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CONDITIONS AFFECTING STRESS ULCERATION

IN ULCER-SUSCEPTIBLE RATS

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ABSTRACT

One of the variables that consistently has an effect on restraint-induced gastric ulceration in laboratory rats is the prior housing conditions of the rats. Group housed rats ulcerate more when physically restrained than individually housed rats, and it was suggested that this difference occurs because the group housed rats are over-crowded in their housing boxes prior to restraint. Over-crowding was also considered the explanation for why the ulcer-susceptible rats bred at the University of New South Wales develop stress ulceration in their home surroundings.

The aim of Experiment 1 was to test two hypotheses: (1) that crowding produces stress ulceration in ulcer-susceptible rats in their home surroundings; (2) that rats group housed under uncrowded conditions ulcerate less when restrained than individually housed rats and rats group housed under crowded conditions. The first hypothesis was partly confirmed. It was found that male rats living in crowded conditions developed more stress ulceration than male rats

housed individually, or male rats housed in uncrowded groups. However, there were no differences among the female groups.

The second hypothesis was not confirmed. With both males and females, the rats housed in uncrowded groups ulcerated more when restrained than the individually housed rats. Furthermore, the male rats housed in crowded groups ulcerated significantly less than the males housed in uncrowded groups.

The sex difference that was found in both sections of Experiment 1 was interpreted as a failure to crowd the female rats sufficiently, since female rats are considerably smaller than male rats. In Experiment 2, female rats were crowded to a degree comparable to that experienced by males in Experiment 1. However, crowded female rats did not develop stress ulceration in their home surroundings, and did not ulcerate less when restrained than uncrowded female rats. It was decided that in any future studies into restraint-induced ulceration, male and female results should be examined separately.

The aim of Experiment 3 was to investigate why the male rats housed in crowded groups in Experiment

1 ulcerated less when restrained than the male rats housed in uncrowded groups. It was hypothesized that, because the crowded males had been suffering mild chronic stress for some time prior to their being restrained, their threshold to stress had been altered such that they reacted less to the severe stressor of restraint. The hypothesis was not supported, and an alternative hypothesis was tested in Experiment 4: that rats adapt to the amount of space made available to them prior to restraint, such that the more space they have available, the more they will react when that space is severely restricted during restraint. The hypothesis was generally supported. However, it is possible that "adaptation to movement permitted" is a more appropriate explanation of the relationship between prior housing and restraint ulceration than "adaptation to space provided".

Weiss has proposed a general theory of stress ulceration, but there are a number of difficulties with it. Therefore, an empirical test of Weiss's theory was initiated in Experiment 5. Weiss's basic design was replicated, but it was found that the design is inadequate for properly evaluating the

theory.

In conclusion, if reliable and valid measures of stress ulceration are desired from the restraint technique, then detailed attention must be paid to standardizing every aspect of experimental procedures. On the basis of the results of Experiment 4, it may be concluded that one important factor that requires standardization is the amount of movement permitted the rat prior to restraint.

CHAPTER 1

INTRODUCTION TO STRESS RESEARCH

The chief concern of this thesis is stress ulceration in rats, induced by the method of physical restraint. Before the main features of the restraint method are described, and the research investigating such stress ulceration is reviewed, it is worthwhile to examine briefly the general development of stress research. Such an analysis, although short, will provide a broad theoretical context in which the primary subject matter of the thesis may be viewed. Perhaps the appropriate place to start is with the work of Hans Selye, the Canadian endocrinologist who introduced the term "stress" to the Life Sciences 40 years ago.

Selye's Model of Stress

In 1926, when he was still studying for his medical degree, Selye became interested in the syndrome of what he called "just being sick" (Selye, 1956, p.16). Selye was fascinated by the persistent appearance of certain common symptoms in patients suffering from a wide range of physical disorders.

These symptoms included loss of muscular strength, loss of weight, loss of appetite, and loss of motivation.

Ten years later, in 1936, Selye reported what he considered to be an experimental analogue of "just being sick". Selye had been subjecting laboratory rats to various noxious manipulations, and he had noticed that as well as the occurrence of specific reactions to specific manipulations, there also occurred a number of reactions that were common to all manipulations. Selye named the common physiological reactions the general adaptation syndrome, or the biological stress syndrome.

Selye borrowed the term "stress" from the applied Science of Engineering, where it had been used to refer to physical forces applied to objects. He reversed the Engineering convention, and used stress to refer to the reaction produced in an organism by exposure to stressors. Further, Selye was interested only in the common features of the reaction. For Selye, stress became the nonspecific response of the body to any demand made upon it (Selye, 1956).

Selye's general adaptation syndrome had three central features: enlargement and overactivity of

the adrenal glands, shrinkage and underactivity of the thymus and the lymph nodes, and appearance of gastrointestinal ulcers. Further, the general adaptation syndrome had three stages. The first stage was the alarm reaction: there was an initial shock phase of lowered resistance, followed by a countershock phase, during which homeostatic mechanisms began to operate. The second stage of the general adaptation syndrome was the stage of resistance or adaptation. During the second stage, homeostatic mechanisms attempted to restore equilibrium in the body. Finally, after prolonged exposure to the stressor, the third stage, that of exhaustion, occurred. The third stage eventuated when the body's homeostatic mechanisms could no longer cope with demands being made upon them for adaptation.

There are certain restrictions on the usefulness of Selye's model of stress. First, because of his preoccupation with only the common elements of all stress reactions, Selye's definition of stress is too limiting. Selye did not concern himself with reactions that vary with the nature of the stressor, or with the organism being stressed. He thus ignored considerable information about the stress reactions

he was studying.

A second, and more important limitation for the behavioural scientist, is that Selye's model is a biological model of stress. Although Selye eventually included psychological stressors among those that could give rise to the general adaptation syndrome (Selye, 1974), Selye himself was basically not interested in behaviour, and stress-induced behavioural changes, common or not, played little part in his model. Thus, any practical use behavioural scientists might make of Selye's model is severely restricted.

Psychological Models of Stress

An entirely different approach to stress, from the one adopted by Selye, has been adopted by psychologists. Apart from the expected emphasis on psychological events rather than on physiological ones, there has been a move away from Selye's preoccupation with common elements in stress reactions, towards an appreciation of all aspects of situations in which stress occurs. This more inclusive approach has led psychologists to an understanding of the complex nature of stress, and of the futility of pursuing a model similar to Selye's in a psychological context. One

problem which has received considerable attention is that of the definition of stress. Most attempts to define the term have involved references to taxing the adaptive processes of the organism, but there is much disagreement about what is considered "taxing".

The early models of psychological stress, formulated chiefly in the 1960s, emphasized the role of mental processes in psychological stress reactions. Accompanying this emphasis was a recognition that stress resulted from an interaction between a particular organism and a particular situation. Thus, little attention was paid in the early theoretical models to aspects of the stressor alone, or to aspects of the stress reaction alone. Rather, emphasis was placed on psychological processes within the person, as determining whether that person would be stressed or not. For example, Appley (in Appley & Trumbull, 1967) discussed stress in terms of "threat perception", and Lazarus (1966) emphasized the role of "cognitive appraisal".

The idea that stress reactions are the result of an interactive process has remained firmly entrenched in the stress literature. Such an approach has led to the rejection of the possibility of finding "a

general model" to account for all stress reactions. More recent approaches to stress have concentrated on specific stress reactions, and any models proposed are limited ones. In addition, the more recent approaches have shifted away from an emphasis on mental processes, and have moved towards an examination of observable behaviour.

Two important attempts to account for specific stress reactions in behavioural terms are Weiss's model of stress ulceration, and Seligman's model of anxiety and depression. Both models are concerned with the coping behaviour of animals in stressful situations. Briefly, Weiss's theory states that stress ulceration is a function of the number of coping attempts an animal makes in a stressful situation, and simultaneously a function of the consequences of such coping attempts (Weiss, 1971a, 1972). Specifically, Weiss claims that stress reactions increase monotonically with coping attempts, but decrease monotonically with improving consequences.

Seligman (1975) is also concerned with coping behaviour in his attempt to account for depressive reactions. Seligman claims that whether animals cope

effectively with stressors or not depends on what they have learned previously about the effectiveness of coping. If animals have learned in one situation that coping attempts are ineffectual, they will transfer that learning to other situations, and behave as if attempts to cope have no effect. For Seligman, the psychological state of depression is the result of learning that coping behaviour in stressful situations is not effective; i.e. the animal "learns to be helpless".

A different approach to stress has been adopted by Holmes and Rahe (1967), who have suggested a model that depends more on stressor events and less on the individual being stressed. Holmes and Rahe propose that stress reactions (represented by physical disorders in the research they have carried out) are a function of the rate of change of events in an individual's life. The change experienced can be either pleasant or unpleasant. What is important is that an adaptation has to be made, and the greater the total adaptation, the greater the stress. Holmes and Rahe have found high correlations between the amount of change experienced and the occurrence of physical disorders. However, in the cumulative change

model, little attention has been paid to individual differences in reactions to situations, and such an exclusion might limit the usefulness of the model.

Measurement of Stress Reactions

Since the 1960s, considerable attention has been given to the problem of establishing reliable and valid indicators of stress reactions. Two main classes of variables have been studied: behavioural variables and physiological variables.

Among the behavioural methods used to measure stress-induced changes are performances on standardized tasks, and observations of motor disturbances such as tremors and speech difficulties. However, the use of such methods involves a number of problems. Observations of motor disturbances, for example, can lack objectivity, and standardized task performance is affected by the motivation of the subject, and also by transient and extraneous events in the environment.

In recent years, physiological measures have gained popularity as measures of psychological stress, partly because they are relatively free from many of the difficulties associated with behavioural measures.

Physiological measures are objective, and are less affected by levels of motivation. The most popular physiological measures used are the peripheral measures of the autonomic nervous system; for example, the galvanic skin response (GSR), respiration rate ($\frac{I}{E}$), heart rate (HR), and blood pressure (BP). Unfortunately, like the behavioural measures, many of these peripheral nervous system measures are affected by trivial changes in the environment that occur at the time the measurement is being taken. Many of the measures are, in fact, part of the physiological orienting response that occurs to novel stimuli. Thus, while the peripheral physiological measures are objective indicators of a reaction, they are not necessarily valid indicators of a stress reaction.

In order to overcome some of the problems associated with peripheral measures, physiological measurements of a more central nature are often made. Measures included in this category are concentrations of the adrenal hormone, hydrocortisone, in the urine and in the blood plasma, and levels of sugar in the blood.

The difficulty posed by the use of any type of physiological measure as an index of stress is

that changes in many physiological systems can be produced by a variety of means, of which stress is only one. For example, physical effort and stress both produce many of the same physiological changes. Furthermore, it is difficult to make distinctions on a physiological basis between different sorts of stress. There is not, as yet, a reliable method of distinguishing physiologically between activity and anxiety, between fear and frustration, between grief and anger, and so on. However, some progress has been made. Ax (1953) and Funkenstein (1955) report that fear and anger can be distinguished physiologically, but Gray (1971) suggests that this differentiation is more likely to be one of states of passivity versus states of activity, rather than specifically one of fear versus anger.

A further difficulty with the use of physiological measures as indices of stress is that individuals differ in the physiological system which is most sensitive to stressors. For example, heart rate might be a sensitive indicator of stress in one individual, and respiration rate might be the most sensitive indicator in another. Thus, valid measurements of stress reactions are often not obtained if only

one physiological system is monitored. Usually, a number of systems must be measured, since it is generally not possible to know which system is sensitive for which individual.

Gastric Ulceration as an Index of Stress

Stress experiments using animals as subjects have been able to use more central physiological measures of stress than have studies using human subjects. The values of using such measures have been outlined above: they are objective, and relatively free of motivational influences; further, the physiological reaction being monitored is unaffected by transitory changes in the environment. The problem of differential sensitivity from organism to organism in the system being monitored still remains, as also does the problem of specifically labelling the cause of the physiological reaction.

A commonly used method in the study of stress reactions in animals is the measurement of gastric ulceration. Such ulceration has often been validated as a stress reaction. One of the earliest examples was the discovery by Curling in the nineteenth century that individuals suffering from extensive superficial

burns develop gastric ulceration soon after suffering the burn. The most notable example of the validation of gastric ulceration as a stress reaction is Selye's identification of it as part of the general adaptation syndrome (Selye, 1956).

The laboratory rat in particular quickly develops gastric ulceration when exposed to certain stressors. Moreover, stress ulceration in rats is a fairly robust phenomenon, and the usual need to monitor more than one system is less important. This thesis is concerned with such stress-induced gastric ulceration in laboratory rats; specifically, it is concerned with the variables that affect the extent of stress ulceration induced by the psychological stressor of physical restraint.

CHAPTER 2

THE NATURE OF THE PSYCHOLOGICAL STRESSORS USED TO INDUCE STRESS ULCERATION IN THE RAT

There are three major psychological methods of inducing gastric ulcers in laboratory rats. The first and most widely used method is immobilization by physical restraint. The second method is exposure to a conflict situation; and the third and most recently developed method is exposure to electric shock stimulation. The restraint technique will be discussed in detail, because of its relevance to the experimental work of this thesis. The conflict and the shock stimulation methods will be discussed more briefly.

RESTRAINT

Hans Selye was the first to demonstrate that immobilization of a laboratory rat induces ulceration in the gastrointestinal tract (Selye, 1956). Selye used a number of methods to achieve immobilization: sectioning the rat's spinal cord, tying the rat's paws together, and wrapping the rat in a towel.

All these methods were successful in producing Selye's general adaptation syndrome, of which gastric ulceration was a central feature.

Although Selye consistently published his work from 1936 onwards, the technique of immobilizing rats to produce gastric ulcers did not become popular until 1956, when a French group, headed by Dr. Serge Bonfils, published reports of experimental work being carried out in Paris.[1] Bonfils and his colleagues reported that gastric ulceration could be induced in the rat's stomach, quickly and reliably, by restraining the rat physically (quoted in Brodie, 1963a). Bonfils' technique was to anaesthetize the rat, and then wrap it in wide-gauge wire screen. The rat's legs protruded through holes cut in the wire, and the protruding legs were held together with tape. Bonfils then suspended the wrapped animal above ground by attaching it to a burette clamp. As the rats were deprived of food and water during the restraint period, Bonfils also administered physiological saline to them to minimize dehydration.

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[1] Details of the French work have, in the main, not been obtained from the original publications; the secondary sources used are provided in the text.

Bonfils' technique rapidly induced "erosion {of the glandular section} of the gastric mucosa which never penetrated into the muscularis mucosa. The lesions were surrounded by an area of local edema and there were numerous capillary pits (areas of intense vasodilatation) in the mucous membrane. Although there was a great deal of hemorrhage in the stomach, perforations never occurred." (quoted in Brodie, 1963a, p.389). The technique produced such gastric pathology in some rats in as short a time as 7 hours, and in 86% of rats within 24 hours.

Some years later, the restraint technique became even more popular with the publication of work in the United States by David Brodie and Harley Hanson (Brodie, 1962, 1963a, 1963b, 1968; Brodie & Hanson, 1960; Hanson, 1963; Hanson & Brodie, 1960). Brodie and Hanson initially replicated the technique instituted by the French group, but they quickly simplified the method, and achieved essentially the same results as Bonfils and his colleagues (Hanson, 1963).

Brodie and Hanson eliminated much of the cumbersome nature of the French method. First, they anaesthetized the rat; then they completely enclosed it in galvanized steel window-screen, and secured the screen

with wire staples or bolts. The rat was allowed sufficient room for normal breathing, but could not move. Its legs did not protrude; it was not suspended; and it was not hydrated with saline.

Nevertheless, ulceration comparable to that found by the French group was produced: superficial erosion of the glandular section of the stomach (Hanson, 1963). Brodie and Hanson also reported that the ulceration varied from small multiple pits to elongated areas of erosion, and that it consistently appeared in the section of the stomach containing the majority of the acid-secreting cells, the fundus (Brodie & Hanson, 1960). Because of the superficial nature of the erosions, they were not considered comparable to human gastric ulcers.

Brodie and Hanson (1960) found that not all species of animals are susceptible to the ulcer-producing effects of restraint. High incidences of ulceration were found in groups of male Wistar mice (92% incidence) and male Holtzman rats (86% incidence), and some ulceration was found in a group of male guinea pigs (46%), but the technique was virtually useless with male hamsters (4%), and quite useless with male albino rabbits (0%), and male and female

rhesus monkeys (0%). Brodie and Hanson also found that spontaneous ulceration occurred in only 10% of the laboratory rat population.

Parameters Investigated by the French and the American Groups

Bonfils and his colleagues investigated a number of the parameters of restraint-induced ulceration, and Brodie and Hanson attempted to replicate many of the experiments the French group had conducted. Both groups studied the time course of the development of restraint ulceration, the time course of the recovery from such ulceration, and the effects of repeated restraint.

Additionally, Bonfils studied the relationship between the volume of restraint and ulceration, the relationship between the frequency of struggling by the restrained rats and ulceration, and the relationship between the prior activity levels of the rats and ulceration. Brodie and Hanson studied the effects of prior food deprivation on restraint-induced ulceration, and the relationship between the weights of the restrained rats and ulceration.

Time Course of Development of Restraint-Induced Ulceration

Bonfils restrained rats for as little as 7 hours, and found that 59% of a group of Wistar rats developed stress ulceration. After 24 hours of restraint, 86% of rats were ulcerating (quoted in Brodie, 1963a).

Brodie and Hanson (1960) found a similar increase in incidence of ulceration with an increase in time of restraint. They destroyed 32 male Holtzman rats after each of four periods of restraint: 6 hours, 12 hours, 18 hours, and 24 hours. After 6 hours of restraint, approximately 20% of rats were ulcerating; after 12 hours, there were 70%; after 18 hours, 80%; and after 24 hours, 90%. Severity of ulceration also increased as duration of restraint increased.

Although there is a difference between the two sets of results in the reaction of rats restrained for 6 to 7 hours, it is clear that the number of rats developing ulcers increases as the time of restraint increases, until after 24 hours, 85% to 90% of rats are ulcerating.

Time Course of Recovery from Restraint-Induced Ulceration

The French group made a detailed study of the recovery from restraint-induced ulceration. Bonfils and his colleagues found that the ulcers "healed by granulation of the base of the crater, until finally the damaged area was covered with gastric epithelium and the only evidence of a lesion was a depressed area in the mucose(sic)" (quoted in Brodie, 1963a, p.389). Bonfils found that after 24 hours of restraint, all rats had signs of active healing within 5 days, and 80% had normal stomachs within 9 days.

Brodie and Hanson (1960) similarly found that rats recovered quickly after 24 hours of restraint. Ulcer incidence in the Holtzman rats used by the Americans declined to the control incidence of 10% after 3 days. Both the French and the American groups found that ulcers healed without scarring, thus reinforcing the opinion that restraint-induced ulcers were not analogous to human gastric ulcers, but rather superficial erosions of the gastric mucosa.

The Effects of Repeated Restraint

The French group subjected their rats to repeated restraint periods of 24 hours each, each period being separated by a rest period of 48 hours during which they had free access to food and water (Bonfils & Lambling, 1963). It had already been established by Bonfils that 86% of rats ulcerated after one period of 24 hours restraint. After two such periods, Bonfils found that the incidence of ulceration decreased to 71%. Further decreases to 51% and 25% occurred after the third and fourth restraint periods respectively. However, Bonfils also found that both weight loss and mortality rate increased with increasing numbers of restraint periods.

Brodie and Hanson (1960) used a different repeated restraint schedule from that used by Bonfils. The Americans restrained rats for 18 hours out of every 24 hours, allowing the rats free access to food and water for the remaining 6 hours. The 24 hour cycle was then repeated five times.

In keeping with Bonfils' findings, Brodie and Hanson found an increase in loss of weight and mortality rate with repeated restraint periods. However, they did not confirm the decrease in incidence of

ulceration. Rather, they found a significant increase in both incidence and severity of ulceration with repeated periods of restraint, until after the fourth period of restraint, 100% of rats were ulcerating. Brodie and Hanson also found that ulcers began to appear in the non-glandular section of the rat's stomach, the rumen, after the third period of restraint.

The difference in findings between the French and the American experiments is difficult to account for. One possible explanation is that the restraint schedule used by Brodie and Hanson was more aversive than that used by the Bonfils group. However, it is questionable whether this factor accounts for the difference. An experiment published in 1964 by Guth and Mendick demonstrated that a repeated restraint schedule apparently more severe than either Bonfils' or Brodie and Hanson's still resulted in decreased ulceration with increasing restraint periods.

Guth and Mendick food deprived male rats for 18 hours, restrained them in wire mesh for 4 hours, then permitted them food for 2 hours. The schedule was repeated daily for 5 weeks, a group of five rats being killed at the end of each of the weeks. Guth and Mendick noticed a similar decrease in ulceration to

that observed by Bonfils. No rumenal ulceration was found. Guth and Mendick also reported the increase in mortality rate, associated with a loss in weight, that had been found by both Bonfils and Brodie and Hanson.

The simultaneous decrease in ulcer incidence and increase in mortality rate found by both Bonfils and Guth and Mendick is interesting. Bonfils hypothesized that the high mortality rate was due to the severe loss of weight suffered by the rats, and the resultant malnutrition. He further hypothesized that the decrease in ulceration was the result of a psychological habituation, or "inurement" (Bonfils & Lambling, 1963, p.157), to the stimulation being imposed. However, if Bonfils is correct, and the rats do habituate to the psychological stressor, it would seem that the adaptation involved is considerable, and such adaptation seriously costs the animals in other ways.

The Relationship Between Volume of Restraint and Restraint-Induced Ulceration

The effect on ulceration of the amount of space available to the restrained rat was systematically

examined by the French group (Bonfils & Lambling, 1963). Bonfils and his colleagues studied the effects of six different restraining volumes: 7,350ml, 1,260ml, 760ml, 560ml, 360ml, and 180ml.

Bonfils found that as the restraining volume decreased, the incidence of ulceration increased. At 7,350ml, ulcer incidence was 12%; at 1,260ml, 21%; at 760ml, 23%; at 560ml, 30%; at 360ml, 46%; and at 180ml, 89%.[2] Bonfils also found that the relationship between the incidence of ulceration and the reciprocal of the restraint volume was a linear one.

These results provided Bonfils with one of the arguments for concluding that restraint is primarily a psychological stressor rather than a physiological one. Bonfils claimed that because the linear relationship between the amount of space available and the incidence of ulceration continued even though the animal was no longer physiologically inconvenienced, then the pertinent stressor for the rat was the psychological effect of the restriction of space,

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[2] The percentage incidences were not provided directly in the report of the experiment; they were obtained by the author by taking the means of the incidences in each restraint-volume group.

rather than any physiological discomfort.

The Relationship Between Struggling and Restraint-Induced Ulceration

Bonfils and his colleagues studied the behaviour of the rats while they were restrained. They found that after recovery from the anaesthesia, the restrained rats engaged in continuous uncontrolled movement, which was soon replaced by intermittent outbursts of activity. Finally, the rats lapsed into prolonged inertia. Bonfils also measured, by the use of electrodes taped to the rats' hind legs, the frequency of struggling engaged in, as measured by kymographic tracings. It was found that there was no correlation between the frequency of struggling and the incidence of ulceration (quoted in Brodie, 1963a).

Bonfils' results have since been confirmed by Ader, Beels, and Tatum (1960a). Ader et al. restrained rats for 20 hours in adjustable galvanized wire cages. They found that there were no differences in struggling and vocalization (either spontaneous or in response to slapping the tail) between those rats that developed ulcers (56% of the group) and those that did not ulcerate.

The Relationship Between Activity Level and Restraint-Induced Ulceration

The French group measured the relationship between spontaneous activity levels prior to the rats being restrained and the extent of ulceration (Bonfils & Lambling, 1963). Measurements were made of a number of activities, but attention was paid only to the number of periods of standing on the hind legs, and the number of trips through a trap door attached to the rat's cage. On the basis of these two measurements, the rats were divided into three activity level groups: an active group, an intermediate group, and a passive group. All rats were then restrained for 7 hours, and the incidence of ulceration within each activity group was measured.

The results for the intermediate activity group were not taken into account because few rats fell into that category (18%), as compared with 52% in the active group, and 30% in the passive group. Bonfils and Lambling report that only 19% of rats in the active group developed ulceration, compared with 76% of rats in the passive group. Thus Bonfils and Lambling conclude that the more passive a rat prior to restraint, the more likely it is to develop ulcers

during restraint.

The relationship between ulceration and activity level has been further explored by other researchers. Mikhail and Broadhurst (1965) measured the activity level of rats in an open field, and then restrained them for 48 hours by wrapping them in gauze and plaster of Paris bandages. Mikhail and Broadhurst found that the less active rats in the open field ulcerated more severely than the more active rats. This result supports the finding of Bonfils and Lambling.

Contrary to the results of Bonfils and Lambling, and of Mikhail and Broadhurst, Sines (1962) found high rank-order correlations between ulceration and measures of activity. However, Sines's results are of dubious value. Sines obtained the high correlation between activity and ulceration with the fourth generation of a group of rats selectively bred for ulcer-susceptibility. When the measurement was repeated with the fifth generation rats, Sines found that the relationship between activity and ulceration had disappeared. Thus, it is probable that Sines's results contain an artifact produced by Sines's use of early generations of selectively bred rats.[3]

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[3]Sines's work will be discussed in more detail in Chapter 5.

While other researchers (e.g. Stern, Winokur, Eisenstein, Taylor, & Sly, 1960; Weininger, 1956; Winokur, Stern, & Taylor, 1959) have measured the activity level of rats in the open field prior to restraining them, no other researcher has directly examined the relationship between activity level and ulceration. Ader (1967b) retrospectively examined his data for an activity-ulceration relationship, but was unable to find one on the basis of the post hoc analysis.

The relationship between restraint-induced ulceration and activity level has been studied from a different point of view by Ader (1964). Ader manipulated the point in the circadian activity cycle of the rat at which it was restrained. One group of male rats was restrained in flexible wire mesh for 6 hours at the beginning of an active period in the circadian cycle, and another group was restrained for an identical period at the beginning of an inactive period. Ader found that more of the rats restrained during activity peaks developed ulcers than did rats restrained during activity troughs.

The difficulty with interpreting Ader's data is that other bodily rhythms might be synchronized with

the activity rhythm. Ader appreciated this problem of interpretation, and he later reported on the study of the cycles of two other bodily rhythms: plasma corticosteroid levels, and plasma pepsinogen levels (Ader, 1967a). Ader found that the plasma corticosteroid rhythm was not synchronized with the activity rhythm. The plasma pepsinogen rhythm, while being synchronized with activity, was found to vary insufficiently to account for the variance in ulceration. However, there are bodily rhythms other than the ones Ader has studied, and the interpretation of his results is still unclear. On the basis of Ader's results, little can be concluded about the relationship between activity itself and restraint-induced ulceration.

Herner and Caul (1972) carried out an experiment in which they pursued Ader's general line of investigation. They selected one group of female rats in estrous, and another group in diestrous (that is, groups of females at maximum and minimum points in the female rat activity cycle), and restrained each rat for 19 hours in a plastic restraining apparatus. Herner and Caul found that there was no significant difference in ulceration between the estrous and the

diestrous females.

The relationship between activity level and restraint-induced ulceration is an interesting one. Bonfils and Lambling (1963) have suggested that restraint is stressful because it limits the normal activity of the rat. This hypothesis has been extended by many researchers (e.g. Ader, 1964, 1967a, 1971; Sawrey & Long, 1962; Wilson, 1966) to include the notion that the more active the rat, the more it will be stressed by restraint. As can be seen from the foregoing discussion, there is little evidence to support this view. In fact, the direct evidence that is available (for example, that from Bonfils and Lambling, and from Mikhail and Broadhurst) seems to support the opposite view, that ulceration is produced more readily in passive rats, rather than in active rats.

The Effect of Prior Food Deprivation on Restraint-Induced Ulceration

Before studying the effect of prior food deprivation on restraint-induced ulceration, Brodie and Hanson (1960) studied the relationship between food deprivation alone and gastric ulceration. They found

that after 30 hours of deprivation, 12.5% of rats had ulcerated, and after 54 hours of deprivation, the incidence had doubled to 25%. From their previous work, Brodie and Hanson knew that 6 hours of restraint would produce an ulcer incidence of approximately 20%. They then sequentially combined the two stressors, food deprivation followed by restraint, to determine whether an additive or a synergistic effect would occur.

Brodie and Hanson found that 6 hours of restraint, preceded by 24 hours of food deprivation (making a total of 30 hours of food deprivation), resulted in an ulcer incidence of 50%, which was not significantly different from an additive effect of 12.5% plus 20%. However, 6 hours of restraint, preceded by 48 hours of food deprivation, resulted in an ulcer incidence of 69%, which was significantly different from an additive effect of 25% plus 20%. Brodie and Hanson thus demonstrated both an additive and a synergistic effect in exposing rats sequentially to the two stressors food deprivation and restraint.

The Relationship Between Age and Weight and Restraint-Induced Ulceration

As age increases, so does weight (at least with adolescent and young adult rats), so it is difficult to consider the relationships between age and ulceration, and weight and ulceration, as separate factors. They will therefore be considered together.

Weight. Brodie and Hanson (1960) food deprived rats of varying weights (50, 92, 185, 265gms) for 48 hours, and then restrained them for 7 hours. They found that as weight increased, ulceration decreased. When Brodie and Hanson repeated the experiment using 24 hours of restraint rather than 7 hours, and different weight categories (90, 150, 350gms), the relationship disappeared, and the incidence of ulceration was the same in all groups.

The relationship between ulceration and weight has also been investigated by Lambert (1968). Lambert restrained three groups of rats of varying weights, in wire corslets suspended from a stand, for 24 hours. Lambert found that as the weight of the rat increased, ulceration decreased: 86% of rats of weight 100-150gms ulcerated; 59% of rats 150-200gms

ulcerated; and 41% of rats over 200gms ulcerated.

Age. Mikhail and Broadhurst (1965) restrained rats by wrapping them in gauze and plaster of Paris bandages. Younger rats (98-131days) ulcerated more severely than older rats (272-328days), even though the younger rats were restrained for 36 hours, and the older ones for 48 hours.

Wilson (1966) restrained male and female rats in plaster of Paris bandage for 24 hours, following 24 hours of prior food deprivation. A second group of rats was similarly deprived of food for a total of 48 hours, but not restrained. In both the restraint and control conditions, Wilson had four subgroups of rats, divided according to age: 42 days, 56days, 70days, and 84 days. Wilson found that as age increased, the difference in ulceration scores between the restraint group and the control group decreased. More specifically, the results demonstrated that older rats developed fewer restraint-induced ulcers than younger rats.

Most of the evidence suggests that the older or heavier a rat, the less it will ulcerate when restrained. However, there may be a very simple

explanation for the results that has nothing at all to do with what appears to be the greater tolerance of older rats for restraint stress. Shay, Komarov, Fels, Meranze, Gruenstein, and Siplet (1945) have reported that the heavier a rat, the longer it takes for the rat's stomach to be emptied of food. Shay et al. found that Wistar rats weighing less than 180gms required 48 hours of starvation before their stomachs were evacuated; rats weighing more than 180gms required up to 72 hours of starvation before evacuation was complete.

These findings by Shay et al. can be used to account for the results of studies investigating the relationship between the age/weight factor and restraint-induced ulceration. If it is assumed that the presence of food in the rat's stomach protects the rat against the development of stomach ulcers, then the longer food remains in the stomach of a rat being restrained, the less likely it will be that ulceration will develop. Since food remains in the stomachs of heavier (and older) rats longer than it remains in the stomachs of lighter (and younger) rats, heavier and older rats will be less likely to develop ulceration. Even though it is known that

restraint increases gastric motility such that the stomachs of restrained rats empty faster than the stomachs of unrestrained rats (see Chapter 3), the general principle still holds that the older or heavier a rat, the longer it will take for that rat's stomach to empty, and the less likely it is that that rat will ulcerate.

One more comment needs to be made before all the results relating to the age/weight factor can be accounted for, and that comment concerns measurement techniques. In the studies that have been discussed in this section, two different measures of ulceration have been used. The first measure is the percentage of rats in a group developing ulcers, i.e. incidence; and the second measure is one of severity of ulceration. The former measure is based on an all-or-none criterion: no distinction is made between rats with few ulcers and rats with many ulcers. However, the severity measure is sensitive to differences among rats that have ulcerated, and is based on the extent of ulceration in each rat.

Now, if the combined food deprivation and restraint periods are of sufficient duration, most rats will eventually ulcerate, irrespective of their

weight/age. Thus, if the food deprivation plus restraint stress continues for long enough, incidences of ulceration in all weight/age groups will eventually approximate 100%. However, with prolonged stress, a severity measure of ulceration would continue to reflect the differences among different weight/age categories, because ulceration can become more severe and more prolific as time progresses.

If one bears in mind the measurement techniques used in each study, all the data discussed in this section can be accounted for in terms of differential emptying of the stomach. However, rather than describe verbally how the data can be explained, the experimental results have been summarized, and explanatory comments added, in Table 1, on page 36.

Other Variables Studied in Relation to Restraint-Induced Ulceration

Relationship Between Sex of Rat and Restraint-Induced Ulceration

A number of investigators have compared restraint-induced ulceration levels in male and female rats. The comparisons indicate either that females ulcerate

Table 1

Summary and Explanation of Results of Experiments Studying the Relationship Between Age/Weight of Rats and Restraint-Induced Ulceration

Experiment	Age or Weight	Food Depr.	Restr.	Total Depr.	Ulcer Measure	Results	Explanatory Comment
Brodie & Hanson Exp.1	50gms to 265gms	48hrs	7hrs	55hrs	incidence	heavier rats ulcerate less	total deprivation period is not sufficiently long for all rats to develop ulceration
Brodie & Hanson Exp.2	90gms to 350gms	48hrs	24hrs	72hrs	incidence	no difference among groups	total deprivation period sufficiently long for incidence to be the same in all groups
Lambert	100gms to 200gms+	0hrs	24hrs	24hrs	incidence	heavier rats ulcerate less	total deprivation period is not sufficiently long for all rats to develop ulceration
Mikhail & Broadhurst	98days to 328days	0hrs	36hrs to 48hrs	36hrs to 48hrs	severity	older rats ulcerate less	severity of ulceration a function of differential emptying of stomach
Wilson	42days to 84days	24hrs	24hrs	48hrs	severity	older rats ulcerate less	severity of ulceration a function of differential emptying of stomach

more than males do, or that there are no differences between males and females.

Sines has compared male and female ulceration rates on a number of occasions. In the first study in which he studied the sex variable, Sines restrained male and female Sprague-Dawley rats in gauze and plaster of Paris bandages for 48 hours, and found that 58% of males developed ulcers, as compared with 68% of females (Sines, 1959). In 1961, Sines confirmed the difference between Sprague-Dawley male and female rats, but could not find any differences between the males and females of a selectively bred stress ulcer susceptible (SUS) strain. In the 1961 experiment, Sines restrained his rats in wire mesh, but for only 12 hours.

In a third experiment, Sines (1962) compared the male and female ulceration rates of five strains of laboratory rats: Sprague-Dawley, August 33322, A x C Irish, Fischer 344, and the fourth generation of the SUS strain. As in the 1961 experiment, Sines restrained the rats for only 12 hours. He found that in all strains, there were no differences between males and females. In this experiment, Sines failed to confirm the sex difference previously found in the Sprague-

Dawley strain.

Sex comparisons have also been made by a number of other investigators; for example, Ader, Beels, and Tatum (1960a), Herner and Caul (1972), Lambert (1968), and Mikhail and Broadhurst (1965). Mikhail and Broadhurst restrained old male and female rats for 48 hours, and young male and female rats for 36 hours, in gauze and plaster of Paris bandages. They found that there were no differences in ulceration between the two sexes.

Ader, Beels, and Tatum restrained male and female rats of three strains (Wistar, Sprague-Dawley, and Long-Evans) for 20 hours in restraining wire cages, and found that females ulcerated more than males. Ader et al. also suggested the possibility of an interaction between strain and sex. However, Ader et al.'s results should be interpreted cautiously, because of a failure by the experimenters to control the age/weight factor, or to control the pre-experimental histories of the rats.

Lambert restrained Wistar male and female rats for 24 hours in wire corslets suspended from a stand. He found that significantly more females (67%) than males (56%) developed stress ulceration. Herner and

Caul restrained males, diestrous females, and estrous females, each in a plastic restraining apparatus, for 19 hours. They found that diestrous females ulcerated significantly more than males, but there were no differences between males and estrous females.

Thus, it has been found by Sines, by Ader, Beels, and Tatum, by Lambert, and by Herner and Caul that females ulcerate more than males; and by Sines, and by Mikhail and Broadhurst that there are no differences between the sexes. It has been suggested by Sawrey and Long (1962) that the greater restraint-induced ulceration in females (in those experiments that have found such a result) can be accounted for by a greater frustration of activity in those female rats in estrous at the time of restraint. Sawrey and Long suggest that of the females restrained, those in estrous are physiologically more prepared to be active, and the complete restriction of activity in restraint will therefore be more stressful for such rats, than it would be for diestrous females or for males.

The hypothesis is a plausible one, but the evidence from the experiment by Herner and Caul does not support it. Herner and Caul found that female rats prepared to be active, i.e. female rats in

estrous, did not ulcerate significantly differently from male rats. It was the female rats in the trough of the estrous cycle that ulcerated more than the males.

There are other factors that should be considered in comparing the results of studies examining sex differences. Methodological conditions are rarely standardized from experiment to experiment: for example, the strain of rat, the method of restraint, and the duration of restraint. Another factor to be taken into account is the difference in weights between males and females within any one experiment. Since the heavier a rat, the less likely it is to ulcerate, the fact that males are heavier than females of the same age presents a problem for the interpretation of any differences in ulceration found between the sexes.

