateral but not unilateral lesions eliminated sexual behavior (34). Long range effects and the absence of functional recovery was reported, provided that the lesion did not spare large portions of the MPOA. If recovery occurred, complete copulation took place within about a month although sexual performance remained inferior (28). The present finding of the strongest effect of these relatively small lesions if they were confined to the SDN, together with the results of the unilaterally SDN-lesioned females which all had the major part of their contralateral lesions falling within the MPOA, may suggest that not only the SDN part of the MPOA is important but that the MPOA, as a whole, plays a role in masculine sexual behavior.

In early studies it was reported that AHA lesions produce cessation of feminine sexual behavior (48) but more recent work reported an increase (46). MPOA lesions have been

reported to facilitate hormone activated feminine sexual responses in both male (54) and female rats (37, 40, 43), to be ineffective (48), or to suppress receptivity, if behavioral activation was accomplished with estrogen implants in the hypothalamus (4). According to a recent view, the potentiation of lordosis in males or females might be caused by an interruption of inhibitory influences originating in the septal area and passing down through the MPOA to the lower brain stem (35, 46, 61, 62). Septal lesions were reported to increase the female rat's behavioral sensitivity with respect to receptivity to both estradiol and testosterone (41). The present results failed to show clearcut effects of SDN lesions upon receptive behavior in testosterone-treated females. The marginal difference in receptivity between bilaterally- and unilaterally-lesioned females, bilaterally-lesioned females failing to show the increase which was noted in unilaterally-lesioned females and controls, however, together with a similar tendency of effects upon proceptivity in both lesioned groups, might be taken as enough evidence to study feminine sexual behavior more thoroughly in future experiments on the SDN and MPOA.

Recently it was indicated that the higher lordosis quotients noted in MPOA-lesioned females are not due to a lesion-induced potentiation of the female's motivation to engage in sexual interaction as proceptive responses were actually reduced if a proper test-situation (i.e., one in which the female could leave the vicinity of the male) was created (20,58). Frequencies of proceptive responses in the present SDN-lesioned females were only marginally affected, both unilaterally- and bilaterally-lesioned females showing a slight reduction of proceptivity whereas the controls showed an increase. If the behavior of the male in these tests is taken as an additional indicator of the female's proceptivity (males tested with bilaterally-lesioned females appeared to be less sexually active), perhaps indeed lesions in the SDN might reduce feminine sexual proceptivity although the effects remained small. It is important to note that all males did mount and have intromissions with these females sufficiently frequently to stimulate significant amounts of receptive and proceptive responses in all females of all groups.

It was recently established that male rats bearing lesions in the MPOA not only abolish sexual behavior but also decrease preference for a receptive female as a motivational factor of behavior (19). Testosterone-treated female rats, frequently tested for masculine sexual behavior, generally develop a preference for receptive females if given the choice between a sexually active male and a receptive female (14). Although partner-preference, as measured preoperatively in the present experiment, did not replicate this finding for all three groups, it was clear that after operation all females showed preference for females and no differences were noted between the groups. These results indicate that even in the group of bilaterally-lesioned females which showed significantly reduced mount frequencies, sexual preference remained unaffected.

In summary, the SDN of the female rat appears to be selectively involved in masculine sexual behavior. The way in which the SDN acts upon this behavior in males apparently needs to be further investigated.

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