AN INVESTIGATION OF THE INVOLVEMENT OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN THE MEDIATION OF BEHAVIOR AFTER STRESS

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ABSTRACT

AN INVESTIGATION OF THE INVOLVEMENT OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN THE MEDIATION OF BEHAVIOR AFTER STRESS

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Three experiments were performed in order to analyse the behavioral and biochemical correlates of four different intensities of the same stressor. In Experiment 1, rats were exposed to heat stress (hot-plate) of varying temperatures for 30 seconds. Activity was recorded in an open field immediately after stress for 30 minutes. The data revealed that the milder temperatures increased, while the higher temperature decreased activity. Experiment 2 assessed the hypothalamic-pituitary-adrenal response to the different temperatures by measuring levels of plasma coticosterone 30 minutes after stress. The four levels of hot-plate temperatures induced differential levels of corticosterone which may best be described as a U-shaped curve, with the extreme temperatures inducing the highest levels of the steroid. Experiment 3 further manipulated the

hypothalamic-pituitary-adrenal axis by administering dexamethasone 25 hours and 1 hour before stress and ACTH 15 minutes before stress. Both affected activity levels by depressing locomotion regardless of the stress intensity. These results are compared to other studies that have addressed the question of stress-induced activation and it is suggested that stress is not a unitary concept, but interacts with the performance of certain behaviors to produce both facilitory or inhibitory results.

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INTRODUCTION

The investigations which are reported in the present thesis were conducted in an attempt to add to the knowledge concerning the biochemical and pharmacological substrates of the effects of stress on subsequent behavior. The studies focus on the anatomical and humoral pathway formally known as the hypothalamic-pituitary-adrenal axis (HPA) and the possible nature of its involvement in modulating behavior under conditions of stress.

The structure of the introduction will be as follows: Initially the hypothesis of stress "intensity" will be discussed in general terms. This will be followed by a brief description of the HPA and its involvement in stress. The next sections will be concerned with the biochemical measurement of stress intensity which will lead into a discussion of specific techniques used to induce different intensities of stress.

STRESS, AROUSAL, AND THE INVERTED U-SHAPED CURVE

The fact that stress affects subsequent behavior has long been surmised by the general public through intuition and informal hypothesis. The general consensus is that

stress has a detrimental effect upon behavior. Some authors within the scientific community, however, have proposed the notion that stress may not neccessarily be of a detrimental nature. For example Selye (1973, 1974, 1980) has distinguished two forms of stress. "Good" stress has been termed eustress while "bad" stress has been given the label of distress. Selye's conceptualization of stress has developed over the years to the point where he perceives that there is a relationship between stress and health. Recently he has diagramed the concept so that it can be represented by a U-shaped curve (Selye, 1974, pg.20) At one end of the continuum are stimulations of an "extremely unpleasant" nature, while at the other end lies the "extremely pleasant" aspect of the same continuum.

Over the years Selye (e.g. 1950, 1973, 1974, 1980) has amassed an extensive list of experimentation on the effects of physical insult (stress) on the organism. According to Selye (1950) the term stress should be reserved for noxious stimuli that would bring about the same physiological syndrome. On the other hand, the term arousal has more often been used to characterize psychological variables. Arousal is usually reserved for such variables as, intensity, frequency, hunger, fear, novelty and conflict. Recently, an attempt has been made to combine the similarities between

arousal and stress and to represent them upon the same continuum. Hennessy and Levine (1979) have pointed out that arousal can be measured both by behavioral as well as physiological variables. Stress, on the other hand has, in the past, been almost excusively measured in terms of pathological variables. But Hennessy and Levine (1979) have argued that the stressors that have pathological effects also have behavioral effects. All of the stressors used by Selye (cold, toxins, heat, restraint) also have behavioral consequences. Animals will learn to avoid the consequences through behavioral means. Therefore Hennessy and Levine (1979) assert that stress can be subsumed under the concept of arousal.

Hebb (1955) formulated views on stress that seem similar to Selye's although they were not concerned with the notion of stress per se, but attempted to deal with the then prominent concept of arousal. Hebb was influenced by the idea that behavior, or the impetus for behavior (drive) was a function of the current arousal level of the organism and its interaction with cue function. Cue function, according to Hebb, served as the guiding or steering mechanism. When the level of arousal was too high or too low, the optimal level of performance could not be attained and behavioral efficiency would decline. As with Selye's (1974)

formulation, Hebb's cue and arousal interaction describes a U-shaped curve.

Malmo's (1959) review of the literature of that period led him to suggest that the internal conditions of the organism by themselves did not produce a direct effect on some motor effector. Appropriate level of stimulation would have the effect of sensitizing the organism. Malmo further speculated that activation (the term that Malmo employed) at any given point might be the interaction between environmental and hormonal conditions. Activation, according to Malmo, had no steering function. He delineated a specific experimental paradigm which included three levels of activation: low, moderate, and high, which would have corresponding expected performance levels of low, optimal, and low, i.e. an inverted U-shaped curve. Malmo stressed that activation levels have meaning only as relative terms and were not to be taken as absolute levels. That is, one level is higher or lower than another level only in relation to the levels that surround it. Therefore studies of activation have to utilize a number of intensity levels so that comparisons can be made within experiments.

The theories breifly described above (except Selye's) are out of date because they were based on available empirical evidence and the physiological knowledge of the

time. Nevertheless, a common theme between Hebb, Malmo, and Selye can be discerned. All of the above authors theorized that there is a functional interaction between the intervening variables of activation, arousal, or stress, and subsequent performance. The functional interaction was viewed as a U-shaped curve.

It has been known for many years that the HPA is functionally involved in the organism's reaction to stress (Selye, 1952). Selye demonstrated that a variety of noxious stimuli would activate the HPA and lead to a number of organic consequences. For example, these stimuli, (i.e. restraint, administration of foreign proteins) would cause hypertrophy and hyperfunction of the adrenal cortex, the involution of the thymus and the nodes and ulcerations in the stomach and intestines. As can be gleaned from these examples, Selye's work has been, in the main, confined to the elucidation of the pathological and immunological consequences of stress. However, the HPA has also been studied within a behavioral context.

Applezweig and Baudry (1955) seem to have been the first to study manipulations of the HPA within behavioral learning paradigms. They found that hypophysectomy retarded the learning of an avoidance response. These authors

expressed the opinion, derived from the interpretation of their studies, that the HPA was involved in the modulation of behavior during and after an aversive event.

The next major body of data elucidating the involvement of the HPA in aversive learning were reported by de Wied (1964). De Wied was able to show that the impairment in the acquisition of a shuttlebox avoidance in response to the noxious stimuli of foot shock were due to the pituitary-hormonal deficiencies caused by removal of the pituitary and one of its major peptides, adrenocorticotropin (ACTH).

