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Effects of Heat-Stress on Behavior and the Pituitary Adrenal Axis in Rats[1]

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GALINA, Z. H., C. J. SUTHERLAND AND Z. AMIT. *Effects of heat-stress on behavior and the pituitary-adrenal axis in rats.* PHARMACOL BIOCHEM BEHAV **19**(2)251–256, 1983.—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 (21, 47, 52°C), while the higher temperature (57°C) decreased activity. Experiment 2 assessed the pituitary-adrenal response to the different temperatures by measuring levels of plasma corticosterone 30 minutes after stress. The four levels of hot-plate temperatures induced differential levels of corticosterone which may best be described as an inverted U-shaped function, with only the extreme temperature (57°) inducing a significant elevation in levels of the steroid. Experiment 3 further manipulated the pituitary adrenal axis by administering dexamethasone 25 hr and 1 hr before stress and ACTH 15 min 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 facilatory or inhibitory results.

Heat-stress Activity Corticosterone ACTH Dexamethasone

THE PRESENT investigators have been involved with the study of stress-induced antinociception (SIA) and pituitary-adrenal involvement in this phenomenon for a number of years [2, 3, 4]. One method of pain assessment often used in these studies consists of a hot-plate heated to specific temperatures on which rodents are placed and their behavior recorded. During the course of experimentation we noticed that variations in the temperature of the hot-plate would not only produce differences in pain responses but would also change other categories of behavior (i.e., locomotion).

Since the introduction of the hot-plate technique by Woolf and Macdonald [46] and the modification by Eddy and his coworkers [15,16] many laboratories have used the procedure to test for the analgesic properties of drugs.

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, it has been found that the sensitivity to analgesic drugs to the pawlick response differed according to the temperature of the plate [38]. Furthermore, in studies of narcotic antagonists it was reported that at 55°C there was a separation between the two prominent behavioral measures; pawlick and jump-off (escape) [29]. At higher temperatures detection of differences between the latencies of the behaviors becomes more difficult. In addition, it has been stated that naloxone was able to modify licking responses when the hot-plate was at 50°C but not at temperatures of 55 or even 80°C [28]. It is interesting, however, to note that these investigators were able to detect differences in jump-off latencies at the higher temperatures.

Changes in corticosterone levels can be detected after a wide variety of aversive events such as: exposure to novel environments [34, 35, 39, 40], predictable vs. unpredictable shock [27], rapid but not gradual blood loss [19], and avoidance paradigms such as conditioned taste aversion [44] to name only a few. Brain levels of corticosterone after novelty stress and pharmacologically induced stress have been found to increase and the magnitude of the increase corresponds to the magnitude of the plasma increase [30].

Corticosterone can also be considered a reliable indicator of the initial intensity of an aversive or noxious event. Studies have been conducted wherein corticosterone levels have been measured after various "degrees" of novelty stress, electric shock [18] and environmental change [26], and the results indicated that there were significant differences as a function of intensity and duration.

In addition to it's usefulness as an indicator of aversion, the corticosterone response can also be used as an indicator of ACTH release. Levels of corticosterone are raised after ACTH is exogenously administered [37] or after noxious stimuli when ACTH is presumed to be released [34].

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Recently, several investigators have reported that at least two forms of SIA seem to exist. For example, Bodnar and his group [10] consistently showed that some stressors are dependent on opiate mechanisms while other stressors are unaffected or at best only partially affected by opiate sensitive systems. Others [32] reported that footshock-induced analgesia and its attenuation by naloxone and dexamethasone was time dependent. They found that 30 min of intermittent footshock could be blocked by both naloxone and dexamethasone; but that continuous footshock of three min duration was not affected by these manipulations. In another experiment [22] also using inescapable shock (applied by electrodes attached to the tail) it was found that 20 shocks (1/min) produced analgesia which was not reversed by naloxone. When the shocks in this paradigm are continued for 80 min the resulting analgesia is naloxone reversible. The results of these two studies [22,32] which report differences in the reversability of SIA as a function of time can also be interpreted in terms of intensity. It is reasonable to assume that the longer the stress is continued the greater its intensity. It seems likely that two separate mechanisms of SIA do in fact exist.

Pituitary peptides (i.e., endorphins and enkephalins) have been shown to modify motor activity in a number of behavioral paradigms [8, 12, 45]. ACTH has also been known to affect motor activity [1, 6, 17, 36]. Hypophysectomized animals that are exposed to stress exhibit reduced activity levels relative to control animals [2] indicating that some pituitary factor plays a functional role in the expression of stress-induced activity. In addition to the studies showing locomotor activating effects, other studies have found that ACTH and the endorphins are released from the pituitary during stressful procedures and are taken up by the systemic circulation [23].

