GLUCOCORTICOID EFFECTS ON OPEN FIELD RUNNING AND AGGRESSION

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ABSTRACT

The effects of glucocorticoid injections on open-field running behavior and aggression were examined in rodents. The first experiment was designed to test the effects of corticosterone injections on food and water dominance and spontaneous aggression in rats. Data suggested that corticosterone may affect certain aspects of dominance in rats, but not all.

The second experiment examined the differences in open-field activity between dominant and subordinate mice. Data verified earlier findings that the dominant male runs significantly more than the subordinate in the open-field situation, and has smaller adrenal glands.

The goal of the third experiment was to test the effects of manipulated blood levels of corticosterone or dexamethasone on open-field running and aggressive behavior of male mice. Different surgical treatments were also utilized to control the amount of endogenous corticosterone and/or testosterone present. The results showed that corticosterone had very little effect on the establishment of dominance. Open field running behavior was depressed by a high dose of corticosterone in castrated animals, while little effect was seen in animals receiving a low dose. Effects of corticosterone on

open-field running were short-lived and reversible. Once injections were discontinued, running jumped significantly in a type of "rebound". The high dose of corticosterone increased open-field running of adrenalectomized animals. Corticosterone had little effect on open-field running in intact animals and animals that were both adrenalectomized and castrated. Unlike corticosterone, dexamethasone did not decrease open-field running behavior in castrated animals.

Since dexamethasone has effects similar to those of corticosterone on metabolism, and pituitary secretion of ACTH, it is unlikely that the corticosterone effect on running is mediated by one of these peripheral actions. On the other hand, since the hippocampus binds corticosterone to a much greater degree than dexamethasone, the motor effects of the hormone may be mediated by this part of the CNS.

It is concluded that, while glucocorticoids apparently are not critically involved in the establishment of dominance relations, they are critically involved in the behavior of the submissive animal once dominance is established.

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INTRODUCTION

The term dominance implies that a given animal gains a high rank and the privileges that accompany that rank (food, mate, territory). Establishment of dominance usually occurs through some type of aggressive behavior ranging from a noninjurious species specific display to obvious injurious aggression, as in the Order Rodentia. Research has shown a correlation between autonomic and endocrine functions in relation to aggressive behavior. However, aggressive behavior is not the function of a single endocrine system but is affected by many. In early investigations, the testes was thought to be the main control system in male aggression. This was based on two main observations: (1) castration reduces aggressiveness while replacement therapy with testosterone restores original aggressiveness, and (2) androgen therapy accelerates onset of aggressiveness in juvenile males (Levy & King, 1953). More recently, adrenal hormones have been studied in their relationship to, and effects on aggressive behavior.

The relationship between hormones of the pituitary-adrenocortical axis and aggressive behavior has been studied primarily during establishment of a dominant/subordinate structure. Bronson & Eleftheriou (1964, 1965) and Baenninger (1970) showed that participation in an agonistic encounter led to increased plasma corticosterone levels in mice. This also occurs in rats (Chapman, et. al., 1969)

with a greater reaction occurring in the subordinate animal. Lough & Higginbotham (1967) demonstrated a time dependency for these effects in mice. When the subordinate animal no longer offered opposition to the dominant, he was usually overlooked by the dominant and his adrenal function returned to normal. Welch (1964) hypothesized that adrenal function increases with any increase in environmental stimulation, but is increased faster in the subordinate. Ely & Henry (1978) suggested that the dominant male responds to social interaction with primarily a sympathetic adrenal-medullary pattern or defense response, whereas the subordinate male reacts with an adrenal-cortical or alarm response.

A recent study by Leshner and colleagues (1973) indicates that, (1) hormones of the adrenal-pituitary axis exert direct influence in the control of aggression, (2) the role played by these hormones in the control of aggression is independent of the effects of the adreno-pituitary axis on gonadal secretion, and (3) there is a long term suppressive effect of high levels of ACTH on aggression, and this is independent of the effects of ACTH on glucocorticoid levels.

Studies of the relationship of endocrine systems to aggression have been directed in two different ways. Some studies have focused on the effects of hormonal change on the agonistic response while others have focused on the effects of the agonistic response on hormonal changes.

The ability of hormones of the adrenal cortex to affect aggression has been observed in various studies. Bilateral adrenalectomy reduces aggressiveness (Harding & Leshner, 1972), while replacement therapy with corticosterone or dexamethasone restores aggressiveness. This suggests that adrenalectomy reduces aggressiveness because it results in decreased corticosteroid levels (Walker & Leshner, 1972). However, it is also necessary to consider the behavioral effects of the changes in ACTH levels which accompany alteration in adrenocortical activity. Bilateral adrenalectomy results in an increase of ACTH (Gemzell, et al., 1951) whereas treatment with exogenous corticosterone results in a decrease (Zimmerman and Critchlow, 1969). Short term treatment with ACTH increases aggressiveness in intact mice, whereas long term treatment results in a decrease of aggressiveness. The short term increase is thought to be a result of increases in corticosterone levels (Leshner, et al., 1973), while the long term decrease appears to be due to an extra-adrenal, extra-testicular effect.

Since aggressive and submissive behavior can be elicited by the same environmental stimuli, it may be that the hormonal state of the animal determines whether it will react aggressively or submissively. For example, an increase in ACTH reduces aggressiveness and increases fearfulness (Brain, 1972, Svare & Leshner, 1973).

Thus, predisposing the animal to be more fearful of a novel opponent may cause it to be less aggressive. However, studies on this facet of behavior, particularly direct studies of the effects of hormones on submissive behavior, have been few.

Factors that might preset an animal to cope with aggression are unclear, but evidence suggests that the baseline hormonal condition of the animal is established early in life and the ability of the adult animal to respond to stress (and perhaps to aggression) is, in part, determined by neonatal interactions of the adreno-pituitary axis (Levine & Mullins, 1966). Repeated studies published by Howard and associates (1965, 1968) indicate that corticosterone treatment during infancy affects the way an animal is able to cope with aggressive encounters as an adult. Post-natal treatment with cortico-sterone affects other behaviors such as open field running, wheel running and maze learning, and it interferes with growth and DNA synthesis in the cerebellum, area dentata of the hippocampus and the olfactory bulbs of the brain.

Although there are several mehcanisms by which hormones could exert their influence on behavior (Beach, 1974), modification of the CNS is perhaps the most important. Corticosterone in the adult affects induction of brain receptors (Lisk, 1971), the development of neurotransmitter systems (Vernadakis, 1971) and gene expression (Schwartz, 1972).

Recent findings of Brain and Poole (1974) showed specific binding of adrenal cortical hormones to the hippocampus, septum and amygdala (areas of the primitive limbic system thought to be involved in emotions such as aggression). Leshner (1975) suggested that under one set of hormonal conditions the responsiveness of the fear or defense circuits is increased and that of the aggressive circuits is decreased, and the animal reacts more submissively. Under reversed hormonal conditions the animal would react more aggressively.

There are also effects of agonistic experiences on the hormonal system. The defeated animal shows a high level of corticosterone, while the dominant seems relatively unaffected (Lough & Higginbotham, 1967). Also, the threat of aggression can elicit increases in corticosterone secretion. Since the peripheral receptors which pick up information concerning agonistic stimuli are not directly connected to endocrine glands, it is probable that the effects of aggressive encounters are mediated through the CNS. The mechanism is perhaps the alteration of protein synthesis in the brain or the state of brain neurotransmitter systems.

In a 1975 review of the relationship of hormones and agonistic behavior, Leshner pointed to a need for further studies on the hypothesis that the hormonal state of an animal influences the way it reacts to a particular

agonistic stimulus. It was the purpose of this research to test this hypothesis. The research was conducted in three separate experiments. Experiment 1 dealt with the effects of corticosterone injections on food and water dominance and spontaneous behavior in rats. In Experiment 2 a possible correlation between open-field running and social rank was examined, as was the previously reported correlation between social rank and various gland and body weights. The purpose of Experiment 3 was to manipulate the level of corticosterone in mice and to test for the effects of this manipulation on open-field running and aggressive behavior.

EXPERIMENT 1

PURPOSE

The purpose of this experiment was to test the effects of corticosterone injections on food and water dominance and spontaneous aggression in rats.

MATERIALS & METHODS

Sixteen adult male Sprague-Dawley-derived rats were castrated and half of those were also bilaterally adrenalectomized. Adrenalectomized animals were maintained on a 1% saline solution and all animals were allowed to recuperate for one week before testing began. Testing consisted of pairing similarly operated animals and observing their interactions. Animals were paired randomly with a new partner each week for five weeks. Each pair was observed four days a week, three times a day in order to determine dominance. On alternate days, the first observation of each cage was preceded by fourteen hours of food deprivation. The paired rats in each cage were then observed for six minutes in competition for a piece of apple. Wire receptacles attached to the side of the cage with a hole cut in one side allowed only one animal at a time to put his head through to obtain the apple. Five hours later, animals were observed for spontaneous behavior during the first ten minutes of the dark cycle (lights out at 9:00 p.m.). Dominance in spontaneous encounters was determined as described by Baenninger (1970) and Grant & Chase (1958) by recording the number of submissive postures. On the two alternate days, water-deprived animals were observed in competition for water using the procedure described above. The time each animal was in control of food or water was recorded on a physiograph.