Since these early studies the HPA and its involvement in learning and memory processes have been studied extensively (for reviews see Bohus & De Wied, 1981; De Wied, 1977, 1980). The relevance of these studies on learning to the present research has to do with the observation that noxious (stressful) stimuli are used either before, after, or during the experimental paradigm and also the that HPA has been found to be intimately involved in the paradigms (Bohus & De Kloet, 1979). Therefore the idea of stress having effects on behavior, other than those directly triggered by the noxious stimuli, has been substantiated. What is needed is research that will delineate a procedure with known effects on the HPA which can then be used in

conjunction with the studies on learning and memory. The experiments about to be described were designed with this in mind.

THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The HPA is an integrated system involved in the regulation of hormonal processes which include elements of both humoral and nervous transmission. Basically the axis can be conceptualized as a negative feedback loop consisting of the hypothalamus, pituitary and the adrenals. It is generally assumed that the hypothalamus exerts control over the pituitary and that the pituitary in turn exerts its effects on the adrenals. Of particular concern is the control of the release by systemic and neurogenic stressors of the sequence of amino acids known as pro-opiocortin in which ACTH and the endogenous opioids are contained (Lowry, Silman, Jackson & Estivariz, 1979; Mains & Eipper, 1981; Watson & Akil, 1981).

Primary control of ACTH release is thought to be mediated by corticotropin-releasing factor (CRF)(Gillham, Insall & Jones, 1979; Smelik, 1981). CRF is synthesized by nerve cells in the area of the medial basal hypothalamus and stimulation of this site releases CRF from axon terminals into the portal system through the median eminence of the

hypothalamus. CRF then travels through the pituitary stalk which joins the hypothalamus and the pituitary. At the level of the pituitary (in particular the anterior portion) CRF then facilitates the release of ACTH into the systemic circulation. (For a detailed review of the most recent evidence for this process see Makara, 1979; Makara, Palkovits & Szentagothai, 1980).

The hypothalamus is by no means the only neural system which is capable of exerting control over the release of ACTH. There are some stressors (e.g. endotoxin, large doses of formaldehyde) which can induce release of ACTH even in the absence of the hypothalamus (Dallman, 1979; Halasz, Slusher & Gorski, 1967; Palkovits, 1977). In general, however, and certainly for the present studies, the hypothalamus occupies the central position under conditions of stress. The classical description of the circuit still stands up to scrutiny and the inconsistencies are probably of minor significance (see Makara et al, 1980).

ACTH released from the pituitary circulates through the blood stream and reaches specific receptors embedded in the adrenals which then cause an increase in the formation of cyclic AMP. Cyclyc AMP tends to act as the "second messenger" which promotes the activity neccessary for the production of the steroid, corticosterone (Sayers, Beall &

Seelig, 1974). The steroid is then released into the blood stream reaching target sites where it exerts immunological (Selye, 1952) and central effects (Bohus & De Kloet,1979). Closing of the HPA loop is accomplished by corticosterone reaching either the hypothalamus, pituitary, or some central site which has yet to be unequivocally demonstrated. This corticosterone can then inhibit the release of ACTH thus producing the feedback regulation (Sayers, Beall & Seelig, 1974).

This feat of biological engineering contains other pathways which seem important in stress. A second mechanism, other than direct humoral feedback is now also being suggested to exert some control over CRF-ACTH release during stress (Ganong, 1977,1980). Catecholamine containing pathways through which feedback could be exercised are now being investigated. The evidence is as yet too preliminary to make definitive statements but the reports are suggestive of the existance of such a neural pathway (Palkovits,1977).

The medial-forebrain-bundle (which contains NE fibers) has access to the hypothalamus through the lateral-retrochiasmatic area from the general area of the medial basal hypothalamus (Palkovits, 1977). These data are very suggestive of the existence of a mechanism of noradrenergic inhibition of ACTH release which would be

disinhibited when norepinephrine (NE) levels fall due to stress. Further evidence for this notion comes from studies that have shown that pharmacological compounds which can effect release of catecholamines centrally can then effect stress induced ACTH release (Ganong, 1977,1980). Catecholamine inhibition of ACTH release gains in conceptual importance when one realizes that catecholamines are differentially affected by many stress producing agents used in psychological laboratories and mediate much of the behavioral changes induced by stress (Anisman, 1978; Anisman, Kokkinidis, & Sklar, 1981)

CORTICOSTERONE AS AN INDEX OF STRESS

The humoral component of the HPA has attracted much attention as a measure of the intensity of stress because it meets the criterion of non-specificity; that is, many and diverse forms of stress, both neurogenic and systemic, produce changes in the system. Corticosterone plasma levels have been used extensively as an indication of the mobilization of the HPA.

Changes in corticosterone levels can be detected after a wide variety of aversive events such as: exposure to novel environments (Mason et al, 1957;Mason, 1968; Pfister,1979; Pfister & King,1976); predictable versus unpredictable shock

(Hennessy et al, 1977); rapid but not gradual blood loss (Gann, 1969); and avoidance paradigms such as conditioned taste aversion (Smotherman et al, 1976) to name only a few. Changes in brain levels of corticosterone have been found to increase as a result of novelty stress and the magnitude of the increase was found to correspond to the magnitude of the plasma increase (Kakihana & Moore, 1978).

Corticosterone can also be considered a reliable indicator of the initial intensity of a noxious event. Friedman et al (1967) conducted a study for the expressed purpose of measuring corticoserone levels after various "degrees" of novelty stress and electric shock. Novelty was represented by the effects of handling unhandled animals for various lengths of time. The different groups of rats were handled for either 30, 120, or 240 seconds. Corticosterone was measured at various intervals after handling and the results indicated that there were significant differences as a function of duration of handling, with longer durations inducing increasingly higher levels of the steroid.

A similar procedure was used to gauge the corticosterone response to shock intensities (Friedman et al, 1967). The shock intensities employed were: 0, 0.2, 0.5, 1.0, 2.0, and 4.0 milliamperes. Not only was the intensity of the shock manipulated, but duration of each intensity was

varied. There was a significant effect of shock intensity and of shock duration. Corticosterone levels rose as intensity and duration were increased. Friedman et al, (1967) interpreted their results to mean that the HPA and specifically the corticosterone response was sensitive to shock and novelty intensity. Smith (1972) has taken exception to this interpretation. Smith argued that the differences in the midrange of intensity were minimal and therefore corticosterone was a poor indicator of the range of intensities between threshhold and maximum. Smith did however agree that corticosterone levels were sensitive to the extreme intensities of shock. Due to the fact that the Friedman et al, study did not include any post hoc statistical tests for comparison between groups the interpretation of the data remains unresolved. Nevertheless, when one considers the data obtained in more recent experiments; the argument by Smith may have to be reassesed.

Hennessy and Levine (1978) varied the degree of novelty to which they exposed mice. Corticosterone was measured after 30 minutes of stimuli. The stimuli used were the progressive change in environmental conditions with respect to the animal's home cage. Each separate group was placed in conditions wherein they would experience different degrees of environmental change. They reported significant

differences among the means of all the groups. As the novelty of the situation increased so did the corticosterone levels, thus adding support for the contention that corticosterone levels can accurately reflect differences in degree of stimulation.

