

The Role of Sexually Dimorphic Medial Preoptic Area of the Hypothalamus in the Sexual Behaviour of Male and Female Rats

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Summary

The neonatally gonadectomized male rats failed to show typical male clasping of the flanks of the female rat in oestrous stage and also could not achieve intromission and ejaculation when studied at 90 days of age. The replacement of testosterone propionate did not abolish the deficit in sexual behaviour observed. The multiunit electrical activity (MUA) in the medial preoptic area (MPOA) of intact males as well as of oestrous females increased significantly in amplitude and frequency from the low basal levels during mounting, but reverted almost to the basal levels after mounting. The basal MUA in the gonadectomized male rats was of relatively high frequency and amplitude when compared to the intact males and oestrous females, and did not show significant changes during and after mounting. It is suggested that the dimorphic organization of MPOA, developing under the influence of neonatal testosterone, plays a significant role in the male sexual behaviour in adulthood.

Key words

Neonatal gonadectomy – Sexually dimorphic medial preoptic area – Multiunit electrical activity – MPOA volume – Testosterone propionate – Sexual behaviour

Introduction

Although many of the consequences of sexual dimorphism such as body size and length have been known for a long time, other differences between males and females such as disease incidence, response to drugs and toxins, the metabolism and assimilation of nutrients have only recently been discovered (Bardin and Catteral 1981). Most spectacular of all such differences is the discovery of sexually dimorphic medial preoptic area of hypothalamus by Gorski *et al.* (1978). Recently Swaab and Fliers (1985) reported the existence of similar sex-related differences also in the MPOA in humans.

It has been reported that the single unit activity of the MPOA is correlated to male sexual behaviour in monkeys (Oomura *et al.* 1983). They also found that the neuronal activity of the MPOA in male rats changed in the presence of an oestrous female rat. More interestingly, Horio *et al.* (1983) observed an increasing firing pattern from the male MPOA during

"pursuit mounting" in rats. Subsequently, Horio *et al.* (1986) recorded multiple unit activity from the MPOA in a freely moving rat and analyzed its relationship to copulatory behaviour.

However, it is not known if the electrical activity from MPOA in the male is different from that of the female during mounting. It needs to be established whether or not the structural dimorphism seen in this area also extends to its function. The present study is therefore an attempt to answer this important question. The effect of neonatal gonadectomy on the electrical activity of MPOA in the male rat was thus investigated.

Materials and Methods

Experimental animals

Normal male and female Wistar albino rats continuously showing 4–5 day oestrous cycle for three weeks were selected and housed for mating in 12 h

light and dark cycles with food and water *ad libitum*. The pregnant females were reared in separate cages. The male and female pups were separated immediately after parturition, cleaned and weighed. The male pups were divided into intact, sham-operated control and neonatally gonadectomized groups.

Bilateral gonadectomy in neonatal male rat pups

Gonadectomy was performed within two hours after birth. The abdominal part of the male pup was cleaned with absolute alcohol and anesthetized with ether. Ventromedial incision was made on the skin in between penile and anal region. Both testes were pulled out from the abdominal cavity and excised. The wound was sutured and dressed with antiseptic ointment. A sham operation was performed in the controls. All pups were returned to their mothers after they had recovered consciousness (Kartha and Ramakrishna, unpublished observations).

Growth profile

Body weight of all experimental animals was followed until 110 days of age at the interval of 5 days. At 21 days, all male pups were weaned and housed in separate cages.

Treatment of gonadectomized male with Testosterone propionate

The neonatally gonadectomized male rats were divided into two groups when adult. The animals of the first group were given testosterone propionate (TP) (500 $\mu\text{g}/\text{kg}$ body weight) and the second group was treated intramuscularly with a carrier (olive oil) on the same day. Twenty-four hours after the injection, different components of mating behaviour were recorded in the presence of oestrous female.

Observation of mounting behaviour in male rats

Each male rat of intact as well as gonadectomized group was adapted at least for 30 min to the mating arena. The circular glass trough of 45 cm diameter and 30 cm height was selected as mating arena for this experiment. The arena was thoroughly cleaned before carrying out the experiment for each animal, in order to eliminate all olfactory cue(s) likely to influence the mating behaviour. The female in oestrous stage, used to test the mating behaviour of one male, was not used for carrying out the experiment with another male on the same day. The experiment was conducted under dim light in an isolated room. The temperature and humidity were kept constant during the experiment. The experiment was repeated on gonadectomized male rats treated with TP or oil.

