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HYPOTHALAMIC CONTROL OF THE ENDOCRINE AND BEHAVIOURAL CHANGES ASSOCIATED WITH OESTRUS IN THE RAT

By G. C. KENNEDY

From the Department of Experimental Medicine, University of Cambridge

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Ovarian secretion is under the control of the anterior pituitary and, in turn, of the hypothalamus (Harris, 1955) and recently the converse influence of ovarian hormones on the hypothalamus has been much discussed (Szentágothai, Flerkó, Mess & Halász, 1962). This feed-back promotes sexual behaviour (Beach, 1948; Sawyer, 1960, 1962) as well as modulating the release of pituitary gonadotrophins (Hohlweg & Junkmann, 1932; Flerkó, 1962; Desclin, 1962) although both functions are also affected by environmental stimuli. The 'centres' concerned with mating may be anatomically separate from those governing the ovarian cycle, and of course each is part of a wider central nervous hierarchy (Sawyer, 1962). There are also species differences, and this paper deals only with hypothalamic localization in the rat.

It will be seen that hypothalamic lesions might inhibit sexual behaviour by preventing the secretion of ovarian hormones, by reducing the sensitivity of the nervous system to them, or by disrupting nervous connexions. It has been shown that ablation of the ventromedial nuclei inhibits mating without, in most cases, interrupting the oestrous rhythm (Kennedy & Mitra, 1963*a*). It does not follow that there is no hormone deficiency, since the lesions might cause a reduced yet normally phased ovarian secretion, and to clear up this point rats with hypothalamic lesions have been spayed and injected with sufficient hormones to promote mating in spayed animals with no hypothalamic lesions.

Oestrus in the normal rat is accompanied by increased locomotor activity, which is probably part of the restlessness or appetitive behaviour that precedes the consummatory stage of an instinct. Similar restlessness is induced by hunger or by D-amphetamine. Ventromedial lesions virtually abolish running activity, which can no longer be stimulated by oestrogen, underfeeding or amphetamine (Kennedy & Mitra, 1963*a*, *b*) but it is not known whether the lesions interfere with 'central perception' of the stimuli or with the motor response.

Mating behaviour can also be inhibited by lesions in the anterior hypothalamus, suprachiasmatic and pre-optic regions (Law & Meagher, 1958; Barraclough & Gorski, 1962) but these generally interfere with ovulation (Hillarp, 1949; Greer, 1953); the behavioural and endocrine mechanisms are thought to be separate, but both effects have been attributed to reduced sensitivity to oestrogen. It is not known whether such lesions reduce running activity. The effect of anterior lesions on running has therefore been compared with that of ventromedial ones, and the response of the rats to oestrogen, amphetamine and underfeeding has been tested. The results indicate differences in the way the two types of lesion interfere with mating, and suggest that the ventromedial region is not directly sensitive to oestrogen.

METHODS

The rats, diet, experimental conditions and activity-measuring cages were described by Kennedy & Mitra (1963*a*). The stereotaxic technique was basically as described in that paper, but the co-ordinates varied. All the lesions were bilaterally symmetrical and 1 mm from the base of the brain. Lesions 7·5 mm in front of the ear bars and 1 mm from the mid line ablated the suprachiasmatic nuclei and part of the pre-optic region. The anterior area and anterior border of the ventromedial nuclei were reached by lesions 6·5 mm in front, and the body and posterior part of the ventromedial nuclei 5·5 mm in front of the ear bars, in both cases 1 mm from the mid line. Groups of rats were prepared with lesions in each of these positions, the positions of which are illustrated by Fig. 1. The brains were all eventually fixed in Bouin's solution, sectioned coronally at 10 μ and examined histologically.

Ovariectomy was performed through separate flank incisions under ether anaesthesia, in most cases in two stages at intervals of a week. Oestradiol benzoate in oil was injected subcutaneously in daily doses of either 5 μg or 125 μg. Progesterone was also given subcutaneously in single doses of 1 mg. The injections were given in the early afternoon, and mating was tested with proved males, habituated to their cages, into which single females were introduced in the evening of the day progesterone was given. Mating behaviour was watched, but not measured quantitatively, and proof of mating was based on the presence of a vaginal plug or of spermatozoa in the vagina next morning.

