

Original article

The role of Medial Preoptic Area in reproductive behavior of male rats

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Abstract

Introduction: It is known that medial Preoptic area (mPOA) of the forebrain exerts a significant influence on reproductive behavior of an animal. The mPOA occupies a key position in the central nervous regulation of male sex behavior. The present study was planned to see whether discrete electrolytic lesions in male rats could have functional deficits in motivational and/or consummatory sexual behavior.

Methods: Thirty adult laboratory bred male Wistar rats weighing around 175 to 240 gms with average age 200 days were selected for the experiment. They were divided into two groups - control group (N=15) and experimental group (N=15). Sexual behavior was observed before lesions and after lesions.

Observations and Result: In lesioned animals sex drive score decreased as compared to control group, also grooming latency, pursuit latency, mount latency, intromission latency and ejaculation latency significantly increased. While grooming frequency, pursuit frequency, mount frequency and intromission frequency significantly decreased.

Conclusion: The present work elucidates that bilateral electrolytic lesions in mPOA in adult male rats severely disrupts the precopulatory and copulatory responses.

Key Words: Medial preoptic area

Introduction:

It is known that medial Preoptic area (mPOA) on the forebrain exerts a significant influence on reproductive behavior of animal. Sexually experienced male rats do not copulate after bilateral lesions in the mPOA. These lesions had very similar effects in a wide range of vertebrates which includes rats, ^(1,2,3,4,5) hamsters, ⁽⁶⁾ mice, ⁽⁷⁾ guinea pigs, ⁽⁸⁾ gerbils, ⁽⁹⁾ goats, ⁽¹⁰⁾ rhesus monkeys, ⁽¹¹⁾ lizards, ⁽¹²⁾ and fishes. ⁽¹³⁾ It is also found that mPOA plays a key role in mediating the effects of sexual experience on male behavior. ⁽¹⁴⁾ Thus mPOA occupies a key position in the central nervous regulation of male sex

behavior. It is a major integrative site for male sexual behavior. ⁽¹⁵⁾

The preoptic nucleus is a region in the basal forebrain that is involved in the control of biorhythms. Medial preoptic nucleus in rat is larger in male than in female. ⁽¹⁶⁾

Male sexual behavior is composed of at least two separate mechanisms, namely sexual arousal and copulatory performance. ⁽¹⁷⁾ Lesions of medial Preoptic area-anterior hypothalamus (POA-AH) severely disrupt typical male copulatory responses. ⁽¹⁸⁾ Males with such lesions, commonly pursue, sniff, palpate and “climb over” stimulus female but they consistently fail to perform any of normal

intromission or ejaculatory patterns requiring mounts with pelvic thrusting. ⁽¹⁹⁾ On the other hand electrical stimulation of mPOA in rats reduced the number of intromissions preceding ejaculation, the time required to reach an ejaculation and the post ejaculatory interval. ^(20, 21) Therefore POA-AH plays a critical role in the control of masculine copulatory patterns. However it is less important for generation of sexual arousal. Male with POA or AH lesions while continuing to seek the company of other animals become much less selective in their choice of partners, preferring sexually receptive females little more non estrous females. This suggests that lesions of POA or AH do affect sexual motivation and any specificity in behavioral targets of such lesions reflects specialization of POA and AH for processing of arousal enhancing hormonal or chemosensory stimuli. The present study was planned to see whether discrete electrolytic lesions in male rats could have functional deficits in motivational and/or consummatory sexual behavior.

Aims & Objectives :

- 1) To observe the sexual behavior of male rat (before lesion of medial Preoptic area)
- 2) To do the electrolytic lesions of medial Preoptic area in male rats.
- 3) To observe the effects of this lesion on sexual behavior (motivational &/or consummatory) of male rats.
- 4) Compare prelesion sexual behavior with post lesion sexual behavior.

Material & Methods:

Thirty adult laboratory bred male Wister rats weighing between 175 to 240 gms with average age 200 days were selected for the experiment. They were divided into two groups - control group (N=15) and experimental group (N=15). They were marked

and housed in separate cages in an animal room having controlled room temperature ($25 \pm 2^{\circ} \text{C}$) and light-dark cycle for 12 hours. They were given food and water ad libitum. Institutional Animal Ethics Committee (IAEC) approval was obtained before beginning the experiment. The care of the animals was done according to the "Guidelines for the Care and Use of animals in Scientific Research" prepared by the Indian National Science Academy, New Delhi.

METHODS EMPLOYED FOR DATA ACQUISITION:

A. Preparation of stimulus female

To produce a fairly stable receptive female which was made to coincide with the time of behavioral testing. Female Wistar rats weighing 200-250 gms were ovariectomized bilaterally under pentobarbital sodium anesthesia (35 mg/kg bw). One week after surgery, animals were primed by administering subcutaneous injection of estradiol benzoate 2ug/day (0.2 ml), which was injected on 4th day by using tuberculin syringe.

B. Test procedure for male sex behavior :

As rats are nocturnal animals and they copulate more rapidly during the dark phase, ⁽²²⁾ tests were conducted in between 2.00 p.m. to 5.00 pm of dark phase under dim red illumination.

