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Oestrous Cycle in Septal Rat Females

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Summary

Bilateral lesions of the dorsal part of the septal area were produced in the brain of female rats of Wistar and Long-Evans stocks. The cytogram of their vaginal smears was evaluated repeatedly from the 30th day after surgery and permanent impairment of their oestrous cycle was found. In comparison with intact control females, oestrous phases were detected more frequently whereas dioestrous phases were observed less frequently. The proportions between the individual periods of the oestrous phase (i.e. pro-oestrus, oestrus and metoestrus) were equal in both septal and intact animals. No difference was found between the rat stocks used. Similarly, the previous life history of the rats which had been housed either in a socially impoverished environment (i. e. single cages) or in large communities (colonies) exerted no influence upon the postoperative impairment of the oestrous cycle.

Key words

Oestrous cycle – Septal lesion – Stock differences – Life history

Introduction

In our previous paper we reported that dorsal septal lesions elicited disturbances of maternal behavior and infanticide in those Wistar females that had lived in a socially impoverished environment. On the contrary, septal Wistar females which had not been submitted to this deprivation as well as all operated Long-Evans females showed neither alterations of maternal behavior nor infanticide (Nováková *et al.* 1993). It is a well-known fact that social behavior is also affected by hormones including the gonadal ones (for review see Blaustein and Olster 1989). It was therefore important to know whether septal injury does not alter the hormonal state of the organism, e.g. by such changes of the oestrous cycle which could participate in alterations of maternal behavior. If there would exist such a causal relation, then changes of the oestrous cycle should occur mainly in those Wistar females which had been reared in socially deprived circumstances.

Methods

Experimental groups. The experiments included females of the same age and genetic origin from either the Wistar (W) or the Long-Evans (LE) stocks. The offspring sisters were always divided into the two sets of intact and operated animals. Each set comprised two groups of females according to their previous life history: one group of animals had lived from birth in a socially impoverished environment, the other group in a socially enriched one. The conventional rearing conditions representing an impoverished environment are as follows: one dam was always housed in a small undivided standard cage together with her offsprings from delivery until weaning on day 30; then the female young were divided by three into monosexual groups and further bred in conventional cages. As a socially enriched environment we considered life in communities. The common breeding space was diversified, constructed from 5 standard cages interconnected by tunnels. The community always consisted of 4 females with their offsprings and, in addition, of 2 adult males that lived there together. When the young were 30 days old, the adult males were removed. At the age of 45 days all rat mothers and young males were also removed and, the community then consisted of young females only. The animals were subjected to the brain operation at 70 days of age. Thereafter, intact and septal females were housed separately. Starting from day 30 after surgery, repeated daily vaginal irrigations were carried out and smear preparations were made.

Following 8 experimental groups were used :

A) Intact animals :
1) cage-bred Wistar females
2) community-bred Wistar females
3) cage-bred Long-Evans females
4) community-bred Long-Evans females

B) Septal animals :
5) cage-bred Wistar females
6) community-bred Wistar females
7) cage-bred Long-Evans females
8) community-bred Long-Evans females

As far as the set of septal animals is concerned, the size of the experimental groups was additionally reduced according to the histological control of the location, shape and extent of the brain lesion. The number of animals used in the particular groups is given in Table 1.

Surgical intervention. Bilateral electrolytic lesions were produced in the dorsolateral regions of the septum (stereotaxic coordinates: anterior 1.0 mm, lateral ± 0.5 mm and vertical 4.4 mm from the bregma). In animals anaesthetized with Nembutal (40 mg/kg i.p.) stainless steel electrode with 0.2 mm in diameter was insulated except for 0.5 mm of the tip. Each electrolytic lesion was performed by the cathode (DC 0.5 mA for 40 s), an injection needle introduced into the skin of the animal's back serving as the indifferent electrode. For other details see our preceding paper (Nováková *et al.* 1993).

