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LIMBIC AND NEOCORTICAL LESION EFFECTS ON  
RESPONSES TO HIGH POPULATION DENSITY

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by  
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# ABSTRACT

The effects of hippocampus, entorhinal cortex and neocortex lesions on behavioural and endocrine responses to a high population density situation were studied. Eighty male and 80 female hooded rats were placed into 4 lesion groups: (1) antero-dorsal hippocampus; (2) entorhinal cortex; (3) neocortex control; (4) sham control. Each group consisted of 20 males and 20 females. Each group was placed in a separate pen and scored on a number of behavioural measures for 30 minutes per day, for 80 consecutive days. At the completion of the study, a series of endocrine measures were taken for each group. The antero-dorsal hippocampal animals demonstrated significantly more sexual, open field and "amicable" behaviour than all other groups. No significant differences were found between groups for blood corticosterone, adrenal and testes weights. The behavioural results were interpreted as reflecting decreased emotionality and increased social interaction in the hippocampal animals in response to high population density.

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## INTRODUCTION

Much of that behaviour which is commonly referred to as adaptive involves some form of adjustment of the organism to stress. In recent years stress has been subjected to an exceedingly wide and diverse range of experimental inquiry. Initiated by the work of Selye (1950), subsequent examination of stress has extended the field of research to include studies upon the precise nature and mechanism of various forms of stress or stressors, the functional and physiological relationship of stress to endocrine functions, and the role of stress in learning situations and in a wide range of other behaviours. Selye distinguished between "general stressors", which involved such things as body injury, biological stress of extreme heat and cold, and "emotional stress" involving sudden changes in light, temperature, sound, environment, or any situation resulting in anxiety, frustration, anger or pain. Both of these types of stress, he pointed out, tended to damage or destroy the homeostasis of the organism. A consistent response to this stress was adrenocortical activation, as measured by blood level of adrenal cortical hormones, adrenal hypertrophy, adrenal ascorbic acid concentration, involution of the thymus and lymph nodes, and decrease in circulating eosinophils. These so-called "stress responses" have thus provided investigators with a number of objective measures with which to gauge the degree of stress in a given experimental situation.

There now exists considerable experimental evidence that high population density functions as a stressor to produce both the typical physiological stress responses as well as a number of marked behavioural responses. In a comprehensive research program designed to investigate the concept of a pituitary-adrenocortical-gonadal mechanism of regulation

of population size, Christian and his co-workers have clearly demonstrated a direct relationship between population density and adrenocortical responses to stress. In a detailed review, Christian, Lloyd, and Davis (1965) have summarized the results of their own ongoing research programs, and have presented further evidence to support their idea of density-dependent behavioural and endocrine mechanisms which account for increased mortality and decreased natality with increased population density. Although the exact nature of the stressful stimuli in high population density situations is far from established, the behavioural and physiological responses to these situations are well documented. In Christian's investigations, the general design used involved placing mice in different sized groups, and recording the relevant measures of endocrine activity. In grouped male mice, the mean adrenal weight increased approximately linearly with the logarithm of population size up to a certain limit (Christian, 1959; Welch & Klopfer, 1961) after 1 week of grouped caging. Bronson and Eleftheriou (1963) have reported decreased thymus weight and adrenal ascorbic acid concentration with increased population size. A similar effect was found by Vandenberg (1960). It has been reported that highly aggressive strains of mice show considerably greater adrenocortical responses to increases in population size (Bronson & Eleftheriou, 1963; Christian, 1955). In female mice, adrenocortical responses to increased population are qualitatively similar but quantitatively less than in males (Christian, 1960). Studies of adrenocortical responses in grouped rats (Barrett & Stockham, 1963) and rhesus monkeys (Mason, 1959) reported adrenal hyperactivity with increased density, as measured by corticosterone and plasma 17-hydroxycorticosteroid

levels respectively.

In a series of studies on freely-growing but confined populations, adrenal hypertrophy and elevated plasma corticosterone levels have been found in house mice (Christian, 1961) with increasing population density. Voles have been shown to respond similarly to population density (Christian, 1959). In one of the few studies upon natural populations, Christian and Davis, (1956) examining a wild rat population in city blocks, found a positive relationship between stages of the density cycle and adrenal weights; a progressive increase in adrenal size was found for each increasing population size. Studies upon woodchucks, deer, and rabbits (cited in Christian et al., 1965) further the evidence for a direct relationship between adrenocortical function and population size.

A number of other effects reported in population density studies and considered to be secondary to increased adrenocortical functioning are the inhibition of inflammation, antibody formation, and diminished resistance to parasitism and infection (Christian, 1963a). In addition, amphetamine is seen to become increasingly toxic when administered to grouped animals, (Thiessen, 1964).

Another major type of endocrine response to high population density is that of gonadal hypoactivity. Weights of testes, seminal vesicles, and preputial glands of brown and albino house mice were reported to decrease in an approximate linear relationship as the log of the population size increased (Christian, 1955). An early study on grouping (cited in Christian, et al. 1965) indicated delayed maturation in brown female mice if grouped prior to puberty. Partial inhibition of reproductive function in mature female mice has also been noted, (Christian,



1960). Christian (1965) reported that depending on the strain, grouping may produce anestrus, reduced time in estrus, pseudopregnancy, or delayed implantation in impregnated mice. Female albino mice, during 6 weeks of grouping, bore no young, and only 77% of these bore young after removal from the crowded conditions. Of these, only 85% successfully reared litters. Pups mothered by previously crowded mothers were stunted at weaning, and offspring of these young were also stunted. Similar effects appear to occur in freely-growing confined, as well as natural, populations of several species. Most prominent of these effects occurred in females, resulting in increased intrauterine mortality and delayed or totally inhibited maturation of the young, (Christian, 1963b). However, similar changes are seen to occur in males, including delayed onset of puberty, and smaller size of seminiferous tubules with delayed and abnormal spermatogenesis (Christian, 1956). In both sexes, growth and development of secondary sex organs is inhibited in all but the oldest and dominant animals (Christian, 1961). In voles, Clarke (1955) showed that birth rates and survival rates of neonates declined as density increased. In rabbits, increased density had similar effects, with a high rate of intrauterine mortality, decreased fecundity, and decreased estrous behaviour associated with high population density. The results from wild rat populations extend and support the data on confined populations of mice. Davis (1951) has shown that the prevalence of pregnancy was greatest in increasing, and lowest in stationary populations. Finally, work by Christian (1963b) reveals a generally negative correlation between density and reproduction, likely the result primarily of inhibition of maturation.

Thus increasing or high population density appears related to at least 2 major endocrine responses - that of adrenocortical hyperactivity, and gonadal hypoactivity which seems to affect almost all stages of reproduction. There is also considerable evidence that these effects are general across a number of species and types of populations, and represent a complex physiological reaction to the stress of high population density.

Behavioural responses to increasing or high density situations have also been examined, although to a more limited extent. In most cases, the behaviour under observation has been treated as a possible cause of the noted endocrine responses to population density, and not as a discrete form of response to population density. However, Calhoun (1952) has reported a number of behavioural responses that appear directly attributable to crowding. In female rats, inability to carry pregnancy to full term, or to survive if they did, in addition to general lack of proper maternal care, was found. Among males, behavioural disorders involved sexual deviations, cannibalism, hyperactivity, and pathological withdrawal when animals refused to eat or drink. In some cases Calhoun reports a "togetherness syndrome" in which animals would crowd together in one pen during feeding periods and almost never eat or drink unless in the presence of other rats. These disturbances served to disrupt the ordered patterns of behaviour necessary in such activity as nest-building, courting, and nursing and care of the young.

With the considerable experimental evidence that high population density situations produce both endocrine and behavioural responses, a question which next suggests itself is that dealing with the nature of

the relationship between the two types of response. That is, can the behavioural changes that occur be related to the changes in endocrine functioning that occur in high population density? Clearly, some of the behavioural disturbances observed by Calhoun appear to be in endocrine-related behaviours, such as maternal and sexual behaviour. Since a major endocrine response to population density stress is that of adrenocortical hyper-activity, it is of value to examine what relationship, if any, exists between this response and various behavioural responses that result from high population density. Little information currently exists on behavioural correlates of high adrenocortical steroid levels produced by stressful situations or on whether behavioural activity under stress is due to elevated ACTH levels or in fact produces these changes in ACTH level. Both of these points of view have, however, been proposed. Aggressiveness and fighting have been shown to result in endocrine responses that typically occur in response to grouping (Bronson & Eleftheriou, 1963; Welch & Klopfer, 1961), and therefore it is suggested that this behaviour, in high population density situations is the primary factor in producing the adrenocortical responses observed. On the other hand, Christian (1964) opposes the view that fighting or aggressiveness is responsible for these endocrine responses, noting that endocrine responses in many cases are identical whether or not there is fighting or injury. As far as the fighting itself goes, little mention is made of it, or any other behaviour, as a form of qualitative or quantitative response to the stress. Christian concludes merely that fighting is another symptom of social competition. The problem of how these symptoms are related has not been critically examined.

That certain behavioural events may be related to ACTH level, has been recognized by a number of researchers. Clinical observations have been made on various fear or anxiety-motivated behaviours produced in patients under ACTH therapy (Mirsky, Miller & Stein, 1953; Cleghorn, 1952). Other studies have attempted to investigate the effect of administering ACTH upon animal performance in avoidance conditioning tasks. Several investigators have reported that ACTH administered at the beginning of acquisition resulted in the ACTH group requiring significantly more trials to extinction than placebo control groups (Murphy & Miller, 1955; Miller & Ogawa, 1962). This effect appeared even in the absence of the adrenals, suggesting a minimal role of the adrenals in this type of avoidance behaviour. This effect was confirmed by De Wied (1966) who found that, as before, the levo-rotatory form of the ACTH molecule delayed the rate of extinction in a shuttlebox and pole jumping experiment, while the dextro-rotatory form significantly enhanced extinction.

In a slightly different approach to the question of behavioural correlates of ACTH levels, Lissak and Endroczi (1961) have examined the variations in the composition and levels of adrenal cortical secretion in relation to different stages of the conditioned alimentary reflex and to experimental neurosis. They found that animals with the longest periods of inhibition of response in a passive avoidance task had high hydroxycortisone-corticosterone ratios compared with the low ratios of those animals with short inhibition periods. They also found that pre-treatment with hydrocortisone resulted in an inhibitory period several times longer than that observed in the same animals many months before

this treatment. In subsequent studies, these investigators found that ACTH considerably facilitated the passive avoidance response in dogs, and in rats they report that the level of adrenal ascorbic acid in response to unilateral adrenalectomy was significantly lower in those animals that were previously better in acquiring a passive avoidance response, again suggesting the facilitating effects of ACTH on passive avoidance. Finally, in an attempt to correlate these learning effects with some behavioural counterparts, a series of stimulation studies were performed in which brain areas were located which resulted in activation of the pituitary-adrenocortical system. These areas were stimulated in dogs, cats, rabbits, and rats with rising intensities of stimulation and the reaction observed. Threshold stimuli elicited clear orientation, which was followed by "emotional reaction" (fear, avoidance) and further elevation of the intensities induced "extreme ferocious behaviour". Upon cessation of stimulation, original behaviour was seen to return at once.

These suggestions of the action of ACTH to enhance passive avoidance have been supported by Levine and Jones (1965) who found that continuous administration of ACTH through the test period resulted in increased passive avoidance in all animals. Recently, Levine and Brush (1966) have attempted to correlate a specific part of the avoidance learning curve with adrenocortical levels, and have hypothesized that the descending arm of the U-shaped behavioural function following fear conditioning might be associated with a homeostatic decrease in the level of circulating plasma steroids. Their results indicate that avoidance learning follows this U-shaped pattern after fear conditioning with

regard to the descending part of this function.

It is apparent from these studies on ACTH and avoidance behaviour that the primary aim is to delineate the precise hormonal antecedent, correlate, or consequence of a specific type of behaviour. Presently, the status of this evidence appears to fit the conclusions of Levine and Brush (1966) who note that the results "while not providing definitive answers, warrant further investigation of the causal status of the relation between avoidance learning and adrenocortical levels and determination of the conditions under which such relations hold". It is felt that this statement should be extended to include not only avoidance learning, but also other types of behaviour that occur in response to stress, and in particular, to population density stress. If the behaviours observed under high population density are in fact manifestations of, or in some way related to, the observed endocrine responses to crowded conditions, it seems likely that those CNS mechanisms involved in the regulation of ACTH would also be involved in these behavioural responses to high population density stress.

In the examination of the neural regulation of ACTH, studies have concentrated, not surprisingly, on those limbic system structures with strong direct connections to the hypothalamus. In the hippocampus this primary efferent pathway is the post-commissural fornix, which establishes direct connections with the mammillary bodies of the posterior hypothalamus. There is also evidence that some fibres may leave the fornix before the mammillary region to continue into the tegmentum (Nauta, 1956). The afferent and efferent connections of the hippocampus have been examined in great detail (Nauta, 1956; Powell, 1954; Green, 1960, 1964) by both

anatomical and electrophysiological techniques. Presently there is also a well-established physiological rationale for the role of the hippocampus in the regulation of ACTH secretion in response to stress. This evidence is based primarily upon studies of hippocampal stimulation and lesion effects upon ACTH levels in animals subjected to a variety of stressful conditions, and is reviewed below.

Mason (1958, 1959) reported that blood plasma and urine 17-hydroxycorticosteroid levels in rhesus monkeys showed a slight acute elevation, followed by a delayed, prolonged suppression of these levels in conditioned avoidance and conditioned emotional response tasks, following stimulation of the hippocampus. During this suppressive phase, even direct hypothalamic stimulation was unable to elicit the typical ACTH response with normal stimulation intensity levels. Hippocampal stimulation in dogs has been shown to decrease the resting level of plasma corticosteroids and prevent the typical adrenocortical hyperactivity in response to stress (Endroczi, Lissak, Bohus, & Kovacs, 1959). Porter (1954) observed that stimulation of the uncus prevented the typical eosinopenic response to injected epinephrine stress. The results of Slusher and Hyde (1961) have also shown that stimulation of the uncus in cats, following acute "encephale isole" preparation, produced a delayed suppression of adrenal effluent corticosteroid levels. Decreased 17-OH CS levels in human patients has been reported to follow hippocampal stimulation (Mandel, 1963).

Hippocampal lesion studies have supported the evidence that the hippocampus functions in an inhibitory capacity to suppress ACTH and consequent corticosteroid levels in response to stressful situations.

Lesions of the hippocampus-fornix system have been observed to significantly elevate 17-OH CS level which had been previously suppressed by lesions of the amygdaloid complex (Mason, 1959). Fendler, Karmos, and Telegdy (1961) found significantly increased adrenocorticosteroid levels in cats one to three months following hippocampal lesions. These results have been confirmed by Knigge (1961) who found that hippocampal lesions did not alter the temporal pattern of corticosteroid response to immobilization stress, but did significantly elevate the resting level of steroids. In a follow-up experiment Knigge (1963) observed that hippocampal lesions negated an existing block of the ACTH response which had been caused by amygdala and reticular formation lesions, and produced an acute ACTH discharge to ether and heart puncture stress. Finally, Kim and Kim (1961) have reported that dorsal hippocampal lesions produced a significant decrease in adrenal ascorbic acid concentration under acute stress, with a gradual increase of this level under chronic stress. These authors conclude, in close agreement with other findings, that the hippocampus exerts a sustained inhibitory influence upon ACTH secretion in response to stress.