Injections of corticosterone were given twice daily: at 10:00 a.m., one hour before the morning observation, and at 5:00 p.m., immediately following the afternoon observation. Corticosterone was suspended in sterile 1% saline and 0.1 cc of the suspension was administered subcutaneously. In each of the eight pairs of the two groups one animal was given a high dose of corticosterone and one a low dose for a one week period. Dosages were changed weekly. Dosages (given as total dose per day) for Weeks 1-5 are listed below.

Week	Total Dose/Day
1	200 ug vs 20 ug
2	400 ug vs 20 ug
3	800 ug vs 20 ug
4	100 ug vs 100 ug
5	saline

Following the testing period the animals were autopsied and examined for adrenal fragments. Both a t-test for paired data and one for independent samples were run on the data using the $p = .05$ level of significance.

RESULTS

Actual injurious aggression in the rats was very limited. No scarring that would indicate fighting was ever visible. Interaction between animals was reduced to sniffing and grooming, with occasional short bouts occurring at lights out.

In neither the adrenalectomized nor the adrenalectomized/castrated group were any significant differences observed between animals competing for food or water in any of the test groups. Likewise, no significant differences were noted in the weights of the animals.

However, a significant difference was noted in spontaneous behavior during Week 3 between castrated animals injected with a high dose of 800 ug corticosterone vs low dose animals given 100 ug per day. Low dose animals were significantly more aggressive in the four nights observed. Also, although not significant, there was a definite trend for the lower dose animals to show more spontaneous aggression than the higher dose animals in Weeks 1 and 2. No trends toward increased aggressive behavior in either animal group were evident during Weeks 4 and 5 in which the paired animals received equal doses of corticosterone or only saline injections.

DISCUSSION

Baenninger (1970) reported that dominance in the rat is multi-dimensional, that is, an animal showing dominance in spontaneous behavior is not necessarily the dominant in food or water competition. The data from this study support Baenninger's position and suggest that corticosterone may affect certain aspects of dominance in rats, but not all. Certainly there was a strong trend toward decreased spontaneous aggression with high corticosterone levels, and increased aggressiveness with lower doses in castrated animals. Unfortunately, good data are lacking and conclusions must be minimal.

It is worth mentioning here that the lack of open aggressiveness and the lack of strong response to corticosterone treatment in white rats may be due to the effects of domestication. Richter (1954) reported differences between the adrenal cortex of wild and domesticated rats. Wild rats have larger adrenals with larger cells capable of increased secretory capacity and greater adrenocortical reactivity (Mosier, 1954). This alteration in the pattern of the adreno-pituitary axis by domestication also has been noted in other animals such as mice. Treiman & Levine (1969) reported a greater adrenocortical response to electric shock by wild mice as compared to inbred, demesticated varieties. Research by Balyaer and Trut (1975) on silver foxes led them to conclude that selection for docility leads to lower plasma corticosterone

levels and to an altered adreno-pituitary system.

Grooming, a behavior associated with aggression in rats, has recently been shown to be directly affected by manipulation of the adreno-pituitary axis. Hypophysectomized rats show a reduced level of grooming in a novel situation. Intraventricular injections of antiserum to ACTH also produced the same effect. The ability of ACTH to produce excessive grooming suggests that ACTH directly affects at least one phase of dominance behavior in rats.

EXPERIMENT 2

PURPOSE

This experiment was designed to use a more aggressive animal, the white mouse, to test for differences in open field activity between dominant and subordinate animals and to verify earlier findings of significant differences in adrenal weights between subordinate and dominant animals (Brain, 1972).

MATERIALS AND METHODS

Forty male mice, three weeks old, were obtained from Mid Continent Research and divided into groups of four. Animals were kept in plastic cages on a 16L:8D cycle (lights on 5:00 a.m. to 9:00 p.m.) and given Purina Lab Chow and water ad lib. When the animals were seven weeks old, each animal was caged with a non-familiar male for an additional two weeks. At the end of the two week period the animals were weighed and marked as subordinate, dominant or no visible social rank. Subordinate animals were easily detected by visible cuts and bites on the tail and rump region.

An open field 15 x 30 cm was constructed of metal mesh flooring with solid metal walls. It was marked into sixty squares of equal area and had metal barriers dividing it into various alleys. Immediately after each animal was ranked, it was weighed and placed individually into the open field. The number of squares entered by a mouse in a five minute period was recorded as was the number of fecal boli deposited. After each testing period the set-up was thoroughly washed with a gamamene solution, then rinsed with a dilute alcohol solution to remove any residual odors.

Following this run all of the animals were castrated and half of the total were also bilaterally adrenalectomized for use in Experiment 3. Adrenals and testes were cleaned and weighed.

A t-test for paired data and for random samples was utilized at $p = .05$ level of significance.

RESULTS

The mean weights of the body, testes and adrenals together with the standard errors for the means are listed in Table 1 for subordinate and dominant mice and those showing no social order. Also included is the mean ($\pm$ standard error) number of squares entered in the open field and the mean number of fecal boli deposited. For comparison purposes, the controls are those animals in which no social rank was noted.

There were no significant differences in body weight between animals at the time they were put together or following one week of pairing. There were no significant differences in testis weight between animals. In cages showing a social rank, the adrenals of the dominant animals were significantly smaller on the left side only than those of the subordinate. The left adrenals of the dominant animals also were significantly lighter than those of the animals with no social rank.

In cages where a definite dominance existed, the subordinate showed significantly less open-field running activity than did the dominant. Interestingly, in those cages where no social rank was visible, open field running was as high as that seen in the dominant group.

Dominant animals deposited significantly more fecal boli than did control animals. No difference in the

Table 1. Comparisons of mean body weight, gland weight, open-field running and fecal boli between animals with no social order and animals with social rank.

	Starting Wt. (g)	Ending Wt. (g)	Testis Wt. g/100 g Body Wt.	Adrenal Wt. g/100 g Bd. Wt. L.	Adrenal Wt. g/100 g Bd. Wt. R.	# of Squares	Fecal Boli
No Social Rank (n=20)	25.7 ± 3.4	28.4 ± 3.0	0.80 ±0.12	.015 ± .003 A	.011 ± .005	278 ± 35 C	0.5 ± .76 B
Ranked Subordinate	25.5 ± 3.5	26.9 ± 4.2	0.82 ± .08	.016 ± .002 B	.010 ± .003	156 ± 39.7 B,C	1.28 ±2.21
Dominant	28.1 ± 2,8	30.3 ± 2.5	0.84 ±0.099	.011 ± .004 A,B	.009 ± .002	276 ± 18 B	2.71 ±2.21 A

(A) Values for the dominant animal are significantly different than control.

(B) Values for the dominant animal are significantly different than subordinate.

(C) Values for the subordinate are significantly different than control.

number of fecal boli was evident between the controls and the subordinate mice.

DISCUSSION

It has been reported that adult mice housed in pairs show a suppression of gonadal function and an increase in adrenocortical activity not seen in mice housed in larger groups (Brain & Nowell, 1970). The effects of "social stress" are readily seen in paired animals; the dominant and subordinate are easily identified, and the hierarchy takes a relatively short time to establish itself (Brain, et al., 1971).

The differential in adrenal weights between dominant and subordinate animals in this study confirms earlier findings by Davis and Christian (1957) and Brain (1972). Although, in this study the difference was limited to the left adrenal. These data suggest that the dominant role may be due to a decrease in adrenal activity. The adrenal size of the subordinate was greater and comparable to that of the control animals. Lough & Higginbotham (1967) suggested that the differential in adrenal weight between subordinate and dominant animals was due to an increase in adrenal activity in the subordinate mice. The length of time the animals were left paired before weights were taken in this study, plus the fact that the controls may have been subjected to prolonged stress due to an unstable social condition, may be responsible for the differences between the data.

This study does not show the differences in testicular weights which might confirm findings by earlier investigators that submissive animals show a definite decrease in the production of testicular androgens. However, testicular weight is not a reliable indicator of androgen production and previous studies were indexed by the weights of sex accessory glands (Lloyd, 1971). Some investigators have suggested that suppression of gonadal function may be a result of increased adrenal function (Desjardins & Ewing, 1971).

The significant decrease in running activity of the subordinates supports previous findings by other investigators. In 1955, Crowcroft observed that wild mice living in a barn established a social heirarchy with the dominant male acting as a "patrol" on the colony. The subordinates were reported not only to move around less, but to actively avoid the dominant. Since avoidance behavior has been associated with an increase in adrenal cortical hormones it is likely the subordinate with its greater adrenal cortical reactivity would exhibit this type of behavior.

Ely and Henry (1978) correlated population-cage motor activity with social hierarchy development. They reported a significant increase in dominant male running. In an attempt to tie in the development of the social hierarchy with physiological parameters, they hypothesized

that the dominant male responds in primarily a sympathetic adrenal-medullary (defense) pattern whereas the subordinate males respond with an adrenal-cortical (alarm) pattern. Once the hierarchy was established (42 days) the subordinate males maintained the Selyean alarm pattern with an elevated pituitary-adrenal cortical activity. The subordinates were still being behaviorally inhibited by the dominant males, but open aggression was mainly replaced by behavioral posturing with the subordinate withdrawing. This is adaptively significant as it minimizes encounters with the dominant, and hence, strengthens the chances for survival of the subordinate.