Table 1

Experimental groups, number of vaginal smears examined and the incidence of determined oestrous and dioestrous phases of the oestrous cycle

Group	Number of animals	Vaginal smears	Incidence of dioestrus	Incidence of oestrus	Index D/OE	Index OE/D
Intact rats						
cage-bred W	10	193	128	65	1.97	0.51
cage-bred LE	12	230	162	68	2.38	0.42
community-bred W	8	115	76	39	1.95	0.51
community-bred LE	9	135	92	43	2.14	0.47
Septal rats						
cage-bred W	24	335	107	228	0.47	2.13
cage-bred LE	29*	395	127	268	0.47	2.11
community-bred W	9×	135	50	85	0.59	1.70
community-bred LE	11*	132	41	91	0.40	2.51

*W = Wistar female rats; LE = Long-Evans (hooded) female rats; D = dioestrous phase; OE = oestrous phase (comprising the pro-oestrous, oestrous and metoestrous periods together in this case) * 1 case of permanent OE, # 1 case of permanent D)*

Identification of the day of the oestrous cycle. Vaginal smears were taken by washing the vagina with about 0.5 ml saline using a glass pipette. The dried fluid stained by the Giemsa method was examined under a light microscope. Irrigations were performed during 5 successive days in 3–4 series. In cage-bred samples of 4 Wistar and 4 Long-Evans septal females daily vaginal irrigations were carried out for control purposes over a period of 12 days without interruption. Irrigations were always made between 09.00–11.00 h under non-regulated light regime conditions. Those preparations containing a vaginal cytogram which could not be unequivocally identified, were eliminated. In general, the vaginal cytology was estimated as dioestrus (D) or oestrus (OE). As seen in Tables 1 to 4, the oestrous phase comprised all three periods of the oestrus, i.e. pro-oestrus, oestrus and metoestrus. In Table 5 each period of the oestrous phase is quoted separately. Fig. 1 shows typical examples of the oestrous cyclicity in some intact and septal females during repeated 5-day or 12-day series of vaginal irrigations.

Cytophotometric measurements of the total RNA content in brain cells. Prior to the end of these experiments two oestrous females were taken from each group (totally 8 intact and 8 septal animals) and the total content of ribosomal RNA in individual cells of their ventromedial and lateral hypothalamus was determined by means of a quantitative microdensitometer (Barr and Stroud, Glasgow). Histochemical preparations consisting of whole cells obtained by a special crushing procedure were stained with gallocyanine-chromalum and measured at 560 nm wavelength. Sets of 80 brain cells for each group were always compared. The values are expressed in arbitrary units of monochrome light absorption.

Histology of the ovaries was examined in animals in which the septal lesions had all the required parameters as regards location, shape and extent and which were therefore included in the results. Histological control was carried out in the sections stained with haematoxylin-eosine. The occurrence of detailed pathological alterations was further not quantified.

Statistics. One-way analysis of variance (ANOVA), Tukey's multiple comparison test, Student's t-test and the chi-square test were used for statistical evaluation.

Table 2
Incidence of dioestrous and oestrous phases in the individual groups of intact females

Group	Dioestrus	Oestrus	Index D/OE
Cage-bred W	12.8±0.5	6.5±.07	2.4±0.5
Cage-bred LE	13.5±0.6	5.7±0.5	2.6±0.2
Community-bred W	9.5±0.4	4.9±0.4	2.0±0.1
Community-bred LE	10.2±0.3	4.8±0.3	2.2±0.2
F	15.52	2.26	0.62
($df_1/df_2=3/35$)	$p<0.0001$	n.s.	n.s.
Tukey's ±D	1.89	2.02	1.16

Data are means ± S.E.M. Tukey's ± D is at p=0.05 level. For further explanation see Table 1.

