COMPENSATORY OVULATION IN BLINDED RATS AND/OR THOSE FROM WHICH THE OLFACTORY BULB HAD BEEN REMOVED

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Summary. Thirty-two prepubertal, female rats (Southern Farms) were allocated to intact control, bilateral optic enucleation (blinded), bilateral olfactory bulb removal (anosmic) and blinded–anosmic groups. Olfactory bulbs were surgically removed between 27 and 29 days of age and eyes were removed at 30 days of age. One ovary was removed from each animal between 107 and 112 days of age on Day 2 (metoestrus) of the oestrous cycle. The number of eggs ovulated was determined by flushing the oviducts with normal saline solution. All rats completed one oestrous cycle and were killed at metoestrus of the following cycle. There was no difference in the number of ova shed between the four groups at the time of removal of the first ovary. One cycle later, compensatory ovulation was found at autopsy to have occurred in all animals. Controls ovulated 10.3 ± 0.5 eggs; blinded, 9.6 ± 0.7 ; anosmic, 10.3 ± 0.3 ; and blinded–anosmic, 9.7 ± 0.8 . Follicular development was quantitatively analysed in both intact and hemispayed blinded and/or anosmic rats. These data suggest that pituitary–ovarian function as evaluated by the number of eggs ovulated is not affected by blinding and/or anosmia.

INTRODUCTION

The observation that compensatory ovarian hypertrophy is partially blocked in rats subjected to bilateral optic enucleation (blindness) and olfactory bulb removal (anosmia) has resulted in extensive use of this preparation as an experimental model for studying pineal–gonadal relationships. Pinealectomy apparently reverses the block imposed by the combined sensory deprivation, perhaps by removing the pineal antigonadal substance, melatonin (Rubin & Traum, 1971). Whether either sensory deprivation or pinealectomy markedly influences the physiological function, i.e. ovulation of eggs, of the ovary has not been evaluated.

Thus, the objective of this study was to determine the effect of bilateral optic enucleation and/or bilateral olfactory bulb removal on ovulation number and follicular development in intact and unilaterally ovariectomized rats.

MATERIALS AND METHODS

Thirty-two prepubertal, female, albino rats (Southern Farms) were divided into intact control, blinded, anosmic and blinded–anosmic groups. Blinding was produced by bilateral enucleation at 30 days of age and animals were made anosmic between 27 to 29 days of age by surgically removing the olfactory bulbs.

Following surgery, rats were housed two per cage under conditions of controlled lighting (fluorescent illumination from 04.00 to 18.00 hours) and temperature ($24 \pm 1^\circ \text{C}$). Purina lab chow and tap water were freely available. Daily vaginal smears were taken, beginning at 60 days of age, and were continued until the animals were killed between the 112th and the 118th day on Day 2 of the oestrous cycle (Day 1 of the cycle refers to oestrus). Each animal was hemispayed on Day 2 (metoestrus) and killed one vaginal cycle later. Each oviduct was dissected from the ovary and flushed with normal saline to determine ovulation number. Ovaries were weighed to the nearest 0.1 mg and were serially sectioned at 10 μm . Each section was examined but the identity of individual ovaries remained unknown until all had been studied. To avoid counting the same follicle twice, only the section containing the nucleolus of the oocyte was measured. Follicular size was calculated by measuring two diameters at right angles to each other, and the follicles were arbitrarily classified into six groups: 294 to 351 μm ; 352 to 394 μm ; 395 to 447 μm ; 448 to 517 μm ; 518 to 570 μm ; > 570 μm .

Statistical probabilities were determined by the Student *t* test; a *P* value of 0.05 or less was considered significant.

RESULTS

The ovarian dynamics of blinded and/or anosmic rats at the time of unilateral ovariectomy (Days 107 to 112) are summarized in Table 1. The first ovary

Table 1. Ovarian dynamics in blinded and/or anosmic rats

	<i>Control</i>	<i>Blinded</i>	<i>Anosmic</i>	<i>Blinded–anosmic</i>
No. of animals	8	8	8	7
Mean ovarian weight (mg/100 g body wt) $\pm$ S.E.	19.8 ± 1.1	$17.1 \pm 1.0^*$	18.4 ± 0.5	$15.8 \pm 1.1^{**}$
Mean no. of ova shed $\pm$ S.E.	4.4 ± 0.2	4.8 ± 0.6	4.4 ± 0.5	4.1 ± 0.5
Total no. of follicles larger than 294 μm $\pm$ S.E.	27.2 ± 2.0 (5)	$18.7 \pm 2.0^{**}$ (6)	22.3 ± 3.2 (6)	$12.8 \pm 2.2^{**}$ (6)

Numbers in parentheses signify numbers of ovaries examined.

* $P < 0.05$. ** $P < 0.01$.

removed from control animals weighed 19.8 ± 1.1 mg/100 g body wt and contained 27.2 ± 2.0 follicles which were larger than 294 μm . By contrast, the ovarian weight and the total number of follicles were reduced in blinded and blinded–anosmic animals; anosmic animals were not different from control rats. It was of interest that all animals ovulated the same number of eggs.