D-amphetamine was mixed with the diet where appropriate to give a daily intake of approximately 5 mg/kg body weight, as described by Kennedy & Mitra (1963*b*).

RESULTS

Ovariectomy and hormone replacement. Female rats of this strain are virtually inactive in the treadmill type of cage before puberty, which occurs between 35 and 40 days of age. Running then increases rapidly to a maximum between 45 and 130 days; a typical long term record was shown in the paper by Kennedy & Mitra (1963*a*). It would be very difficult to ovariectomize a rat, allow time for healing and restore activity by injecting hormones with any certainty that the 'natural' level had not changed meanwhile. It seemed most reasonable therefore to perform the experiment at different ages. In a series of nineteen rats, ovariectomy was carried out between 80 and 120 days after birth.

Figure 2 shows the result in a rat which had probably not yet reached its highest spontaneous activity. Running was unaffected by the removal of the first ovary, but fell to a low level when the second was taken out. Oestradiol (125 μg) for 3 days, followed by progesterone on the 4th and repetition of this cycle a number of times, quickly restored running to the pre-operative level and eventually to a higher level. There were day-to-day fluctuations, but these bore no relation to the days on which progesterone

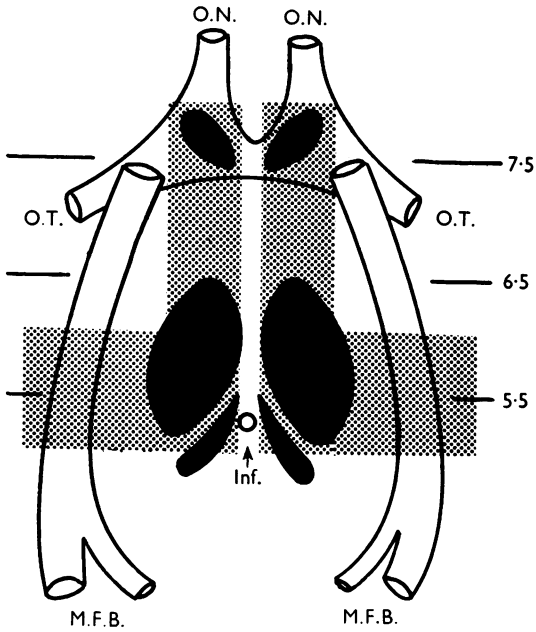

Fig. 1. Diagram to illustrate distribution of hypothalamic lesions. The numbers indicate the distance in mm anterior to the ear bars of the stereotaxic instrument. O.N., optic nerves; O.T., optic tracts, with the suprachiasmatic nuclei indicated in black between them. M.F.B., medial forebrain bundles, with the large ventromedial nuclei and small arcuate nuclei between them. Inf., infundibulum. The parallel stippled areas were the sites of lesions, and delineate a region regulating the sex cycle and sex behaviour. The overlapping, laterally directed areas are concerned mainly with energy balance, and are included to emphasize the multiple functions of the ventromedial nuclei.

was given, and were seen also in rats given oestrogen alone. The large dose of oestrogen was given because preliminary tests showed that the more physiological dose of 5 μg did not reliably stimulate running, particularly if the interval after ovariectomy was at all prolonged. At 120 days the rat was put with a male and immediately mated, as it did again at 140 days, just after injections were stopped. Three weeks later the vaginal smear was anoestrous and the rat refused to mate.

Figure 3 illustrates rather a different response to hormone treatment. Although the rat was no older than the previous one it had apparently reached its maximum activity before the ovaries were removed, and it did not exceed this when injected. The rat in Fig. 4 was older at ovariectomy, and oestrogen subsequently brought activity only to the lower level that experience had led one to expect in a normal rat of this age. The later parts of these two figures illustrate the effects of hypothalamic lesions and will be discussed below.