In view of some of the similarities between stress-induced analgesia and stress-induced activation we decided to employ one stressful procedure (hot-plate) and vary its intensity dimension in order to examine its effect on stress-induced activation. Using various intensities of the same stressor may reveal that activation by stressful manipulations may also be a function of activating different mechanisms as do diverse forms of SIA. Specifically we asked: First, can hot-plate stress induce different levels of activity in an open field and is there a relationship between the stress intensity and activity. Secondly, does corticosterone reflect the differences in activity and intensity and, thirdly, do manipulations of the hypothalamic-pituitary-adrenal (HPA) system through pharmacological means affect the stress induced activation.

EXPERIMENT 1

The temperature of the hot-plate has been associated with the onset of different behaviors. The first experiment used four different levels of hot-plate stress in an attempt to determine if stress-induced activation follows a predictable pattern.

METHOD

Subjects

The subjects were 32 male Wistar rats (Canadian Breeding Farms and Laboratories Ltd., Que.) weighing 200–300 g. 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.

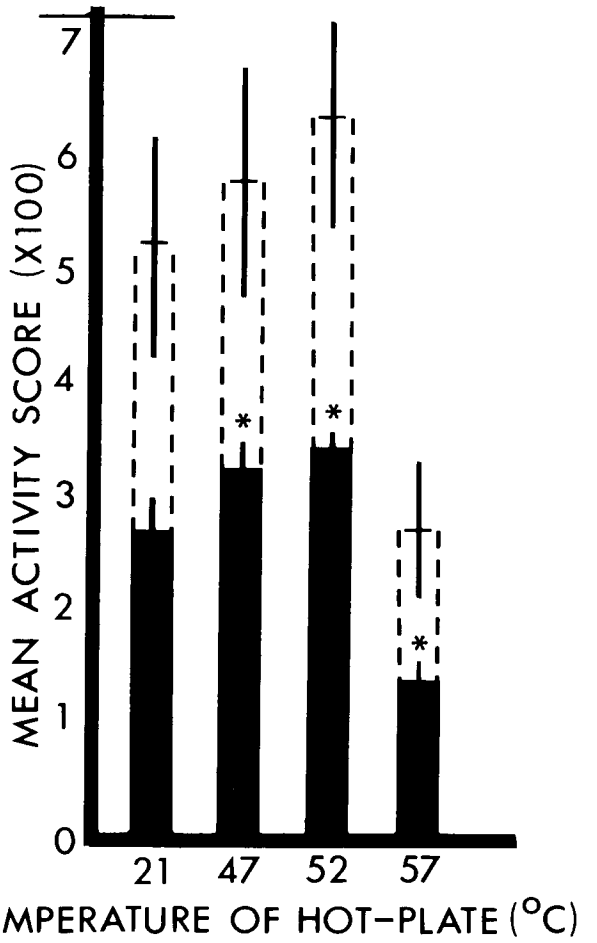

FIG. 1. Mean (±SEM) activity counts after acute hot-plate stress. Solid bars indicate activity levels after 30 min in open fields. Dashed bars indicate cumulative levels after 120 min of same group. (n=8) (*) Represents significant difference from controls (21°C) of at least ($p<0.05$).

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 Plexiglas box and served as the actual hot-plate. A round Plexiglas tube 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 (45.7×45.7×39.5) 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 electronic monitoring apparatus and would yield 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 Plexiglas 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 AND DISCUSSION

Activity levels of the four different temperature groups are depicted in Fig. 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. 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°) ($p<0.05$). A trend analysis revealed a significant quadratic trend, $F(1,28)=302.16$, $p<0.00001$, which accounted for 66% of the variance [31].

Different intensities of the hot-plate induced levels of activity which closely resemble an inverted U-shaped curve. Some authors [7,25] 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 delibilitating effects of "severe" stress.

EXPERIMENT 2

In Experiment 1 we 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 [20].

