HYPOTHALAMIC SEXUAL PRECOCITY
IN FEMALE RATS OPERATED SHORTLY AFTER BIRTH

By
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ABSTRACT

Electrolytic lesions were placed in the anterior hypothalamus of 3–4 day-old female rats; vaginal opening was hastened in comparison with blank-operated littermates in 12 of 17 rats bearing a lesion in the basal supra- and post-chiasmatic area. In the animals with the earliest vaginal opening, lesions reached upward towards the region of the anterior commissure and the paraventricular nuclei. The degree of advancement of puberty in rats operated at the age of 3 or 4 days was similar to that caused by lesions made at 10, 14 or 15 days. This finding suggests that the effect of a lesion upon gonadotrophin secretion does not begin to take place until after the age of at least two weeks.

Clinicians have long been aware of the fact that sexual precocity may sometimes be related to intracranial disorders. As early as 1862 Kussmaul summarized two types of intracranial disease which had been shown to be associated with sexual precocity, one being rickets. Discussing this curious coincidence, Kussmaul suggested that the rhachitic process in the skull modified brain development and thereby advanced sexual maturation. The basic tenet of this hypothesis has recently received support from experimental investigations into the mechanisms which determine the time of onset of sexual maturation. These have given rise to the view that a maturation process takes place in the hypothalamus during childhood, which results in a decreasing sensitivity of the hypothalamus to the feed-back action exerted by gonadal hormones, via the hypothalamus, upon the adeno-hypophysis. It has been suggested that destruction (by pathological processes or experimental procedures) of the hypo-
thalamic region which is sensitive to gonadal hormones, could remove the inhibitory action upon the pituitary gland in early childhood and thus initiate gonadal maturation (Donovan & Van der Werff ten Bosch 1959).

The first experimental proof that cerebral lesions could accelerate sexual maturation in the rat was obtained after the placement of lesions in the anterior hypothalamus of rats aged 14 or 15 days (Donovan & Van der Werff ten Bosch 1956). It was subsequently shown that operations on 10-day-old rats would likewise advance sexual maturation (Donovan & Van der Werff ten Bosch 1959). The present experiment was carried out with the object of ascertaining whether lesions placed in the hypothalamus at the age of 3 or 4 days would be effective, and whether such lesions would advance puberty to a greater extent than lesions placed at later ages. The latter point seemed of particular interest in view of: a) the fact that ovaries are generally believed to be relatively insensitive to gonadotrophic hormones during the first ten days or so, and b) the problem whether a lesion is effective through some immediate triggering influence which sets off a chain of events once and for all, or through its permanent destruction of pathways which may not be utilized until some later date.

METHO DS

The rats used in this study were of the inbred albino Great Wistar strain which was also used in the second and third experiment of a previous study on the effect of cerebral lesions on sexual maturation (Donovan & Van der Werff ten Bosch 1959). The animals were born in 1960, in the months of January to May inclusive, and transferred, within four days of birth, to a room illuminated for eight hours per day by artificial light only, and with a constant temperature of about 24°C (range 22-27°C). Litters were left with their mothers until the age of four weeks. Food consisted of a standard commercial diet and water ad libitum. At about the time of puberty, the male and female young were separated. Female young were examined daily for vaginal opening from the age of three weeks.

Operations were carried out under ether anaesthesia on the third or fourth day of life. All the young of each litter were subjected to the operative procedure, i. e. alternate members of either sex receiving a lesion, whilst a blank-operation was performed in the others. In view of the small size of the head it was impossible to utilize the regular stereotaxic machine employed in the previous studies. Instead, a small head holder was constructed of wood which enabled fixation of the head in a reproducible manner; the animal thus fixed was placed vertically below a rack and a pinion carrying a platinum wire glass-insulated electrode with a bare tip of about 1 mm. After incision of the skin the electrode was lowered through the bregma over a distance of 6 mm. The neutral electrode was contained in cotton wool soaked in saline on which the rear end of the animal rested. Anodal lesions were made by passing 3 mA DC for 30 sec. The only difference of a blank-operation was that no current was passed while the electrodes were in position. After recovery from anaesthesia, litters were replaced with their mothers.