A number of studies have examined the effects of hippocampal lesions upon different types of behaviour. Most of these studies have looked at behaviour in a non-stress situation. For example, Kim (1960) has studied the effects of hippocampal lesions upon nest-building, general activity, and salt preference; Rasmussen, Kaada, and Bruland (1960) observed changes in heterosexual drive as a result of different limbic system lesions in rats; Roberts, Dember and Brodwick (1962) and Leaton (1965) investigated the effects of hippocampal lesions upon



exploration in a novel situation. Since the hippocampus has been clearly implicated in the regulation of adrenocortical responses to stressful situations, it would appear reasonable to examine its role in the regulation of behavioural responses to stress. In this way the role of the hippocampus in mediating endocrine responses to stress could conceivably provide a rationale for any behavioural changes in response to stress that might occur as a result of hippocampal lesions.

In summary, there is considerable evidence that high population density can function as a stressor to produce adrenocortical hyperactivity, gonadal hypoactivity, and a number of behavioural disturbances. Since the hippocampus has been shown to play a major role in the regulation of ACTH, in response to stress, and since a number of learning and behavioural responses appear related to ACTH and corticosteroid levels, it is suggested that the hippocampus may also function in behavioural responses to stress. The present study has therefore attempted to examine what role, if any, the hippocampus plays in various behavioural responses to the stress of high population density, and its possible relationship to the role of the hippocampus in the regulation of ACTH in response to stress.

#### METHOD

##### Subjects

The Ss were 80 male and 80 female hooded rats of the Quebec Breeding Farms strain, 75-80 days of age at the beginning of the experiment. Prior to surgery, Ss were randomly assigned to the following 4 groups, with 20 males and 20 females to each group:

- (1) Antero-dorsal hippocampal lesions (ADHL).
- (2) Entorhinal cortex lesions (ENTO).
- (3) Cortical control lesions (CORT).
- (4) Sham-operated control group (SHAM).

### Surgery

All surgery was performed under clean surgical technique, using ether anesthesia. Bilateral electrolytic lesions were placed with the aid of a Krieg-Johnson stereotaxic instrument, using a current of 3 ma. for a duration of 30 sec. The coordinates used were as follows:

ADHL - 3.5 mm. posterior to bregma, 2 mm. lateral to midline,  
4 mm. ventral.

ENTO - 5 mm. posterior to bregma, 5 mm. lateral to midline,  
6 mm. ventral.

CORT - 3.5 mm. posterior to bregma, 2 mm. lateral to midline,  
2 mm. ventral.

SHAM - 3.5 mm. posterior to bregma, 2 mm. lateral to midline,  
with holes drilled in the skull and the dura pierced.

The openings in the skull were packed with Gelfoam. Animals were allowed 10 days for post-operative recovery before being placed into the experimental pens.

### Apparatus

Following recovery, Ss were placed into 4 large group pens by an assistant. In this way the observer had no prior knowledge of the location of each group. The pens were designed as illustrated in Fig. 1.

Figure 1

## Schematic diagram of group pens

The food bins, tops, and floors of the cages were made of hardware cloth. Three plywood shelter boxes, measuring 1' x 1' x 6" were placed in 3 of the corners of each pen, with hinged tops to permit periodic observation of litters. A hinged top on each group pen allowed access for the filling of food bins and examination of the shelter boxes. Water was supplied automatically by a modified Furnasman humidifier apparatus which maintained a constant water level by means of its float assembly. The drinking trough allowed 2 rats to drink at one time. The food bins extended from the floor to the top of the cage to prevent the Ss from entering the food bin.

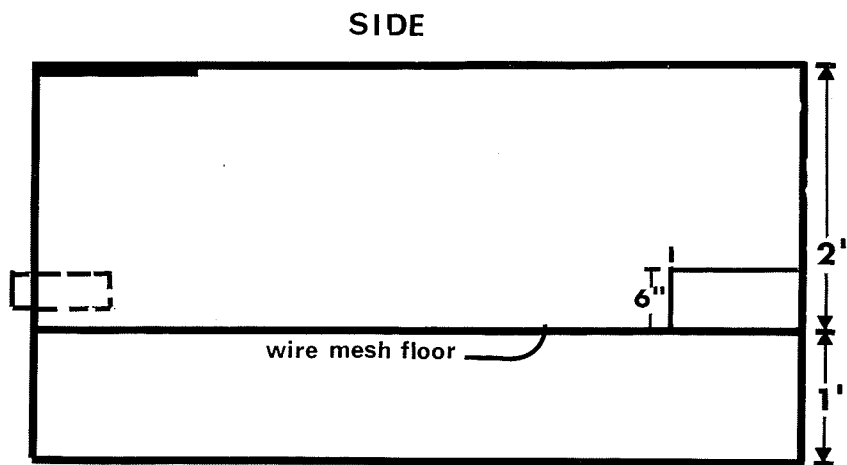
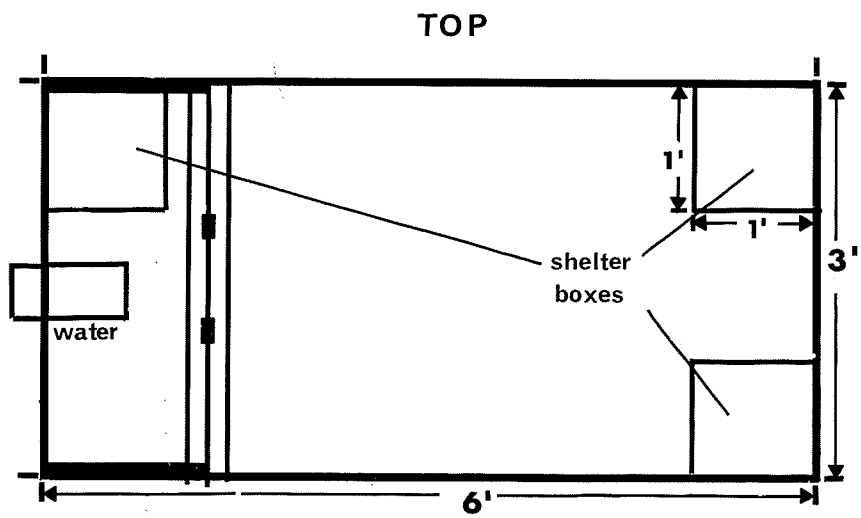


Fig. 1

### Procedure

All 4 cages were in the same air-conditioned room with the temperature maintained between 72° - 76° F. A reversed 12-hour light (8pm-8am) - 12-hour dark (8am-8pm) cycle was used throughout the observation period. Observation took place from mid-July to mid-October. To allow observation during the dark cycle, a 10-watt dark-room type red lamp was suspended 6 feet above the centre of each cage. During the light cycle the red lamps remained on, and the light source was a ceiling fluorescent light. Light metre readings (using a Yashica photographic light metre) were taken at the floor level of each cage to insure equal lighting over each cage in both dark and light cycles.

The Ss had free access to food and water throughout the entire 80-day observation period.

### Behavioural observations

The behavioural observations that were recorded were selected and defined as follows:

Ritual Fighting. Two or more animals rearing on hind legs and assuming the "boxing" position, feinting, with very light or no physical contact; adoption of "threat" posture, usually flank to flank with arched back and sideways motion, often with the forepaw of one animal on the other's back.

Physical Fighting. Two or more rats engaged in active physical combat, involving such activities as biting, scratching, forceful pushing, squealing, and stepping on and forcefully holding down the other animal.

Gnawing. Animal engaged in chewing on any wooden or wire part of the pen other than food or water containers.

Male-Female Mounting. Involved one male rat mounted on the hind quarters of other female rat, with its front paws firmly grasping the flanks of the mounted animal.

Male-Male Mounting. Involved one male rat mounted on the hind quarters of other male rat, with its front paws firmly grasping the flanks of the mounted animal.

Grooming. Animal licking, scratching, washing itself, rubbing its head with forepaws.

Social Exploration. One animal licking, sniffing, grooming or lightly nibbling, pawing or nuzzling the other animal. Also frequently involved sniffing of the other animal's genitalia.

Crossing the Open Field. The number of animals making a continuous trip across the approximate centre of the pen floor, either walking or running, and beginning at any side of the cage and uninterrupted by exploratory behaviour.

Exploring. Number of animals making short, erratic trips, frequently stopping, nosing and sniffing into openings and parts of the cage, often standing on hind legs and sniffing the air.

Rats in the Open Field. Number of rats with their entire body outside of the shelter boxes.

Rats Mobile in the Open Field. Number of rats engaged in running or walking on cage floor or on shelter boxes.

Natality. Number of pups found alive in the shelter boxes every 2nd day.

Mortality. Number of adults and pups found dead every 2nd day.

Hermits. Number of rats remaining crouched in corners or on mesh wall throughout the daily testing session. The position of these animals was

noted 9 times over the 30 min. observation period. Only those animals in the same positions for all of the 9 scoring intervals were recorded as hermits.

#### Observation Procedure

Each cage was observed for a 30 minute period each day for a period of 80 days. The observations were recorded over the same time period (10 am - 1 pm) each day. The order of the cages observed each day was randomized to control for possible time-of-testing effects on behaviour. During the first week after the animals were placed in their pens, observations were made by 2 observers independently to check the precision of the defined behavioural categories. This time also allowed the Ss to adapt to their cages and the reversed dark-light cycle.

A checklist of all the behaviours to be observed was used (see App. D). The 30 minute observation period for each cage was marked off into 180 10-sec intervals, with a space for each interval on the checklist. Two methods of recording the behaviour were used:

(1) Those observations recorded every interval. This was done for ritual fighting, physical fighting, gnawing, male-female and male-male mounting, grooming, and social exploration. At the start of each interval if any of these behaviours were occurring, a check mark next to these behaviours, and in the appropriate interval space on the list, was made. At the end of the observation period, the number of check marks for each behavioural measure was totalled, and this total served as the daily score for each of these behaviours. Using this method, the maximum daily score was 153. The remaining 27 time intervals were used for recording the observations below.

(2) Those observations recorded at every 20th interval. This method was used for crossing the open field, exploring, rats in the open field, rats mobile in the open field, and hermits. At every 20th interval, a numerical count was made of the animals involved in each of the above behaviours. This count was thus made 9 times throughout every 30 min. observation period. As shown on the checklist in App. D, 27 of the 180 time intervals were used for recording these behaviours. At the end of the observation period the count was totalled for each of the behaviours recorded in this way, and this total served as the daily score for these behaviours.

A numerical count was also used for the natality and mortality measures. These counts were made every second day for each cage, following the daily testing session.

To mark off the 10-sec. intervals, a modified Gerbrandt tape programmer was used which made an audible click every 10 seconds. The observer would sit before the cage, holding the checklist, switch on the timing apparatus, and record the appropriate behavioural activity when the click was heard. Completion of the checklist indicated the end of the 30 min. observation period for each cage.

At day 50, each cage was observed for a 10 minute period (60 10-sec intervals) every 3 hours over a 24-hour period, to determine if there were obvious behavioural activity differences between groups.

#### Endocrine Studies

Upon completion of the 80-day observation period, all animals were sacrificed by decapitation. Ten males from each group were selected at random, and their blood corticosterone level, adrenal weights, and testes weights were determined.



## Histology

Brains were fixed in 10% formalin and frozen sections were cut at 30 $\mu$ . Every 6th section was saved and stained with cresyl violet. Stained sections were projected on to cross-sectional brain diagrams (De Groot, 1959) and the extent of tissue damage was traced on the diagrams. Composite cross-sectional diagrams at levels A 3.4 and A 3.0 were made for the ADHL and ENTO groups. Dorsal views of representative CORT and ENTO lesions were constructed by projecting every 2nd section throughout the lesion area on to millimeter paper, and locating the extent of the lesion (lateral to midline) and the maximum lateral extent of each section. The proportional measurements were then transferred to a dorsal-view brain diagram.

## RESULTS

### Anatomical Results

Antero-dorsal hippocampus lesions. (ADHL) Fig. 2 shows a cross sectional diagram of a composite lesion based upon 28 brains of the ADHL group, at levels A 3.4 and A 3.0 in DeGroot's atlas (1959). The blackened area represents tissue destruction common to the majority (19) of the brains, while the stippled area indicates the area and maximal extent of damage in those brains with the largest lesions. The damage represented by the outer boundary of the stippled area occurred in 2 brains, while the other 7 brains sustained damage between this boundary and that of the blackened area.

In all animals the hippocampal lesions damaged the dorso-medial section of the hippocampus, the overlying cortex and corpus callosum, and the fascia dentata. The damage extended approximately 3 mm. on each

side of the midline, and in the anterior-posterior dimension the lesions extended from approximately 3 mm. posterior to bregma to 6-7 mm. posterior to bregma. The stria medullaris and the lateral and medial habenular nuclei were damaged or destroyed in all but 4 cases. In the posterior direction, slight damage in several cases extended to the area pretectalis, lateral thalamic nuclei pars posterior, and to the superior colliculus. In addition to this damage, the majority of the brains sustained slight damage to the antero-dorsal thalamic nuclei. In the 9 brains with the greater damage, the antero-ventral and lateral thalamic nuclei also were invaded. In the 3 cases with the most extensive thalamic damage, the paraventricular nuclei, nuclei parataenialis, nuclei parafascicularis, and part of the dorsomedial thalamic nuclei were damaged. One of these also involved some damage to nucleus ventralis pars dorsomedialis and nucleus posterior thalami (using the terminology of Konig & Klippel, 1964).

Cortical lesions. (CORT) Fig. 3 shows diagrammatic cross-sections at levels A 3.4 and A 3.0 (deGroot, 1959) plus a dorsal view of a representative cortical lesion. The damaged cortex was localized in Krieg's (1946) area 17 and area 18, the striate and associated visual cortex. In the anterior cases, area 7 was entered. The corpus callosum was damaged in all Ss. With the exception of 2 cases with very slight bilateral, and one case unilateral, damage to the most dorsal part of the anterior hippocampus, the hippocampus was not invaded. The cingulate cortex, cingulum, and splenium corpus callosi were bilaterally damaged in all but one case.

Entorhinal cortex lesions. (ENTO) Fig. 4 illustrates a diagrammatic cross-sectional view of a composite entorhinal cortex lesion, and a dorsal view of a representative ENTO lesion. Dorsally, the lesions were centred in the striate visual vortex (area 17) and occasionally extended into area 18a of Krieg (1946). Ventrally the majority (20) of these lesions were concentrated in the entorhinal cortex region and generally included the subiculum, tapetum and in several cases the gyrus dentatus. In only 3 cases was the posterior hippocampus proper substantially damaged. In 4 cases when the lesion extended in an antero-dorsal direction (2 of these unilaterally) damage was sustained to the splenium of the corpus callosum, dorsal hippocampal commissure and forceps major. In the 2 unilateral cases, the cingulum as well appeared damaged.

Sham control group. (SHAM) The SHAM group showed no sign of cortical damage, which could have occurred as a result of penetration of the drill into cortical tissue.

#### Data Analysis

Analysis of the behavioural scores was performed with the aid of a computer. A randomized blocks design was employed, with 4 main treatments and 80 replications, each day being considered as a replication. Grouping by days served to remove a generally large day-to-day error variance and thus enhance any main treatment effects that were present. Certain behavioural measures were not statistically analyzed. These measures, which are referred to below, yielded data which, due to problems of definition and scoring, could not be meaningfully analyzed statistically. Summaries of the analyses of variance are given in tables 1-10 of Appendix C, together with a post-hoc analysis of these

## Figure 2

## ADHL lesions

Composite cross-sectional diagram of ADHL brains at levels A 3.4 (upper section) and A 3.0 (lower section) of De Groot's atlas (1959). The blackened area represents tissue destruction common to the majority of the brains. The stippled area represents the area and maximal extent of damage in the brains with the largest lesions.