File (1982) has found similar results in a recent experiment using different stimuli. Though this study was designed to examine the effect of an agent (chlordiazepoxide) known to reduce the corticosterone response to stress, close examination of the control groups (saline only) reveals a differential release of corticosterone after various stressors. The stressors used were 10 minutes of exposure to a novel environment, 20 minutes exposure to a tone (noise stimuli) and two hours exposure to both restraint and cold. It was assumed that the stressors were progressively more severe and in fact the corticosterone response seemed to reflect this. There was a progressively higher release of corticosterone in response to the stressors in presumed order of severity.

In addition to its usefullness as an index of aversion (e.g. stress intensity), the corticosterone response can also be used as an indicator of ACTH release. Levels of corticosterone are raised when ACTH is exogenously administered (Moncola, Peron & Dorfman, 1959) or after

noxious stimuli when ACTH is presumed to be released (Mason,

THE HOT-PLATE METHOD:

INTENSITY AND DURATION OF HEAT STRESS

Since the introduction of the hot-plate technique by Woolf and Macdonald (1944) and the modifications by Eddy and his coworkers (Eddy, Touchberry & Lieberman, 1950; Eddy and Lembach, 1953) many laboratories have used the procedure to test for the analgesic properties of drugs. The procedure consists of preinjecting the subject (rodent) with a pharmacological agent and then, at various intervals, placing it on a hot-plate which is preheated to a temperature which is thought to be aversive. The reaction of the subject is then recorded. Latency to lick a paw and/or escape are the behavioral measures most often recorded. Differences in the latencies to initiate one or the other of the behaviors is taken to indicate the effectiveness of the pharmacological treatment.

Placement on the hot-plate induces pain as evidenced by the animal's subsequent behavior. The animal will begin licking its paws shortly after being placed on the heated plate. Latency to the initial pawlick will depend on the temperature and the effectiveness of the pharmacological agent. It is assumed that pawlicking behavior is an attempt

by the rodent to spread saliva on the affected area thereby decreasing the pain through evaporative cooling. This supposition comes from the demonstration that saliva spreading is is one of the most prominent behavioral methods available to the rodent when placed in heated environments (Adolphe, 1947; Hainsworth, 1967). The second major behavior, escape, has long been considered to designate an aversive event (Fantino, 1973; Herrnstein, 1968).

There seems to be no single temperature which is used across laboratories. Nevertheless there is a range of temperatures which are commonly used: 45-60°C. Use of different temperatures has been found to change the behavioral reaction when used to test the effectiveness of narcotic and non-narcotic analgesics. For example, O'Callahan and Holtzman (1975) found that the sensitivity to analgesic drugs on the pawlick response differed according to temperature. The hot-plate at 49.5°C was sensitive to dose response analysis. All the narcotic antagonists used exhibited dose specific effects on the pawlick response when the temperature was 49.5°C. Yet these same drugs had no effect on the pawlick response at 54.5°C. In addition to this they found that the pawlick latencies were lower after the higher temperatures.

Janicki and Libich (1979) used a 55°C hot plate in

their study of narcotic antagonists. They report that at 55° C they were able to get separation between the two prominent behavioral measures; pawlick and jump-off (escape). At higher temperatures they were unable to detect differences between the latencies of the behaviors.

Jacob, Tremblay and Colombel (1974) state that the narcotic antagonist naloxone was able to modify licking responses when the hot-plate was at 50°C but not after temperatures of 55°C or even 80°C. However, they were able to detect differences in the jump-off latencies at higher temperatures (55, 65, and 80°C). Jacob and Ramabadran (1981) suggest that the reason for the failure to find differences in the licking response at temperatures of 55 C or higher was because of a "floor" effect. That is, the pawlick latency was already at a minimum and therefore no differences could be detected.

In addition to the examples that indicate that different temperatures of the hot-plate affect the response, there is some indication that duration of exposure also affects responses. When animals are initially placed on a hot-plate of 51°C for up to a maximum of 120 seconds, the first response to appear is pawlicking, which is then followed by escape attempts. Upon repeated testing, the pawlick response usually does not appear, instead the

animals first response is to make an escape attempt (Amir & Amit, 1978, 1979). However when animals are confined to the hot-plate for up to seven minutes both pawlicking and escape as well as bellying (lying prostrate on the plate) behavior appear in succession. First pawlicking dominates. This is followed by escape attempts and when this fails, bellying becomes the predominant response (Hunt, Switzman & Amit,note 1). These data indicate that changing the duration of heat stress affects subsequent behavior as does the intensity.

There have been suggestions that the pawlick and escape responses are differentially mediated and reflect different aspects of the nociceptive system (Grevert & Goldstein, 1977; Fredrickson, Burgus & Edwards, 1977; Amir & Amit, 1979). Frederickson, Burgus and Edwards (1977) reported that the jump or escape response was more readily affected by opioid compounds (e.g. morphine and naloxone) than was the pawlick response. In the study by Amir and Amit (1979) a separation between pawlick and escape was also observed. When they measured analgesia on the hot-plate they found no differences between pawlick latencies but they did find significant differences in escape latencies. On the basis of these experiments and the evidence from human studies (Jaffe & Martin, 1976; Melzack, 1973) both Amir and Amit (1979) and Frederickson, Burgus & Edwards (1977) suggested

that the pawlick and escape responses were representative of separate aspects of the nociceptive system. Pawlicking in response to a hot-plate is considered to reflect the perception of pain (superficial nociception) because the narcotic antagonist naloxone does not modify the response and also because the effects of morphine are less sensitive to the pawlick response than to the escape response (deep or pathalogical pain) (Jacob & Ramabadran, 1981).

The pawlick response may be akin to the tailflick response (elicited by noxious radiant heat to the tail) in that the tailflick response is thought to be spinally mediated. The tailflick response can be initiated even in the absense of segments of the spinal cord which interupt descending transmission from higher centers (Irwin et al, 1951). The escape response would need a more integrated response and is probably mediated at the brain stem or higher centers.(Irwin et al 1951).

Collectively, these data and observations strongly support both the fact that different intensities and durations of temeratures can induce differences in latencies of behavior and the suggestion that these differences reflect various aspects of the nociceptive system.

STRESS AND BEHAVIOR: ANALGESIA

The hot-plate technique has not only been used to assess analgesic drugs, but has also been used in the assessment of the phenomenon of stress-induced analgesia (SIA). SIA is manifested when animals are subjected to noxious stimuli and subsequently show signs of increased pain threshold. Some of the manipulations which have been shown to induce a transient analgesia are foot shock (Madden et al, 1977; Hayes et al, 1978) immobilization/restraint (Amir & Amit, 1978, 1979) centrifugal rotation (Hayes et al, 1978) cold water swims (Bodnar et al, 1978), warm water swims (Christie, Chester & Bird, 1981) and conditioned fear (Rosencrans & Chance, 1976).