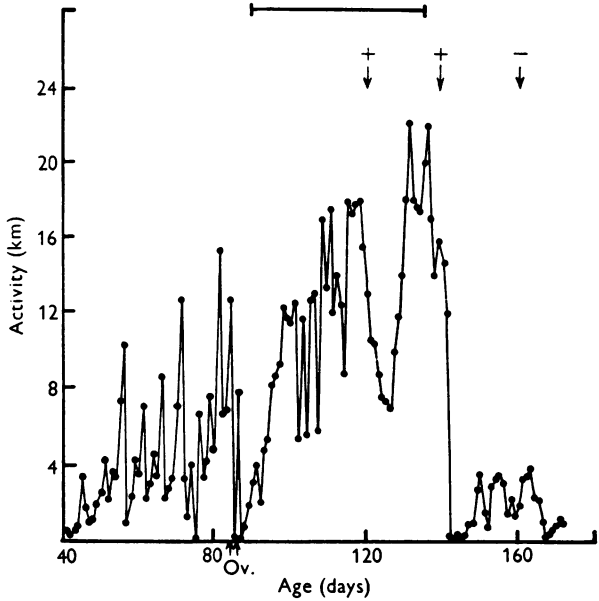

Fig. 2. Running activity and mating behaviour of a young ovariectomized rat treated with ovarian hormones during the period shown by the bar at the top of the figure; the arrows below indicate mating (+) or refusal (−). Activity during hormone treatment continued the natural rise before ovariectomy (Ov.).

In separate tests oestrogen was injected into intact rats at different ages. Even massive doses never promoted higher activity than spontaneous oestrus. To emphasize this point, six intact and six ovariectomized weanlings were injected from 21 days after birth with oestrogen and progesterone as described above. Activity did not begin until the rats were over 40 days old. In other weanlings running was readily induced even before 30 days by fasting overnight.

Hypothalamic lesions in ovariectomized rats. Figure 3 shows the effect of lesions in the ventromedial nuclei upon the running activity stimulated by ovarian hormones in ovariectomized rats. Eight rats showed the same response. Obesity, which would otherwise have followed the lesions, was prevented by giving the animals only sufficient food to maintain a steady

weight, yet activity never rose above the level of the untreated castrate in spite of continued hormone treatment. The inactivity was accompanied by refusal to mate.

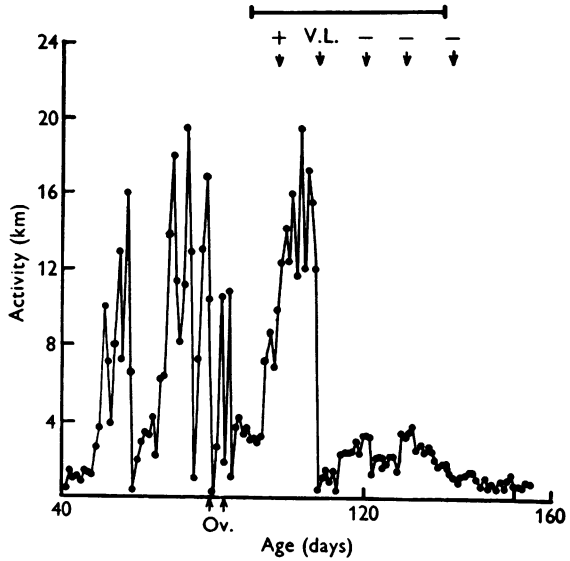

Fig. 3. Effect on running activity and mating behaviour of bilateral ventromedial lesions (V.L.) in a rat treated in other respects as in Fig. 2. Symbols as before. The activity restored by the hormones was abolished by the lesions.

A different response followed most anterior hypothalamic lesions, as shown in Fig. 4. Although activity returned only slowly after the hypothalamic operation, it eventually recovered completely. Mating behaviour was not interrupted in this way; the rat copulated normally even before running began to be restored. Five of eight operated rats mated and recovered the whole or the greater part of their activity, one mated but did not run after the hypothalamic operation, and the other two neither mated nor ran. No obvious difference could be detected in the position of the lesions, or in the degree of damage to the anterior part of the ventromedial nuclei.

Hypothalamic lesions in intact rats. Twelve young active females, running at least 10,000 revolutions at oestrus, had lesions placed in the suprachiasmatic region, and a similar group had lesions in the anterior hypothalamic area. No significant differences in endocrine or behavioural responses were seen between the groups, so they will be described together. All the rats showed periods of persistent vaginal cornification, and although this was not always continuous, more than 60% of the smears were cornified.

Fourteen rats showed little or no reduction in running activity. Of these twelve mated spontaneously without oestrogen treatment, but only four had normal pregnancies and reared litters. The remainder showed periods of dioestrus after mating, varying from 3 to 18 days, during which activity was greatly reduced. No laparotomies were done, but it seems probable that most of these rats ovulated and some became pseudopregnant.