Electrophysiology

Electrodes were prepared by etching stainless steel pins into 0.3 to 0.6 mm tip diameter (Nerve Goodman Ltd, Birmingham U.K.). The electrodes of 8.0 mm length were fixed to male plugs, insulated with 10 % W/V polymethyl methacrylate in chloroform and dried at 37 °C. The prepared electrodes were implanted in MPOA (anterioposterior 6.5 mm; lateral 0.5 mm; vertical 7.8 mm) (Thompson 1978). A miniature screw implanted in the frontal sinus served as negative terminal for recording electrical activity.

Recording of EEG. The animals were adapted to the recording arena at least one hour before starting the experiment. The female plugs, fixed to the cords, were connected to the implanted male plugs. Plugs from the MPOA served as the positive pole; the female plug from the frontal sinus served as the negative terminal and the cord connected to the aluminium sheet serving as the base of the recording cage, formed the ground during the EEG recording. The electrical potentials were amplified (amplifier AB 620G) and recorded by a polygraph system (Nihon Kohden, Japan).

The electrical activity from MPOA was recorded in males aged 90 days under various experimental conditions including the presence of female in oestrous stage. In oestrous females of the same age, electrical activity of the same area was recorded in the presence of a normal male. The electrical activity from MPOA of the 90-day-old gonadectomized males was also recorded under the same experimental conditions as that used for intact males. Generally, each recording session lasted for 30 min. The electrical activity was recorded for at least 10 s continuously during sexual behaviour. The mean frequency of electrical activity per second during this period was taken to represent the activity that could be correlated with the behaviour and was compared with the mean basal frequency. Activity above 50 μV was considered as high-amplitude activity and that below this level was considered as low-amplitude activity. The changes in frequency and amplitude of the activity from the basal level were noted.

After completing EEG recording, 1 mA anodal electrical current was passed through the electrode under ether anaesthesia. The animal was perfused intraventricularly by 0.9 % NaCl solution followed by 10 % formalin. The brain was isolated and fixed in 10% buffered formalin for histological examination to confirm the localization of the implanted electrode.

Volume of MPOA

The brain of 90-day-old intact and gonadectomized male as well as oestrous female rats of

the same age were separated and histologically analyzed by silver impregnation method of Ramon Y Cajal modified by Favorsky (1930). The diameter of MPOA from slices of 6 μm thickness from brain, impregnated with silver was measured by micrometer under 10x magnification. The total number of sections cut from the anterior part where the MPOA first appeared to the posterior region where the MPOA was last present were taken into account. This was considered as the length of MPOA. The volume of MPOA was calculated as shown below.

Volume of MPOA = $\pi r^2 l$ where 'r' is the radius of the region and 'l' is the total length of MPOA.

From the serial sections cut, the smallest diameter and the largest diameter of MPOA were added and the 'r' was calculated as the mean diameter.

Statistics

The values are expressed as mean \pm SE and statistical evaluation of the results were carried out using one-way ANOVA test. P values less than 0.05 were considered as significant. Generally, littermates were used as controls for all experiments unless otherwise stated. Each group comprised of six animals.