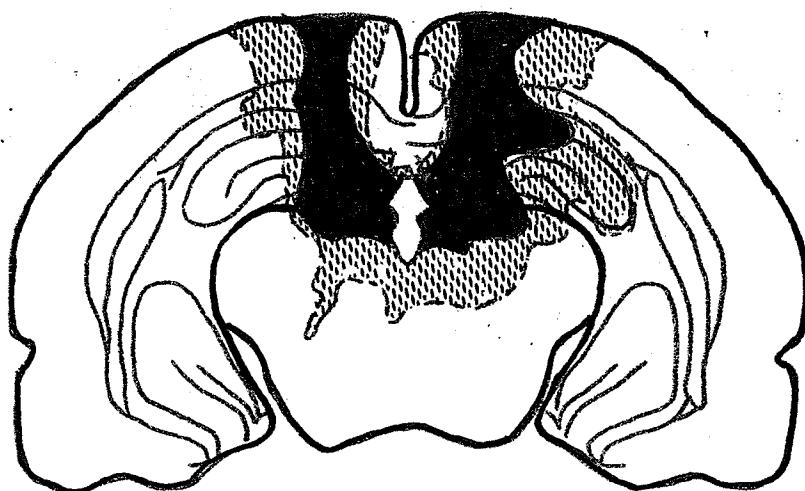
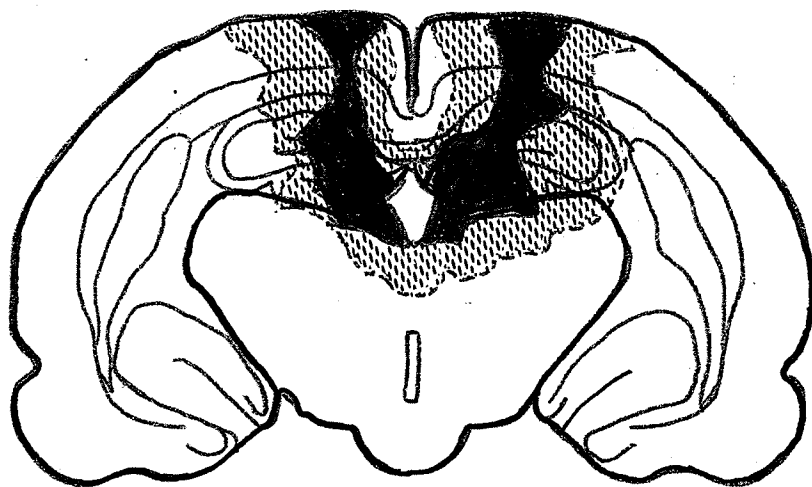


Fig. 2

## Figure 3

## Cortical lesions

Diagrammatic cross-section of a representative cortical lesion at levels A 3.4 (upper left) and A 3.0 (upper right) in De Groot's atlas (1959). The lower diagram illustrates the dorsal view of this lesion. Cortical destruction is indicated by the blackened areas.

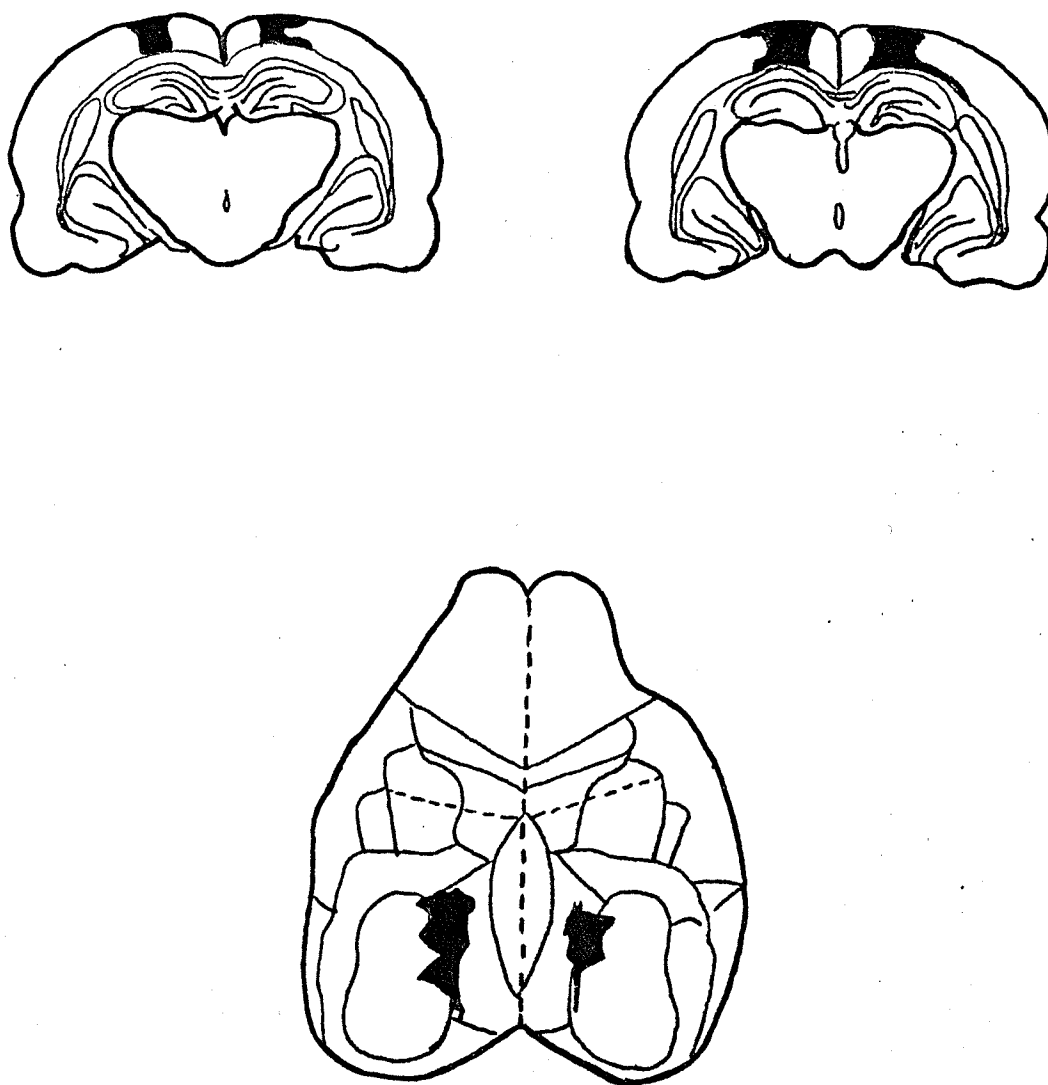


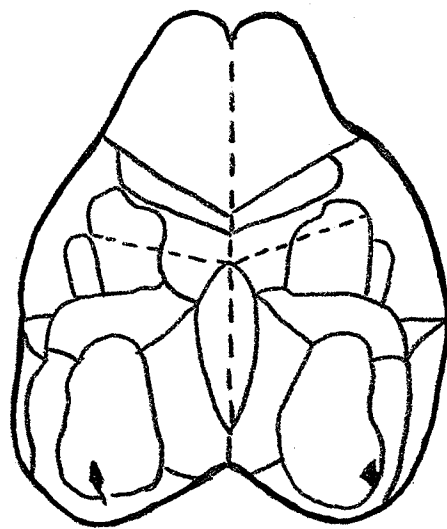
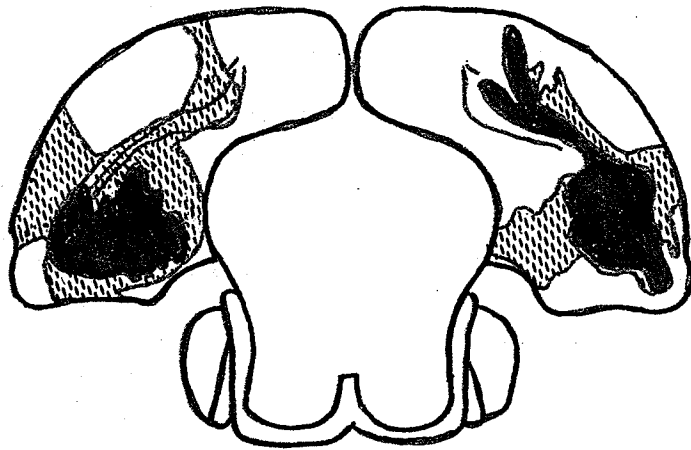
Fig. 3

## Figure 4

## Entorhinal cortex lesions

Upper section: composite cross-sectional diagram of entorhinal cortex lesion at level A 350  $\mu$  in Konig & Klippel's atlas (1964). The blackened area represents tissue destruction common to the majority of the brains. The stippled area represents the area and maximal extent of damage in the brains with the largest lesions. Lower section: dorsal view of a representative entorhinal cortex lesion. Cortical destruction is indicated by the blackened areas.





.Fig. 4

F-values that were found to be statistically significant. A linear regression analysis for each behavioural measure was also performed to determine possible differences between the groups in trends over the 80-day observation period. The results of this analysis are summarized in tables 1-10 of Appendix B. Both of these analyses were programmed to detect differences at both the  $p < .05$  and  $p < .01$  level. As a consequence only these levels of significance are referred to below. It will be obvious from the summary tables, however, that a great many of the differences reported well exceed these levels of significance. Therefore, unless otherwise specified, reference to significant differences will refer to the  $p < .01$  level of statistical significance.

Each behaviour analyzed statistically is also represented graphically. In each case, the mean daily score has been plotted against four 20-day blocks to indicate differences both within and between groups over the 80-day observation period. For purposes of reference, results of analyses of variance, and the levels and direction of statistical significance have been placed opposite their respective graphical description of each behaviour.

Since the daily scores for each behaviour were based upon the daily group scores, the slight variations in the numbers of each group, due to deaths post-operatively and during the course of the observation period, should be noted. Post-operative deaths necessitated the original differences in group size. At the start of the observation period, the group sizes were: ADHL - 17 males, 16 females (33); SHAM - 16 males, 12 females (28); ENTO - 15 males, 16 females (31); CORT - 17 males, 15 females (32). The deaths which occurred over the observation period

were relatively equally distributed between groups and throughout the observation period, the first death occurring day 3, and the last occurring day 63. The final group sizes were: ADHL - 16 males, 13 females (29); SHAM - 16 males, 8 females (24); ENTO - 15 males, 14 females (29); CORT - 16 males, 13 females (29). Thus, while the total number of deaths per cage did not differ noticeably between groups, the deaths were concentrated among the females in each group, and accounted for 11 of the 13 deaths that occurred over the observation period. In the statistical analysis, since the original and final group sizes were relatively large, and the deaths fairly equally distributed among the groups, it was estimated that on a purely mathematical basis these differences in group size would not greatly affect the probabilities of a given behaviour being scored as "occurring" or "not occurring". Since it was almost impossible in advance to accurately assess either the direction or the degree of effect that these slight differences in group size might have on any given behavioural measure, no mathematical corrections of the scores (e.g. log transformations) were attempted. However, since the greatest difference in group size was between the ADHL and SHAM groups (5) it was of considerable interest to examine whether the scores obtained by the ADHL group were not only significantly different from the SHAM animals but also from either the entorhinal or cortical control groups as well. The original numbers of these groups differed from the ADHL group by 2 and 1, respectively, and their final numbers were identical with the ADHL group. In this regard, it is relevant to note that on every behavioural measure where the ADHL group differed significantly from the SHAMs, it also differed significantly from either the ENTO or

CORT groups, and in most cases from both. This would suggest it highly unlikely that the differences observed were spurious, or due to differences in population sizes.

### Behavioural Results

Ritual Fighting. The ADHL group showed significantly higher ritual fighting scores than the SHAM and ENTO groups, which did not differ over the 80-day period (see Fig. 5). The CORT animals displayed significantly higher scores than both the SHAM and ENTO groups. There was a significant decrease in ritual fighting over time in all groups except the SHAMs, which after an initial decrease in the first 20 days continued to increase over the rest of the observation period.

Physical Fighting. The physical fighting scores (see Fig. 6) appeared to closely parallel those of ritual fighting. As in ritual fighting, the ADHL group scored significantly higher than SHAMs, although it did not differ from the ENTO and CORT groups. These latter 2 groups were statistically similar, although both showed significantly more physical fighting than the SHAM group. The level of physical fighting was higher than for ritual fighting in all groups except the SHAMs whose initial scores on both "fighting" measures were similar. The trends for physical fighting for all groups generally paralleled those for ritual fighting, showing significantly decreasing fighting in all groups except the SHAMs which sustained its initial level of fighting.

A number of unquantified general observations were noted regarding fighting behaviour. As already noted, the number of fights generally decreased over time. The intensity and duration of these fights also appeared to decrease over the observation period. Fighting between male

Figure 5  
Ritual Fighting

Mean daily score on ritual fighting for each lesion group over an 80 day period. Days are presented in 4 successive blocks of 20 days each. Maximum possible daily score was 153.

$F = 10.93^{**}$   $df = 3/237$

ADHL > SHAM<sup>\*\*</sup>; ADHL > ENTO<sup>\*\*</sup>;

CORT > SHAM<sup>\*\*</sup>; CORT > ENTO<sup>\*\*</sup>.

Note: <sup>\*\*</sup>  $p < .01$

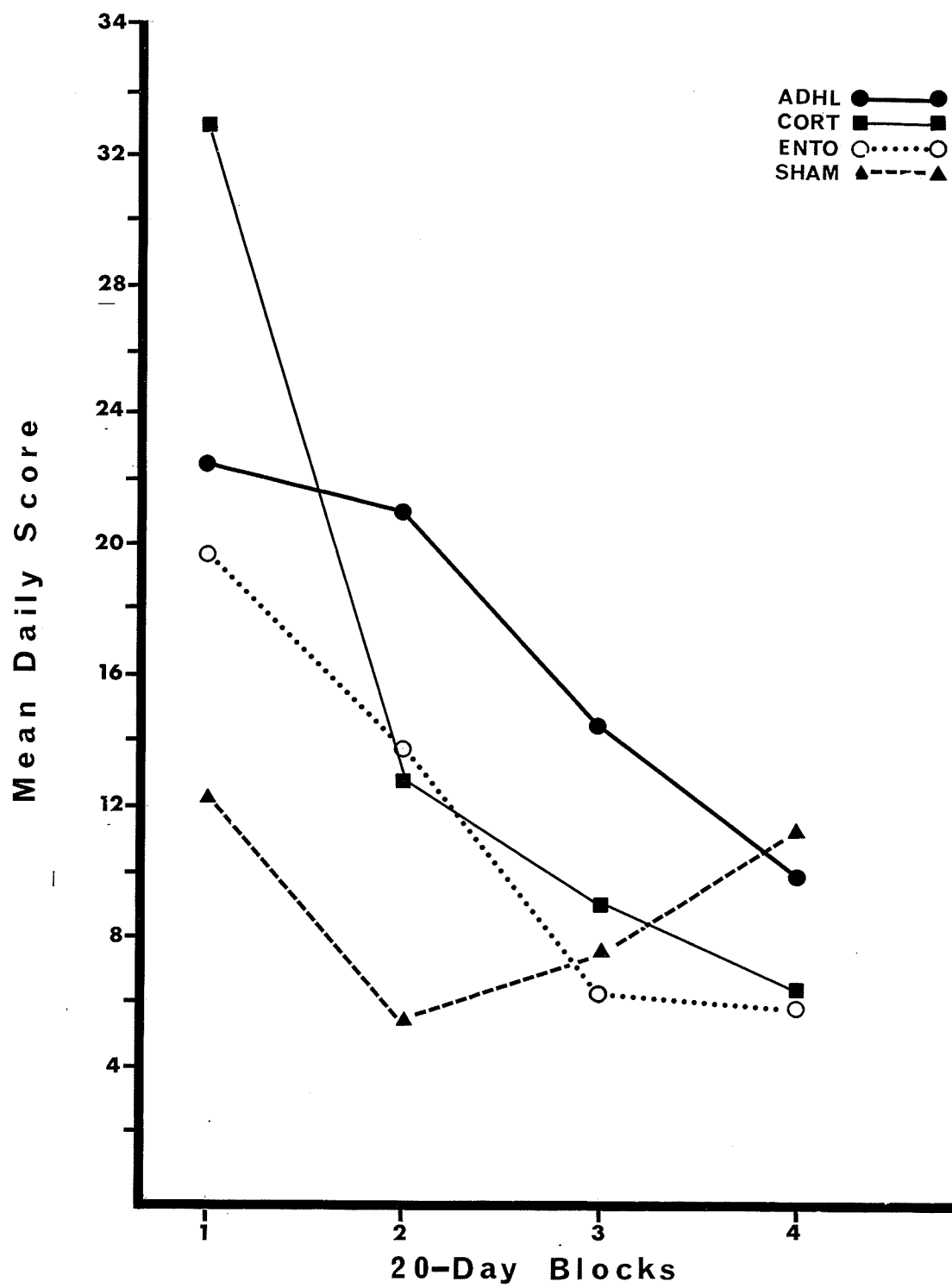


Fig. 5

Figure 6  
Physical Fighting

Mean daily score on physical fighting for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Maximum possible daily score was 153.

