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Pituitary/Adrenal Hormones Do Not Influence Bulbectomy-Induced Behavioural Changes[1]

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HULL, E. M., G. l'HOMMEDIEU, C. KASTANIOTIS AND J. R. FRANZ. *Pituitary/adrenal hormones do not influence bulbectomy-induced behavioral changes.* PHYSIOL. BEHAV. 22(3) 417–421, 1979.—The possible causal role of increased adrenal/function in olfactory bulbectomy-induced irritability and hyperactivity was investigated. Bilateral olfactory bulbectomies or sham operations were performed on sixty male Mongolian gerbils. Five days later daily injections of dexamethasone phosphate (which suppresses ACTH and, indirectly, cortisol), metyrapone (which inhibits production of cortisol and indirectly increases ACTH) or saline were begun and continued throughout the period of testing. Bulbectomized animals were more active in an open field apparatus, more aggressive in grouped and paired social interaction tests, and initiated more non-aggressive contacts in paired social interaction tests. Both dexamethasone and metyrapone led to increased activity in the open field but did not significantly affect any other measure. Neither dexamethasone nor metyrapone significantly decreased any of the behavioral changes resulting from bulbectomy. These results are taken as evidence that physiological levels of the hormones of the pituitary/adrenal axis are not essential for bulbectomy-induced behavioral changes in the male gerbil.

Olfactory bulbs Hormones Cortisol ACTH Aggression Activity

CONSIDERABLE discussion in recent years has centered on the question of whether and under what conditions differential effects are produced by removal of the olfactory bulbs as opposed to removal or inactivation of the main olfactory receptors in the nasal cavities (see Alberts [1], Cain [4], Edwards [7] and Murphy [17] for reviews). Spector and Hull [20], Alberts and Friedman [2] and others have reported increased mouse killing by bulbectomized animals but not those rendered anosmic by peripheral manipulations. Irritability and activity are also differentially affected by centrally and peripherally induced anosmia, as demonstrated by Alberts and Friedman [2], Carter [5], Hull and Homan [11], and Hull, Hamilton, Engwall and Rosselli [10]. While bulbectomy does not always produce a more emotional, active or aggressive animal, there seems to be little question that in certain circumstances olfactory bulbectomy does produce more profound, or at least different, effects on behaviour than does peripheral anosmia, and that frequently these effects are characterized as increases in irritability, activity, and/or aggressiveness.

Since loss of the main olfactory sense modality cannot adequately account for the behavioral changes following bulbectomy, it has been suggested that bulbectomy removes a tonic inhibitory influence on some limbic system structure(s). Whether this influence is mediated neurally at all stages of the system or whether a hormonal change occurs as an intermediate step is not known. For example, the amygdala, with which the olfactory bulb is connected anatomically, is known to play a role in the control of both pituitary/adrenal and pituitary/gonadal function. (See Zolovick [22] for review.) Furthermore, hormones of both these systems have been shown to affect aggressive (and/or "emotional") behavior, at least under some conditions. (See Leshner [13] for review.)

A previous experiment in this lab suggested a role for the adrenal glands in mediating bulbectomy-induced behavioral changes [10]. Bulbectomized gerbils, but not peripherally deafferented ones, were excessively aggressive in a crowded home cage, killing 60% of the animals in that experimental condition. The adrenal glands of these experimental animals were significantly heavier than those of sham operated or peripherally deafferented controls; there was no significant difference among groups in testis or seminal vesicle weight. In addition, Eichelman, Thoa, Bugbee and Ng [8] reported that bulbectomy resulted in heavier adrenal glands in rats, and King and Cairncross [12] found increased levels of corticosterone in bulbectomized rats. On the other hand, Loyber, Perassi, Lecuona and Peralta [16] reported lower levels of corticosterone in female albino rats, although adrenal weights were the same as in controls.