Relationship Between Strain of Rat and Restraint-Induced Ulceration

It has been demonstrated by Sines (1962), and by Ader, Beels, and Tatum (1960a), that ulceration can vary with the strain of laboratory rat being used. Sines restrained male and female rats from

different strains for 12 hours. The strains he selected from were: Sprague-Dawley, Fischer 344, August 33322, A x C Irish, and the fourth generation of selectively bred stress ulcer susceptible (SUS) rats. Among the males, Sines found that the SUS rats ulcerated significantly more than the rats of all other strains; A x C Irish ulcerated more than Sprague-Dawley, and Fischer 344 ulcerated more than August 33322 and Sprague-Dawley. Among the females, the SUS rats ulcerated significantly more than the rats of the Sprague-Dawley, August 33322, and A x C Irish strains. There were no other differences among the females.

Ader, Beels, and Tatum also found that strain differences in restraint-induced ulceration vary with the sex of the rat. Ader et al. restrained rats from the Wistar, Sprague-Dawley, and Long-Evans strains for 20 hours in adjustable restraint cages. Ader et al. found that Wistar females ulcerated significantly more than Sprague-Dawley and Long-Evans females. There were too few males in the experiment to make any reliable comparisons. Ader et al. later replicated the experiment using only males of the three strains, but there were no significant strain differ-

ences in ulceration. A further replication with females demonstrated again that Wistar females ulcerated more than Sprague-Dawley females, but there was no confirmation of the difference between Wistar females and Long-Evans females.

Ader et al.'s results confirm the general trend of Sines's results; i.e. that there are strain differences in restraint-induced ulceration, but these differences appear to vary with the sex of the rat. However, Ader et al.'s results should be interpreted cautiously, because, as was stated in the previous section, Ader, Beels, and Tatum did not control for the age/weight factor, or for the pre-experimental histories of the rats.

Weinstein and Driscoll (1972) restrained wild rats (Rattus norvegicus) in wire mesh cocoons. The rats had to be food deprived prior to restraint for at least 48 hours, and restrained for an additional 24 hours before an incidence of ulceration occurred comparable to that obtained with laboratory rats after 24 hours restraint but with no prior food deprivation. There were no differences between males and females. Thus, laboratory rats are far more susceptible to the ulcer-producing effects of restraint than are wild rats.

The most obvious problem that arises in investigating strain differences in restraint-induced ulceration is that similarly aged rats of different strains are invariably of different weights. Thus, the relationship between weight and ulceration is again a factor in the interpretation of differences in reaction to restraint.

Relationship Between Season and Restraint-Induced Ulceration

Lambert (1968) and Wilson (1971) have both studied the effect of the time of year when the rat is restrained on ulceration levels. Lambert restrained Wistar rats for 24 hours, in wire corslets suspended from a stand, during the months Februarly to July. Lambert found that 78% of rats restrained during April and May (spring) developed ulcers. Of rats restrained during February and March (late winter), 59% developed ulcers; and of rats restrained during June and July (summer), 46% ulcerated.

Wilson carried out an experiment similar to Lambert's, but he obtained different results. Wilson's experiment was more sophisticated than Lambert's. Wilson restrained 64 rats, 32 males and 32 females,

each month, for 12 months. Experimental conditions were well standardized. At the time of restraint (always the 15th or 16th day of the month), the rats were 110 to 120gms, and were housed eight to a cage. Room temperature was maintained during the 12 months at 22°±1°C. The rats were initially food deprived for 24 hours, and then restrained for another 24 hours in plaster of Paris bandage.

Wilson found that severity of ulceration in both male and female rats decreased from January through to June, and then increased again through to December. In other words, ulceration was most severe during winter, and least severe during early summer. Restraint during spring and autumn produced ulceration intermediate in severity.

The results of both Lambert's and Wilson's experiments demonstrate that there is seasonal variation in the ulcerative response to restraint. However, it is also clear that the phenomenon is not a robust one. Its appearance possibly depends on the strain of rat being used, and the laboratory conditions of the experiment.

The Effect of Cold on Restraint-Induced Ulceration

It has been demonstrated that restraint-induced ulceration can be exacerbated if the restrained rat is simultaneously exposed to cold. However, the results of the cold plus restraint experiments are not entirely consistent.

Brodie and Valitski (1963) food deprived male rats for 24 hours, and then restrained them for either 15 minutes, or 30 minutes, or 60 minutes. During the restraint period, the rats were kept in a cold room set at 5°C to 6°C. Brodie and Valitski found that 30 minutes and 60 minutes of cold plus restraint synergized to produce more gastric pathology than the sum of the stressors acting alone. However, Brodie and Valitski also found that the only gastric pathology that occurred was gastric hemorrhage; there were no gastric erosions.

Senay and Levine (1967) restrained female rats in plastic restraint boxes, following 24 hours of food deprivation. The restrained rats were then placed in a cold room, set at 4°C to 7°C, for 2 hours. Senay and Levine found that such conditions produced significantly more gastric erosions in the rats than did the sum of the two stressors acting alone.

Goldenberg (1973) later confirmed the finding by Senay and Levine that cold plus restraint does produce gastric erosions, and not just gastric hemorrhaging as found by Brodie and Valitski. Goldenberg food deprived male rats for 27 hours, then restrained them in a cylindrical metal can, and placed them in a cold room (7°C) for 3 hours.

Both Goldenberg and Senay and Levine used stress periods longer than that used by Brodie and Valitski. This factor might account for the failure by Brodie and Valitski to produce gastric erosions with the combination of the cold and the restraint stressors.

Methods of Restraint Other Than the Bonfils and the Brodie and Hanson Methods

Many investigators studying restraint-induced ulceration have used either the Bonfils method - for example, Lambert (1968), Menguy (1960), and Sawrey and Sawrey (1964) - or Brodie and Hanson's more simplified method - for example, Ackerman, Hofer, and Weiner (1975), Ader (1963, 1964, 1965, 1970a), Ader and Plaut (1968), Guth and Mendick (1964), Lovibond (1969), Sawrey and Sawrey (1968), and Weinstein and Driscoll (1972). New techniques have

also been introduced, and the number of methods is rapidly increasing.

It is important to know whether different methods of restraint yield similar ulcerogenic effects. Some researchers (e.g. Brodie, 1971; and Dubrasquet, Sergent, Lewin, and Bonfils, 1971) have claimed that there are no differences in ulceration produced by different restraint methods. Bonfils and Lambling (1963) in particular claim that the "technique used to establish complete immobilization is of little importance, as the results are always the same: the erosions occur in the glandular mucous membrane, their incidence ranges from 85 to 100 per cent after twenty-four hours of restraint" (p.154). In the following section, techniques other than the Bonfils and the Brodie and Hanson ones will be briefly reviewed, to ascertain whether any differences in ulceration produced by the various methods do exist.

Weininger (1954, 1956) carried out a study in restraint-induced ulceration even prior to the publication of the French work. Together with a group of Japanese workers who published a study in restraint-induced ulceration in Tokyo in 1953 (Pfeiffer, 1971),

Weininger was among the first after Selye to use the restraint method. Weininger restrained rats by wrapping them in soft gauze bandage, binding them with adhesive tape, then placing them on their backs for 48 hours. Unfortunately, Weininger does not give any details as to the nature of the ulceration produced with this method, other than that "bleeding points" resulted (Weininger, 1954, p.285).

Early in the development of restraint procedures, a number of investigators began to use plaster of Paris bandage; among them were Mikhail and Broadhurst (1965), Sines (1959, 1960), Stern, Winokur, Eisenstein, Taylor, and Sly (1960), and Winokur, Stern, and Taylor (1959). The procedure usually adopted was to wrap the rat in gauze bandage first, and then cover it in plaster of Paris bandage. The rat's head and tail were left free. Researchers using this method usually restrained their rats for 48 hours; they found gastric ulceration similar in nature to that found by Brodie and by Bonfils: erosion of the fundus of the gastric mucosa.

Mikhail and Holland (1966) simplified the plaster of Paris technique used by Sines and the others. Mikhail and Holland made open-ended plaster of Paris

corslets, which were then cut down one side. The corslet was placed around the rat's abdomen and thorax, and was then secured with rubber bands. Using a prior food deprivation period of 12 hours, Mikhail and Holland found a 0% incidence of ulceration after 12 hours encorsletation, 75% after 24 hours, and 50% after 36 hours.

Although Mikhail has consistently used and recommended this technique (Mikhail, 1969, 1972, 1973), it varies from other methods of restraint in one important respect. Encorsleting rats does not produce ulceration in the fundus of the glandular section of the stomach, the usual site of restraint-induced ulcers. Rather, encorsleting produces ulcers chiefly in the antrum of the rat's stomach, an area of the glandular section of the stomach close to the duodenum. The antrum consists of paler and thinner mucosal tissue than the fundus, and contains few of the acid-secreting parietal cells which proliferate in the fundus (Lambert, 1965).

Mikhail and Holland have described the lesions as "discrete circular white areas of less than 1.5 mm in diameter, often with tiny brown centres" (Mikhail & Holland, 1966, p.346). This description is differ-

ent from that given by Bonfils and by Brodie and Hanson of the gastric erosions produced by their methods of restraint. Therefore, it is important to be cautious in making comparisons between the results of Mikhail's studies and the results of studies obtaining more conventional stress ulcers.

In more recent years, there has been a move among some researchers towards using rigid, but adjustable constructions, rather than flexible materials, in order to restrain rats. Mezinskis, Gliner, and Shemberg (1971), Price (1972), and Senay and Levine (1967) have all used restraining boxes or cages, in which the space available to the rat is restricted by adjustable panels or by removable rods. The description of the gastric ulceration that results from the use of such restraining cages indicates that the ulceration produced is comparable to that of standard restraint. However, the technique does not always reliably produce ulceration. Price, for example, when he used a restraining box, found blood clots and inflammation, but no erosions of the gastric mucosa.

Caul and Buchanan (1971) have developed a rat restrainer which operates on the basis of leg clamps

and body plungers. Caul and Buchanan have found the technique to produce ulceration in approximately one-third of male Holtzman rats, after 19 hours of restraint and 24 hours of prior food deprivation. Caul, Buchanan, and Hays (1972) describe the nature of the resultant gastric pathology, and claim that it is comparable to that produced by other standard methods of restraint.

The difficulty with making cross-study comparisons to ascertain the effectiveness of a particular method of restraint is that experimental designs and procedures are not standardized. Although it is claimed that most restraint procedures produce ulceration similar to that produced by the standard Brodie and Hanson method, many investigators do not give a clear description of what they regard as a standard restraint effect. For example, some researchers use the terms "lesion" and "ulcer" interchangeably. Lesions however refer not only to ulcers, but also to other types of gastric pathology, such as gastritis, petechiae, and large hemorrhagic spots. While many of the methods discussed above might produce gastric lesions, it is another matter whether they also consistently produce stress ulcers.

If a method of restraint is established as one which reliably produces stress ulceration, it is then another matter whether that method efficiently produces such ulceration. Unfortunately, it is difficult to make decisions about the efficiency of restraint procedures, because factors such as strain and age of rat, duration of restraint, and duration, if any, of a prior food deprivation period, are never held constant. To answer these questions, it is necessary to compare different methods of restraint within the one experimental design. Singh (1971) has carried out such an experiment.

Singh compared six types of restraint procedures for their effectiveness in producing stress ulceration. The methods used were the following:

(1) the rat was taped to a thick board with adhesive; its head and tail were free;

(2) the rat was clamped in three places (head, middle, end) to a wire mesh sheet;

(3) the rat was placed in a wire mesh cage, and its space was restricted by the insertion of wire rods;

(4) the rat was wrapped in flexible wire mesh, and its position secured with staples; the

tail was free;

(5) the rat was placed into a tight-fitting wire mesh cylindrical tube with a wire mesh cone for its head;

(6) the rat was placed into a sheet metal cylindrical tube with a wire gauze cone for its head.

Singh found that after 24 hours of restraint, only those rats restrained using methods (4), (5), and (6) developed stress ulceration. Rats restrained using methods (1), (2), and (3) developed pinpoint, superficial hemorrhage, but ulcers were not present. Of those methods effective in producing ulceration, method (4) induced an ulcer incidence of 60%, method (5) an incidence of 90%, and method (6) an incidence of 100%. Severity of ulceration also changed with the method used, and the most severe ulceration was produced by method (6).

On the basis of Singh's results, it is legitimate to conclude that there <u>are</u> valid differences in the ulcerative effects of different methods of restraint. Certainly, there is substantial leeway for variation, but not as much as some investigators have suggested. One important variable appears to be whether the rat (particularly its head) is enclosed

or not, or whether it is simply not permitted to move. In making cross-study comparisons, consideration should always be given to the methods of restraint employed. Furthermore, new methods of restraint should be carefully examined to establish the exact nature of their ulcerogenic effects.

The Use of Restraint-Induced Ulceration to Measure the Effects of Superimposed Stimuli

The restraint method of inducing stress ulceration has been used as a methodological device for ascertaining the properties of other psychological variables. The paradigm is a simple one: it involves measuring changes in restraint-induced ulceration produced by presenting other stimuli to rats while they are restrained. Any change in ulceration above or below a restraint-only control level is assumed to reflect the excitatory or inhibitory arousal properties of the stimulus being presented. The assumption is made that excitatory stimuli increase the general stress the rat is experiencing, and thus will increase the level of ulceration; and conversely, inhibitory stimuli decrease the general stress, and thus the subsequent ulceration.

The technique was first used by Sawrey and Sawrey in 1964. Sawrey and Sawrey classically conditioned rats by pairing a conditioned stimulus (CS) of light and buzzer with an unconditioned stimulus (UCS) of shock. Sawrey and Sawrey later restrained the rats for 48 hours, and presented the CS during restraint. A group of rats that received 80 such conditioning trials showed an ulceration rate significantly above that of rats not exposed to the prior conditioning trials. Rats that received only 20 conditioning trials did not ulcerate differently from rats that were restrained but not conditioned.

Unfortunately, it is difficult to interpret Sawrey and Sawrey's results. Sawrey and Sawrey did not control for the possible ulcerogenic effects of shock during the conditioning period, which was held only 47 hours prior to restraint and while the rats were food deprived. The difference in restraint-induced ulceration between the no-conditioning group and the group exposed to 80 conditioning trials could have resulted from the 5.3 hours of shock received by the conditioning group prior to restraint, rather than from the effects of conditioned fear superimposed on restraint.

In a second experiment, Sawrey and Sawrey (1968) presented both the conditioning trials and the CS-test trials while the rats were restrained. Sawrey and Sawrey used a light CS, and paired it with different intensities and durations of electric shock. After 80 presentations of the CS, half of which were followed by the UCS, the CS was presented alone for the remainder of a 48 hour restraint period. Sawrey and Sawrey found that rats receiving the most intense shocks ulcerated more than rats receiving lesser intensities. As with the Sawrey and Sawrey 1964 study, it is not possible to isolate the relevant contributions made by shock and by conditioned fear to the changes in ulceration.

Lovibond (1969) and Weiss (1970) have also used restraint to demonstrate the effects of conditioned stimuli. Lovibond trained rats in a runway to avoid shock at the sound of a buzzer, and to approach food at the sight of a light. The rats were then restrained in wire mesh for 24 hours, following 24 hours food deprivation; different groups (all of which received the same runway training) received different patterns of buzzer and light stimulation. Lovibond demonstrated that restraint ulceration could be

inhibited by the presentation during restraint of the signal for food, the light; and could be exacerbated by the simultaneous presentation of light and buzzer, which Lovibond saw as representing a conflict situation. Lovibond was not able to demonstrate exacerbation of ulceration through presentation of the signal for shock alone, the buzzer.

Weiss (1970) lightly restrained rats in tube hardware cloth for 19 hours. One group of rats was subjected to signalled (i.e. predictable) shock; another group to random presentations of the CS and the UCS (unpredictable shock); and a third group to restraint only, without any signals or shocks. Weiss found that rats receiving predictable shock ulcerated significantly more than rats simply restrained; and rats receiving unpredictable shock ulcerated more than rats receiving an equivalent amount of predictable shock.

Weiss's design of presenting an equivalent amount of either predictable or unpredictable shock to restrained rats has been used by other researchers as well. Mezinskis, Gliner, and Shemberg (1971) and Price (1972) both found less ulceration in restrained rats able to predict shock than in restr-

ained rats not able to predict shock.

Caul, Buchanan, and Hays (1972), while using a similar design to that of Weiss's, found somewhat different results. Caul et al. kept rats in plastic restrainers for 19 hours, and found that rats receiving unpredictable shock ulcerated more than rats receiving predictable shock. However, rats receiving predictable shock did not ulcerate more than rats not receiving any shock at all.

Mikhail (1969, 1971, 1972) has been very critical of attempts to demonstrate the effects of superimposed stimuli, particularly conditioned fear stimuli, by measuring stress ulceration. Mikhail has conducted a number of experiments to show that conditioned stimuli do not alter restraint-induced ulceration rates. In one set of experiments, Mikhail (1969) exposed rats to conditioning trials, and then presented the conditioned stimulus while the rats were encorsleted for 24 hours in plaster of Paris cylinders. Mikhail demonstrated that the presentation of conditioned stimuli did not increase ulceration above a control level, even though in one experiment, Mikhail presented additional shock while the rat was encorsleted.

Mikhail supports his experimental results by claiming that it is theoretically impossible to increase gastric ulceration by the presentation of fear stimuli during ulcer-inducing procedures. Mikhail bases this claim on the assumption that conditioned fear results in sympathetic nervous system activity, which is incompatible with the appearance of gastric ulceration. Mikhail assumes that such ulceration is primarily the result of parasympathetic nervous system activity (Mikhail, 1969, 1971, 1972).

A number of criticisms can be made of Mikhail's experimental work and of his arguments. First, Mikhail's method of inducing initial ulceration cannot be considered comparable to standard restraint. As was noted in an earlier section of the Chapter, encorsletation produces ulceration primarily in the antrum of the rat's stomach. Therefore, Mikhail's results cannot be considered as evidence that superimposed stimuli do not exacerbate standard restraint-induced ulceration.

Mikhail can be further criticized because of his claim that it is physiologically impossible to exacerbate ulceration through the presentation of conditioned fear stimuli. The physiological mediation

of stress ulcers is by no means clear, and it is unlikely to be as simple as Mikhail suggests. Even though parasympathetic activity is probably involved in the production of stress ulcers, and sympathetic activity is involved in the production of conditioned fear, there is no justification for rejecting experimental evidence that the two stressors (restraint and fear) can combine, perhaps even synergize, to produce an increased stress ulceration effect.

The results presented in this section do demonstrate that restraint-induced ulceration can be exacerbated by superimposed stimuli. Some studies demonstrate that psychological factors, in conjunction with the physical stressor shock, have an effect on restraint-induced ulceration. Other studies have shown that psychological factors alone are sufficient to exacerbate stress ulceration. Contrary to the claim that Mikhail persistently makes, the fact that the superimposed stressors are different in nature from the restraint stressor appears to be irrelevant.

CONFLICT

The conflict technique of inducing gastric ulcers in laboratory rats was introduced at about the same time Bonfils and his colleagues were popularizing the restraint method. In 1956, Sawrey and Weisz published the details of a study in which they had placed rats in an approach-avoidance conflict situation for 30 days, and had found ulcers "in the lower region ... of the stomach" after that time (Sawrey & Weisz, 1956, p.269).

The method Sawrey and Weisz used was the following. They placed rats in a runway, in which food was available at one end, and water at the other end. The sections of the runway floor immediately adjacent to the food and water receptacles were electrically charged, such that the rat had to endure shock in order to eat or drink. The middle section of the runway floor was not charged. Every 48th hour, the shock was turned off, thus allowing the rat free access to food and water for that hour. Groups of three rats at a time lived in the runway for 30 days. A control group of rats, living in home cages, was kept on 47 hours food and water deprivation for the duration of the experiment. Sawrey and Weisz found

that "lesions" (presumably glandular) developed in six out of nine of the conflict rats, whereas no lesions developed in the food deprivation control rats.

Sawrey and Weisz's study in conflict-induced stomach ulceration, while being suggestive of the ulcerogenic effects of conflict, did not control for the possible contribution of shock to the ulceration observed. A further study was planned to separate both the independent and the interactive effects of shock, hunger, and thirst (Sawrey, Conger, & Turrell, 1956). A similar paradigm to that used by Sawrey and Weisz was employed, except that rats lived in the conflict situation alone rather than in triplets.

In their experiment, Sawrey, Conger, and Turrell found that all ulceration developed in the upper, non acid-secreting section of the rat's stomach, the rumen, and not in the "lower" section as had been found by Sawrey and Weisz. Sawrey et al. found further that 76% of rats exposed to a conflict situation, with its associated shock and deprivation schedules, developed such rumenal ulceration.

Through an intricate system of control groups,

Sawrey et al. established that exposure to shock alone contributed nothing to this 76% incidence; similarly, exposure to thirst alone, or in combination with the shock, contributed nothing. Food deprivation alone resulted in an ulcer incidence of 20%, and when combined with shock, the incidence increased to 40%. When thirst was added to food deprivation and shock, the incidence dropped back to 30%. (Thirst, it appeared, had an attenuating effect on conflict-induced ulceration.) Thus, on the basis of these results, Sawrey, Conger, and Turrell concluded that conflict itself is ulcerogenic.

Two problems arise in the interpretation of Sawrey et al.'s data. The first problem is that differential amounts of food were probably consumed by the different groups of rats, particularly since the rats in the conflict group lost significantly more weight than the rats in the control groups. Now, it is well established that long term interruptions to feeding schedules produce rumenal ulceration in rats (Lambert, 1965; Mikhail, 1973; Paré & Temple, 1973). Therefore, the differences in ulceration could be attributed to the differences in the amount of food consumed during the conflict period, rather

than to the stress engendered by the conflict itself.

The second problem is that the control groups receiving shock, either alone or in conjunction with food and/or water deprivation, were wired in series with the conflict group, such that the shock control rats were receiving what was to them unpredictable shock. On the other hand, the conflict group was receiving shock that was contingent on the rats in that group approaching the food and water receptacles; i.e. they were receiving predictable shock. Some researchers (e.g. Dykman, Gantt, & Whitehorn, 1956; and Paré, 1964) have suggested that chronically predictable shock is more stressful than chronically unpredictable shock. Thus, the control groups may have ulcerated less because the chronic shock they were receiving was unpredictable rather than predictable. However, in spite of these objections, Sawrey et al.'s results are still suggestive that conflict, in interaction with shock and food and water deprivation, might be ulcerogenic.

Paré (1962) introduced a different experimental paradigm to study the ulcerogenic effects of conflict situations. He conducted a conflict study where rats had to shuttle backwards and forwards across an

electrified grid to obtain water, but they were never shocked while they were drinking. The rats lived in the conflict situation for 8 hours a day, every day for 4 weeks. They were also maintained on a 23 hour food deprivation schedule.

Paré compared the gastric pathology in the approach-avoidance conflict group with that in a control group which received water only when the conflict group received it; and that in a control group which received both water and shock when the conflict group received them. Gastric erosions were not found in any rats. However, Paré did find that mild gastric pathology (as measured by loss of mucous and clotted blood) occurred more often in the water and shock control group than in any other group. Paré's results offer no support at all for the claim that conflict itself induces stress ulceration.

In a later experiment, Paré (1972a) reverted to the runway method introduced by Sawrey and Weisz in 1956. A control group of rats was maintained on a 47 hour food and water deprivation schedule, and was wired in series to a conflict group to receive identical shocks. A second control group received no shocks, but was maintained on the 47 hour depriv-

ation schedule. Paré found that ulceration developed mainly in the rumens of the rats' stomachs; he also found that there were no differences in ulceration between the conflict and the control groups. As in the 1962 experiment, Paré's results provided no support for the idea that conflict itself is ulcerogenic.

Moot, Cebulla, and Crabtree (1970) introduced yet another paradigm to investigate conflict-induced ulceration. Moot et al. used a Skinner box, in which the rat was required to bar press to obtain food. In the conflict group, bar-pressing was accompanied by shock; in the control group, there were presentations of food and shock which were always contingent on the food and shock presentations in the conflict group. The rats were kept in the conflict situation for three test sessions, each session lasting 20 hours. Unlike most other conflict studies, Moot et al.'s study produced ulceration in the glandular section of the stomach. However, as in the Paré 1962 and 1972 experiments, there were no differences in ulceration between the conflict group and the group controlling for the effects of shock and food deprivation.

Using the paradigm introduced by Moot, Cebulla,

and Crabtree, Paré (1972b) investigated another aspect of the conflict situation. Paré was interested in the temporal relationship between the positive and negative consequences of action in a conflict situation. He exposed rats to three conflict sessions of 22 hours each. During the conflict session, each rat was placed in a Skinner box, and was required to press a lever in order to obtain food. Paré then compared the effects of four different temporal relationships between the administration of food and the administration of an accompanying shock.

In group one, the rats were shocked when they pressed the lever; in group two, they were shocked as food was being delivered to the food hopper; in group three, shock came as the rats opened the hopper door; and in group four, the rats were shocked as they were eating. Each conflict group had two control groups: one control group received the same shock and food presentations as the conflict group, and the second control group received the same food presentations. Neither control group had access to a lever.

Unlike Moot, Cebulla, and Crabtree, Paré found that ulceration was chiefly rumenal. Furthermore,

the only condition where the conflict group ulcerated more than its control groups was the one where rats were shocked as they were eating. However, although Paré provided the conflict and the control rats with equivalent amounts of food, there still remains the problem of whether differential amounts of food were ingested by the rats in the two groups. Ader, Tatum, and Beels (1960), in an experiment in conflict-induced ulceration, found that conflict rats who developed ulcers ate and drank significantly less than conflict rats who did not develop ulcers. In his 1972b experiment, Paré does present evidence to show that the amount of weight lost by the rats in the conflict group was not significantly different from the amount lost by the rats in the control groups, but this does not necessarily mean that the amount of food ingested by the two groups was sufficiently different to affect the extent of rumenal ulceration.

Paré's finding that a significant difference in ulceration between the conflict group and the control groups only occurs if the shock is administered at the same time as the food is being eaten, has been indirectly supported by results from other studies.

Paré (1962) found that rats able to drink after they had crossed an electrified grid did not ulcerate more than the control rats. Similarly, Moot, Cebulla, and Crabtree (1970) found that rats shocked when they pressed a bar for food did not differ in ulceration from rats in a control group.

Comment on the Conflict Technique

The evidence so far has not demonstrated conclusively that conflict itself is ulcerogenic. Although some studies have found a difference in ulceration between conflict groups and control groups, the control groups have never been completely adequate. First, there has not been sufficient control over the amount of food consumed in the different groups. Secondly, control groups invariably receive chronic shock that is unpredictable, whereas the conflict group receives chronic shock that is predictable.

A further difficulty is that most conflict studies produce ulceration in the rumen of the rat's stomach. The rumen in the rat is functionally not a true stomach, but a storage cavity covered internally with an esophageal type of tissue: non acid-secreting squamous epithelium. Ulcers in the rat's rumen are

typically produced by the method of pylorus ligation, by which acid accumulates in the glandular section of the stomach, and eventually refluxes past the limiting ridge into the rumen, and erodes the tissue (Lambert, 1965). Rumenal ulcers are also produced in the rat by starvation, or by interfering in some other way with feeding schedules (Lambert, 1965; Paré & Temple, 1973; Sun & Chen, 1963). True stress ulcers are typically found in the glandular section of the stomach (Lambert, 1965), and there has never been any reason to suggest - apart from the evidence from the conflict studies - that psychological stress induces rumenal ulceration in rats.

It can be tentatively suggested that in conflict situations typically employed, rumenal ulceration is initially induced by a severe and chronic disruption of normal feeding schedules, including being required to eat while being shocked. This ulceration might then be exacerbated because of a concomitant exposure to other stressors; for example, shock, if it is sufficiently aversive, and its associated properties (predictability/unpredictability). Psychological conflict might prove to be one such exacerbatory stressor, but as yet, the evidence to say that it is,

is not very strong.

Parametric Studies in Conflict-Induced Ulceration

Even though there is doubt as to the ulcerogenic nature of conflict itself, a number of parametric studies have been conducted to investigate the factors that affect conflict-induced ulceration. These studies will be described very briefly under three headings: duration of conflict, testing conditions, and characteristics of the rat.

Duration of Conflict

Ader, Beels, and Tatum (1960b) found that rats living in a conflict situation for 8 days developed significantly more rumenal ulceration than rats living in a conflict for 4 days. Paré (1972a) found that after 7 days of conflict, ulceration levelled off: 10 days of conflict did not produce significantly more ulceration than 7 days; and after 21 days, ulceration was significantly reduced below both the 7 and the 10 days level. Paré also found that a feeding schedule of 1 hour every 48 hours led to more ulcers in the conflict situation than a schedule of 1 hour

every 24 hours.

Testing Conditions

Conger, Sawrey, and Turrell (1958) found that rats tested in a runway together (i.e. more than one rat living in the runway) ulcerated less than rats tested alone. Sawrey and Sawrey (1966) also found that rats tested alone ulcerated more than rats tested together. However, Ader, Beels, and Tatum (1960b) found that rats tested in pairs ulcerated the same as rats tested alone.

Characteristics of the Rat

Sawrey and Long (1962) used the runway situation to examine the relationship between sex and strain of rat, and conflict-induced ulceration. Sawrey and Long found that, of four strains of rats (Long-Evans, Wistar, Sprague-Dawley, and Nebraska Hooded), male and female Nebraska Hoodeds were very resistant to ulceration. So also were Wistar females. On the other hand, Paré (1972a) found that Long-Evans rats ulcerated more than Sprague-Dawleys and Wistars.

Sawrey and Sawrey (1966) found that older rats

(164 days) ulcerated more than younger ones (84 days and 124 days). However, this experiment varied from other conflict experiments in that no food and water were available during the period when the runway grid was electrified. Therefore, it is questionable whether the rats were in conflict or not, as they had no motivation to endure the shock. Paré (1972a), in a standard conflict situation, found no differences in ulceration between 50 day old rats and 120 day old rats.

SHOCK

The third paradigm used to induce gastric ulcers in laboratory rats involves the use of the aversive stimulus electric shock, and the manipulation of its associated properties. The technique originated in a study by Brady, Porter, Conrad, and Mason in 1958. Brady et al. were investigating avoidance-escape behaviour in rhesus monkeys. They found that after the monkeys had been maintained on an avoidance-escape schedule for 6 to 7 weeks, the monkeys developed gastrointestinal ulcers. Control monkeys, who received the same shocks as the avoidance-escape monkeys, did not develop any ulcers.

Since the time of Brady et al.'s study, shock paradigms have become popular as methods of inducing ulceration in laboratory rats. The usual procedure is to manipulate one or more of the psychological properties of the stressor. The variables investigated most frequently are shock controllability and shock predictability.

Shock Controllability

A number of different paradigms have been used

to study the ulcerogenic nature of the controllability factor. Those most commonly used are: active avoidance-escape situations, escape only situations, and passive avoidance-escape situations.

Active Avoidance-Escape Paradigms

Rice (1963) investigated a feature of the avoidance-escape paradigm that had been used by Brady, Porter, Conrad, and Mason. Brady et al. had used a 6 hours on/6 hours off stress routine with their rhesus monkeys; i.e. the monkeys were required to lever press for 6 hours to control shock, but were then given 6 hours rest free from any shock, followed by another 6 hours of stress, and so on. Brady et al. found that no other stress-rest ratio was successful in producing gastrointestinal ulcers in the monkeys.

Rice placed rats in an avoidance-escape situation for 21 days, and permitted them free access to food and water. The rats were required to turn a wheel to escape or avoid a 2 second shock. Rice then compared the 6 hours on/6 hours off ratio with other stress-rest ratios - 1/1, 2/2, 3/3, 4/4, 5/5, ... 7/7, 8/8, 9/9, 10/10, 11/11, 12/12. Rice found that while

ulceration in the avoidance-escape rats peaked at the 5/5 and 6/6 ratios, ulceration was also produced in the other ratios, with the exception of the 1/1 and 12/12 ratios. The 1/1 and 12/12 ratios did not produce ulceration significantly different from that in a control group of rats, which lived shock-free in the apparatus for the duration of the experiment. Rice does not describe either the nature or the placement of the ulceration that developed in the avoidance-escape rats, other than to refer to it as stomach ulceration.

Paré (1971) conducted an experiment similar to Rice's. He had rats working an avoidance-escape lever on a 6 hours on/6 hours off schedule for 21 days, with free access to food and water. Paré also used a number of different shock intensities: .4mA, 1.0mA, 1.5mA, and 2.0mA. He found that ulceration developed in the glandular section of the stomach, and it occurred only when the shock was 2.0mA. When Paré introduced a 23 hours food and water deprivation schedule, ulceration also occurred when the shock level was 1.5mA. Paré did not find any support for the finding by Brady et al. and by Rice that a 6/6 stress-rest ratio provides the optimal condition for

inducing stress ulceration.

Weiss (1971a) did not use a stress-rest schedule, but required rats to work continuously for 48 hours to avoid and escape tail shock of 1.6mA to 3.4mA. Food was not allowed, but the rats had continuous access to water. Each avoidance-escape rat had a control rat wired in series to receive identical shocks, but the control rat had no control over the shock's occurrence.

Weiss found that control rats developed a considerable amount of glandular ulceration, while rats with control over the occurrence of the shock developed much lower levels of ulceration. Weiss's results demonstrated that a stress-rest schedule was not necessary to produce stress ulceration in an active avoidance-escape situation. Weiss also found, contrary to the results of Brady et al., that uncontrollability of shock is more ulcerogenic than controllability.

Barbaree and Harding (1973) did not confirm Weiss's findings. Barbaree and Harding found that rats with control over the occurrence of shock developed more glandular ulceration than rats with no such control. Rats were food deprived for 4 to 6 hours, and then required to wheel turn to avoid shock,

during a stress period of either 6 or 12 hours. Barbaree and Harding found that the avoidance rats (the shock duration was too short for escape to be possible) turned the wheel so often, that few shocks were experienced by them or the control group wired in series. In the absence of a significant shock stressor, it is to be expected that the control group developed little ulceration.

Escape Only Paradigms

Wald, MacKinnon, and Desiderato (1973), using an escape only situation, supported Weiss's finding that uncontrollability of shock is more ulcerogenic than controllability. Rats were required to alternate between a platform grid and a continuously charged grid floor in order to escape intermittent shock on the platform grid. Wald et al. found that rats able to escape the platform shock ulcerated more than rats wired in series with the escape rats, but without any control over the shock. The rats were food deprived for 48 hours prior to a 6 hours stress session, and the resultant ulceration developed in the glandular section of the stomach.

In a later experiment, Desiderato, MacKinnon,

and Hissom (1974) found that stress ulceration produced by the platform grid/floor grid alternation could be increased by permitting rats 2 hours rest in their home cages following the termination of the stress session. Rats rested in this way ulcerated more than did rats exposed to a further 2 hours of stress. However, the difficulty in interpreting these results is that rats permitted 2 hours rest following the stress period were also allowed free access to water, whereas rats exposed to 2 hours of further stress were not permitted water; i.e. the provision of water for the resting rats may have been the ulcerogenic exacerbator in Desiderato et al.'s experiment rather than the provision of a home cage resting period. Desiderato et al. found that extension of the post-stress rest period to 6, 12, and 24 hours did not significantly increase ulceration above the level obtained with 2 hours of post-stress rest.

Passive Avoidance-Escape Paradigms

Experimenters using passive avoidance-escape situations have obtained more equivocal results than those using situations where the rat can take

direct action to control a stressor. Buchanan and Caul (1974) used a situation in which rats had to remain immobile in order to escape and avoid shock. The rats were food deprived for 24 hours prior to the stress session, and then placed in the passive avoidance-escape situation for 20 hours.

Buchanan and Caul found that glandular lesions developed in rats required to make such passive avoidance responses, but not significantly fewer than the number which developed in rats receiving the same amount of shock, but allowed free movement. (It is interesting to note that in addition, Buchanan and Caul found that the passive avoidance rats ulcerated more than rats subjected to shock and mild body restraint, but less than rats subjected to shock and leg restraint.)

Goesling, Buchholz, and Carreira (1974) similarly used a passive avoidance response with rats. Goesling et al. food deprived rats for 23.5 hours, then stressed them for four sessions of 23.5 hours each. In this experiment, rats able to control shock by remaining immobile ulcerated less than rats receiving shock without having any control over it. Ulceration developed in both the rumenal and the glandular

sections of the rats' stomachs, but chiefly in the glandular section.

Controllability Without Avoidance or Escape

Other aspects of controllability are also relevant to the development of shock-induced ulceration. Gliner (1972) gave rats a choice of receiving predictable shock or unpredictable shock, by allowing them to choose the side of a shuttle box they preferred to occupy. Gliner maintained his rats on a food deprivation schedule for the 5 days of the experiment, and for 3 days prior to its commencement.

During the experiment, rats were stressed for 6 hours a day. Gliner used two levels of shock, .5mA, and 2.5mA, and he found that 2.5mA was significantly more ulcerogenic than .5mA. Rats able to choose between predictable and unpredictable shock ulcerated less than rats who were not able to choose between the schedules. Most ulcers were found in the glandular section of the stomach; a few ulcers were located in the rumen, but these ulcers were not included in the results.

Shock Predictability

The second variable that has been consistently investigated is the predictability or unpredictability of shock. The usual procedure is to present rats with unavoidable shock, and to vary the extent to which the shock can be predicted by the rat.