That the HPA is involved in SIA can be ascertained from the following observations. Injections of pituitary peptides such as B-endorphin have raised pain thresholds (Loh et al, 1976). Hypophysectomy eliminated restraint-induced analgesia (Amir & Amit, 1979) and attenuated swim stress-induced analgesia (Bodnar et al., 1979). Hypophysectomy was also able to abolish tail shock (Maclennan et al., 1982) and footshock-induced analgesia (Guillemin et al., 1977). Since hypophysectomy deprives the organism of its major scource of endogenous peptides, it seemed reasonable at the time to attribute this form of analgesia to these peptides. More recent research has implicated not a pituitary peptide per

se, but the adrenal steroid, corticosterone, in the mediation of SIA.

Bodnar et al. (1979) stressed rats by forcing them to swim in water for 3.5 minutes at various temperatures. Analgesia was evident at 2, 8, and 15 C in normal rats. In hypophysectomized rats no analgesia could be detected after any water temperature. However, of particular interest was the observation that hypophysectomized animals that were given corticosterone supplements did show analgesic reactions in response to swims of 2, 8, 15, and 21°C (but not 28 and 35°C). Corticoserone not only reinstated analgesia in hypophysectomized rats but in fact enhanced the swim-induced analgesia.

In another experiment, Maclennon et al (1982) also reported that corticosterone may play a functional role in SIA. These investgators found that adrenalectomy (which abolishes corticosterone systhesis) completely blocked the elavation in pain threshold induced by inescapable shock (80, 5 second, 1 mA shock administered to the tail). When corticosterone was injected 15 minutes before stress into adrenalectomized animals, these animals again exhibited elevated pain thresholds. This finding is consistent with the suggestion that corticosterone release plays a crucial role in SIA. These findings also put into question the

notion that the pituitary peptide B-endorphin mediates SIA since levels of B-endorphin are elevated after adrenalectomy (Rossier et al., 1979).

Recently, several investigators have reported that two forms of SIA seem to exist. For example, Bodnar and his group (Bodnar et al., 1980) have consistently shown that some stressors are dependent on opiate mechanisms while others are unaffected or at best only partially affected by opiate sensitive systems. Bodnar et al. (1980) found that naloxone, even at extremely high doses, only partially reduced swim-stress induced analgesia. Lewis, Cannon & Liebeskind (1980) reported that footshock-induced analgesia and its attenuation by naloxone or dexamethasone is time dependent. They found that the analgesia produced by 30 minutes of intermittent foot shock could be blocked by both naloxone and dexamethasone; but that continuous foot shock of 3 minutes duration was not affected by these manipulations. Dexamethasone blocks the release of the pituitary peptides B-endorphin and ACTH (Guillemin et al 1977) while naloxone antagonizes the analgesic effect of both B-endorphin (Jacquet, 1978) and the hyperalgesic effect of ACTH (Bertolini, Poggioli & Ferrari, 1979). In another experiment (Grau et al., 1981) also using inescapable shock (applied by electrodes attached to the tail) it was shown

that 20 shocks (1/minute) produced analgesia which was not naloxone reversable. When the shocks in this paradigm are continued for 80 minutes, the resultant analgesia is naloxone reversable.

The results of these two studies (Lewis, Cannon & Liebeskind, 1980; Grau et al., 1981) which report differences in the reversability of SIA as a function of duration of stress can also be interpreted in terms of intensity. It is reasonable to assume that the longer the stress is continued the greater its intensity. Once again it seems that when either the intensity or the duration of a stressor are varied there are differences in subsequent behavior.

STRESS AND BEHAVIOR: LOCOMOTOR ACTIVITY

The activity levels of animals placed in novel environments (open field) is thought to reflect emotionality and stress (Denenberg, 1969). The procedure is simple yet ethologically valid (Archer, 1973; Walsh & Cummins, 1976) and the HPA has been implicated in the mediation of locomotor activity in open field experiments (Katz,1979). Hypophysectomized animals that are exposed to stress exhibit reduced activity levels relative to control animals (Amir & Amit, 1979) indicating that some pituitary factor may play a

role in the expression of stress-induced activity. The administration of ACTH effects activity in a dose related manner (Amir et al, 1980) as does the administration of endorphins and enkephalins (Browne & Segal, 1980a, 1980b). In addition to the studies that have directly administered pituitary peptides, other studies have shown that ACTH and the endorphins are released from the pituitary during stressful procedures and are taken up by the systemic circulation (Guillemin et al., 1977). In view of the demonstrations that HPA manipulations can affect locomotor activity in a dose dependent fashion, the open field paradigm along with stressful procedures can be used as a sensitive measure of HPA involvement.

In summary, the various observations and demonstrations expounded upon above lead to the following suggestions:
(1) Stress or aversive stimulation may not neccessarily have detrimental consequences, they may vary along an intensity dimension. The implication is that stress may have both negative and positive features. Experimentation may lead to the generation of U-shaped curves depicting the interaction between stress and behavior.
(2) The HPA is intimately involved in the organism's reaction to stress, both along behavioral and biochemical

dimensions.

(3) An integral part of the HPA, that of corticosterone release, can in some aversive paradigms be a useful indicator of stress intensity and of the mobilization of the axis.

(4) Different temperatures used to induce hot-plate stress may be used to induce different behavioral responses to both pharmacological and aversive stimulation.

(5) Measures of activity can be used to assess the reaction to stress and the concomitant changes in the HPA.

These observations led to the following studies. They were conducted for the purpose of answering a number of related questions. First, can hot-plate stress induce different levels of activity in an a open field and is there a relationship between the stress intensity and activity? Secondly, does corticosterone reflect the differences in activity and thirdly, do manipulations of the HPA through pharmacological means affect the stress induced activation? In addition, the results may help shed more light on the phenomenon of SIA.

Experiment 1

The temperature of the hot-plate has been associated with changing the latencies of the onset of different behaviors. These changes are found when measuring analgesic responses. The first experiment used four different levels of hot-plate stress in an attempt to determine if stress induced activation follows a predictable pattern alluded to by some researchers (Hebb, 1955; Malmo, 1959; Selye, 1974).

We observed in a preliminary study that temperatures above 57°C tended to cause observable physical damage to the paws of some of the rats. Therefore 57°C was chosen as the highest temperature and decrements of 5°C were used to make up the other two heat stress groups (i.e. 52 and 47°C). The control group (21°) was placed on a plate that remained at room temperature throughout the experiment.

Method

Subjects. The subjects were 32 male Wistar rats (Canadian Breeding Farms and Laboratories Ltd., Que.) weighing 200-300 gms. Animals were received ten days before experimentation began and were handled each day. They were allowed free access to food (Purina Lab Chow) and tap water and were kept in individual steel cages under standard

laboratory conditions (lights on: 8:00 A.M. to 8:00 P.M.). All experiments were conducted between 10:00 A.M. and 1:00 P.M.

Apparatus. A Plexiglas box was constructed to hold the distilled water that heated the hot plate; and a circulating water pump and heater (Haake, model E2) was attached to one side of this box. An aluminum plate was cut to fit snugly on top of the plexiglass box and served as the actual hot-plate. A round plexiglass cylinder was placed on the hot-plate (25 cm.dia., 30 cm. high) so that the rat could not escape. The control hot-plate (21°C) was nearly identical but had no heating or pumping apparatus.

