The effect of septal lesion on sexual behavior, sperm count and testicular activity in Young male albino rats

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Abstract
The aim of this study was to assess the effect of septal lesion on sexual behavior, sperm count and testicular morphology in male albino rats. 24 adult sexually experienced male Haffkine albino rats, aged between 90-120 days and weighing between 200 – 250 grams were selected for the study. They were divided into three groups Group I (n=8) as controls, Group II (n-8) were sham operated and Group III (n=8) were septal lesioned. The components of male sexual behavior are pursuit, mount, intromission and ejaculation. The latencies of different components of male sexual behavior were recorded by using separate stop watches for each of the parameters studied. Simultaneously frequencies of the different components of sexual behavior was also recorded in 10 minutes. The occurrence of each pursuit, mount, intromission and ejaculation was scored according to classical criteria. Testicular morphology and sperm count was estimated The results indicate that septal lesions are effective in suppressing the sexual behavior in rats as indicated by increase in pursuit, mount, intromission and ejaculation latencies ($P<0.01$) and shows significant decrease in intromission and ejaculation frequency ($p<0.01$) , decrease in sex drive (0.01) but no change in pursuit and mount frequency. There is statistically significant decrease in testicular weight in septally lesioned rats as compared to control and sham group. The sperm count is markedly reduced ($p<0.01$) in septally lesioned rat as compared to control and sham group. The present study shows that the bilateral lesion of the septal nuclei decreases the sexual behavior in male rats. The bilateral septal lesion also alters the testicular weight and morphology along with decrease in sperm count. This indicates that septal nuclei control the functions of reproductive organ in male rats. It would be worthwhile to estimate testosterone levels in male rats following septal lesions to elucidate the exact mechanism of action of septal lesion.

Keywords: Septal Nuclei Lesion, Male Rats, Sexual Behavior, Sperm Count, Testicular Morphology.

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Introduction

Every component of reproductive activity of vertebrate depends on interplay between neural and endocrine events.[1-3] Our knowledge of the neural mechanism controlling the closely related functions of gonadotrophins secretion and mating behavior is still incomplete. Nevertheless, during the last few decades a considerable amount of information has become available which has begun to elucidate at least the localization of the central nervous substrates of the reproductive functions and to some extent mechanism of their interactions. Naturally most of the relevant data has come from neurophysiological and neuroendrocrinological experimentation. Largely as an outcome of anatomical analysis by the aid of modern experimental techniques, the hypothalamo-pituitary complex can now be conceived as a centrally placed component of a farlung and diversified neural mechanism, the 'Limbic system' which extends from the cerebral hemisphere caudally at least to the upper pontine level.[4] Despite the fact that the septum occupies strategic position in the limbic system, so far no specific have been attributed to it, except for its role in self stimulation, aggression and drinking behavior.[5] It is known that the lesions of the medial preoptic area (mPOA) inhibit the display of male sexual behavior[6-10] whereas electrical stimulation of mPOA facilitates it.[11,12] Swanson and Cown[13] have demonstrated that various parts of the septal nuclei project substantially to mPOA, laieral preoptic area and anterior hypothalamic area. It is possible therefore, that the lesions in the septum may modify sexual behavior and morphology of gonads.

It has been reported that destruction of lateral septum facilitates lordosis and soliciting behavior not only in females[14,15] but also in male rats.[5,16] Conversely electrical stimulation of sexual area has been found to diminish lordotic activity in female hamsters.[17] Thus it is postulated that a lordosis inhibiting influence may exist in septal area. Yamanouchi et al have demonstrated that a large horizontal cut made between the septum and preoptic area strongly facilitated lordosis and soliciting behavior in both female and male rats.[18] Sharma et al[19] demonstrated that septal lesioned rats have continuous estrous and increased estrogen excretion in urine and inhibition of new follicular growth. Similarly septal nuclei exert influence over ovaries as is manifested by reduction in ovarian and uterine weights, marked disruption of estrous cycle and suppression of sexual activity in lesioned animal.[20] Evidence is also accumulating to indicate that the septum may be involved in male sexual behavior.[5,21] Thus it is possible to think that septal lesion may influence the release of gonadrotropic releasing hormone in hypothalamus which in turn influence the release of gonadrotropic hormone from anterior pituitary and therefore affect sexual behavior and fertility.