Subsequent follow-up of the animals was not uniform. Some of the rats were killed
on the day of vaginal opening, to enable a study of the ovarian histology, whilst others were kept alive longer for a study of the vaginal smears between the ages of two and three months; the second category of rats was killed at the ages of 82 to 98 days. The rats were killed with ether and the body weights were recorded and the tail length was measured by lowering the tail into calibrated glass tubing which was rested against the ischium to ensure constancy of a measuring technique. Ovaries and uteri were dissected and weighed fresh; in the older animals the adrenal glands were also weighed. The skulls were opened and the brain fixed in situ in 10% formalin, as were the ovaries and uteri. The brains of lesioned rats were embedded in low viscosity nitrocellulose, serially sectioned and stained with Luxol Fast Blue and cresyl violet (Klüver & Barrera 1953). Ovaries of lesioned rats were embedded in paraffin and sections stained with haematoxylin-eosin. Figures are presented as means ± standard error of the mean; significance of difference was calculated with Student's t-test.

RESULTS

Time of vaginal opening

Mean age at time of vaginal opening. – Twenty-two (approximately one-third of all rats thus operated) lesioned rats survived for a sufficiently long period of time; 5 of these are not included in the lesioned group described below because their hypothalami were intact, although the optic chiasma had sustained slight or severe damage. Ages at vaginal opening of these five rats were 39, 40, 43, 50 and 63 days respectively. Individual ages at time of vaginal opening of the remaining 17 lesioned rats and their 34 blank-operated litter-mates may be seen in Fig. 1. It is clear that vaginal opening was hastened in lesioned rats: on day 37 over 50% of the lesioned animals and only 2 controls had an open vagina. Mean age at vaginal opening was 45.0 days in the blank-operated controls and 40.6 days in 17 rats with a hypothalamic lesion. The difference of 4.6 days is significant at the 5% level.

The present study did not include intact, unoperated control animals, but data are available for 75 such rats which were reared under identical lighting conditions in the autumn of 1958; their mean age at vaginal opening was 47.1 ± 0.9 days (Van der Werff ten Bosch 1959). In comparison with these intact controls the blank-operations in the present experiment did not show a significantly advanced age of vaginal opening.

Influence of litter size on age at vaginal opening. – Litter size is known to influence the rate of somatic growth and of sexual maturation (Engle et al. 1937; Kennedy 1957; Widdowson & McCance 1960). Fig. 2 includes all the rats depicted in Fig. 1; the litter size is the total number of male and female members, blank-operated and lesioned, which survived operation and the immediate post-operative period. The figure demonstrates a tendency for blank-operated members of large litters to be older at the time of vaginal opening than blank-operated members of small litters. Although their numbers
### Fig. 1.

Ages at the time of vaginal opening in lesioned rats (identified by the numerals within the blocks) and their own blank-operated littermates (hatched blocks).

Dotted lines indicate average ages for all 17 lesioned (a, 40.6 ± 1.93 days) and all 34 blank-operated (b, 45.0 ± 1.00 days) rats included in this figure.

### Fig. 2.

The influence of litter size (= all surviving males and females in a litter) upon age at which the vagina opened in the rats included in Fig. 1.

Dotted lines indicate average ages for the blank-operated rats derived from litters of a size; these averages are 47.4 (8 in a litter), 45.7 (7), 43.3 (6), 43.0 (5) and 42.0 (4 in a litter) days.
are small, it would seem that the age at which the vagina opens in lesioned rats is not related to size of the litter in which the animals are reared.

**Body and organ weights**

Table 1 gives data collected at post-mortem. It may be seen that the average body weight and tail length are significantly smaller in lesioned rats killed at the time of vaginal opening (numbers 1, 2, 3, 4, 6, 7, 8, 10, 12, 13, 16) than in controls killed at that stage. It should be noted, however, that the lesioned rats were also younger when these data were obtained (mean age 38.5 vs. 42.3 days). From the body weight and tail length data obtained at the age of about three months, it appears that neither growth in length nor gain in weight seems to have been affected by the lesions. The ovaries were significantly smaller at the time of vaginal opening in lesioned than in control rats, although the relative weights of the ovaries were almost identical.