Sawrey (1961) shocked two groups of rats for 20 hours a day for 14 days. In one group, the shock (2.5mA) always followed a conditioned stimulus (CS), but never a discriminative stimulus (DS). In the second group, the shock followed the CS on 50% of the trials, but also followed the DS on 50% of the trials. Food and water were not permitted during the stress sessions. Ulcers developed in both the rumenal and the glandular sections of the rats' stomachs, but chiefly in the glandular section. Both groups of rats developed ulcers, but rats receiving the relatively unpredictable shock ulcerated more than rats receiving the totally predictable shock.

Paré (1964) was not able to confirm Sawrey's results. He subjected rats to signalled shock of 3mA for 22 hours a day for 23 days. Another group received unsignalled shock for a similar period. The rats were permitted free access to food and water for the

duration of the experiment. Paré found few ulcers in the group receiving predictable shock, and none at all in the group receiving unpredictable shock. Paré considered the absence of feeding restrictions the explanation for why so few ulcers developed.

Gliner and Shemberg (1971) tried to increase shock-induced ulceration by manipulating the presentation of conditioned fear stimuli. Rats were food deprived for 48 hours, then subjected to 36 hours of stress, on a 2/2 stress-rest schedule. During the 2 hours stress, rats were exposed to either predictable or unpredictable shock; during the 2 hours rest, some rats were unstimulated, and others were exposed to the conditioned fear stimulus without the shock. Gliner and Shemberg could not demonstrate that receiving conditioned fear stimuli during the rest period affected ulcer levels; but, as in other experiments, the basic shock paradigm did produce glandular (and some rumenal) ulceration in all the groups of rats.

Comment on the Shock Technique

The shock paradigm has proved a useful one for inducing glandular stress ulceration in rats. However, it is not possible to separate the ulcerogenic effects

of the physical stressor from those of the psychological stressors associated with the shock. At this stage, the evidence suggests that uncontrollable shock is more ulcerogenic than controllable shock, and that unpredictable shock is more ulcerogenic than predictable shock. It can also be concluded that, as with the other paradigms of stress ulceration, gastric pathology can be exacerbated by depriving the rat of food, both prior to and during the stress session. Finally, as might be expected, ulceration increases with an increase in the intensity of the shock being delivered.

CHAPTER 3

THE MEDIATION OF RESTRAINT-INDUCED ULCERATION

The nature of the stress experienced by rats when they are restrained is largely a matter of speculation. There are some investigators - for example, Sawrey (1961), Hartry (1962), Mikhail and Holland (1966), and Wilson (1966) - who have mused over the possible stress states involved. The two states most often discussed in relation to restraint are "fear" and "frustration".

One suggestion is that the restrained rat experiences intense fear because it perceives a serious threat to its survival. Another suggestion is that the restrained rat becomes severely frustrated, and subsequently enraged, at having its normal movement and activity restricted. A third possibility, so far not considered in the literature, is that the restrained rat experiences some combination of fear and frustration. For example, the rat might initially experience fear when it discovers it cannot move, but this fear might eventually give rise to frustration as the rat discovers that its attempts to remove itself from the situation are ineffectual. Alternat-

ively, the rat might initially experience frustration at not being able to move, and then fear when it discovers that nothing can be done to remove the stressor.

Such speculations are anthropomorphic, as there is no direct way of determining the psychological state induced in the rat by restraint. However, there has been a substantial interest in using indirect means, especially data from physiological studies, to make inferences about the nature of the stress experienced by the restrained rat. Considerable evidence exists to show that fear and rage produce different patterns of physiological arousal. For example, Pavlov collected pure gastric juice in isolated pouches of dogs' stomachs, and found that dogs exhibiting fearful behaviour (e.g. cringing and whining) showed a decrease in gastric functioning; dogs engaging in behaviour typical of rage showed signs of increased gastric functioning (Barnett, 1963).

Similar observations to those made by Pavlov have been made by people working with patients with gastric fistulas, the most famous cases being Beaumont and his patient Alexis St. Martin (Beaumont, 1833), and Wolf and his patient Tom (Wolf & Wolff, 1947).

Wolf, for example, noticed that when his patient was anxious, frightened, or depressed, the gastric mucosa became pale, the secretion of HCl decreased, and the muscular movement of the stomach slowed. When the patient became angry, frustrated, or hostile, the gastric mucosa became engorged with blood, gastric secretory activity increased, and there was an increase in gastric motility. Furthermore, the fragility of the mucosa increased to the extent where strong muscular movement sometimes produced small lesions in the mucosa (Wolf, 1965).

The observations made by Pavlov and by Wolf have been partly supported by the results of more carefully controlled experiments; for example, those by Ax (1953), and Funkenstein (1955). Ax and Funkenstein both found that the physiological reaction induced by states of fear and anxiety is similar to that produced by injections of adrenaline. (Adrenaline is a hormone secreted in large amounts by the adrenal medulla.) However, the physiological state induced by anger is similar to that produced by simultaneous injections of adrenaline and noradrenaline. (Noradrenaline is a trans-

mitter substance of the sympathetic nervous system, and is very similar in structure to adrenaline; it is also secreted in large amounts by the adrenal medulla.)

Now, it is also known that one of the actions of adrenaline is to inhibit gastric activity by reducing the blood supply to the mucous membrane of the stomach. On the other hand, noradrenaline possesses minimal inhibitory properties (Stedman's medical dictionary, 1972). Thus, decreased gastric activity appears to be part of the physiological reaction induced by fear, but not part of the reaction induced by anger.

In interpreting the significance of the Ax and Funkenstein data, Gray (1971) has pointed out that the distinction between the two physiological reactions may well represent a general psychological distinction between states of emotional passivity and states of emotional activity, rather than specifically a distinction between fear and anger. Nevertheless, the evidence is suggestive that states such as frustration and rage are more likely to result in gastric pathology than states such as fear and anxiety.

The evidence discussed so far points to the possible role of noradrenergic activity in the development of gastric ulceration. However, most excitatory gastric activity is under the influence of the parasympathetic branch of the autonomic nervous system, mediated in the stomach via the fibers of the 10th cranial nerve, the vagus, and it seems unlikely that the parasympathetic branch is uninvolved in the production of gastric pathology.

The problem is that parasympathetic activity presumably assumes a minor role when animals are emotionally aroused. Such stress is usually dealt with by an increase in sympathetic activity, whether it be predominantly adrenergic or noradrenergic (Milner, 1970). If parasympathetic nervous system activity <u>is</u> involved in the production of gastric pathology, the manner in which it becomes involved is still largely a mystery. Perhaps homeostatic forces produce intense parasympathetic compensatory activity following strong sympathetic action; alternatively, autonomic reflex arc activity, of a very complex nature, might be operating.

In an attempt to account for the development of stress ulceration, some researchers have suggested

general physiological schemes, in which possible routes of mediation are broadly outlined. Selye, for example, proposed that stress operates on the posterior hypothalamus to secrete an adrenocorticotrophic hormone (ACTH) releasing factor, and simultaneously on the anterior hypothalamus to stimulate the vagus nerve. The ACTH releasing factor stimulates the anterior pituitary to release ACTH, which in turn acts on the adrenal cortex to secrete corticosteroids into the blood stream. The action of the corticosteroids results in gastric erosion. At the same time, the vagal hyperactivity, produced by stimulation of the anterior hypothalamus, also results in increased gastric activity (Selye, 1956).

Different aspects of Selye's global model have been discussed by other researchers; for example, the role of vagal activity has been considered by Brodie and Hanson (1960), Goldenberg (1973), and Mikhail (1969), and the role of the adrenal glands by Brodie (1963a). In addition, the importance of vascular changes has been discussed by Bonfils (quoted in Brodie, 1963a), and Brodie (1962).

In assessing the role of various factors in the mediation of restraint-induced ulcers, it is import-

ant to consider what is known specifically about the physiological changes produced by restraint. In the following section, the evidence concerning the role of gastric secretory activity, gastric motility, vascular activity, adrenal-pituitary activity, and vagal activity will be reviewed. Mention will also be made of central amine function, and the contribution that drug studies make to understanding the physiological mediation of restraint-induced ulcers.

Gastric Secretory Activity

Gastric Acid

In clinical gastroenterology, acid gastric juice is considered essential for the development of human peptic ulceration (Truelove & Reynell, 1972), but pathologically more superficial conditions, such as gastritis and stress ulceration, can occur in an achlorhydric stomach (Thompson, Berkowitz, & Polish, 1967; Truelove & Reynell, 1972). Experiments have been carried out to determine whether restraint-induced stress ulcers in rats are dependent on acid gastric juice for their development, or whether they can develop independently of gastric secretory act-

ivity.

Menguy (1960) examined the role of gastric secretion in restraint-induced ulcers by using the method of pylorus ligation. (This method is one where the section of the small intestine known as the pylorus is tied off, such that gastric secretions cannot pass through the intestine, but are forced to accumulate in the stomach.) The rats in Menguy's experiment were fasted for 48 hours; then half of them were restrained for 6 hours in wire corsets suspended from a stand, and the other half remained in individual cages. Menguy calculated the free acid output for each group, and found that restrained pylorus ligated rats had a significantly lower free acid output than pylorus ligated rats not restrained. Menguy thus postulated that "acid-peptic digestion is not the mechanism responsible for restraint ulcers of the rat" (Menguy, 1960, p.915).

The validity of Menguy's conclusion is questionable. In his experiment, Menguy relied on a measure of gastric acidity -"free acid"[4]- that has been

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[4] "Free acid" is the amount of acid measured up to the point when Töpfer's reagent changes colour; the amount of acid measured between the change of Töpfer's

described as having "no scientific basis" and serving "no useful purpose" (Truelove & Reynell, 1972, p.116). Physiologically, it would have been more useful if Menguy had measured the proportion of total acid in the sample of gastric juice, rather than the proportion of free acid; or alternatively, if he had measured the pH of the gastric contents.

Brodie, Marshall, and Moreno (1962a) carried out a more sophisticated experiment than Menguy's. Brodie et al. used two methods for collecting gastric juice. In one group, they collected gastric samples by means of pylorus ligation, and in another group they collected it by means of chronic gastric fistulas implanted in the rumens of the rats' stomachs. Brodie et al. compared the gastric secretions of the chronic fistula rats with that of pylorus ligated rats, under conditions of both restraint and non-restraint.

The chronic fistula rats were first food deprived for 24 hours, and then gastric secretions were collected during the following 24 hour period. After 6 days, the rats were again food deprived for 24

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reagent and the change of phenolphthalein is known as "combined acid". The sum of the values for free acid and combined acid is known as "total acidity" (Truelove & Reynell, 1972).

hours, and then restrained for another 24 hours. During both the 24 hour restraint and the 24 hour non-restraint periods, gastric juice was sampled every 4 hours. A similar procedure was instituted with the pylorus ligated rats, with the exception that a group of rats was sacrificed at the end of each 4 hour period.

Brodie et al. found that with both the chronic fistula and the pylorus ligation techniques, the restraint condition produced significantly less gastric secretion than did the non-restraint condition (although the inhibition of secretion was less consistent in the pylorus ligated group than it was in the chronic fistula group). There the similarity between chronic fistula rats and pylorus ligated rats ended. In the chronic fistula rats, restraint produced an increase in the concentrations of free and total acid, and no change in the free acid output, as compared with the effects of non-restraint. In contrast, the restrained pylorus ligated rats exhibited no change in free and total acid concentrations, and a decrease in free acid output, compared with the pylorus ligated rats not restrained. Although the decrease in free acid output for restrain-

ed pylorus ligated rats confirms the finding by Menguy, it should also be disregarded along with Menguy's, because of the doubtful validity of the free acid measure.

In discussing their results, Brodie et al. drew attention to the fact that unrestrained pylorus ligated rats secrete high levels of gastric juice. For this reason, Brodie et al. claimed that increases in acid concentration in pylorus ligated rats would be difficult to detect. Brodie et al. therefore disregarded the results of the pylorus ligation condition in assessing the changes in gastric secretory activity produced by restraint. (This high level of gastric activity in pylorus ligated rats also provides another reason for discounting Menguy's results.) On the basis of the results of the chronic fistula rats alone, Brodie et al. suggested the hypothesis that it is the concentration of acid rather than the volume that is the critical factor in the development of restraint ulcers.

Hartry (1962) conducted a study which employed 48 hours restraint together with doses of the tranquilizing drug reserpine (two doses of .1mg/kg, 24 hours apart) to induce gastric ulcers in female rats.

Hartry collected the stomach contents of the rats immediately they were sacrificed, and then titrated the contents with NaOH to a pH of 7. The results indicated that the stomach contents of ulcerated rats were significantly less acid than the stomach contents of non-ulcerated rats.

Although the comparison made in the Hartry study was different from the one made in the Brodie, Marshall, and Moreno study (the former compared ulcerated and non-ulcerated rats, and the latter compared restrained and unrestrained rats), the Hartry study apparently provides negative evidence for the hypothesis by Brodie et al. that increased acid concentration is a mediating factor in restraint-induced ulceration.

However, it is questionable whether the methodology of Hartry's experiment is adequate for investigating the role of acid in the formation of restraint ulcers. It has been suggested by Brodie, Marshall, and Moreno, in a report on an experiment investigating the effect of ulcerogenic drugs on gastric acidity (Brodie, Marshall, & Moreno, 1962b), that the mechanism of reserpine-induced ulcers is different from that of restraint-induced ulcers. In support of

this position, Brodie et al. (1962b) refer to experimental results showing that reserpine-induced ulcers are not affected by injection of the anticholinergic drug atropine, or by the surgical procedure of vagotomy. On the other hand, restraint-induced ulcers can be inhibited by both procedures. For this reason, conclusions about the role of gastric acid in the formation of restraint ulcers should not be made on the basis of the results of Hartry's experiment.

Lack of support for an emphasis on acid concentration is also apparently provided by the results of a study by Boles and Russell (1970). Boles and Russell restrained a group of rats in plastic restraining devices for 36 hours, and then studied the changes in gastric ulceration, gastric motility, and total acidity. However, Boles and Russell also used reserpine as an additional stressor, and thus, most of the results of their experiment are not relevant. However, in a group designed to control for the effects of restraint, Boles and Russell found that rats restrained for 36 hours without receiving any reserpine did not differ in measures of total acidity (as measured by a pH meter at the end of 36 hours) from control rats left in their home cages.

Although the results of the Boles and Russell study appear to disconfirm the acid concentration hypothesis put forward by Brodie, Marshall, and Moreno, it is necessary to point out a major difference in procedure between the experiment by Boles and Russell and that by Brodie et al. Boles and Russell examined the stomach contents for total acidity at the completion of the 36 hour restraint period. On the other hand, Brodie et al. made such measures every 4 hours during a 24 hour restraint period.

An examination of the data from the experiment by Brodie et al. shows that the difference in total acidity between restrained and unrestrained rats had disappeared by the 24th hour. Therefore, the finding by Boles and Russell that there is no difference in total acidity between restrained and unrestrained rats after 36 hours of restraint is not contrary to the findings of Brodie, Marshall, and Moreno. The main difference between the two sets of results is that Brodie et al. have studied the course of events during a 24 hour stress session; Boles and Russell know only the end result after a 36 hour stress session.

Support for the emphasis by Brodie et al. on acid concentration comes from the results of a study by Levine and Senay (1970). Female rats were restrained in plastic restraining devices, and then refrigerated for 2 hours. After the rats were sacrificed, the stomach contents were collected, and the pH values of the gastric secretions determined. Levine and Senay found a strong negative relationship between the incidence of stress ulcers and intragastric pH values; that is, the more acidic the rat's gastric contents, the more likely the rat was to have developed stress ulcers. Some caution is needed in using these results as support for the acid concentration hypothesis, as Levine and Senay used a physical stressor (cold) as well as restraint.

Many of the experiments investigating the role of acid in the development of restraint ulcers have to be regarded cautiously, because of the introduction of other stressors, or because of the use of unsatisfactory measurement techniques. The only study that makes a reasonable contribution to determining the role of acid in the formation of restraint-induced ulcers is the chronic fistula study by Brodie, Marshall, and Moreno. They isolated the effect of

restraint, and measured gastric secretory activity regularly across a 24 hour stress session. Although the use of the chronic gastric fistula has been criticized by some researchers on the grounds that some loss of secretion may occur through the pylorus, the technique is regarded as superior to the pylorus ligation technique (Lambert, 1968). Thus, at this stage of investigation, the evidence tentatively suggests that restraint-induced ulceration is accompanied by increased acid concentration.

Pepsinogen Activity

Pepsinogen is a proenzyme secreted by the gastric mucosa. In the stomach, it is converted to pepsin, which is instrumental in the digestion of protein (Stedman's medical dictionary, 1972). In the rat, pepsinogen is formed and secreted by the zymogenic cells in the fundus of the glandular mucosa of the stomach (Lambert, 1965).

Blood pepsinogen and uropepsin levels have been used as predictors of susceptibility to duodenal ulcers in humans. However, patients with gastric ulcers cannot be discriminated from those without gastric ulcers on the basis of pepsinogen and pepsin

levels (Ader, Beels, & Tatum, 1960a). Ader and his colleagues have conducted a series of experiments (Ader, Beels, & Tatum, 1960a; Ader, 1963a, 1963b) to investigate whether a relationship exists between plasma pepsinogen levels and restraint-induced ulceration in the rat.

Ader, Beels, and Tatum carried out a number of experiments, in which they restrained male and female rats of three different strains (Wistar, Sprague-Dawley, and Long-Evans) for 20 hours, in adjustable restraining cages. Ader et al. found that there were no differences in plasma pepsinogen levels between restrained rats and control rats that had remained unmanipulated in their home cages. However, Ader et al. did find differences in pepsinogen levels between restrained rats that ulcerated, and restrained rats that did not ulcerate. The rats that developed ulcers had significantly higher plasma pepsinogen levels than the rats that did not develop ulcers. The conclusion implicit in the discussion by Ader et al. of their results is that while high pepsinogen levels per se are not predictive of the occurrence of gastric erosions, such pepsinogen levels <u>occurring under stress conditions</u> are indicative of a rat's susceptibility

to ulceration.

Ader (1963b) has presented support for the finding that restrained rats that ulcerate have higher pepsinogen levels than restrained rats that don't ulcerate. Ader presented a summary of plasma pepsinogen levels collected from rats used in a number of stress ulceration studies. The methods used to induce ulceration in these studies included both the restraint and the conflict techniques. An analysis of his data indicated that rats that develop ulcers in the glandular mucosa of the stomach (i.e. the acid-secreting section) consistently have higher plasma pepsinogen levels than rats that do not develop glandular mucosa ulcers, irrespective of the technique used to induce ulceration. An analysis of plasma pepsinogen levels of rats that develop ulcers in the rumen of the stomach only (ulcers generally induced by the conflict technique) demonstrated no relationship between the occurrence of rumenal ulcers and plasma pepsinogen levels.

Ader (1963a) conducted another series of experiments to determine whether basal (i.e. pre-stress) plasma pepsinogen levels are predictive of the occurrence of gastric erosions in the restrained rat. Ader

was able to demonstrate such a predictive relationship, but only under certain stringent conditions. A period of restraint minimally conducive to ulceration (in this case, 6 hours) was required, and predictions could be made only about rats with basal plasma pepsinogen levels falling in the upper and lower 15% of the distribution of basal plasma pepsinogen levels of all rats used in the experiment. Even then, the prediction had a margin of error, as not all rats in the "high" basal group developed ulcers, and not all rats in the "low" basal group remained ulcer-free. Thus, Ader concludes: "on a statistical basis, a high plasma pepsinogen level may be considered a biological indicator of an increased liability to gastric erosions" (Ader, 1963a, p.231).

It is interesting to note that, in his 1963a series of experiments, Ader was not able to demonstrate the differences in plasma pepsinogen levels between rats with ulcers and those without ulcers, when periods of restraint of 6 hours and 12 hours were used. These periods of restraint are much less than the 20 hours restraint used by Ader, Beels, and Tatum in the experiment which initially demonstrated the difference in plasma pepsinogen levels between

ulcerated rats and nonulcerated rats.

Although Ader has been successful in identifying plasma pepsinogen level as a relevant variable in the development of restraint ulceration, the effect of the variable appears to be somewhat elusive, and its role is probably a minor one. Nevertheless, the results of Ader's experiments have reinforced the view that gastric secretory activity is important in the development of restraint-induced ulceration.

Gastric Motility

Gastric motility is another aspect of gastrointestinal activity whose excitation is under the control of the parasympathetic nervous system. Brodie, for one, has specifically implicated gastric motility in the development of restraint ulceration by suggesting that "immobilization of a rat produces a marked central nervous system disturbance, which produces ... an increase in gastric acidity and motility" (Brodie, 1962, p.108).

Brodie has empirically investigated the relationship between gastric motility and restraint ulceration. Brodie and Hanson(1960) compared the gastric

motility of restrained rats with the gastric motility of free moving rats. Brodie placed two groups of rats on a reduced feeding schedule for 14 days. During this time, the rats were fed for 2 hours a day, and allowed water continuously. At the end of the 2 weeks, one group was restrained in wire mesh for 24 hours, and the other group was maintained in its home cage. Neither group had access to food or water during the stress period.

At the end of the 24 hours, Brodie weighed the stomach contents of all the rats. He found that there was significantly less food in the stomachs of restrained rats than there was in the stomachs of the free moving rats.

Eagleton and Sines (1962) also investigated the relationship between restraint-induced ulceration and gastric motility. In their experiment, Eagleton and Sines restrained a group of sixth generation stress ulcer susceptible (SUS) rats and a group of Sprague-Dawley rats (from which the SUS rats had initially been derived) for 30 minutes. Prior to restraint, all rats were food deprived for 24 hours, and a mixture of chromic oxide and water was introduced into the rats' stomachs.

Eagleton and Sines found that the chromic oxide traversed a greater intestinal distance in the SUS rats than in the Sprague-Dawley rats. Previous work by Sines (1959) had demonstrated that SUS rats develop significantly more restraint ulceration than Sprague-Dawley rats. Thus Eagleton and Sines demonstrated, indirectly, that increased restraint-induced ulceration is associated with increased gastric motility.

Another investigation into gastric motility and restraint ulceration has been carried out by Boles and Russell (1970), who measured gastric motility by changes in gastric bioelectrical potentials recorded by the electrogastrogram (EGG). Unfortunately, Boles and Russell's study is of little value in understanding the mechanism of restraint-induced ulceration, as they used reserpine as well as restraint to induce gastric ulceration in their rats. Boles and Russell found that rats restrained for 36 hours in plastic restraining devices and simultaneously injected with reserpine showed a <u>decrease</u> in gastric motility as compared with rats subjected to restraint alone. The decrease in gastric motility in restraint plus reserpine rats accompanied an increase in ulceration, compared with the ulceration in the restraint-

only group.

Boles and Russell state that their results are directly contrary to the claim by Brodie that restraint increases gastric motility. However, Boles and Russell's results are confounded by their use of reserpine, and their results should be interpreted cautiously. At this stage, what evidence is available seems to suggest a positive relationship between gastric motility and restraint-induced ulceration.

Vascular Activity

Both Bonfils (Bonfils & Lambling, 1963) and Brodie (1962) have noted that pronounced mucosal vascular changes occur when rats are restrained. Bonfils went further and suggested that restraint-induced ulcers are primarily caused by a "functional capillary disturbance" (quoted in Brodie, 1963a, p.389).

Guth and Hall (1966) carried out an experiment in which they investigated the vascular changes that accompany restraint. Guth and Hall restrained a group of rats in wire mesh for periods ranging from 30 minutes to 24 hours. They found that after only

30 minutes of restraint, there was marked vascular engorgement immediately below the surface epithelium of the glandular section of the stomach. Such hyperemia preceded the appearance of any stress ulceration. Guth and Hall also found that when ulceration began to develop, it did so in those sections of the gastric mucosa where the hyperemia had occurred. Guth and Hall interpreted their results as being compatible with the hypothesis that "engorgement of the mucosa is a primary factor leading to a decrease in tissue resistance and permitting subsequent erosion by the peptic acid secretion" (Guth & Hall, 1966, p.569).

In a later experiment, Guth and Kozbur (1968) hypothesized that the mucosal hyperemia observed in the 1966 experiment was produced by degranulation of the mast cells. (Mast cell degranulation is a process which causes the release of serotonin, histamine, and heparin into the tissues (Katz & Siegel, 1968); serotonin inhibits gastric secretion, histamine stimulates it, and heparin acts as an anticoagulant (Stedman's medical dictionary, 1972).) However, after studying the effects of bilateral subdiaphragmatic vagotomy on restraint-induced ulceration, Guth

and Kozbur concluded that the mucosal engorgement that accompanies restraint is produced by parasympathetic activity, rather than by mast cell degranulation.

While there is strong support for the claim that considerable vascular activity accompanies restraint-induced ulceration, there is no direct evidence at this stage to say whether such vascular changes are instrumental in producing stress ulceration or not.

Adrenal-Pituitary Activity

Most of the activity of the adrenal cortex is under the control of adrenocorticotrophic hormone (ACTH), which is secreted by the pituitary. Selye considered that the adrenal-pituitary axis played an important part in all stress reactions. In particular, he thought that stress ulcers are caused, in part, by the action of certain corticosteroids secreted by the adrenal cortex. (There are two main groups of corticosteroids: the anti-phlogistic (anti-inflammatory) hormones, also known as the gluco-corticoids, and the pro-phlogistic (pro-inflammatory) hormones, also known as the mineralo-corticoids (Selye, 1956).) Selye claimed that the anti-phlogistic properties of the glucocorticoids have the effect of weakening the

internal "inflammatory barricade" of the stomach, and thus producing digestion of the gastric mucosa (Selye, 1956, p.180).

Early in his work, Selye noted that surgical removal of either the adrenal glands (adrenalectomy), or the pituitary gland (hypophysectomy) reduces an animal's resistance to a stressor. Some experimenters have attempted to assess the role of adrenal-pituitary activity in the development of restraint-induced ulceration by carrying out surgical procedures similar to Selye's.

Adrenalectomy

Menguy (1960) performed bilateral adrenalectomy on one group of rats, and a sham operation on a second group. He then restrained both groups in wire mesh corsets, suspended from stands, for 20 hours. On the basis of the restraint-induced ulceration found in the two groups, Menguy concluded that there was no "significant qualitative or quantitative difference between the lesions of the adrenalectomized and control animals" (Menguy, 1960, p.913).

Such a conclusion is odd in the light of an admission by Menguy that only three of the adrenal-

ectomized rats survived the 20 hours restraint.

Menguy does not state the cause of death of the remaining 17 experimental rats; nor does he indicate whether the stomachs of these 17 were inspected for ulceration and included in the analysis. Given that Menguy does not clearly report on what basis he came to his conclusion, it is best to treat his statement of "no difference" between adrenalectomized and control rats with considerable caution.

Brodie and Hanson (1960) showed that bilateral adrenalectomy, performed 8 days before restraint, increased the incidence of restraint-induced ulceration. In their experiment, Brodie and Hanson compared two different periods of restraint: 6 hours and 24 hours. They found that after 6 hours of restraint, both incidence and severity of ulceration were increased in the adrenalectomized rats. After 24 hours of restraint, only severity of ulceration was increased. Like Menguy, Brodie and Hanson had a high mortality rate in the adrenalectomized rats that were restrained. In the 6 hour restraint group, 25% of adrenalectomized rats died; and in the 24 hour restraint group, 52% died.

The Bonfils group, as reported by Brodie and Hanson (1960), found that adrenalectomy had no effect

on restraint-induced ulcers. Bonfils found that after 7 hours of restraint, there were no differences in ulceration between an adrenalectomized group of rats and a control group.

Hypophysectomy

Menguy (1960) removed the pituitaries of a group of rats, then 14 days later he restrained them for 20 hours. The incidence of ulceration in the hypophysectomized group was compared with that in a group of control rats similarly restrained, but not hypophysectomized.

Menguy's description of his results is excessively sketchy. His only statement concerning the differences in ulceration between the hypophysectomized and control groups is as follows:

> After 20 hr. of restraint stress there was a 100 per cent incidence of gastric erosions in the hypophysectomized rats. There was no apparent difference in degree from the lesions of the control rats. (Menguy, 1960, p.913)

Menguy's conclusion that removal of the pituitary has no effect on restraint-induced ulcers should be treated with suspicion. Once a 100% incidence is achieved, then incidence is no longer a discriminatory measure.

Some other measure (e.g. number of ulcers, or total length of ulceration) is necessary to discriminate among groups. Menguy's statement that there was "no apparent difference in degree" is inadequate as a quantitative assessment of the difference between the two groups.

Brodie and Hanson (1960) restrained one group of hypophysectomized rats for 6 hours, and a second group for 24 hours. Brodie and Hanson found that the removal of the pituitary had no effect on either the incidence or the severity of the stress ulcers. On the basis of this evidence, Hanson (1963) concluded that "the etiology of the lesions ... is not dependent on the intactness of adrenal-pituitary axis" (p.393).

Contrary to the Menguy and the Brodie and Hanson data, Bonfils and Lambling (1963) report that "under certain precise experimental conditions {hypophysectomy} aggravates the restraint ulcer" (p.161). Unfortunately, Bonfils and Lambling do not specify the relevant experimental conditions that result in hypophysectomy producing increased stress ulceration.

The value of surgical interventions such as adrenalectomy and hypophysectomy is doubtful in assessing the role of the adrenal-pituitary axis in

the development of restraint-induced ulceration. The removal of both the pituitary and the adrenal glands causes considerable trauma to the organism. Best and Taylor (1958) claim that animals that have had both adrenal glands removed are dead within 10 to 15 days after the operation, as animals cannot survive without the adrenal cortex. Removal of the pituitary also has dramatic consequences, because of its importance to the endocrine system as a whole; for example, most of the activities of the adrenal cortex are prevented by hypophysectomy because of the absence of the pituitary hormone ACTH.

One particular problem is that the activity of the nervous system is probably seriously disrupted when such operations are performed. Bilateral adrenalectomy involves the removal of both the adrenal cortex and the adrenal medulla. While the adrenal medulla is not essential to life, it is an extension of the nervous system, and secretes large amounts of adrenaline and noradrenaline at times of stress. Hypophysectomy presumably involves removal of both the anterior and the posterior lobes. The secretion of ACTH is under the control of the anterior lobe only (the adenohypophysis); the posterior lobe, like

the adrenal medulla, is intimately connected with the functioning of the nervous system. Therefore, changes that are observed after adrenalectomy and hypophysectomy are probably attributable to a number of interacting factors, rather than to the absence of ACTH and the corticosteroids alone.

Plasma Corticosteroid Levels

An alternative method of establishing the role of the corticosteroids in the development of restraint-induced ulceration is to study the hormonal levels in the blood. It is known that corticosteroids are secreted by the adrenal cortex when an organism is stressed, but it is not known to what extent the endogenous steroids act directly to produce stress ulceration. The glucocorticoids for example suppress inflammation in the body, and it is probable that some pathologically superficial conditions, such as gastritis, would be attenuated rather than exacerbated by the action of such corticosteroids.

The evidence so far about the relationship between the superficial condition of restraint-induced ulceration and plasma corticosteroid levels is minimal. Little is known other than that restraint

is accompanied by adrenal hypertrophy (Stern, Winokur, Eisenstein, Taylor, & Sly, 1960), and that a significant increase in blood corticosterone levels occurs within 1 hour of rats being restrained (Knigge, Penrod, & Schindler, 1959).

The effect of exogenously administered corticosteroids has also been considered. Hanson and Brodie (1960) administered prednisolone to rats daily after they had been released from restraint. Hanson and Brodie found that the effect of the steroid was to delay recovery from restraint-induced ulcers. On the other hand, Bonfils found that the administration of cortisone over several days preceding restraint significantly decreased the frequency of restraint ulcers (quoted in Brodie, 1963a).

The role of the corticosteroids in the development of restraint-induced ulcers is apparently a complex one. The organism cannot survive without the activity of the adrenal cortex: the gland acts to maintain the status quo of body chemistry, and generally to protect the organism from the harmful effects of stress. However, the effects of corticosteroids are sometimes undesirable ones, and the production of stress ulceration may be one of these undesirable

consequences. The evidence suggests that prolonged exogenous administration of corticosteroids may lead to gastric ulcers through impairment of the normal secretion of protective mucus (Wolf, 1965), and it is known that corticosteroid drugs can exacerbate already existing ulcers (Wingate, 1972). However, the role of endogenously produced corticosteroids in the development of the superficial condition of stress ulceration is still not fully understood.

Parasympathetic Nervous System Activity

The evidence concerning the role of the parasympathetic branch of the autonomic nervous system in the development of restraint ulcers is less equivocal than that concerning the role of corticosteroid activity.

Menguy (1960) partially parasympathectomized one group of rats by cutting the fibers of the vagus nerve; a second group of rats simply had the vagus nerve exposed. Menguy then restrained both groups for 20 hours. Only 36% of the vagotomized rats developed ulceration, compared with 100% of rats with the vagus nerve intact.

The Bonfils group also found that vagotomy

reduced the incidence of restraint ulceration. In Bonfils' study, the incidence of ulceration in a normal group was reduced by 49% in a vagotomized group, after 24 hours of restraint (quoted in Brodie, 1963a). In this experiment, Bonfils and his colleagues also found that as the volume in which the rat was restrained was increased, the protective effect of blocking the parasympathetic nervous system decreased, and even disappeared. On the basis of these findings, Bonfils and Lambling (1963) concluded that while the parasympathetic nervous system plays a role, it is by no means the only factor, and not even the primary factor.

Brodie and Hanson (1960) also investigated the effect of vagotomy on restraint-induced ulceration. Brodie and Hanson restrained a vagotomized group and a normal group for 24 hours, and found a reduction of 42% in the incidence of erosions. This reduction was not statistically significant. However, in the light of the finding by Bonfils that the amount of protection afforded by vagotomy is dependent on the volume in which the rat is restrained, the reduction in ulceration found by Brodie and Hanson should not be ignored.

The evidence suggests that the parasympathetic nervous system plays an important part in the development of stress ulcers. However, as Bonfils pointed out, it is clearly not the only factor. In all the experiments discussed above, considerable restraint-induced ulceration still occurred even though there was no parasympathetic innervation of the stomach.

Central Amine Activity

In recent years, there has been much interest in the relationship between central amines and behavioural and physiological reactions. The central amines of particular interest are norepinephrine, serotonin (which is known to inhibit gastric secretion), and dopamine.

Some investigations have already been carried out into the relationship between restraint-induced ulceration and such central amine levels. For example, Nagura (1972) placed rats in restraining cages, and then lowered them into water baths, where they remained for 20 hours. Nagura found that, together with stress ulcers, there occurred a depletion in brain norepinephrine. The level of dopamine in the brain remained unaltered.

Paré and Livingston (1970) report that restraint has been known to result in both an increase and a decrease in brain norepinephrine levels. Paré and Livingston suggest that noxious stimulation results in an initial decrease in brain norepinephrine levels, but with continued stimulation the level returns to normal and may even elevate above the basal level. Because of the complex nature of central amine functioning, little is known as yet of the relationship between central amine levels and restraint-induced ulceration.

Contribution Made by Drug Studies to Understanding the Mediation of Restraint-Induced Ulcers

Numerous drugs have been administered to rats, both prior to and during restraint, to ascertain the effects such drugs have on stress ulceration. The contribution these studies make to elucidating the mechanism of restraint-induced ulceration is limited. Drugs act in a variety of ways, and particular pathways of operation are often difficult to identify. Nevertheless, drug studies have contributed to determining the general nature of the physiological mediation of restraint ulcers, without being very help-

ful in identifying the specific action of restraint.

The data on the interaction between restraint and drugs can be divided into two groups: data from studies where ulceration has been attenuated by the action of a drug; and data from studies where ulceration has been exacerbated by a drug. The difficulty with the interpretation of these studies is that the drug used may not have acted through the same pathways as restraint, but may have affected gastric activity through some additional route. Thus, while results from drug studies are useful, they should be interpreted cautiously.

Attenuation Effects

Two large groups of drugs have been found consistently to inhibit restraint-induced ulceration. These groups are the anticholinergics, and the central nervous system depressants. Hanson and Brodie (1960) have carried out a considerable amount of research with drugs from both groups. Hanson and Brodie showed that atropine sulfate, scopolamine methiodide, mepiperphenidol, and propantheline (all anticholinergics), administered prior to restraining rats, all

reduced ulceration to below the level of a restraint-alone control group. Similarly, the central nervous system depressants chlorpromazine,[5] benactyzine, and pentobarbital all reduced ulceration to below that of a restraint-alone group.

The action of anticholinergics has been described by Brodie (1963b) as chemical vagotomy. Thus, the results from studies looking at the effects of anticholinergics are in accordance with the results of other studies that suggest that the parasympathetic nervous system (via the vagus) plays an important part in the development of restraint-induced ulceration.

Exacerbation Effects

Exacerbation of restraint ulcers has been consistently obtained with the tranquilizing drug reserpine. An increase in restraint-induced ulceration with the administration of reserpine has been demonstrated by Bonfils (Bonfils & Lambling, 1963), and Hartry (1962). Reserpine is a drug that induces

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[5] Although Hanson and Brodie describe chlorpromazine as a central nervous system depressant, it is more usually known as an anticholinergic.

an adrenergic blocking effect; that is, it produces a parasympathetic bias. However, reserpine is also known to stimulate the gastric mucosa directly. Thus, its ulcerogenic effect could be due to either increased parasympathetic activity, or direct stimulation of the gastric mucosa, or a combination of both activities.