$F = 17.20^{**}$   $df = 3/237$

ADHL > SHAM\*\*

ENTO > SHAM\*\*

CORT > SHAM\*\*

Note: \*\*  $p < .01$

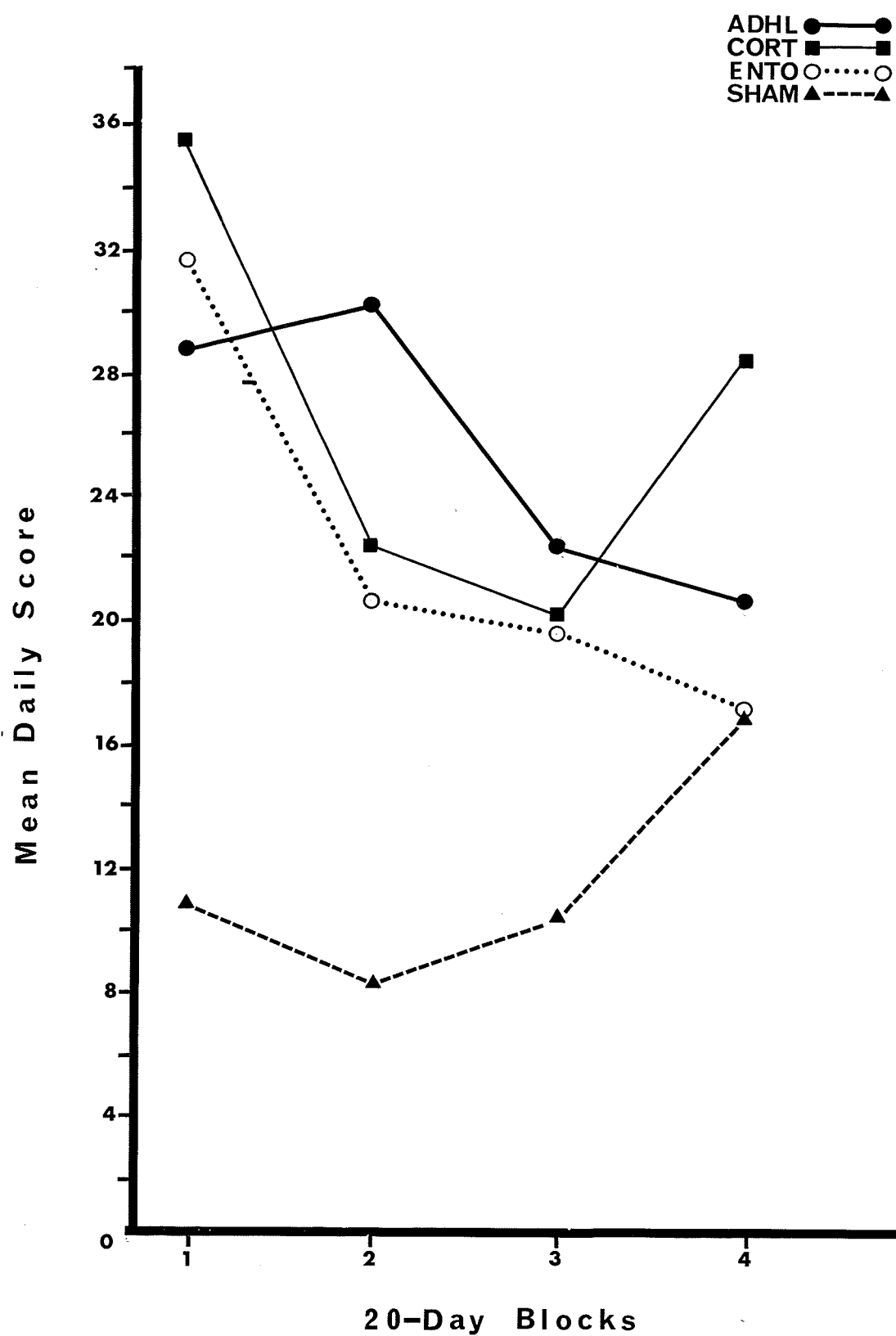


Fig. 6



and female occurred a number of times during bouts of high sexual activity. This generally occurred when a number of males attempted simultaneously to mount a resisting female, who would then lie on her back, often kicking, while being forcibly held down by a male and often violently "nosed" by other surrounding males. This type of behaviour was scored, according to the pre-defined criteria, as physical fighting. Although in these cases, it is clear that fighting was related in occurrence to sexual activity, it should be noted that while fighting generally declined over time, sexual activity, specifically male-female mounting, tended to increase for all groups over time. This would suggest that factors other than sexual activity cycles were involved in producing the fighting behaviour. Indeed, fighting was seen to occur most often between the dominant male and other males in cases where a dominant male was clearly distinguishable. A phenomenon, also reported by Barnett (1964) was seen to occur where the excitement during a fight "irradiated", often producing a chain reaction of fighting between the original antagonist and several other males. In several instances, the excitement appeared to spark the initiation of several new fights. This behaviour was observed primarily in the CORT and ENTO groups, and to some extent in the SHAM group, but rarely if at all in the ADHL animals. Fighting also ensued in a number of instances when a male entered a shelter box other than his own, or attempted to mount a female in the presence of a dominant male. Again, this was apparent only when there was evidence of a dominant male.

In the present study, there was no indication of dominance-subordination hierarchies in the ADHL group but there was an obviously dominant

animal in the SHAM group. Typically, after the first few weeks, when the dominant SHAM animal was present very few males either left the shelter boxes or approached the dominant male or the females in the open field. If the latter did happen, violent fighting almost inevitably ensued. The dominant animal would often follow its opponent into a shelter box or remain just outside and attack any animal that dared to venture out. One of the results of this was that relatively little activity occurred when this dominant male was present. It is significant to note that on one day when the dominant male was not present throughout the entire testing session, the greatest number of animals in the open field was recorded, as well as considerably higher open field and sexual activity than usual. On only one other occasion when the dominant male was present was there any comparable sexual activity. Interestingly, this male was observed on only one occasion to engage in mounting behaviour.

No similar, strongly established dominance order was seen in any of the other groups, although the ENTO group contained a male that was considerably larger than the others and often initiated spells of violent fighting with any and all nearby males. In the ADHL group, however, a rat that at one moment had overpowered one opponent was seen submissive and subordinate in another encounter. With time, a noticeable decrease in intensity and duration of fighting was most apparent in the ADHL group, with few, if any, outbreaks of fighting as severe or prolonged as those seen in the other groups.

Male - Female Mounting. One of the most marked effects observed was in sexual activity, both heterosexual and homosexual. In male -

female mounting (see Fig. 7) the ADHL group was found to greatly exceed all other groups throughout the entire observation period. Once again, the ENTO and CORT groups did not statistically differ from each other. The SHAM group displayed significantly less male - female mounting than all other groups. Over the 80-day period, all groups tended to increase in male - female mounting, although only the SHAM and ENTO groups had significantly increasing trends.

Male - Male Mounting. Differences in sexual behaviour were even more marked for male - male mounting (see Fig. 8). As with male - female mounting, the ADHL group showed a very significantly greater occurrence of male - male mounting than all other groups, consistently over the observation period. In this behaviour, however, there were no significant differences between ENTO, CORT, and SHAM groups. All of these latter groups displayed low levels of male - male mounting initially, with the ENTO and CORT animals decreasing significantly throughout the observation period to almost negligible levels. This was in sharp contrast to the initial high level of male - male mounting in the ADHL group which maintained this level throughout.

It is perhaps of some interest to note the sexual activity patterns that occurred in various groups at different times. In both male - female and male - male mounting, the typical mounting pattern appeared similar for all groups. Mounting was often observed to follow an attack or chase, with the pursued rat often making a rapid, usually incomplete attempt to mount a nearby animal, male or female. Tinbergen (1951) and Barnett (1961) refer to such behaviour as typical of "displacement" activity which may serve to prevent aggressive contact. Another observa-

## Figure 7

## Male - Female Mounting

Mean daily score on male-female mounting for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Maximum possible daily score was 153.

$F = 54.38^{**}$   $df = 3/237$

ADHL > SHAM<sup>\*\*</sup>; ADHL > ENTO<sup>\*\*</sup>; ADHL > CORT<sup>\*\*</sup>

ENTO > SHAM<sup>\*\*</sup>

CORT > SHAM<sup>\*\*</sup>

Note: <sup>\*\*</sup>  $p < .01$

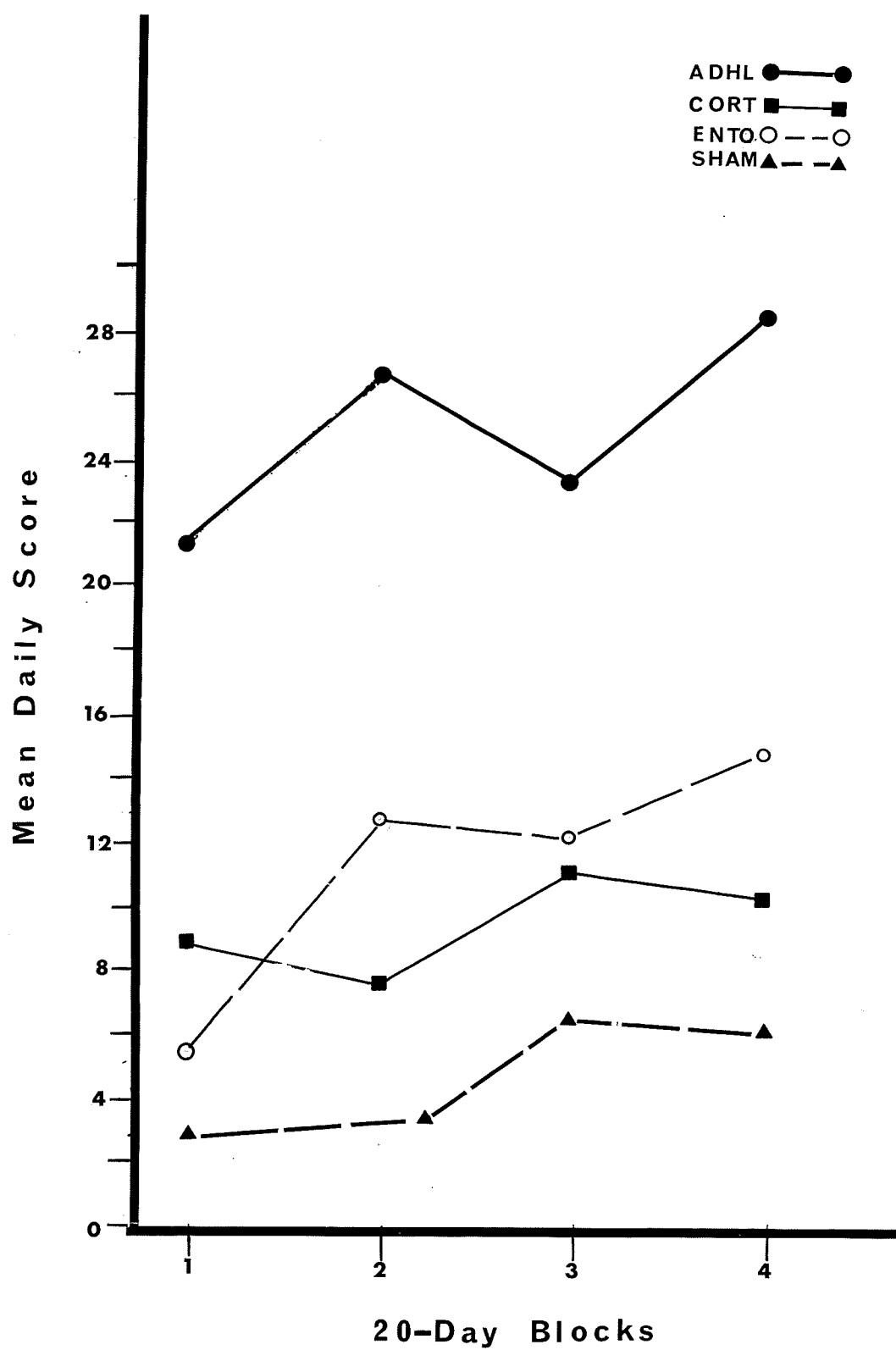


Fig. 7

## Figure 8

## Male - Male Mounting

Mean daily score on male - male mounting for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Maximum possible daily score was 153.