The individual uterine weights of these rats are plotted in Fig. 3. In both the lesioned and the control group these weights show great variations. Fig. 3 A shows the relative weights, in mg per 100 g body weight. The lesioned rats killed at this time had an average age of 38.5 days and an average relative uterine weight of 169 mg; the blank-operated animals averaged 42.3 days and their uteri weighed 117 mg. The difference in average uterine weight is due to the very heavy uteri (of over 230 mg) of four rats in the lesioned group. However, the four lesioned rats are amongst the six smallest in weight of their group. The absolute weights, in Fig. 3 B, do not show any differences between lesioned and blank-operated groups. This figure further suggests that the weights of the uteri at vaginal opening fall into two classes, a class with pre-

**Table 1.**

Data obtained at post-mortem of blank-operated and lesioned rats; figures are mean ± SEM.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Operation</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Tail length (mm)</th>
<th>Ovaries (mg)</th>
<th>Uterus (mg)</th>
<th>Adrenals (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28–55</td>
<td>blank</td>
<td>15</td>
<td>104 ± 3.4</td>
<td>137 ± 1.5</td>
<td>30 ± 1.8</td>
<td>121 ± 12.4</td>
<td></td>
</tr>
<tr>
<td>(time of</td>
<td>lesion</td>
<td>11</td>
<td><strong>72 ± 4.8</strong></td>
<td><strong>119 ± 4.2</strong></td>
<td><strong>17 ± 2.1</strong></td>
<td>118 ± 20.6</td>
<td></td>
</tr>
<tr>
<td>vaginal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>opening)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>82–98</td>
<td>blank</td>
<td>17</td>
<td>171 ± 6.7</td>
<td>*165 ± 1.3</td>
<td>65 ± 4.7</td>
<td>240 ± 25.1</td>
<td>42 ± 2.4</td>
</tr>
<tr>
<td>lesion</td>
<td>6</td>
<td>159 ± 8.2</td>
<td>163 ± 1.1</td>
<td>60 ± 10.6</td>
<td>159 ± 36.1</td>
<td>42 ± 3.6</td>
<td></td>
</tr>
</tbody>
</table>

* Of 16 rats only.
** Significantly different from controls (P < 0.001); for all others: P > 0.05.
Scatter diagram showing: A, relative weights of the uterus per 100 g body weight – and: B, absolute weights of the uterus, of rats killed at the time of vaginal opening. Black dots: 15 blank-operated rats; open circles: 11 lesioned rats. The high figures for 4 lesioned rats in A are due to the low body weight, their absolute values (in B) falling within the normal range. Note the »free« interval of about 40 mg between the classes of prepubertal weights and of post-pubertal weights in B.

Pubertal values which slowly increase with age (from about 30 mg in the younger to about 100 mg in the older animals) and a class with post-pubertal values (over 100 mg) which likewise seem to increase with age.

No difference occurred in ovarian weights at the age of three months. The apparent difference in weight of uteri of these older animals is due to the extremely small uteri of two lesioned rats (no. 15: 69 mg, no. 17: 60 mg).

The weights of the adrenal glands failed to show an effect of the lesions upon this organ.

**Ovarian histology and vaginal smears**

Histological examination of the ovaries of 10 lesioned rats killed on the day of vaginal opening (those of the eleventh animal got lost) showed normal complements of maturing and mature follicles and corpora lutea in 7 instances (numbers 2, 3, 6, 7, 10, 13, 16). In 3 cases mature follicles were found but no corpora lutea (numbers 4, 8, 12). Early vaginal opening had thus been associated with early follicular maturation in all rats whereas luteinization, and presumably ovulation, had occurred in over half of them.
Vaginal smear studies of 6 lesioned rats showed regular cycles in numbers 5, 11 and 14; these animals had normal ovaries. Vaginal smears were almost continuously oestrous in rat 9 and almost continuously dioestrous in numbers 15 and 17. The ovaries of these three rats contained numerous maturing and mature follicles but had no corpora lutea; their uteri were very small (no. 15 and 17: see above) or relatively small (135 g in number 9).