Since hormones of the pituitary/adrenal axis have been implicated in some forms of aggression, and since bulbectomy apparently results in increased adrenal activity in gerbils and some rats, the present experiment was designed to

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assess the roles of ACTH and cortisol in the development of bulbectomy-induced behavioral changes. (Cortisol is the major corticoid of the gerbil.) Dexamethasone was administered to bulbectomized and sham operated gerbils to inhibit synthesis of ACTH (thereby also decreasing cortisol, indirectly). Metyrapone was similarly administered to inhibit 11-β-hydroxylation of 11-deoxycortisol to cortisol. (ACTH levels would be elevated because of lack of cortisol's negative feedback.) Saline was administered to bulbectomized and sham operated animals as a control. Open field activity, social interaction behavior, group aggression and irritability were assessed. Pharmacological, rather than surgical, intervention was utilized in order to restrict physiological changes largely to the specific hormones under consideration, as well as to minimize the death rate.

METHOD

Animals

Sixty male gerbils, 90 days of age, were obtained from Tumblebrook Farms, Inc. and were divided into six equal groups: Bulbectomy-Dexamethasone, Bulbectomy-Metyrapone, Bulbectomy-Saline, Sham-Dexamethasone, Sham-Metyrapone, Sham-Saline. Animals were housed individually in clear plastic cages (28×18×12.5 cm) throughout the experiment with ad lib access to food and water. Lights were on from 7 a.m. to 7 p.m.

Apparatus

Both activity and social interaction tests were conducted in a 1 m^2 open field apparatus, made of plywood, painted gray, and lined off into 16 equal squares. Pegs made of plastic dowels 2 cm high were inserted into holes at the corner of each square except along the boundary of the field.

Procedure

All operations were performed under Avertin anesthesia (IP, 0.7 cc/100 g body weight, 2.5% tribromoethanol in isotonic saline). Bulbectomy was accomplished by aspiration after drilling a rectangular hole in the skull over the bulbs. Gelfoam was administered for hemostasis, and Sulfathiozole was sprinkled over the sutured wound. Sham operations consisted of drilling holes over the bulbs with no damage to underlying brain tissue. Drug administration was begun 5 days after surgery. Either 0.4 mg dexamethasone phosphate (dexamethasone 21 phosphate disodium salt, Merck, Sharp, and Dohme), 10 mg metyrapone (Metopirone, Ciba), or isotonic saline was injected IP between 9:00 and 9:30 a.m. every day for 10 days. Behavioral testing was conducted from 1:00 to 4:00 p.m., beginning 7 days after surgery. Fourteen animals died before behavioral testing was completed; none of their scores was used in data analysis. Three additional animals died before physiological measures were assessed; their scores were retained in the behavioral data though no physiological data were available for them.

Activity of each animal was assessed individually in the open field apparatus for 5 min. Number of lines crossed with all four feet was tabulated on an electromechanical counter. The apparatus was washed with an Alconox solution after each animal was tested. Next, each animal was marked on the tail with a felt tip pen and allowed to interact for 10 min in the same open field apparatus with one of two standard test animals, also tail marked, selected from our colony for their high levels of aggressive behavior in previous tests.

Numbers of ventral gland rubs, aggressive encounters, and nonaggressive contacts initiated by each animal were recorded separately by means of electromechanical counters. A ventral gland rub was scored when an animal perceptibly lowered its abdomen onto either a peg or the floor. Aggressive behavior included fighting, biting, pushing and chasing. Nonaggressive contacts included any face or body contacts not listed above as aggressive behavior. If both animals appeared to initiate a behavior simultaneously, both received the appropriate score. Next, irritability was assessed in the home cage by blind ratings of animals' responses to being approached by a gloved hand and to being picked up and held on three separate attempts. A score of 0 was assigned if the animal did not react at all; 1, if the animal strugggled slightly or actively avoided the gloved hand; 2, if the animal struggled consistently and vigorously on all attempts to pick it up; 3, if the animal consistently showed extreme agitation, vocalized, and/or jumped from the tester's hand; 4, if the animal in addition to the criteria for 3, bit the gloved hand. Finally group aggression was measured by placing all animals of a treatment group, for example, Bulbectomy-Dexamethasone, together for 10 min in a standard 10×40 cm clear plastic cage. All instances of boxing, chasing, and fighting were tabulated by two observers and summed by each to yield total aggression scores. Grooming (self or other) and ventral gland marking were also tabulated. Since the scores recorded by the two observers were nearly identical, the average of the two was reported. All behavioral tests were run blind.