An experiment which exemplifies the sorts of problems that arise in interpreting the results of drug studies is one by Sines (1963). Sines found that administration of the anticholinergic drug chlorpromazine exacerbated restraint-induced ulcers. This finding is opposite to that obtained by Hanson and Brodie (1960), who demonstrated an attenuation effect with this particular drug. An examination of the methodologies of the two studies revealed a crucial difference. Sines used a dosage level of 10mg/kg, and Hanson and Brodie used a level of 24mg/kg. Thus, it is important that the results of drug studies be used cautiously to support a particular hypothesis about the mediation of restraint-induced ulcers.

Conclusions

The physiological evidence points to a multifactorial account of the development of restraint-

induced ulceration. One of the factors that is clearly involved is parasympathetic nervous system activity. Not only does vagotomy reduce the incidence of restraint ulceration, but also local activity under the control of the parasympathetic system (gastric secretory activity, gastric motility, and vascular engorgement) has been shown to accompany, and sometimes precede, restraint-induced ulceration.

It is probable that, of the local gastric activity that does occur, no one factor is responsible for the erosions that develop. Restraint is accompanied by a number of changes: an increase in gastric secretory activity (both acid and pepsinogen activity), an increase in gastric muscular movement, and a hyperemic reaction occurring close to the surface of the mucosa. Restraint-induced ulceration probably results from an interaction of these (and other) changes. An additional factor that might be involved is decreased production of the protective mucus that normally lines the gastric mucosa of the stomach, and helps protect it from digestion (Hollander, 1962).

While parasympathetic nervous system activity has a key role in the development of restraint-induced ulcers, other activity is also involved, since vagotomy

fails to prevent the ulceration completely. However, the nature of any other activity is not very clear. Corticosteroid activity may be involved, and the roles of such factors as noradrenergic activity and central amine functioning are still to be fully investigated.

One important problem that has received little attention is how vagal activity is initiated when rats are restrained. It was pointed out early in the Chapter that psychological stress is usually accompanied by sympathetic nervous system activity, which is presumably incompatible with parasympathetic arousal. The solution to this problem should be pursued experimentally. It is a fundamental aspect of fully understanding the psychological and physiological mediation of restraint-induced ulcers.

CHAPTER 4

THE EFFECT OF PREVIOUS EXPERIENCES ON SUSCEPTIBILITY TO RESTRAINT-INDUCED ULCERATION

In stress research, there has always been considerable interest in studying the relationship between the previous experiences of an animal and its subsequent resistance to stress. Particular attention has been paid to the effect of early experiences on the resistance to stress in later life. In animal studies, the procedure is usually adopted where animals are treated either benignly or traumatically when they are young, and then exposed to stressors as adults to gauge whether the threshold for stress has been altered. (Unfortunately, the methodology of these experiments is usually such that any changes observed in pre-treated animals may only be attributed to previous experience, and not to early experience in particular.)

The most usual way of benignly treating young rats is to handle them gently, and it is usually found that such benign treatment results in an increased stress threshold in adult life (Bovard & Newton, 1956; Denenberg, 1964; Weininger, 1956). Bovard (1958),

after a thorough review of the literature, argued strongly that "early handling raises the threshold for response to stress, thus conserving the metabolic resources or adaptation energy ... of the organism" (p.259). On the other hand, the effects of traumatic treatment (the most usual being electric shock stimulation) appear to be less consistent. Ader (1959) reports that some investigators have found an increased stress threshold following early traumatic experiences, others have found a lowered threshold, and still others have found no change at all in the stress threshold.

A number of theories have been proposed which outline the nature of the relationship between early experience and later resistance to stress (e.g. Ader, 1970b; Bovard, 1958; Denenberg, 1964; Levine, 1962). Levine and Denenberg, in their theories, make no distinction between early experiences that are benign and those that are traumatic. Levine claims that all early stimulation constitutes stress, and he claims further that such stress will increase the animal's resistance to chronic stressors in later life, but will decrease its resistance to acute stressors. Denenberg makes no distinction between acute and

chronic stressors, but claims that early stimulation should increase resistance to all subsequent stressors.

Bovard has adopted a different approach from Levine's and Denenberg's. Bovard discriminates between the effects of early benign experiences and early traumatic experiences, suggesting that the former increase resistance to later stress, and the latter decrease resistance, irrespective of the nature of the subsequent stressor.

Ader has adopted an entirely different position from all three. He suggests that the reaction to a stressor, following a particular early experience, is not related to either the pre-stress experience per se, or the adult stressor per se, but rather to an interaction between the two. Ader claims that any stress reaction is a function of the interaction between the psychophysiological changes produced by pre-stress experiences and the particular response that a stressor evokes. Ader's theory has the advantage of being able to account for a great deal of the experimental data.

In the context of studying the general relationship between life experiences and stress reactions,

some details have emerged about the specific relationship between previous experiences and resistance to stress ulceration. Many of the experiments from which such information has been collected have been concerned with the effect of early experiences on physiological and psychological development. Stress ulceration is generally included in these experiments as one of many dependent variables, rather than as the primary focus of investigation. However, even in this indirect manner, much information has been collected about the relationship between life events and later susceptibility to restraint-induced and conflict-induced ulceration. In the following section, the experiments in which stress ulceration was induced by restraint will be considered in detail; the experiments which employed the conflict technique will be mentioned only briefly.

Pre-Natal Events

Ader and Plaut (1968) investigated the effect of in utero stimulation on the later resistance of adult rats to restraint-induced ulceration. In Ader and Plaut's experiment, one group of pregnant females received benign treatment, in the form of handling

twice a day every day, throughout gestation; a second group of pregnant females was left unmanipulated. After the birth of the pups, there were no further manipulations, and all pups were fostered to unmanipulated mothers.

The pups were weaned at the normal age of 21 days, and then housed in groups of three to four. At 83 days of age, they were individually housed for 1 week, and then restrained in wire mesh for 6 hours, following 18 hours of food deprivation.

Ader and Plaut found a sex difference in the way offspring of handled and unhandled mothers reacted to restraint. Female rats that had received the in utero stimulation ulcerated more than the female rats that had not received the stimulation. On the other hand, there were no differences in ulceration between the males that had been stimulated and those that hadn't.

Ader and Plaut repeated their study. This time, as well as manipulating pre-natal handling, they also manipulated the type of housing the rat was exposed to immediately prior to restraint. (The housing variable was included for study because Ader had previously identified it (Ader, 1965) as relevant

in determining the effects of early stimulation.) As in the first study, immediately after weaning, half the rats were individually caged, and half were group caged three to four per laboratory cage. However, unlike the first study, rats were maintained in this fashion (i.e. either group or individually housed) until they were restrained.

In the results of their second study, Ader and Plaut found that the sex difference had disappeared, but that there was an effect due to housing. Of those rats individually housed, the rats stimulated in utero ulcerated more than the rats not stimulated. However, of those rats group housed, there was no significant difference between the stimulated rats and the unstimulated rats. Ader and Plaut interpreted their results as suggesting that the type of housing in which the rat lives affects differences in ulceration that can be produced by pre-natal manipulations.

Post-Natal Events

Most of the experimental work investigating the effect of immediate post-natal manipulations on later resistance to stress ulceration has been carried out

by Ader and his colleagues. However, the first experimenter to investigate the problem was McMichael, in 1961. McMichael was interested in comparing the effects of handling and shock stimulation in early life on later resistance to stress. McMichael's experiment was prompted by claims by Bovard (1958) that early handling produces a physiologically and psychologically superior animal, and by similar claims by Levine (1957, 1958) about the effects of electric shock stimulation.

In McMichael's experiment, one group of rats was handled for 3 minutes a day, from the 1st to the 21st day of life; a second group was shocked for the same period of time, and a third group remained untouched. When the rats were 22 days old, they were housed individually. Then, at age 52 days, they were restrained in canvas cocoons for 2 hours, following 12 hours of prior food deprivation. McMichael found no differences in ulceration among the three groups.

Ader (1965) carried out a similar study, in which the post-natal manipulations were identical to McMichael's. In his experiment, Ader was primarily concerned with whether there is an interaction between early experiences and subsequent housing

conditions of animals. As in McMichael's experiment, one group of rats was handled daily for the first 3 weeks of life, a second group was shocked daily, and a third group was left unmanipulated. Following the stimulation period, Ader housed half the rats individually, and half in groups of six to seven. Then, at age 120 days, the rats were restrained in wire mesh for 18 hours.

Although Ader used the same stimulation schedules as McMichael, he obtained different results. Among the group housed rats, Ader found that the rats shocked as pups ulcerated more than the handled rats or the unmanipulated rats. Among the individually housed rats, the shocked and the unmanipulated rats ulcerated more than the handled rats. As in the later Ader and Plaut study of 1968, Ader interpreted this result to mean an interaction between post-weaning housing and early experience. Ader also found that overall, more group housed rats developed ulcers than did individually housed rats.

There are a number of methodological differences between Ader's experiment and McMichael's experiment which probably account for the differences in the experimental results. First, Ader used rats that were

120 days old, and McMichael used rats that were only 52 days old. Secondly, Ader restrained his rats for 18 hours, while McMichael restrained his for only 2 hours, during which time McMichael's rats had their stomachs loaded with water. Finally, McMichael distinguished between ulcers and bleeding points in measuring the extent of gastric pathology, while Ader made no such distinction.

In another experiment, Ader (1970a) again studied the interaction between early experiences and subsequent housing. Ader manipulated the handling received by young pups, and then noted the effect of the manipulation on later resistance to restraint-induced ulceration. Ader used three different handling conditions: one group was handled for 3 minutes a day, every day during the first 3 weeks of life; a second group was similarly handled during the second 3 weeks of life; and a third group was not handled at all.

At weaning age (21 days), all rats were group housed, irrespective of the handling condition to which they belonged. At 42 days of age, half the rats were kept housed in groups of four to five, and half were individually housed. Then, at age 120 days, all rats were restrained in wire mesh for 6 hours, follow-

ing 18 hours of food and water deprivation. As in his previous studies, Ader found an interaction between early experience and subsequent housing condition. In the individually housed group, the rats handled after weaning (i.e. during the second 3 weeks of life) ulcerated more than the rats not handled at all, and both groups of rats ulcerated more than the rats handled prior to weaning. In the group housed condition, there were no differences in ulceration among the three groups.

The results of Ader's experiments suggest that early experiences affect later resistance to restraint-induced ulceration. However, this effect is only detected if rats are <u>individually</u> housed prior to being restrained. If rats are group housed, the effect of the early experience is either altered or completely masked. Ader's results concerning the housing variable are interesting, and the role of prior housing in the development of restraint-induced ulceration will be taken up again later.

<u>Conflict studies</u>. Ader, Beels, and Tatum (1960b) found that conflict-induced rumenal ulceration is also affected by post-natal experiences. Ader et al.

found that rats whose mothers were removed from time to time during the first 10 days of life ulcerated less than rats receiving such manipulations during the second 10 days of life. The second 10 days of life group ulcerated the same as an unmanipulated control group.

Age of Weaning

The normal age at which rats are weaned is 21 days (Rowett, 1960). If they are weaned prematurely, rats generally show physiological and psychological impairment (Erdösová, Flandera, Křeček, & Wiener, 1967). Some experiments have been carried out to determine whether changing the age of weaning affects later susceptibility to restraint-induced ulceration (Ackerman, Hofer, & Weiner, 1975; Erdösová, Flandera, Křeček, & Wiener, 1967). Ader and his colleagues have also investigated the relationship between age of weaning and conflict-induced ulcers (Ader, Tatum, & Beels, 1960; Ader, 1962).

Erdösová et al. weaned one group of rats at 16 days of age, and a second group at 30 days of age. A number of rats from each weaning group were then restrained for 7 hours in metal sheet cylinders at

the following ages: 23, 30, 35, 50, 70, and 100 days. Erdösová et al. found that prematurely weaned rats ulcerated more than the later weaned rats if they were restrained at a very young age (at 23, 30, or 35 days of age). However, prematurely weaned rats restrained at ages 50, 70, and 100 days ulcerated the same as later weaned rats.

Ackerman, Hofer, and Weiner carried out an experiment similar to that by Erdösová et al. One group of rats was weaned at 15 days of age, a second group at 21 days of age, and a third group at 25 days of age. Rats from each weaning group were then restrained for 24 hours in wire mesh, at the following ages: 10, 17, 22, 30, 42, 62, 100, and 200 days. The results obtained by Ackerman et al. were similar to those obtained by Erdösová et al. Prematurely weaned rats restrained at an early age ulcerated more than normal and later weaned rats. By the time the rats were 200 days old, there was no difference in restraint-induced ulceration among the rats weaned at different ages.

A major difficulty in interpreting the results of the experiments by Erdösová et al. and by Ackerman et al. is that in neither experiment was there a control for the possible contribution of nutritional

factors to the differences in ulceration among the different weaning groups. Each group had access to the mother's nutritional supplies for different periods of time, and this factor alone might have been sufficient to affect later resistance to stress ulceration.

Conflict studies. Ader, Tatum, and Beels (1960) found that male rats weaned at 15 days of age were more susceptible to conflict-induced ulcers than male rats weaned at 22 days or at 35 days of age. There were no differences among female rats weaned at different ages. Ader later established (Ader, 1962) that the differences in conflict ulceration in males weaned at different ages were not attributable to nutritional factors. Ader later reported that the greater susceptibility of early weaned males to stress ulceration could not be demonstrated when ulceration was induced in adult rats by restraint rather than by conflict (Ader, 1967c).

Post-Weaning Events: Handling

Both Weininger (1956) and Winokur, Stern, and Taylor (1959) have studied the effects of post-weaning

handling on later resistance to restraint-induced ulceration. In his experiment, Weininger weaned two groups of rats when they were 23 days of age, and housed them individually. One group of rats was handled 10 minutes every day, for 21 days. The second group was not handled at all. When the rats were 79 days of age, Weininger restrained them for 48 hours by wrapping them in gauze and adhesive bandages. He found that handled rats developed less stress ulceration than unhandled rats.

However, Weininger also found that at the time they were restrained, handled rats weighed significantly more than unhandled rats. Weininger repeated his experiment, this time restraining handled and unhandled rats of the same weight. He found that handled rats were still less susceptible to gastric erosions than were unhandled rats.

Winokur, Stern, and Taylor carried out an experiment similar to Weininger's to ascertain whether Weininger's results could be confirmed for group housed rats. Winokur et al. weaned 22 day old rats, and housed them in groups of 11 or 12. One group of rats was handled for 5 minutes a day, and a second group was handled for 10 minutes a day, every day for

21 days after weaning. A third group was not handled at all. When the rats were 79 days old, they were restrained in gauze and plaster of Paris bandages for 48 hours. Winokur et al. found that handled rats ulcerated less than unhandled rats, but there were no differences between the 5 minute and the 10 minute groups.

The evidence from both these experiments supports the view that handling at an early age reduces susceptibility of rats to restraint-induced ulceration. It is interesting to note that although Weininger housed rats individually, and Winokur et al. housed rats in groups, they obtained similar experimental results. This similarity of results across different housing conditions is contrary to what was found by Ader. Ader consistently demonstrated that differences in ulceration found in individually housed rats could not be detected in group housed rats. The effect of prior housing conditions on the susceptibility of rats to restraint-induced ulcers appears to be an important one, and the problem will now be taken up in greater detail in the next section.

Post-Weaning Events: Housing

As well as the investigations carried out by Ader, there have been two other experiments that have directly studied the effect of prior housing on restraint-induced ulceration: one by Stern, Winokur, Eisenstein, Taylor, and Sly (1960), and one by Sines (1965). In both experiments, the effect of group housing on restraint-induced ulceration was compared with that of individual housing.

Stern et al. housed rats either individually or in groups of 15. At about 80 days of age, the rats were restrained in gauze and plaster of Paris bandages for 48 hours. Stern et al. found that the group housed rats ulcerated more than the individually housed rats. This result was later confirmed by Ader, who found in his 1965 study that more group housed rats developed ulcers than did individually housed rats.

The experimental result that group housed rats ulcerate more when restrained than individually housed rats is counter-intuitive. Rats prefer to associate with other rats, and they often huddle together in groups (Barnett, 1963; Rowett, 1960). In addition, group housed rats are reported as being less emotional

than individually housed rats (Ader, Tatum, & Beels, 1960; Stern, Winokur, Eisenstein, Taylor, & Sly, 1960). On the basis of what is known about the preference of rats for group conditions, it would not be unreasonable to expect that group housed rats would ulcerate less when restrained than individually housed rats.

Stern et al. suggested an hypothesis to explain why their group housed rats ulcerated more when restrained than the individually housed rats. Stern et al. claimed that the individually housed rats ulcerated less than the group housed rats because living in the individual cages represented a partial adaptation to restraint. However, the evidence in favour of this hypothesis is not very strong. First, Bonfils demonstrated that the restraint ulceration effect virtually disappears once the volume of the restraining cage exceeds 7,000cc (Bonfils & Lambling, 1963). The individual cage used by Stern et al. had a volume of approximately 23,000cc. Therefore, it is difficult to think of the individual housing condition used by Stern et al. as even partly restraining.

Secondly, even if one assumes that an approximation to restraint did exist in the individual cage condition, it is not certain that adaptation to restr-

aint occurs with repeated exposure. Some experimenters have found reduced ulceration with repeated restraint, but there has been a report of increased ulceration as well. Further, experiments on repeated restraint have shown that rats become vulnerable in other ways, and a large percentage of them lose weight and die. Thus, the hypothesis put forward by Stern et al. suggesting a partial adaptation to restraint in the individual housing condition to account for the lesser ulceration in that group is not a very plausible one.

An alternative hypothesis can be suggested which depends on the conditions under which the rats are group housed. In the group housed condition used by Stern et al., 15 rats were housed in a cage 10ins. x 36ins. x 12ins. (25cm x 91cm x 30cm), and in the individually housed condition, each rat was in a cage 10ins. x 12ins. x 12ins. (25cm x 30cm x 30cm); that is, there was a floor space of 24sq.ins. (155sq cm) per rat in the group housed condition, and a floor space of 120sq.ins. (775sq cm) per rat in the individually housed condition.

The same calculations can be made for the rats in Ader's experiment. Ader housed the group housed

rats, six or seven to a group, in cages 11.5ins. x 16.5ins. x 7ins. (29cm x 42cm x 18cm), and the individually housed rats in cages 11.5ins. x 7ins. x 7ins. (29cm x 18cm x 18cm); that is, a floor space of approximately 29sq.ins. (187sq cm) per rat in the group housed condition, and a floor space of approximately 80sq.ins. (502sq cm) per rat in the individual housing condition.

Now, it is possible that the group housed rats in both the above experiments ulcerated more than the individually housed rats because the stress threshold of the group housed rats had been lowered by their chronic exposure to stress in the form of crowded housing conditions. Thus, an alternative hypothesis to account for the greater ulceration found in group housed rats is that if group housed rats are stressed through crowding, they will react more to restraint than will rats not exposed to crowding stress.

Another investigation into the effect of housing has been carried out by Sines (1965). Sines housed half of a group of stress ulcer susceptible rats individually, and the other half in groups, three to four to a group. When they were 65 days of age, all the rats were restrained in wire mesh for 6 hours.

In keeping with the results of Stern et al. and Ader, Sines found that group housed rats ulcerated significantly more than individually housed rats. However, Sines housed his group housed rats in cages 7.5ins. high x 12ins. x 15.5ins. (19cm x 30cm x 39cm), which provided an average floor space of 46-62sq.ins. (293-390sq cm) per rat. It is very unlikely that these housing conditions constituted crowding stress for Sines's rats. Thus, the crowding hypothesis suggested above to account for the difference in ulceration between group housed and individually housed rats cannot account for Sines's results.

Sines himself suggested an hypothesis to account for the difference in ulceration between the group housed rats and the individually housed rats. Sines based his hypothesis on activation theory as expounded by Fiske and Maddi (1961). Fiske and Maddi hypothesized that each animal has a characteristic level of activation, which is determined in part by the habitual stimulation to which the animal is exposed. If the amount of stimulation (and therefore activation) suddenly varies from that which the animal is adapted to, then stress is produced. The greater the difference between real activation level and normal

requirements, the greater the stress.

Sines used this theory to explain why group housed rats ulcerate more than individually housed rats. Sines made a number of assumptions about the stimulation levels provided by group housing, individual housing, and restraint. He claimed first, that group housing provides more stimulation than individual housing, and secondly, that both forms of housing each provide more stimulation than restraint. Then, since the transfer from group housing to restraint provides a greater move away from normal activation level than the transfer from individual housing to restraint, group housed rats will be more stressed by restraint than individually housed rats.

The chief difficulty with Sines's theory is that it is not amenable to empirical investigation. It is not possible to determine how much stimulation rats are provided with by various situations, and although it might be intuitively obvious that group housing is more stimulating than individual housing, it is not intuitively obvious that restraint provides very little stimulation. It could be argued quite strongly, for example, that restraint provides far more kinesthetic stimulation than could be provided

by any housing condition. Since Sines's theory cannot be tested experimentally, it is of limited use as an explanation of the housing effect.

Conclusions

The evidence demonstrates conclusively that restraint-induced ulceration can be affected by the rat's previous experiences. (To what extent these experiences have to be early ones, rather than just previous ones, is not yet known.) The evidence suggests that handling raises the threshold for restraint stress, such that handled rats ulcerate less than unhandled rats. The effect of shock has been studied less extensively than that of handling, but what evidence is available tends to suggest that the rat's susceptibility to restraint ulceration is increased following previous experience with electric shock stimulation. As might be expected, results vary from experiment to experiment, but there are so many aspects of the methodologies that are not standardized (e.g. restraint technique, duration of restraint, and prior food deprivation periods), that this lack of consistency in the results is not surprising.

One variable that does appear to have a consistent effect, irrespective of the methodology of the experiment, is the housing variable. In every experiment that has compared housing conditions, it has been found that group housed rats ulcerate more when restrained than individually housed rats. The result is an interesting one, as it is contrary to what one might expect on the basis of the preference by rats to live in groups. One of the purposes of the experimental work carried out for this thesis was to investigate the effect of prior housing on restraint-induced ulceration. The problem will be taken up again in the latter part of Chapter 5.

CHAPTER 5

SINES'S STRAIN OF STRESS ULCER SUSCEPTIBLE RATS

Early in the development of the restraint technique, Jacob Sines in the United States became interested in developing a strain of rats that was particularly susceptible to the ulcerogenic effects of restraint. Sines instituted a selective breeding program, and was able to demonstrate that certain selection procedures resulted in a strain of rats that was more susceptible to the ulcerogenic effects of restraint than was the original parent strain. Sines labelled the in-bred rats stress ulcer susceptible (SUS) rats, and he spent many years studying their behavioural and physiological characteristics. Descendants of Sines's SUS strain were used in the experimental work carried out for this thesis; consequently, some time will be spent examining Sines's work.

Development of the SUS Strain

Sines (1959) selected normal Sprague-Dawley male and female rats, 80-100 days old, and restrained them in gauze and plaster of Paris bandages for 48

hours. At the end of the restraint period, Sines did not destroy the rats to ascertain whether they had ulcerated or not. Rather, he anaesthetized each rat, opened its abdominal cavity, and lifted out the stomach. Sines then inflated the stomach with 2-5cc of air, injected through the forestomach. With the aid of a light shining through the distended stomach, Sines made a decision as to whether gastric ulceration had occurred or not. Using this method, he decided that 58% of the males and 68% of the females had developed standard gastric ulceration.

Sines validated the technique by killing a number of the rats so treated, and examining the internal linings of the stomachs for ulceration. He found that decisions made about the ulcerative state of a stomach using the method of inflation and transillumination were correct in 12 out of 13 cases; (the one error made was in the direction of a "no ulceration" decision when in fact ulceration had occurred).

Those rats not killed in the validation procedure were given time to recover from the abdominal surgery. Sines then mated those males and females that had been identified as developing restraint ulceration. When they were 80-100 days old, the offspring of the

first selective matings were restrained in the same way as the parent generation had been restrained. Sines again used the inflation and transillumination technique to identify which rats had ulcerated, and he found a significant increase in the incidence of ulceration: 21% more males and 20% more females in the selectively bred group developed restraint ulceration than had done so in the parent group.

Sines then continued the selective breeding with those of the first generation which had developed ulcers. When restrained, the second generation showed further increases in ulceration: 3% more males and 9% more females ulcerated than in the first generation. Further selective breeding of ulcerated rats led to a 100% incidence of gastric ulceration in both males and females in the third generation (Sines, 1960).

Once a 100% ulceration incidence had been achieved, Sines was forced to alter his method of selecting rats for breeding. Still using the method of inflation and transillumination, Sines started to make decisions about the <u>severity</u> of the ulceration produced by restraint. However, judgements of severity proved considerably more difficult to make than all-or-none

judgements, and after the fourth selectively bred generation, Sines attempted to inhibit the development of restraint ulceration by administering anticholinergic drugs (Sines, 1961). Sines assumed that only those rats that were especially susceptible to stress ulceration would become ulcerated under such conditions, and judgements could be made on the basis of incidence once again, rather than on the basis of severity.

Since his 1961 report, Sines has provided few details of the course of his selective breeding program, but at last reports (Sines & McDonald, 1968), 20 generations of SUS rats had been bred, with continued increases in susceptibility. More recently, Ader (1970a) has reported using rats of the 23rd generation of Sines's SUS stock.

Characteristics of the SUS Rats

Ulceration

Sines (1962) compared the ulceration rate of SUS rats with that of other strains of rats. Sines selected male and female rats of five different laboratory strains, and kept them in restraining cages

for 12 hours. The strains he selected from were Sprague-Dawley, Fischer 344, August 33322, A x C Irish, and the fourth generation of the SUS rats.

Sines found that the SUS males ulcerated significantly more than the males of all other strains. The SUS females ulcerated significantly more than the females of the Sprague-Dawley, August 33322, and A x C Irish strains, but not more than the Fischer 344 females.

Weight

Sines (1961) compared the weights of third, fourth, and fifth generation SUS rats with the weights of stock Sprague-Dawley rats. He found that all the SUS rats, irrespective of generation, were significantly lighter than the Sprague-Dawley rats. However, Sines noted a trend in the data for the weights of the SUS rats to regress with successive generations to the weights of the Sprague-Dawley rats. Unfortunately, Sines did not pursue the investigation beyond the fifth generation.

Activity and Emotionality

Sines (1961) compared the levels of activity and emotionality in the third, fourth, and fifth generations of the SUS rats with the levels in the Sprague-Dawley rats. Sines measured activity level by the number of square crossings and the amount of rearing in the open field, and emotionality by the number of defecations in the open field.

The results indicated that the third and fourth generations of SUS males entered a significantly greater number of squares, and reared more often than the Sprague-Dawley males. However, the fifth generation of SUS males did not differ in measures of activity from the Sprague-Dawley males. In the case of females, there were no differences between SUS rats and Sprague-Dawleys on the number of squares entered in the open field. Third generation SUS females reared more often than Sprague-Dawley females, but there were no differences between fourth and fifth generations and Sprague-Dawleys.

A similar trend was apparent when the males were compared on measures of emotionality. Third and fourth generation SUS males defecated more often than Sprague-Dawley males, but there were no differ-

ences between the fifth generation and the stock rats. With females, the trend was different. All three generations of SUS females defecated more than Sprague-Dawley females.

In another study, Sines (1962) replicated some of the findings of the 1961 study. He compared fourth generation male and female SUS rats with the rats of four other strains (including stock Sprague-Dawleys) on measures of activity and emotionality. As in the 1961 study, Sines found that fourth generation males entered more squares, reared more often, and defecated more often than Sprague-Dawley males. Fourth generation SUS males also entered more squares than August 33322 males, reared more often than Fischer 344 males, and defecated more than both of them. There were no differences on any of the measures between SUS males and A x C Irish males.

Again, as in the 1961 study, there were no differences between fourth generation SUS females and stock Sprague-Dawley females on measures of activity. Furthermore, there were no differences between the SUS females and the females of all the other strains on either activity measure. However, the female SUS rats defecated more often than the Sprague-Dawley

females, as in the 1961 study, and also more often than the August 33322 females.

It is interesting that Sines has consistently found no differences in measures of activity and emotionality between fifth generation SUS rats and stock Sprague-Dawley rats (with the single exception that SUS females defecated more than stock Sprague-Dawley females). Sines himself noted that the behaviour of the fourth and fifth generations of the SUS rats tended to regress towards the behaviour of the stock Sprague-Dawley rats. Rather than interpret the regression as a direct consequence of continued inbreeding for ulcer susceptibility, Sines (1961) suggested an hypothesis, based on his particular selection procedure, to account for the disappearance of the differences between SUS rats and Sprague-Dawley rats on the measures of activity and emotionality.

Sines pointed out that fifth generation SUS rats were the offspring of rats selected for their susceptibility on the basis of severity of ulceration, rather than on the basis of an all-or-none criterion. Because of the lesser sensitivity of a severity rating (under the conditions of stomach inflation and transillumin-

ation), Sines claimed that the offspring of such matings would be less sensitive to the selection pressures than had their parents been selected on an all-or-none basis.

Sines's hypothesis is vague, and does not really explain why the fifth generation SUS rats should be less active and less emotional than the earlier generations of SUS rats. Sines has not tested the hypothesis empirically, and he has not continued his study of activity and emotionality levels past the fifth generation to ascertain whether the differences in activity and emotionality would return or not once he had reverted to the selection procedure based on incidence. Nevertheless, Sines has continued to assume that the differences in activity and emotionality found between the third and fourth generations and the Sprague-Dawleys are legitimate differences between SUS rats and Sprague-Dawley rats generally.

For example, Sines carried out a number of studies in which he compared the behaviour of SUS rats with that of Sprague-Dawley rats in particular learning situations. In one experiment, he found that SUS males traversed a runway for a water reward more slowly than Sprague-Dawley males, but the SUS males

learned an avoidance from shock response faster (Sines, Cleeland, & Adkins, 1963). In another experiment, he found that sixth generation SUS rats lost less weight when water deprived for 24 hours than Sprague-Dawley rats, but were less dominant than the stock rats in gaining access to a water tube (Sines & Eagleton, 1961). In both experiments, Sines interpreted the differences between SUS and Sprague-Dawley rats in a theoretical framework that depended on SUS rats having a higher activity level than Sprague-Dawley rats.

On the basis of the interpretation of his own data, Sines has consistently proposed that ulcer susceptibility is related to activity and emotionality levels (Sines, 1963, 1966). (It is odd that Sines considered that ulceration was related to both activity and emotionality, as the usual measures of activity and emotionality (ambulation and defecation in the open field) are often negatively correlated.) Sines's insistence on the existence of such a relationship has meant that undue emphasis has been placed on corroborating Sines's findings. For example, Mikhail and Broadhurst (1965) restrained two groups of rats, one selectively bred for eliminative emotionality, and the other for unemotionality. On the basis of

Sines's theory, it was predicted that the emotional rats would ulcerate more than the unemotional, but no differences in ulceration were found between the two groups.

More generally, an extensive study has been made of the relationship between activity level and ulceration. This data was reviewed in Chapter 2 (see pp.25-29), and it was concluded that there is very little experimental evidence to support the claim that activity level and ulceration are positively related. On the contrary, some evidence suggests that passive rats are more likely to develop restraint ulceration than active rats.

Physiological Mediation of Ulcer Susceptibility

Whatever the behavioural characteristics of the SUS rats, there is no question that they are more susceptible to developing restraint-induced ulceration than are the rats of the parent Sprague-Dawley strain. Sines has suggested that the SUS rats are functionally sympathectomized, or parasympathetically dominant (Sines, 1961). In the light of the evidence reviewed in Chapter 3 about the physiological mediation of restraint-induced ulceration, it is clear that

such a parasympathetic bias, if it did exist, would predispose the SUS rats to developing restraint ulceration. In support of his hypothesis, Sines demonstrated that SUS rats have greater gastric motility when restrained than Sprague-Dawley rats (Eagleton & Sines, 1962). However, Sines failed to demonstrate any difference in free gastric acidity between the SUS and the Sprague-Dawley rats, although it was pointed out in Chapter 3 (see pp.92-93) that the free acid measure is not a useful measure of gastric secretory activity.

Sines has reported one other important finding about the SUS rats. He has consistently found that the SUS females are not good breeding rats (Sines & McDonald, 1968). Sines initially noted the problem with fourth generation SUS rats, when female rats had difficulty in delivering their litters. Those females that did deliver their pups successfully often refused to mother them. Sines found that the problem continued to be a serious one up until the 9th generation of SUS females, and still persisted to some extent with the 20th generation.

Ader has confirmed Sines's observations about the SUS females being poor breeders. Ader obtained

rats from the 23rd generation of Sines's SUS stock, and had considerable difficulty in getting the females to either breed or provide adequate mothering (Ader, 1970a).

MAINTENANCE OF ULCER-SUSCEPTIBLE RATS AT THE UNIVERSITY OF NEW SOUTH WALES

In 1967, Sines provided Lovibond with a group of 17th generation SUS rats (Lovibond, 1969). Since that time, the rats have been maintained by Lovibond, first at the University of Adelaide, and subsequently at the University of New South Wales. Since the arrival of the SUS rats in Australia, no new stock has been introduced to the colony. Sines's selective breeding program has not been continued; instead, the SUS rats have been mated randomly among themselves, without regard to degrees of susceptibility in the breeders. Because Sines's selective breeding program has not been continued, the rats maintained at the University of New South Wales will be referred to as ulcer-susceptible rats rather than as SUS rats, to maintain a distinction between the Australian rats and Sines's rats. Some space will now be given to

describing the conditions under which the ulcer-susceptible rats are bred and maintained at the University of New South Wales.

Breeding Program

Male and female rats, aged 100-120 days, and in good health, are selected for the breeding program. Three females and one male are housed together in a breeding box made of dense white polyethylene. The floor of the box is covered with sawdust, and the rats live on the sawdust. A metal grate lid, with an indented food hopper and two water bottles, is fitted to the top of the box. The breeding rats have free access to standard rat pellets and tap water; in addition, twice a week, they are provided with milk instead of water, and the pellet is supplemented with fresh meat (liver and heart).

After the male and females have been together for 10-15 days, the male is removed from the box and a new male introduced. This procedure guards against one or more of the females not becoming pregnant because of rejection of the first male. Eighteen days after the commencement of breeding, the females are examined for gravidity. Those females obviously

gravid are removed from the breeding box, and isolated in another box with some nesting material. The gravid female is then left undisturbed until after the birth of the litter.

Up until the time the pups are 14 days old, the mother and litter are not disturbed in any way, even for routine cleaning procedures. Once the pups are 2 weeks old, the routine change of box and sawdust is resumed. The pups are then left with the mother until they are 23 days of age, at which time they are weaned. Prior to the litter being weaned, both mother and pups are maintained on the supplemented diet outlined above.

As was found by Sines (Sinès & McDonald, 1968), and by Ader (1970a) with the SUS females, the ulcer-susceptible females are not good breeders. The problems encountered at the University of New South Wales are similar to those encountered elsewhere. Ulcer-susceptible females are slow to conceive; they sometimes have difficulty delivering their litters; and they appear to become infertile after the age of about 8-9 months. However, the major problem encountered is that the females do not provide adequate mothering for those litters delivered successfully. The mothers

often fail to retrieve the pups, or to build appropriate nests. In addition, new born litters are often eaten by the mothers, even though adequate nutrition is provided them in the form of meat and milk.

All possible precautions have been taken to correct the problem. The mothers and litters are undisturbed until the pups are 14 days old; noise is kept at a minimum; and the diet is supplemented. Nevertheless, the problem has persisted, and it is only by sheer perseverance that the stock has been maintained. It should be added that the extent of the problem appears, to some degree, to be governed by the prevailing season. The ulcer-susceptible rats are the most difficult to breed during late winter and early summer, but during late summer and early autumn, the breeding program is reasonably successful.[6]

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[6] It is possible that the seasonal changes in breeding efficiency are related to the nutritional value of the food pellet at particular times of the year. More by-products of grains, rather than the whole grains themselves, are used in manufacturing stock feeds during winter, thus resulting in a nutritionally inferior product at that time of the year. Such alterations in the nutritional value of stock feed might affect the efficiency of the reproductive processes of the rats.

Maintenance Program

When they are weaned, the young rats are immediately group housed according to sex. Groups of approximately six like-sexed rats are housed in white plastic boxes identical to those used for breeding. The rats are then maintained in this fashion until they are required for experimental purposes.

Twice a week, all rats living in boxes are transferred to clean boxes with clean sawdust. Each rat is transferred by an animal attendant picking it up behind the shoulders and carrying it to the clean box. In this way, all the ulcer-susceptible rats receive consistent handling throughout their lives, and as adults are very docile rats. Rats are generally maintained in the same social groups, and reorganization from box to box occurs only for specific reasons. When the rats are used for experimental purposes, they are usually transferred to metal grate cages, one rat per cage, for the duration of the experiment.