The open field chambers were constructed of wood and were painted with black enamel. Four photocells were strategically embedded in the chamber walls dividing each chamber into nine equal squares. Each photocell was connected to an electric monitoring apparatus and yielded a single count each time the rat crossed a beam. Chambers were wiped clean after each test.

Procedure. The hot-plates were heated to specific temperatures (21, 47, 52, and 57°C) before animals were brought to the test room containing the hot-plates. They were carried in a box which consisted of eight separate compartments and were left undisturbed for 30 minutes. At

the end of the 30 minute period each rat was separately placed on a hot-plate for 30 seconds. The top of the plexiglass tube was covered with styrofoam allowing no escape. At the 30 second mark animals were picked up by hand and brought to the open field room (approximately 5 feet down the hall) and placed in individual open fields. Activity was recorded for two hours and sampled every 30 minutes.

Results

Activity levels of the four different temperature groups are depicted in Figure 1. Both the first 30 minutes and the total of 120 minutes of open field activity are shown so that comparisons could be made between this experiment and those that followed. (This was done because the peak corticosterone response occurs at approximately 30 minutes after stimulation). A one-way ANOVA revealed a significant group effect, $F(3,28) = 151.72$, $p<0.0001$. Post hoc Tukey tests revealed that all three experimental groups were significantly different from the control temperature group (21^{O}) ($p<0.05$). A trend analysis revealed a significant quadratic trend, $F(1,28)= 302.16$, $p<0.00001$, which accounted for 66% of the variance (Kirk, 1968). The trend analysis also revealed a significant linear trend,

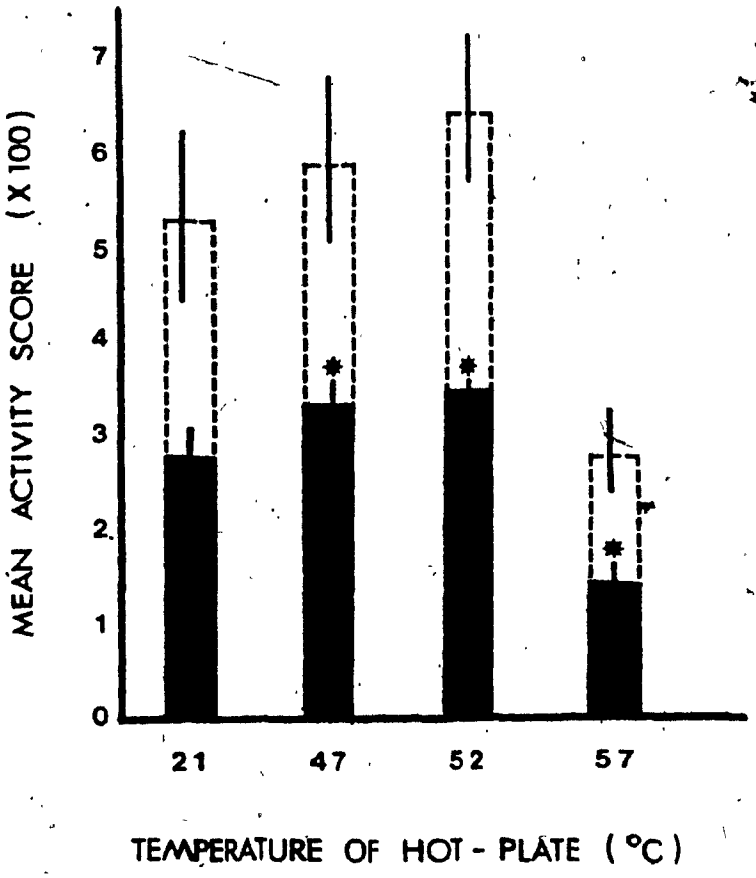

Figure 1. Mean (±SEM) activity counts after acute hot-plate stress. Solid bars indicate activity levels after 30 min. in open fields. Dashed bars indicate levels after 120 min. of the same group. (n=8) (*) represents significant difference from controls (°21) of at least ($p<0.05$).

$F(1,28)=122.31$, $p<0.005$, which can account for 26% of the data.

Discussion

Different intensities of the hot-plate induced levels of activity which closely resemble an inverted U-shaped curve. Some authors (Hebb, 1955; Malmo, 1959; Selye, 1974) have predicted such a relationship between arousal and different forms of behavioral activity. The present paradigm may therefore prove useful in further investigations of the activating effects of "mild" stress and the debilitating effects of "severe" stress.

Experiment 2

In Experiment 1 it was found that different temperatures could elicit varying levels of activity. These activity levels fluctuated as a function of hot-plate temperature. Experiment 2 was designed to explore the possibility that the HPA was also affected by hot-plate manipulations. An assay for plasma corticosterone was utilized to answer this question in the hope that it may yield an index of the intensity of the stressor and magnitude of HPA involvement.

Method

Subjects. As in the previous experiment, 32 male Wistar rats were used. Housing and handling conditions were as previously described in Experiment 1.

Procedure. The procedure was identical to that described in Experiment 1 except that after 30 minutes in the open field the rats were quickly removed, decapitated and trunk blood was collected in heparinized tubes and frozen to be assayed at a later date. Plasma corticosterone levels were determined fluorometrically by the method of Glick, Von redlick and Levine (1964).

Results

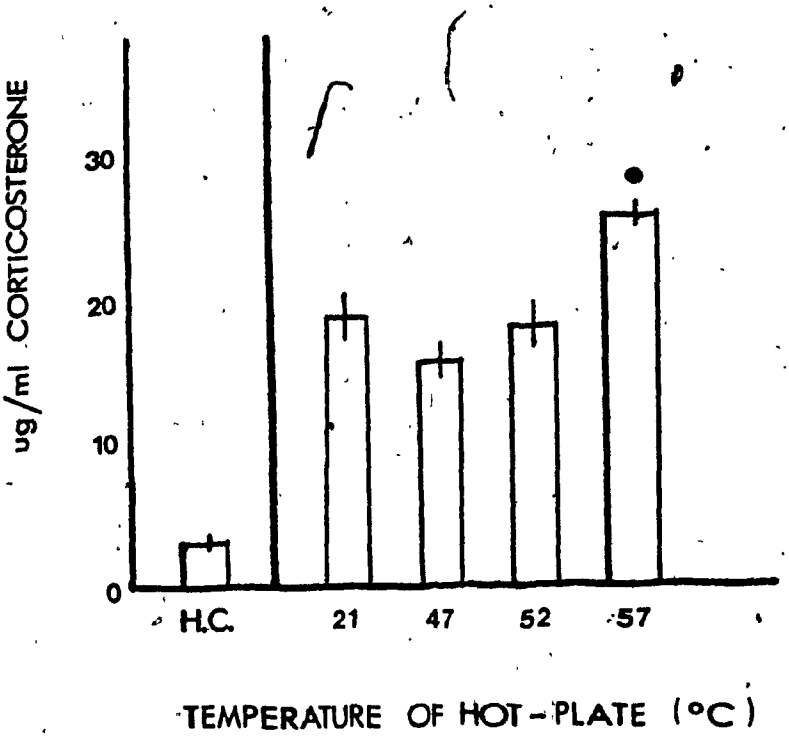

Figure 2. Mean (±SEM) levels of corticosterone after acute hot-plate stress and 30 min. in open fields. (H.C. indicates home cage controls). (●) represents significant differences from controls (21^{o}) of at least ($p<0.05$).