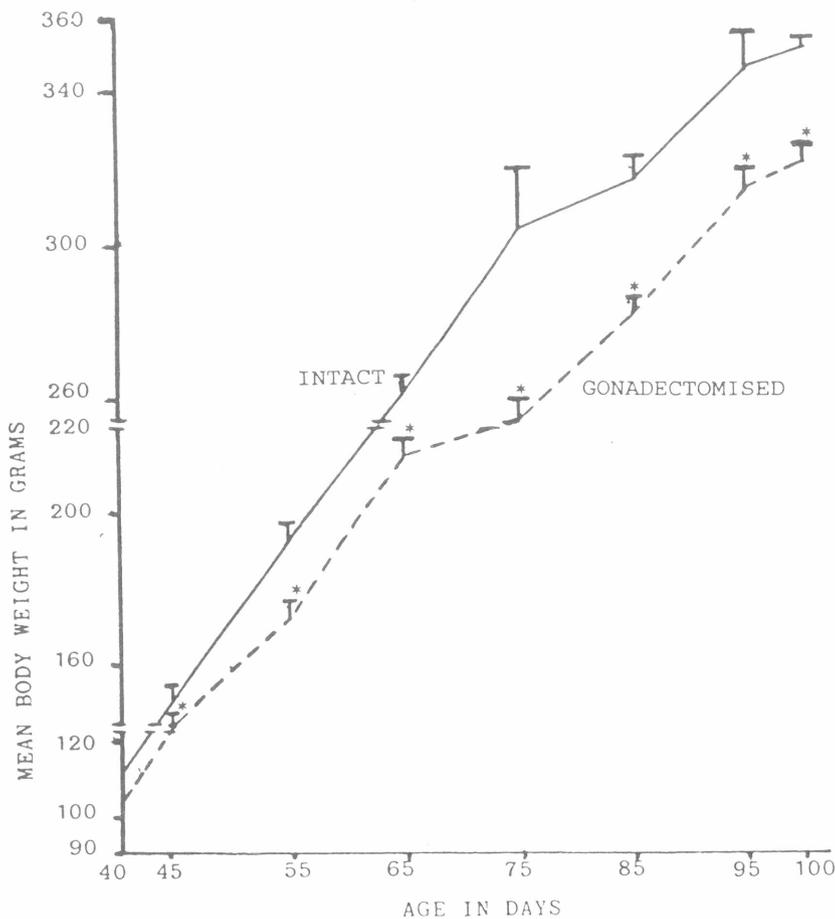


Fig. 1
Profile of body weight

Results

Growth profile

The body weight was significantly lower in gonadectomized males than in intact males from the age of 45 days. However, the reduction of body growth in gonadectomized males became more pronounced from day 75 onwards until the end of experiment (Fig. 1).

Mounting behaviour

Intact male

The intact male clasped the flanks of female in oestrus with forepaws to draw her close towards his perineal region. The male straddled the dorsal surface of the female, grasped her flanks with forepaws near her hind legs and contacted her thighs with his hind legs. In response to the mounting, the oestrous female stood immobile with her legs extended as her rump

elevated in lordosis. The male attempted rapid repetitive pelvic thrusts which culminated in the vaginal penetration, intromission and ejaculation.

Gonadectomized male

The gonadectomized male failed to clasp the flanks of the female unlike the intact male. Hence, the

gonadectomized male could not palpate the flanks and dorsal surface of the female. It was noted that the female in oestrous behaved aggressively by kicking and biting the gonadectomized male and they failed to show lordosis. Moreover, the gonadectomized male could not achieve either vaginal penetration or ejaculation.

Table 1

Mean range of amplitude of electrical activity from MPOA of normal males, gonadectomized males and oestrous female rats aged 90 days.

Group	Mean range of amplitude of electrical activity (μV)					
	Before mounting		During Mounting		After mounting	
Normal males	33 \pm 2.25	46 \pm 1.96	35 \pm 5.10	56* \pm 2.76	34 \pm 2.66	45 \pm 3.11
Gonadectomized males	35 \pm 3.10	58 \pm 5.12	38 \pm 4.11	63 \pm 3.21	36 \pm 3.43	61 \pm 2.98
Oestrous females	28 \pm 4.11	34 \pm 3.23	35 \pm 3.14	51* \pm 4.11	31 \pm 5.44	38 \pm 3.92

$n = 6$, * $P < 0.05$.

Table 2

Mean frequency of low- and high-amplitude electrical activity from MPOA of normal males, gonadectomized males and oestrous female rats aged 90 days.

Group	Mean frequency of electrical activity					
	Before mounting		During mounting		After mounting	
	<50 μv	>50 μv	<50 μv	>50 μv	<50 μv	>50 μv
Normal males	23.00 \pm 0.72	0	11.30 \pm 0.92	26.36* \pm 1.87	18.63 \pm 1.63	0
Gonadectomized males	33.13 \pm 1.10	12.47 \pm 1.23	36.14 \pm 1.43	15.50 \pm 2.11	29.72 \pm 2.36	11.81 \pm 0.32
Oestrous females	28.91 \pm 1.34	0	18.97* \pm 2.11	33.23* \pm 2.24	22.43 \pm 1.44	0

$n = 6$, * $P < 0.05$

Gonadectomized male treated with testosterone

The treatment with TP did not abolish the deficits in the sexual behaviour observed in the gonadectomized males. However, the frequency of mounting tended to increase in the TP-treated group. The vaginal penetration and ejaculation was not achieved by these animals (data not shown).