However very little information is available on effect of septal lesion on sexual behavior and fertility in male rats. Therefore it is decided to undertake this study to delineate the role of septal nuclei in controlling the sexual behavior.

Material and Methods

Selection of Animals: 24 adult sexually experienced male Haffkine albino rats, aged between 90-120 days and weighing between 200 – 250 grams were selected for the study. The animals were housed in separate polyvinyl cages, under 12 hours light and 12 hours dark regime (light on at 18.00 hours and off at 6:00 hours) and controlled room temperature.

The present study was designed to explore the role of septal nuclei in altering the sexual behavior, sperm count and testicular morphology in rats. To study the effect of septal lesions on sexual behavior, testicular morphology and sperm count, rats were divided into three groups

Group I (n=8) as controls

Group II (n=8) were sham operated

Group III (n=8) were septal lesioned

Screening: All male rats were screened for sexual behavior in an arena measuring 30×40×40 cm. the male rats were placed in the arena for 5 minutes adaptation period prior to the introduction of sexually receptive female. Only those rats which ejaculate within 5 minutes of introduction of female rats into the arena were selected for the study. The behavioral studies were conducted under dim light illumination during dark phase of dark/light cycle.

Stereotaxic Instrument: The stereotaxic instrument allows the precise placement of electrodes and cannula into specific areas of brain on the basis of co-ordinates specified in the brain atlas. Usually measurements are taken from zero point in the skull called Bregma. The implanted electrodes can be used for recording the electrical activity in the brain, making lesions or stimulating the implanted areas.

Stereotaxic Technique: Rats were anaesthetized with sodium pentobarbitone 35mg/Kg of body weight intraperitoneally and then given atropine sulphate 25mg/cc to minimize any respiratory discomfort. The anaesthetized rats were fixed properly in the apparatus through the ear bars. The holes are drilled at the mark points through the skull. The unipolar (anodal) electrode(28 guage) varnished except at the tip was lowered stereotaxically in predetermined sites using co-ordinates from Paxinos and Watson.[22] The other electrode (neutral) was fixed to the ear of the animal. By using research stimulator S.S. 44 (Medicare), a D.C. anodal current of 1.5 mA intensity was passed for 20seconds in lesion groups. The lesions were made bilaterally.

In sham group the electrode was lowered to septal nuclei but no current was passed. After the procedure was over, electrodes were removed from the skull and skin was sutured and the site was cleaned with spirit.

Test Procedure for Male Sexual Behavior

Male rats were introduced into the test arena 5 minutes before introduction of the stimulus female. Introduction of the sexually receptive female marks the beginning of the experiment. The components of male sexual behavior are pursuit, mount, intromission and ejaculation as shown in Fig.1. The latencies of different components of male sexual behavior were recorded by using separate stop watches for each of the parameters studied. Simultaneously frequencies of the different components of sexual behavior was also recorded in 10 minutes. The occurrence of each pursuit, mount, intromission and ejaculation was scored according to classical criteria.

Sex drive score: Weightage was given to individual components of male sexual behavior for quantification of sex behavior as mentioned below in table 1.

Table 1

S. No.	Parameter	Sex drive Score
1	Pursuit	1
2	Pursuit + mount	2
3	Pursuit + Intromission	3
4	Pursuit + Ejaculation	4

The sequence of arrangement of 4 parameters indicates an increasing of sex drives. The order of arrangement is justified by the circumstances that these parameters appear in this sequence during sexual maturation.[23] Weightage was given to all counts of the four events occurring during the test period and all the values were added up.