Localization of brain lesions

The lesions were remarkably constant in size and localization, considering the small size of the brain at the time of operation. Table 2 summarizes the degree to which various structures which serve as landmarks in the region of the anterior hypothalamus were involved. The rats are arranged in the order in which they showed vaginal opening. It is clear from this table that damage to none of the structures listed can be regarded as essential by itself for the production of precocious vaginal opening. The table does show, however, that the earliest vaginal opening occurred in rats bearing a lesion which extended upward towards the anterior commissure and the paraventricular nuclei. In all rats the lesion caused major damage to the tissue between the optic chiasma and the anterior commissure; a very uniform type of lesion (Fig. 4) damaged
the midline and adjacent tissue between the optic chiasma and the anterior commissure in eight rats (1, 3, 8, 9, 12, 13, 15, 17), a small midline lesion was noted in six (4, 5, 7, 10, 14, 16) while the lesion in this area was predominantly unilateral in three rats (2, 6, 11). The majority of the lesions also involved basal pre-optic structures, unilaterally (2, 6, 13) or in the midline (3, 4, 7, 9, 12, 16).

DISCUSSION

The concurrent existence of cerebral pathology and true sexual precocity in children has often been described (Bing et al. 1937; Weinberger & Grant 1941; Lange-Cosack 1951, 1952; Bauer 1954; Benoit & Assenmacher 1955; Jolly 1955; Klees & Fetzer 1957; Wilkins 1957; Schmidt et al. 1958; Van der Werff ten Bosch 1959), especially with pathological changes posteriorly in the hypothalamus (Driggs & Spatz 1939; Weinberger & Grant 1941; Bauer 1954; Morley 1954). The first attempts at mimicking the clinical condition by placement of experimental lesions in the brain were therefore focussed on the mammillary region. These studies, on infantile rabbits, proved negative (Bustamante et al. 1942; Gaupp 1950), which is understandable in the light of more recent work on the rabbit (Saul & Sawyer 1957; Sawyer et al. 1960).

Lesions in the anterior hypothalamus of Lister rats aged 14 or 15 days caused early vaginal opening and early luteinization (Donovan & Van der Werff ten Bosch 1956), whilst further work showed that similar lesions placed in Wistar rats at ages 10 or 14 days again advanced the time of vaginal opening whereas preoptic lesions were not effective (Donovan & Van der Werff ten Bosch 1959). These findings have been confirmed by several investigators. Bogdanove & Schoen (1959) placed anterior lesions in rats aged 18–19 days and noted two varieties of precocious sexual development when the rats were killed 10–15 days later: corpora lutea were found only in rats bearing lesions which involved the arcuate nucleus. Elwers & Critchlow (1960) also placed anterior lesions, in 18–20 day-old rats, and killed them at the age of 33 days. At death 5 of 9 rats considered to possess effective lesions had an open vagina; the present work (see Fig. 3) sheds some doubt on the validity of the criterion used by these workers for the establishment of effective lesions.

The work reported here concerns rats operated at a very early age, the third or fourth day after birth. Of 17 rats which received a lesion in the anterior hypothalamus 12 displayed vaginal opening before any of their own blank-operated littermates. From Table 2, in which the animals are arranged in order of age at vaginal opening, it appears that seven of the eight earliest animals had sustained damage to the fornix or the paraventricular nuclei. The lesioned area that all rats shared, was the basal hypothalamic tissue just

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Table 2.

Summary of structures damaged by lesions (+, slightly damaged; ++, markedly damaged; ++++, completely destroyed). Double sets of symbols indicate bilateral injury.

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Optic chiasma</th>
<th>Anterior commissure</th>
<th>Suprachiasmatic nuclei</th>
<th>Supra-optic nuclei</th>
<th>Fornix</th>
<th>Paraventricular nuclei</th>
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<tr>
<td>1</td>
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<td>++++</td>
<td>++++</td>
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<td>11</td>
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</tbody>
</table>
above the caudal portion of the optic chiasma. In an earlier study (Donovan & Van der Werff ten Bosch 1959, expt. 2 A) four rats with lesions wholly or almost wholly confined to the dorsal part of the anterior hypothalamus had an average age of vaginal opening similar to blank-operated controls; in the only one of these rats that showed early opening of the vagina the lesion had completely destroyed one paraventricular nucleus. Gellert & Ganong (1960) placed lesions in the brain of 24-day-old rats and found that when these were situated in the anterior hypothalamus they had no effect on the age of vaginal opening or of the first oestrus. The description and demonstration of these lesions suggest that they did not include the basal tissue and that they were situated more caudally than the lesions which have been found to be effective by other workers.