Figure 2 illustrates the data obtained from the corticosterone assay. A one-way ANOVA indicated that there was a significant effect of temperature, $F(3,28) = 6.39$, $p<0.005$. Also depicted in Figure 2 are the corticosterone levels of the home cage control subjects (H.C.) which were part of the same batch delivered by the breeders (n=5). They were simply removed from their cages on test day, immediately decapitated, and their trunk blood was assayed at the same time as the other groups. They are presented for comparison purposes and were not included in the statistical analysis. Further analysis with post hoc Tukey tests revealed that the 57°C group differed significantly from every other group ($p<0.05$). Trend analysis revealed a significant quadratic trend, $F(1,28)= 11.48$, $p<0.005$, which accounts for 55% of the variance. There was also a significant linear trend, $F(1,28)= 9.40$, $p<0.005$, which can account for 44% of the data.

Discussion

As seen in Experiment 1, when the behavioral activation was measured, the levels of corticosterone measured in Experiment 2 also fluctuated in conjunction with the different levels of heat stress. This finding seemed to support the notion that the HPA and specifically

corticosterone are activated by stress.

The results of this experiment are also in agreement with the results of Friedman et al. (1967), Hennessy and Levine, (1978), and File, (1982) in that there was a graded release of corticosterone as a result of different intensities of stress. Since there were no significant differences between groups after post hoc analysis between the midrange intensities, the results also are in agreement with Smith (1972) in that corticosterone discriminates between maximum and minimum of intensity. The 47°C group did not differ statistically from either the 21 or 52°C groups.

Having established that plasma corticosterone was differentially affected by temperature manipulations, further analysis of the HPA was warranted. The previous experiment did not indicate whether the changes in the HPA mediated the activity or was only concomitant to it.

Experiment 3

One way of assessing the contribution of the HPA to a particular behavioral event is the use of pharmacological blockade of one of the organs in the axis. Blockade of the release of pituitary hormones can be affected by the administration of the drug dexamethasone (DEX), a synthetic corticosteroid. A number of reports have shown that DEX can block or greatly attenuate the release of both ACTH and B-endorphin into the blood stream under certain circumstances. Under conditions of foot shock and/or restraint stress, the release of these peptides is blocked by prior DEX administration (Guillemin, 1977; Rossier et al, 1979, 1980).

Since we decided to block the release of ACTH and B-endorphin with DEX the exogenous administration of ACTH could further elaborate the role of ACTH. Reinstatement of a behavior previously blocked by DEX through ACTH administration would implicate ACTH in the mediation of the observed behavior.

Method

Subjects. Sixty-four male Wistar rats were housed and handled in the same manner as that described in Experiment 1.

Drugs. Dexamethasone sodium phosphate (Merck Sharp & Dohme) and ACTH (lyphilyzed porcine ACTH (1-39) Armour Pharmaceuticals) were dissolved in 0.09 % saline solution.

Procedure. Once again the same procedure used in Experiment 1 was used except for the following changes. DEX was administered (i.p.) to the animals (0.04 mg/kg/ml) 25 hrs. and (0.02 mg/kg/ml) 1 hr. before placement on the hot-plate. Some animals also received ACTH (20 IU/kg/ml, s.c.) 10 minutes before placement on the hot-plate. The various drug and temperature combinations can be seen in Table 1. Locomotor activity was measured for 30 minutes. Only two levels of hot-plate temperature were used because they are the extreme ends of the temperature range of the previous studies.

Results

The various drug and temperature combinations and their resultant activity levels are represented in Figure 3. A three-way ANOVA, (ACTH x DEX x Temperature) was performed and revealed the following results: There was a significant main effect of temperature, $F(1,56) = 42.16$, $p<0.00001$, ACTH, $F(1,56) = 4.18$, $p<0.04$, and a significant ACTH x DEX interaction, $F(1,56) = 8.04$, $p<0.006$. There were no other significant interactions. Post hoc analysis using Tukey

Table 1: Time of drug administration for the various groups.

GROUP DESIGNATION	DRUG ADMINISTERED 25 hrs BEFORE	DRUG ADMINISTERED 1 hr BEFORE	TEMPERATURE OF HOT-PLATE
SS21	saline	saline	21°C
SS57	saline	saline	57°C
SA21	saline	ACTH	21°C
SA57	saline	ACTH	57°C
DS21	DEX	saline	21°C
DS57	DEX	saline	57°C
DA21	DEX	ACTH	21°C
DA57	DEX	ACTH	57°C