$F = 153.60^{**}$   $df = 3/237$

ADHL > SHAM<sup>\*\*</sup>; ADHL > ENTO<sup>\*\*</sup>; ADHL > CORT<sup>\*\*</sup>

Note: <sup>\*\*</sup>  $p < .01$

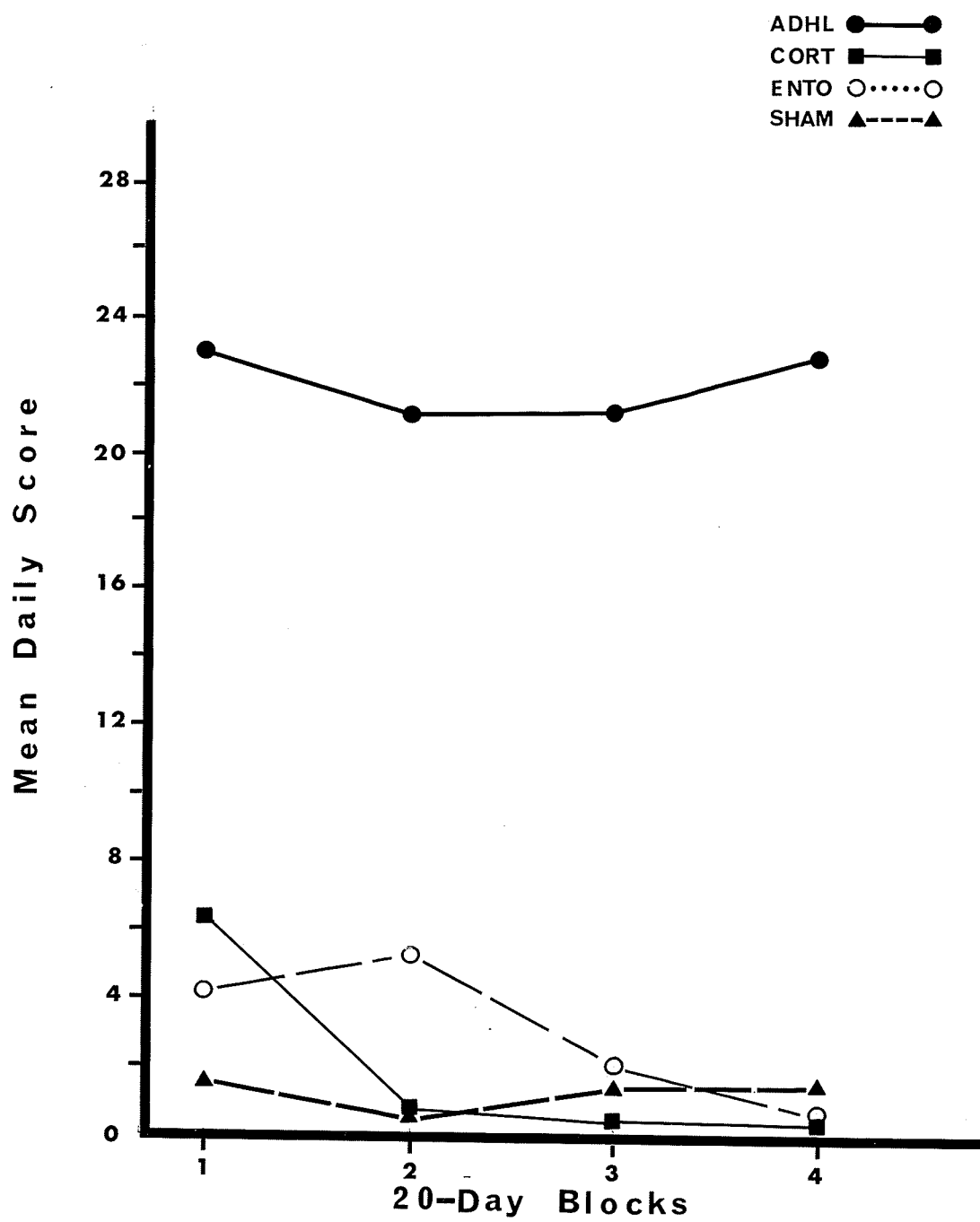


Fig. 8

tion, perhaps best described as "mass-mounting", involved from 2 to as many as 10 males doggedly pursuing a single female which often resisted attempts to mount her. This female would often be persistently pursued, even into the shelter box, while the other males remained outside and took up pursuit when the female emerged once again. When such a female attempted to resist, often assuming a "submissive" posture, i.e. on her back, she was often forcibly "nosed" and sniffed by other males, and was sometimes kept in a corner unable to escape. This type of behaviour occurred in all cages to some degree, but was most prevalent in the CORT group, where after several weeks, very little sexual activity other than this type appeared to occur. Other sexual "abnormalities" observed were "tandem mounting", also reported by Schreiner and Kling (1953) in cats, in which as many as 4 rats would mount one another simultaneously. This behaviour occurred relatively few times, and was observed in the first few weeks in the ADHL group only. Inverted mounting, pictorially depicted by Barnett (1961) also occurred, although infrequently, only in the SHAM group. When this did occur, it almost always involved 2 males, the one mounted generally showing little response.

Gnawing. As seen in Fig. 9, all groups displayed considerable gnawing. Interestingly, this behavioural pattern contrasts sharply with almost all other measures. It is the only behaviour in which the ADHL and SHAM groups do not differ significantly from one another. Both of these groups are significantly lower on gnawing than the ENTO and CORT groups, which once more do not differ statistically from one another. No significant trends over time were observed in any of the groups, with each group maintaining its initial level of responding.



## Figure 9

## Gnawing

Mean daily score on gnawing for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Maximum possible daily score was 153.

$F = 22.89^{**}$   $df = 3/237$

ENTO > ADHL<sup>\*\*</sup>; ENTO > SHAM<sup>\*\*</sup>

CORT > ADHL<sup>\*\*</sup>; CORT > SHAM<sup>\*\*</sup>

Note: <sup>\*\*</sup>  $p < .01$

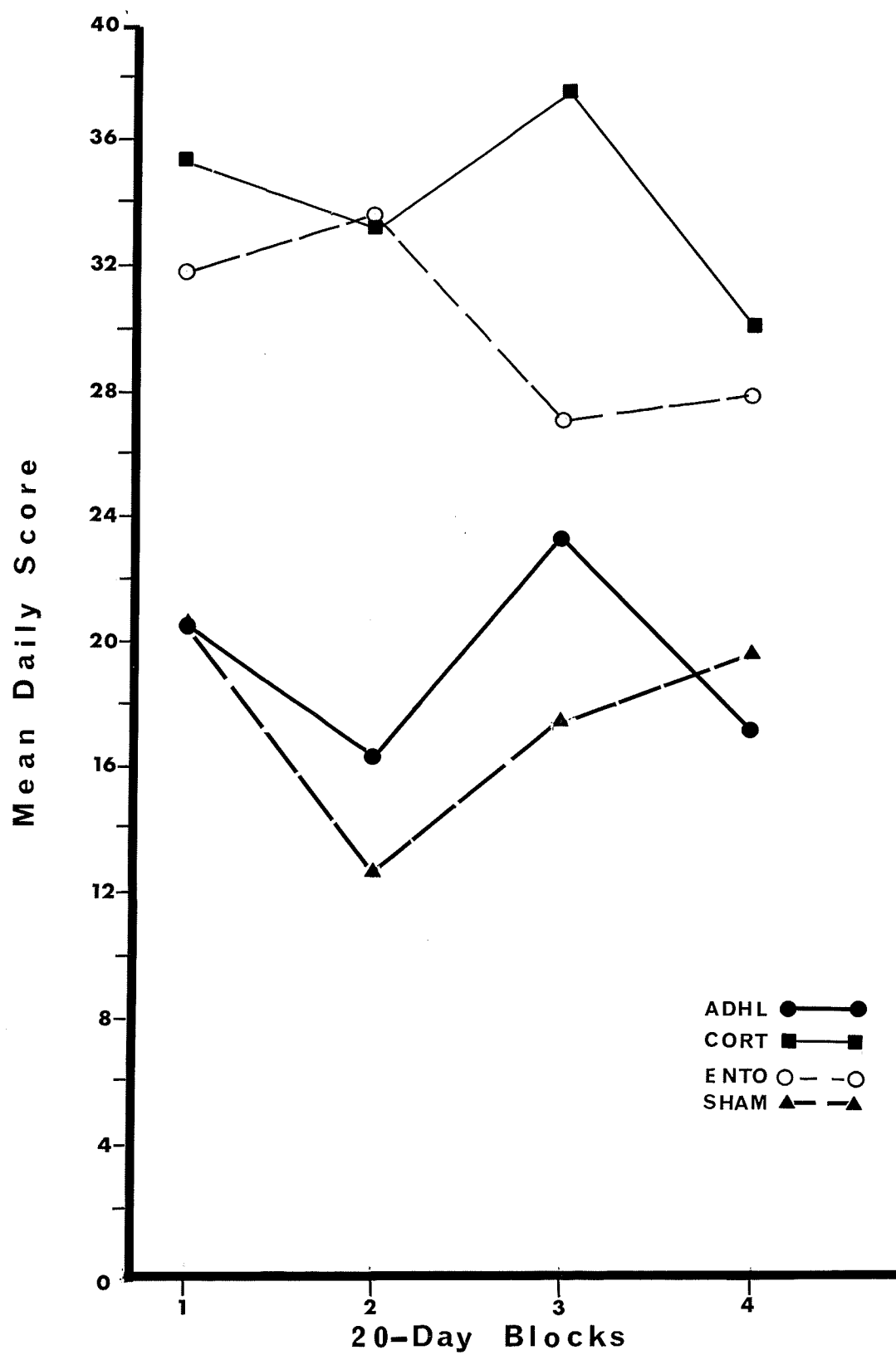


Fig. 9

Grooming. Fig. 10 represents the grooming scores. Both ADHL and ENTO groups indicate similar levels of grooming over all 4 blocks of days. Statistically, these groups did not differ from one another although both showed significantly higher grooming scores than the CORT and SHAM animals. CORT animals also groomed significantly more than SHAMs. All groups displayed strong, significant increases in grooming over time.

Social Exploration. Social exploration is presented in Fig. 11. The ADHL group showed significantly higher scores, and the SHAM group significantly lower scores, than all other groups. The ENTO and CORT scores were found to differ from one another at the  $p < .05$  level of significance. There was no indication of significant trends for any of the groups, with the regression coefficients of the ADHL, SHAM and CORT groups indicating statistically parallel slopes for these groups. One aspect of the "social exploration" category, sniffing of genitalia, often occurred during "mass-mounting" behaviour. Examination of the curves and trends for both social exploration and male - female mounting does not, however, indicate any apparent parallel between the scores for these 2 behaviours. This is possibly because sniffing of genitalia comprises only one of several aspects of social exploration, or possibly because these two behavioural factors occur relatively independent of one another.

Rats in the Open Field. One of the earliest and most marked differences in behaviour observed was the extremely large degree of variation between groups in the number of animals that were observed outside of the shelter boxes, and engaging in other activity. From the

## Figure 10

## Grooming

Mean daily score on grooming for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Maximum possible daily score was 153.

$F = 82.47^{**}$   $df = 3/237$

ADHL > SHAM<sup>\*\*</sup>; ADHL > CORT<sup>\*\*</sup>;

ENTO > SHAM<sup>\*\*</sup>; ENTO > CORT<sup>\*\*</sup>;

CORT > SHAM<sup>\*\*</sup>

Note: <sup>\*\*</sup>  $p < .01$

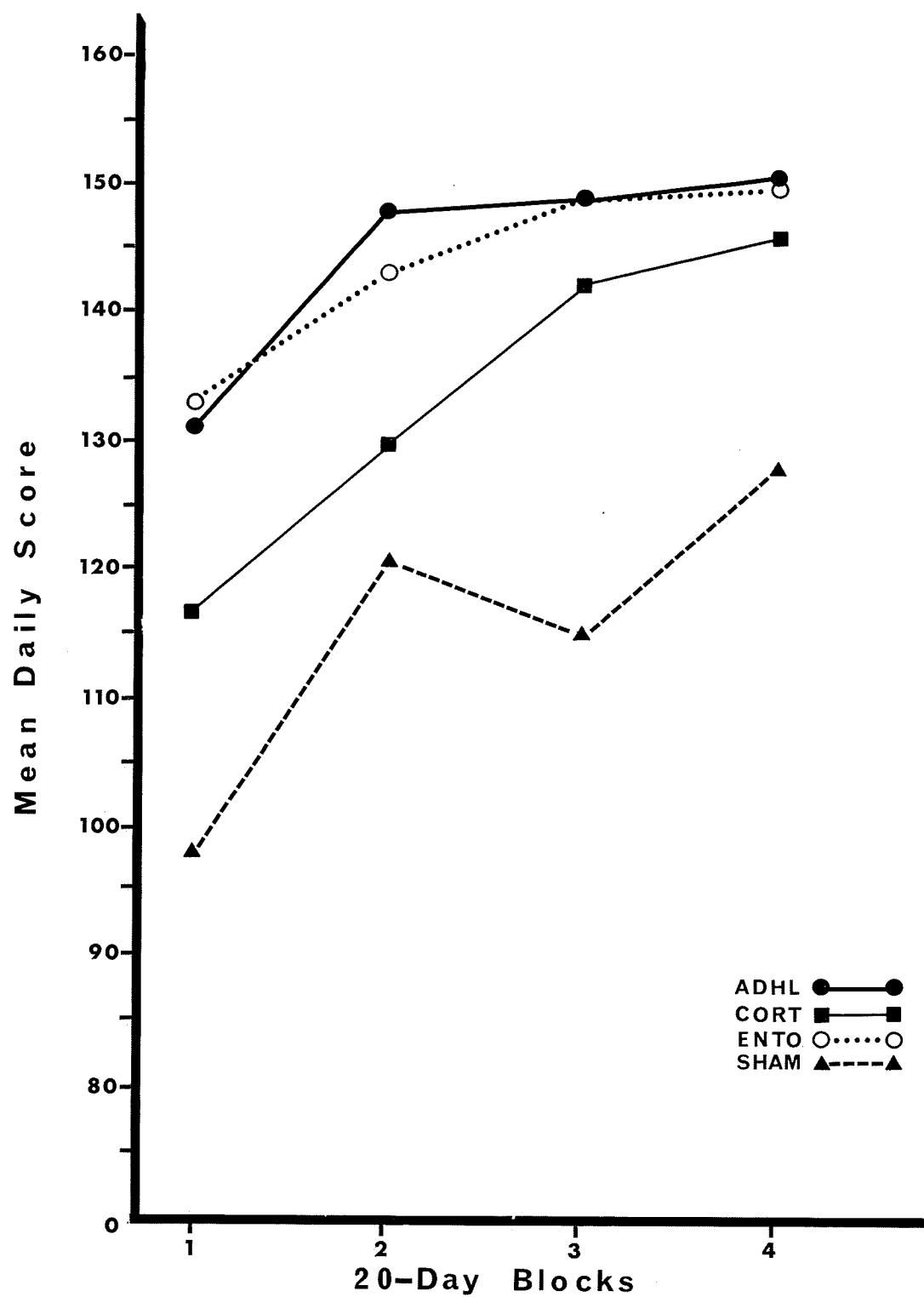


Fig. 10

Figure 11  
Social Exploration

Mean daily score on social exploration for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Maximum possible daily score was 153.