Lesions outside the anterior hypothalamus may also cause sexual precocity in the rat; the two structures implicated so far are the medial portion of the amygdaloid complex (Elwers & Critchlow 1960) and the arcuate nucleus in the posterior tuberal region of the hypothalamus (Gellert & Ganong 1960). The various effective lesion sites may constitute different sections of a single nervous pathway, or different pathways which converge onto a final common pathway, e.g. the arcuate nucleus. Elwers & Critchlow (1960) commented that "the effective lesions in the amygdala centered in the converging fiber contributions to the stria terminalis. In addition, the extensive 'effective' region in the anterior hypothalamus corresponds approximately to the diffuse terminations of this fiber tract in the diencephalon".

A point of particular interest is the fact that lesions of identical localization placed at different ages cause an approximately identical advancement of the age at vaginal opening. Table 3 summarizes data from the present and pre-

<table>
<thead>
<tr>
<th>Age at operation (days)</th>
<th>Age at vaginal opening of earliest rat (days)</th>
<th>Average age at vaginal opening of earliest 1/3 of all rats</th>
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<tbody>
<tr>
<td></td>
<td>blank</td>
<td>lesion</td>
</tr>
<tr>
<td>14-15</td>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td>14*</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>10*</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>3-4*</td>
<td>35</td>
<td>28</td>
</tr>
</tbody>
</table>

* Rats of identical inbred albino strain.

Table 3.
Advancement in age at vaginal opening caused by lesions at different ages. Between brackets: number of rats included in calculation.
vious work (Donovan & Van der Werff ten Bosch 1959). In order to avoid the difficulty of distinguishing 'effective' from 'ineffective' lesions, the effect is here recorded of the age at operation on the earliest age at which any one member of a group, and also the average age at which the earliest one-third of each group showed vaginal opening. The differences between lesioned and blank-operated animals are more or less constant, which indicates that the lesion does not actively stimulate the secretion of gonadotrophins by the pituitary gland. Instead, it would appear that the lesion is effective by its interference with some physiological process which—in the rat—occurs relatively shortly before puberty, the effect depending on the fact that the lesion is there at that particular stage of development of the young animal. In other words: rather than triggering off a chain of events at and from the time of its production, a lesion seems to act by virtue of its having destroyed structures which would, if the animal had remained intact, have played a role at a later age. Alternatively, the lesions may in fact have initiated a rise in the output of pituitary gonadotrophins, the apparent delay of an effect being due to the relative insensitivity of the ovaries to gonadotrophins during the first few weeks after birth (Saunders & Cole 1936; Price & Ortiz 1944; Eayrs 1951; Picon 1956). However, daily pituitary implants from the age of 14 days cause sexual maturation in 8–10 days (Smith 1927), a latent period which is shorter than any observed after the placement of a hypothalamic lesion.

The possible mechanism which causes sexual precocity in the presence of a lesion has previously been discussed in some detail (Donovan & Van der Werff ten Bosch 1959). It has been argued that in the normal infantile subject a fundamental change toward sexual maturity would seem to be a reduction in the sensitivity of the hypothalamus toward gonadal hormone, and that lesions in the anterior hypothalamus may cause sexual precocity through a blockade of the indirect negative feed-back action exerted by gonadal hormone secreted by infantile gonads upon pituitary gonadotrophin secretion.

It is remarkable that relatively few rats with sexual precocity show persistent oestrus with apparent inhibition of ovulation. Many workers have now demonstrated that lesions in the anterior hypothalamus may cause this abnormality of the oestrous cycle in adult rats, and it has recently been shown that in such animals the release of luteinizing hormone from the pituitary gland is blocked although the storage—and presumably its production—within the pituitary gland appeared to be unimpaired (Van der Werff ten Bosch et al. 1962). The essential area to be destroyed in order to obtain this effect in adult rats, seems to be the basal midline region overlying the optic chiasma (Van Rees et al. 1962). In rats with sexual precocity following lesions, this region is usually damaged, but such lesions extend further upward. It appears from these findings and from the work of Flerkó & Bárdos (1959) that ovulations are less frequently inhibited when both the immediate supra-chiasmatic and the higher structures
are damaged than when the lesion is confined to the basal tissue. The higher lesion may be involved in the gonadal feed-back upon secretion of FSH whilst the lower lesion interferes with the release of LH (Flerkó & Bárdos 1959). The presence of the higher lesion appears in some measure to compensate for the effect of the lower lesion, in that ovulations may occur.

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