In a study on the long term effects of corticosterone treatment during infancy, those animals which received a subcutaneous implant of corticosterone during the period of postnatal brain growth were significantly less active as adults in a 10 minute exploratory open-field period than were controls (Howard & Granoff, 1968). The reduced activity in the open field combined with increased defecation in mice given corticosterone in infancy suggests a role of corticosterone in creating timidity in mice. Later behavioral studies by Howard (1973) suggested that mice treated with corticosterone in infancy are hyper-emotional as adults. In the present experiment, the fact that the subordinate males have larger left adrenals, and therefore, greater adreno-cortical activity suggests

possible alterations by corticosterone of neuroendocrine pathways associated with running activity and possibly aggressive behavior.

This study did not support findings by Howard (1968) that more timid animals deposit significantly more fecal boli. In this experiment the dominant animals deposited more fecal boli than did the subordinates or controls, although the number of fecal boli deposited was not significantly different between dominant and subordinate animals.

Just how the hormones of the adrenal cortex mediate their effects on neuroendocrine systems is not known, but may range from complex neural feedback systems to target organ and brain receptor sensitivity. A more detailed discussion of the possible avenues for effects of glucocorticoids on the CNS will be included in the Discussion following Experiment 3.

EXPERIMENT 3

PURPOSE

The goal of Experiment 3 was to manipulate the blood levels of corticosterone or dexamethasone and to test for the effects of this manipulation on the open-field running behavior and aggressive behavior of male mice. Different surgical treatments were also utilized to control the amount of endogenous corticosterone and/or testosterone present.

MATERIALS AND METHODS

Male albino CFW mice, three weeks of age, were obtained from Mid Continent Research. Animals were housed in plastic cages in groups of four and maintained on a 16L:8D cycle (lights on 5:00 a.m. to 9:00 p.m.). Animals were given Purina Lab Chow and water ad lib and maintained in this manner for four weeks. Animals were randomly divided into nine test groups and treated as indicated in Table 2.

Animals were treated with antibiotics (1.5 mg/lb body weight with streptomycin) one week prior to and one week following surgery.

The ten castrated animals in Group A and the ten castrated and adrenalectomized animals in Group B were retained from Experiment 2. Animals were housed singly for one week before being placed into the experimental routing.

The basic experimental design for each group was as follows: Following the four weeks maturation period animals were weighed and surgically treated as indicated in Table 2. Adrenalectomized animals were maintained on a 1% saline solution. Animals were housed individually for a period of one week then placed individually into an open field set-up and the number of squares entered in five minutes were recorded, as were the number of fecal boli deposited. The cage was cleaned as reported in

Table 2. Surgical treatment and hormonal treatment administered to white mice in Experiment 3.

Group	# of Animals	Surgical Treatment	Hormonal Therapy
A	10	Castrated	Corticosterone
B	10	Castrated & Bilaterally Adrenalectomized	Corticosterone
C	20	Castrated	Corticosterone
D	20	Castrated-Reversed	Corticosterone
E	10	Intact	Corticosterone
F	20	Bilaterally Adrenalectomized	Corticosterone
G	10	Castrated	Dexamethasone
H	10	Castrated	Saline
I	10	Bilaterally Adrenalectomized	Saline

Experiment 2. On a randomly assigned basis, half of the animals in treatment groups A-F were given a high dose of corticosterone (200 ug in 0.2 cc 1% saline) injected in two injections: one at 10:30 a.m., the second at 4:30 p.m. The other half was given a low dose of corticosterone (20 ug in 0.2 cc 1% saline) at the same times. Injections were given daily for one week. At the end of that week a second open-field run was made. Following that run, one high- and one low-dose animal were placed together and allowed to remain paired with continued injections for another week. Animals were paired so that the mean running values for the high-dose group were not significantly different from those of the low-dose group. At the end of that week a third open-field test was made.

On days one and two of the pairing period animals were observed at lights out for aggressive encounters. Observations were ten minutes per cage. The behavioral items were weighted as to significance of actual injurious aggression. Responses were recorded on an event recorder and the following behavioral items were scored (Grant & Mackintosh, 1963).

1.	Exploration.	X touches Y with snout = .5 pt.
2.	Pushing.	X threatens Y, pushing it with the head or knocking it with the forepaws = 1 pt.
3.	Attacking.	X scratches and bites Y, usually accompanied by squealing and rolling over = 1 pt.
4.	Tail rattling.	Rapid vibrating movements = .5 pt.

Behavioral scores were tabulated for each animal and social rank was assigned on the basis of these scores. Scores for each pair were put into ratios and the dominant was said to be any animal with a 3/1 or higher ratio.

Following the two-week period animals held over from Experiment 2 were removed from the experiment. With Groups C-I injections were discontinued and animals were housed singly for a period of one week. At the end of this time a fourth open-field run was made.

The experimental procedure for Group G (castrated) was exactly as listed above except half of those animals received a high dose of dexamethasone (20 ug in 0.2 cc 1% saline) and the other half a low dose (2 ug in 0.2 cc 1% saline) administered in two daily injections.

In Group D (castrated-reversed) animals were run through the experiment as given one time, allowed to rest for one week, then run through the entire experiment again with the dosages being reversed (i.e. those animals originally receiving a high dose of corticosterone were given a low dose the second time around).

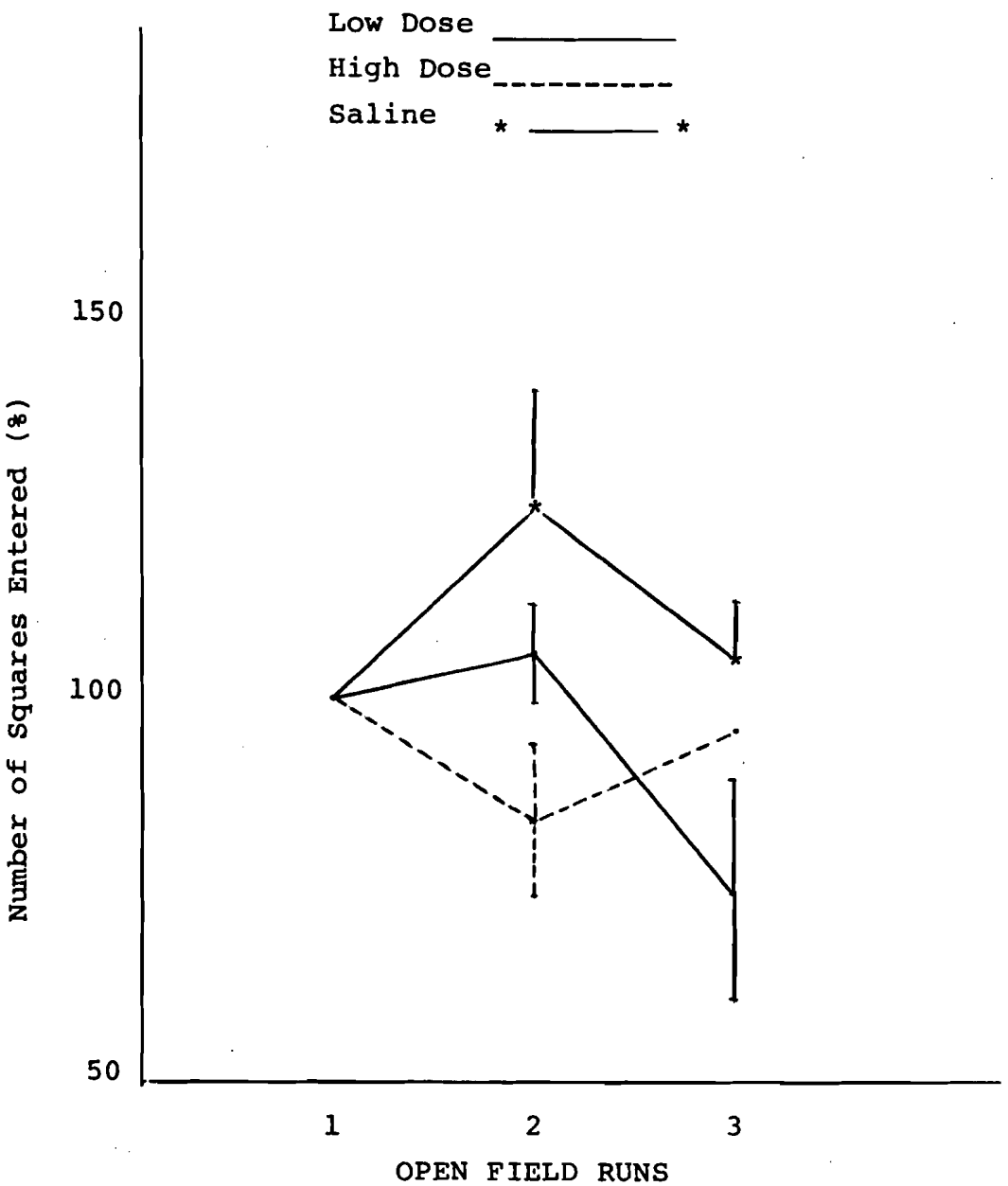
Both a t-test for paired data and one for independent samples were utilized using the $p = .05$ level of significance. A correlation coefficient was also run.