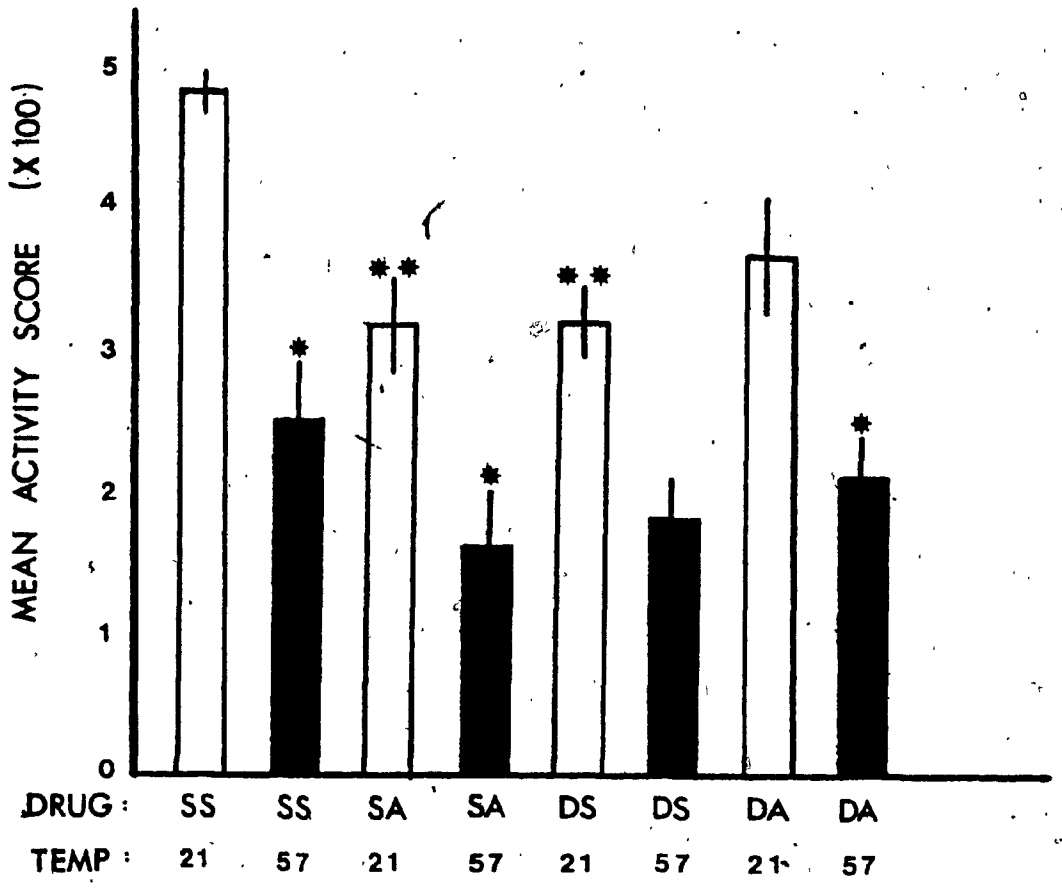

Figure 3. Mean (±SEM) activity counts after acute hot-plate stress of either (21^{O}) or (52^{O}). Drugs: Saline (S), ACTH (A), Dexamethasone (D). The first letter indicates which drug was given 25 hrs. and 1 hrs. before test. The second letter indicates which drug was given 10 min. before. (*) indicates significant differences of at least ($p<0.05$) from its (21^{O}) control. (**) indicates significant differences ($p<0.05$) from (SS21).

tests indicated significant differences between groups ($p<0.05$). These can be seen in Figure 3.

Discussion

As in the two previous experiments the heat of the hot plate generated significant differences in activity levels. The significant effect of temperature replicates some of the effects of Experiment 1.

General Discussion

Experiment 1 demonstrated that four intensity levels of the same stressor could produce differential spontaneous locomotor behavior within an open field apparatus. In Experiment 2, measurement of plasma corticosterone levels under the same conditions as Experiment 1 revealed that corticosterone levels also fluctuate in response to stress intensity. Finally, in Experiment 3, using pharmacological manipulations of the HPA it was found that ACTH administration inhibits the expression of activity following the stressors regardless of intensity.

It is of particular interest that the data generated in the first study resembled an inverted U-shaped function of stressor intensity and subsequent activity. Several decades ago it was postulated that a similar function would be present for the interaction between arousal and behavior (Hebb, 1955; Malmo, 1959; Selye, 1974). Though the present experiments did not directly address the question of whether stress is detrimental or not; they may indirectly clarify some findings from learning experiments involving ACTH. For example, Gold and Van Buskirk (1976) found that the effects of ACTH on memory are dose related. Exogenous administration of various doses of ACTH (0.03, 0.3, 3.0 IU/animal) in a

passive avoidance paradigm resulted in the generation of an inverted U-shaped curve on memory performance. The lower doses enhanced memory while the higher doses impaired it. Also the effects of shortening the chain of amino acids contained in the ACTH sequence in conjunction with increasing molecular weight of the ACTH compound resulted in an inverted U-shaped curve when attentional processes were studied (Sandman, Beckwith & Kastin, 1980). Similar results were obtained by Sands and Wright (1979) in their study of memory processes and ACTH. Since the corticosterone response reflects a concomitant release of ACTH, use of the present paradigm which effects a differential release of corticosterone, may be used as part of learning experiments, in place of (or in addition to), pharmacological manipulation. Instead of placing animals into open fields after stress, placement in learning situations where their responses can be measured may add credence to the pharmacological studies.

The effect of ACTH in the present paradigm is not surprising since it is in line with previous studies. ACTH, given before placement in the open field can evoke biphasic responses. Low doses produce excitation, while high doses are suppressive (Amir et al., 1980). The dose used in the present experiment (20 IU/kg/ml) is the same as the dose

employed in the previous experiment where it produced a suppression in activity. The 21 C hot-plate (which represents room temperature) had no measurable effect over that which was previously observed after open field alone. That is, ACTH administration again reduced behavioral activity. The addition of ACTH to heat stress (57°C) in the pesent experiment did not further reduce activity.

Examination of the data of Experiment 3 suggests that ACTH alone was able to supress behavior; that DEX did not significantly supress behavior and that the combination of ACTH and DEX attenuated the ACTH induced depression. The reasoning behind the use of the combination of ACTH and DEX was that there was evidence to suggest that the dose regimen used in the present paradigm would effectively reduce the release of ACTH. Since ACTH reduced activity and DEX attenuated this reduced activity, it may be that exogenous ACTH in combination with the endogenous levels reduces activity. However, when DEX was administered it reduced the endogenous levels of ACTH. Therefore the exogenous levels would not be enough to cause the same degree of suppression as does the combination of exogenous and endogenous ACTH. These speculations remain to be tested. Assays to determine the magnitude of DEX suppresion are now being carried out in order to resolve these questions.

The three milder stress intensities raise the activity level of the animal in a manner analagous to a dose response curve. The drastic change in activity seen with the 57°C group would suggest that perhaps a pain threshold had been passed. It is possible that 57°C is sufficiently painful, making locomotion aversive to the rat. There is in fact some evidence supporting this contention. Hardy et al (1957) and Cunningham, Benson and Hardy (1957) have done some experiments that indicate that a pain threshold may have been surpassed. They have observed that the "reflex twitch" and "flight reaction" occured at different skin temperatures. Thermal radiation was used as the stimulus to evoke these reactions. In contrast to studies of antinociception where the animal is subjected to a stimulus of known temperature, these investigators actually studied the skin temperature at the spot of stimulation. (The location on the skin where the radiant heat was focused). The reflex twitch (i.e. tail flick) is initiated when the temperature of the skin is between 45-46°C and the flight reaction (escape) occurs between 51-52°C. In the present studies, temperatures of up to 52°C induced increases activity while 57°C reduced activity. This may be a reflection of different intensity of heat on pain sensitivity. As Jacob and Ramabadran (1981) have surmised,

the lower temperatures may only have caused superficial pain while 57°C induced a deep pathological pain which was then reflected in activity.

The behavior of the rat on and off the hot-plate is considerably different and warrants discussion. One would assume that painful paws would elicit vigourous pawlicking behavior, however observations of the animals in a near identical situation to that reported here indicates that this was not the case (Galina, Sutherland & Amit, note 1). In that study, while the stressors were exactly the same, five behaviors were recorded including pawlicking. Subsequently pawlicking had to be dropped from the data analysis because the amount of pawlicking from any of the temperature groups was negligible. This is in contrast to the behavior while on the hot-plate where vigorous paw-licking is seen at 57°C. Also when escape attempts were analysed (using simple dichotomous analysis, i.e., escape or no escape) none of the 21 or 47°C group made any attempt to escape; the 52°C group was inconsistent (3 yes, 5 no), whereas each rat in group 57°C attempted many vigourous escape attempts during the exposure to the hot-plate. This data lends support to the notion that the different temperatures induce different levels of pain sensitivity. Also, the fact that there was no negligible pawlicking

behavior during the observation period (immediatly after stress) may indicate that the pain induced is of a transient nature.

In the two studies which have shown that corticosterone plays a role in SIA (Bodnar et al, 1978; Maclennon et al 1982) only one dose of corticosterone was employed thus making it impossible to ascertain a dose response relationship. Since the corticosterone response parameters of the present experiment are known, experiments are now under way to find out if heat stress also induces analgesia, and if so, is the magnitude of analgesia related to the magnitude of corticosterone release.