Table 3
Incidence of dioestrous and oestrous phases in the individual groups of septal females

Group	Dioestrus	Oestrus	Index D/OE
Cage-bred W	4.5±0.4	9.5±0.6	0.53±0.05
Cage-bred LE	4.4±0.4	9.2±0.5	0.54±0.07
Community-bred W	5.6±0.4	9.4±0.4	0.62±0.08
Community-bred LE	3.7±0.6	8.3±0.6	0.53±0.12
F	1.43	0.67	0.16
($df_1/df_2=3/69$)	n.s.	n.s.	n.s.
Tukey's ±D	1.94	2.43	0.33

Data are means ± S.E.M. For further explanation see Tables 1 and 2.

Results

Table 1 presents a general survey concerning the determination of the phases of the oestrous cycle (without statistical evaluation) as well as the numbers of animals in the experimental groups and of the vaginal smears examined (Table 1). No significant difference was found between incidences of the oestrous phase in groups of intact females from both stocks ($F=2.26$; df/df=3/35). On the contrary, the dioestrous cytogram was seen less frequently in those W and LE females which have been bred in communities ($F=15.52$; df/df=3/35; $p<0.0001$) (Table 2). In septal females, the incidence of the dioestrous cytogram was nearly identical in all groups of both rat stocks ($F=1.43$; df/df=3/69); just the same result was observed in the case of the oestrous phase ($F=0.67$; df/df=3/69) (Table 3).

In all septal females, the incidence of cytograms of the dioestrous type decreased independently of their strain and their previous life history as compared with the non-operated ones (Fig. 1). On the contrary, the incidence of cytograms of the oestrous type in septal females increased simultaneously. The D/OE index (the quotient given by dividing the dioestrous by oestrous smear values) ranged from 2 to 2.4 in the intact females and from 0.4 to 0.6 in the septal ones. The reciprocal OE/D index then offers ranges from 0.4 to 0.5 for the intact rats and from 1.7 to 2.5 for the septal animals, thus yielding a perfect mirror pattern of the oestrous cycle following this type of brain injury (Table 4).

No significant differences between proportional participation of pro-oestrus, oestrus and metoestrus in the total oestrous phase were observed in intact and septal females (chi-square = 1.57 for cases, 0.47 for percentage; df=2; n.s.). The most frequently observed cytogram exhibited properties typical for the oestrous pattern whereas cytograms of the metoestrous pattern were seen least frequently (Table 5).

Histological control of the ovaries showed different degrees of atrophy with the formation of follicular and luteinic cysts in all septal rats. In non-operated females no pathological changes were found.

In 2 septal females from the LE stock permanent oestrus was observed, another W female showed permanent dioestrus.

Cytophotometric measurements of the total RNA content in the ventromedial hypothalamic cells showed higher values in septal animals. Their mean RNA values (M±S.E.M.) were 197.1±3.2 arbitrary units while in intact animals these values were 185.3±3.5 only, the difference being significant ($p<0.01$). In the cells of the lateral hypothalamus the analogous difference of the total RNA content was not significant (181.8±3.8 after septal lesion, 182.6±4.1 in the controls).