Male rats were introduced into the testing arena 5 minutes before introduction of the female to facilitate their adjustment to the experimental conditions. Initially in the pre-adjustment phase the frequency of defecation and urination was an indicator of their emotional reactivity to the new conditions in the behavioral recording cage.

After 5 minutes, introduction of sexually receptive female rat into the test cage was marked the beginning of the experiment. 5 stopwatches were started at the same time for calculating the latency

of grooming, mount, pursuit, intromission & ejaculation.

Components of male sexual behavior:

The occurrence of each grooming, pursuit, mount, intromission & ejaculation was scored according to classical criteria.^(23, 24) Behavioral parameters comprised of determination of latency of grooming, pursuit, mount, intromission & ejaculation and frequency of grooming, pursuit, mount & intromission in addition to measurement of sex drive score.

Sex drive score:

Weightage was given to individual components of male sex behavior for quantification of sex behavior as mentioned below:

Parameters	score
1. Grooming	1
2. Pursuit	2
3. Mount	3
4. Mount + intromission	4
5. Intromission + ejaculation	5

The duration of sex drive test was 10 minutes

The sequence of arrangement of the 5 parameters indicates an increasing degree of sex drive.

Experimental Operation Group (Stereotaxic Electrolytic Lesions):

The lesions were made using stereotaxy apparatus and electrodes. Rats were anaesthetized with sodium pentobarbitone (35 mg/kg body weight) intraperitoneally and then received atropine sulphate 2.5 mg to minimize any respiratory discomfort. Stereotaxic coordinates for different lesions were used as per Paxinos and Watson,⁽²⁵⁾ and a unipolar (anodal) electrode (28 gauges) varnished except at tip, was used for lesioning. The other electrode (neutral) was fixed to the ear of the animal. In the lesion groups, a research stimulator SS 44 (Medicare) was used to pass

a D.C. anodal current of 1.5 mA. intensity for 20 seconds. The lesions were made bilaterally. After removal of electrodes from the skull, the skin was sutured and an intramuscular injection of benzathin penicillin 10,000 I.U. was given, nebasulf powder was sprinkled on stitched area and the animal was transferred to its cage.

SHAM OPERATION IN CONTROL GROUP:

Animals in other group were subjected to sham operation, in this group only electrodes were passed in mPOA but no current was passed. Except this rest of procedures was same as in experimental group. Similarly postoperative care was taken as in the experimental animals

POST OPERATIVE OBSERVATION IN GROUP I AND II:

Seven days were allowed for animals to recover from operative trauma. From 7th day onwards sexual behavior was recorded in the same way as during preoperative period.

5. Histological examination of lesion site:

As the end of recording session, the animal was anesthetized with pentobarbital sodium (35 mg /kg B.W.). The ascending aorta was exposed and through it the brain was perfused with 0.9 % saline followed by 3 % potassium ferrocyanide in 10 % formal saline for fixation of the brain tissue and development of Prussian blue reaction at the lesion site. The skull was opened and brain was taken out and kept in 10 % formal saline and processed later for histological examination to confirm the lesion site.

Statistical analysis was done by applying unpaired 't' test.

Observations & Results:

Results of the study are presented under the following headings:

1. Results are described in the groups

Group I - Control group

Group II - Experimental group

Each group contained 15 animals.

2. Record of sex drive score in experimental and control group.
3. Record of Grooming Latency (GL), Pursuit Latency (PL), Mount Latency (ML), Intromission Latency (IL) and Ejaculation Latency in experimental and control group.

4. Record of Grooming Frequency (G), Pursuit Frequency (PF), Mount Frequency (MF) and Intromission Frequency (IF) in experimental and control group.

Record of Grooming latency (GL), Pursuit latency (PL), Mount Latency (ML), Intromission latency (IL) and Ejaculation latency (EL) in experimental animals was markedly increased as compared to control (Table I).

Table I: Changes in sexual behavior parameters after electrolytic lesion into the mPOA of rats

Measurement of male sex behavior	Control group	Experimental group
Sex drive score	76.2 ± 9.08	21.06 ± 5.33 *
Grooming latency (sec)	60.6 ± 14.94	106.93 ± 26.18 *
Pursuit latency (sec)	38.33 ± 9.44	115.73 ± 21.12 ***
Mount latency (sec)	51.26 ± 10.81	224.66 ± 111.54 ***
Intromission latency (sec)	45.86 ± 8.32	279.06 ± 86.58 ***
Ejaculation latency (sec.)	309.86 ± 21.47	> 600

* P< 0.05 , *** P< 0.001

By applying unpaired 't' test it was observed that difference in sex drive and GL was statistically significant (P < 0.05) and GL, PL, ML, IL, and EL of control and experimental group was highly significant (P < 0.001). Record of Grooming

Frequency (GF), Pursuit Frequency (PF), Mount Frequency (MF), and Intromission Frequency (IF) in experimental animals was decreased significantly as compared to control group (Table II).