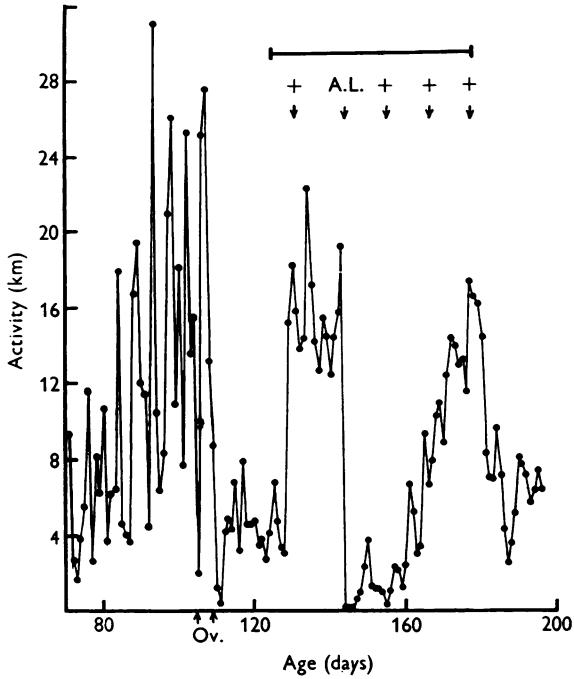

Fig. 4. Effect of bilateral anterior hypothalamic lesions (A.L.) under similar conditions to Fig. 3. Activity was only temporarily inhibited by the lesions, and there was no interference with mating behaviour. Symbols as in Fig. 2.

Ten rats showed a reduction of running to 4000 revolutions/day or less, about the castrate level. Only two of these mated without oestrogen, and neither showed any break in cycle. Since it has been shown that lesions of this type may lead to ovarian atrophy (Greer, 1953) these ten rats were all subsequently injected for 3 weeks with oestrogen and progesterone as described above. In no case was there any significant increase in activity, although two rats mated which had not done so before. Hormone injection was stopped, and the rats were deprived of food for 3 days. In every case the pre-operative activity returned within 2 days, and disappeared again on refeeding. None of the rats was significantly obese.

Four inactive rats were fed *ad lib.* with diet in which amphetamine was incorporated. Pre-operative activity returned more slowly than during starvation, a maximum being reached in 7–10 days.

The ovaries of the active rats were weighed and sectioned post mortem. None of them contained fresh corpora lutea, and very few contained any at all. Follicles were numerous and very variable in size, as described by previous authors. The mean weight was 23·4 mg compared with 43·0 mg in normal rats of the same age ($P < 0{\cdot}001$).

DISCUSSION

These experiments confirmed that lesions in the ventromedial region of the hypothalamus inhibited both copulatory and running activity, and since neither was restored by large doses of oestrogen and progesterone the effects were not due to hormone deficiency. On the other hand the hyperphagia showed that the ablated region is not specifically a sex centre. This hyperphagia only appears under optimal conditions; if the rats have to work for food they eat less than normal, i.e. they show reduced hunger drive (Miller, Bailey & Stevenson, 1950). It has been suggested (Kennedy, 1961) that the region is concerned with 'general drive', i.e. the facilitation of the motor components of a variety of motivated acts, in the same way as Dell (1957, 1958) proposed for the closely associated reticular activating system.

There is no evidence that the ventromedial region is the anatomical substrate for motivation itself, for food, sex or 'spontaneous' activity. Baillie & Morrison (1963) emphasized the distinction between motivation and drive by showing that rats which had previously been conditioned to press a bar to obtain food continued to do so after hypothalamic lesions that abolished normal eating altogether. In a similar way the rats with ventromedial lesions may well have felt sexual excitement, but their motor performance was certainly impaired, and that is all that is meant by reduced drive.