The rats are maintained on a standard diet throughout their lifetimes. Rats not involved in experimental programs have free access to standard rat pellets (provided initially by Allied Feeds, but

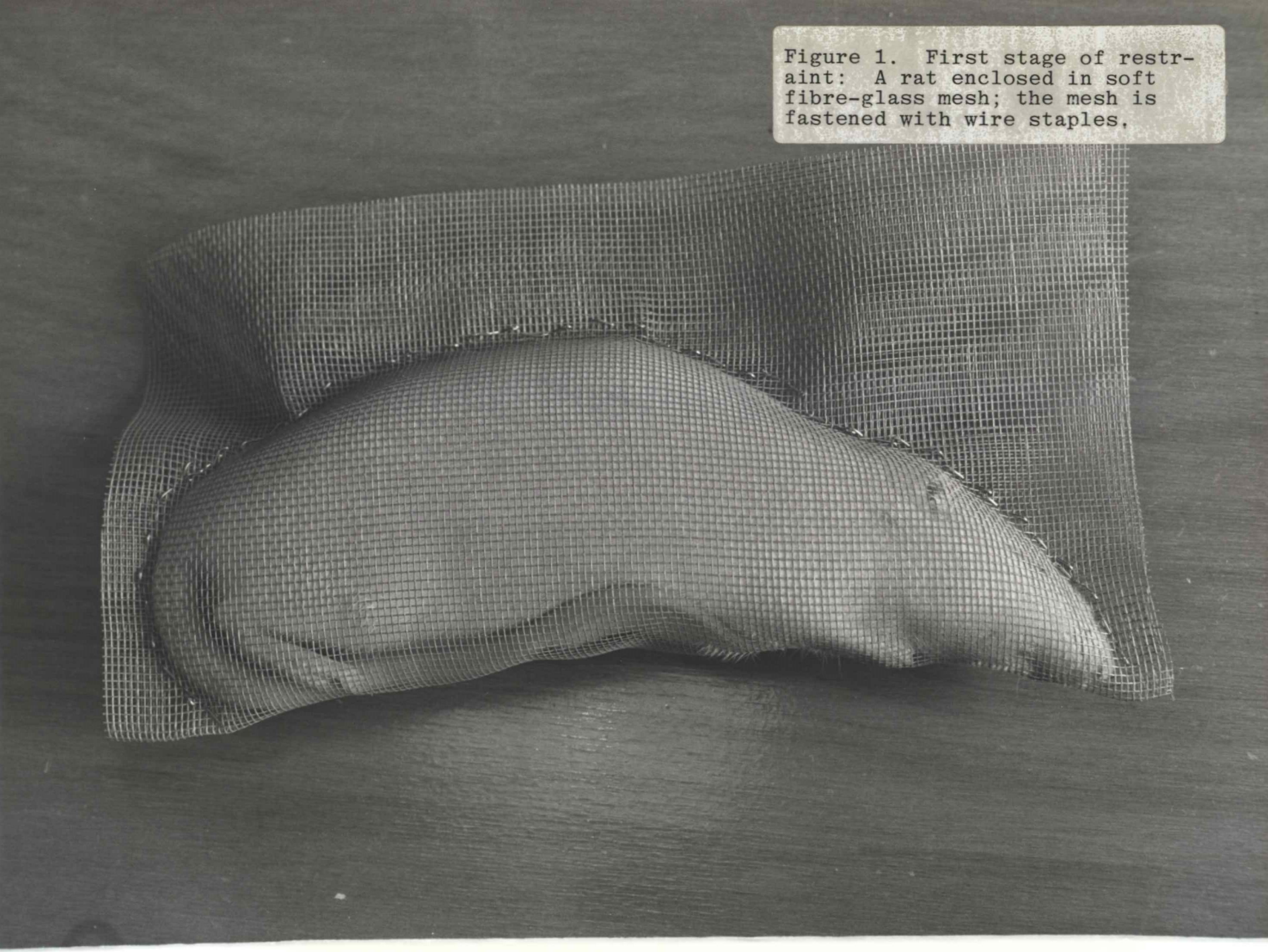

Figure 1. First stage of restraint: A rat enclosed in soft fibre-glass mesh; the mesh is fastened with wire staples.

now by Doust and Rabbidge) and tap water. As was mentioned above, breeding rats, gravid females, and pre-weaned pups receive supplementary milk and meat. After the rats are weaned, they continue to receive supplementary meat twice a week, but no milk.

The rats are maintained in an animal holding room where the light-dark cycle is artificially controlled, and set at 8 hours light and 16 hours dark. The light cycle commences at 8.00a.m. The animal holding room is air-conditioned, and the temperature is set at 22°±1°C (70°±2°F). Atmospheric humidity is partly controlled by means of a humidifier, set to maintain the relative humidity between the limits of 40% and 60%.

Use of the Ulcer-Susceptible Rats in Experimental Research

At the University of New South Wales, the stressor most frequently used to induce gastric ulceration in the ulcer-susceptible rats is restraint. The method of restraint employed is similar to that introduced by Brodie and Hanson (1960). Rats are first wrapped in soft fibre-glass mesh (gauge: 49 squares per sq. in.) as shown in Figure 1, facing page 166, and then

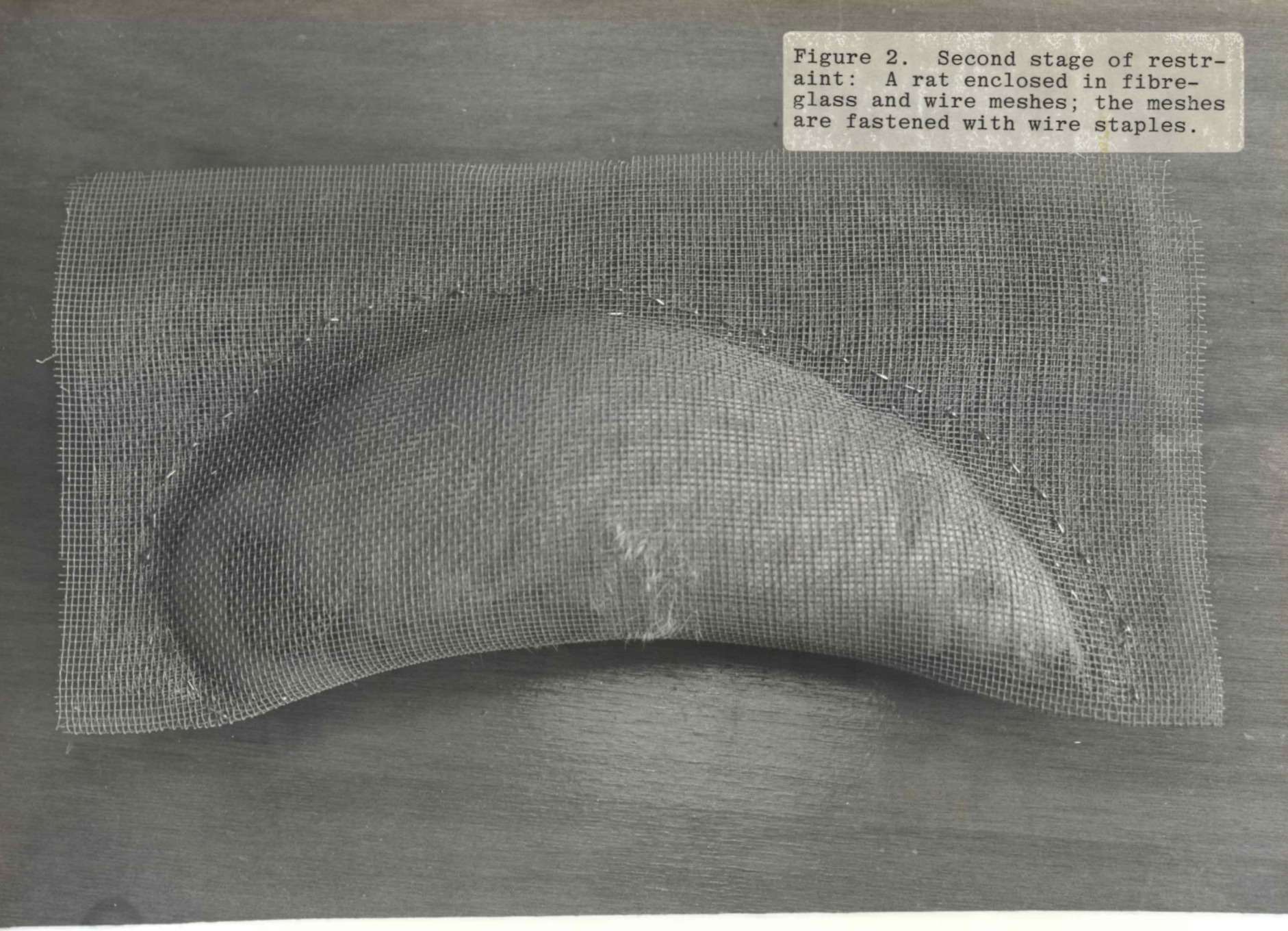

Figure 2. Second stage of restraint: A rat enclosed in fibreglass and wire meshes; the meshes are fastened with wire staples.

in wire mesh (identical size gauge) as shown in Figure 2, facing page 167; both layers of mesh are secured with wire staples. (The rat is wrapped in the soft mesh first to prevent the harsh wire mesh causing the rat any physical damage.)

The period of restraint most commonly used is 24 hours. Prior to restraint, the rats are always food deprived for 24 hours. The food deprivation is not introduced suddenly; instead, the feeding hours are gradually reduced over a 5 day period, until by the 5th day, the rats have been deprived for 24 hours. The following schedule is usually adopted:

Day 1: Feeding 9.00a.m. - 6.00p.m.
Day 2: Feeding 9.00a.m. - 3.00p.m.
Day 3: Feeding 9.00a.m. - 12.00noon
Day 4: Feeding 9.00a.m. - 10.00a.m.
Day 5: At 10.00a.m., 24 hours deprivation from 10.00a.m. on Day 4.

As would be expected, the method of restraint adopted results in stress ulceration in the fundus of the glandular section of the stomach, identical to that described by Brodie and Hanson: superficial erosion of the gastric mucosa which does not extend past the muscularis mucosa. It is sometimes accompanied by hemorrhage, and it is often surrounded by local edema. It varies from multiple pits to elongated

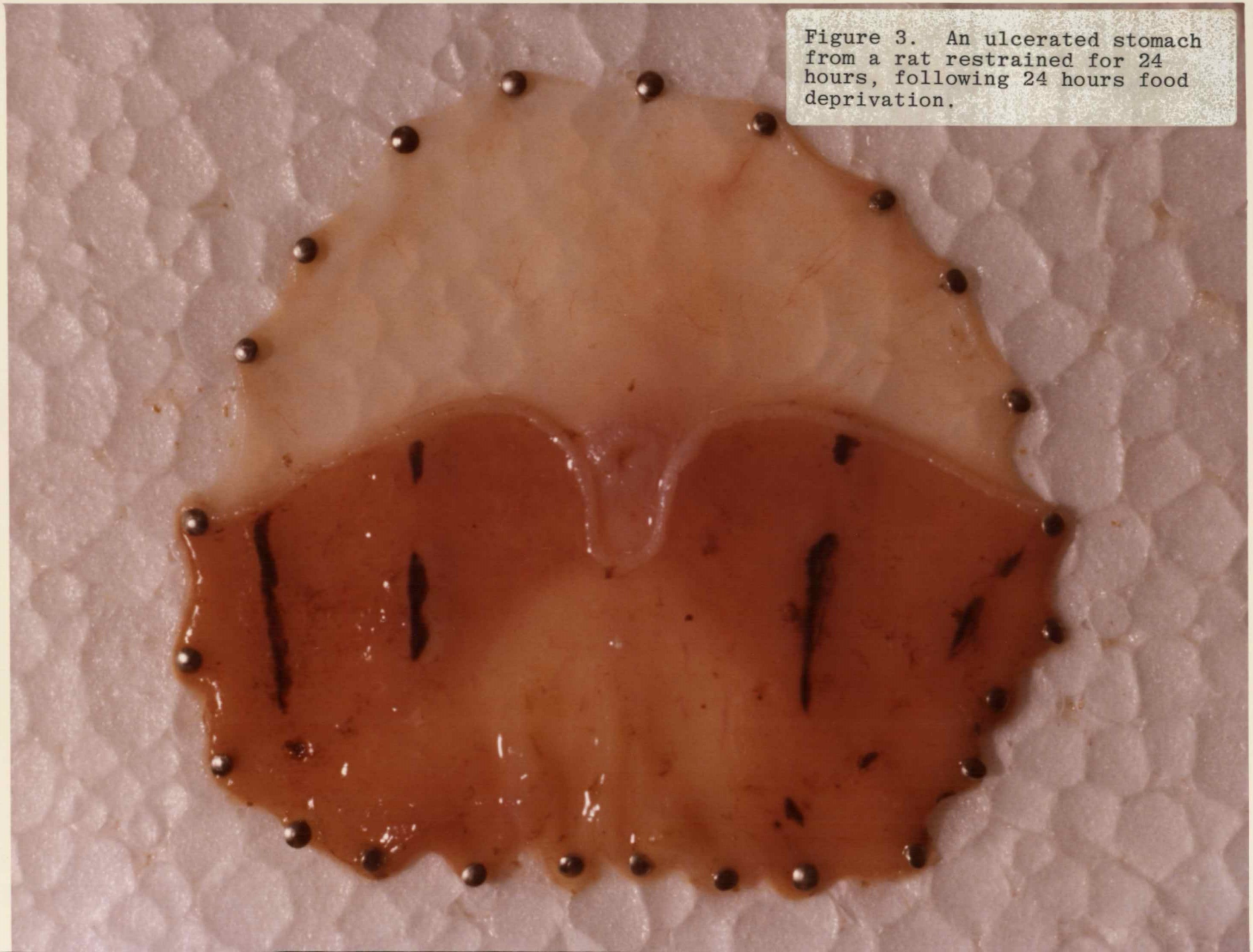

Figure 3. An ulcerated stomach from a rat restrained for 24 hours, following 24 hours food deprivation.

areas of erosion. An example of an ulcerated stomach is shown in Figure 3, facing page 168.

The specific criteria used for measuring restraint ulceration are the following: (1) an ulcer is any erosion of the fundal mucosa that can be detected with the naked eye; (2) the erosion may or may not be accompanied by hemorrhage; and (3) each separate erosion, irrespective of size, is counted as one ulcer. The ulcer-susceptible rats also have a propensity for developing ulceration in the rumen, but this ulceration is regarded as different from true stress ulceration, and is not included in measurements of restraint-induced effects.

Pilot Studies Investigating Parameters of Restraint-Induced Ulceration

In an effort to identify the contribution of various factors to the development of restraint-induced ulceration in the ulcer-susceptible rats, a number of short parametric studies was conducted. In these studies, the rats were examined for gastric ulceration at the following times:

(1) prior to being transferred from their group housing to the individual cages;

(2) after living in the individual cage for 7 days, and prior to the introduction of the food deprivation schedule;

(3) after the end of the food deprivation period, and prior to its being restrained.

The studies carried out were pilot studies, and most of the results will not be described here. However, one particular result was somewhat alarming. It was found that the incidence of ulceration in the rats examined for gastric pathology prior to being removed from their group housing was particularly high. In addition, the incidence of ulceration in rats that had been individually housed for 7 days was lower than that in the group housed rats.

It was decided that the group housed rats were being exposed to some particular stressor that was not affecting the individually housed rats, and a closer examination was made of the conditions under which the rats were group housed. In particular, the possibility that the group housed rats were crowded was investigated, given the fact that crowding stress had previously been suggested as a possible cause of other differences between group and individually housed rats (namely, the finding that group

housed rats ulcerate more when restrained than individually housed rats). As was stated earlier, six like-sexed rats were housed in one box, and the dimensions of the box were 42cm x 28cm x 15cm (16.5ins. x 11ins. x 6ins.), providing an average floor space of 196sq cm (30sq.ins.) per rat. This figure is comparable to the 24sq.ins. per rat provided by Stern et al. in their 1960 experiment, and the 29sq.ins. per rat provided by Ader in his 1965 experiment.

It was decided that the effect of the housing variable on stress ulceration should be investigated empirically. There were two questions that needed answering. First, do crowded housing conditions produce stress ulceration in ulcer-susceptible rats? Secondly, do crowded housing conditions increase susceptibility to restraint-induced ulceration? The aim of Experiment 1 was to answer both these questions.

MEASUREMENT OF ULCERATION IN ULCER-SUSCEPTIBLE RATS

Before Experiment 1 is described, a brief comment should be made about the measurement techniques used to quantify stress ulceration. A number of techniques

are presently in use, ranging from a simple count of the number of rats in a group that develop ulceration, to more complicated measures such as calculating the proportion of ulcerated gastric tissue in individual rats.

When Bonfils and Brodie and Hanson introduced the restraint method, they used a simple incidence measure to quantify the ulcerogenic effect. However, the incidence measure has two problems. The first problem is that it ignores much of the information contained in the data: there is no distinction made between rats with few ulcers and rats with many ulcers.

The second problem is that the measure is useless once the majority of rats are developing ulcers. Given the fact that nearly 100% of ulcer-susceptible rats ulcerate when restrained, the incidence measure is clearly not a useful tool with these rats.

There are a number of other measures in use that rely on the extent of ulceration in individual rats, rather than on the number of ulcerated rats in a group. There are three such severity measures commonly being used: number of ulcers per rat, total length of ulceration per rat, and individual ulcer ratings based on the holistic appearance of the stomach. Each

of these measures has advantages and disadvantages.

Counting the number of ulcers per stomach is an objective method of quantifying stress ulceration. Once a criterion is established as to what constitutes an ulcer, stomach lesions are rejected or accepted on the basis of whether they meet the criterion. Unlike the incidence measure, counting the number of ulcers discriminates among rats that have all ulcerated as well as between ulcerated and nonulcerated rats. This method is currently the most popular way of quantifying stress ulceration.

The disadvantage with the method of counting numbers is that no account is taken of the size of each erosion. One small pitted erosion represents the same ulcerogenic action as a large hemorrhaged fissure. The second severity method, counting the total length of ulceration in each stomach, overcomes this problem. Counting lengths makes use of all the information there is about the extent of stress ulceration. However, counting the length of ulceration is a tedious and a time consuming task, and only warrants being used if it is a more sensitive indicator of the differences between groups. Some experimenters (e.g. Ackerman, Hofer, & Weiner, 1975;

Lovibond, 1969) have found that measuring total length of ulceration provides no more information than counting numbers of ulcers. However, Weiss (1971a), using shock as a stressor, has found measuring lengths marginally more sensitive than counting numbers.

The third method used to measure ulcer severity involves assigning scores or ratings to stomachs on the basis of the extent of the ulceration. This method has been used by Sines (1961, 1962, 1965); Stern, Winokur, Eisenstein, Taylor, and Sly (1960); and Wilson (1966). Rating stomachs on, for example, a 5-point scale, has the definite advantage of being time saving. Furthermore, variance in the data is controlled by artificially nominating what the limits of that variance will be.

However, the rating method has a number of disadvantages. First, it lacks standardization. Each experimenter invents a different rating method, and cross-study comparisons are rendered more difficult. Secondly, by artificially imposing a set of scores, precise measures of the ulceration are not made; instead, approximations to a fixed and limited model are calculated. Finally, the rating method sometimes lacks the objectivity of the other two severity

measures, as raters often make holistic judgements according to criteria that cannot be specifically defined. Thus, inter-rater reliability is not as high as with the other two methods.

On the basis of these considerations, the procedure has been adopted with the ulcer-susceptible rats at the University of New South Wales to measure the number of ulcers per rat. However, it was considered that there were strong reasons for further investigating the usefulness of measuring length of ulceration. Therefore, in Experiment 1, both numbers of ulcers and total length of ulceration were used to measure stress ulceration.

CHAPTER 6

EXPERIMENT 1: THE EFFECT OF THREE DIFFERENT HOUSING CONDITIONS ON STRESS ULCERATION

It was concluded in Chapter 5 that the effect of the housing variable on stress ulceration should be examined more thoroughly. The aim of Experiment 1 was to answer two questions relating to the housing effect. First, does crowding stress produce gastric ulceration in ulcer-susceptible rats? Secondly, does crowding stress have the effect of increasing gastric ulceration induced by restraint?

The experiment was planned partly to solve problems surrounding the housing of the ulcer-susceptible rats at the University of New South Wales. Therefore, the experiment was designed around the type of housing currently in use. In the first stage of the experiment, the individual cage condition was compared with the group housing condition to see whether either was sufficiently stressful by itself to induce gastric ulceration. In addition, a third housing condition was introduced in which rats lived in groups, but with more space available to them than in the standard boxes generally used for group housing.

It was predicted that the rats living in the spacious, uncrowded boxes would develop less gastric ulceration in their home surroundings than the rats living in the crowded boxes. It was not known whether or not the rats living in the individual cages would develop more ulceration than the rats living in the uncrowded boxes; neither was any prediction made about the differences in ulceration between the rats living in the individual cages and those living in the crowded boxes.

In the second stage of the experiment, rats living in all three housing conditions were restrained. Given that under normal circumstances, rats prefer group conditions, it was predicted that rats living in the uncrowded boxes would develop less restraint ulceration than either the rats living in the crowded boxes or the rats living in the individual cages. Furthermore, it was predicted that rats living in the crowded boxes would ulcerate more when restrained than rats living in the individual cages. Both males and females were included in the study, and because of the tendency for females to develop more restraint-induced ulceration than males, the two sexes were compared for overall differences in ulceration.

Method

Subjects

The subjects were 108 ulcer-susceptible Sprague-Dawley rats, 54 of which were males, and the other 54 females. A group of 18 rats, 9 males and 9 females, was assigned to each of six groups. In the course of the experiment, three rats died: two females from one group, and a male from another group. All three rats died from causes unrelated to the experiment.

The rats were bred and maintained until they were 23 days old as described in Chapter 5 (see pp.162-164). The treatment of the rats after the age of 23 days was part of the experimental procedure, and will be described in detail in the procedure section. All rats were 23 days of age at the beginning of the study, and between 120 and 150 days of age at the end. Immediately prior to ulceration assessment, male rats had a mean weight of 330gms, and female rats a mean weight of 230gms.

Apparatus

The apparatus consisted of three types of housing constructions for rats. The first type was a

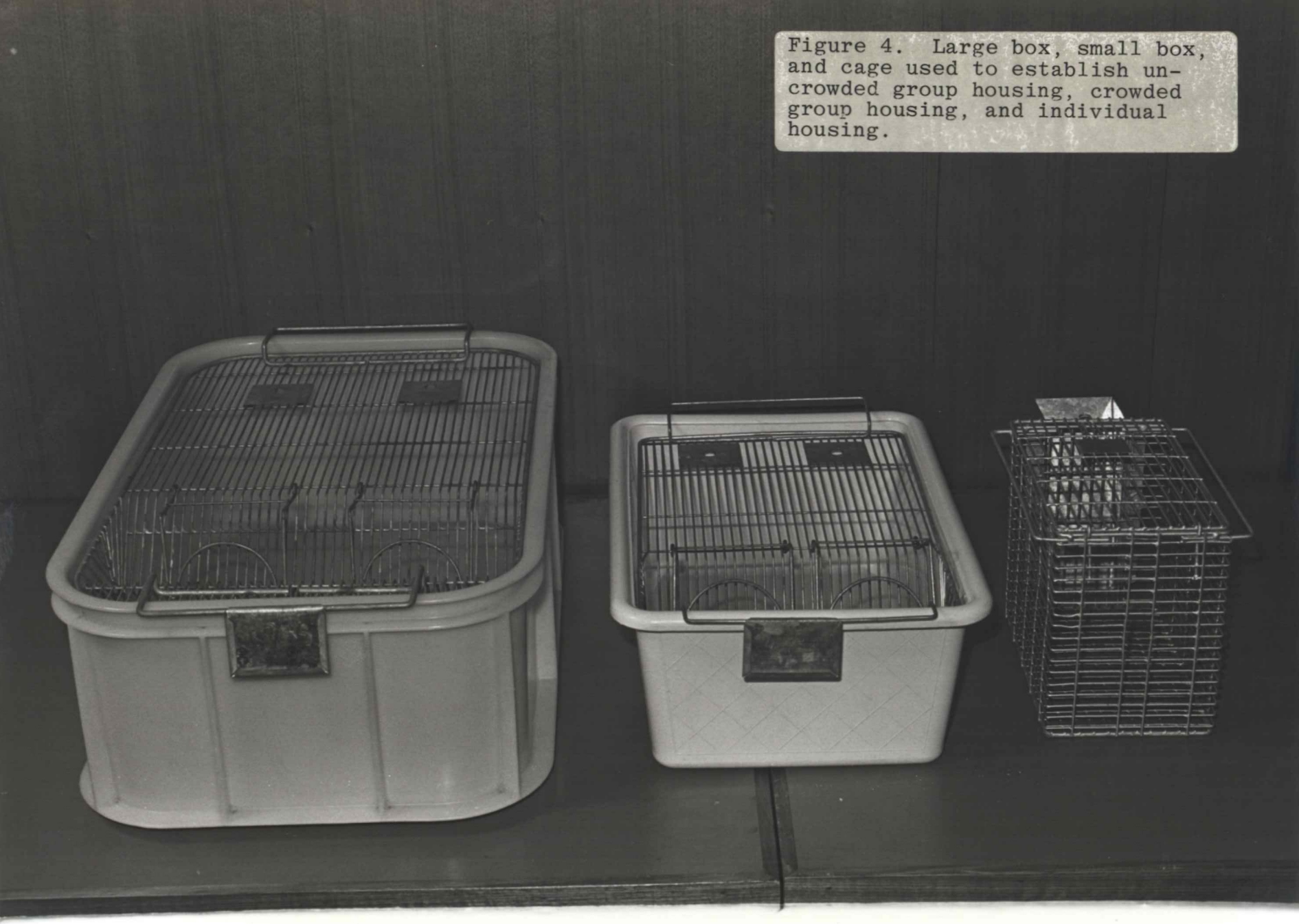

Figure 4. Large box, small box, and cage used to establish uncrowded group housing, crowded group housing, and individual housing.

large plastic box, made of high density polyethylene, with internal dimensions of 60cm x 37cm x 21cm (24ins. x 14.5ins. x 8.5ins.). The box was fitted with an aluminium grate lid, which had an indented food hopper, and two cradles for holding water bottles. The second type of housing was also a high density polyethylene box, but smaller, with internal dimensions of 42cm x 28cm x 15cm (16.5ins. x 11ins. x 6ins.). The box was fitted with a metal grate lid identical in design to that described above. There were six of each type of box. In the plastic boxes, sawdust covered the floors, and the rats lived on the sawdust.

The third housing construction was a metal grate cage, measuring 24cm x 15cm x 19cm (9.5ins. x 6ins. x 7.5ins.). A food hopper was attached to the front of the cage, and water was available from an automatic watering system attached to the back of the cage. The cages were suspended in racks, above trays of sawdust, such that the rats lived on the open weave metal floors. The relative sizes of the three housing constructions are shown in Figure 4, facing p.178.

The rats were weighed on an Ohaus Triple Beam Balance. A desiccator jar with ether in the bottom was used to anaesthetize the rats prior to restraint,

and to kill them at the end of the experiment. Rats were restrained in fibre-glass mesh (gauge: 49 squares per sq.in.), and wire mesh (gauge: 49 squares per sq. in.), both fastened with wire staples.

The stomachs were pinned out on slabs of polystyrene, and an Olympus stereoscopic microscope, Model SZIII, set at a magnification of 7 diameters, was used to count the number of ulcers and measure the total length of ulceration.

Procedure

Allocation of rats to housing conditions. At 23 days of age, 36 rats (18 males and 18 females) were allocated to each of the three housing conditions: individual housing in a wire cage, group housing in a small plastic box (crowded group housing), and group housing in a large plastic box (uncrowded group housing). Within each housing condition, half of the rats in that condition (i.e. 9 males and 9 females) were assigned to a no-restraint group, and the other half were assigned to a restraint group.

From age 23 days to 42 days, the individually housed rats were maintained in their cages, one rat to a cage; the group housed rats (both crowded and

uncrowded) were maintained in the small boxes, six like-sexed rats to a box. When they were 42 days old, the rats in the uncrowded housing group were transferred to the large boxes; the rats in the crowded housing group continued to live in the small boxes; and the individually housed rats continued to live in the cages. The rats then lived in their respective housing conditions until they were approximately 120 days of age. The rats in the individual cages were provided with 360sq cm (57sq.ins.) of floor space per rat, those in the crowded group housing with 196sq cm (30sq.ins.) per rat, and those in the uncrowded group housing with 370sq cm (58sq.ins.) per rat.

Maintenance of rats during the experiment. Throughout the experiment, all rats were handled twice a week as part of the routine cleaning schedule of the animal holding room. As described in Chapter 5, the rats housed in plastic boxes were transferred twice a week to clean boxes containing clean sawdust. Sawdust trays under the individual cages were changed at the same time as were the plastic boxes. Rats in the cages were picked up twice a week as were the group housed rats, but they were not placed in clean cages. The

cages themselves were changed once every 3 weeks. Other details of maintenance during the experiment are provided in Chapter 5 (see pp. 165-166).

No-restraint group. At age 120 days, all rats in the no-restraint group were weighed and then killed. Their stomachs were removed, opened along the greater curvature, and pinned out on slabs of polystyrene. The food in the stomach was removed by gently washing the stomach under cold tap water. The stomachs were inspected for erosions by two research assistants, neither of whom had any knowledge of the groups to which the stomachs belonged. Both the number of ulcers and the total length of ulceration were measured for each stomach. Where the counters disagreed about a measurement, a mean of the two independent measures was taken.

Restraint group. The rats in the restraint group were aged between 120 and 150 days at the time of restraint. In this condition, the rats were first weighed, and then placed on a graduated food deprivation schedule for 5 days (as described in Chapter 5 on p.167) until they had reached 24 hours deprivation.

Following the final fasting period, the rats were weighed again, lightly anaesthetized, and then restrained for 24 hours in fibre-glass mesh and wire mesh (as shown in Figures 1 and 2, facing pp. 166 and 167). Since the extent of ulceration varies with the tightness of restraint, the rats were restrained by a research assistant who was unaware of the groups to which the rats belonged. While the rats were restrained, they were kept in a quiet, air-conditioned room, where the temperature was set at 22°±1°C. The light-dark cycle during restraint was the same as that experienced by the rats in the animal holding room.

At the end of the restraint period, the rats were killed with an overdose of ether. The stomachs were removed, opened along the greater curvature, and pinned out on slabs of polystyrene (as shown in Figure 3, facing p.168). The stomachs were then examined for number of ulcers and total length of ulceration. The measurements were made by two research assistants, neither of whom had any knowledge of the groups to which the stomachs belonged. Where the counters disagreed about a measurement, a mean of the two independent measures was taken.

Results

No-Restraint Group

All ulceration occurred in the glandular section of the stomach; the inter-rater reliability was +.97. The mean number of ulcers and the mean length of ulceration in mm were calculated for each of the housing conditions, and are presented in Table 2.

Table 2

Mean Number of Ulcers and Mean Length of Ulceration Found in Rats Living in Different Housing Conditions

		Measurement	
Housing	n	Mean number of ulcers	Mean length of ulceration (in mm)
Individual	17	0.4	0.71
Crowded group	18	0.8	2.03
Uncrowded group	18	0.2	0.48

On close inspection of the data, it became clear that the males had reacted differently from the females, at least in the crowded group housing condition. Therefore, it was decided to compare the three housing conditions separately for males and females. The separate means for male and female rats within each housing condition are presented in Table 3.

Table 3

Mean Number of Ulcers and Mean Length of Ulceration Found in Male and Female Rats Living in Different Housing Conditions

Group	n	Measurement	
		Mean number of ulcers	Mean length of ulceration (in mm)
Males			
Individual	8	0.2	0.17
Crowded grp	9	1.4	3.22
Uncrowded grp	9	0.1	0.15
Females			
Individual	9	0.5	1.18
Crowded grp	9	0.2	0.84
Uncrowded grp	9	0.3	0.80

Because of the difference between the sexes in their reactions to crowded group housing, the data were analyzed separately for males and females. The data contained a large proportion (70%) of zero scores; therefore, nonparametric statistics were selected to analyze the results. Kruskal-Wallis one-way analyses of variance (Siegel, 1956) were carried out to detect any overall difference among either the males or the females on both measures of ulceration. The statistical values are presented in Table 4; the Type I Error Rate(α) was set at .05.

Table 4

Kruskal-Wallis Analysis of Variance *H* Values: Overall Differences Among Male and Female Rats on Each Measure of Ulceration

		Measurement	
Sex	*df*	Number of ulcers	Length of ulceration
Males	2	10.78*	12.89*
Females	2	.46	.29

*$p < .01$

The Kruskal-Wallis H values demonstrated that there was a significant overall effect among the three male groups, but that there was no effect among the three female groups. A number of Mann-Whitney U tests were then carried out to establish which particular differences among the males were significant. The Mann-Whitney U values (expressed as z scores) are presented in Table 5; α was set at .05.

Table 5

Mann-Whitney U Values (Expressed as z Scores) for Housing Comparisons Among Male Rats on Each Measure of Ulceration

	Measurement	
Housing comparison	Number of ulcers	Length of ulceration
Individual vs.Crowded group	2.37*	2.76**
Individual vs.Uncrowded group	.17	.09
Crowded group vs.Uncrowded group	2.83**	2.91**

*p<.01
**p<.005

Weights. The mean weights (taken prior to sacrifice) of male and female rats were calculated for each housing condition, and are presented in Table 6.

Table 6

Mean Weights in gms of Male and Female Rats Living in Different Housing Conditions

	Sex	
Housing	Males	Females
Individual	354.4	243.8
Crowded group	322.4	221.1
Uncrowded group	314.6	216.7

The weight data were analyzed separately for males and females. Planned comparisons (Hays, 1963) were written to compare all group housed rats with individually housed rats, and among the group housed rats to compare crowded rats with uncrowded rats. The summaries of the analyses of variance for males and females are presented in Table 7; α was set at .05.

Table 7

Analysis of Variance Summary Tables (Planned Comparisons): Weights of Males and Females Living in Different Housing Conditions

Source	SS	df	MS	F
	Males			
Between	52590.6	25		
Individual vs.All grouped	7067.5	1	7067.5	3.60
Crowded grp vs.Uncrowded grp	288.0	1	288.0	.15
Error	45235.1	23	1966.7	
	Females			
Between	7944.3	26		
Individual vs.All grouped	3733.4	1	3733.4	21.71*
Crowded grp vs.Uncrowded grp	84.5	1	84.5	.49
Error	4126.4	24	171.9	

*$p < .01$

Restraint Group

The majority of the ulceration occurred in the glandular section of the stomach; the few rumenal ulcers that did develop were not included in the results. (The majority of the rats that developed rumenal ulceration were in the individual housing condition.) The inter-rater reliability was +1.0. The mean number of ulcers and the mean ulceration length in mm were calculated for each housing condition, and are presented in Table 8.

Table 8

Mean Number of Ulcers and Mean Length of Ulceration Found in Restrained Rats Living in Different Housing Conditions

		Measurement	
Housing	n	Mean number of ulcers	Mean length of ulceration (in mm)
Individual	18	3.2	7.4
Crowded group	16	9.8	13.3
Uncrowded group	18	13.9	18.2

As in the no-restraint condition, it became clear from inspection of the data that the females had reacted differently from the males. Again, it was decided that the results for the males and the females should be examined separately. The mean number of ulcers and the mean length of ulceration for each housing condition are presented separately for males and females in Table 9.

Table 9

Mean Number of Ulcers and Mean Length of Ulceration Found in Restrained Male and Female Rats Living in Different Housing Conditions

Group	n	Measurement	
		Mean number of ulcers	Mean length of ulceration (in mm)
Males			
Individual	9	1.3	3.6
Crowded grp	9	6.2	8.7
Uncrowded grp	9	11.3	15.2
Females			
Individual	9	5.0	11.1
Crowded grp	7	14.3	19.3
Uncrowded grp	9	16.5	21.3

At the outset of the experiment, a set of planned comparisons had been written to answer the following questions:

(1) do the rats in the uncrowded group housing condition ulcerate less when restrained than the rats in the crowded group housing condition?

(2) do the rats in the uncrowded group housing condition ulcerate less when restrained than the rats in the individual housing condition?

(3) do the rats in the crowded group housing condition ulcerate more when restrained than the rats in the individual housing condition?

On inspection of the data, it was found that the rats in the uncrowded group housing condition had ulcerated more and not less than the rats in the other two groups. The crowding-stress hypothesis to explain why group housed rats develop more restraint-induced ulceration than individually housed rats had not been supported, and there was no point in testing the planned comparisons. However, it was decided that the differences in the data were of sufficient size and interest to warrant further investigation.

The ideal choice of statistical analysis would have been to carry out a two-way analysis of variance,

examining the overall effect of the housing variable, the overall effect of the sex variable, and the interaction between the two. Individual comparisons could then be made using Scheffé's method of post-hoc comparisons (Hays, 1963). However, the correct use of the analysis of variance depends on certain assumptions being met: namely, that errors are normally distributed, and that distributions of errors for each cell of observations have the same variance. Considerable departure from normality of distribution is permitted so long as the number of observations in each treatment-combination cell is relatively large. In addition, the requirement of homogeneity of variance is relatively unimportant so long as there are equal numbers of observations per treatment-combination cell (Hays, 1963).

The data in the restraint condition violated both the assumption of normality of distribution and that of homogeneity of variance. Furthermore, since it had been decided that the male and the female data should be examined separately, the number of observations per cell had been reduced to only nine (in one case it was seven). It was considered that such a sample size was far too small to overcome the problem

of a non-normal distribution. Therefore, the analysis of variance was decided against as an appropriate means of analyzing the data.

The only alternative was to use nonparametric procedures. Kruskal-Wallis analyses of variance were carried out to detect any overall differences among the males or among the females in the three housing conditions. The procedures were carried out for both numbers of ulcers and length of ulceration. The Kruskal-Wallis *H* values are presented in Table 10; α was set at .05.

Table 10

Kruskal-Wallis Analysis of Variance *H* Values: Overall Differences Among Restrained Male and Female Rats on Each Measure of Ulceration

		Measurement	
Sex	*df*	Number of ulcers	Length of ulceration
Males	2	15.67**	11.09*
Females	2	11.09*	5.41

*$p < .01$
**$p < .001$

In those groups where the H value was significant, individual comparisons between means were carried out using the Mann-Whitney U procedure. The Mann-Whitney U values (expressed as z scores) are presented in Table 12, on page 195; α was set at .05.