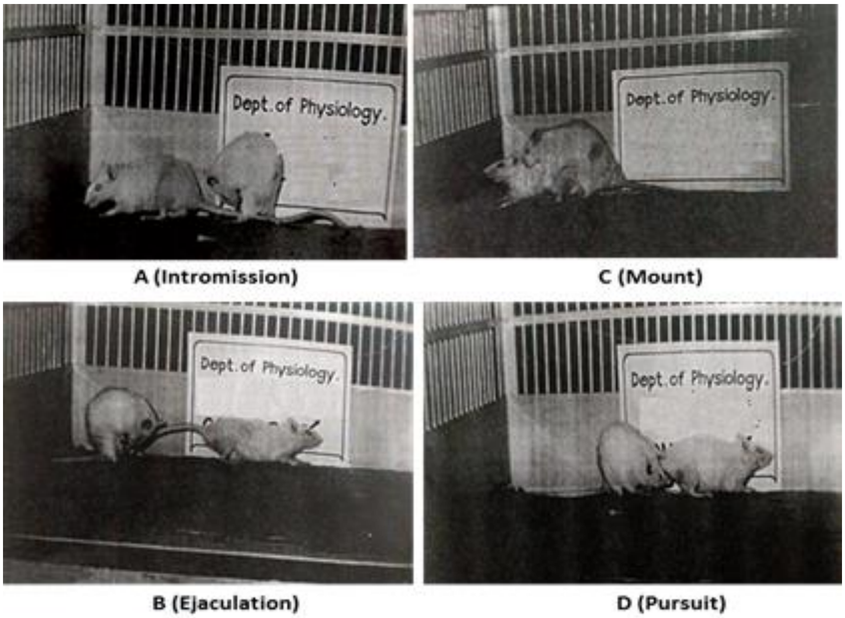

Fig. 1: Shows different components of Sexual behavior

Method of sperm count and Testicular morphology

Testis was exposed by taking incision over the skin and testicular fluid was aspirated from caudal portion of epididymis by needle aspiration method. For sperm count testicular fluid is taken into WBC pipette mixed with diluents fluid and Neubaurs chamber was charged it was focused under low power of microscope and finally sperms were counted. For determination of sperm motility drop of testicular fluid is taken over the warm slide and is mixed with 0.9 % saline. Motility was determined under high power with reduced illumination. Testicular tissue was processed, stained with haematoxylin and eosin and activity is observed.

Statistical analysis

The mean of sexual behavioral score before surgical procedure and after septal lesioned rats were calculated and ANOVA was applied to see if the difference observed in individual group were significant. This was followed by Tukey's multiple comparison tests to test the level of significance of observed differences in individual groups.

Results

The effect of bilateral septal lesions on sexual behavior, sperm count, sperm motility and testicular weight was studies in 24 male rats which are divided into group I as a control (n=8) and Group II (n=8) as sham lesioned and group III(n=8) were septal lesioned. Sexual behavior is significantly decrease in septally lesioned rat (Table 2) as compared to control and sham group as indicated by decrease in intromission and ejaculation frequency ($p<0.01$) and decrease in sex drive score ($p<0.01$). However there was not much change in the latencies of Pursuit, Mount, Intromission and ejaculation in control, sham and septal lesioned group (Table 3). By studying the Fig No.2 it is seen that sperm count is markedly reduced ($p<0.01$) in septally lesioned rat as compared to control and sham group. We have tried the sperm motility also in all the three groups. However, the result were not accurate due to technical difficulties therefore not worth commenting upon.

Testicular weights were compared in all the three groups. There is statistically significant decrease in testicular weight in septally lesioned rats as compared to sham group. (Fig. 3) Histologically, the lesioned rats showed depressed spermatogenesis as demonstrated by a general decrease in the number of cells from different generation of germinal epithelium in some rats, empty spaces i.e. total absence of germinal epithelium in some rats and maturation arrest in few rats in comparison with those of control and sham group rats which had healthy germinal epithelium and high population of spermatozoa.

Table 2: Effect of bilateral septal lesions on Pursuit, Mount, Intromission and ejaculation frequencies and Sex Drive score (S.D.S.) in 10 minutes (mean ± SD)

Sexual behavior parameters	Group I (n=8)	Group II (n=8)	Group III (n=8)
Pursuit	8.31 ± 1.04	7.75 ± 0.59	8.31 ± 0.98
Mount	4.00 ± 0.37	3.88 ± 0.48	2.44 ± 0.34*
Intromission	11.75 ± 0.96	11.86 ± 0.75	7.38 ± 0.56 *
Ejaculation	1.12 ± 0.08	1.19 ± 0.13	0.63 ± 0.12*
Sex drive score	81.25 ± 3.08	78.93 ± 2.20.50	55.93 ± 2.35*