Electrical activity from MPOA

The basal electrical activity in the MPOA of intact male before introducing the oestrous female is of

low amplitude and low frequency (Tables 1 and 2). In contrast, the activity is of higher frequency and amplitude in the neonatally gonadectomized male. During mounting, the frequency increased in both intact and gonadectomized males. However, high-amplitude activity was seen in the intact males during mounting only. In both intact and gonadectomized males, the frequency of activity returned to the basal level after mounting. It is noteworthy that basic values of electrical activity of MPOA in intact and gonadectomized male rats were different (Fig. 2).

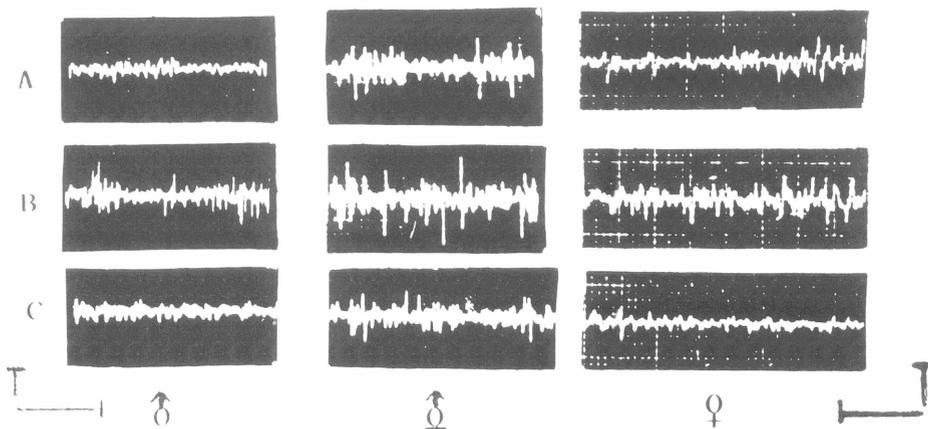


Fig. 2 Electrical activity from MPOA of normal, gonadectomized male rats as well as oestrous female rat before (A), during (B) and after (C) mounting. Calibration 0.2 s, 200 μ v.

The most unique feature of the electrical activity of MPOA seen in oestrous females and gonadectomized males aged 90 days was that the basal activity in both groups was characterized by similar high-amplitude levels. However, the frequency of high-amplitude activity of MPOA increased in oestrous females during mounting. After mounting, the frequency of high-amplitude activity returned to almost basal level in all the groups.

The volume of MPOA

The MPOA of gonadectomized male rat was found not to be well organized due to the scattered distribution of neurones in the area. The volume of MPOA was found to be six times smaller in gonadectomized males when compared to the intact males (Table 3). But the volume of MPOA from females in oestrus was eight times smaller than that of intact males. Both these differences were statistically significant.

Table 3

Volume of MPOA in normal males and gonadectomized males as well as in oestrous female rats aged 90 days.

Group	Volume of MPOA ($\text{mm}^3 \times 10^{-3}/\text{g}$ brain weight)
Normal males	23.00 ± 2.30
Gonadectomized males	$3.60 \pm 1.01^*$
Oestrous females	$2.76 \pm 1.68^*$

$n = 6$, * $P < 0.05$.

Discussion

Gorski *et al.* (1978) demonstrated that the volume of sexually dimorphic MPOA in adult rat is significantly influenced by the prior perinatal hormonal environment. Though they indicated the age of

castration as day one, the exact age at which the gonads were removed from neonatal pups was not mentioned. An eight-fold decrease in the volume of MPOA was found after castration compared to intact/sham operated male rats, when measured in adulthood. However, one critical factor which was later revealed by Corbier *et al.* (1978) was not taken into account in these studies. Corbier *et al.* (1983) demonstrated that there was an instantaneous surge in serum testosterone levels in male rat pups exactly 2 h after birth. The present investigation must be viewed as significant in this context because the gonadectomy was performed with the clear objective of nullifying such a surge in order to study the effect of gonadectomy on the dimorphic organization of MPOA.

Growth profile

The body weight of gonadectomized males was found to be significantly lower on day 45 when compared to the intact males. The decrement in body weight became more pronounced on day 75 onwards till the end of the study. This marked difference in body weight may be partially due to the retardation of the development of accessory sex organs.