At the termination of behavioral testing animals were sacrificed by exsanguination under ether anesthesia between 1:00 and 4:00 p.m. (the normal time for behavioral testing), after the usual morning injections. Ether was administered by placing each animal into a bell jar containing a beaker with ether-soaked cotton, for 45–60 sec. Immediately after removal from the bell jar, blood samples were obtained by cutting the brachial artery. All samples were obtained within 1.5 min after removal from the home cage. Blood samples were collected in heparinized tubes and centrifuged for 15 min at 1000 g. Following the radioimmunoassay procedure outlined by Clinical Assays, Inc., denaturation of plasma proteins was accomplished using 100 microliter plasma samples in 2 mls of borate buffer heated in a water bath to 60°C for 30 min. Cortisol standard dilutions were then prepared ranging from 1 to 48 mg% and assay of standards and experimental samples was performed simultaneously in the antibody-coated tubes provided. Following a 10 min incubation, 100 microliters of iodine-125 labelled cortisol was added to all tubes. The subsequent 45 min incubation at 37°C assured maximum binding of the labelled and unlabelled cortisol to the antibody. The contents of all tubes were then aspirated and the tubes rinsed twice with tris buffer. Each tube was then counted in a gamma counter for one minute under conditions optimum for iodine-125.

Brains of bulbectomized animals were exposed to Formalin, removed and cut into 40 μ sections. They were then mounted and stained with cresyl violet. Slides were then projected and the most rostral section without damage was noted and compared with the atlas of the gerbil brain by Loskota, Lomax and Verity [15]. Extent of surgical damage was rated separately for each side on a 6-point scale. A score of 1 indicated incomplete removal of the main olfactory bulb. A 2 was assigned if the entire main olfactory bulb was re-

TABLE 1

BEHAVIORAL MEASURES

	Aggression (mean per 10-min social interaction test)		Nonaggressive Contacts (mean per 10-min s.i. test)		Activity (mean lines crossed in 5 min)		Group Aggression (total scores per 10-min group test)*
	Mean	SE	Mean	SE	Mean	SE	Total
Bulbectomy-Dexamethasone	6.13†	4.43	55.87‡	3.19	318.00‡§	18.71	13.5
Bulbectomy-Metyrapone	2.13†	0.87	59.75‡	9.45	247.57‡§	19.41	23.0
Bulbectomy-Saline	7.50†	4.00	76.75‡	6.42	236.00‡	16.14	54.0
Sham-Dexamethasone	0.63	0.42	50.75	6.89	218.43§	23.81	14.5
Sham-Metyrapone	0.50	0.27	42.37	4.13	188.33§	10.83	40.0
Sham-Saline	0.86	0.44	29.43	6.33	94.71	20.44	1.0

* Mean for 2 observers.
† $p<0.05$, Bulbectomy vs sham surgery.
‡ $p<0.01$, Bulbectomy vs sham surgery.
§ $p<0.01$, Drug vs saline.

TABLE 2

PHYSIOLOGICAL MEASURES

	Cortisol ($\mu g\%$)		Absolute Adrenal Wt. (mg)		Relative Adrenal $Wt\left(\frac{\text{mg adrenal}}{\text{g body}}\right)$		Body Wt. (g)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Bulbectomy-Dexamethasone	1.94*	0.46	30.86*	1.11	46.17*	1.84	67.13†	1.73
Bulbectomy-Metyrapone	31.03	4.51	44.55*	1.83	56.57*	2.22	77.50	2.86
Bulbectomy-Saline	33.56	5.94	37.78	2.99	52.88	3.41	72.00	3.79
Sham-Dexamethasone	1.04*	0.03	31.30*	1.28	48.38*	2.70	65.50†	2.49
Sham-Metyrapone	33.00	3.86	43.83*	1.39	59.25*	1.28	73.75	2.26
Sham-Saline	22.53	8.58	30.43	1.04	49.00	4.32	62.67	3.08

* $p<0.01$, Drug vs saline.
† $p<0.05$, Drug vs saline.

moved but at least part of the anterior olfactory nucleus (AON) remained. If the entire AON was removed, but the olfactory tubercle (TULC) was intact, a score of 3 was assigned. Some damage to TULC in addition to complete removal of AON resulted in a score of 4. A score of 5 indicated complete destruction of TULC, but no damage to overlying frontal cortex. A 6 was assigned if in addition to TULC destruction, the frontal cortex was damaged. Lesion scores for the two sides of each brain were then summed.