The overall means for males and females are presented in Table 11. Females ulcerated significantly more than males on both the number of ulcers measure, t (50) = 2.86, $p<.005$ (one-tailed), and the length of ulceration measure, t (50) = 2.97, $p<.005$ (one-tailed).

Table 11

Mean Number of Ulcers and Mean Length of Ulceration for Male and Female Rats

		Measurement	
Sex	n	Mean number of ulcers	Mean length of ulceration (in mm)
Males	27	6.3	9.2
Females	25	11.8	17.1

Table 12

Mann-Whitney U Values (Expressed as z Scores) for Housing Comparisons Among Restrained Male and Female Rats on Each Measure of Ulceration

	Measurement	
Housing comparison	Number of ulcers	Length of ulceration
Males		
Individual vs.Crowded group	3.17***	2.08*
Individual vs.Uncrowded group	3.35***	2.97**
Crowded group vs.Uncrowded group	1.68*	1.90*
Females		
Individual vs.Crowded group	2.13*	(H>.05)
Individual vs.Uncrowded group	3.19***	(H>.05)
Crowded group vs.Uncrowded group	.64	(H>.05)

*p<.05
**p<.01
***p<.001

Weights. The mean weights (taken prior to food deprivation) of male and female rats were calculated for each housing condition, and are presented in Table 13.

Table 13

Mean Weights in gms of Male and Female Rats Living in Different Housing Conditions

	Sex	
Housing	Males	Females
Individual	376.9	245.1
Crowded group	341.6	209.9
Uncrowded group	348.0	239.0

The weight data were analyzed separately for males and females. Planned comparisons were written to compare individually housed rats with all group housed rats, and among the group housed rats to compare crowded rats with uncrowded rats. The summaries of the analyses of variance for males and females are presented in Table 14; α was set at .05.

Table 14

Analysis of Variance Summary Tables (Planned Comparisons): Weights of Males and Females Living in Different Housing Conditions

Source	SS	df	MS	F
	Males			
Between	28170.7	26		
Individual vs.All grouped	6186.7	1	6186.7	6.81*
Crowded grp vs.Uncrowded grp	186.9	1	186.9	.21
Error	21797.1	24	908.2	
	Females			
Between	13049.8	24		
Individual vs.All grouped	2449.9	1	2449.9	7.43*
Crowded grp vs.Uncrowded grp	3344.1	1	3344.1	10.14**
Error	7255.8	22	329.8	

*$p < .05$

**$p < .01$

Discussion

No-Restraint Group

Among the males, rats housed in groups in crowded conditions developed significantly more stress ulceration in their home surroundings than rats housed in groups in uncrowded conditions or rats housed in individual cages. Male rats housed in uncrowded groups and in individual cages both developed insignificant amounts of stress ulceration. All female rats, irrespective of their housing condition, developed insignificant amounts of ulceration in their home surroundings.

The failure of the crowded female rats to develop stress ulceration is interesting. One possible explanation is that the females were sufficiently smaller than the males (by approximately 100gms) for the small box used in the experiment not to constitute a crowded condition for them. Before it can be concluded that females are not stressed by crowded living conditions, the experiment should be repeated with females occupying a proportionately smaller living compartment.

The two measures of ulceration (number of ulcers and length of ulceration) provided the same informat-

ion about the differences among the groups. However, there were differences in the sensitivity with which the two measures detected effects, and on the whole, length of ulceration was marginally more sensitive than number of ulcers.

There were few significant differences among the weights of the rats housed in the three different housing conditions. Among the males, there were no significant differences at all. Among the females, individually housed rats weighed more than the group housed rats, but there was no difference between rats housed in crowded groups and rats housed in uncrowded groups.

Restraint Group

The results did not confirm the hypothesis that crowding stress is responsible for group housed rats ulcerating more when restrained than individually housed rats. As was expected, rats in the crowded groups ulcerated more than rats in the individual cages; but rats in the uncrowded groups also ulcerated more than rats in the individual cages. These two findings established unequivocally that group housed rats, irrespective of whether they are crowded

or not, ulcerate more than individually housed rats.

The most unexpected result was that crowded male rats ulcerated less when restrained than uncrowded male rats. With the females, this was not the case. Crowded females did not develop significantly less restraint ulceration than uncrowded females. It was suspected that this difference between male and female reactions in the crowded housing condition reflected the probable failure of the apparatus to establish a crowded condition for females.

The data on number of ulcers and length of ulceration both provided the same basic information as regards the trends of the differences among the groups. However, it was clear that the length of ulceration measure was less sensitive in detecting these differences than was the number of ulcers measure. With α set at .05, the length of ulceration measure did not even detect an overall effect among the female groups, whereas the number of ulcers measure detected it at the .01 level. It was thought that the lesser sensitivity in the length of ulceration measure was probably a function of the greater variability in the scores as compared with the variability in the numbers measure.

There were more significant differences in weights in the restraint condition than there were in the no-restraint condition.[7] Group housed males weighed significantly less than individually housed males, but there was no difference between males in crowded groups and those in uncrowded groups. Among the females, the weight results were somewhat unexpected. Group housed females weighed significantly less than individually housed females, but females housed in uncrowded groups weighed significantly more than females housed in crowded groups.

The interpretation of the results of Experiment 1 poses a number of difficulties. First, any differences in ulceration between groups must be considered in the context of differences in weights of the rats

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[7] One possible explanation for the larger number of significant differences in weights across different housing conditions in the restraint section is that the restraint rats were older (maximum age of 150 days) than the no-restraint rats (maximum age of 120 days) at the time the weight measurements were taken. The rats living in the plastic boxes had an opportunity to engage in limited exercise, whereas rats living in the individual cages had no opportunity to exercise at all. As the rats became older, differences in weight between the individually housed rats and the group housed rats would have increased because of the greater accumulation of surplus body fat in the caged rats as compared with the boxed rats.

in those groups. For example, while individually housed rats (both males and females) ulcerated significantly less than the group housed rats, the individually housed rats also weighed significantly more than the group housed rats. As was pointed out in Chapter 2 (see pp.31-36), there is a considerable amount of evidence to suggest that heavier rats ulcerate less than lighter rats. Therefore, the difference between the individually housed rats and the group housed rats could be a function of the difference in weight rather than the difference in housing. The interpretation of the difference in ulceration between the males housed in crowded groups and the males housed in uncrowded groups is less equivocal. There was no significant difference in weight between the two groups, and therefore the difference in ulceration is more likely to be a function of the housing difference per se.

A further problem with the interpretation of the results is that the methodology of the experiment itself was not ideal. The chief problem was that the type of housing construction was not standardized. Rats in the individually housed condition lived in open grate metal cages, whereas rats in the group

housed condition lived in opaque plastic boxes. It would have been preferable had all the housing apparatus been constructed of the same material, but as was pointed out in the introduction to Experiment 1, one of the aims of the experiment was to compare housing conditions currently in use at the University of New South Wales.

There were a number of problems associated with the use of different housing materials. First, rats in boxes were transferred to clean boxes twice a week, whereas rats in cages occupied the same cage for 3 weeks. Secondly, rats in boxes were able to engage in coprophagy, and rats in cages were not. It is known that rats obtain nutritional benefit (in the form of essential fatty acid and vitamin supplementation) from feeding on their own excrement (National Academy of Sciences National Research Council, 1962); therefore it is not unreasonable to assume that the group housed rats were in a nutritionally superior position to the individually housed rats, particularly during the 5 day food deprivation period. This factor does not explain why group housed rats ulcerated more than individually housed rats, but it is possible that it accounts for why

individually housed rats developed the majority of the rumenal ulcers, since nutritional disturbances are notorious for producing such ulceration in rats.

One final problem with the methodology, although probably a trivial one, is that rats in individual cages and rats in crowded groups occupied their respective housing from the age of 23 days, whereas rats in uncrowded groups did not occupy their housing until they were 42 days of age.

Conclusions

A number of conclusions can be made on the basis of the results of both the no-restraint and the restraint sections of the experiment. First, male rats in crowded groups develop more stress ulceration in their home surroundings than male rats individually housed or those housed in uncrowded groups. It was decided that whether the result is also true for females required further investigation.

Secondly, the manner in which rats are housed during their lifetimes has an effect on their weights as adult rats. Therefore, within any one experiment, it is important to standardize housing conditions, or else systematically control the weights of rats in

different experimental groups.

Thirdly, it can be concluded that for restraint-induced ulceration at least, counting the number of ulcers is a more sensitive measure of differences among groups than counting the length of ulceration. While measuring the length might seem, intuitively, to provide a more complete picture of the ulcerogenic effect, differences between groups are best reflected by counting the number of ulcers.

Fourthly, it has been confirmed that females develop more restraint-induced ulceration than males. Sines (1961) found that in the parent Sprague-Dawley strain, females developed more restraint ulceration than males. However, Sines could not demonstrate the sex difference in the early generations of the SUS rats. In Experiment 1, it was found that the ulcer-susceptible females do ulcerate more when restrained than the ulcer-susceptible males.

Finally, it is clear that the housing variable does have an effect on restraint-induced ulceration. Group housed rats do ulcerate more than individually housed rats, though this effect is possibly confounded by the weight differences between the rats occupying the different housing conditions. Furthermore,

crowded male rats ulcerate less when restrained than uncrowded male rats - a finding that was quite contrary to expectations. As in the no-restraint section of the experiment, it was decided that whether this result could be found with crowded and uncrowded females or not required further investigation.

CHAPTER 7

EXPERIMENT 2: THE EFFECT OF TWO DIFFERENT HOUSING CONDITIONS FOR FEMALE RATS ON STRESS ULCERATION

The results of Experiment 1 revealed a number of differences between male and female ulcer-susceptible rats. The first difference was that female ulcer-susceptible rats ulcerated significantly more when restrained than male ulcer-susceptible rats. This result confirmed the finding by many other experimenters (e.g. Sines, 1959; Lambert, 1968; Herner & Caul, 1972) that females develop more restraint-induced ulceration than males.

The other differences between males and females in Experiment 1 occurred because of the different ways the two sexes reacted to the experimental manipulations. First, it was found that males living in crowded groups developed considerable stress ulceration in their home surroundings, but that females living under the same conditions developed very little stress ulceration. Secondly, males housed in crowded groups ulcerated less when restrained than males housed in uncrowded groups, but there was no

significant difference in restraint ulceration between crowded and uncrowded females.

It was suggested in Chapter 6 that the most likely reason for there being no difference in stress ulceration between the crowded females and the uncrowded females in either the no-restraint or the restraint sections was because of the probable failure of the apparatus to establish crowded conditions for females. Although it was considered that the small plastic box used to group house rats at the University of New South Wales was generally having a crowding effect, it was not taken into account at the time of the design of the experiment whether females would be less adversely affected by the conditions or not. The females in Experiment 1 weighed, on the average, 100gms less than the males, and it is possible that while the males were subjected to crowding stress, the females were not.

The aim of Experiment 2 was to compare female rats in an adjusted crowded condition with female rats in an uncrowded condition. Crowded housing for females was established by using a box that was smaller than the small box used in Experiment 1. It was decided that the reduction in size of the female

crowded box, compared with the crowded box used in Experiment 1, should be determined by a comparison of the sizes of male and female rats. The individual housing was not included in Experiment 2, as the primary interest was in the comparison between crowded groups and uncrowded groups. As in Experiment 1, the rats in Experiment 2 were compared under conditions of both no-restraint and restraint.

It was predicted that females living in groups in properly crowded conditions would develop more stress ulceration in their home surroundings than females living in groups in uncrowded conditions. Furthermore, it was predicted that group housed females properly crowded would ulcerate significantly less when restrained than uncrowded group housed females. Because of the lesser sensitivity of the length of ulceration measure found in Experiment 1, it was decided that only numbers of ulcers would be counted.

Method

Subjects

The subjects were 48 female ulcer-susceptible

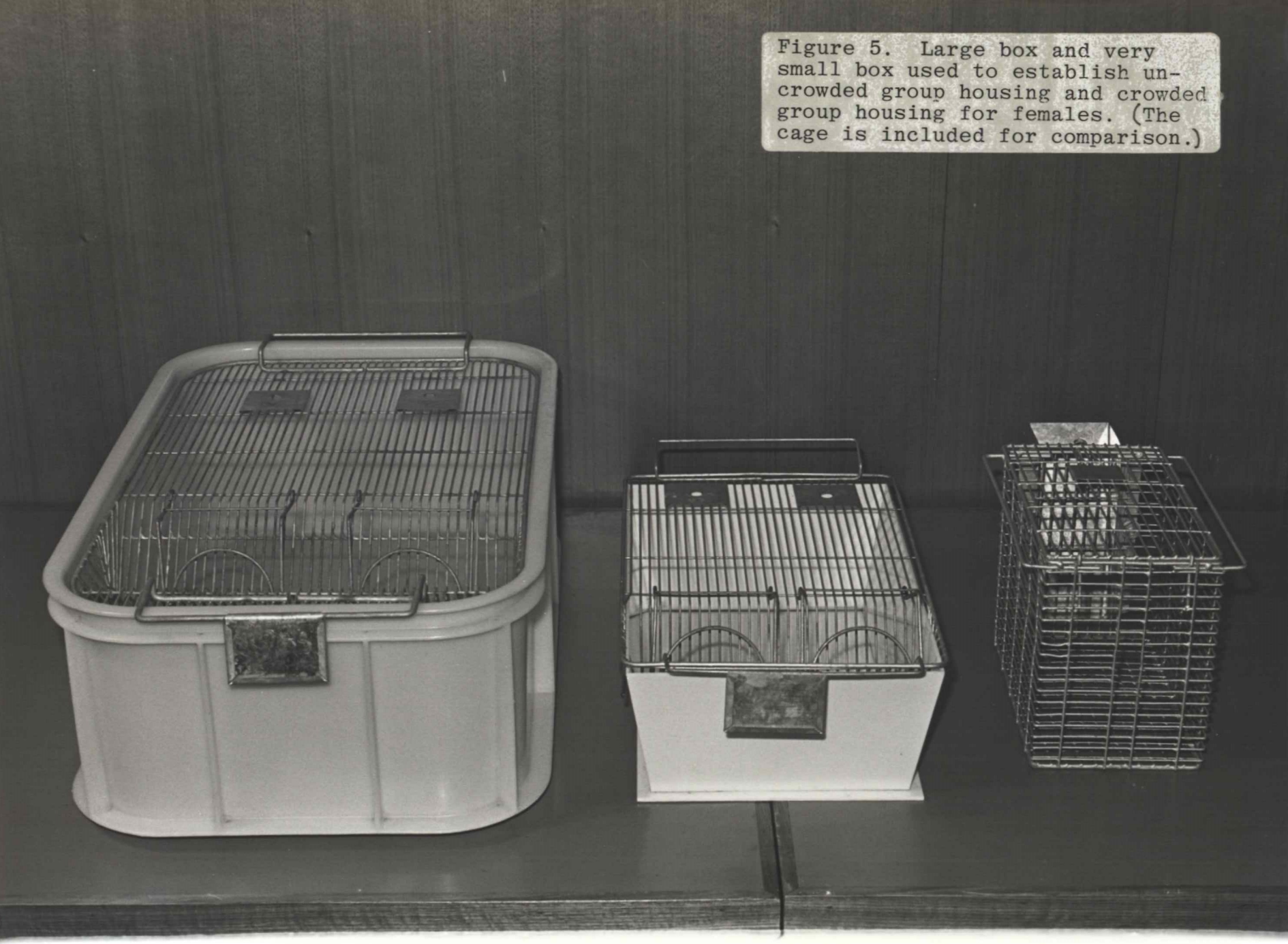

Figure 5. Large box and very small box used to establish uncrowded group housing and crowded group housing for females. (The cage is included for comparison.)

Sprague-Dawley rats. They were divided into four groups, 12 rats in each group. The rats were bred until weaning age as described in Chapter 5 (see pp. 162-164). All rats were 23 days old at the beginning of the experiment, and aged between 115 and 125 days at the time of examination for ulcers. Immediately prior to ulceration assessment, the female rats had a mean weight of 230gms.

Apparatus

The apparatus was similar to that used in Experiment 1. The housing apparatus consisted of two types of housing boxes for rats. Both boxes were made of high density polyethylene, and they were fitted with metal grate lids with indented food hoppers and two water bottle cradles. One box had internal dimensions of 60cm x 37cm x 21cm (24ins. x 14.5ins. x 8.5 ins.); the other box had internal dimensions of 39cm x 25cm x 13cm (15.5ins. x 10ins. x 5ins.). There were eight boxes in all, four of each type. Sawdust covered the floors of both the plastic boxes, and rats lived on the sawdust. The relative sizes of the two housing boxes are shown in Figure 5, facing page 210. (The individual cage is included in Figure 5 for purposes of

comparison only; it was not used in Experiment 2.)

An Ohaus Triple Beam Balance was used to weigh the rats, and a desiccator jar with ether in the bottom was used to anaesthetize the rats prior to restraint, and to kill them at the end of the experiment. Rats were restrained in fibre-glass and wire meshes of the same gauge used in Experiment 1. Both meshes were fastened with metal staples. The stomachs were pinned out on slabs of polystyrene.

Procedure

Determination of the size of the box that would establish crowded conditions for females. The volumes of 120 day old male and female ulcer-susceptible rats were determined by a displacement of water test. The average volume of water displaced by male rats was 400ml, and that displaced by female rats was 280ml. The ratio of the volume of a female rat to the volume of a male rat was thus calculated to be .7. Consequently, a plastic box was constructed such that the volume of the box was reduced to .7 of the volume of the crowded box used in Experiment 1. To achieve this reduction in volume, all dimensions of the box were reduced by about 2.5cm (1in.).

Allocation to housing conditions. The rats were bred and maintained until weaning age as described in Chapter 5. At age 23 days, 24 rats were allocated to each of the two housing conditions: group housing in a large plastic box (uncrowded group housing), and group housing in a very small plastic box (crowded group housing). There were four large boxes, each housing 6 female rats, and four very small boxes, each housing 6 females. Within each housing condition, half the females (i.e. 12 rats) were allocated to a no-restraint group, and the other half were allocated to a restraint group.

As in Experiment 1, rats in the uncrowded group housing condition lived until they were 42 days in the smaller boxes. At age 42 days, they were transferred to the large boxes. (While this procedure was not methodologically ideal, it was included in Experiment 2 so as to maintain a procedure parallel with that of Experiment 1.) The rats remained in their respective housing conditions until they were approximately 120 days old. The rats were reared and maintained throughout the experiment in the same manner as described in Experiment 1 (see p.180).

No-restraint group. At age 120 days, all rats in the no-restraint group were weighed and then killed with an overdose of ether. Their stomachs were removed, opened along the greater curvature, cleared of food, and pinned out on slabs of polystyrene. The numbers of ulcers were counted by two research assistants, neither of whom had any knowledge of the groups to which the stomachs belonged. If the counters disagreed about any measurement, a mean of the two independent measures was taken.

Restraint group. The rats in the restraint group were aged between 115 and 125 days at the time of restraint. The rats were first weighed, and then placed on a food deprivation schedule for 5 days (as described in Chapter 5, on p.167). Following the final 24 hours deprivation, the rats were weighed again, and then restrained in fibre-glass mesh and wire mesh for 24 hours. The rats were restrained by a research assistant who was unaware of the groups to which the rats belonged. (Both stages of the restraint procedure are shown in Figures 1 and 2, facing pp.166 and 167.)

During restraint, the rats were kept in a room

where the temperature varied from 20°C to 26°C, and where the light-dark cycle was identical to that experienced in the animal holding room. At the end of the restraint period, the rats were killed, and their stomachs removed, opened along the greater curvature, and pinned out on slabs of polystyrene (as shown in Figure 3, facing p.168). The numbers of ulcers were counted by two research assistants, who were unaware of the groups to which the stomachs belonged. A mean count was taken where the counters disagreed about a measurement.

Results

No-restraint Group

All the ulcers occurred in the glandular section of the stomach; the inter-rater reliability was +.95. The mean number of ulcers was calculated for both the crowded group housing condition and the uncrowded group housing condition. The means are presented in Table 15, on page 215.

The data indicated that the females living in the crowded groups had not developed more stress ulceration in their home surroundings than the females

Table 15

Mean Number of Ulcers Found in Female Rats Living in Different Housing Conditions

Housing	n	Mean number of ulcers
Crowded group	12	.4
Uncrowded group	12	1.2

living in the uncrowded groups. The prediction made prior to commencing the experiment had not been met; therefore, no statistical analysis of the data was made.

Restraint Group

The majority of the ulcers occurred in the glandular section of the stomach; the few rumenal ulcers that did develop were not included in the results. The inter-rater reliability was +.99. The mean number of restraint-induced ulcers was calculated for the females living in crowded and uncrowded groups, and both means are presented in Table 16.

Table 16

Mean Number of Ulcers Found in Restrained Female Rats Living in Different Housing Conditions

Housing	n	Mean number of ulcers
Crowded group	12	25.4
Uncrowded group	12	16.3

Again, it was clear from inspection of the data that the females living in the crowded groups had not developed less restraint ulceration than the females living in the uncrowded groups. However, the difference in ulceration between the two groups was so large in the direction opposite to that predicted, that it was decided to carry out a two-tailed t test of significance, rather than the one-tailed directional test originally planned: t (22) = 2.62, $p<.05$.

As in Experiment 1, it was found that the females living in the crowded groups weighed significantly less than the females living in the uncrowded groups, t (22) = 2.81, $p<.05$ (two-tailed). The means of the

weights are presented in Table 17.

Table 17

Mean Weights in gms of Female Rats Living in Different Housing Conditions

Housing	Mean weight (in gms)
Crowded group	209.3
Uncrowded group	226.8

Discussion

The results from the no-restraint section of the experiment demonstrated that ulcer-susceptible females housed in crowded groups do not develop more stress ulceration in their home surroundings than ulcer-susceptible females housed in uncrowded groups. Thus the results of Experiments 1 and 2, considered together, indicate a sex difference in the reaction of the ulcer-susceptible rats to crowded housing conditions. Males appear to be sufficiently stressed by such crowding to develop stress ulceration; females

appear not to be.

Such a sex difference in reaction to housing conditions is not counter-intuitive. Establishing territoriality is an important part of the behaviour pattern of male rats (including laboratory rats), but is much less important for female rats (Barnett, 1963). Therefore, reducing the amount of space available to rats is probably more stressful for male rats than for female rats.

The results from the restraint section of Experiment 2 are much more puzzling. On the basis of the results of Experiment 1, it was expected that the females group housed in crowded conditions would ulcerate significantly less when restrained than the females group housed in uncrowded conditions. This result was not obtained. Instead, the crowded females ulcerated significantly more when restrained than the uncrowded females. There were no obvious differences between the methodologies of Experiments 1 and 2 to explain why this reversal of trend had occurred. Although the crowded females weighed less than the uncrowded females in Experiment 2, thus predisposing the crowded females to more restraint ulceration, a similar difference in weight between the crowded

and the uncrowded females had occurred in Experiment 1 without resulting in more ulceration in the crowded females.

At this stage, all that can be suggested is that male and female ulcer-susceptible rats react differently to restraint depending on their prior housing conditions. If such an interaction between housing and sex does exist, then a number of practical steps should be taken by those carrying out empirical research in the area. First, it is preferable to use either males or females in an experiment on restraint-induced ulceration. Secondly, it is preferable to use males rather than females, because of what appears to be increased variability of response on the part of the females. Finally, if both males and females are used in the same experiment, allocation of the sexes to the experimental groups must be carefully controlled, and larger than normal samples chosen to help overcome the problem of increased within-group variance.

CHAPTER 8

EXPERIMENT 3: A STUDY OF THE EFFECT OF EXPOSURE TO PRIOR STRESS ON LATER RESISTANCE TO RESTRAINT-INDUCED ULCERATION

The results of Experiment 1 indicated that group housed rats, whether crowded or not, ulcerated significantly more when restrained than individually housed rats. This result was found for both males and females. The results of Experiment 1 also indicated that male rats living in crowded groups ulcerated significantly less when restrained than male rats living in uncrowded groups.

For female rats, the effect of crowding on restraint-induced ulceration is less clear. In Experiment 1, slightly crowded female rats did not develop a significantly different amount of restraint ulceration from uncrowded female rats. In Experiment 2, female rats subjected to a degree of crowding that was considered comparable to the crowding experienced by male rats in Experiment 1 ulcerated significantly more when restrained than uncrowded females. On the basis of all these findings, the decision was made in Chapter 7 that in the future, restraint-

induced ulceration should be studied separately for male and female rats.

Rather than pursue the puzzling differences found between the male and the female ulcer-susceptible rats, it was decided to study what was considered the most interesting result of Experiment 1: the finding that crowded males ulcerate less when restrained than uncrowded males. The difference between crowded and uncrowded males in their reactions to restraint was particularly interesting because the results of Experiment 1 also established that crowded male rats were stressed by their living conditions, and uncrowded male rats were not. In other words, if the two sets of results were considered together, it appeared as if male rats exposed to a mild, chronic stressor (crowding) reacted less to an acute, severe stressor (restraint) than did male rats who had not been exposed to a stressor prior to the restraint. On the basis of these considerations, it was hypothesized that the stress reaction to restraint could be reduced in male rats if such rats had prior experience with mild, chronic stressors.

Hypotheses of this type are not new. Many researchers working in the stress area have examined

the possibility that prior experience with stressors protects the organism against later stress reactions. Some of these experiments were discussed in Chapter 4, and it was pointed out in that Chapter that the results from this type of research have been equivocal. Nevertheless, the data from the group housing section of Experiment 1 suggested the possibility that such a protective mechanism might be operating, and it was decided to examine the possibility empirically in Experiment 3.

The experiment was designed along the following lines. One group of rats experienced a mild, chronic stressor for some weeks prior to being restrained, and a second group had no such experience with a stressor prior to restraint. For two reasons, electric shock stimulation was chosen as the mild, chronic stressor. First, it was assumed that experience with stressors generally was the factor that was providing the protection against the restraint effect, and not the crowding stressor per se. Therefore, it was necessary to choose a mild, chronic stressor that was unrelated to the rats' housing conditions. Secondly, the mild stressor chosen had to be one that was universally accepted as a valid means of produc-

ing stress reactions in laboratory rats. Electric shock stimulation filled both these requirements. A further advantage of using shock was that the severity of the stress produced could be quantitatively controlled.

At this stage of planning the experiment, it became apparent that a problem in the interpretation of the results would arise if only the shock stress and the no shock stress groups were employed in the study. The difficulty was that the group of rats being stressed prior to restraint was not only being exposed to stress, but it was also being exposed to novel stimulation (e.g. being removed from the animal holding room, and being handled by the experimenter). In other words, any difference in restraint-induced ulceration between the group being exposed to a prior stressor and the group not being exposed could not be unequivocally interpreted as due to the effect of the prior experience with stress. To overcome this problem of interpretation, a third group was added to the experimental design, a group which was subjected to the same procedure as the shock stress group, but which was not actually shocked.

It was predicted that the male rats experiencing

mild, chronic stress prior to being restrained would develop significantly less restraint ulceration than the male rats not stressed prior to restraint and also less than those receiving only novel stimulation prior to restraint. Although the experiment was primarily investigating the factors controlling the development of restraint ulceration in male ulcer-susceptible rats, it was thought worthwhile to include females in the experiment so that their behaviour could continue to be observed. The results of the males and the females were analyzed separately.

Method

Subjects

The subjects were 79 ulcer-susceptible Sprague-Dawley rats, 39 of which were males, and 40 of which were females. The rats were allocated to one of three groups, 13 males and 12 females in one group, and 13 males and 14 females in each of the other two groups. Of the 39 males, 10 were eliminated from the results; and of the 40 females, 7 were eliminated. Of the 17 rats eliminated, 12 ate their way out of the restraining mesh, and 5 died from causes related

to the experimental procedure.

The rats were bred and maintained until the commencement of the experiment as described in Chapter 5 (see pp.162-166), with the exception that they were all group housed, six or seven to a group, in large plastic boxes rather than small ones. All rats were aged between 115 and 145 days at the beginning of the study, and aged between 155 and 185 days at the end. At the time of assessment for ulcers, the male rats had a mean weight of 362gms, and the female rats a mean weight of 233gms.

Apparatus

Throughout the experiment, the rats were housed in large plastic boxes 60cm x 37cm x 21cm. The experimental apparatus consisted of six shock boxes, all modelled on the design of a shock box used by Weiss to administer tail shock to rats (Weiss, 1971a, 1972). Each shock box consisted of a small compartment with three clear perspex sides, a clear perspex top, and an aluminium door. The floor was a grid floor made of eight perspex bars, each with a diameter of 6mm (.25ins.), and each set 19mm (.75ins.) apart. The box was sufficiently large to contain the rat comfort-

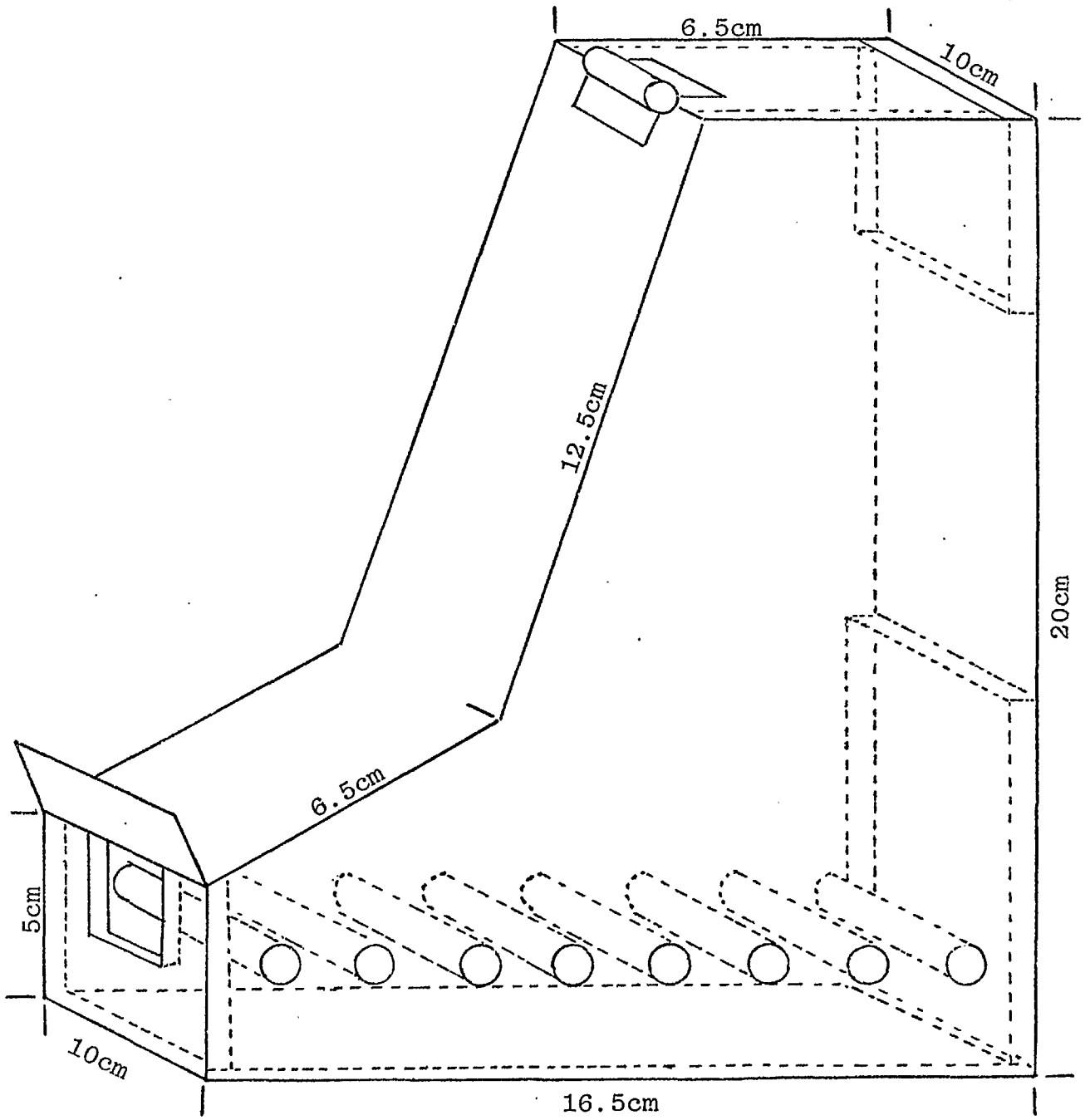

Figure 6. Appearance and dimensions of the shock box used in Experiment 3 to deliver tail shock to rats. (The box was modelled on the design of a box used by Weiss (1971a); there was an opening in the wall of the box facing the door, but this opening was covered with a piece of perspex during Experiment 3.)

ably, but the rat had little room in which to move around. The dimensions of the box are presented in Figure 6.

The rat's tail protruded through an opening underneath the door of the box. A rubber disc, 50mm (2ins.) in diameter, was placed on the base of the rat's tail, and fixed in place with Elastoplast elastic adhesive bandage, which was attached to the rat's tail on either side of the disc. The shock was received through subdermal stainless steel electrodes, manufactured by the Grass Instrument Company, Model E2B.

A shock generator was constructed in the laboratory, and set to deliver a .2mA pulsing shock (.5 seconds on, .5 seconds off). An Ohaus Triple Beam Balance was used to weigh the rats, and a desiccator jar was used to anaesthetize the rats prior to restraint, and to kill them at the end of the experiment. The rats were restrained in fibre-glass and wire meshes of the same gauge used in Experiment 1.

Procedure

Allocation to experimental groups. At the beginning of the experiment, all rats were randomly

allocated to new housing groups, 6 or 7 to a group. The 39 males were then randomly allocated to one of three groups: the shock stress/restraint group, the novel stimulation/restraint group, or the restraint only group. There were 13 males in each group. Similarly, the 40 females were randomly allocated to one of the three groups, 12 in the shock stress/ restraint group, and 14 in each of the novel stimulation/restraint and the restraint only groups.

Shock stress condition. Five of the six shock boxes were used in the shock stress condition. The 13 male rats were shocked in two groups of 5, and one group of 3; the 12 female rats were shocked in two groups of 5, and one group of 2. The rats in the shock stress group were removed from their home boxes, and placed 1 to a box in the shock boxes. The rats' tails were secured outside the box with the rubber disc, and the electrodes inserted under the skin of the rats' tails. Mild pulsing shock was then delivered to the rats for 3 minutes. At the completion of the shock period, the electrodes and the rubber disc were removed, and the rats were returned to their home boxes. This procedure was carried out

once a day, for 5 days of the week, for 4 weeks. On the remaining 2 days of each week, the rats were rested. The rest days were included because it was considered that the stress regimen would be too aversive without them.

Novel stimulation condition. The rats in the novel stimulation group were removed from their home boxes for the same 5 days of the same 4 weeks as the rats in the shock stress group. Each rat in the novel stimulation group was placed in the sixth shock box, and left to sit there for 7 minutes. These rats did not receive any shock; their tails were not fixed outside the box; and no electrodes were inserted. A duration of 7 minutes was selected because each shock stress rat was away from its home box for that amount of time. The sixth shock box was used for the novel stimulation group only; it was never used for the shock stress procedure.

Restraint only condition. The third group, the restraint only group, was left unmanipulated for the duration of the shock stress and the novel stimulation periods. Like all the rats in the study, they

lived in groups in uncrowded conditions, and were involved in the routine cleaning schedule of the animal holding room.

Rest period and restraint. At the end of the 4 week manipulation period, the rats in the shock stress and the novel stimulation groups were rested for 14 days to allow them to recover from any stress ulceration that might have been induced by the experimental procedures. At the end of the rest period, a food deprivation schedule was commenced. Over 4 days, food intake was gradually reduced, until by the 5th day the rats had been food deprived for 23 hours. The rats were then maintained on 1 hour of food a day, for 4 to 6 days.

Following the final food deprivation period, the rats were restrained for 24 hours in fibre-glass mesh and wire mesh (as shown in Figures 1 and 2, facing pp.166 and 167). The rats were restrained by a research assistant who was unaware of the groups to which the rats belonged. During restraint, the rats were kept in an air-conditioned room where the temperature was set at 22°±1°C. The light-dark cycle was identical to that experienced in the animal hold-

ing room.

At the end of the restraint period, the rats were killed, and their stomachs removed, opened along the greater curvature, and pinned out on slabs of polystyrene (as shown in Figure 3, facing p.168). Numbers of ulcers were counted by two research assistants who operated without any knowledge of the groups to which the stomachs belonged. A mean count was taken where there was disagreement about a measurement.

Results

The majority of the ulcers occurred in the glandular section of the stomach; the few rumenal ulcers that did develop were not included in the results. The inter-rater reliability was +.98.

The results of the experiment were analyzed separately for males and females. Only 29 of the 39 males and 33 of the 40 females in the experiment were included in the analysis. The sources of the loss of the 10 males and the 7 females, and the groups that were affected by the losses are presented in Table 18.

Table 18

Number of Rats Eliminated From Different Pre-Restraint Stimulation Conditions and Reasons for Elimination

Group	Reason for elimination	
	Ate way out of mesh	Death due to experimental procedure[a]
Males		
Shock stress	0	2
Novel stimulation	5	1
Restraint only	2	0
Females		
Shock stress	2	0
Novel stimulation	3	0
Restraint only	0	2

[a] Includes death during the food deprivation period, during the pre-restraint anaesthesia, and during the restraint itself.

For those rats remaining in the experiment, the mean numbers of ulcers in each of the three groups are presented separately for males and females in Table 19.