The role of MPOA in sexual behaviour

The foetal MPOA implanted from male to the female rats enhanced masculine sexual behaviour (Arendash and Gorski 1982). The bilateral lesion of the same area in prepubertal male rat completely disrupted the masculine behaviour in adulthood (Meisel 1982, Twiggs *et al.* 1978). However, the active role of MPOA in male sexual behaviour is further elucidated by the studies which showed that the bilateral single-stage lesions (Giantonio *et al.* 1970, Ginton and Merari 1977, Heimer and Larsson 1967, Lisk 1968) or two-stage ablations of MPOA (Finger *et al.* 1973, Klaric and Hendricks 1986), not only abolished the complete pattern of male copulatory behaviour, but also attenuated the surge of plasma LH, FSH and prolactin levels in male rats (Preslock and McCann 1987). It is also known that the electrical stimulation of MPOA enhanced the male sexual copulatory behaviour by its frequency as well as by the stimulus-born responses (Hanada *et al.* 1980, Malsbury 1971, Merari and Ginton 1975, Van Dis and Larsson 1871, Vaughan and Fisher 1962), including the elevation in plasma LH level in male rats (Jamieson and Fink 1976, Kawakami *et al.* 1973). On the contrary, the electrical stimulation of MPOA abolished lordosis in female rats (Moss *et al.* 1974).

Horio *et al.* (1983) observed an increased firing pattern in multiple unit activities (MUA) from the male MPOA during pursuit mounting in rats. Later studies (Horio *et al.* 1986) demonstrated that the baseline firing rate of MPOA neurones increased by

36.7% when the female was introduced. However, maximum rate of firing was observed during pursuit mounting followed by intromission and ejaculation. Soon after the ejaculation, the frequency of firing decreased considerably so that it attained minimum rate. In monkeys, the single unit activity of MPOA was found to correlate with the male sexual activity (Oomura *et al.* 1983). Mink *et al.* (1983) observed increase in neuronal firing of MPOA from the male rat in the proximity of oestrous female.

In the present study, the electrical activity of MPOA from intact males shows a higher frequency during mounting. There is also an upward trend in the frequency of electrical activity of MPOA in oestrous females during mounting. However, in gonadectomized males there is no such significant change in the frequency of electrical activity in MPOA during mounting. It is important to note that even the basal level of electrical activity in gonadectomized males is generally of high frequency in contrast to intact males or females in the oestrous stage.

It is possible to assume that the neurones in this area are rather prematurely primed after gonadectomy and hence the reinforcement of the stimulus such as the presence of oestrous female and mounting does not further enhance activity of the area. How much of this is due to poor performance of the gonadectomized male during copulation, such as its failure to clasp the female and/or the lowered number of pelvic thrusts, remains to be answered. Nevertheless, it appears that the dimorphic organization of MPOA during development, under the influence of testosterone, plays a significant role in male sexual behaviour in adulthood. This assumption is based on the following factors: (1) MPOA is poorly organized in the gonadectomized male (2) electrical activity in MPOA of the gonadectomized male remains relatively high in frequency and does not rise significantly during mounting, and (3) there are behavioural deficits in the copulatory behaviour of such animal. Persistence of deficits in copulatory behaviour of gonadectomized males even after testosterone treatment lends credence to the primacy of structural integrity in the sexually dimorphic MPOA of the male rat for successful mating behaviour.

Functional interrelationship of MPOA with other limbic areas

It is interesting to note that the bilateral amygdaloid lesions could effectively block male copulatory behaviour (Schwartz and Kling 1964, Bermant *et al.* 1968, Giantonio *et al.* 1970) while these lesions mainly disrupted the cyclicity and the gonadotropic (LH) secretion in female (Kawakami *et al.* 1980). Although the hippocampal region showed no direct involvement in sexual behaviour, this region did display changes in excitability during the oestrous cycle

in female rats. The ablation of the hippocampus elevated LH and FSH levels (Terasawa and Kawakami 1973) but delayed the vaginal opening including gonadal atrophy (Riss *et al.* 1963) in female rats. Electrical stimulation of the medial amygdala resulted in ovulation in female rats (Kawakami *et al.* 1973) while the stimulation of the amygdala or hippocampus caused excitation in more neurones of MPOA and also caused inhibition in smaller number of neurones of this area (Kawakami *et al.* 1980). This evidence thus suggested the existence of neural connectivity between

medial preoptic area, basolateral amygdala and dorsolateral hippocampus which modulated the sex-related changes in endocrine and behavioural functions in males and females. Further studies in establishing the functional relationship between medial preoptic area, basolateral amygdala and dorsolateral hippocampus can be attempted by recording the electrophysiological activities from these areas during copulatory behaviour in freely behaving rats. The work is now in progress.

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