Both the endocrine and behavioural effects of suprachiasmatic and anterior hypothalamic lesions differed from those of ventromedial ones. Whereas the latter disturbed the oestrous cycle in less than half of a series of rats (Kennedy & Mitra, 1963*a*) the more anterior group almost invariably caused constant vaginal cornification but interfered much less with either mating or running. Although running activity was not essential to mating, lesions that interfered with one generally inhibited the other. Some of the suprachiasmatic lesions that reduced running were too far forward to have involved the ventromedial nuclei, but regional ablation is not a sufficiently selective technique to decide how closely the anterior

hypothalamic control of mating behaviour and running are associated. Barraclough & Gorski (1962) claimed that one must ablate a larger portion of the suprachiasmatic region to prevent mating than to block spontaneous ovulation. It may be that the same is true of the anterior hypothalamic area, and that reduction of activity also requires extensive damage. Michael (1962) showed that uptake of radioactive oestrogen by hypothalamic cells is very widespread, and it is reasonable to suppose that lesions of a given size in many different positions might have much the same effect in reducing the oestrogen sensitivity of the region. The smaller size of lesions blocking spontaneous ovulation may simply mean that this mechanism has a higher 'threshold' to oestrogen feedback than has the mating response. This is viewing the problem in its simplest terms, however, and it is unlikely that it offers a complete explanation.

Szentágothai *et al.* (1962) suggested that persistent vaginal cornification was caused by interruption of the oestrogen feed-back that normally inhibits follicle stimulating hormone (FSH) release, so preventing fluctuations in oestrogen level that would release luteinizing hormone (LH) and cause ovulation. The rats can release LH if their ovaries are grafted into the spleen to reduce the oestrogen level in the general circulation (Flerkó & Bárdos, 1961) and as we saw they may ovulate reflexly if mating is not blocked. This last observation does not support the suggestion by Desclin, Flament-Durand & Gepts (1962) that the failure to ovulate rhythmically is due to insensitivity to environmental stimuli. On the other hand, most of these rats have very small ovaries, and their oestrogen secretion is probably reduced, and Bogdanove (1963) recently questioned whether the hypothalamic-pituitary response to normal levels of oestrogen was necessarily impaired by anterior lesions.

The argument in favour of reduced feed-back interfering with mating seems stronger, although still not conclusive. In spite of the reduction in ovarian weight, hormone lack was not responsible for either the frigidity or the inactivity—the ovaries were small even in rats that behaved normally, and in most of the others even large doses of oestrogen and progesterone did not restore normal behaviour. There is unequivocal evidence that mating results from direct sensitivity to oestrogen of the anterior region of the hypothalamus, for Lisk (1960) showed that minute implants of oestradiol in these same areas induced a lordosis response in ovariectomized rats. With the same reservations as were made earlier about general drive, one can conclude that an extensive, oestrogen sensitive area in the anterior hypothalamus and suprachiasmatic region is concerned in the 'specific drive' for sex in the female and for the control of ovulation, both reflex and spontaneous.

After ablation of a sufficient portion of this area of known oestrogen

sensitivity, the ventromedial region was unable to respond to oestrogen by promoting running activity or mating behaviour. Running could still be induced by underfeeding or amphetamine. This suggests that the ventromedial nucleus is insensitive to oestrogen, receiving its stimulus indirectly from the anterior hypothalamus. In infancy activity could also be promoted by starvation but not by oestrogen, and subsequently oestrous activity was a function of age and could not be enhanced by giving large amounts of oestrogen, although underfeeding increased running still further (Kennedy & Mitra, 1963*a*). A limited capacity of the anterior hypothalamus to relay stimuli to the ventromedial nuclei, changing with age, could explain these findings and the apparent paradox that the hypothalamus is hypersensitive to oestrogen in the infant rat (Harris, 1961), yet the hormone does not then cause running. Interruption of the relay as well as reduction of oestrogen sensitivity could contribute to the inactivity caused by the anterior lesions.

SUMMARY

1. The effect of ovariectomy upon the running activity of rats of different ages, and upon its restoration by ovarian hormones was studied.

2. Lesions in the ventromedial region of the hypothalamus always prevented the arousal of running activity or mating behaviour by ovarian hormones, whereas anterior hypothalamic lesions did so less frequently.

3. When activity was reduced by anterior lesions, it could be restored by underfeeding. This was not so after ventromedial lesions.

4. Normal infant rats also became active when underfed but not when injected with ovarian hormones.

5. It is suggested that the ventromedial region is not directly sensitive to oestrogens, but facilitates the locomotor components of a number of instinctive acts, while the anterior hypothalamus is concerned specifically with sexual behaviour and relays facilitatory stimuli to the ventromedial centres.

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