Closer analysis of follicular development (Table 2) revealed that the difference in the total number of follicles between control and blinded and blinded–anosmic animals was only present in the 352- to 394- μm and 395- to 447- μm follicular-size groups. In addition, anosmic animals had fewer follicles of 352 to 394 μm in size than controls. Other differences in the number of follicles of a particular size between control and experimental animals were not apparent.

Table 2. Follicular development in one ovary from blinded and/or anosmic rats

Size of follicles (μm)	Control	Blinded	Anosmic	Blinded–anosmic
294 to 351	7.8 $\pm$ 2.0 (5)	5.5 $\pm$ 1.0 (6)	4.8 $\pm$ 0.9 (6)	4.0 $\pm$ 1.5 (6)
352 to 394	6.6 $\pm$ 0.7 (5)	4.2 $\pm$ 0.8 (6)*	4.0 $\pm$ 0.6 (6)**	2.2 $\pm$ 0.6 (6)***
395 to 447	7.0 $\pm$ 0.6 (5)	4.3 $\pm$ 0.8 (6)*	6.2 $\pm$ 1.2 (6)	3.7 $\pm$ 0.8 (6)**
448 to 517	4.2 $\pm$ 1.2 (5)	3.7 $\pm$ 0.9 (6)	6.0 $\pm$ 1.9 (6)	3.3 $\pm$ 0.9 (4)
Mean $\pm$ S.E.	25.6 $\pm$ 2.0 (5)	17.7 $\pm$ 1.2 (6)*	21.0 $\pm$ 2.8 (6)	12.0 $\pm$ 2.6 (6)***

Numbers in parentheses signify numbers of animals in groups with follicles of the respective size ranges.

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.005$.

In anosmic and blinded–anosmic rats which had been unilaterally ovariectomized for one oestrous cycle, the weight of the remaining ovary was significantly less when compared with the value from control animals (Table 3). All animals provided evidence of compensatory ovulation in a doubling of the number of ova shed by the remaining ovary following unilateral ovariectomy.

Blinded–anosmic rats had a decrease in the total number of follicles larger than 294 μm when compared with control animals (18.0 versus 24.0, respectively). In comparing the total number of follicles in the first ovary removed with the number in the remaining ovary, a significant increase was observed in the blinded–anosmic rats.

All three experimental groups (blinded, anosmic, blinded–anosmic) had fewer follicles in the size group 395 to 447 μm . Other differences in follicle-size groups were not apparent (Table 4).

Table 3. Ovarian dynamics in blinded and/or anosmic unilaterally spayed rats

	Control	Blinded	Anosmic	Blinded–anosmic
No. of animals	8	8	8	7
Mean ovarian weight (mg/100 g body wt) $\pm$ S.E.	23.1 $\pm$ 1.5	21.6 $\pm$ 1.0	19.7 $\pm$ 1.1†	19.8 $\pm$ 1.3*
Mean no. of ova shed $\pm$ S.E.	10.3 $\pm$ 0.5	9.6 $\pm$ 0.7	10.0 $\pm$ 0.3	9.7 $\pm$ 0.8
Total no. of follicles larger than 294 μm $\pm$ S.E.	24.0 $\pm$ 2.3 (6)	21.7 $\pm$ 1.6 (6)	22.7 $\pm$ 2.0 (6)	18.0 $\pm$ 1.8* (6)

Numbers in parentheses signify numbers of ovaries examined.

* $P < 0.05$. † $P < 0.25$.

Table 4. Follicular development in the remaining ovary from blinded and/or anosmic rats unilaterally spayed for one cycle

Size of follicles (μm)	Control	Blinded	Anosmic	Blinded-anosmic
294 to 351	4.5 ± 0.6 (6)	4.5 ± 0.9 (6)	4.0 ± 0.5 (6)	4.8 ± 0.9 (6)
352 to 394	4.3 ± 0.8 (6)	4.2 ± 0.6 (6)	4.0 ± 0.8 (6)	4.2 ± 0.8 (6)
395 to 447	8.7 ± 1.4 (6)	6.0 ± 0.4 (6)*	4.8 ± 0.8 (6)†	3.7 ± 0.7 (6)†
448 to 517	4.8 ± 0.8 (6)	4.7 ± 0.5 (6)	6.0 ± 0.5 (6)	3.5 ± 0.6 (6)
Mean $\pm$ S.E.	22.3 ± 2.4 (6)	19.3 ± 1.0 (6)	18.8 ± 2.1 (6)	16.2 ± 1.8 (6)*

Numbers in parentheses signify numbers of animals in groups with follicles of the respective size ranges.

* $P < 0.05$. † $P < 0.025$.

A comparison of the body weight and various organ weights in blinded and/or anosmic hemispayed rats showed that the body weight of blinded-anosmic animals was significantly less than that of the controls and the uterine weight of anosmic animals was decreased. There was, however, no difference in adrenal and thyroid weights between the groups (Table 5).