Table 19

Mean Number of Ulcers Found in Male and Female Rats in Different Pre-Restraint Stimulation Conditions

Group	n	Mean number of ulcers
Males		
Shock stress	11	36.8
Novel stimulation	7	41.3
Restraint only	11	23.5
Females		
Shock stress	10	33.5
Novel stimulation	11	61.5
Restraint only	12	31.8

At the beginning of the experiment, a set of planned comparisons had been written to answer the

specific questions being considered. Primarily, it was expected that the rats in the shock stress group would develop less restraint ulceration than the rats in the restraint only group. However, the data did not confirm the prediction; therefore the planned comparisons were not tested. Some thought was given to testing the significance of the differences in the data that did occur, but this action was decided against for reasons which will be discussed in the next section.

Discussion

The results of the experiment were quite contrary to expectations on a number of grounds. First, the data did not support the hypothesis that male rats will react less to restraint if they are exposed beforehand to a mild, chronic stressor. Secondly, the novel stimulation group reacted more to restraint than either of the other two groups; and thirdly, the ulceration scores were unusually high in virtually all the groups. Even though certain unpredicted differences were found in the data, no trend was pursued statistically, because it was considered that the sample statistics obtained from Experiment

3 were both unreliable and invalid. The reasons for arriving at this conclusion are presented below.

First, a large number of rats had to be eliminated from the experiment (chiefly from the novel stimulation group) because they ate their way out of the restraining mesh. The implication of this elimination of data is that the mean ulceration counts (as presented in Table 19) do not reliably represent the relative effects of the experimental manipulations. The problem of rats eating their way through the restraining mesh is not a new one. Brodie (1962) has found that rats eat their way through wire mesh. The problem has also been previously encountered by the author, but not with the amazing frequency with which it occurred in Experiment 3. If the mesh method of restraining rats is to continue to be useful, a solution to the problem must be found.

A second problem with the interpretation of the results of Experiment 3 is that the food deprivation schedule on which the rats were maintained prior to restraint was too stringent. Neither was the schedule sufficiently controlled. The rats were maintained on interrupted diets for from 7 to 10 days before being restrained, and it is probable that a large

food deprivation component is being reflected in the ulceration counts. It is also possible that the food deprivation schedule contributed to the very high numbers of ulcers found in the data. Following the completion of Experiment 3, it was demonstrated in a pilot study that rats which are introduced to 24 hours food deprivation by having their food intake gradually reduced over a number of days develop significantly more restraint ulceration than rats which are simply deprived of food for the 24 hours immediately preceding restraint.

The results of the food deprivation pilot study indicate the importance of the deprivation schedule in the development of restraint-induced ulceration. First, fasting should be stringently controlled such that all rats in an experiment are placed on identical deprivation schedules. Secondly, the author suggests that if valid stress ulceration measures are desired, food deprivation periods prior to restraint be kept to a minimum, particularly where ulceration counts are expected to be high.

The final problem with the interpretation of the results of Experiment 3 is that during the restraint period it was not possible to keep the rats in a quiet

room. Bonfils has found that noise exacerbates restraint-induced ulceration (quoted in Mikhail & Holland, 1966), and it is possible such an exacerbation occurred in Experiment 3, thus producing even higher and more uninterpretable ulceration counts. In conclusion, it would seem that for valid interpretations to be made of restraint-induced effects, the conditions under which rats are restrained and the prior conditions to which they are exposed should be carefully controlled.

CHAPTER 9

EXPERIMENT 4: A STUDY OF THE EFFECT OF THE AMOUNT OF SPACE OCCUPIED BY A RAT PRIOR TO RESTRAINT ON RESTRAINT-INDUCED ULCERATION

The hypothesis tested in Experiment 3 to account for why the male rats housed in crowded groups in Experiment 1 ulcerated less when restrained than the male rats housed in uncrowded groups was abandoned. Instead, the possibility was considered that crowding affects restraint-induced ulceration not because the mild, chronic stress induced by crowding immunizes against later stress, but because both situations - crowding and restraint - manipulate the amount of space available to the rat. On the basis of this observation, it was hypothesized that there is a relationship between the amount of space available to the rat prior to restraint and the reaction of the rat to severe restriction of space during restraint.

More specifically, the hypothesis predicts that the more space the rat has available to it prior to restraint, the more will be the stress reaction when

the rat is restrained. Such a hypothesis not only accounts for why the crowded males ulcerated less when restrained than the uncrowded males, but it also accounts for the general finding that individually housed rats (both males and females) ulcerate less when restrained than group housed rats. The aim of Experiment 4 was to begin investigating this hypothesis. As in Experiment 3, both male and female rats were included in the study, but their results were analyzed separately.

It was decided that the first step should be to house rats individually in all the housing spaces used in Experiments 1 and 2 (i.e. large box, small box for males, small box for females, and cage). Such a procedure would eliminate the effect of group housing per se, and at the same time independently vary the amount of space available to individual rats prior to restraint. It was considered that the rats would have to occupy these spaces for a short period of time only, in order for them to become adjusted to the amount of space available.

So as to overcome some of the problems of data interpretation encountered in the previous three experiments, a number of improvements were made to

the methodology of Experiment 4. First, the food deprivation period prior to restraint was carefully controlled. The procedure of removing the food 24 hours immediately prior to restraint was adopted, rather than that of gradually reducing intake over a number of days. Secondly, the routine cleaning schedule in the animal holding room was altered such that the rats in the wire cages were transferred to clean compartments at the same rate as were the boxed rats. Thirdly, the restraint procedure was modified to make it more difficult for rats to eat their way through the mesh.

The final change that was made was not associated with improving the methodology, but was concerned with increasing the efficiency of the procedural work. Since the inter-rater reliabilities in the previous three experiments had been so high (ranging from +.95 to +1.00), it was decided to use only one person to count the number of ulcers in each stomach.

Method

Subjects

The subjects were 108 ulcer-susceptible Sprague-

Dawley rats, 54 of which were males and 54 of which were females. There were 18 males and 18 females allocated to each of three groups. Of all the rats in the study, 8 females were eliminated from the results: 2 ate their way out of the restraining mesh, 3 died during the anaesthesia prior to restraint, and 3 died from causes unrelated to the experiment.

The rats were bred and maintained until the beginning of the experiment as described in Chapter 5 (see pp.162-166), with the exception that after the rats were weaned, they were group housed in large boxes rather than small boxes. The rats were aged between 300 and 350 days at the time of the experiment. When the rats were assessed for ulceration, the male rats had a mean weight of 460gms, and the female rats a mean weight of 300gms.

Apparatus

The housing apparatus was the same as that used in Experiments 1 and 2. There were four different housing constructions: a large plastic box 60cm x 37cm x 21cm, a small plastic box for males 42cm x 28cm x 15cm, a smaller plastic box for females 39cm x 25cm x 13cm, and a metal grate cage, 24cm x 15cm

x 19cm. Other details of the housing apparatus are given in Chapter 6 (see pp.177-178).

The remainder of the apparatus was also the same as that used in Experiments 1 and 2: an Ohaus Triple Beam Balance, a desiccator jar, fibre-glass and wire meshes, wire staples, and polystyrene slabs (see pp. 178-179).

Procedure

Allocation to living space. At the beginning of the experiment, the rats were weighed, then randomly allocated to one of three groups. It was assumed that at the beginning of the experiment there would not be any systematic differences in weights due to housing among the males or among the females, since all rats had been housed in a standardized fashion since birth. Consequently, it was assumed that randomized allocation of rats to groups would also result in randomized allocation of weights.

The rats were allocated to either a large living space, a medium living space, or a small living space. The housing box 60cm x 37cm x 21cm provided the large living space for both male and female rats. The housing box 42cm x 28cm x 15cm provided the medium

living space for males, and the housing box 39cm x 25cm x 13cm the medium living space for females. The metal grate cage 24cm x 15cm x 19cm provided the small living space for both males and females. The area of floor space and the total volume of space provided by each housing condition are presented in Table 20.

Table 20

Amount of Space Provided Male and Female Rats Occupying Different Housing Conditions

Housing condition	Space provided	
	Floor area (in sq cm)	Total volume (in cc)
Large box (males and females)	2,220	46,130
Small box (males only)	1,180	15,850
Small box (females only)	980	11,090
Cage (males and females)	360	6,840

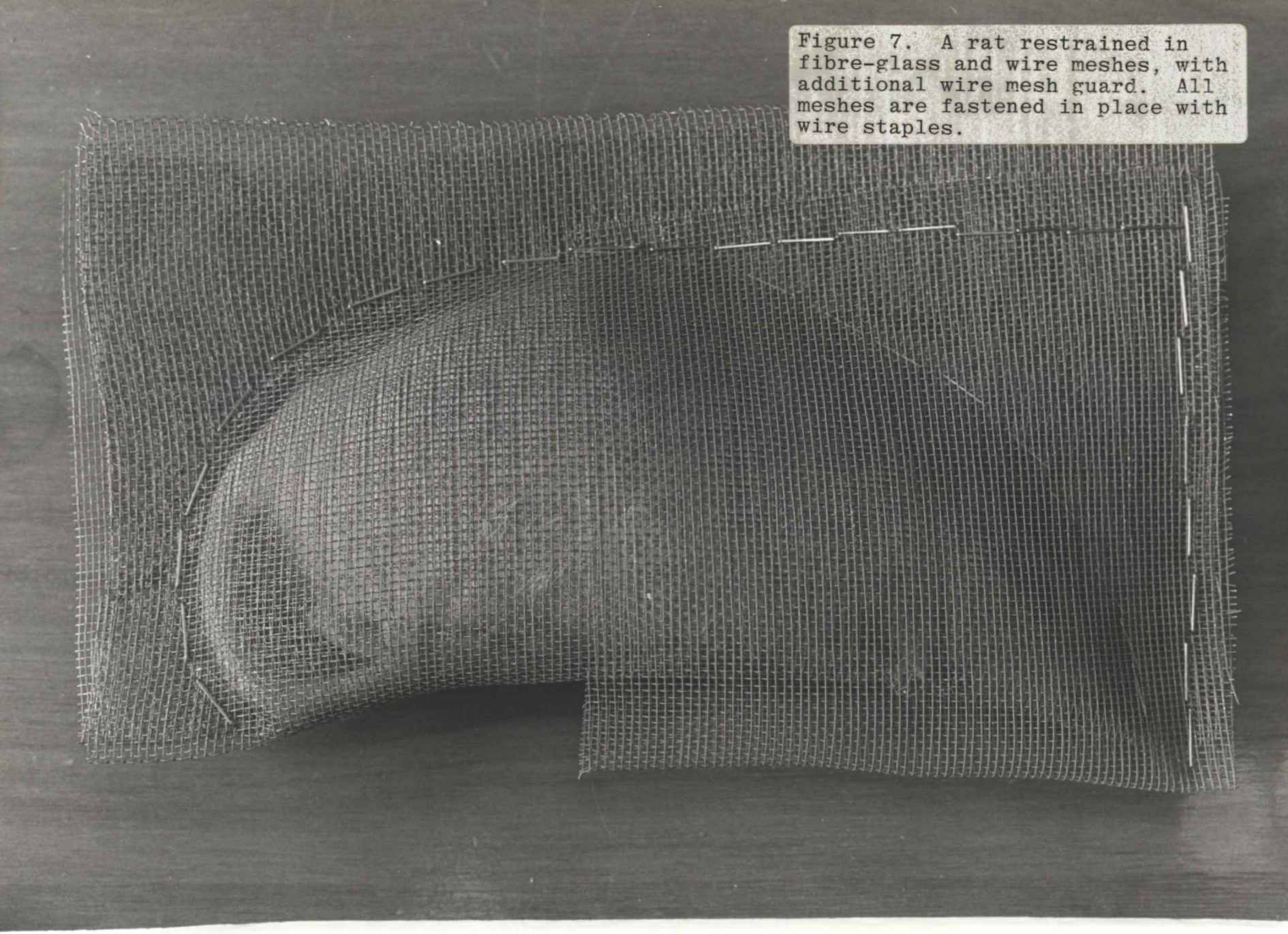

Figure 7. A rat restrained in fibre-glass and wire meshes, with additional wire mesh guard. All meshes are fastened in place with wire staples.

Unlike Experiments 1 and 2, there was no group housing; each rat lived alone in the box or cage to which it had been allocated. The rats were kept in their individual boxes or cages for 14 days. The routine cleaning schedule in the animal holding room, as described in Chapter 5 (see pp. 165-166), continued throughout the experiment. As part of the cleaning schedule, all rats were transferred to clean housing conditions twice a week, irrespective of whether they occupied the plastic boxes or the metal cages.

Restraint. On the 14th day of the isolated housing, the rat was weighed, then deprived of food for 24 hours. Following the deprivation, the rat was weighed again and restrained in fibre-glass and wire meshes as described in Chapter 5 (see pp.166-167). In addition, an extra piece of wire mesh was placed around the head of the rat, about 19mm (.75ins.) away from the actual restraining mesh, and then stapled in place above and behind the rat's head. This procedure was carried out to discourage the rat from attempting to eat its way out of the restraint. A restrained rat with the additional guard is shown in Figure 7, facing page 244. As in previous experiments, the rats

were restrained by a research assistant who was unaware of the groups to which the rats belonged.

The rats were restrained for 24 hours. During restraint, they were kept in a quiet room, where the temperature varied between 21°C and 25°C. The light-dark cycle was the same as that experienced in their home surroundings. At the end of the restraint period, the rats were killed, and their stomachs were prepared for inspection as in previous experiments. The numbers of ulcers were counted by the experimenter, who operated without any knowledge of the groups to which the stomachs belonged.

Results

The majority of the ulcers developed in the glandular section of the stomach; the few rumenal ulcers that did occur were not included in the results. As in Experiment 1, the majority of the rats with rumenal ulceration were those living in the metal grate cages. The results for males and females were examined separately, although total male ulceration was compared with total female ulceration.

The mean number of ulcers for males and females in the three space groups are presented in Table 21.

Table 21

Mean Number of Ulcers Found in Restrained Male and Female Rats Occupying Different Amounts of Pre-Restraint Space

Group	n	Mean number of ulcers
Males		
Large space	18	28.9
Medium space[a]	18	24.7
Small space	18	11.5
Females		
Large space	15	23.0
Medium space[a]	15	11.8
Small space	16	7.2

[a] The medium space used for males was larger than the medium space used for females (see Table 20 for exact dimensions of spaces provided).

At the beginning of the experiment, a set of planned comparisons had been written to answer the specific questions being considered. These questions

were:

(1) do rats living in large and medium spaces ulcerate more when restrained than rats living in small spaces?

(2) do rats living in large spaces ulcerate more when restrained than rats living in medium spaces?

The questions were considered separately for males and females, and the analysis of variance summary tables are presented in Table 23, on page 248; α was set at .05.

It was found that female rats as a group ulcerated significantly less than male rats as a group, t (98) = 3.19, $p<.002$ (two-tailed). The mean number of ulcers for males and females are presented in Table 22.

Table 22

Mean Number of Ulcers Found in Restrained Male and Female Rats

Sex	n	Mean number of ulcers
Males	54	21.7
Females	46	13.6

Table 23

Analysis of Variance Summary Tables (Planned Comparisons): Numbers of Ulcers in Restrained Males and Females Occupying Different Amounts of Pre-Restraint Space

Source	SS	df	MS	F
	Males			
Between	10611.7	53		
Large and medium vs.Small	2800.9	1	2800.9	18.67*
Large vs.Medium	160.4	1	160.4	1.07
Error	7650.3	51	150.0	
	Females			
Between	5006.7	45		
Large and medium vs.Small	1018.4	1	1018.4	14.94*
Large vs.Medium	1056.1	1	1056.1	15.49*
Error	2932.2	43	68.2	

*$p < .01$

Weights. The random allocation of rats to groups at the beginning of the experiment was successful in eliminating significant differences in weights across groups. The mean weights of male and female rats in each space condition (taken prior to the 24 hours food deprivation) are presented in Table 24.

Table 24

Mean Weights of Male and Female Rats Occupying Different Amounts of Pre-Restraint Space

Group	Mean weight (in gms)
Males	
Large space	464.6
Medium space	462.9
Small space	456.0
Females	
Large space	294.8
Medium space	293.7
Small space	290.5

Discussion

The hypothesis that the more space a rat has available to it prior to restraint the more will be its reaction to a severe restriction of space during restraint was generally supported. Among the males, rats living in large and medium spaces ulcerated significantly more when restrained than rats living in small spaces. However, there was no significant difference in restraint ulceration between rats living in large spaces and those living in medium spaces. Among the females, the hypothesis was fully supported. Females living in large and medium spaces ulcerated more when restrained than those living in small spaces, and females in large spaces ulcerated more than those in medium spaces.

Although efforts were made to improve the methodology of Experiment 4, two substantial criticisms can still be made. The first is that, as in Experiment 1, the rats in the small space group occupied housing compartments that were substantially different from the housing compartments occupied by the rats in the other two groups. The small space group lived in open grate cages whereas the other two groups lived in opaque plastic boxes.

The second criticism is that previous experience with the living space allocated to each rat was not held constant across the groups. Rats living in the boxes providing the large space had prior experience with that housing from the time they were 23 days of age to the beginning of the experiment, and males living in the boxes providing the medium space had prior experience with that housing as young pre-weaned pups. Females living in the boxes providing their medium space, and rats living in the small space cages had no prior experience at all with their housing.

In spite of the criticisms that can be made of the methodology, the experiment served its purpose of initiating investigation into the "adaptation to space" hypothesis. While the early results are encouraging, there are still some inconsistencies that have to be accounted for. First, the male rats adapted to the medium space in Experiment 4 did not ulcerate significantly less than the male rats adapted to the large space. Secondly, if it is assumed that the "adaptation to space" hypothesis can account for female ulceration levels as well, it is difficult to explain why the females occupying the small plastic

boxes in Experiment 1 did not ulcerate significantly less than the females occupying the large plastic boxes in that Experiment.

Since the "adaptation to space" hypothesis cannot account for these inconsistencies in the data, perhaps an alternative hypothesis can be suggested that will. In the introduction to Experiment 4, it was claimed that housing conditions and restraint both manipulate the amount of space made available to a rat. Now, it can also be claimed that both manipulate the amount of movement allowed the rat. At first sight, there may appear to be no difference between the amount of space made available and the amount of movement permitted. However, a subtle distinction can be made between the two in terms of the behaviour of the rat.

In the laboratory, space made available to the rat is controlled by the experimenter, and theoretically, a limitless amount of space can be made available. However, how much of that space is actually used by the rat (i.e. how much movement the rat engages in) is determined by the rat. Therefore, it is possible to imagine a situation where a large amount of space is available, but where the rat moves

no more than had a lesser space been provided.

If an additional assumption is made that the amount of movement engaged in by a rat is determined not only by the amount of living space provided, but also by the number of other rats occupying that living space, then such an "adaptation to movement" hypothesis can account for all the results of both Experiments 1 and 4. In Experiment 1, if a female rat occupying a large box with five other female rats moved about no more than a female rat occupying a small box also with five other female rats, then no difference in restraint ulceration would be expected. In Experiment 4, if a male rat occupying a large space alone moved about no more than a male rat occupying a medium space alone, then again no difference in restraint ulceration would be expected.

The author is well aware of the inherent danger in proposing an hypothesis to account for experimental results that depends on "what the rat does". Such hypotheses can be invoked to account for all sorts of data, and are virtually impossible to disprove. However, the data of Experiments 1 and 4 do suggest that "adaptation to movement" is a more appropriate explanation of the reaction to restraint

than "adaptation to space".

A final comment needs to be made about restraint-induced ulceration in females. In both Experiments 3 and 4, the trend of the results of the female rats was virtually identical to that of the male rats. In Experiment 4, the female results met the predictions of the hypothesis even better than did the male results. On the basis of these findings, it seems likely that the result obtained in Experiment 2 that crowded females ulcerate more when restrained than uncrowded females is unreliable. Nevertheless, male and female results should continue to be analyzed separately until the mechanisms of restraint-induced ulceration are more fully understood.

It is interesting to note that in Experiment 4, the females ulcerated significantly less than the males. This result has never been reported before in an experiment on restraint-induced ulceration, and it is unlikely that the difference is due to sampling variance. The relevant factor causing this reversal of trend is more likely to be the age of the rats used in Experiment 4. The rats were approximately 12 months old at the time they were restrained, and it is possible that the female ulcer-susceptible

rats were either approaching or experiencing menopause at this time. While the usual menopausal age for laboratory rats is 15-18 months (Rowett, 1960), it is probable that the ulcer-susceptible females reach menopause earlier because of their tendency to become infertile at about 8-9 months. Therefore, endocrinological factors may be responsible for the lesser restraint ulceration in the older female ulcer-susceptible rats.

One other factor that is probably relevant to the interpretation of the sex difference found in Experiment 4 is that males and females were not exposed to identical treatments: males in the medium space group occupied a larger box than did females in the medium space group. This meant that males in the medium space group were able to engage in more movement than were the females, thus predisposing the males to more restraint-induced ulceration.

The interpretation of any difference in restraint ulceration between male and female rats is probably a very complex question. Endocrinological differences, weight differences, and differences in adaptation to space and movement might all be involved, either independently or in interaction with each other.

CHAPTER 10

WEISS'S THEORY OF STRESS ULCERATION

Experiments investigating restraint-induced ulceration (and stress ulceration in general) have mainly studied the conditions under which such ulceration develops. Relatively little attention has been paid to formulating general theories of stress ulceration, and those theories that have been presented deal with physiological events rather than behavioural ones. In 1971, Jay Weiss in the United States published the first comprehensive behavioural theory of stress ulceration (Weiss, 1971a). In this chapter, Weiss's work will be considered in some detail, and the contribution his theory makes to understanding the development of stress ulceration will be evaluated.

The Nature of the Theory

Weiss's theory states that stress ulceration is a function of two variables: (1) the number of responses (coping attempts) an animal makes in a stressful situation, and (2) the amount of relevant feed-

back provided the animal by these coping attempts. Weiss defines relevant feedback as response contingent stimuli that are not associated with the stressor, and he nominates two criteria for establishing the degree of relevant feedback: the temporal distance of the stimulus from the onset of the stressor, and the conspicuousness of the change produced in the total stimulus situation.

The two variables (responding and relevant feedback) operate in the following way. Ulceration tends to increase monotonically as the number of responses increases, and tends to decrease monotonically as the amount of relevant feedback increases. Furthermore, the expression of the relationship between responding and ulceration is dependent on the amount of relevant feedback provided by such responding. Thus, as relevant feedback increases, the tendency for ulceration to increase as the number of responses increases diminishes, such that at very high relevant feedback, the number of responses is not related to ulceration.

Weiss generated his theory from the data of an experiment (Weiss, 1971a) designed to investigate the failure of experimenters to replicate the Brady

"executive monkey" effect. As described in Chapter 2 in the section on Shock (see p.74), Brady, Porter, Conrad, and Mason (1958) found that monkeys able to control the occurrence of a shock stressor developed severe duodenal ulceration, whereas monkeys without control over the stressor did not develop ulcers. Since the publication of the study, other experimenters have found it very difficult to confirm the results obtained by Brady et al.

Weiss designed a study in which he examined three conditions: (1) avoidance-escape from unsignalled shock, (2) avoidance-escape from shock preceded by a 20 second pulsing beep, and (3) avoidance-escape from shock preceded by a 170 second tone-plus-beep sequence (150 seconds of ascending tones followed by the 20 second beep as in condition (2)). The response-shock interval was 200 seconds; there was no shock-shock interval, as the shock continued until the animal made an escape response. The stress period lasted 48 hours, during which time the animals were food deprived.

Weiss used rats as the experimental subjects. Each rat was enclosed in a small experimental chamber into which a wheel manipulandum protruded.

The rats in all three conditions were able to make standard escape and avoidance responses: the rats could terminate or postpone the shock and the signals by turning the wheel 45° (Weiss, personal communication).

In the three conditions, each avoidance-escape rat had two matched controls. One rat was wired in series ("yoked") to the experimental rat, and therefore received the same shocks and signals, but was unable to control the occurrence of the stressor. The third rat acted as a non-shock control for the effects of food deprivation and 48 hours confinement in the apparatus. All the control rats in the yoked and the non-shock groups were provided with a wheel manipulandum.

Weiss used two measures of ulceration: number of ulcers and length of ulceration. He found that in all three signal conditions, the yoked control rats ulcerated more than the avoidance-escape rats, and the avoidance-escape rats ulcerated more than the non-shock control rats. The largest difference between the avoidance-escape and the yoked rats occurred in the tone-beep sequence (progressive signal) condition; the second largest difference between the

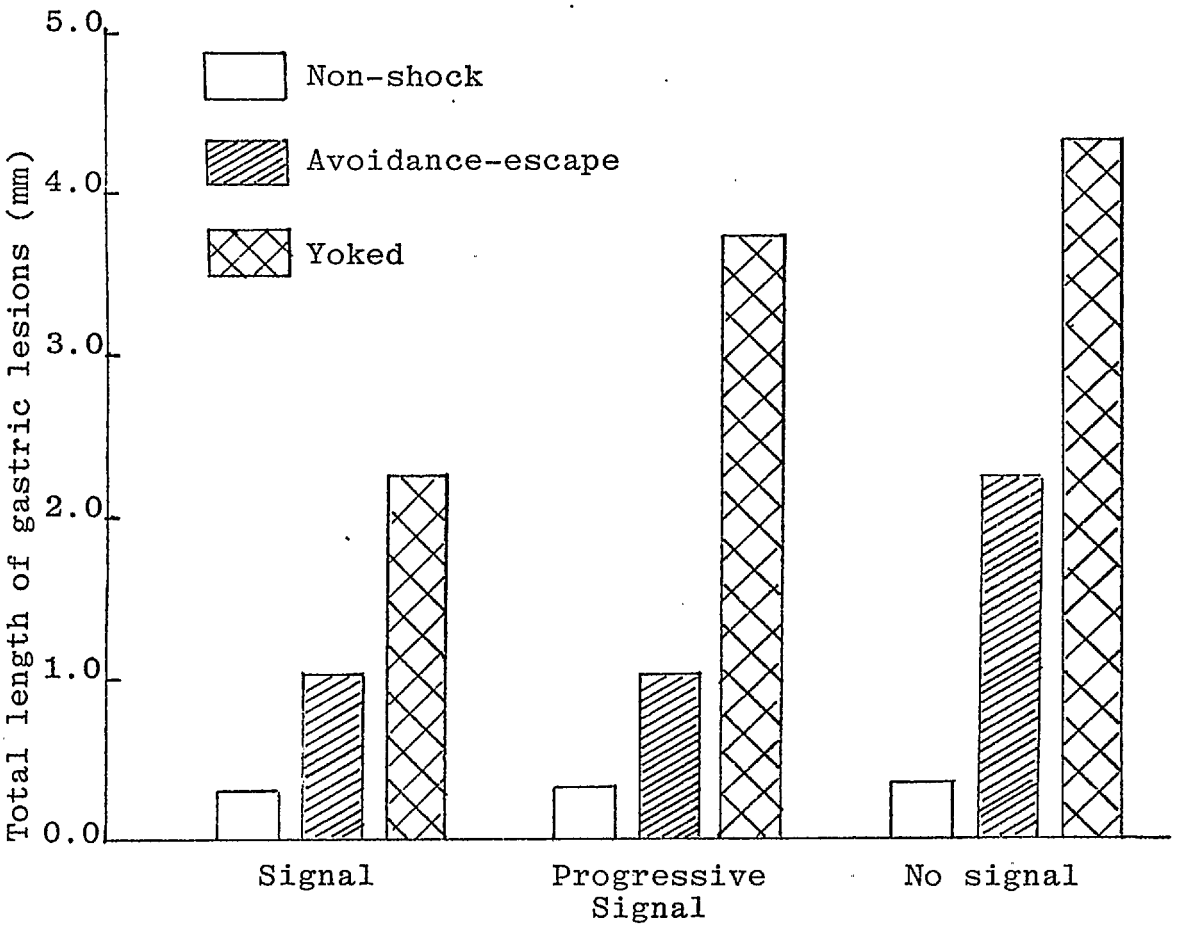

Figure 8. Median length of ulceration in the non-shock, avoidance-escape, and yoked control groups in the signal, progressive signal, and no signal conditions (after Weiss, 1971a, p.6).

two occurred in the no signal condition; and the smallest difference occurred in the signalled group. The bar diagram in Figure 8, taken from Weiss (1971a), illustrates these results.

Weiss then explained how the experimental data were consistent with his theory. He pointed out that the nine groups (three avoidance-escape, three yoked controls, and three non-shock controls) could be ranked in terms of how much relevant feedback the wheel turn response provided the rats in each group.

In the avoidance-escape groups, an avoidance response made in the absence of a signal produced internal stimulus change (kinesthetic cues) that was always at least 200 seconds removed from the onset of the stressor. As kinesthetic cues do not represent a conspicuous change in total stimulation (since the external stimulus situation remains unchanged), Weiss defined such feedback as <u>low</u> relevant feedback. <u>High</u> relevant feedback was provided by the termination of a tone or beep, because it produced a conspicuous stimulus change (internal <u>and</u> external) that was always unassociated with the onset of the stressor. Weiss regarded shock termination as even better relevant feedback than that provided by tone termin-

ation, because of the higher salience of the change in the stimulus situation.

Thus, the unsignalled shock condition provided low relevant feedback for all avoidance responses; the signal condition provided high relevant feedback for avoidance responses made during the 20 seconds signal, and low relevant feedback for avoidance responses made during the 180 seconds preceding the signal; and the progressive signal condition provided high relevant feedback for avoidance responses made during the 170 seconds signal, and low relevant feedback for avoidance responses made during the preceding 30 seconds. Therefore, among the three avoidance-escape groups, the progressive signal group provided the greatest amount of relevant feedback, the unsignalled group provided the least amount, and the signal group provided an intermediate amount.

Responses made by rats in the three yoked control groups all provided zero relevant feedback, since responses made in these groups did not consistently produce stimuli unassociated with the stressor. In the three non-shock control groups, zero relevant feedback was also provided by coping responses, since such responses did not produce stimuli unassociated

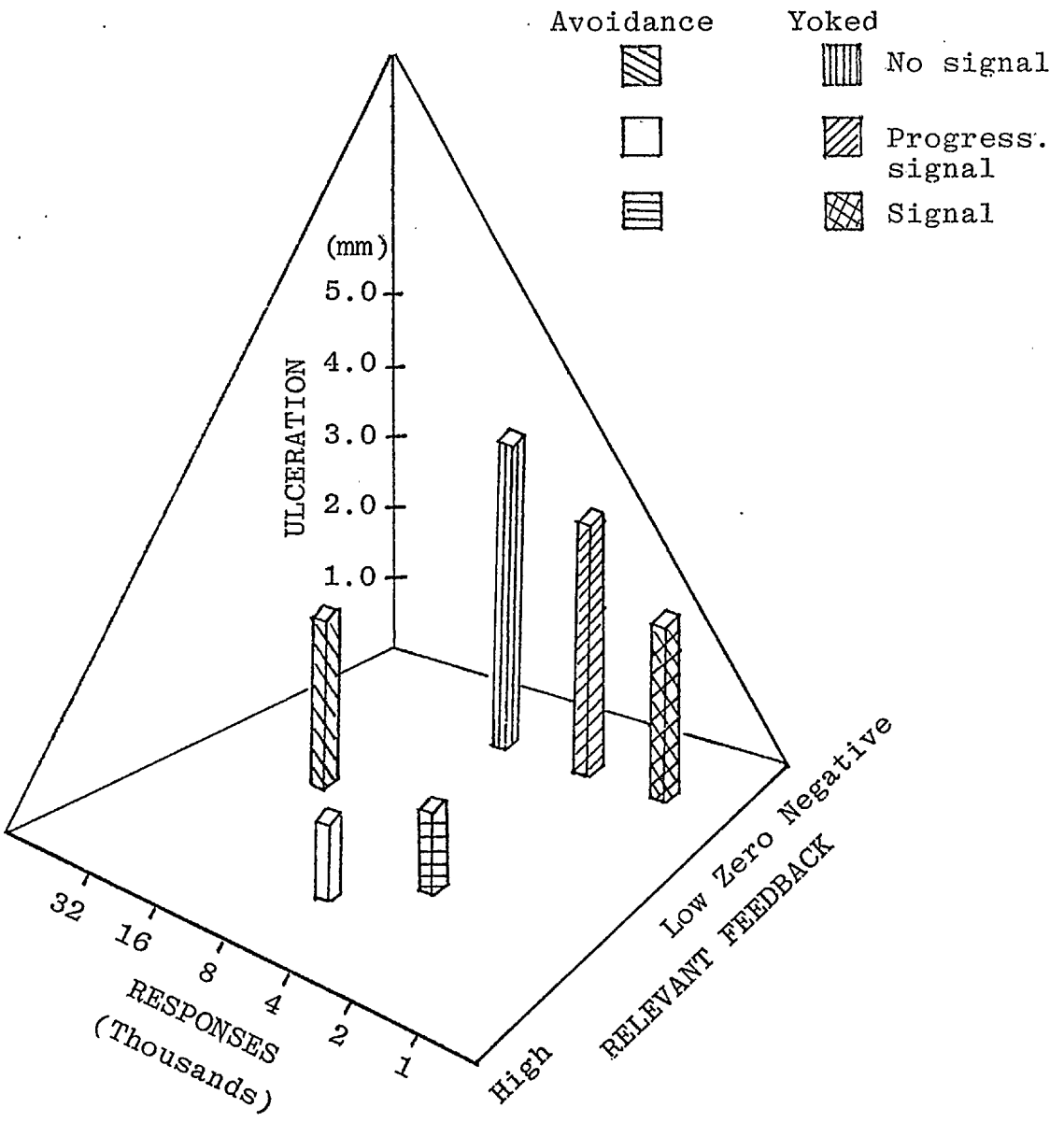

Figure 9. Data from Weiss's 1971a experiment plotted on Weiss's three dimensional figure. The position of each bar is determined by the amount of relevant feedback received and the number of responses made in that group. The height of the bar is determined by the amount of ulceration that occurred in the group (after Weiss, 1971a, p.12).

with the stress of confinement in the apparatus or the stress of food deprivation.

Weiss then graphically represented the relationships among relevant feedback, responding, and ulceration as a three dimensional figure. Relevant feedback was plotted along a horizontal axis, responding along a second horizontal axis perpendicular to the first, and ulceration along a vertical axis perpendicular to both horizontal axes. Next, Weiss showed how the plotted experimental data (shown in Figure 9, on p.263) matched the model predicted by his theory. The theoretical model (shown in Figure 10, on p.265) illustrates how ulceration decreases with an increase in relevant feedback, and increases with an increase in responding, with the latter correlation approaching zero as relevant feedback increases.

The first experiment (which resulted in the generation of the theory) was followed by two other studies, both intended to test the theory directly (Weiss, 1971b, 1971c). In the second study (Weiss, 1971c), Weiss extended the concept of relevant feedback to include "negative" relevant feedback, a condition where responding produced stimuli <u>associated</u> with the stressor. Weiss's model predicts that negative

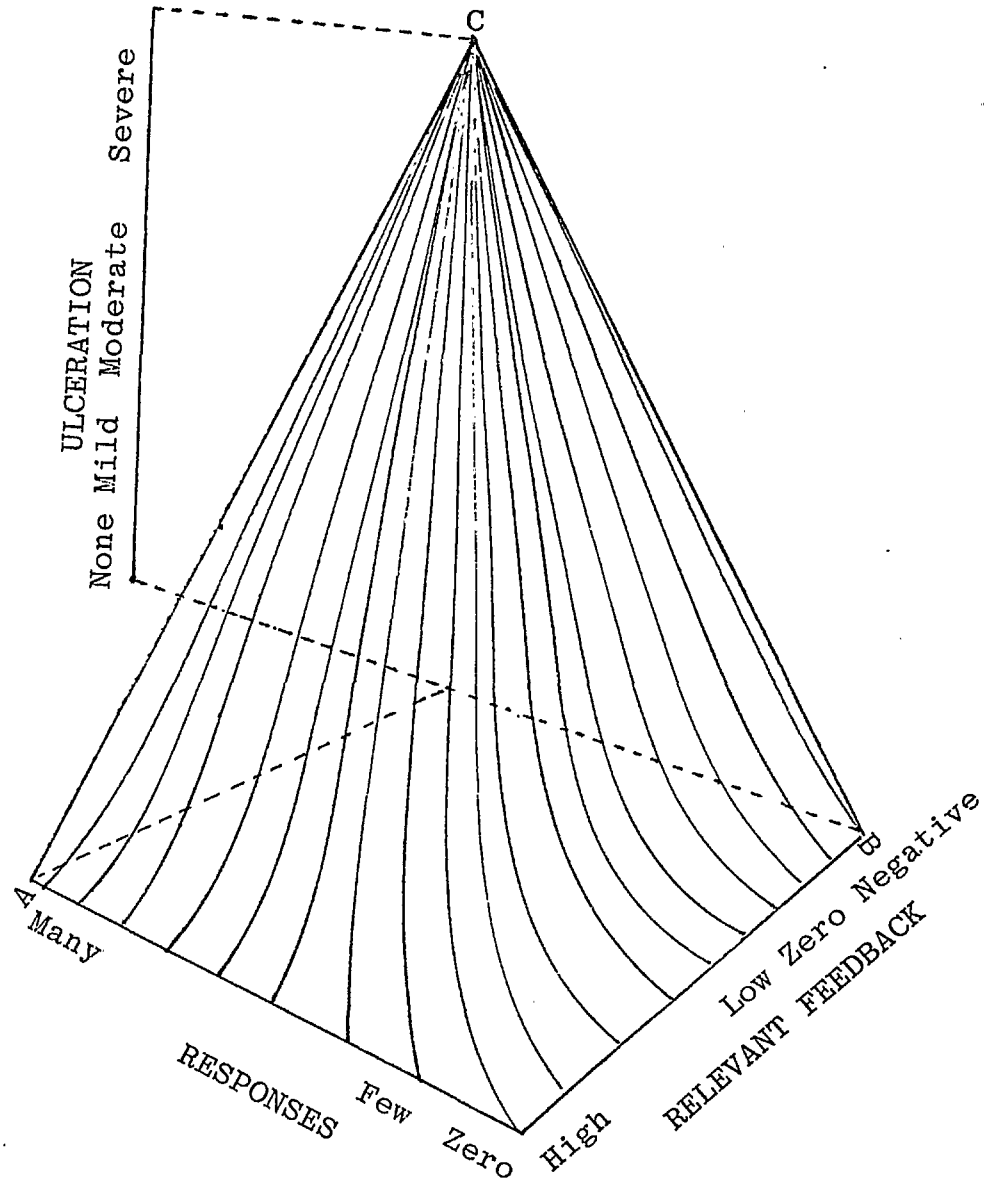

Figure 10. The three-dimensional model predicted by Weiss's theory, depicting the relationships between responding, relevant feedback, and ulceration (after Weiss, 1971a, p.10). The model operates in the following way: "Where a hypothetical number of responses and amount of feedback intersect, the amount of ulceration is determined by the height of the plane above this point." (Weiss, 1971a, p.10).

relevant feedback is extremely ulcerogenic (see Figure 10). Weiss investigated this prediction using the same basic design as in the first experiment. The avoidance-escape group could control signalled shock by turning a wheel; a second group was yoked to the experimental group to control for the ulcerogenic effects of the shock schedule.

