Developmental and Functional Aspects of the Human and Rat Sexually Dimorphic Nucleus of the Preoptic Area

F.H. De Jonge\textsuperscript{a}, D.F. Swaab\textsuperscript{a}, M.P. Ooms\textsuperscript{b}, E. Endert\textsuperscript{c},
N.E. Van de Poll\textsuperscript{a,1}

\textsuperscript{a}Netherlands Institute for Brain Research, Amsterdam; \textsuperscript{b}Department of Endocrinology, Erasmus University, Rotterdam, and \textsuperscript{c}Laboratory of Endocrinology, Faculty of Medicine, University of Amsterdam, The Netherlands

Sexual differentiation of reproductive physiology and behavior in mammals is primarily under the control of gonadal hormones that act upon the brain during a critical period early in development [1]. In the adult animal, the medical preoptic area (MPOA) in particular has been implicated as a structure of central importance for the expression of these functions. Studies using different experimental approaches such as lesions and electrical stimulation have implicated the MPOA in the regulation of masculine sexual behavior, feminine sexual behavior and gonadotropin release in several species [2–6].

Within the MPOA of the rat, the sexually dimorphic nucleus (SDN-POA), being 3–8 times larger in volume in males as compared to females, has been identified and first described by Gorski et al. [7, 8], and analogous sexually dimorphic structures now have been identified in a variety of species such as the rat, gerbil, guinea pig, ferret, toad and quail [7–16]. Sexual differentiation of this nucleus is controlled by perinatally circulating androgens, at least in rats, gerbils and guinea pigs [8–10, 17, 18]. Circulating hormones in adulthood influence SDN-POA size to a minor extent in these species [11, 19–21], but possibly are more important, in species such as the quail [13], toad [12] and ferret [15]. It is therefore shown that sexual

\textsuperscript{1} We wish to thank H. Stoffels and G. van der Meulen for preparing the figures and M.A. Hofman for his comments on the manuscript.
differentiation of SDN-POA size, at least in mammals, is controlled by the same factors that control the development of sexually dimorphic functions, and neuroanatomical sex differences within the MPOA might therefore underly sex differences in reproductive physiology or behavior.

There are only a few studies investigating neuroanatomical sex differences in the human [22–28]. However, we recently discovered a sexual dimorphic cell group in the human hypothalamus (SDN-POA), corresponding to the intermediate nucleus as described by Braak and Braak [29] which, according to its sexual dimorphism, its location within the MPOA and its location relative to the supra-chiasmatic nucleus (SCN) and paraventricular nucleus (PVN), can be seen as the human analog of the SDN-POA as described in several mammalian species. We will describe here the ontogeny of the human SDN-POA, and we will discuss these data in relation with data obtained in animal studies. In addition, we investigated in the rat the possibility that the conspicuous anatomical sex difference in SDN-POA volume might underly sex differences in reproductive behavior and gonadotropin release.

*Ontogeny of the SDN-POA in the Human Hypothalamus*

SDN-POA volume, cell number, cell density and nuclear cell size were measured in 100 human brains coming from the autopsy of subjects ranging from fetal week 22 up to 93 years. Included in these subjects were 9 men without overt signs of neurological disorder, who died from AIDS and were homosexual according to own reports, and 11 subjects that had been diagnosed clinically and pathologically as suffering from Alzheimer’s disease (see table 1 for specification of subjects). General procedures used are described in detail elsewhere [24, 28]. In short, the hypothalamic area was dissected, dehydrated and embedded in paraffin, following 1 month of fixation in formaldehyde. Serial 6-μm frontal sections were cut and area measurements were performed on every 25th section of the SDN-POA and every 10th section in its most rostral and caudal parts. The number of SDN-POA cells per unit volume (= cell density) was estimated by counting the total number of nuclear profiles per unit in the center of the SDN-POA followed by a discrete unfolding procedure [29].