Table 5. Body and organ weights in blinded and/or anosmic unilaterally spayed rats

	Control	Blinded	Anosmic	Blinded-anosmic
No. of animals	8	8	8	7
Mean body wt (g)	247.0 ± 5.4	235.9 ± 5.2	249.9 ± 6.8	206.9 ± 4.6 †
Uterus (mg/100 g body wt)	131.3 ± 7.0	130.1 ± 6.7	112.5 ± 5.7 †	121.4 ± 13.1
Adrenals (mg/100 g body wt)	27.6 ± 1.3	28.6 ± 0.9	28.5 ± 0.8	27.8 ± 0.9
Thyroid (mg/100 g body wt)	7.0 ± 0.3	6.7 ± 0.3	7.2 ± 0.3	6.9 ± 0.5
Nose-anal length (cm)	21.0 ± 0.2	20.6 ± 0.2	21.0 ± 0.2	19.6 ± 0.3 ***

† $P < 0.025$. *** $P < 0.005$.

Comparison of the nose-anal length indicated that blinded-anosmic rats were significantly shorter than intact controls. Other groups showed no difference.

In comparing the weight of the first ovary with that of the second in this study, all groups except that of the anosmic animals showed a significant increase when considering group change, whether the data were reported as absolute, relative, absolute % or relative %. When analysing the data and considering only those animals in each group which showed a change, all groups displayed compensatory ovarian hypertrophy and to the same degree.

DISCUSSION

A recent study (Peppler, 1971) has shown that ovarian weight is a poor end-point in evaluating compensatory mechanisms and that ovulation number is more indicative of ovarian function. The present study demonstrates that

blinded and/or anosmic hemispayed rats do display compensatory ovulation and appear to exhibit normal ovarian function.

The reduced number of follicles larger than 294 μm and decreased ovarian weight in blinded and blinded–anosmic intact rats suggests lower gonadotrophin plasma levels. This marked decrease in follicular development correlated with the finding that these rats showed fewer days of vaginal cornification during a 30-day period than did control rats. However, the fact that these animals ovulated the same number of eggs as intact controls demonstrates that all animals had adequate gonadotrophin stimulation for the normal ovulation process and number.

Unilateral ovariectomy before Day 4 of the oestrous cycle in intact rats with 4- and 5-day cycles results in the remaining ovary doubling the number of ova shed at the next oestrus (Peppler & Greenwald, 1970a). In the present study, blinded and/or anosmic rats displayed compensatory ovulation following unilateral ovariectomy for one oestrous cycle.

Compensatory ovulation in the rat is caused by an increase in the number of large follicles which mature during the oestrous cycle (Peppler & Greenwald, 1970b). However, the total number of follicles is the same in intact and hemispayed rats at metoestrus after one cycle, three cycles or 3 months (Peppler, 1971). In this study, follicular development at metoestrus in control hemispayed rats was the same as that in control intact rats and, similarly, there was no difference in blinded and anosmic hemispayed rats. Although the remaining ovary of blinded–anosmic hemispayed rats had fewer follicles when compared with ovaries from controls, it was the only one which had a significant increase in the total number of follicles when compared with the ovary removed from blinded–anosmic rats during the previous cycle. Such a finding suggests an increase in the gonadotrophin stimulation following unilateral ovariectomy in these animals. Whether this increase is one of concentration as proposed by Benson, Sorrentino & Evans (1969) or duration of exposure to a minimal amount (Peppler, 1972) has not been determined.

Contrary to the findings of Dickson, Benson & Tate (1971), bilateral optic enucleation did not block the hypertrophy of the remaining ovary in this study. The remaining ovary of control, blinded and blinded–anosmic animals showed a significant increase in weight one cycle later. The increase in anosmic rats was significant when only those individual animals having a change (4/8) were considered. This demonstrates the unreliability of using ovarian hypertrophy as an endpoint in hemispayed animals and emphasizes the necessity of ovulation number as an index of ovarian function.

Neither blinding nor anosmia influenced the final body weights in this study. When the procedures were combined, however, body weight and nose–anal length were substantially less than those of normal rats. This latter finding may reflect decreased levels of plasma growth hormone since Sorrentino, Reiter & Schalch (1971) reported that male rats subjected to blinding and anosmia showed decreased pituitary growth hormone levels.

Only the uterine weight of anosmic animals was lower in this study. This confirms a previous finding by Reiter & Ellison (1970). In contrast, however, uteri in blinded–anosmic rats were not smaller in the present study. This can be

explained by the fact that blinded-anosmic animals showed a significant increase in follicular development following unilateral ovariectomy. This increase in number of follicles may have produced more oestrogen which stimulated uterine growth in these animals.

The data of the present study indicate that ovarian function is not compromised by blinding and/or anosmia. Only follicular development appeared to be affected by photic and olfactory deprivation. Differences in the number of eggs ovulated were not observed among the various groups either before or after unilateral ovariectomy. Collectively, these findings shed doubt on the usefulness of the blind and anosmic rat as an experimental model for studying pineal-gonadal relationships and corroborates previous findings that eggs ovulated and not weight changes are indicative of 'normal' ovarian function (Peppler, 1971).

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