The design differed from that of the first experiment in that half way through the 48 hours stress session, Weiss changed the consequences of the coping response. During the first 24 hours, the standard wheel turn resulted in successful avoidance and escape. During the second 24 hours, a short shock pulse immediately followed the response before avoidance or escape became effective, thus creating a situation where responding produced stimuli (shock) associated with the stressor (shock), that is, negative relevant feedback. Weiss found that the negative relevant feedback group ulcerated more than the zero relevant feedback yoked control group. This result supported Weiss's prediction that negative relevant feedback is extremely ulcerogenic, since in his theory <u>zero</u> relevant feedback is itself already considerably ulcerogenic.

Weiss next compared zero relevant feedback with very high relevant feedback (Weiss, 1971b). An experimental design was constructed again using an avoidance-escape group with a yoked shock control, but in which each avoidance or escape response (from unsignalled shock) was followed by a 5 second tone. The inclusion of the tone met the criteria for very high relevant feedback in that the tone was temporally unassociated with the onset of the stressor, and at the same time it provided conspicuous stimulus change with every response. Weiss demonstrated that under such feedback conditions, rats ulcerated much less than the yoked controls receiving unsignalled shock but no tonal feedback. This finding provided support for Weiss's hypothesis that as relevant feedback increases, ulcerogenic stress decreases.

Criticisms of Weiss's Experiments and Theory

There are a number of difficulties with Weiss's work. Criticisms can be made of his interpretation of the experimental data, and of his choice of experimental design. More importantly, the theory itself has a number of shortcomings that make empirical investigation of it extremely difficult. In the foll-

owing sections, each of the criticisms will be dealt with in turn.

Interpretation of the Data

On a number of occasions, Weiss does not give a full explanation of what the data mean in the context of the theory. An example is Weiss's failure to recognize that although his three signal conditions (progressive signal, signal, and no signal) offered different opportunities for relevant feedback in the avoidance-escape condition, these opportunities were not necessarily taken. Weiss argues that the progressive signal avoidance-escape group offered more relevant feedback for responding than the signal avoidance-escape group because of the 170 seconds of conspicuous stimulus change in the progressive signal group as compared with only 20 seconds of conspicuous change in the signal group. However, Weiss does not take into account the actual response patterns of the rat, as opposed to the theoretically possible response patterns. Weiss had no control over when the rats wheel turned. His distinction between the two signal conditions (in terms of the amount of relevant feedback provided) only makes sense if the rats responded

consistently throughout the pre-shock interval. This did not happen. Weiss reports in his 1971a article:

> Approximately 70% of the avoidance-escape animals in these two signaled-shock conditions did not often respond during the beep signal preceding shock but responded quickly after the shock began, thus terminating it; ....The remaining 30% of the animals in each of these conditions, on the other hand, consistently responded during the beep prior to the shock, terminating this signal and avoiding the impending shock. (p.5)

Thus, while the progressive signal group had, theoretically, more opportunities for relevant feedback, these opportunities were not taken. Even more importantly, both groups responded either during the shock, or during the beep immediately preceding the shock. Both obtained a high amount of relevant feedback, and both obtained the same amount of relevant feedback. Weiss does not recognize the significance of there being no difference in the response patterns of the two groups. That is, he does not recognize that there is no difference in the amount of relevant feedback received by the rats in the two signalled avoidance-escape groups.

Although there was not better relevant feedback in the progressive avoidance-escape group, Weiss uses the assumption of such a difference to account for the large difference in ulceration between that group

and its yoked control group. Weiss claims that the larger difference between these two groups (larger relative to the differences between the avoidance-escape groups and the yoked groups in the other conditions) can be accounted for by the higher relevant feedback obtained from responding by the progressive signal avoidance-escape group.

Again, Weiss is misinterpreting his data. The differences can be accounted for by the higher ulceration in the yoked control group, and not by a decreased ulceration in the avoidance-escape group. In fact, the progressive signal avoidance-escape group developed the same amount of ulceration as the signal avoidance-escape group. The difference in ulceration is only between the two yoked groups (see Figure 8,p.260).

Another problem with the interpretation of the results is that Weiss does not deal with the difficulty posed by the combination of different amounts of relevant feedback in any one avoidance-escape group. As noted above, 70% of the rats in the two signalled avoidance-escape groups consistently escaped shock, and the remaining 30% consistently avoided it. In the unsignalled avoidance-escape condition, there was also both escape and avoidance behaviour. Such com-

binations of degrees of relevant feedback are difficult to interpret, and Weiss does not suggest a way of assigning a total value of relevant feedback to groups composed of different ordinally scaled values of relevant feedback.

There is one other result that Weiss fails to deal with satisfactorily. Weiss (1971a) found that "the ulceration of avoidance-escape animals which consistently terminated the beep signal before shock did not differ markedly from those avoidance-escape animals in the same signal conditions which did not do so" (p.6). It is reasonable to assume that those rats which did not terminate the beep were rats which consistently terminated the shock, since Weiss claims that rats either escaped shock or terminated the beep. It can be concluded then that rats consistently avoiding the shock did not ulcerate differently from rats escaping the shock. This result appears directly contrary to Weiss's theory, since escape responses provide more relevant feedback than avoidance responses. However, it is not possible to use this lack of difference in ulceration between escapers and avoiders as evidence against the theory because the response totals of the two groups are not known.

Methodology of the Experiment

The basic methodological difficulty with the design of Weiss's experiments arises out of his use of a yoked procedure to control for the effects of the shock schedule. The problem with this type of shock-control group is that yoked animals receive a shock schedule which has different psychological properties from those of the shock schedule received by avoidance-escape animals. In Weiss's experiment, the schedule in the avoidance-escape group was a predictable one: signal plus shock occurred every 200 seconds after a wheel turn, and a wheel turn terminated both signal and shock. However, the yoked group was unaware of the effect on shock presentations of wheel turns made by the avoidance-escape animals. The schedule for yoked animals thus became much less predictable.

Weiss cannot justifiably claim that the two groups were receiving identical shock schedules; predictability of the schedule was an uncontrolled variable. Since Weiss himself has found that unpredictable shock schedules are more aversive than predictable shock schedules (Weiss, 1970), it is to be expected on this ground alone that the yoked control

group would ulcerate more than the avoidance-escape group. However, Weiss is claiming in his 1971 articles that it is the zero relevant feedback for coping responses that is producing the increased ulcerogenic effect in the yoked control group. Unfortunately, Weiss has created an experimental situation which is biased in the direction of confirming his theory.

Formulation of the Theory

The chief problem with Weiss's theory is that it lacks precision in its theoretical treatment of the two variables, coping behaviour and relevant feedback. For example, Weiss's only definition of coping behaviour is an operational one: a coping response is a 45° turn of the wheel manipulandum. Apart from the fact that the definition excludes other direct attempts to handle the stressor (e.g. tail biting), such a specific operational definition is too limiting to enable one to arrive at a general understanding of the concept of coping behaviour.

In stress situations generally, coping behaviour can refer to a number of activities, ranging from obvious attempts to terminate a stressor, to random disorganized behaviour. Weiss does not attempt to

delineate this range; nor does he indicate what particular responses he considers relevant to coping. Weiss's concentration on a single operational definition thus creates serious difficulties for any attempt to test his theory experimentally.

Weiss's treatment of the concept of relevant feedback also causes problems. Weiss claims that one of the advantages of his theory of stress ulceration is its quantitative nature. However, the mathematical properties of the relevant feedback scale are very elementary. Weiss has constructed an ordinal scale of relevant feedback: escape responses provide more relevant feedback than avoidance responses during a signal, and avoidance responses during a signal provide more relevant feedback than avoidance responses prior to a signal. However, the size of the difference in relevant feedback from condition to condition is not specified.

This limitation becomes serious if it is taken into account that an animal obtains more than one type of relevant feedback during a stress session (namely, by performing both escape responses and avoidance responses), or that any one group of animals includes some which predominantly escape and others

which predominantly avoid. In such situations, it becomes very difficult to determine the total amount of relevant feedback obtained by the animal or the group, without "adding together" two distinct types of feedback. Since Weiss's scale of relevant feedback is ordinal, addition may not be performed without making independent assumptions about the interval properties of the scale.

One of the contradictions in Weiss's work is that, while he assumes an ordinal scale in expounding his concept of relevant feedback, he assumes an interval scale in plotting the experimental data in his three dimensional figure (see Figure 9, on p.263). Weiss arbitrarily identifies where different amounts of relevant feedback fall along the relevant feedback dimension without stating what the relationships among the different points are. Crediting an ordinal scale with unspecified interval properties detracts from the credibility of Weiss's diagram.

Conclusions

Weiss's theory has been met with considerable enthusiasm by those working in the area of stress ulceration, and by stress researchers generally. Since

its first appearance in 1971, and its later publication in Scientific American in 1972, investigators have been accepting the theory at its face value, and they have been interpreting their experimental results in terms of the theory's two basic premises. The prominent experimental psychologist Martin Seligman has recently added to this acceptability by discussing the theory at some length in his book Helplessness (Seligman, 1975), but without examining the real problems associated with its formulation.

Weiss's theory has not yet been subjected to any independent empirical investigation. The only evidence that is available is indirect evidence from studies investigating restraint-induced ulceration. Restrained rats are in a position where any coping behaviour necessarily results in zero relevant feedback, since no coping response produces stimuli unassociated with the stressor of restraint. Therefore, Weiss's theory would predict that as coping behaviour increases, so does ulceration. If it can be assumed that struggling by restrained rats is coping behaviour, then the amount of struggling and the extent of ulceration should be positively correlated. Evidence has been presented by both Bonfils (quoted in Brodie,

1963a) and by Ader, Beels, and Tatum (1960a) to show that no such relationship exists. Therefore, at this stage, Weiss's theory is certainly not useful in accounting for the development of restraint ulceration.

It can be seen from the discussion presented in this Chapter that Weiss has overlooked a number of problems associated with the derivation and the organization of his theory. Given the rapid acceptance it has received, it is important that the theory be empirically tested as soon as possible, so that a proper assessment of it can be made. It was decided to embark on such a project, and the aim of Experiment 5 was to initiate an experimental investigation into Weiss's account of stress ulceration.

CHAPTER 11

EXPERIMENT 5: A TEST OF WEISS'S THEORY OF STRESS ULCERATION

The aim of Experiment 5 was to test Weiss's theory of stress ulceration. However, considerable difficulty was encountered in designing an experiment that would test the specific propositions being put forward by Weiss. First, on close inspection of the theory, it appeared that neither of the variables Weiss proposed as being related to stress ulceration - number of coping responses and relevant feedback - could be independently manipulated. The number of coping responses emitted by an animal in a stressful situation depends on what the animal itself does, and is outside the control of the experimenter. Furthermore, the amount of relevant feedback provided an animal by coping attempts depends on when the animal responds, and again, this factor is outside the direct control of the experimenter.

Secondly, although a number of intriguing predictions could be made on the basis of Weiss's theory, it appeared that they, too, could not be directly tested. For example, Weiss's theory predicts that

animals that do not respond at all will not ulcerate. But how is such a prediction tested? One possibility is to construct a stressful situation which one expects, on a priori theoretical grounds, to lead to an absence of responding by the animal. But the animal may in fact not behave in the expected manner, and no test of the hypothesis would be possible.

Another prediction made by Weiss's theory is that animals that permit the stressor to arrive, and then turn it off (i.e. escape from it) will be less stressed than had they taken action to prevent it from arriving in the first place (i.e. avoid it). But again, testing such a prediction depends on what animals do in a stressful situation, and constructing situations in the hope that animals will behave in a particular manner is not a very satisfactory way of proceeding.

It was decided to abandon the strategy of trying to test hypotheses derived from the theory, or of trying to manipulate the independent variables proposed by Weiss. Instead, as a first step, it was decided to replicate one of Weiss's stressful situations, accurately observe the behaviour of the animals in that situation, and then ascertain whether the relat-

ionships postulated by Weiss really do hold. The situation selected was escape-avoidance from signalled shock, in which all types of relevant feedback were possible: low relevant feedback from responses made during silence, medium relevant feedback from responses made during the signal, and high relevant feedback from responses made during the shock.

In carrying out such an experiment, there were a number of mathematical problems to solve. First, Weiss's theory predicts that there is a negative correlation between amount of relevant feedback and amount of ulceration, and a positive correlation between number of coping responses and amount of ulceration. However, Weiss complicated the situation by further claiming that as relevant feedback increases, the correlation between responding and ulceration approaches zero.

The second problem was to obtain a total measure of relevant feedback for each rat during the stress period by "adding together" different amounts of ordinally scaled feedback (low, medium, and high). To make such an addition, assumptions would have to be made about the interval properties of the scale, assumptions that Weiss did not provide.

The mathematical quandary was resolved by deciding to integrate what was hypothesized by Weiss about the ulcerogenic properties of relevant feedback and the ulcerogenic properties of responding. Weiss claimed that responses made during low relevant feedback are very ulcerogenic, that responses made during medium relevant feedback are less ulcerogenic, and that responses made during high relevant feedback have little ulcerogenic effect. Thus, the total ulceration that occurs in any one rat is the sum of the three ulcerogenic effects for that rat.

The situation can be described in another way. A logical extension of Weiss's theory is that the responses made during the three categories of relevant feedback can be weighted for their ulcerogenic value, such that the ulcerogenic weight for low relevant feedback responses is greater than the weight for medium relevant feedback responses, which in turn is greater than the weight for high relevant feedback responses. Since ulceration is the result of the sum of the three ulcerogenic effects, a single "ulcerogenic effect" score can be calculated for each animal, using the formula $X_1R_1 + X_2R_2 + X_3R_3$, where X_1, X_2, and X_3 are the three weightings in the

low, medium, and high feedback categories respectively, and R_1, R_2, and R_3 are the total number of responses made in each of those categories. On the basis of Weiss's theory, it would be predicted that there would be a significant positive correlation between "ulcerogenic effect" score and actual amount of stress ulceration.

There was still a major problem. Appropriate weights would have to be assigned to each of the relevant feedback categories. It was decided that a meaningful choice of weights was probably impossible, since all that was known about them on the basis of Weiss's theory was that $X_1 > X_2 > X_3$. It was also known that none of the weights equalled zero (i.e. had no ulcerogenic effect at all), since Weiss had experimented with a condition that was even less ulcerogenic than any of the conditions included in Experiment 5 (namely, the condition in which a tonal feedback was included with every response). Therefore, it was decided to adopt a statistical solution to the problem. Rather than try and guess at the correct weightings, it was decided to use a multiple regression analysis to calculate the actual weightings in the sample data, and then ascertain whether Weiss's primary condition of $X_1 > X_2 > X_3 > 0$ had been met.

Thus, the experiment was carried out in the following way. A replication of Weiss's "avoidance-escape from signalled shock" group was designed. Actual response totals for each of the three relevant feedback categories were calculated, and a multiple regression equation was calculated to determine the combination of weightings of response totals that was the best predictor of stress ulceration in the sample data.

Although Weiss's design was replicated as closely as possible, a number of procedural changes were made to improve the performance of the animals on the avoidance task. First, the avoidance-escape training period was extended from the 30 minutes used in Weiss's experiment to 3 hours. The animals were given two training periods, each of 90 minutes duration, and separated by 24 hours. Secondly, the 20 second pulsing beep signal prior to the shock was replaced by a 20 second buzzer. The buzzer was considered a more salient stimulus, and it was expected that the animals would respond more frequently during a buzzer than during a beep.

An additional change that was made to the procedure was to use subdermal electrodes rather than

skin surface electrodes. This substitution was made to overcome the problem caused by differences in skin resistance. Because of the change from surface electrodes to subdermal electrodes, the shock intensities used by Weiss also had to be adjusted.

Method

Subjects

The subjects were 84 male, ulcer-susceptible, Sprague-Dawley rats. The rats were bred and maintained until the beginning of the experiment as described in Chapter 5 (see pp.162-166). From the time they were weaned, and during the experiment, the rats were group housed, six to a group, in large plastic boxes. The rats were 130 to 180 days at the time of the experiment, and they had a mean weight of 394gms. Six rats were eliminated from the results of the experiment because they were suffering from physiological disorders unrelated to the nature of the experiment.

Apparatus

The apparatus used in this experiment was modelled on the apparatus used by Weiss (1971a), and was

the same as that used in Experiment 3. Six shock boxes were constructed on the basis of diagrams published by Weiss (1971a, 1972). Each box had three clear perspex sides, a clear perspex top, and an aluminium door. The floor consisted of a grid of eight perspex bars, each bar 6mm (.25ins.) in diameter, and each 19mm (.75ins.) apart. The dimensions of the shock boxes are provided in Figure 6, on page 226.

In the perspex wall opposite the door to the box, there was an opening through which a large wheel manipulandum protruded.[8] The shock box was very small, and there was little room in it for anything other than the rat and the wheel. The wheel was made of aluminium, and had a diameter of 102mm (4ins.). It measured 64mm (2.5ins.) across its rungs, of which there were 30. The wheel had sufficient inertia acting on it to prevent it from free wheeling, but insufficient to make turning it aversive for the rat.

The rat's tail protruded through an opening underneath the door of the box, and was fixed outside the box by a rubber disc, 50mm (2ins.) in diam-

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[8] In Experiment 3, the opening had been closed with a piece of perspex.

eter. The disc was held in place with Elastoplast elastic adhesive bandage attached to the rat's tail on either side of the rubber disc. Subdermal stainless steel electrodes, manufactured by the Grass Instrument Company, Model E2B, were used to deliver the shock, and were held in place with Elastoplast. A shock generator was constructed in the laboratory, and was set to deliver shock intensities ranging from .2mA to 1.3mA.

Each Weiss box was placed in an individual sound attenuating chamber, which was continually lighted by means of a 24 watt bulb situated away from the direct gaze of the rat. The buzzer signal prior to the shock was electrically simulated, and delivered through a 50mm (2ins.) diameter speaker attached to the wall of the chamber immediately facing the rat. An electric suction fan was inserted in the wall of the chamber facing the rat to provide a constant level of white noise, and to maintain air circulation within the chamber. The db of the fan noise and the simulated buzzer was measured to be 90db by a Brüel and Kjær Sound Level Meter, Type 2203. A plastic water bottle was attached to the side of the shock box to provide the rat with continuous access to water

throughout the stress period.

Wheel turn responses were recorded by the action of magnets (which were attached to the wheel) passing in front of reed switches. There were eight magnets and two reed switches in all, four magnets and one reed switch on either side of the wheel. The magnets were arranged such that a response was recorded every 45° turn. Responses were recorded on electro-mechanical numerical counters. There were three counters for each of the six rats: the first counter to record responses made during the shock, the second to record responses made during the buzzer, and the third to record responses made during silence.

As in previous experiments, an Ohaus Triple Beam Balance, a desiccator jar, and polystyrene slabs were also used in the Experiment.

Procedure

Avoidance-escape training: day 1. Six rats were trained at a time. At the beginning of training, each of the rats was allocated to one of the six shock boxes. Each rat was weighed, placed in the shock box, and had its tail fixed outside the box by means of the rubber disc. The electrodes were then inserted

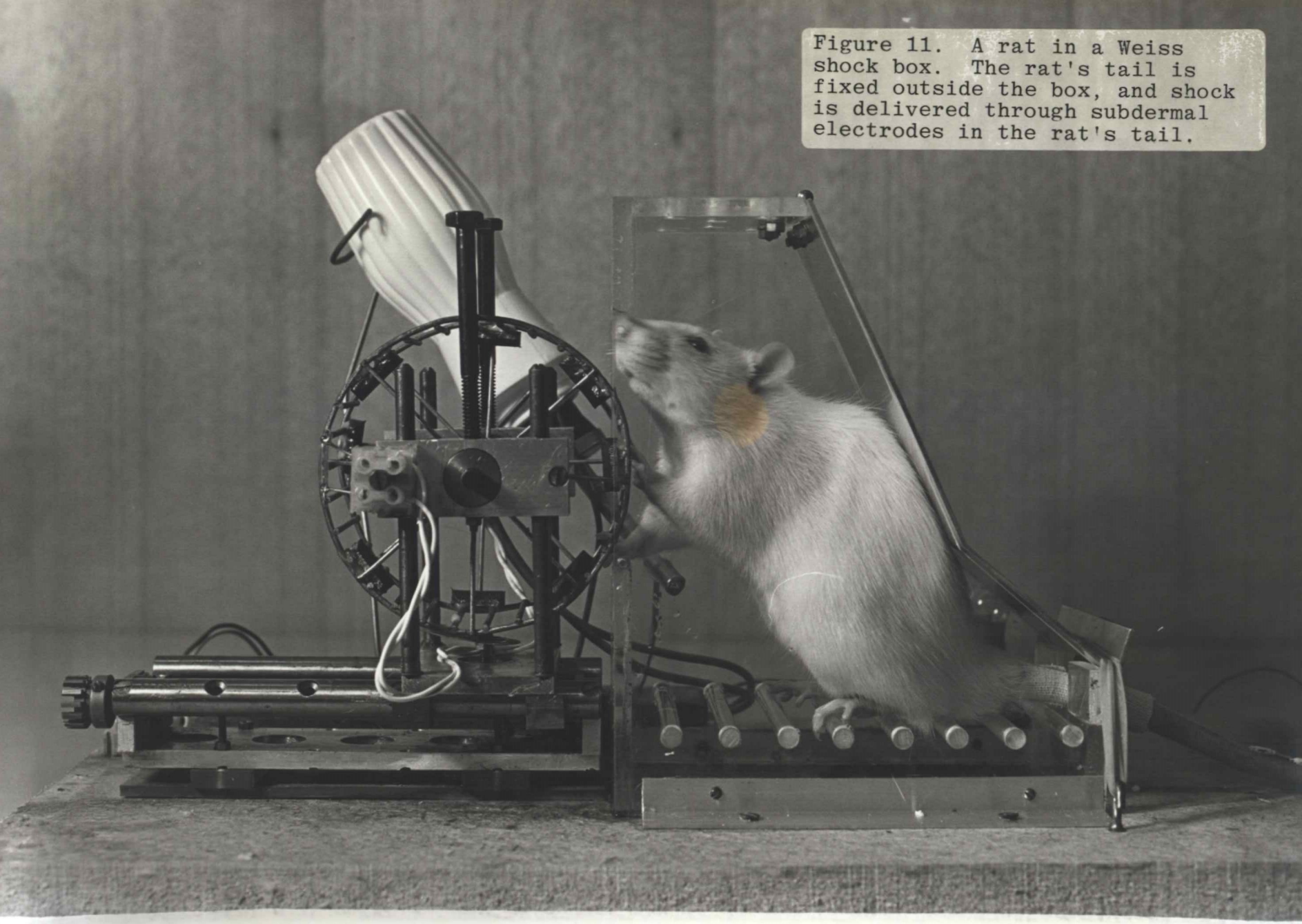

Figure 11. A rat in a Weiss shock box. The rat's tail is fixed outside the box, and shock is delivered through subdermal electrodes in the rat's tail.

under the skin of the rat's tail. Water was continuously available, but there was no food. Figure 11, facing page 288, shows a rat in one of the shock boxes.

On day 1 of training, the rats were given 90 minutes of avoidance-escape training. The shock level was initially set at .2mA, but was increased to .4mA during the training period. Each shock was preceded by a 20 second buzzer. As in Weiss's experiment, the duration of the shock was not fixed, but remained on until the rat made an escape response. The rat could escape the shock only by turning the wheel manipulandum 45°. The rat could also avoid the shock by turning the wheel prior to or during the signal. The intertrial interval was set at 200 seconds, and making a 45° wheel turn any time during that 200 seconds reset the timing at the beginning of the intertrial interval.

The rats learned to escape the shock very quickly, usually on the first half a dozen trials. If the rat was slow at learning, the escape response was modelled for him by the experimenter. All rats had learned to escape the shock by the end of the first training period.

At the completion of the 90 minute training period, the rats were returned to their home boxes, and their tails marked with dye for easy identification. The rats were then deprived of food for 24 hours, but allowed continuous access to water.

Avoidance-escape training: day 2. At the end of the 24 hour food deprivation period, the rats were weighed again, and each rat was returned to its own shock box for a second 90 minute training period. Initially, the shock was set at .4mA, but then quickly increased to .5mA. The aim of the second training period was to teach the rats to avoid the shock as well as to escape it. The method used to achieve this end was modelling of avoidance responses (primarily during the buzzer signal) by the experimenter. The modelling was much less effective in teaching rats to avoid than it was in teaching them to escape. However, irrespective of whether the rats had learned to avoid shock or not, the second training period was terminated at the end of 90 minutes, and the 48 hour stress period was begun immediately.

Stress session. The stress session commenced

immediately the second training period finished, and it lasted for 48 hours. As in the training period, the rat was presented with signalled shock, and the rat could either escape or avoid by turning the wheel manipulandum 45°. The intertrial interval was 200 seconds, and the shock was continuous until a response was made. There was no further modelling by the experimenter. The shock intensity was initially set at .6mA, and was increased to .8mA after 6 hours, to 1.0mA after 20 hours, to 1.1mA after 30 hours, and to 1.3mA after 44 hours. No food was permitted during the stress session, but water was continuously available.

The responses made by each rat were recorded on one of three counters, depending on whether the response was made during the shock, during the buzzer, or during silence. If more than one response was made at any one time, the first response was recorded in the feedback category during which the responding commenced, and the other responses were recorded in the silence (low relevant feedback) category. This procedure was adopted because once the first response had been made, the stimulus situation immediately changed, such that any further responses provided

only low relevant feedback.

At the end of the 48 hour stress period, the response tallies were noted, and rats were removed from their shock boxes. They were killed and their stomachs were prepared for inspection in the usual manner. The number of ulcers in each stomach was counted by the experimenter, who counted the ulcers without any knowledge of the response tallies for the individual rats.

Results

The majority of the ulcers developed in the glandular section of the stomach; the few rumenal ulcers that did occur were not included in the results. The stress ulceration was not severe; it consisted of small superficial pitting that was rarely accompanied by hemorrhaging.

Four measures were obtained for each rat:

(1) number of ulcers

(2) number of responses in the low relevant feedback category (R_1)

(3) number of responses in the medium relevant feedback category (R_2)

(4) number of responses in the high relevant feed-

back category (R_3).

On the basis of these measures, a multiple regression equation was calculated to determine the combination of the response totals that was the best predictor of ulceration. The equation was presented in the form

$$U = A + X_1R_1 + X_2R_2 + X_3R_3$$

where U = number of ulcers

R_1, R_2, R_3 = number of responses in the low, medium, and high feedback categories respectively

X_1, X_2, X_3 = coefficients for R_1, R_2, R_3 categories respectively

A = a constant (the Y-axis intercept)

The coefficients for the relevant feedback categories and the value of the constant A are presented in Table 25, on page 293; α was set at .05 to test the hypotheses that these values are significantly different from zero.

The respective contributions of the regression equation and the residual to the variance in the data are presented in Table 26, on page 294; α was set at .05.

Table 25

Regression Equation Coefficients for Different Relevant Feedback Categories

Category	Value of coefficient
Low relevant feedback (X_1) (Responses during silence)	.0007*
Medium relevant feedback (X_2) (Responses during signal)	-.0032
High relevant feedback (X_3) (Responses during shock)	-.0021
Constant A (Y-axis intercept)	4.8

*$p < .01$

The mean number of ulcers found in the rats was 6.8 (standard deviation = 7.5). The mean number of responses per feedback category and the correlation co-efficients between number of ulcers and number of responses in each feedback category are presented in Table 27, on page 294.

Table 26

Analysis of Variance Summary Table: Partition of Variance into Regression and Residual Components

Source	SS	df	MS	F
Regression	454.6	3	151.5	2.86*
Residual (Measure of error component)	3916.7	74	52.9	

*$p < .05$

Table 27

Mean Number of Responses and Correlation Coefficients Between Responding and Ulceration in Different Relevant Feedback Categories

Relevant feedback category	Mean number of responses[a]	Correlation (respond.ulcer)
Low	4715.2	+.3
Medium	84.7	-.007
High	491.0	-.08

[a] $n = 78$

Finally, an overall correlation between weight of animal (prior to food deprivation) and number of ulcers was computed. Pearson's r was found to be +.10.

Discussion

The multiple regression analysis indicated that the coefficients X_2 and X_3 were not significantly different from zero. In other words, neither the medium nor the high relevant feedback category made a significant contribution to the variance in the ulceration scores. The multiple regression analysis found that the only significant contribution to the variance in ulceration came from the low relevant feedback category. Thus, on the basis of the results of Experiment 5, Weiss's theory was not supported. Medium and high relevant feedback were found to be irrelevant to the development of stress ulceration.

Such a conclusion is, however, over-simplified. If one examines the mean number of responses in each of the relevant feedback categories (in Table 27), it becomes clear that in comparison with the amount of low relevant feedback that occurred, the amounts of medium and high relevant feedback were utterly trivial. If a common sense approach to the data is

adopted, it would be expected that a trivial amount of medium and high relevant feedback would result in a trivial contribution to ulceration. Therefore, it might be concluded that the experiment was not a fair test of the role of medium and high relevant feedback in the contribution to stress ulceration.

There is a further complication to the interpretation of the results of Experiment 5. The design of the experiment itself actually imposed the limits on the number of responses that could be recorded for each rat in the medium and the high relevant feedback categories. It will be recalled that if a series of responses were made by a rat at any one time, only the first of the series was recorded in the category during which the responding commenced. All other responding was recorded in the low relevant feedback category, because those further responses only provided the rat with low relevant feedback. It was the first response that turned off the buzzer or the shock, and it was the first response that provided the rat with the medium or high relevant feedback.

The adoption of this procedure meant that the number of responses recorded in the medium and the high relevant feedback categories could be no greater

than the total number of buzzers or shocks that occurred in the 48 hour stress period. Since the buzzer-shock sequence occurred every 200 seconds provided the rat made no intervening responses, the maximum number of responses possible in the medium relevant feedback category was 960, and the maximum number possible in the high relevant feedback category was 864. On the other hand, the number of responses that could be recorded in the low relevant feedback category was limited only by the number of responses a rat could make in the 48 hour stress session.

Thus, the design of the experiment itself created the situation where the contribution made by medium and high relevant feedback categories was trivial. However, if this criticism can be made of the design of Experiment 5, it must also be made of Weiss's experiments themselves, since Experiment 5 was a replication of Weiss's basic design. What happened in Experiment 5 undoubtedly happened in Weiss's experiment; that is, irrespective of the signal condition Weiss's rats received, they obtained a trivial amount of medium and high relevant feedback, and a comparatively overwhelming amount of low relevant feedback. It is therefore nonsense for Weiss to talk of one experim-

ental situation providing low relevant feedback, and another providing medium or high relevant feedback.

The charge can be made that it is being overly pedantic to allocate responses to feedback categories in the manner in which they were allocated in Experiment 5. The rat probably makes no distinction between the first 45° wheel turn and all the 45° turns that follow it in the one sequence. For the rat, the appropriate response is most likely "wheel turning" generally, rather than specific 45° turns. But the pedantic nature of the methodology of Experiment 5 is a product of Weiss's own concentration on peripheral events. Weiss does not concern himself with "what the situation means to the rat". Weiss is concerned with quantifying coping behaviour and relevant feedback, and for that reason he defines each coping response as one 45° wheel turn, and he defines relevant feedback as the change brought about in the total stimulus situation by that response.

If a common sense approach is adopted towards the data of both Experiment 5 and Weiss's experiments, then it must be concluded that the experimental design used is not an appropriate test of the role of medium and high relevant feedback in the development of stress

ulceration. Weiss's theory has not been supported; but neither has it been disconfirmed. It simply hasn't been given an adequate test. Whether such a test can even be designed is doubtful, and Weiss's theory might not ever be subjected to proper empirical investigation.

CHAPTER 12

CONCLUSIONS

In this thesis, the factors that affect restraint-induced ulceration have been closely examined. One of those factors, prior housing conditions, has been investigated experimentally, and the obtained results have led to certain speculations about the mechanism of restraint ulceration. In Chapter 10, Weiss's general theory of stress ulceration was reviewed in some detail, and an empirical test of deductions from the theory was carried out in Experiment 5. It was decided that Weiss's theory as presently formulated is not a useful account of stress ulceration in general or of restraint ulceration in particular.

It is worthwhile reiterating some of the more important findings of the literature research into restraint-induced ulceration, particularly those that are contrary to widely held views. First, the method of restraint used to induce ulceration is not irrelevant. It was concluded in Chapter 2 that the type of gastric pathology produced does vary with the method of restraint employed, there being some methods that do not even produce true stress ulceration.

Secondly, there is little evidence to support the claim that activity level and restraint-induced ulceration are positively correlated. The evidence suggests instead that passive rats are more likely to develop restraint ulceration than are active rats. In particular, the hypothesis that female rats ulcerate more when restrained than male rats because some female rats are in estrous (and therefore more active) at the time of restraint has not received any experimental support.

It was also concluded that the parasympathetic nervous system is actively involved in the physiological mediation of restraint-induced ulceration, although factors other than parasympathetic activity are also involved. A critical question is how the parasympathetic activity is initiated when rats are restrained, as stress usually results in activation of the sympathetic nervous system.

The experimental work of the thesis itself was concerned primarily with the effect of prior housing conditions on restraint-induced ulceration. The charge that earlier researchers had overcrowded their group housed rats, with the result that individually housed rats ulcerated less when restrained than group

housed rats, proved to be irrelevant. In Experiment 1, it was found that group housed rats ulcerated more when restrained than individually housed rats irrespective of whether the group housed rats were crowded or not. In fact, crowded male rats ulcerated significantly less when restrained than uncrowded male rats.

In Chapter 9, a hypothesis was suggested which accounts for why group housed rats develop more restraint ulceration than individually housed rats. Initially, the hypothesis was formulated in terms of the amount of space rats have available to them in their living quarters prior to restraint, and it was predicted that the more space rats have available, the more will be the stress reaction when that space is severely restricted during restraint. The hypothesis was later modified to predict the extent of restraint ulceration on the basis of adaptation to previous movement permitted rather than on the basis of adaptation to previous space provided. In other words, it is now predicted that the more movement the rat is adapted to prior to restraint, the greater will be the stress reaction when that movement is restricted during restraint.

The adaptation to movement/space hypothesis includes the general notion of change: in restraint, the rat is required to adapt to a change in either the movement permitted or the space provided. It has been suggested by some researchers (mainly Holmes & Rahe, 1967) that change itself (of whatever quality) is inherently stressful, and some of the relevant research was discussed briefly in Chapter 1. To what extent change itself is stressful or not still requires further empirical investigation. However, implicit in the designs of Experiments 1 to 4 of this thesis was the belief that any additional adaptations expected of the rat, above and beyond that already demanded by restraint, would have an exacerbatory effect on ulceration. Whenever rats were restrained, attempts were made to maintain them under conditions that were as similar as possible to those of the animal holding unit in which the rats had been housed prior to restraint. The restrained rats experienced the same light-dark cycle and the same temperature conditions as they had in their living quarters. Efforts were also made to keep extraneous stimuli such as noise and light to a minimum.

Note that it is not being suggested that any of

these additional changes were particularly stressful in and of themselves. Rather, it is being suggested that given the intensity of the stress the rat was experiencing during restraint, any additional adaptations that had to be made, however trivial, became disproportionately stressful.

The most important contribution that this thesis as a whole has to offer is the judgement that obsessive standardization of conditions is imperative if valid and reliable ulceration measures are desired from the restraint technique. There are, of course, the obvious sources of variation: for example, sex of rat, weight of rat, and manner in which the rat is food deprived. Through scrutiny of the literature and experimentation, this thesis has been able to suggest some of the less apparent sources: method of restraint, size of prior housing compartment, number of rats occupying the same housing compartment, and conditions under which the rat is restrained. If particular attention is paid to the standardization of such conditions, the results of studies into restraint-induced ulceration should, in the future, be more valid and more reliable.

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