

PRELIMINARY OBSERVATIONS ON THE REPRODUCTIVE EFFECTS OF THE PINEAL GLAND IN BLINDED, ANOSMIC MALE RATS

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Removal of the eyes (Reiter, 1968) or of the olfactory bulbs (Orbach & Kling, 1966) slightly delays gonadal maturation in rats. The gonadal inhibitory effect of blinding is prevented if animals are pinealectomized (Reiter, 1968). It was found recently that if weanling male rats have both their eyes and olfactory bulbs removed, the testes are less than half their normal size at 70 days of age; this entire effect is reversed if the animals also have their pineal gland removed. This indicates that another external factor, in addition to the photo-period, may influence directly or indirectly the antigonadotrophic activity of the pineal gland. The experiment was repeated and the following data illustrate some interrelationships between olfaction, photo-period, the pineal gland and reproduction.

Sixty-four 21- to 23-day-old male Sprague-Dawley rats were divided into seven groups (Table 1). Although not indicated in the Table or mentioned hereafter, all non-pinealectomized animals were subjected to a sham pinealectomy; this operation had no apparent effect on the results. The methods for performing real pinealectomy (Hoffman & Reiter, 1965) and blinding (Reiter, Sorrentino, Hoffmann & Rubin, 1968) have been previously described. Olfactory bulbs were aspirated with mild suction through two holes (2 mm diameter) which were drilled in the skull overlying these structures. Animals were kept two to a cage in temperature- $(23 \pm 2^\circ \text{C})$ and light-(14 hr of light/day) controlled rooms. Light was provided by 40-watt 'cool white' fluorescent bulbs with an intensity of 20 to 85 footcandles within the cage. Food was placed on the floor of the cages to ensure that it could be found by all animals. Rats were killed, between 10.00 and 13.00 hours, at 68 days of age and the testes, seminal vesicles and coagulating glands were weighed and prepared for microscopy. Pineal glands of non-pinealectomized rats were weighed and assayed for their content of hydroxyindole-O-methyltransferase (HIOMT) according to the method of Axelrod, Wurtman & Snyder (1965). HIOMT, an enzyme found only within the pineal gland of mammals, is required for the synthesis of melatonin, a pineal antigonadotrophic substance (Wurtman, 1967).

Blinded rats (Table 1, Group 2), as expected, had smaller than normal reproductive organs (absolutely and relatively) unless they were pinealectomized (Group 3). Anosmia, alone (Group 4), or in combination with

pinealectomy (Group 5), did not influence the size of the gonads. If rats were both blinded and anosmic (Group 6), body growth and maturation of the reproductive organs were severely retarded. These organs were also microscopically immature compared to those of all other animals. By comparison, if dual sensory deprivation was combined with removal of the pineal gland (Group 7), the testes grew at the normal rate and were histologically mature; although removal of the pineal gland did not completely prevent inhibition

TABLE 1

MEAN (\pm STANDARD DEVIATION) BODY, TESTICULAR, ACCESSORY ORGAN (SEMINAL VESICLES AND COAGULATING GLANDS) AND PINEAL GLAND WEIGHTS OF 68-DAY-OLD RATS SHOWING EFFECTS OF BLINDING, ANOSMIA AND PINEALECTOMY EITHER ALONE OR IN COMBINATION

Group and treatment	N	Body wt (g)	Mean organ weights*			HIOMT†
			Testes	Accessory organs	Pineal	
1. None	10	295 \pm 21	3159 \pm 175 (1071 \pm 84)	337 \pm 41 (141 \pm 35)	(1.64 \pm 0.17) (0.55 \pm 0.12)	99.4 \pm 8.9 (60.9 \pm 5.6)
2. Blinded	9	280 \pm 14	2749 \pm 218‡ (982 \pm 91)§	218 \pm 29‡ (78 \pm 18)§	1.26 \pm 0.19 (0.45 \pm 0.13)	110.3 \pm 10.1 (88.6 \pm 6.3)
3. Blinded; pinelectomized	8	291 \pm 16	2984 \pm 141 (1025 \pm 85)	310 \pm 62 (107 \pm 42)	—	—
4. Anosmic	10	282 \pm 20	3017 \pm 162 (1070 \pm 104)	299 \pm 71 (106 \pm 29)	1.54 \pm 0.24 (0.55 \pm 0.14)	87.4 \pm 6.7 (60.3 \pm 6.0)
5. Anosmic; pinelectomized	8	288 \pm 15	3074 \pm 220 (1067 \pm 95)	333 \pm 48 (116 \pm 25)	—	—
6. Blinded; anosmic	10	188 \pm 38‡	1279 \pm 187‡ (680 \pm 109)§	58 \pm 10‡ (31 \pm 6)§	1.24 \pm 0.27 (0.66 \pm 0.16)	105.3 \pm 8.8 (85.5 \pm 5.7)
7. Blinded; anosmic; pinelectomized	9	270 \pm 12	2965 \pm 252 (1098 \pm 125)	263 \pm 75‡ (77 \pm 34)§	—	—

HIOMT expressed as $\mu\mu\text{moles}$ of ^{14}C -labelled melatonin formed/hour.

* Absolute weights (mg) with relative weights (mg/100 g body wt) in parentheses.

† HIOMT per pineal with relative (HIOMT/mg pineal) amount in parentheses.

‡ $P < 0.001$ compared with absolute mean weight of untreated controls (Group 1).

§ $P < 0.001$ compared with relative mean weight of untreated controls (Group 1).

|| $P < 0.05$ compared with relative amount HIOMT of untreated controls (Group 1).

of accessory organ growth in these animals, they were much larger than those of non-pinealectomized controls and were microscopically indistinguishable from those of untreated animals. Normal body growth was also restored by pinealectomy.

Darkness or blinding reportedly increases pineal HIOMT activity (Wurtman, 1967). When expressed relative to the weight of the gland, pineals of blinded rats (Group 2) had elevated levels of HIOMT. Anosmia alone (Group 4) had no influence on the pineal content of this enzyme. Similarly, HIOMT activity in blinded, anosmic rats (Group 6) was no higher than in animals that were eyeless only.

Blinding modifies reproduction by activating the pineal to produce an antigonadotrophic substance (Reiter *et al.*, 1968). When blindness is coupled with anosmia, the gonadal response is greatly exaggerated, but melatonin synthesis based on levels of HIOMT, is not. At least two explanations for the role of anosmia exist. First, if anosmia does work through the pineal, it may cause the production of an antigonadotrophic substance other than melatonin, which acts synergistically with melatonin to induce greater gonadal atrophy than melatonin alone can cause. Other pineal antigonadotrophic substances exist (McIsaac, Taborsky & Farrell, 1964; Soffer, Fogel & Rudavsky, 1965). The second possible explanation is that olfactory bulb removal may not change the activity of the pineal gland but may sensitize the neural site at which the pineal antigonadotrophic substance normally acts. In addition to the obvious inhibitory effects on gonadotrophins, other pituitary hormones may have been suppressed since the mean body weight of blinded anosmic rats was less than that of rats in all other groups.

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