RESULTS

The results of hormonal injections on open-field running are presented in Figures 1 - 7. Each figure shows the number of squares (± standard deviation) entered in a five minute open field testing period in two weeks of testing. As each animal was run against itself, the initial run was taken as 100%. The values for Runs 2, 3 & 4 are listed as percentages of the initial run which occurred before injections began. The animals represented in Figs. 1 & 2 differed from other test groups in that they had been held over from Experiment 2, and thus, were three weeks older at the onset of Experiment 3 and had been handled differently. The animals of Group D, represented in Fig. 4 were also handled differently as they were being run through the experiment a second time with the dosages of corticosterone reversed.

Fig. 1 shows that after one week of corticosterone injections (Run 2) open-field running in high-dose castrated animals dropped significantly, whereas the low-dose animals elevated slightly, but not significantly. The control group increased also, perhaps due to their improved physical state two weeks following surgery. Following two weeks of injections, running in the high dose animals returned to approximately it's original value. Standard deviation on the point was extremely large. Running in low-dose animals dropped significantly on the third run

FIGURE 1. TREATMENT GROUP A - CASTRATED. MEAN OPEN FIELD ACTIVITY OF MICE DURING A TWO WEEK TESTING PERIOD OF HIGH VS LOW DOSES OF CORTICOSTERONE.


Low Dose
High Dose
Saline
150
100
50
Number of Squares Entered (%)
1
2
3
OPEN FIELD RUNS

as did the running of the controls.

Basically the same pattern is seen in Fig. 2 which also shows running behavior of castrate-only animals. High-dose animals showed a slight decrease in running at Run 2, but not as sharp as that seen in Fig. 1. Following an additional week of treatment, running in the low-dose group dropped significantly. The high dose group dropped slightly, but not significantly. When the animals were separated with no injections for a period of one week there was a significant increase, or "rebound", in running behavior in both treatment groups that was not exhibited by the controls.

After animals from treatment group C were allowed to rest one week they were run through the experimental procedure again, this time with the doses reversed. Those animals previously receiving high doses of corticosterone were given low doses, and vice versa. The results are shown in Fig. 3. Interestingly, there was still a decrease in running behavior in both low- and high-dose groups, but the high-dose drop was significant and much more rapid. At Run 2 the low dose also had dropped. This was in contrast to the slight, but non-significant, elevation in running previously seen in low-dose animals at Run 2 in Figs. 1 & 2. Following two weeks of hormonal therapy the low-dose group dropped even further, while high-dose running remained at a low level. Following the cessation

FIGURE 2. TREATMENT GROUP C - CASTRATED. MEAN OPEN FIELD ACTIVITY OF MICE DURING A TWO WEEK TESTING PERIOD OF HIGH VS LOW DOSES OF CORTICOSTERONE FOLLOWED BY ONE WEEK OF RECOVERY.

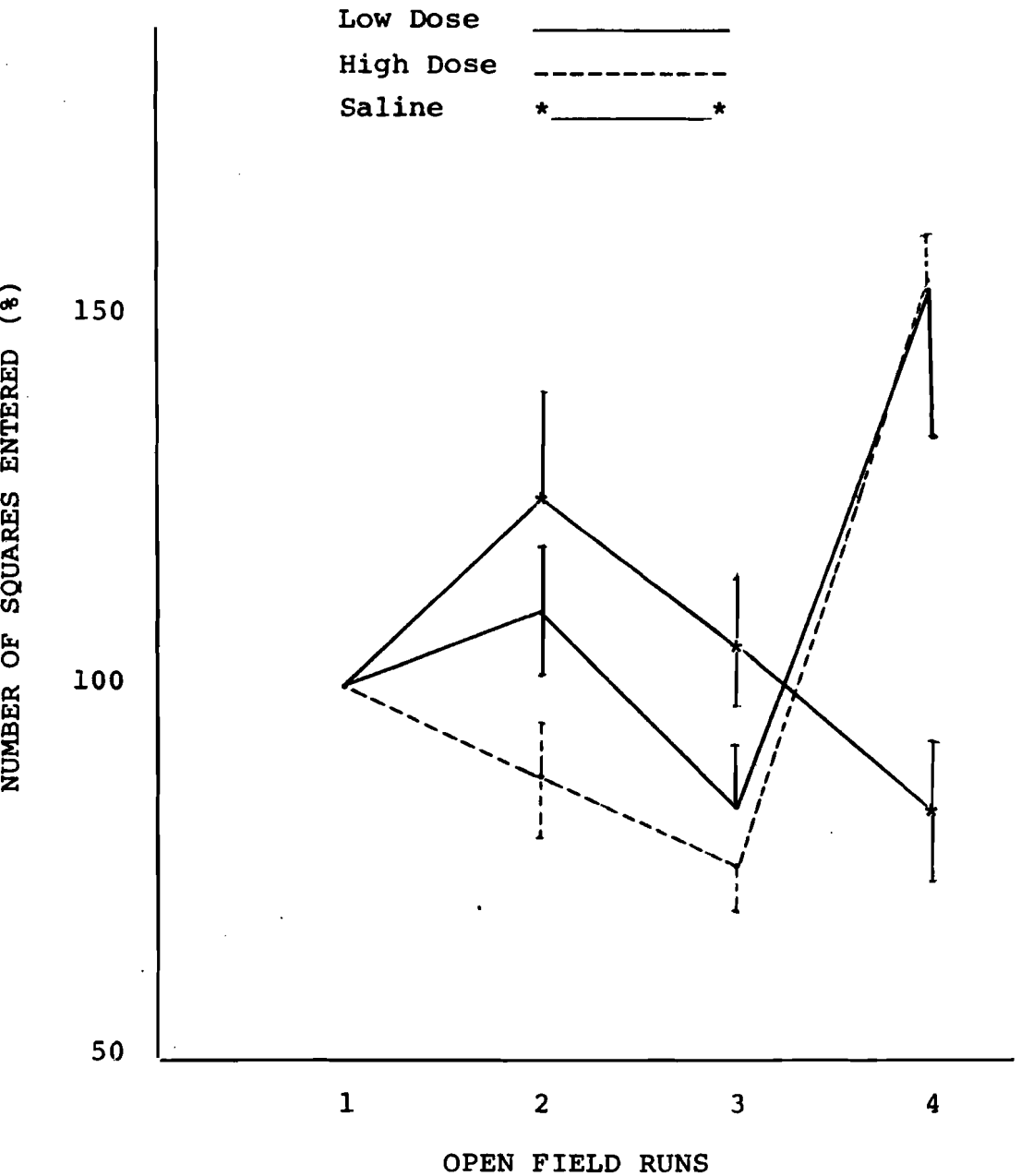
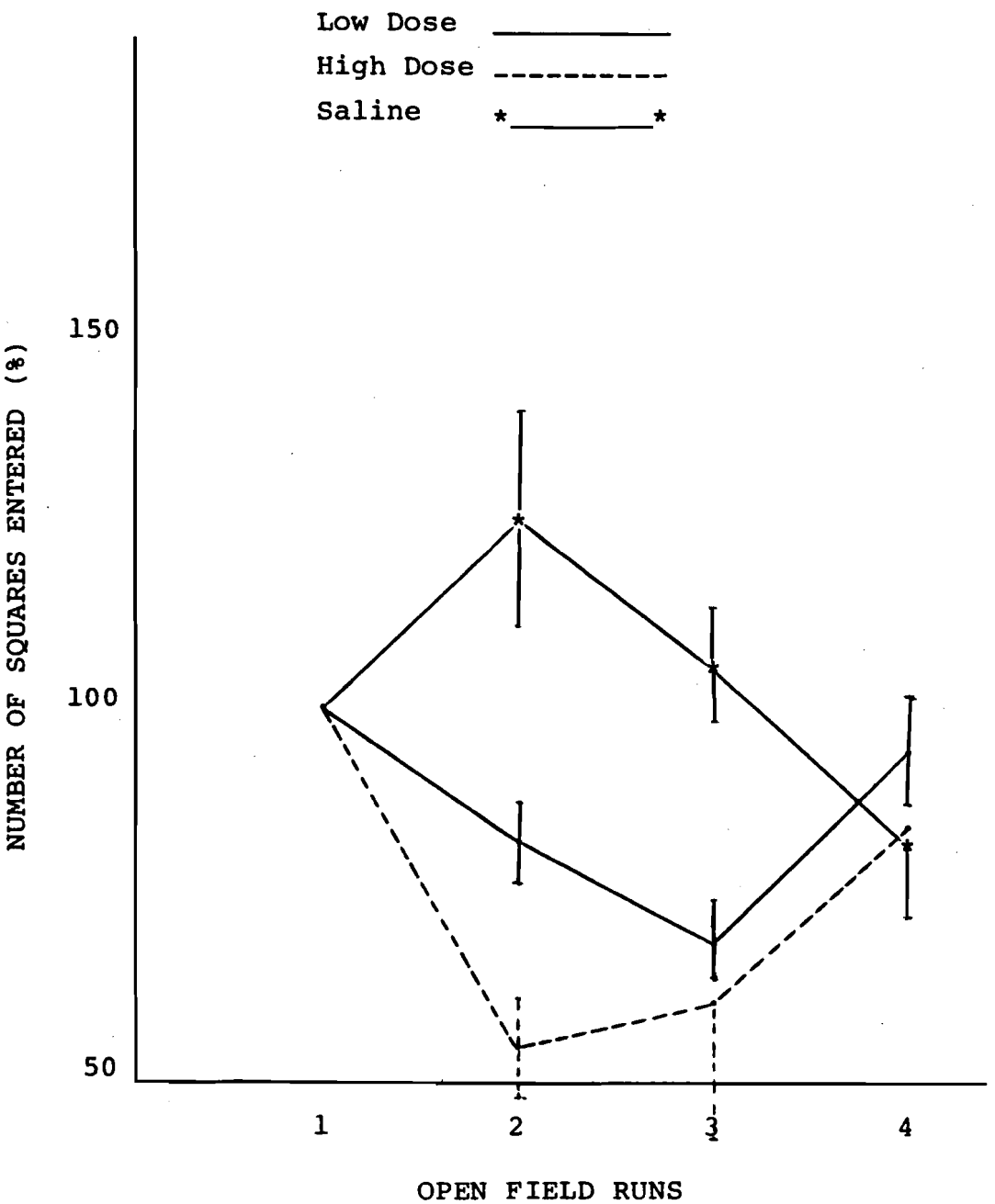