RESULTS AND DISCUSSION

Figure 2 illustrates the data obtained from the corticosterone assay. A one-way ANOVA indicated that there was a

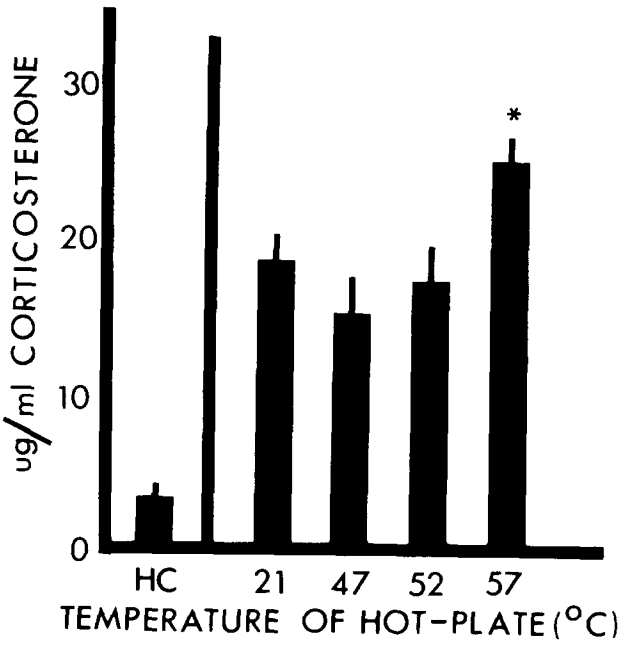

FIG.2. Mean (±SEM) levels of corticosterone after 30 min in open field. (H.C. indicates home cage controls.) (*) Represents significant difference from control (21°C) of at least ($p<0.05$).

significant effect of temperature, $F(3,28)=6.39$, $p<0.005$. Also depicted in Fig. 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 ($p<0.05$). The 47° and 57°C groups did not differ significantly from the controls. Trend analysis revealed a significant quadratic trend, $F(1,28)=11.48$, $p<0.005$, which accounts for 55% of the variance.

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.* [18], and Hennessy and Levine, [26], in that there was a graded release of corticosterone as a result of different intensities of stress.

EXPERIMENT 3

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. Dexamethasone (DEX) is known to inhibit pituitary hormonal responses to stress [23] and ACTH has been shown to influence activity in our open fields [1]. Experiment 3 was de-

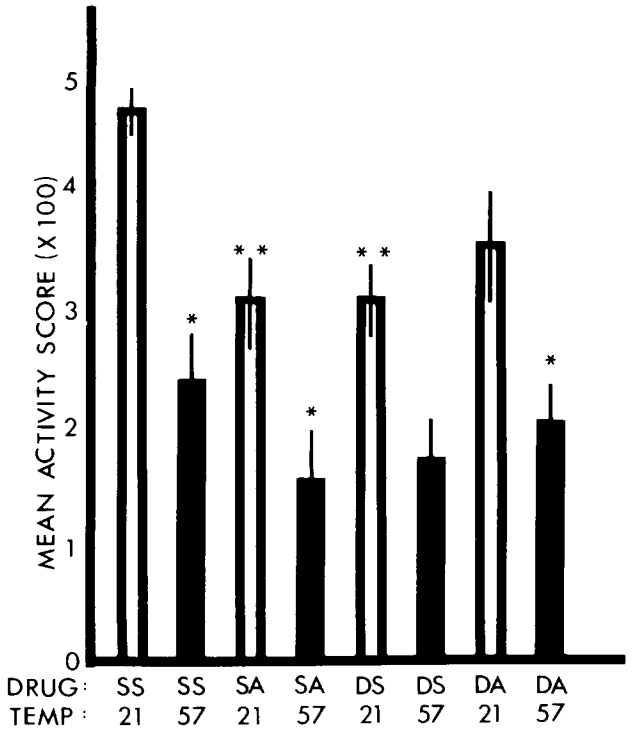

FIG.3. Mean (±SEM) activity counts after acute hot-plate stress of either (21°) or (57°C). Drugs: Saline (S), ACTH (A), Dexamethasone (D). The first letter indicates which drug was given 25 hr and 1 hr before test. The second letter indicates which drug was given 10 min before. (*) Represents significant difference of at least ($p<0.05$) from its (21°) control. (**) represents ($p<0.05$) from (SS21).

signed to examine the possible modifying effects of both DEX and ACTH on "stress-induced activation."

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 and Dohme) and ACTH (lyphilyzed porcine ACTH (1–39) Armour Pharmaceuticals) were dissolved in 0.09% saline solution.

Procedure

Once again the same protocol as described in Experiment 1 was used except for the following changes. DEX was administered (IP) to the animals (0.4 mg/kg) 25 hr and (0.02 mg/kg) 1 hr [23] before placement on the hot-plate. Some animals also received ACTH (20 IU/kg SC) 10 min before placement on the hot-plate.

RESULTS AND DISCUSSION

The various drug and temperature combinations and their resultant activity levels are represented in Fig. 3. A three-way ANOVA, (ACTH × DEX × 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 × DEX interaction, $F(1,56)=8.04$, $p<0.006$. There were no other significant interactions. Post hoc analysis using Tukey tests indicated significant differences between groups ($p<0.05$). These can be seen in Fig. 3.