In figure 1, total SDN-POA cell number (= cell density × SDN-POA volume) is presented as a function of age (log-log scale) for 55 males and 45 females. As illustrated, the human SDN-POA could already be distinguished
Table 1. Specification of subjects used in the developmental study on the human SDN-POA (see text for further explanation)

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>Males</th>
<th></th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>reference group</td>
<td>homosex. (AIDS)</td>
<td>Alzheimer</td>
<td>total</td>
<td>reference group</td>
<td>Alzheimer</td>
<td>total</td>
<td></td>
</tr>
<tr>
<td>A &lt; 10</td>
<td>17</td>
<td></td>
<td>17</td>
<td></td>
<td>15</td>
<td></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>B 10–20</td>
<td>3</td>
<td></td>
<td>3</td>
<td></td>
<td>5</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>C 20–40</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>16</td>
<td></td>
<td>6</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>D 40–60</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>10</td>
<td></td>
<td>6</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>E 60–80</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td></td>
<td>3</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>F 80–100</td>
<td>3</td>
<td></td>
<td>3</td>
<td></td>
<td>5</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>9</td>
<td>4</td>
<td>55</td>
<td>38</td>
<td>7</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Development and sexual differentiation of the human SDN-POA (log-log scale). Note that at the moment of birth the SDN-POA is equally small in boys (▲) and girls (○). □ = Homosexual men who died from AIDS. The curves are quintic polynomial functions fitted to the data of 55 males (drawn line) and 45 females (dashed line) with F(5, 49) = 10.05, p < 0.001 and F(5, 39) = 7.32, p < 0.001, respectively [from 28].
in the fetal brain around midpregnancy. SDN-POA cell number rapidly increased up to the age of 2–4 years postnatally for both boys and girls. It is only after this age that the human SDN-POA differentiated according to sex, due to a decrease in both SDN-POA volume and cell number in women. In men, the SDN-POA remained unaltered up to the age of about 50 after which a marked decrease in cell number was observed as well. At each stage of development, the SDN-POA volume followed a course similar to that of SDN-POA cell number. Significant differences in SDN-POA cell number were observed when males and females of age categories B to F (10–93 years) were compared (t test, two-tailed, \( p < 0.001 \)) and the same results were obtained when homosexuals and demented patients were included in the analysis. However, no sex differences in cell density or nuclear cell size were observed. Further analysis of the reference group from 10 to 93 years indicated that the nonlinear, sex-dependent aging of the human SDN-POA results in sex differences in SDN-POA cell number which are greatest at 10–50 years of age and 80–100 years of age, and smallest at 50–80 of age [see also 23].

By analogy with many mammalian species, the human brain is believed to undergo sexual differentiation during development due to an organizing effect of gonadal hormones [1, 27]. As can be seen from figure 1, however, sexual differentiation of the SDN-POA occurs after 4 years postnatally due to a loss in cell number in the female, well after the occurrence of the perinatal testosterone peak. This observation corroborates observations from animal experiments indicating that perinatal circulating hormones do not influence SDN-POA volume by affecting neurogenesis [9, 10, 17], and one developmental study in the rat showing that SDN-POA cell number decreased with increasing age in females, but not in males [31]. This and our observations therefore suggest the possibility that the perinatal testosterone peak promotes cell survival a few years later by preventing the 'preprogrammed' cell death which normally occurs in the female SDN-POA. Postnatal cell death leading to sexual differentiation seems to be a phenomenon that has in the hypothalamus only been observed in the SDN-POA, since SCN and PVN failed to show sexual dimorphism in the same subjects. Figure 2 illustrates this point by showing the PVN-, SCN- and SDN-POA volume of 16 human subjects of the reference group aged 20–40 (6 females and 10 males). Homosexuals were excluded from this analysis, since preliminary results from our lab suggest that extremely large SCNs may occur in this particular group of subjects [26]. In addition, volumetric data on the rat hypothalamus were used for interspecies comparison. These data, which are based
Fig. 2. Volumes of hypothalamic regions in young adult rats (right: 2–2.5 months) and in humans (left: 20–40 years). Values represent the mean volume (±SEM) from one hemisphere. The variance of the SCN volume in females could not be determined. For data sources and abbreviations, see text [from 23].

on paraffin-sectioned brain material (≤12 μm), were compiled from van den Pol [32] and Gülnder [33] for the SCN, from Hsu et al. [31] for the SDN-POA, and from Hsu and Peng [34] and Peng and Hsu [35] for the PVN.

Is the Human SDN-POA Sexually Dimorphic?