RESULTS

Bulbectomized animals exhibited more aggressive, $F(1,41)=4.84$, $p<0.05$, and nonaggressive, $F(1,41)=20.08$, $p<0.01$, contacts in social interaction tests and more aggression in group testing. (See Table 1 for results of all behavioral tests. Since group aggression scores do not represent independent samples within the group, statistical comparisons were not made.) In addition, standard animals exhibited more aggressive contacts when paired with bulbectomized animals than with shams. Bulbectomized animals were also more active in the open field apparatus, $F(1,41)=20.02$, $p<0.01$, but were not more irritable than controls in the handling test. Both dexamethasone and metyrapone increased activity in the open field, $F(2,41)=7.09$, $p<0.01$, but did not significantly affect any other measure. There were relatively low frequencies of ventral marking behavior in all groups, and no significant differences among groups on this measure.

Histological examination indicated that one bulbectomized animal had remnants of both main olfactory bulbs and another had a remnant of one main olfactory bulb. Six additional animals had incomplete destruction of the anterior olfactory nucleus on one side. All others [17] gave evidence of complete bilateral AON destruction with more or less damage to the olfactory tubercles and frontal cortex. One animal exhibited bilateral damage to frontal cortex; three additional ones had unilateral damage. Mean extent of lesion scores were 7.7, 8.0 and 7.8 for Bulbectomy-Dexamethasone, Bulbectomy-Metyrapone, and Bulbectomy-Saline groups, respectively. Extent of lesion scores were plotted against the various behavioral and physiological indices for each group in an attempt to discern a linear relationship between them; no such relationship was evident for any group on any measure. In addition, bulbectomized animals were divided into Complete (bilateral destruction of both MOB and AON) and Incomplete (either unilateral or bilateral remnants of MOB and/or AON) Lesion groups. Data from Complete and Incomplete groups were pooled across drug treatments on social interaction aggression and

nonaggressive contact scores and on irritability scores (since there were no significant differences among drug treatment groups on these measures), and compared by means of *t*-tests. Since there was a significant drug effect on open field activity, analysis of variance was used to assess the effects of complete vs incomplete bulbectomy on activity scores. Animals with complete bulbectomies scored significantly higher than did those with incomplete ablations on the irritability tests (means=2.7 and 1.3 for complete and incomplete bulbectomy groups, respectively; $t(22)=2.50$, $p<0.05$); there were no significant differences on any other measure.

Radioimmunoassay of cortisol indicated that dexamethasone had almost completely suppressed cortisol production by blocking ACTH; however, measured cortisol levels of metyrapone-treated and control animals were not different according to Newman-Keuls comparisons, $F(2,38)=26.54$, $p<0.01$. (See Table 2 for all physiological measures.) Metyrapone increased absolute and relative adrenal weights and dexamethasone decreased them, relative to controls, $F(2,38)=18.70$, $p<0.01$. Body weights of dexamethasone treated animals were also decreased, $F(2,38)=4.91$, $p<0.05$.