$F = 122.83^{**}$   $df = 3/237$

ADHL > SHAM<sup>\*\*</sup>; ADHL > ENTO<sup>\*\*</sup>; ADHL > CORT<sup>\*\*</sup>;

ENTO > SHAM<sup>\*\*</sup>; ENTO > CORT<sup>\*</sup>

CORT > SHAM<sup>\*\*</sup>

Note: \*  $p < .05$

\*\*  $p < .01$

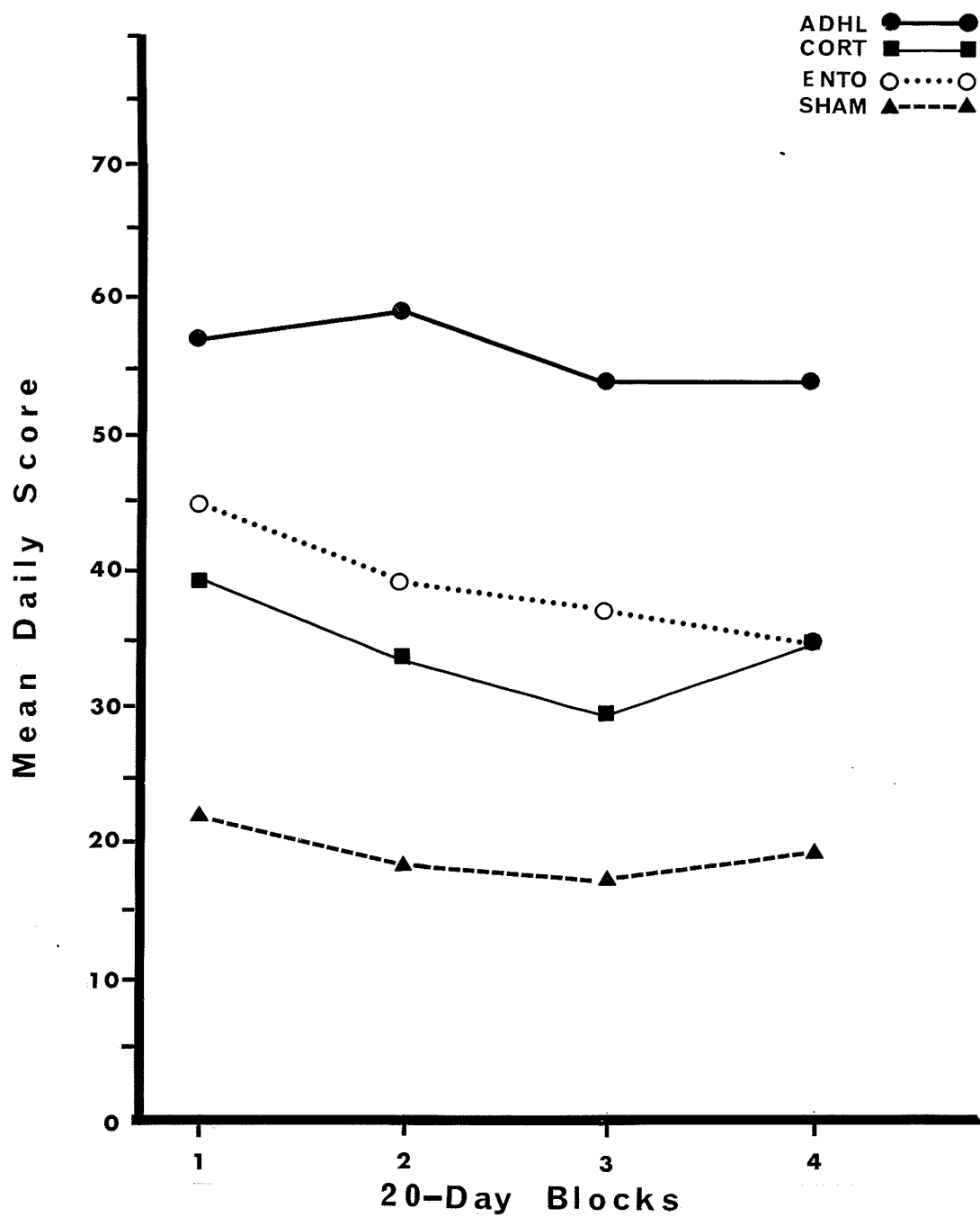


Fig. 11

first week, differences in overall general activity and in numbers of animals engaged in various activities were clearly in evidence. As can be seen by Fig. 12, these differences in the numbers of animals remaining outside of the shelter boxes were maintained over the entire observation period. The ADHL scores were very significantly greater than the scores of all other groups. In what appeared to be a common finding, the ENTO and CORT scores did not differ significantly, but both were considerably and significantly greater than the SHAM scores. In addition, both of these latter groups displayed significantly decreasing scores over time, while the ADHL and SHAM groups did not vary a great deal in their scores over the 80-day period.

Exploring. The ADHL group exhibited significantly more exploratory behaviour than did all other groups (see Fig. 13). Once more, the ENTO and CORT groups did not differ, and both were significantly higher than the SHAM scores. All groups showed significant negative trends over time, indicating a generally decreasing amount of exploratory behaviour over the observation period for all groups.

Rats Mobile in the Open Field. Fig. 14 represents mobility in the open field. The pattern of mobility was similar to that of a number of other behavioural measures in that the ADHL group was significantly more mobile than the ENTO, CORT, and SHAMs. The CORT and ENTO groups did not differ statistically from each other and both groups showed significantly higher open-field mobility than the SHAMs. Only the CORTs showed a significant decrease over time, while the other groups did not display any clear trends.

Crossing the Open Field. This measure was not statistically



## Figure 12

## Rats in Open Field

Mean daily score on the number of rats in the open field for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Values represent the number of rats in the open x 9 (no. of times recorded per session).

$F = 389.29^{**}$   $df = 3/237$

ADHL > SHAM<sup>\*\*</sup>; ADHL > ENTO<sup>\*\*</sup>; ADHL > CORT<sup>\*\*</sup>

ENTO > SHAM<sup>\*\*</sup>

CORT > SHAM<sup>\*\*</sup>

Note: <sup>\*\*</sup>  $p < .01$

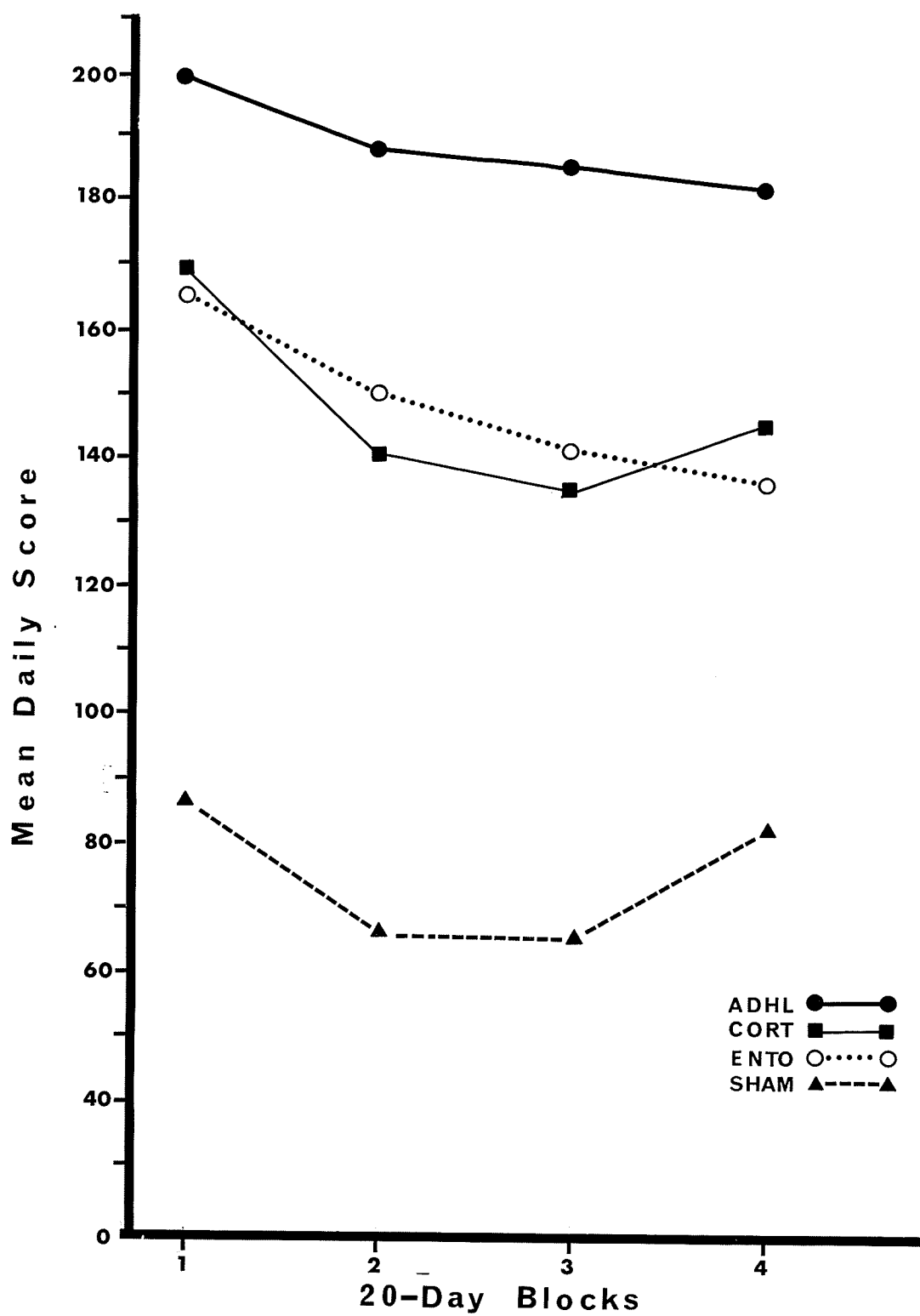


Fig. 12

## Figure 13

## Exploring

Mean daily score on the number of rats exploring for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Values represent the no. of rats exploring x 9 (no. of times recorded per session).

$F = 51.82^{**}$   $df = 3/237$

ADHL > SHAM<sup>\*\*</sup>; ADHL > ENTO<sup>\*\*</sup>; ADHL > CORT<sup>\*\*</sup>

ENTO > SHAM<sup>\*\*</sup>

CORT > SHAM<sup>\*\*</sup>

Note: <sup>\*\*</sup>  $p < .01$

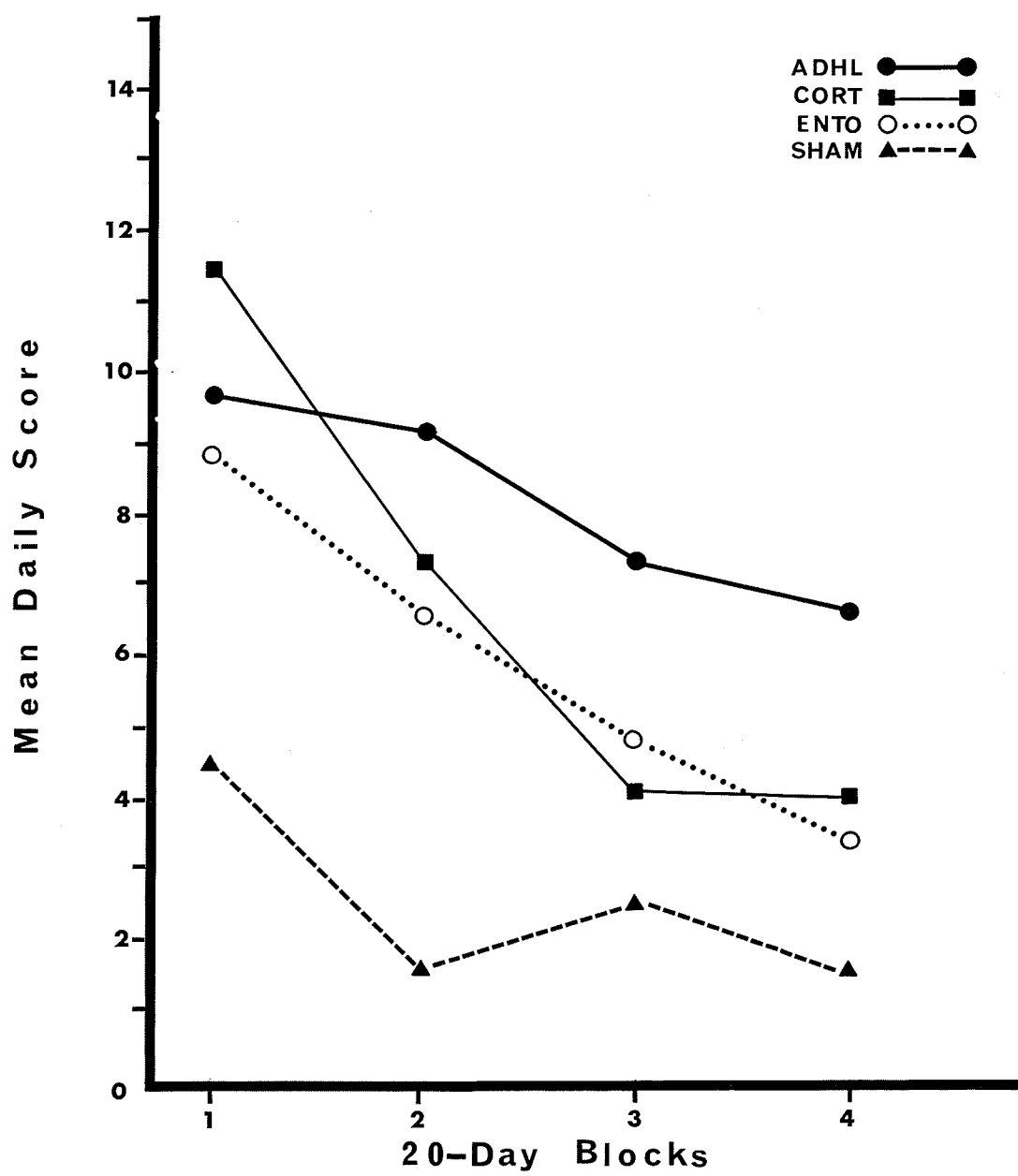


Fig. 13

Figure 14

## Rats Mobile in Open Field

Mean daily score on the number of rats mobile in the open field for each lesion group over an 80-day period. Days are presented in 4 successive blocks of 20 days each. Values represent the no. of rats mobile x 9 (no. of times recorded per session).

$F = 30.76^{**}$   $df = 3/237$

ADHL > SHAM<sup>\*\*</sup>; ADHL > ENTO<sup>\*\*</sup>; ADHL > CORT<sup>\*\*</sup>

ENTO > SHAM<sup>\*\*</sup>

CORT > SHAM<sup>\*\*</sup>

Note: <sup>\*\*</sup>  $p < .01$

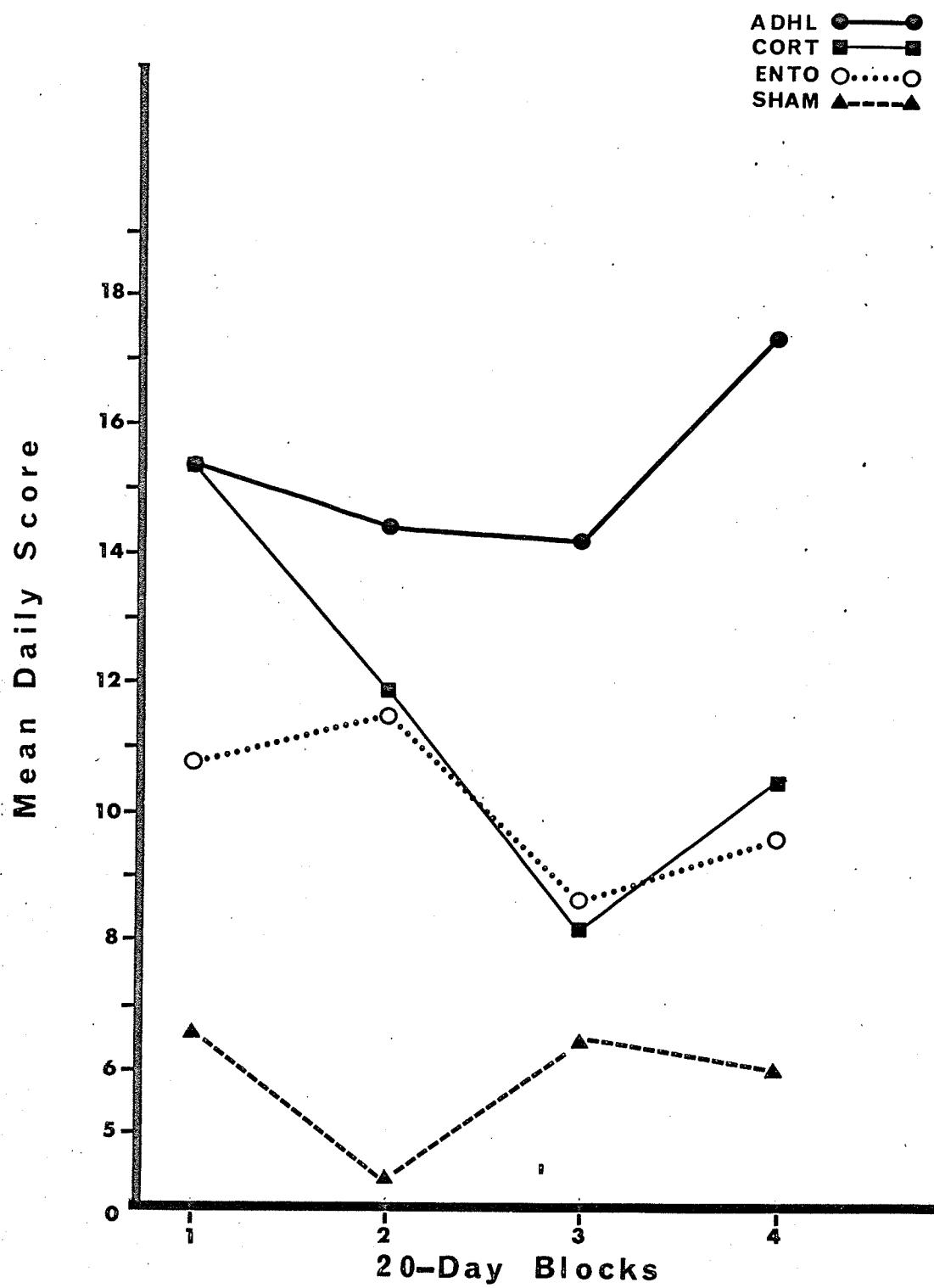


Fig. 14

analyzed due to the very low scores obtained for all groups and the obvious overlap of the scores. It appeared that this measure, as defined and scored, was not a suitably sensitive measure.