Recently, Allen et al. [22], using 60-μm serial sections, described four cell groups (INAH-1–4) within the human hypothalamus, two of these (INAH-2 and INAH-3) showing sexual dimorphism in volume. However, contrary to our results, INAH-1, which is, according to their description, the same cell group as the human SDN-POA described in our study, was not found to be sexually dimorphic, although a similar decrease in volume with aging was found. One of the reasons for discrepancy between results mentioned by the previous authors, is that the females of our sample as a whole were over 10 years older than the males. Therefore, according to the author's suggestion, the decrement in SDN-POA volume due to aging, might underly the sex differences observed in our sample. This is, however, unlikely to be the case,
since SDN-POA volumes of young adult males and females selected for a narrow age range (20–40 years, mean age 29 ± 0.43 for males and 33.5 ± 0.58 for females) differed significantly between the sexes as well.

It could also be speculated that overrepresentation of age-matched males and females from 50 to 80 years of age may obscure sex differences in SDN-POA volume which are clearly present at both a younger and an older age. Inspection of the sample of Allen et al. [22] shows indeed that almost half of their subjects are aged 50–80. However, also in the remaining subjects, no evident sex difference in SDN-POA volume is present. Alternatively, while sex differences can be observed in serial 6-μm sections as we used, sex differences may not be detectable in thicker, 60-μm sections. A third explanation for the different results might be found in the great variability in fixation duration in the sample of Allen et al. [22], which in particular affects volumetric measures, but not cell number.

*Functional Aspects of the Human SDN-POA*

The 9 homosexual men who died from AIDS were statistically compared with age- and sex-matched controls, since smaller SDN-POAs could be expected on the basis of indirect evidence from animal experiments: treatments that affect SDN-POA volume, like perinatal stress or neonatal hormonal manipulation, profoundly influence both sexual performance and sexual orientation [1, 36–38]. However, these homosexuals were not different from controls (Mann-Whitney U test, p > 0.2, see also fig. 1) and preliminary results on SDN-POA volumes of 5 additional homosexuals further corroborate this finding. The suggestion that homosexuals have a femal hypothalamus therefore seems unjustified.

*The SDN-POA and Reproductive Behavior in Male Rats*

Functional aspects of the SDN-POA have been investigated in only a few experiments until now. However, because of the sex difference in volume, its afferent and efferent connections [39, 40], and its presence within the MPOA, the SDN-POA has been hypothesized to be particularly involved in the regulation of reproductive behavior. Moreover, since perinatal hormonal manipulations affecting SDN-POA volume did not correlate with comparable changes in feminine sexual behavior [18], a possible involvement of the
SDN-POA in feminine reproductive behavior is less likely than in masculine reproductive behavior.

Masculine sexual behavior of 36, bilaterally SDN-POA lesioned, sexually naive male rats was therefore investigated in four biweekly tests for sexual behavior with a highly receptive and proceptive stimulus female. Stereotaxic surgery and behavioral testing were previously described elsewhere [5, 6, 41]. Sexually naive males were deliberately selected, since sexual experience is known to prohibit the manifestation of deleterious effects of manipulations on sexual performance in males [42].

After histological analysis of serial 6-μm paraffin sections, the following groups were discerned: (1) SDN-POA lesioned males with bilateral destruction of 50–100% of the SDN-POA (lesion SDN-POA >50%, n = 14); (2) SDN-POA lesioned males with minor bilateral damage to the SDN-POA (lesion SDN-POA <50%, n = 17); (3) lesioned males with the SDN bilaterally intact (incorrectly located lesion, n = 4, excluded from statistical analysis), and (4) control animals (CTL, n = 24), which included both sham lesioned and males that were only anesthetized (see figure 3 for representative coronal sections).

SDN-POA lesioned animals showed longer ejaculation latencies than controls on the first, but not on three subsequent tests (fig. 4, p < 0.05). However, no differences were observed between SDN-POA lesioned animals with major or minor damage to the SDN-POA. Ejaculation latencies of males with an incorrectly located lesion were well within the range of controls. In a similar way, SDN-POA lesioned males initially showed longer mount and intromission latencies than controls (data not presented), but all differences had disappeared on the fourth sex test. No differences in ejaculatory efficiency, postejaculatory interval, mount or intromission frequency were found. These data show that SDN-POA lesioned males show a deficit in masculine sexual behavior which, however (1) is independent from the extent of damage in the SDN-POA, and (2) rapidly disappears after repeated testing.