Since measured cortisol of metyrapone-treated animals did not differ significantly from saline controls, additional groups of bulbectomized and sham operated animals received injections of metyrapone 4 hr, or daily for 10 days, before sacrifice and subsequent assay for cortisol. The 4-hr and 10-day injections were given between 9:00 and 9:30 a.m., as were the injections in the original experiment. Animals were sacrificed between 1:00 and 2:00 p.m., in the same counterbalanced order as injections were given. Animals were killed by decapitation within 20 sec after removal from the home cage, and within 60 sec after initial disturbance of removing home cage to adjoining room. RIA was performed utilizing a Gamma Coat Kit (Clinical Assays) identical to the original kit, except that the antibody cross-reacted only 7% with 11-deoxycortisol instead of the 20% cross-reactance of the original antibody. The 4-hr metyrapone values were significantly depressed relative to saline controls, $t(11)=5.92$, $p<0.01$; metyrapone: 3.5 μg%; saline: 17.6 μg%. Since values of bulbectomized animals were not significantly different from those of sham controls, their scores were combined. By Day 10, however, sham metyrapone animals' cortisol levels had returned to normal (sham-metyrapone: 12 μg%, sham-saline: 10.6 μg%) and bulbectomized saline animals' cortisol levels were approximately twice those of sham-saline controls (bulbectomy-saline: 20.5 μg%). Attrition in the bulbectomized metyrapone group rendered interpretation of that comparison difficult. Operations×drug interaction, $F(1,11)=9.86$, $p<0.02$.

DISCUSSION

In behavioral tests bulbectomized animals were more aggressive in both the grouping and the social interaction tests, and were more active in the open field than were sham operated animals. The increased irritability when handled was not observed in these animals, although it was observed in a pilot experiment. Furthermore, none of these measures (including irritability in the pilot experiment) was decreased as a result of either drug treatment. Activity, in fact, was increased by both treatments. It would appear, therefore, that neither ACTH nor cortisol is a necessary mediator of the observed behavioral changes.

According to our radioimmunoassay results, dexamethasone blockade of ACTH almost completely suppressed adrenal cortisol output, while metyrapone treatment appeared not to have blocked cortisol production. Two alternative interpretations of this lack of effect are possible: our dose may simply have been ineffective, or it may have been effective early in the experiment, leading to decreased negative feedback to the pituitary, which in turn produced more ACTH, thereby largely overcoming the blockade of cortisol production. It was for this reason that the additional experiment was done, demonstrating that the dose was indeed effective on the first administration, but that after 10 days of daily administration the pituitary appeared to overcome the blockade by increased secretion of ACTH. One observable effect of metyrapone treatment was the increased size of adrenal glands in these animals. This hypertrophy is consistent with other reports of metyrapone suppression of corticoids in rodents [6, 18, 19] and with the effects of metyrapone in humans [14]. The lower absolute values of cortisol in the second experiment may have resulted from use of a more specific antibody in that RIA procedure and/or from the lack of daily social interaction tests which were part of the original experiment. Thus, our metyrapone treatment produced not so much a diminution of cortisol levels as an enhancement of ACTH titers. In summary, our dexamethasone treatment resulted in unmeasurably low quantities of cortisol and presumably similarly low quantities of ACTH. Metyrapone, on the other hand, was associated with relatively normal levels of cortisol, but presumably with large quantities of ACTH.

Brain and Leshner have proposed that ACTH acts to reduce aggressiveness. Administration of adrenal corticoids frequently increases aggressiveness, possibly as a result of their negative feedback on ACTH production. However, Harding and Leshner [9] found that while adrenalectomy, with its resultant increase in ACTH, decreased aggressiveness in both isolated and grouped animals, the difference between these two types of animals remained significant.

A previous experiment in our lab on effects of environmental enrichment and crowding on normal unoperated gerbils failed to disclose a significant correlation between basal levels of cortisol and aggression scores in social interaction tests or in the home cage. Spencer, Gray and Dalhouse [21], reported a similar lack of correlation between adrenal corticoids and gerbils' behavior. In the present experiment, even gross manipulations of adrenal/pituitary hormones produced only slight, nonsignificant differences in aggressive behavior. Furthermore, the bulbectomy vs sham differential was not abolished. We therefore propose the behavioral changes which result from olfactory bulbectomy are not mediated by hormones of the pituitary/adrenal axis. We must also conclude that in gerbils, at least, basal levels of the adrenal/pituitary hormones play at best a minor role in differences in aggression, and that changes in basal levels of these hormones after bulbectomy are not responsible for the heightened aggression and activity scores of our bulbectomized animals.

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