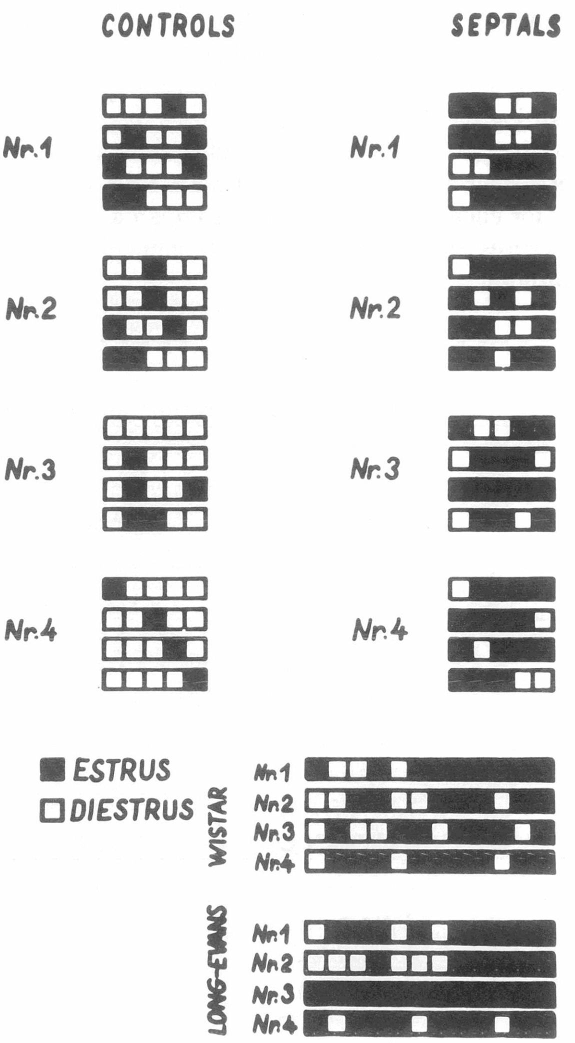

Fig. 1
Incidence of the oestrous (black squares) and dioestrous phase (empty squares) in randomly selected 4 control (No. 1 and No. 2 = Wistar, No. 3 and No. 4 = Long-Evans rats) and 4 septal females (again 2 equally numbered Wistar and 2 Long-Evans ones) from the respective cage-bred groups. Upper part of the figure: Each square represents 1 day, each short bar 5 consecutive days. Lower part: Analogically, the incidence of both phases of the oestrous cycle during 12 consecutive days in other groups of 4 septal Wistar and 4 septal Long-Evans females is depicted by long bars. The figure serves as illustration of the oestrous cycle course in individual control and in septal lesioned animals.

Table 4
Incidence of dioestrous and oestrous phases in the individual groups of intact and septal females

Group	Brain	Dioestrus	Oestrus	Index D/OE
Cage-bred W	Intact	12.8±0.5	6.5±0.7	2.41±0.49
	Lesioned	4.5±0.4	9.5±0.6	0.53±0.05
Cage-bred LE	Intact	13.5±0.6	5.7±0.5	2.56±0.21
	Lesioned	4.4±0.4	9.2±0.5	0.54±0.07
Community-bred W	Intact	9.5±0.4	4.9±0.4	2.01±0.41
	Lesioned	5.6±0.4	9.4±0.4	0.62±0.08
Community-bred LE	Intact	10.2±0.3	4.8±0.3	2.22±0.18
	Lesioned	3.7±0.6	8.3±0.6	0.53±0.12
F $(df_1/df_2=7/104)$		62.13 $p<0.0001$	10.87 $p<0.0001$	31.64 $p<0.0001$
Tukey's ±D		2.38	2.87	0.79

Data are means ± S.E.M. For further explanation see Tables 1 and 2.

Table 5
Proportional occurrence of individual oestrous periods in intact and septal females

	Pro-oestrus		Oestrus		Metoestrus		Chi-square		
	Cases	%	Cases	%	Cases	%	Cases	%	
Intact	62	28.8	137	63.7	16	7.4	156.2	72.72	$p<0.00$
01									
Septal	222	33.0	409	60.9	41	6.1	453.5	67.58	$p<0.00$
01									
Chi-square	1.32 n.s.	0.41 n.s.	0.56 n.s.	0.18 n.s.	0.49 n.s.	0.14 n.s.	1.57 n.s.	0.47 n.s.	– –

Both the intact and the septal groups include females from 4 subgroups, i.e. cage- and community-bred animals of the Wistar and Long-Evans stocks.

Discussion

The present study revealed that the septum takes part in the control of the oestrous cycle in rat females. Following dorsal septal lesions, the incidence of the oestrous phase was more frequent, whereas the dioestrous phase occurred less frequently.