In order to investigate whether or not deficits in these SDN-POA lesioned males had really disappeared on the fourth sex test, a fifth test was run under suboptimal conditions. The males were therefore (a) not adapted to the test environment prior to testing, and (b) confronted with a stimulus female that was only marginally receptive and proceptive, being brought into artificial heat by a small dose of estradiol benzoate (EB 4 μg, i.m. [39]). SDN-POA lesioned male rats again showed longer ejaculation latencies than controls when retested under suboptimal conditions (fig.4, test 5, p < 0.05). These data therefore indicate that deficits in sexual behavior of SDN-POA
Fig. 3. Representative 6-μm paraffin-embedded serial sections of (a) an incorrectly lesioned male rat (SDN-POA intact); (b) SDN-POA lesioned male rat with >50% damage to the SDN-POA; (c) incorrectly lesioned female rat (SDN-POA intact), and (d) SDN-POA lesioned female rat with >50% damage to the SDN-POA. AC = Anterior commissure; OC = optic chiasm. Arrows indicate the intact SDN-POA (a, c) or the lesion sites (b, d). Small arrows indicate remaining cells of the SDN-POA (b).
Fig. 4. Ejaculation latency of SDN-POA lesioned male rats during tests with a maximally receptive and proceptive stimulus female (tests 1–4) and during a subsequent test with a marginally receptive stimulus female (test 5). Ejaculation latency has been set to the maximum possible value (i.e. 1,800 s) if ejaculation did not occur in that particular test. ○ = Lesion SDN-POA >50% (n = 14); △ = lesion SDN-POA <50% (n = 17); □ = SDN-POA not lesioned (n = 4); * = control (n = 24).

Lesioned male rats are long-lasting, but sensitive to experiential factors. Since deficits in sexual behavior were concentrated on the parameters mount latency, intromission latency and ejaculation latency, the data furthermore suggest that SDN-POA lesions in particular affected aspects of sexual arousal.

SDN-POA and Sexually Stimulated Gonadotropin Release

A recent study [44] showing inhibiting effects of lesions in the area of the SDN-POA on gonadotropin release in castrated male rats, suggests that SDN-POA lesions may disturb masculine sexual behavior through a deleterious effect on hormonal feedback mechanisms. However, analysis of basal plasma levels of testosterone (T) of our lesioned animals indicated no significant differences as compared to controls (Mann-Whitney, p>0.42). In order to find out whether hormonal mechanisms related to sexual activity
might be affected by SDN-POA lesions, we investigated whether SDN-POA lesions disrupt the sexually stimulated release of luteinizing hormone (LH), T and prolactin which were previously shown in several species to be associated with sexual arousal [45–47]. Trunk blood was collected from 22 sexually experienced, SDN-POA lesioned male rats with either major or minor damage to the SDN-POA, which were either confronted with a sexually receptive female (15 min) (sexually stimulated group, n = 14) or directly taken from their home cage (without sexual stimulation, n = 8) prior to decapitation. As illustrated in figure 5, plasma levels of T, LH and prolactin were significantly increased after sexual stimulation of SDN-POA lesioned male rats, well within the range of sexually stimulated hormone release previously reported [45, 46]. Although the data do not exclude the possibility that the sexually stimulated hormone release in SDN-POA lesioned animals is lower than in intact rats, they do not provide any evidence for the suggestion that deficits in sexually stimulated hormone release might underly deficits in sexual behavior of SDN-POA lesioned male rats.

The SDN-POA and Reproductive Behavior in Female Rats

In a second study, effects of SDN-POA lesions on masculine sexual behavior (mounting) in 36 female rats (250–300 g) were investigated. In order to increase basal levels of mounting behavior, these females were ovariectomized and subcutaneously implanted with a Silastic implant containing testosterone propionate. Four preoperative and 4 postoperative tests
Fig. 6. Mean mount frequencies of bilaterally (△) and unilaterally (□) SDN-POA lesioned female rats and controls (○) on 4 pre- and 4 postoperative tests of 10 min. Both lesioned groups differ from controls on the postoperative tests (ANOVA, p < 0.05) [from 41].