* Statistical significance

Table 3: Effect of bilateral septal lesions on Pursuit, Mount, Intromission and ejaculation latencies in seconds (mean ± SD)

Sexual behavior parameters	Group I (n=8)	Group II (n=8)	Group III (n=8)
Pursuit	13.06 ± 2.54	8.81± 1.46	15.50 ± 2.50
Mount	55.87 ± 9.82	34 ± 6.11	48.68 ± 10.32
Intromission	83.25 ± 18.05	58.31 ± 9.59	89.62 ± 18.50
Ejaculation	255.43 ± 19.47	291.75 ± 38.11	344.93 ± 60.59

* Statistical significance

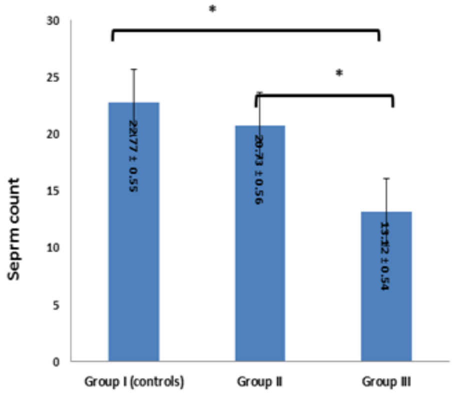

Fig. 2: Effect of Bilateral septal lesion over sperm count (mean ± SEM)

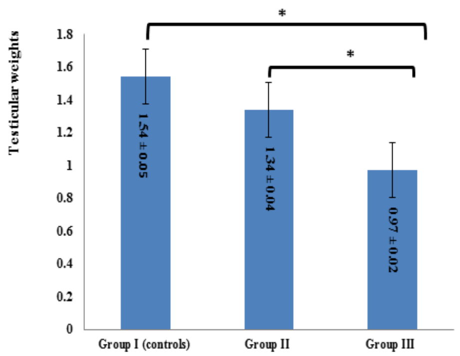

Fig. 3: Effect of Bilateral septal lesion over testicular weights (mean ± SEM)

Discussion

Beach et al has suggested that copulatory behavior in rat requires a successive activation of two excitatory mechanisms, the sexual mechanism (SAM) and the intromission and ejaculation mechanism (IEM). In sexual behavior first there is need for sexual arousal. When this occurs, intromission can be achieved and copulation follows. Intromission and copulation then provide mew additional source of stimulation so that the animal passes ejaculation threshold and ejaculation[24] The bilateral electrolytic lesion in the septum in present study causes increase in the pursuit, mount, intromission and ejaculation latencies; decrease in the mount, intromission, ejaculation frequency and decrease in the sex drive score, indicating that SAM and IEM are impaired. These findings are consistent with the earlier report[5,21,25,26] and suggested that septum has facilitatory influence on masculine sexual behavior in rats.

The septal region has attracted attention of many because of its apparent involvement in variety of motivational, emotional and associative processes. Unfortunately the neural basis of these behavioral activities is poorly understood. The known connections of the septal region relate to it most directly to hypothalamus and those parts of the brain stem and telencephalon that share significant connections with the hypothalamus. Swanson and Cowan have demonstrated that the ventral part of the lateral septum projects massively to the medial preoptic area and the adjoining area. The importance of POA in regulation of masculine behavior is well known in mammals.[8,27] Electrical stimulation of POA, dorsomedial hypothalamus and the lateral hypothalamic area evoke a typical sequence of sexual behavior. It is seen that lesions of preoptic area anterior hypothalamus severely disrupt male copulatory response.

Some studies have demonstrated that a large horizontal cut made between the septum and preoptic area strongly facilitates lordosis and soliciting behavior both in female and male rats and these suggest the importance of ventral neural output of septum in mechanism of inhibition of female sexual behavior in rats.[18,28] Thus it is likely that lateral septal area together with mPOA and median forebrain bundle may play a role in integrating limbic influences for expression of total masculine behavior.