Low Dose
High Dose
Saline
NUMBER OF SQUARES ENTERED (%)
150
100
50
1
2
3
4
OPEN FIELD RUNS

FIGURE 3. TREATMENT GROUP D - CASTRATED DOSES REVERSED. MEAN OPEN FIELD ACTIVITY OF MICE DURING A TWO WEEK TESTING PERIOD OF HIGH VS LOW DOSES OF CORTICOSTERONE FOLLOWED BY A ONE WEEK RECOVERY PERIOD.


Low Dose
High Dose
Saline
NUMBER OF SQUARES ENTERED (%)
150
100
50
1
2
3
4
OPEN FIELD RUNS

of treatment at the end of Run 3, there was a significant increase in running during Run 4. This rebound was not nearly as pronounced as that seen during Run 4 in Fig. 2.

Fig. 4 represents the only group that was both castrated and bilaterally adrenalectomized. No control was run for this group. As in the castrated-only groups (Figs. 1 & 2) there was a slight elevation in open-field running in the low-dose group after one week of injections, but this decreased slightly after an additional week of treatment. In the high-dose group there was a nonsignificant decrease in running after one week, but open-field activity returned to normal after an additional week. None of the points between treatment groups or between runs were significantly different from each other.

The effects of hormonal injections on the open-field running behavior of adrenalectomized animals is shown in Fig. 5. The pattern of the controls seen in Fig. 5 for adrenalectomized animals was not unlike that of the control castrated animals seen in Figs. 1, 2 & 3. Both high-and low-dose animals differed in their response to hormonal therapy from the castrated animals. A significant increase (rebound effect) was seen on the fourth run. This was significantly different from Run 3 of those animals and from Run 4 of the controls. The high-dose group increased their running drastically throughout the experiment, leveling out somewhat between Runs 3 & 4 and thereby not exhibiting the rebound effect seen in the low dose group.

FIGURE 4. TREATMENT GROUP B - ADRENALECTOMIZED AND CASTRATED. MEAN OPEN FIELD ACTIVITY OF MICE DURING A TWO WEEK TESTING PERIOD OF HIGH VS LOW DOSES OF CORTICOSTERONE.

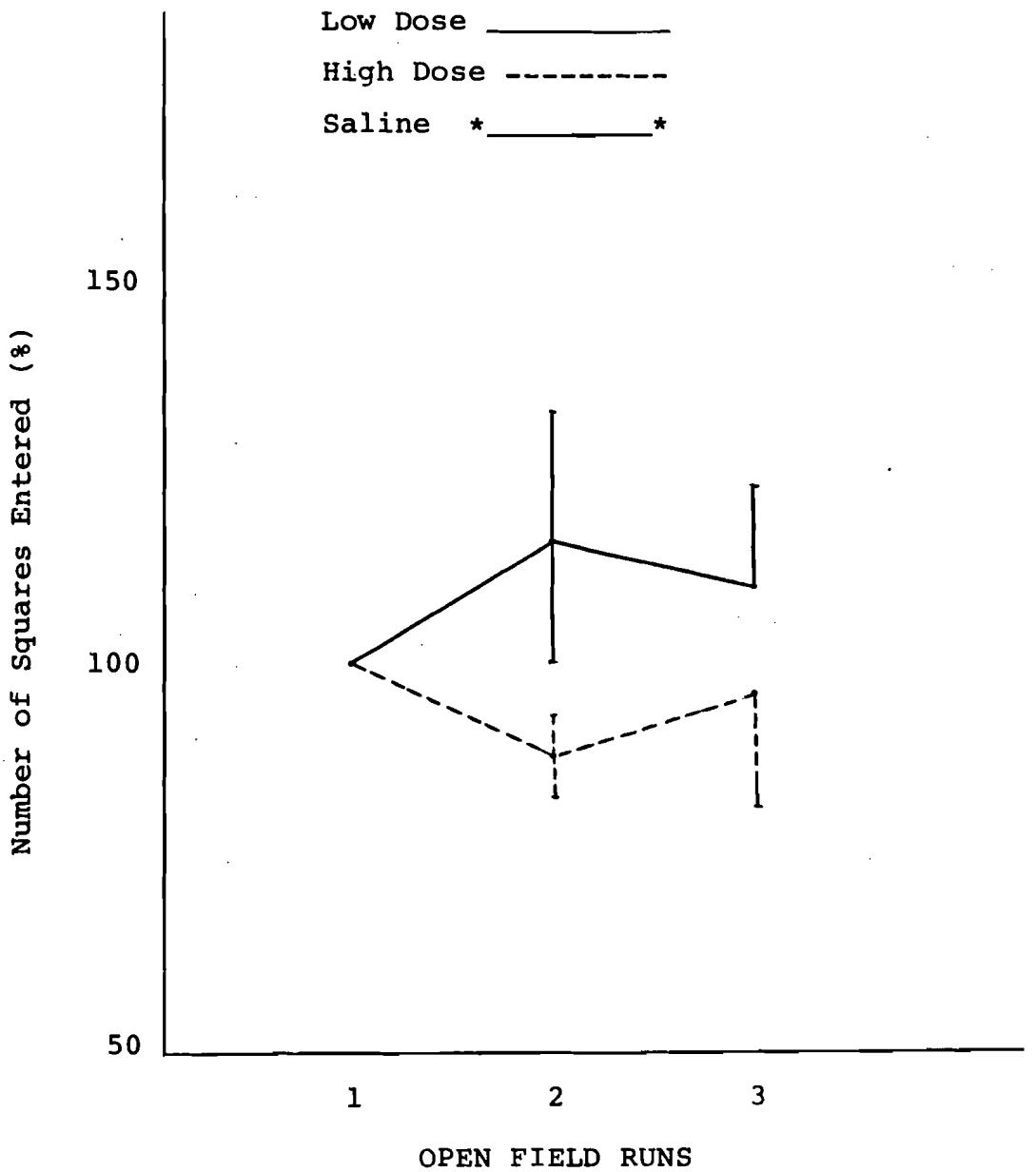
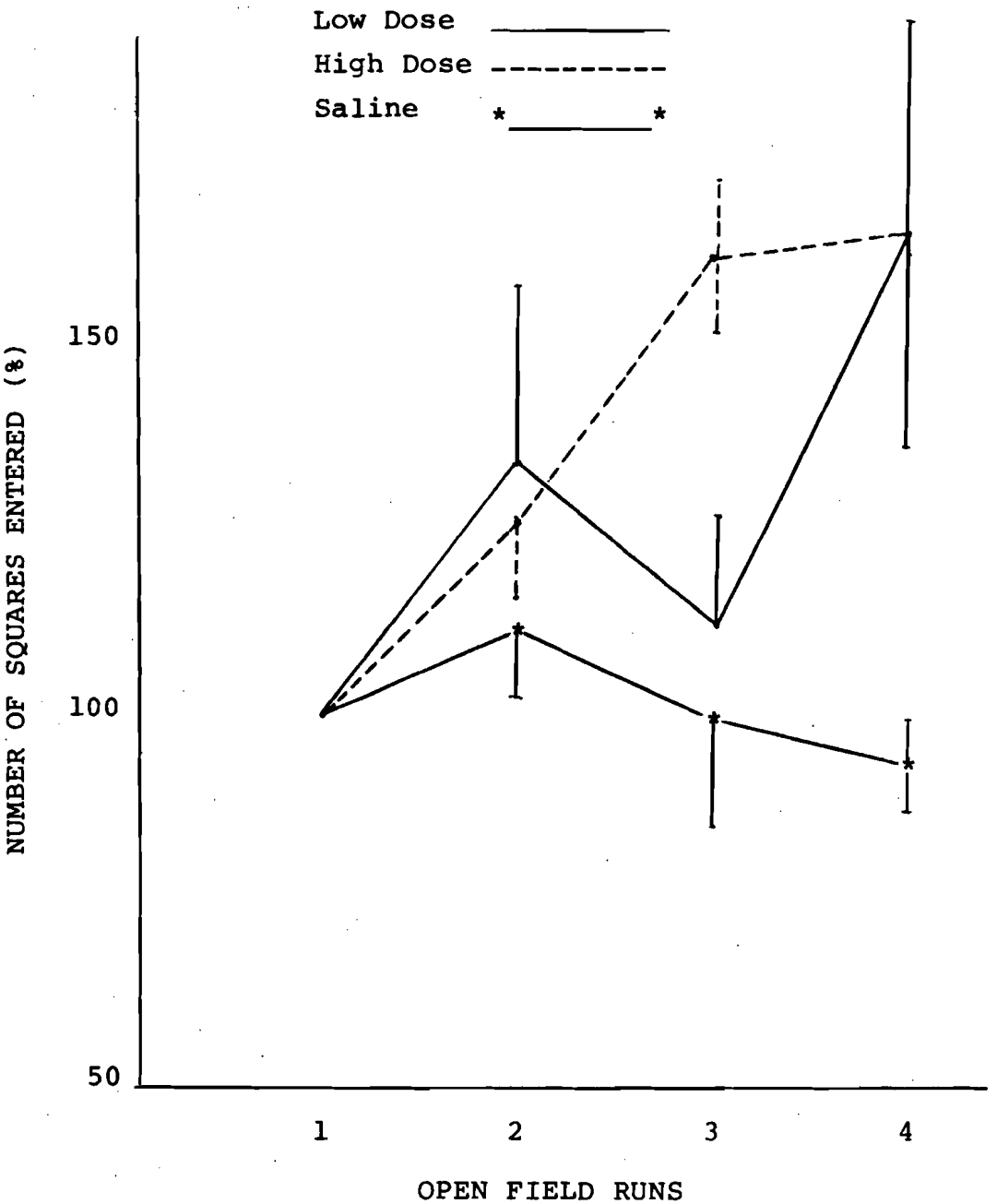