Table II: Changes in sexual behavior parameters after electrolytic lesion into the mPOA of rats

Measurement of male sex behavior	Control group	Experimental group
Grooming frequency (nos./600sec)	3.13±0.48	2.33±0.48 ***
Pursuit frequency(nos./600sec)	3.13±0.83	2.26±0.59 ***
Mount frequency(nos./600sec)	3.86±0.83	2.33±0.61 ***
Intromission frequency(nos./600sec)	13.52±1.64	2.66±0.89 ***

*** P<0.001

By applying unpaired 't' test it was observed that difference in GF, PF, MF & IF of control and experimental group is highly significant ($P < 0.001$).

Discussion

Sex behavior can be analyzed broadly into 2 major components –libido-(indicative of sexual arousal) or motivation and potency manifested in copulatory performance. Libido is defined as desire to copulate and may be measured in terms of reciprocal of time spent on copulatory activity. Increase in latencies of parameters like pursuit, mount and intromission after the lesion of mPOA indicates inhibition of sexual arousal.

Copulatory performance is measured in terms of mount, intromission till ejaculation and ejaculation latency. The significant increase in the mount latency (ML), intromission latency (IL) and ejaculation latency (EL) in experimental group of animals with lesions in mPOA of the present series establishes the role of mPOA in the control of masculine copulatory patterns.

It has been observed that male rats with lesion in this area, showed less preference for a receptive female than control males. This suggests that there is decline in sexual motivation in mPOA lesioned rats.⁽⁴⁾ which correlates with our findings that Grooming latency (GL),Pursuit Latency (PL), Mount latency(ML),Intromission latency (IL) and Ejaculation latency (EL)increases and Grooming frequency (GF),Pursuit frequency(PF),Mount frequency(MF),and Intromission frequency (IF) decreases as compared to control animals. By this reasoning intromission and ejaculatory patterns are more likely to be affected by mPOA lesions than mounts. These acts typically appear at later stages in normal copulatory sequence indicating their dependence on higher levels of sexual arousal. In our

studies ejaculation latency in lesioned animals was very much increased (refer table I),the animals failing to ejaculate within 10 minutes .This proves beyond doubt that sexual arousal mechanism is severely disrupted in mPOA lesioned rats.

Various hypotheses have been put forth regarding changes in precopulatory and copulatory behavior in mPOA lesioned rats (I) it has been observed that the decline in mating behavior in the mPOA lesioned rats is caused by a decrease in the sexual motivation.⁽⁴⁾

(II) Some studies however suggest that lesions have sexual motivation unaffected since precopulatory behavior persists in male rats after mPOA lesions. Reduced motivation is not the only consequence of mPOA lesions. It has been postulated that the copulatory performance mechanism is also impaired. Moreover incomplete mounts have been observed in the mPOA lesioned rats.^(19,26) Similarly Rhesus monkeys with lesions in this area do not copulate but continue to masturbate and also press bars to obtain the company of female.⁽¹¹⁾ It was suggested that after lesion, the animal is unable to perform the normal pelvic thrusting pattern associated with mounting.⁽²⁷⁾

(III)A third hypotheses considers that mPOA is involved in the mechanism related to the initiation of copulation and also to those related to the execution of the behavior itself.^(28,29) It was further supported by the neural transplantation studies in which fetal brain transplant induces the recovery of male sex behavior in the mPOA lesioned rats ,where both precopulatory as well as copulatory behavior are restored.⁽³⁰⁾ Our results also show that both copulatory and precopulatory components of masculine performance are significantly affected by mPOA lesions. Evidence from unit activity studies shows definite involvement of the mPOA in sexual arousal in rats⁽³¹⁾ and in monkeys.⁽³²⁾

To the best of knowledge gained about male mPOA the functions of various parts of this area are not clearly delineated. It is however quite likely that the different parts may modulate copulatory and motivational responses. The present study was not aimed to work out whether there is such a functional dissociation within the mPOA. The electrolytic lesions carried out in the present work inadvertently involved large area and probably the cell efferent fibres reducing mPOA. It is therefore likely that deficits in both precopulatory (motivational) and copulatory responses were observed.

The present work elucidates the role of mPOA in regulatory reproductive behavior. It should however be realized that electrical and/or chemical stimulation of mPOA will give more meaningful responses. It is known that the neurotransmitter acetylcholine plays an important role in modulating reproductive behavior. In conclusion the present work elucidates that bilateral electrolytic lesions in mPOA in adult male rats severely disrupts the precopulatory and copulatory responses.

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Conclusion:

- 1) Longer grooming latency, lesser grooming frequency, longer pursuit latency and lesser pursuit frequency observed in mPOA lesioned rats indicate deficits in precopulatory behavior.
- 2) Longer- mount latency, intromission latency, ejaculation latency and lesser mount frequency, intromission frequency were suggestive of deficits in copulatory behavior in mPOA lesioned rats.
- 3) Present work elucidates that bilateral electrolytic lesions of mPOA in adult male rats severely disrupts the precopulatory and copulatory responses.

Acknowledgements:

We are thankful to the Principal of our Institute for permitting us to utilize the instruments and other facilities in department of Physiology. We also thank the head of department of Physiology and other teaching and nonteaching staff members for their kind support and help during completion of our research.

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