Mortality and Natality. Due to the high degree of crowding, the relatively large openings in the wire mesh floor, plus the eating of young pups by adults, it was not possible in the present study to keep an accurate and meaningful record of the number of litters and/or pups that were born. None of the pups in any cage, however, survived more than a single day, often being trampled, inadequately nursed, and falling through the floor. The adult deaths, as earlier noted, were highly concentrated among the females, accounting for 11 of 13 deaths. This finding is consistent with the high rate of maternal deaths found by Calhoun (1962) and appears highly related to intrauterine deaths during pregnancy and difficult delivery as a result of the crowded conditions.

Hermits. The data obtained from this measure were also not amenable to statistical analysis, and difficulties in scoring prevented any meaningful interpretation of the scores.

24-Hour Observations. Observation of the groups over a 24-hour period to determine whether any obvious overall behavioural activity differences were present during the light cycle revealed very little activity in any of the light-cycle observation periods. All activity declined rapidly in the hour preceding the onset of the light, and showed a similar upsurge before the beginning of the dark cycle. During the light-cycle, there was generally not more than 2 or 3 rats outside of the shelter boxes at one time in any pen, and the almost negligible activity of any kind precluded any meaningful analysis of this data.

Other Observations. During the course of the observations, it became apparent that the groups were responding noticeably differentially to sudden noises during feeding and cleaning, to the view of the observer in the light, and to puffs of air blown through the top of the pen to observe reactivity. Although no quantitative measures were recorded, it appears of value to note these differences in response since they were obvious and general enough to draw the attention of the observer to them, and were also observed by several others in the laboratory. It was observed that the ADHL group showed the least responsiveness to these stimuli. These animals appeared undisturbed by the presence of the experimenter poised over the top of the pen, and when a puff of air was directed at those rats which had climbed up the sides of the food bin, these animals would often blink and flinch but not attempt to flee the source of apparent irritation. The most responsiveness to such stimuli was seen in the SHAM group whose animals would immediately flee into shelter boxes or drop to the floor of the pen and run to the other side. Although ENTO and CORT groups were clearly more responsive than the ADHL animals, they did not appear as reactive as the SHAMs. Once such differences were noted, these informal tests of reactivity were performed at various times throughout the 80-day period, and these differences appeared to hold up. In this regard, it is interesting to note that at the conclusion of the study, the ADHL animals offered considerably less resistance to being retrieved from the pen.



## DISCUSSION

### Behavioural Results

Analysis of the behavioural responses to high population density following antero-dorsal hippocampus, entorhinal cortex, and neocortex lesions suggests that significant changes have occurred in the areas of sexual, social and emotional behaviour.

Sexual Behaviour. Possibly the most dramatic lesion effect observed was the state of hypersexuality that was produced in the ADHL group. This group evidenced a constant elevation in both heterosexual and homosexual mounting behaviour which significantly exceeded that of all other groups. While the role of the hippocampus in sexual behaviour is not yet clearly determined, recent research has suggested that the hippocampus is in some way involved in the regulation of sexual activity. Kim (1960) reported that male rats with ablation of the dorsal hippocampus and overlying neocortex displayed more frequent sexual mounting than that observed pre-operatively. The present findings of marked hypersexuality in the ADHL group appears similar in several respects to the excessive sexual activity reported by Kluver and Bucy (1937) in monkeys which had undergone temporal lobotomy, and the findings of Schreiner and Kling (1953) of hypersexuality produced in male and female cats as a result of amygdalectomy. The findings of these latter authors suggests the possibility that the hypersexuality found in the present study in the ADHL group may have been in part effected by damage to amygdaloid pathways and interconnections, which involve the circuitous stria terminalis and a second closely overlapping major efferent sub-cortical pathway from the amygdala. Also, there is electrophysiological

evidence (Gloor, 1960) of indirect connections between the hippocampus and amygdala, which would be severely interrupted by hippocampal damage. A further possibility which cannot be discounted is the lesion damage to the stria medullaris and habenular nuclei in the ADHL group. Such damage was also present in the study of Rasmussen, Kaada, and Bruland (1960) which reported a large and persistent increase in heterosexual drive in rats (as measured by number of crossings of an electrified grid to a sex incentive) as a result of bilateral removal of the dorso-medial part of the diencephalon. Since no data on the behaviour of individual rats could be kept in the present study, it was unfortunately not possible to correlate the degree of brain damage in each animal with measurements of its behaviour.

The present findings, in which the SHAM group displayed the lowest sexual activity of all groups and the ADHL group demonstrated the highest sexual activity of all groups, suggests that if sexual hypoactivity is a major response to population-density stress, this effect is attenuated or reversed by antero-dorsal hippocampal lesions. It would also appear that this change is not a direct function of altered gonadal function since no differences in testes weights between groups were found.

Emotional Behaviour. Comprising the very general category of "emotional" behaviour are the more specific classifications of (a) open field activity (b) "amicable" behaviour, and (c) conflict behaviour.

In the present study, "open-field activity" would include "rats in the open field," "exploring," "crossing the open field," and "mobile in the open field." Responses to open-field situations in both natural

and experimental rat populations have been widely utilized in experimental studies as indices of "emotional" or stressful behaviour. In the present study, the ADHL group were significantly more active than any other group on every open-field behaviour. Several previous studies are in agreement with this finding of increased general locomotor and exploratory activity following hippocampus lesions. Kimble (1963) and Teitlebaum and Milner (1963) observed increased activity in rats following hippocampal lesions. Similar findings were reported by Douglas and Isaacson (1964) who noted that hippocampal animals were more active than controls in exploration and in an activity wheel. Roberts, Dember and Brodwick (1962) found that hippocampal animals failed to reduce, or were significantly impaired in their ability to reduce, their activity with continued exposure to a new environment. A similar effect has been reported by Leaton (1965) who found that the running speeds of hippocampal rats in a T-maze declined at a significantly slower rate than that of cortical and sham groups. In another open-field measure - "rats in the open field" - a very early-occurring and marked difference, particularly between the ADHL and SHAM groups, was noticed in the differing numbers of animals that remained outside of the shelter boxes. This is highly interesting in the light of Calhoun's report (1962) that pathological withdrawal was a typical behavioural disturbance of normal rats in response to high population density. Also, Moyer (1963) found that rats subjected to prolonged shock stress took significantly longer to leave a "timidity" box and were more emotional in the open field than the non-stressed animals. Observation of natural and experimental situations has clearly established avoidance of the open field as a signifi-

cant component of fearful or emotional behaviour in rats. The strong differences on this measure between the ADHL and SHAM groups would indicate that the ADHL animals displayed behaviour contrary to that expected for normal animals, with the ADHL animals showing a significantly lower level of fearful or emotional behaviour than that seen in any other group, as measured by the open-field behaviour.

The behavioural syndrome defined as "social exploration" consisted of several behaviours, often closely related in occurrence. All of these behaviours have been described as "amicable" insofar as they are non-aggressive, are clearly directed toward other rats, and often involve forms of mutual cutaneous stimulation (Barnett, 1961). These behaviours appear to reduce or prevent aggression in rat colonies. In the "amicable" behaviour category, the ADHL group displayed a significantly higher level of "social exploration" than all other groups. Such behaviour is as noted directed toward social interaction, and is clearly not typical of stressed or highly emotional animals. Recently, Clark and Schein (1966) reported that dominant mice, which characteristically display the least signs of emotional response, engaged in significantly more social grooming, social and genital sniffing than did subordinate mice.

Grooming and gnawing have less clearly established significance in rat populations. Grooming, aside from its occurrence in bodily cleaning, has been seen to occur when exploratory, fighting, or coitus have been interrupted (Barnett, 1961). It may in part serve to prevent aggressive contact and possible injury. The ADHL group, although it did not differ significantly from the ENTO group on this measure, groomed significantly

more than did the CORT and SHAM groups. Gnawing also appears to occur independently of such apparent needs as hunger, but its functional significance is not well understood. It is interesting to note, however, that only on this measure were the ADHL and SHAM groups statistically similar, and only on this measure did the ADHL group show lower scores than the ENTO and CORT groups.

On the basis of the open-field and "amicable" behaviours, the ADHL group displayed significantly more social and "non-emotional" or non-stressful behaviour than did any other group. This appears highly consistent with the observations during the test period that the ADHL group appeared noticeably less reactive to sudden noises, puffs of air, and being retrieved than did the other groups, in particular the SHAMs. However, the ADHL group also displayed higher levels of "conflict" behaviour than the SHAMs. Conflict behaviour encompasses both ritual and physical fighting, and it will be recalled that the ADHL group showed significantly more ritual fighting than both ENTO and SHAM groups, and significantly more physical fighting than the SHAMs. Ritual fighting, however, appears to function as an injury or aggression-reducing behaviour in rats, much in the manner of "amicable" behaviour. Physical fighting, though, is generally regarded as indicative of stressful situations, and although the ADHL group did not fight significantly more than the ENTO and CORT groups, it fought more than the SHAMs. It is possible that the physical fighting scores of the ADHL group were influenced upward by the high sexual and other behavioural activity which tended to bring these animals into more frequent contact with one another. It is also of interest to note that while the ADHL, CORT and ENTO groups showed a general decline

in fighting over time, the SHAMs maintained their original level of fighting throughout the 80-day period.

Considering these results, it is suggested that the antero-dorsal hippocampal lesions have resulted in a decreased or attenuated level of emotional reactivity and, as a possible consequence or concomitant of this, a resultant increase in various forms of social and non-emotional behaviour. Such an interpretation might reasonably account for the observed high levels of activity in all the major behavioural categories in the ADHL group. One way in which decreased emotional reactivity could act is through the disruption of dominance-subordination relationships. As noted earlier, there was a virtual absence of any form of dominance relationships among the ADHL animals while the SHAMs displayed clear evidence of a dominant animal. In the SHAM group, when the dominant male was established and present, almost all the other males remained in the shelter boxes or far from this male. This resulted in relatively little ongoing activity. Significantly, when this male was absent activity levels in almost all other behaviours were considerably elevated. Conceivably, part of the "social pressure" of high population density may be an increase or enhancement of fear responses, upon which dominance relationships are highly dependent. Removal or attenuation of these fear responses would then likely weaken dominance relationships among animals and possibly necessitate continual or more frequent establishment and re-establishment of superiority in conflict situations. This fact might account for the significantly higher levels of fighting behaviour seen in the ADHL animals than in the SHAMs. Attenuation of fear responsiveness in the ADHL group could at the same time generate higher levels of

various other activities that are generally seen in non-stress situations.

One of the most important questions to be asked is how the hippocampus is related to these seemingly diverse behaviours. Pribram and Kruger (1954) noted that support for Papez' implication of the hippocampus (and their own "third system") in emotion still required considerable experimental substantiation. Today, although no integrated theory of hippocampal function has yet been proposed, some reasonable evidence involving the hippocampus in emotion and emotional expression does exist. Perhaps the most direct evidence comes from stimulation and lesion studies. Gellhorn (1963) has reviewed these studies in great detail. For example, Ranson (1936) found that stimulation of the fornix where it penetrates the lateral hypothalamic area evoked many manifestations of rage, and MacLean (1955) reported that hippocampal seizures apparently lowered the rage threshold in a cat, producing wild excitement and viciousness. Prolonged stimulation of the rostral hippocampus in the monkey has led to generalized agitation (Brady & Hunt, 1955) while alertness and uneasy mewling were found by De Molina and Hunsperger (1959) upon stimulation of the hippocampus, the descending columns of the fornix, anterior nucleus of the thalamus, and the mammillary bodies in the cat. Kaada, Jansen and Andersen (1953) reported fear, anger, and fury in cats following hippocampal stimulation. In the lesion studies, lesions of the mammillary bodies and the post-commissural fornix were reported by Ranson (1932, 1936, 1939) to result in the taming of wild monkeys and the absence of emotional reactivity. Ranson also reported that lesions in the cingulate cortex of monkeys, which is intimately connected with the hippocampus (Pribram & Kruger, 1954) led to reduced

emotionality and tameness. These results have been confirmed by Glees, Cole, Whitty and Cairns (1950) and by Ward (1948). Lastly, Green, Clemente, and DeGroot (1957) reported that behaviour patterns which suggested fear in the cat were related to hippocampal seizures. These studies, while not conclusive, certainly suggest the involvement of the hippocampus and its interconnections with emotional behaviour and re-activity. Some of the parallel effects reported as a result of damage to different limbic system structures and their interconnections are not altogether unexpected. The involvement of the limbic system in emotional behaviour is now well documented, if not as well understood. The anatomical integration of the limbic system structures as earlier noted by Papez (1937) prompted his theory of an "emotional circuit". The electrophysiological integration of limbic structures has already been substantiated by many researchers (Kaada, 1951; Gloor, 1960; Green, 1964). Recent work already cited by Mason (1958, 1959, 1961), Knigge (1961, 1963) and Slusher and Hyde (1961) have presented a considerable foundation for limbic system integration in neuroendocrine function. Until recently, the lack of experimental data prevented the formulation of any general statements about the role of the hippocampus in behaviour. With behavioural data now forthcoming, one might not unreasonably expect to find reflection of this integration in behavioural activity as well.

An overall examination of the present results indicated what appeared to be a general pattern in the behavioural observations: the ADHL group evidenced significantly higher scores than the ENTO or CORT groups, which in turn had significantly higher scores than the SHAMs in every major behavioural category.