Low Dose
High Dose
Saline
150
100
50
Number of Squares Entered (%)
1
2
3
OPEN FIELD RUNS

FIGURE 5. TREATMENT GROUP F - BILATERALLY ADRENALECTOMIZED. MEAN OPEN FIELD ACTIVITY OF MICE DURING A TWO WEEK TESTING PERIOD OF HIGH VS LOW DOSES OF CORTICOSTERONE FOLLOWED BY ONE WEEK OF RECOVERY.


Low Dose
High Dose
Saline
150
100
50
NUMBER OF SQUARES ENTERED (%)
1
2
3
4
OPEN FIELD RUNS

Even so, running of the high-dose animals at Run 4 was significantly different from that of the control animals.

The effects of dexamethasone injections on the open-field running behavior of castrate animals is shown in Fig. 6. Standard deviation were large on all points. In the low-dose group there was a significant increase in running following one week of injections. After two weeks the running was decreased, but not as low as it was originally. Also, there was no rebound effect seen at Run 4. The high-dose animals continually climbed, although in small increments, throughout the testing period, but none of the increases were significant. This contrasts with the depression of running seen in animals treated with a high dose of corticosterone (Figs. 1, 2 & 3).

The last graph, Fig. 7, represents the effects of corticosterone therapy on intact animals. No controls were run on this group. As with the dexamethasone group, the intact animals showed a different pattern of response than either the castrated or adrenalectomized groups. There was very little effect seen of corticosterone therapy on intact animals. Also, the rebound seen in Run 4 was nonsignificant.

It should be pointed out here that early in the experimentation it was questioned whether the effects seen after two weeks of injection therapy were due entirely

FIGURE 6. TREATMENT GROUP G - CASTRATED WITH DEXAMETHASONE TREATMENT. MEAN OPEN FIELD ACTIVITY OF MICE DURING A TWO WEEK TESTING PERIOD OF HIGH VS LOW DOSES OF DEXAMETHASONE FOLLOWED BY ONE WEEK OF RECOVERY.

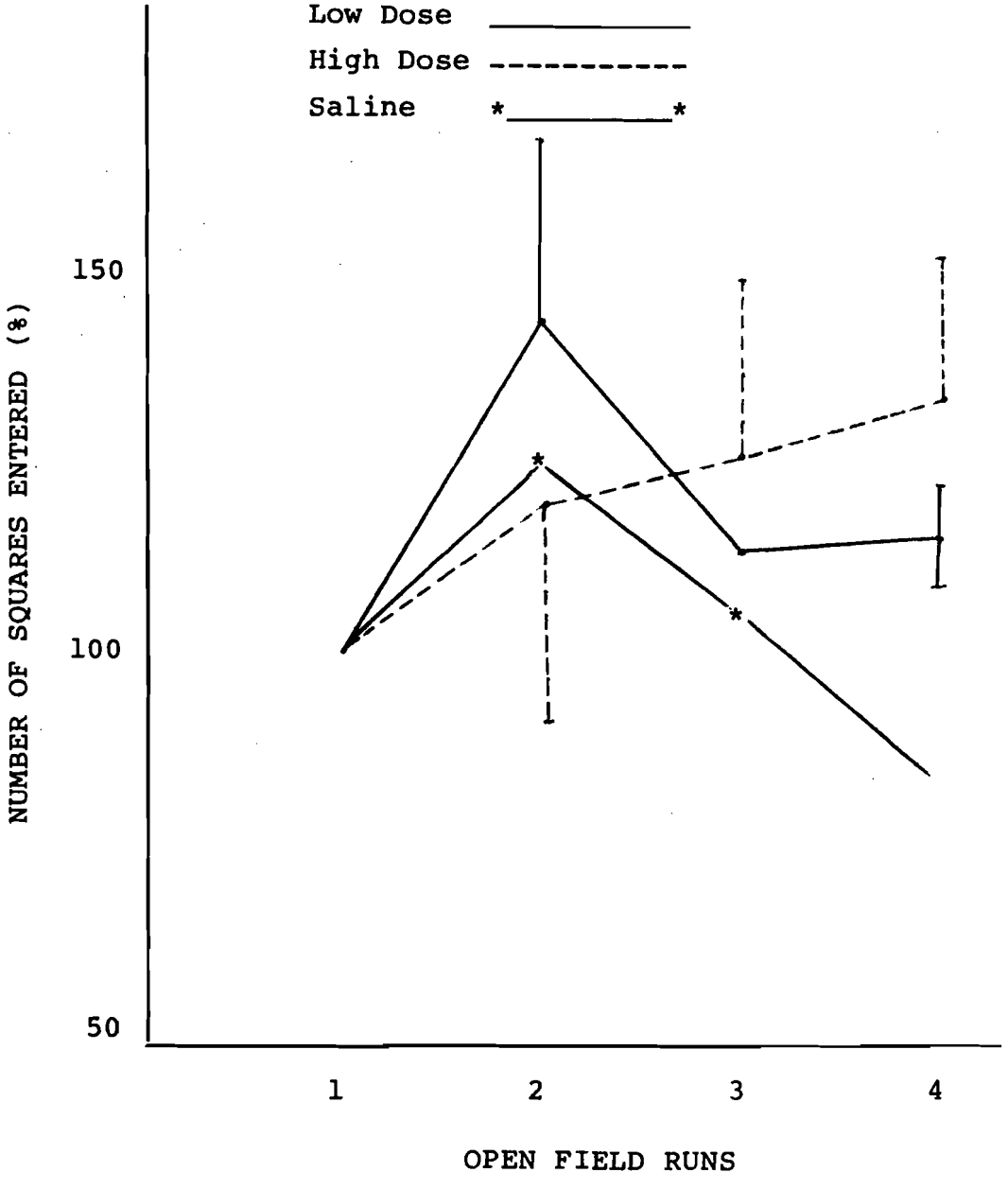
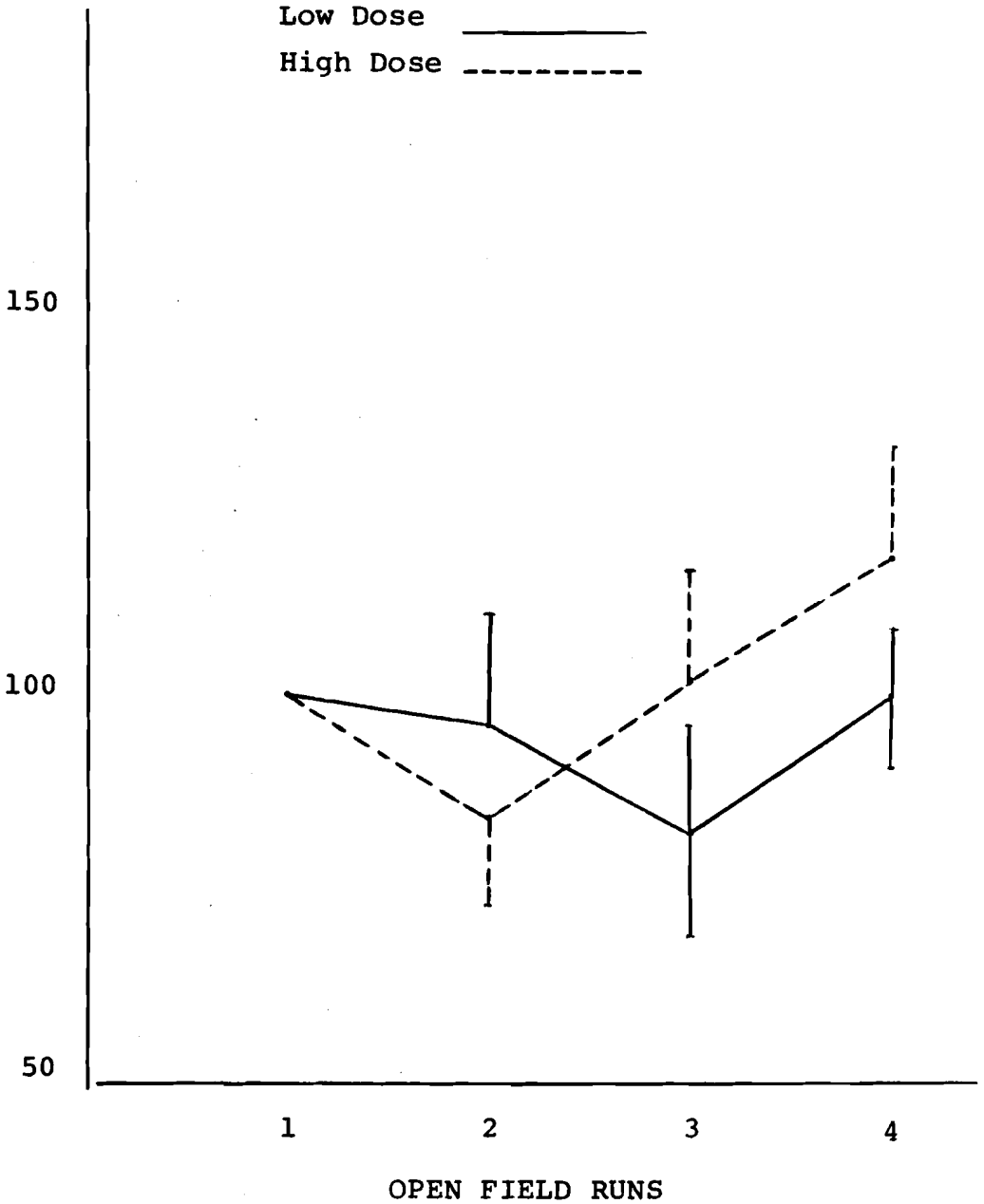