In our study septal lesions not only suppresses sexual behavior but also affect the testicular morphology and sperm count of the animal indicating that these effects of septal lesions may be mediated through hormones like FSH,LH and testosterone. It is known that the activity of the pituitary gonadotrophs is governed by the central nervous system, secretory neurons terminating in the medial eminence of the hypothalamus produce controlling factors which arrive by the local portal vessels. To date only one hormone, gonadotrophin releasing hormone (GnRH) has been isolated from the hypothalamus which has a properties of dictating gonadotrophin secretion. This peptide stimulates the synthesis and secretion of both LH and FSH by the gonadotrophins.

GnRH has been detected in the blood draining the hypothalamus in the rat[29] and rhesus monkey[30] and changes in its secretion are thought to occur during puberty and during the seasonal sexual cycle. Gnrh is secreted in pulses and judging from the concentrations achieved in the portal blood, these represent the secretion of neumorous secretory neurons which are in some way synchronized in their activity. It is generally accepted that it is the pulsatile GnRH secretion that causes the pusatile pattern of FSH & LH secretion. Since one of the most conspicuous change during puberty or the seasonal sexual cycle is a change in the frequencies of episodic peaks of LH in the blood, it can be expected that frequency control of pulsatile GnRH release is one of the principal ways by which the hypothalamus regulates pituitary and testicular activity.[31]

The dependence of the male gonads on the pituitary gland was demonstrated quite long back in the rat, when it was shown that testes regress after hypophysectomy and that anterior extracts more specifically FSH & LH or testosterone maintained or restored the testicular weight of hypophysectomized rats. Only a few papers have dealt with the quantitative analysis of spermatogenesis in the adult and immature animals.

In the rat in which almost all work on hypophysectomy has been carried out, removal of the pituitary gland induces a severe regression in spermatogenesis, as shown by a decrease in the number of germ cells in seminiferous tubules until 25 days after hypophysectomy, after which the number of germ cells remains fairly constant. Hypophysectomy reduces the number of stem spermatogonia and the yield of all the subsequent steps of spermatogenesis. However, some round spermatids are still produced but they all degenerate.[32] In hypophysectomized rats, the stages which are more sensitive than other to hormonal deprivation are from A_0 to A_1 stem spermatogonia, the different spermatogonial generations meiotic prophase.[33] In our study, histological findings are maturation arrest and /or total absence of germinal epithelium. Experimental data reveals that the basal compartments cells of seminiferous tubules are more rapidly affected by any change in hormonal balance which suggests that they are a direct target for gonadotrophins and testosterone. This seems likely as FSH receptors have been demonstrated on sertoli cells[34] and spermatogonia[35] of the rat. It is difficult to determine whether gonadotrophins and/or androgens act directly on spermatogonia or indirectly by causing sertoli cells to secrete substances that enhance spermatogenesis. At present sertoli cells are considered to be the main target cells for hormones in seminiferous tubules. Their response to these hormones is well documented for the immature rats[34] and such a response could end in information being translated to the germinal cells, especially those of adluminal compartment. Androgen binding protein (ABP) which transports and concentrates androgens around the germinal cells[34] is an end product of hormone stimulated sertoli cells. The gonadotrophins directly dictate the activity of somatic cells of the testis and thus create an environment suitable to the normal development of the germ cells during maturation division.

Thus review of physiology of testes suggests that mechanism for the reduced fertility and aletered testicular morphology in septally lesioned rats operates through hypothalamus. It is possible that the bilateral septal lesion disrupt septal connections with the part of hypothalamus which are responsible for production of GnRH. The decreased GnRH secretion in turn may reduce the fertility, sperm count and alter the testicular morphology. Though at the moment there is no direct evidence for the hypothesis, further studies on effect of septal lesions on the hormonal levels in the blood would help to resolve the issue.

Conclusion

The present study shows that the bilateral lesion of the septal nuclei decreases the sexual behavior in male rats. The bilateral septal lesion also alters the testicular weight and morphology along with decrease in sperm count. This indicates that septal nuclei control the functions of reproductive organ in male rats. It would be worthwhile to estimate testosterone levels in male rats following septal lesions to elucidate the exact mechanism of action of septal lesion.

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