The findings described in this paper indicate that surgical intervention into the limbic system had a definite effect on the oestrous cycle in females of different rat stocks, independently of whether they had lived in an enriched or impoverished environment. A qualitatively entirely different effect of the same operation was observed with respect to maternal behavior. Its pattern was disturbed in cage-bred Wistar females only, in which infanticide regularly appeared and natural maternal behavior was paradoxically replaced by a xenoparental one (Nováková *et al.* 1993). Thus, the septal syndrome displayed a marked dichotomy. On one hand, disturbances of maternal behavior were dependent on the stock adherence, on the other hand, irregularities of the oestrous cycle after septal brain injury appeared in all female subjects independently of their genetic and phenotypical properties. Hence, for the stability of maternal behavior the properties of the neurones of the limbic system were decisive, i.e. whether this had developed in an optimal or suboptimal way. Only the suboptimally developed limbic system was so labile that it was not able to compensate for the deficit of the integrated behavioral function elicited by the partial septal injury. However, even an optimally developed limbic system was not able to attenuate disturbances of cyclic ovarian function, though ovulation also appears to be the result of the integration of complex hormonal and neural effects, some of them being facilitatory and some inhibitory (for review see Gorski *et al.* 1975). The hypothalamic structures seem to be really dominant in such a case. Thus, the septal syndrome always implies impairment of the oestrous cycle but need not necessarily impair behavior.

Our data raise considerations on the role of the medial hypothalamus in septal symptomatology because a relatively small intervention into the limbic system resulted in an increased total content of ribosomal RNA in ventromedial hypothalamic cells. Therefore, in the mechanism of septally initiated disturbances of oestrous cycle, even activated ventromedial hypothalamic structures may participate. This effect may have been caused either by the attenuation of the modulatory (in general inhibitory) action of the septum on regulatory hypothalamic regions, or by a long-lasting influence of higher oestrogen levels. In both cases activation of the neurones could appear. Oestrogen acts at the cellular level by binding their receptors and increases mRNA levels only in those brain regions that express oestrogen receptors (Maggi *et al.* 1989). Our data on higher total RNA contents in ventromedial hypothalamic cells indicating their enhanced activity in septally damaged animals are consistent with this report.

Disturbances of the oestrous cycle were also evoked in a reverse situation, e.g. if the activity of the ventromedial hypothalamus was decreased either by surgical injury or by a genetic lesion in obese females. Overfeeding and weight gain were associated with a longer dioestrous phase and a less frequent oestrous phase, duration of the oestrous cycle being increased (Glick *et al.* 1990). According to Sclafani (1971) exclusive destruction of the ventromedial hypothalamus resulted in a prolonged (14 days) dioestrus immediately after the operation, then followed by a permanent oestrus. Independently of the kind of deformation of the oestrous cycle, sexual receptivity and fertility decreased in females with hypothalamic injuries (for review see Bray and York 1979). These findings present a substantial difference between septally and hypothalamically operated females: the "septal" females displayed ovulatory oestrus, while oestrus in "hypothalamic" animals had anovulatory properties. Histopathological alterations in the ovaries of septal females were obviously not as extensive as to decrease their fertility, at least during this postoperative period.

In addition, a difference in the incidence of the dioestrous phases between the two stocks of intact females was found in our experiments. In community-bred females the incidence of dioestrus was lower in comparison with cage-bred animals. This could be interpreted by the generally known phenomenon of synchronization of the individual cycle phases in a close community and dormitories, based probably on the pheromone (olfactory) principle (Horský and Pressl 1981). Such effects might result in more homogeneous animal groups bred in large communities as compared to females reared in isolated small cages. In septal females this phenomenon could not be observed since the oestrous phase of the cycle was prevailing.

In conclusion, since the lesion-induced disturbance of the oestrous cycle was affected neither by the strain (Wistar vs Long-Evans) nor by breeding conditions (single cage vs community), it cannot explain the earlier reported failure of maternal behavior and infanticide observed under similar conditions in cage-bred Wistar rats only as septal symptoms of disturbances of mothering and of oestrous cycle in laboratory rats are independent of each other.

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Reprint Requests

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