Within this pattern there was another consistent observation. Both ENTO and CORT appeared to behave similarly. Both groups displayed statistically similar levels of activity in all but one behaviour (grooming). Both groups typically had significantly higher scores than the SHAMs and significantly lower scores than the ADHL group. In addition, examination of the regression analysis for these two groups revealed similar slopes (i.e. both positive or both negative) on every measure. There was a general tendency in both groups for the activity levels to decrease over the 80-day observation period. One possible explanation for these similarities might be that the important common factor in both of these groups was the removal of overlying cortex despite the difference in location of the lesions. However, closer examination of the damaged areas in these groups indicated that in the cortical control group the cingulum was bilaterally damaged in almost every case. The close interconnections between the cingulate cortex and hippocampus via the cingulum, and between the entorhinal cortex and the hippocampus via the temporo-ammonic pathway are well established (Papez, 1937; Pribram & Kruger, 1954). In addition, these interconnections have been classified as part of the same functional sub-system of limbic system structures (Pribram & Kruger, 1954). The ENTO group had originally been intended to serve as a postero-ventral hippocampus lesion group, but histological examination had found the lesions to be too far posterior, and centred in the entorhinal cortex. Because of these interconnections with the hippocampus, it was decided to retain the ENTO group in the present study. With the damage to the cingulum in the CORT group, it thus appeared that both ENTO and CORT groups had sustained damage to afferent anatomical

connections with the hippocampus. Supporting this view is the fact that both of these groups, similar to the ADHL group, showed significantly higher levels of activity than the SHAMs in every measure but one (male - male mounting). Such evidence adds consistency to the conclusion that the degree and direction of the observed behavioural activity changes are primarily a function of damage to the hippocampus system.

### Endocrine Results

Blood Corticosterone and Adrenal Weights. No statistically significant differences in blood corticosterone levels between the ADHL, CORT, and SHAM groups were found, nor were there any differences between groups in adrenal weights. These findings are in disagreement with the experimental evidence already reviewed that dorsal hippocampal lesions effect elevated ACTH and adrenalcorticosteroid levels in animals subjected to various forms of stress. What may be a possible factor in this lack of agreement is the nature of the stress situation itself. The present study has examined adrenocortical responses following a prolonged or chronic stress situation. Therefore, data concerning the function of the hippocampus in endocrine regulation in chronic stress situations would appear to be most relevant to the present findings. Many of the studies examining hippocampal regulation of pituitary-adrenocortical responses to stress have studied the acute stress situation. Some evidence, however, is available on the pattern of adrenocortical response to periods of more prolonged stress in hippocampus-lesioned animals. Knigge (1961) found that while resting or basal plasma corticosterone levels in hippocampal animals were significantly higher than controls,

the temporal pattern of adrenocortical response to immobilization stress at one-half and 4 hours after continuing stress, did not differ significantly from non-lesioned controls. Kim and Kim (1961) studying the effects of dorsal hippocampal lesions upon the adrenal ascorbic acid response to chronic stress (6, 10, and 11 days), found that the adrenal ascorbic acid content generally recovered over time after an initial depletion and was not significantly different from normal controls. In both of these studies, as in the present one, no significant differences in adrenal weights were found. These results suggest that while initial corticosteroid elevations result from hippocampal lesions, these differences may tend to disappear with prolonged stress. This could possibly represent a form of physiological adaptation or homeostatic response to chronic stress. The degree to which such an effect occurs may depend upon the nature of the stress and the measure of adrenal response that is used. Possibly these adrenocortical measures were not sufficient in themselves to reflect the effects of chronic stress. Other physiological and/or behavioural measures might therefore be more meaningful indices of stress in the chronic stress situation.

Analysis of corticosterone levels did, however, reveal a significantly lower level ( $p < .05$ ) in the ENTO than in the SHAM group. Since little evidence exists on the involvement of the entorhinal cortex in neuroendocrine function, it is somewhat difficult to interpret these findings. A possible anatomical basis for these effects might exist in the temporo-ammonic pathway of the entorhinal cortex. Electrophysiological studies have indicated specific responses in the hippocampus upon stimulation of the entorhinal cortex as well as the reverse effect,

indicating both efferent and afferent connections between the hippocampus and the entorhinal cortex. It seems possible that the entorhinal cortex-posterior hippocampus and the anterior hippocampus-fornix system could comprise anatomically and physiologically distinct systems in the regulation of ACTH secretion.

Gonadal Weights. The present study found no differences in relative testes weights between the 4 groups. Knigge (1961) also reported no significant changes in testicular weights in dorsal hippocampal animals in response to immobilization stress. This latter view is supported by the findings of Peretz (1967) who found that ablation of the postero-ventral hippocampus weakened estrous behaviour in cats, but that injection of female hormones did not restore the original estrous behaviour. The behavioural findings of very large, significant differences between groups in both heterosexual and homosexual activity would suggest that either testes weights do not accurately reflect differences in sexual activity or alternatively, that such changes in sexual behaviour were more directly a function of altered CNS activity than a function of altered gonadal hormone level.

#### SUMMARY

The present study examined the effects of antero-dorsal hippocampus, entorhinal cortex, dorsal neocortex and SHAM lesions upon behavioural and endocrine responses to the stress of high population density. The major findings were:

- (1) The ADHL group displayed significantly higher levels of activity in social and "non-emotional" behavioural measures than did all other groups.

(2) The ADHL group displayed marked hypersexuality. Both heterosexual and homosexual activity were significantly higher in this group than in all other groups.

(3) Both ENTO and CORT groups behaved similarly. Both groups generally had significantly lower scores than the ADHL group, and significantly higher scores than the SHAMs.

(4) No differences between groups in adrenal or testes weights were found. No differences in blood corticosterone levels were found between ADHL, CORT, and SHAM groups. The ENTO group showed a significantly lower blood corticosterone level than the SHAM group.

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APPENDIX A  
ENDOCRINE RESULTS

TABLE 1

MEAN BODY WEIGHTS, ADRENAL AND TESTES WEIGHTS, AND  
BLOOD CORTICOSTERONE LEVELS FOR ALL GROUPS

	GROUPS			
	ADHL	SHAM	ENTO	CORT
Body Weights (gm) (N = 12)	386.7	368.5	392.9	366.5
Adrenal Wt/Body Wt (N = 12)	.000141	.000130	.000136	.000142
Testes Wt/Body Wt (N = 12)	.00947	.00933	.01072	.00945
Blood Corticosterone (mg/100 ml plasma) (N = 12)	16.65	18.60	9.78	14.30

APPENDIX B

REGRESSION ANALYSES OF BEHAVIOURAL MEASURES

TABLE 1

## REGRESSION ANALYSIS FOR "RITUAL FIGHTING"

Group	Function	F-values	t-values	
			<u>Groups</u>	<u>t</u>
1. ADHL	$Y = 25.61 + -0.21X$	25.74**	1-2	29.44**
2. SHAM	$Y = 9.45 + -0.01X$	0.10	1-3	5.23**
3. ENTO	$Y = 21.07 + -0.24X$	56.92**	1-4	21.94**
4. CORT	$Y = 31.01 + - .40X$	42.31**	2-3	38.09**
			2-4	45.50**
			3-4	19.51**

TABLE 2

## REGRESSION ANALYSIS FOR PHYSICAL FIGHTING

1. ADHL	$Y = 31.52 + -0.15X$	5.42*	1-2	18.15**
2. SHAM	$Y = 10.42 + -0.03X$	0.30	1-3	8.25**
3. ENTO	$Y = 31.65 + -0.24X$	16.27**	1-4	2.67**
4. CORT	$Y = 31.23 + -0.12X$	2.16	2-3	27.67**
			2-4	13.24**
			3-4	10.0 **

TABLE 3

## REGRESSION ANALYSIS FOR MALE-FEMALE MOUNTING

1. ADHL	$Y = 20.57 + 0.10X$	2.12	1-2	3.52**
2. SHAM	$Y = 1.45 + 0.07X$	4.93*	1-3	4.74**
3. ENTO	$Y = 5.04 + 0.15X$	7.34**	1-4	6.87**
4. CORT	$Y = 8.02 + 0.36X$	0.49	2-3	10.87**
			2-4	5.39**
			3-4	13.60**

NOTE: \* =  $p < .05$ \*\* =  $p < .01$

TABLE 4

## REGRESSION ANALYSIS FOR MALE-MALE MOUNTING

Group	Function	F-values	t-values	
			<u>Groups</u>	<u>t</u>
1. ADHL	$Y = 18.35 + 0.06X$	1.03	1-2	8.43**
2. SHAM	$Y = 0.89 + 0.00X$	0.03	1-3	20.04**
3. ENTO	$Y = 5.97 + -0.08X$	15.79**	1-4	23.40**
4. CORT	$Y = 5.76 + -0.10X$	29.15**	2-3	30.15**
			2-4	40.0 **
			3-4	7.11**

TABLE 5

## REGRESSION ANALYSIS FOR GNAWING

1. ADHL	$Y = 14.82 + 0.07X$	1.82	1-2	3.09**
2. SHAM	$Y = 16.83 + 0.04X$	0.31	1-3	13.82**
3. ENTO	$Y = 33.93 + -0.10X$	1.10	1-4	11.07**
4. CORT	$Y = 35.80 + -0.06X$	0.43	2-3	10.41**
			2-4	7.60**
			3-4	3.03**

TABLE 6

## REGRESSION ANALYSIS FOR GROOMING

1. ADHL	$Y = 132.21 + 0.30X$	37.59**	1-2	10.09**
2. SHAM	$Y = 95.89 + 0.44X$	15.33**	1-3	3.85**
3. ENTO	$Y = 132.53 + 0.27X$	30.25**	1-4	23.31**
4. CORT	$Y = 112.93 + 0.51X$	70.93**	2-3	12.26**
			2-4	4.43**
			3-4	26.73**

NOTE: \* =  $p < .05$ \*\* =  $p < .01$

TABLE 7

## REGRESSION ANALYSIS FOR SOCIAL EXPLORATION

Group	Function	F-values	t-values	
			Groups	t
1. ADHL	$Y = 56.90 + -0.02X$	0.13	1-2	0.01
2. SHAM	$Y = 20.30 + -0.02X$	0.24	1-3	9.76**
3. ENTO	$Y = 44.33 + -0.13X$	3.64	1-4	1.95
4. CORT	$Y = 36.20 + -0.05X$	0.45	2-3	10.99**
			2-4	2.19*
			3-4	7.78**

TABLE 8

## REGRESSION ANALYSIS FOR RATS IN OPEN FIELD

1. ADHL	$Y = 193.53 + -0.18X$	3.69	1-2	4.92**
2. SHAM	$Y = 78.91 + -0.10X$	0.94	1-3	23.01**
3. ENTO	$Y = 169.86 + -0.55X$	26.45**	1-4	10.62**
4. CORT	$Y = 160.44 + -0.37X$	8.14**	2-3	26.47**
			2-4	14.32**
			3-4	9.24**

NOTE: \* =  $p < .05$   
 \*\* =  $p < .01$



TABLE 9  
REGRESSION ANALYSIS FOR EXPLORING

Group	Function	F-values	t-values	
			Groups	t
1. ADHL	$Y = 10.66 + -0.06X$	12.55**	1-2	9.77**
2. SHAM	$Y = 3.96 + -0.04X$	17.92**	1-3	18.10**
3. ENTO	$Y = 10.20 + -0.10X$	46.30**	1-4	23.75**
4. CORT	$Y = 11.45 + -0.12X$	51.71**	2-3	33.27**
			2-4	38.88**
			3-4	6.73**

TABLE 10  
REGRESSION ANALYSIS FOR RATS MOBILE IN OPEN FIELD

1. ADHL	$Y = 13.89 + 0.06X$	1.61	1-2	7.75**
2. SHAM	$Y = 5.55 + 0.01X$	0.29	1-3	14.26**
3. ENTO	$Y = 11.18 + -0.03X$	1.10	1-4	24.93**
4. CORT	$Y = 15.56 + -0.10X$	10.02**	2-3	9.99**
			2-4	25.07**
			3-4	15.91**

NOTE: \* =  $p < .05$   
 \*\* =  $p < .01$

## APPENDIX C

POST-HOC ANALYSES OF VARIANCE BY METHOD OF LEAST  
SIGNIFICANT DIFFERENCES (LSD)

TABLE 1  
RITUAL FIGHTING

Groups	F value	LSD (p<.05)	LSD (p<.01)	Differences between group means	
1. ADHL	10.93	3.08	4.04	1-2	+ 8.13
2. SHAM				1-3	+ 5.82
3. ENTO				1-4	+ 2.08
4. CORT				2-3	- 2.31
				2-4	- 6.05
				3-4	- 3.74

TABLE 2  
PHYSICAL FIGHTING

1. ADHL	17.20	4.43	5.82	1-2	+13.47
2. SHAM				1-3	+ 3.21
3. ENTO				1-4	- 1.00
4. CORT				2-3	-10.26
				2-4	-14.47
				3-4	- 4.21

TABLE 3  
MALE-FEMALE MOUNTING

1. ADHL	54.38	3.27	4.30	1-2	+20.40
2. SHAM				1-3	+13.55
3. ENTO				1-4	+15.34
4. CORT				2-3	- 6.85
				2-4	- 5.06
				3-4	+ 1.79

TABLE 4  
MALE-MALE MOUNTING

1. ADHL	153.5	2.12	2.78	1-2	+19.68
2. SHAM				1-3	+17.83
3. ENTO				1-4	+18.92
4. CORT				2-3	- 1.85
				2-4	- .76
				3-4	+ 1.09

TABLE 5

## GNAWING

Groups		F-values	LSD (p<.05)	LSD (p<.01)	Differences between group means	
1.	ADHL	22.89	4.68	6.16	1-2	- .81
2.	SHAM				1-3	-12.37
3.	ENTO				1-4	-16.00
4.	CORT				2-3	-11.57
					2-4	-15.19
					3-4	- 3.63

TABLE 6

## GROOMING

1.	ADHL	82.47	4.35	5.72	1-2	+30.63
2.	SHAM				1-3	+ .9
3.	ENTO				1-4	+11.0
4.	CORT				2-3	-29.73
					2-4	-19.63
					3-4	+10.1

TABLE 7

## SOCIAL EXPLORATION

1.	ADHL	122.83	3.78	4.97	1-2	+36.47
2.	SHAM				1-3	+16.83
3.	ENTO				1-4	+21.55
4.	CORT				2-3	-19.64
					2-4	-14.92
					3-4	+ 4.72

TABLE 8

## RATS IN OPEN FIELD

1.	ADHL	389.29	6.53	8.58	1-2	+111.48
2.	SHAM				1-3	+ 38.58
3.	ENTO				1-4	+ 40.88
4.	CORT				2-3	- 72.90
					2-4	- 70.60
					3-4	+ 2.30

TABLE 9  
EXPLORING

Groups	F-value	LSD (p<.05)	LSD (p<.01)	Differences between group means	
1. ADHL	51.82	0.96	1.26	1-2	+ 5.89
2. SHAM				1-3	+ 2.25
3. ENTO				1-4	+ 1.68
4. CORT				2-3	- 3.64
				2-4	- 4.21
				3-4	- 0.57

TABLE 10  
RATS MOBILE IN OPEN FIELD

1.	ADHL	30.76	2.12	2.78	1-2	+10.24
2.	SHAM				1-3	+ 6.25
3.	ENTO				1-4	+ 4.88
4.	CORT				2-3	- 3.99
					2-4	- 5.36
					3-4	- 1.37

Behavioral Measures	10-Sec. Intervals										Date _____ Cage # : _____ Testing order : _____					
	1	2	3		18	19	20		38	39	40		58	59	60	
Ritual fighting	✓															
Physical fighting		✓														
Gnawing																
Male-female mounting	✓															
Male-male mounting		✓														
Grooming	✓															
Social exploration																
Crossing open field					2											
Exploring					1											
Rats in open field						15										
Rats mobile in open field							4									
Hermits							1									
Natality																
Mortality																

APPENDIX D

SAMPLE CHECKLIST USED IN

RECORDING BEHAVIOURAL ACTIVITY