Low Dose
High Dose
Saline
150
100
50
NUMBER OF SQUARES ENTERED (%)
1
2
3
4
OPEN FIELD RUNS

FIGURE 7. TREATMENT GROUP E - INTACT. MEAN OPEN FIELD ACTIVITY OF MICE DURING A TWO WEEK TESTING PERIOD OF HIGH VS LOW DOSES OF CORTICOSTERONE FOLLOWED BY ONE WEEK OF RECOVERY.


Low Dose
High Dose
150
100
50
1
2
3
4
OPEN FIELD RUNS

to the effects of the hormones on behavior or due in part to the fact that the animals were paired during this time and the hierarchy established after the first week in some way affected the running behavior. To test for this, animals in Groups C & F (castrated only and adrenalectomized only) were subdivided into two groups. The animals in one group were paired following the first run while animals in the second group were housed singly until after the second run and then paired. There was no significant difference in the running behavior of the two groups. It was concluded that the point at which pairing occurred had no effect on the subsequent increase or decrease of running behavior and data from both subgroups were averaged together.

No significant difference in the number of fecal boli deposited in any of the groups was noted between high- and low-dose animals or between high- and low-dose groups and controls.

The outcome of aggressive encounters for experimental groups C - I are listed in Table 3. For Groups C & F where experimentation was originally started with twenty animals, there were two subdivisions in each group as stated earlier. All other groups originally contained only ten animals and aggression scores were taken on days one and two of the pairing which immediately followed the initial run. In some groups (C_1, C_2, and E) there

Table 3. Aggression scores of Treatment Groups C through G.

Group	Surgical Treatment	% Dom. High Dose	% Dom. Low Dose	% No Dom.
C_1	Castrated	0	80	20
C_2	Castrated	20	40	40
F_1	Adrenalectomized	25	75	0
F_2	Adrenalectomized	50	0	50
D	Castrated-Reversed	20	20	0
E	Intact	25	50	25
G	Castrated - Dex.	40	40	20

were deaths that decreased the total number of pairs from five to four. In the dexamethasone treatment group (G) dominance was equally divided between the low- and high-dose animals. In the case of the intact animals (Group E) more low-dose than high-dose animals attained dominance, however, the data were not significant. In Group C_1, which were castrated, animals paired immediately after the first run, all dominant animals, in those pairs exhibiting dominance dominance, were in the low-dose group. In the animals kept separate until after the second run (Group C_2) the low-dose dominants were reduced to 66%.

In Group D, where castrated animals had been tested under one set of conditions then again under reversed conditions, most of the pairs showed no dominance. The dominance that was present was equally divided between the high- and low-dose animals.

In the adrenalectomized animals paired after Run 1 (Group F_1) low-dose animals exhibited most of the dominance present. In the second group (F_2) which was paired and tested after Run 2, there was a reduction in the number of low-dose dominants from 75% to 0%. All of the dominance here was in the high-dose animals and there was a 50% increase in the number of pairs showing no dominance.

A correlation coefficient was run on the aggression data from the above pairings to test for a correlation

between aggression scores and open-field running behavior. No correlation was seen.

DISCUSSION

Attempts have been made to relate both agonistic behavior and running activity to fluctuations of the adrenal-pituitary system. The effects of alterations in adrenal functioning on the activity levels of rodents and alternately the effects of changes in activity levels on endocrine function have been examined (Durant, 1924; Riss et al., 1959). Earlier investigators concluded that the glucocorticoids and not ACTH are responsible for alterations in running behavior (Kendall, 1970). Leshner (1971) suggested that since the endocrine system is one of the main controlling systems for intermediary metabolism, it may be that the endocrine system affects activity through its effects on metabolism.

Part of the difficulty in working with hormones of the adrenal-pituitary axis is that there is no particular class of behaviors that they influence. They influence many. Also, it is difficult to distinguish the direct from the indirect effects.

Experiment 2 showed that the dominant animal exhibits significantly more open-field running behavior than does the subordinate. This observation supported earlier findings that the dominant is more mobile and "patrols" more. This appeared to be due to a decreased level of glucocorticoids found in the male dominant. If this is true, creating a high level of corticosterone in the peripheral

circulatory system might predispose an animal to act more submissively.

In the castrated only test groups (Figs. 1 & 2) the animals treated with a high dose of corticosterone showed a decrease in running behavior after only one week of injections. On the other hand, it required two weeks for the low dose to depress running behavior. This suggests that there may be a physiological mechanism that responded quickly to large quantities of the hormone, but required a build-up of the effects of the smaller quantities before a response was seen. The effect was short-lived and reversible. This can be seen when hormonal therapy is discontinued. After a period of one week, open-field running was no longer depressed.

The data in Fig. 3 indicate that earlier hormonal therapy alters the subsequent responses of animals to a given stimulus. In castrated animals that were tested twice (with the doses being reversed the second time), running in the high dose group dropped drastically after one week (Run 2). This contrasted with the slight increase seen in these same animals (then low dose) at Run 2 in Fig. 2. The low dose animals in Fig. 3 also showed a significant decrease in running after only one week of injections, while the previously untreated low-dose animals shown in Figs. 1 & 2 showed a slight, but insignificant increase in running during this time period.

The decline in running of the low-dose animals continued over the second week and, as seen in Fig. 4, the running behavior of both high and low-dose groups was approximately equal at Run 3. Evidently, the mehcanisms needed to decrease running behavior were still present in the animals after one week without injections. It was necessary only to reactivate the system, which was then capable of altering the running behavior more drastically or in a shorter period of time.

In the saline injected controls for the adrenalectomized group (Group F, Fig. 4), running dropped only slightly during the experiment. This may have been due to the decreasing physical condition of the animals when no replacement for lost glucocorticoids was given. There was a slight, but not significant decrease in body weight that was seen only in this group. In the experimental animals, a low dose of corticosterone increased the running behavior after one week of injections, but running was down to it's original level after two weeks of injections. Although running behavior in high dose animals seemed to increase at an unusual rate, this may be explained by the fact that adrenalectomized animals were in a weakened state at the time of the original run (Run 1) and the original run score (which was taken as 100%) was lower than those seen in other groups. Nevertheless, at least part of the effect on running behavior was probably due

to the fact that corticosterone injections acted as replacement therapy for lost glucocorticoids. This is in keeping with the report by Leshner (1971) that replacement therapy with corticosterone restores running wheel activity in adrenalectomized mice.

Animals in Group B (Fig. 4) which were both adrenalectomized and castrated maintained a fairly constant level of open-field running. Injections of corticosterone seemed to have little to do with actively increasing or decreasing the level of open-field running.

Throughout the entire experiment the intact animals (Fig. 7) showed minimal effects of the corticosterone treatment on open-field running. Running in high-dose animals did decrease, but not significantly, and at Run 3 running was back up to the original level. The presence of both the adrenals and the testes in the intact animals seemed to have a buffering effect on the hormones given. Leshner (1971) noted that corticosterone in dosages of 5 mg/kg decreased running wheel activity in intact mice, but restored running wheel activity in adrenalectomized mice.