Perhaps a clue to the nature of the observed suppression of behavior may be found in those studies suggesting that B-endorphin can induce immobility (Browne, Derrington & Segal, 1979). The decrease in activity seen in the present experiment after the stressors may be due to the release of B-endorphin from the pituitary. However, two observations argue against this suggestion. First, a reversal of the depression was not found after DEX treatment which should have blocked the stress induced release of B-endorphin (Guillemin et al., 1977). Secondly, our subjective observations revealed neither muscular rigidity nor loss of righting reflex which are usually associated

with B-endorphin related immobility (Browne, Derrington & Segal, 1979). (However, in support of the above suggestion it is worth noting that B-endorphin has been found in the brain independent of the pituitary (Watson, Akil & Walker, 1980). It is premature to exclude the possibility that brain opioids may induce immobility). An investigation into the possible effects of opioid antagonists in the present paradigm should be carried out to determine opiate participation.

A drop in motor performance in stressful paradigms (such as inescapable electric shock) in avoidance studies have been correlated with lower levels of the neurotransmitter norepinephrine (NE) (Anisman et al., 1979). These studies use electric shock to induce aversion and in one study using various durations of electric shock a correlation between NE and activity was found (Weiss et al, 1980). NE levels in the brainstem and hypothalamus, after stress, correlated with the reduction in activity after the various intensities of stress. As mentioned earlier (see Introduction) NE may exert a tonic inhibitory effect on CRF release. It is interesting to speculate, that within the present paradigm the intensity of heat stress differentially affected NE which would then affect CRF-ACTH and finally corticosterone release. The data reported here are in line

with that of Weiss et al. (1980).

Certainly, in terms of the psychopharmacology of analgesics, the results suggest that some re-evaluation of the results obtained from the hot-plate technique may be neccessary. In many studies that utilize the hot-plate animals are retested every 15 or 30 minutes after the initial trial. The results demonstrated in the preceeding experiments show that by the second or third time on the hot-plate the animal's physiology has drastically changed. The 57°C hot-plate causes a serious decrement in motor movement after 30 seconds of exposure which is still evident two hours later. Since some categories of motor movement is the measure of analgesia it may be that at that point (second or third trial) the supposed analgesic agent may be having an effect on the motor system and not the nociceptive system. It would seem, however, that the measure itself, that the animal has to make a movement which the experimenter then evaluates, is itself inseparable from the technique.

Attempts to reconcile the results from the present studies with similar studies reported in the literature present numerous anomalies which exist concerning stress induced activation. In most available reports locomotor activity was only measured when it became apparent that

motor debilitation may underly stress-induced analgesia. For example, exposure to cold water swims (3.5 minutes at 2°C) increased subsequent activity levels of rats (Bodnar et al, 1979). These alterations were not affected by hypophysectomy or corticosterone supplements. Forced swimming in warm water (25°C for 15 minutes) can result in immobility (Porsolt et al., 1978). Initially while in the water the rats make vigorous movements and then they exhibit increasing periods of immobility. Subdermal formalin injection in combination with hypophysectomy was found to reduce behavioral activity (Amir & Amit, 1979). When immobilization stress is applied before formalin, hypophysectomized animals increased thier activity relative to control animals which received injection alone. Immobilization for 30 minutes did not affect locomotion (Blair et al, 1982). In experiments which utilized foot shock as a stressor it was reported that animals initially exhibited a transient increase in activity which was followed by a reduction in activity (Anisman, De Catanzaro & Remington, 1978).

At present it is difficult to reconcile all the above data to form a unitary hypothesis to describe and explain all known phenomena concerning stress induced activation and its neural and hormonal mechanisms. Many of the discrepancies which exist in the literature may be explained

in terms of methodological differences in the execution of the respective studies. These differences include such factors as the time relationship between onset of stress and the measurement of activity. The effects of different environments on activation, and also the type of measurement used to quantify activity. Perhaps of greater importance is the notion that on the basis of the data obtained in these studies, the concept of stress does not appear to be alinear unified concept which follows an established psychophysical relationship between the stressor and behavior. Instead it would seem that stress may interact with certain behaviors in complex multifaceted, both facilitory and inhibitory, fashion. To summarize, we have found that different intensity of stress evokes varying effects on behavior and biochemistry of the HPA. The HPA response is not an all or none phenomenon, but is sensitive to gradient of stress intensity.

Alterations in HPA functioning have been implicated in human studies of stress and pathology. Stress provoking situations faced by humans have resulted in concomitant elevations in cortisol (Francis, 1979; Mason, 1968) and other studies show impaired HPA functioning which may lead to or be correlated with depression and other psychopathologies (Shlesser et al, 1980; Von Zerssen &

Doerr, 1980). Recently, Anisman and Zacharko, (1982) have speculated that stress may have a precipitative influence on depressive illness. The studies which were conducted here can be extended to chronic stress manipulations and may aid in understanding the variable human response to stress.

The studies reported in the present thesis were designed to help gain information in a number of related areas. We have found that locomotor activity, though encompassing a wide range of gross movement, was able to differentiate between different intesities of stress. Secondly we have ascertained that the reaction of the HPA (corticosterone) to the stressors is rate sensitive. And thirdly, we have found that the HPA is only partly involved in the modulation of the activity after stress. Further research must more closely examine these findings in order to put them into a wider context within the behavioral study of stress. "Stress" is ubiquitous in the present society and it is through well defined experimentation, such as has been begun here, that stress will be understood and finally controlled.

Reference Notes

1. Hunt, A., Switzman, L. & Amit, Z. Unpublished observations.

2. Galina, Z.H., Sutherland, C.J. & Amit, Z. Unpublished observations.

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APPENDIX 1

Experiment 1: Activity level of the various groups after hot-plate stress and 30 min. of open fields.

21	47	52	57
213	225	359	202
163	248	349	176
389	247	348	135
320	285	321	124
255	341	306	121
396	375	366	113
247	381	371	85
200	496	356	141

APPENDIX 2

Experiment 2: Corticosterone levels after hot-plate stress and 30 min. in open field.

21	47	52	57
24.4	16.9	16.9	23.7
22.0	18.8	10.3	30.0
23.0	21.6	20.4	28.4
23.2	5.4	22.3	28.6
21.6	14.5	15.0	26.5
7.5	16.4	20.4	22.3
19.4	16.4	20.6	23.7
14.3	16.1	22.5	22.7

APPENDIX 3

Experiment 3: Activity levels after 30 min. in open field after hot-plate stress and drug treatment.

AD21	AD57	AS21	AS57
269	160	427	68
262	351	190	322
514	141	382	176
400	186	260	81
277	173	418	74
265	347	200	330
515	150	380	170
421	192	271	90

SD21	SD57	SS21	SS57
216	127	518	222
295	156	432	382
394	129	477	344
367	289	457	55
218	134	513	225
300	149	439	376
390	126	480	341
364	277	451	61