For mounting behavior (10 min) were run in test cages containing a highly receptive and proceptive stimulus female (fig. 6). Preoperative tests were run, since previous experiments indicated that this experience facilitates the expression of facilitating or inhibiting effects of various manipulations on mounting behavior in the female rat [48]. The following groups were based upon analysis of serial 6-μm paraffin sections: (1) lesioned females with bilateral damage of more than 50% of the SDN-POA (n = 8); (2) lesioned females with unilateral damage of more than 50% of the SDN-POA (n = 13); (3) incorrectly located lesion (n = 2, excluded from analysis) and controls, including sham-operated females and females that were only anesthetized (n = 13). From the unilateral lesioned females, 10 were lesioned on the right side, and 3 at the left side. No behavioral differences between these subgroups could be detected. Both bilaterally and unilaterally SDN-POA lesioned females showed inhibited mounting postoperatively (ANOVA, p < 0.05), but unilaterally and bilaterally lesioned females did not differ from each other [see also 41].

In addition to the tests for mounting behavior, tests for receptivity, proceptivity and sexual orientation (towards a sexually active male as opposed to an estrous female) were run, but no effects of SDN-POA lesions on these aspects of reproductive behavior were found [see also 41, 43, 48–50 for a more detailed description of these behavioral aspects].
Discussion

The present results indicate that SDN-POA lesions cause deficits in masculine, but not feminine sexual behavior in both male and female rats. Although it is impossible to differentiate between effects due to damage to the SDN-POA proper and effects attributable to damaged fibers of passage, the results suggest a particular involvement of the SDN-POA in masculine sexual behavior and are, therefore, in line with experiments in male gerbils, indicating that bilateral lesions disrupt masculine sexual performance and scent-marking [51]. Anderson et al. [36, 37], moreover, reported significant correlations between the volume of the SDN-POA, plasma T levels and sexual performance in male rats. However, our results are clearly at odds with those of Arendash and Gorski [52] in male rats, indicating that lesions just dorsal to the SDN-POA inhibit masculine sexual performance, while ablation of the SDN-POA proper was reported not to affect masculine sexual behavior. Sexually experienced male rats however were used in that study, and it was suggested that effects of the lesions might have been found if sexually naive males had been used. Indeed, the fact that normal male sexual performance was readily restored after repeated testing in our study, suggests that effects of SDN-POA lesions may indeed only become manifest in males that are sexually naive and/or tested under suboptimal conditions. This conclusion may seem to be in conflict with our results in females, in which masculine sexual behavior was inhibited in mount-experienced females. However, test procedures to be chosen for females are fundamentally different from those for males [43, 48–50] and the results therefore suggest that experienced as opposed to naive subjects is not a variable essential to the mechanism by which the SDN-POA may regulate sexual performance, but rather a methodological variable which influences the sensitivity of a particular test.

Further procedural differences between our study and that of Arendash and Gorski [52] includes the use of intact male rats in our study as opposed to castrated and T-implanted males in theirs. However, no indication so far suggests that SDN-POA lesions affect sexual behavior by disturbing hormonal mechanisms, since SDN-POA lesioned intact males showed basal T levels well within the range of controls, and a sexually stimulated hormone release not different from that previously reported [45, 46].

In our lesion study, we investigated whether neuroanatomical sex differences underlie sexual dimorphisms in behavior and the results indicate that the SDN-POA, which shows a conspicuous neuroanatomical sexual di-
morphism, is involved in the regulation of masculine sexual behavior, which is typically sexually dimorphic in nature. It should be stressed however that the deficits in sexual behavior of SDN-POA lesioned rats are only small relative to those seen in males after complete ablation of the MPOA, resulting in complete loss of sexual activity [46]. Although our results indicate a clear involvement of the SDN-POA in the regulation of masculine sexual behavior in male and female rats, they only show minor impairments in masculine sexual behavior and they do not, at present, give a satisfactory explanation for the conspicuous neuroanatomical sexual dimorphism in SDN-POA volume.

References


F.H. De Jonge, Netherlands Institute for Brain Research, Meibergdreef 33, NL-1105 AZ Amsterdam ZO (The Netherlands)