Amounts of dexamethasone used were carefully calculated to equal as closely as possible the therapeutic value of corticosterone. Although dexamethasone treatment has been reported to restore aggressiveness and running wheel activity in adrenalectomized mice (Walker and Leshner, 1972), it did not have the same effects as corticosterone on open-field running. While running was decreased in

animals receiving corticosterone, in those animals given dexamethasone running did not diminish, but increased (Fig. 6).

The fact that dexamethasone did not elicit the same response as corticosterone in reducing open-field running suggests that the corticosterone effect is mediated by a direct action on the brain. Dexamethasone is a powerful glucocorticoid and its metabolic effects are very similar to those of corticosterone. Therefore, it seems unlikely that the different effects of these two compounds on running behavior could be due to their effects on intermediary metabolism. Similarly, since ACTH secretion is known to be decreased by both corticosterone and dexamethasone injections (Brain, 1971) it is unlikely that the decrease in open-field running following corticosterone injections is mediated by a subsequent decrease in the levels of ACTH. On the other hand, there is an area of the brain which binds corticosterone to a greater degree than dexamethasone. That area is the hippocampus (McEwen, 1975) and could, therefore, be responsible for mediating the motor effects of the hormone. Differences in the effects of dexamethasone and cortisol were also noted by Kendall (1970). He reported that administration of dexamethasone in drinking water in supraphysiological doses markedly stimulated running wheel activity. Implantation of a cortisol acetate pellet into the median eminence of the hypothalamus depressed running wheel

activity which was restored by the addition of dexamethasone to the drinking water. It was concluded that since the pellet suppressed ACTH, increased glucocorticoid rather than depressed ACTH was responsible for the increase in activity. It should be pointed out that spontaneous running wheel behavior may be physiologically regulatory, such as eating and drinking behavior, and possibly is not affected by the same mechanisms affecting open-field running.

Interestingly, in the castrated groups (both high and low doses) and in the low-dose adrenalectomized group, open-field running not only returned to the original level once hormonal therapy was discontinued, but increased significantly. As far as this author knows, this "rebound" effect has never before been reported. This response was not seen in any of the saline control groups, and therefore, is not due to the cessation of injections or changes in handling. Rather, it suggests that once the exogenous source of the glucocorticoid was removed there was an actual shift in the physiological pathways controlling open-field running. Since there was no rebound effect in the dexamethasone group, it is unlikely that ACTH is directly involved in this phenomenon. The exact length of time necessary for this response to occur, and the length of time it lasts is uncertain, as animals in this study were tested only once, one week following cessation of injections.

Exactly where the hormones are exerting their effects in the CNS is unknown. As seen in this study, corticosterone treatment can alter open-field running behavior in castrated or adrenalectomized mice and may do so by a direct action on the brain. It is known that the brain of higher vertebrates contain populations of cells that are sensitive to steroid hormones, including those of the adrenal cortex. When labeled corticosterone is injected into adrenalectomized rats or monkeys the steroid shows up in the brain in two main places: the hippocampus and the septum, both parts of the primitive cortex (McEwen, 1975).

There appears to be a characteristic "hormone architecture" for each hormone in its binding to brain receptor sites, but there is some overlap. In the septo-hippocampal region androgen and corticosterone concentrating cells are found in identical areas. Also, a single cell may have receptors for more than one steroid hormone (Stumpf & Sar, 1978). They proposed that the selective action of androgens on given areas of the brain activates agonistic behavior patterns. Since glucocorticoid receptors are located in the same region, the adrenal hormones may be essential for the full activation or expression of the behavior. This might be a possible explanation as to why the effects of hormonal injections are the greatest in castrated or adrenalectomized animals. The receptors in these animals would be unoccupied by endogenous hormones.

Therefore, injected hormone would be bound more readily and have a more profound effect.

One theory of the role of hormonal binding is that the binding proteins regulate genetic activity by combining directly or indirectly with the genome. In peripheral tissues the steroids work by coupling to a receptor and causing portions of the DNA molecule to become accessible to enzymes that can form new m-RNA. The m-RNA in turn directs the formation of new protein molecules, which will eventually reach the target area and carry out the response (McEwen,1972b). The final result would be a selective increase in the activity of those genes leading to the manufacture of protein molecules needed for cell function.

Paired animals were used in this study. Earlier studies have shown that the effects of social stress are most marked in paired animals since the dominant and subordinate can be easily identified (Brain & Nowell, 1971). It is thought, therefore, to be more useful to study the effects of hormonal therapy on social rank of paired animals.

Data from this study are insufficient to determine whether manipulations of corticosterone levels can predispose an animal to a specific social rank. When animals in Group C (castrated only) were paired immediately following the first run and tested for aggression after only one or two days of hormonal therapy, the dominance

was found in the low-dose group. Despite the lack of correlation between open-field running and aggression in general, in this particular group aggression was correlated with running behavior seen at Run 2 in Fig.2. Low-dose animals (dominants) ran more than high-dose animals (subordinates). In those animals paired after one full week of injections, low dose dominance was down by half - as was the running activity of the low-dose group seen at this point. In none of the other test groups were any significant differences noted. The low number of pairs may have been partially responsible for this. It is more likely, however, that while corticosterone therapy is able to affect open-field running, it has little effect on the social rank of the animal.

Evidence from other studies suggests that hormonal responses to novel stimuli may affect subsequent behavioral reactions, possibly through alteration of memory pathways (Gold & van Buskirk, 1977). For example, corticosterone seems to make hippocampal nerve cells less sensitive to stimuli that bring on the theta rhythm associated with memory and learning behavior (Turner & Bagnara, 1971). Also, post-training treatment of rats and mice with vasopressin or adrenocorticotrophin increases the retention of avoidance responses and submissive responses can be viewed as a type of avoidance reaction (Lorenz, 1967). Recent studies by Roche & Leshner (1979) show that prior

defeats increase the future submissiveness of an animal and that administration of ACTH immediately after an initial defeat also leads to increased submissiveness. Thus, while results from this current study indicate that ACTH is not directly involved in open-field running behavior, it does seem likely that ACTH plays a definite role in the establishment of the social rank of an animal.

Finding from the data on open-field running indicates that the hormonal state of the animal can prepare or pre-set the sensory receptors or central neural processing mehcanisms that control the pattern of response to a given stimulus. The experience of the animal in the situation and the behavior it exhibits probably produce further changes in the hormonal state which will alter its reaction to further stimuli. Research has shown that early experience can affect adrenocortical reactivity and the subsequent baseline hormonal level of an animal. Rats handled as infants show lower concentration of plasma corticosterone upon exposure to a given stimulus than unhandled controls (Levine, 1967). It has been suggested that this differential response to stress is a result of increased concentration of steroids produced in the young animal by handling. Howard and associates (1968) observed that animals treated with corticosterone in infancy showed altered patterns of open-field running. Mice given corticosterone at two days of age and tested as adults

showed lasting alteration of operant behavior coupled with a decrease in cerebral weight and DNA content. When the corticosterone was given at 22 days there was no change in cerebral weight, DNA content, or operant behavior although there was a decrease in open field activity (Howard, 1968).

The differences between permanent responses to steroids in infancy and the temporary responses seen in adults may be due to differences in the target neurons at the time of impact (McEwen, 1975). Possibly, corticosterone may reach the nuclei of hormonally sensitive differentiating nerve cells during a critical period in brain development and hence affect the pattern of neural connections. Once formed, the neurons may no longer be susceptable to other hormonal influence. In the adult, the role of the hormone might be to alter the efficiency of the synapses in the circuits.

Conclusions from this research would indicate that the baseline hormonal state of an animal is responsible for the way it will behave in an open field situation. Also, while glucocorticoids apparently are not critically involved in the establishment of dominance relations, they are critically involved in the behavior of the submissive animal once dominance is established. Prior studies have shown that following competition, defeated animals show an increased level of adrenocortical activity (Bronson & Eleftheriou, 1964) and that the threat of

defeat by itself will elicit increases in adrenal activity in a previously defeated animal. Defeat also affects the state of the central nervous system circuits which control endocrine function by altering protein synthesis in the brain and the state of brain neurotransmitter systems (Eleftheriou, 1971). Therefore, once an animal becomes subordinate, its adrenals enlarge resulting in increased secretion of glucocorticoids. This high level of glucocorticoids could then act back on the brain to cause the low level of open-field running and perhaps other behaviors associated with the subordinate animal such as avoidance behavior. Since submissive responses can be viewed as naturally occurring avoidance reactions, this is consistent with earlier findings that show that raising the level of adrenal-pituitary hormones following a stressful encounter enhances later adaptive responding (Gold & van Buskirk, 1977). This increase in adrenal functioning may then be an adaptive response which would result in less contact with the dominant and a greater chance for the survival of the subordinate.

The data cited suggest that it is possible to predispose an animal to react in a given way to certain agonistic stimuli. This predetermination may be genetically programmed into the animal or may be altered by conditions occurring early in life. The possible mechanisms of action are varied and numerous and probably include effects

on induction of brain receptors (Lisk, 1971), developing neurotransmitter systems (Philipson & Sander, 1975), myelination of developing neurons (Granich & Timeras, 1971), gene expression (Schwartz, 1972), DNA content (Howard, 1965) and neuronal morphogenesis (Shapiro & Vukovich, 1970).

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