The effect of ACTH in the present paradigm is not surprising since it is in line with previous studies. ACTH, given before placement in open field can evoke biphasic responses. Low doses produce excitation, while high doses are suppressive [1]. The dose used in the present experiment is the same as the dose employed in our 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 present experiment did not further reduce activity.

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 [7,25]. 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 [21] 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 [42]. Similar results were obtained by Sands and Wright [43] 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 three milder stress intensities raise the activity level of the animal in a manner analagolus 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 to the

rat making locomotion aversive. There is in fact some evidence supporting this contention. Hardy *et al.* [24] and Cunningham, Benson and Hardy [14] have done some experiments that indicate that a pain threshold may have been surpassed. They have observed that the "reflex twitch" and "flight reaction" occurred 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 in activity while 57°C reduced activity. This may be a reflection of different intensities of heat on pain sensitivity.

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 and Amit, submitted). 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 negligable. This is in contrast to the behavior while on the hot-plate where vigourous pawlicking is seen at 57°C. Also when escape attempts were analysed (using simple dichotimous analysis, i.e., yes 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, since there was no negligable pawlicking behavior during observation after stress may indicate that the pain induced is of a transient nature.

There have been two studies which have shown that corticosterone plays a role in SIA [11,38], however, only one dose of corticosterone was employed thus making it impossible to ascertain if a dose response relationship existed. 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 β-endorphin can induce immobility [13]. The decrease in activity seen in the present experiment after the stressors may be due to the release of β-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 β-endorphin [23]. Secondly, our subjective observations revealed neither muscular rigidity nor loss of righting reflex which are usually associated with β-endorphin related immobility [13]. (However, in support of the above suggestion it is worth noting that β-endorphin has been found in the brain independent of the pituitary. 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 are being carried out to determine opiate participation.

Attempts to compare the results from the present studies with similar studies reported in the liturature 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 or learning after stress. For example, exposure to cold water swims (3.5 min at 2°C) increased subsequent activity levels of rats [11]. These alterations were not affected by hypophysectomy or corticosterone supplements. Forced swimming in warm water (25°C for 15 min) can result in immobility [41]. Initially while in the water the rats make vigourous movements and then they exhibit increasing periods of immobility. Subdermal formalin injection in combination with hypophysectomy was found to reduce behavioral activity [2]. When immobilization stress is applied before formalin, hypophysectomized animals increased their activity relative to control animals which received injection alone. Immobilization for 30 min did not affect locomotion [9]. In experiments which utilized footshock as a stressor it was reported that animals initially exhibit a transient increase in activity which was followed by a reduction in activity [5].

At present it is difficult to reconcile all of the above data to form a unitary hypothesis to describe and explain all known phenomenon concerning stress-induced activation and its neural and hormonal mechanisms. Many of the discrepancies which exist in the liturature 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 a linear unified concept which follows an established psychophysical relationship between stressor and behavior. Instead it would seem that stress interacts with certain behaviors in complex multifaceted, both facilatory 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 intensity.

REFERENCES

1. Amir, S., Z. H. Galina, R. Blair, Z. W. Brown and Z. Amit. Opiate receptors may mediate the suppressive but not the excitatory action of ACTH on motor activity. *Eur J Pharmacol* **66:** 307–313, 1980.
2. Amir, S. and Z. Amit. The pituitary gland mediates acute and chronic pain responsiveness in stressed and non-stressed rats. *Life Sci* **24:** 439–448, 1979.
3. Amir, S. and Z. Amit. Endogenous opiate ligands may mediate stress-induced changes in the affective properties of pain related behavior in rats. *Life Sci* **23:** 1143–1152, 1978.
4. Amir, S. and Z. Amit. Enhanced analgesic effects of stress following chronic administration of naloxone in rats. *Eur J Pharmacol* **59:** 300–307, 1979.

5. Anisman, H., D. De Catanzaro and G. Remington. Escape performance following exposure to inescapable shock: Deficits in motor response maintainance. *J Exp Psychol (Anim Behav Proc)* **4:** 197–218, 1978.
6. Beckwith, B. E. and C. A. Sandman. Behavioral influences of the peptides ACTH and MSH: Methodological Review. *Neurosci Biobehav Rev* **2:** 311–338, 1978.
7. Berlyne, D. E. Arousal reinforcement. In: *Nebraska Symposium on Motivation*, edited by D. Levine. Lincoln. University Nebraska Press, 1967, pp. 1–110.
8. Bhargava, H. N. Effects of methionine-enkephalin and morphine on spontaneous locomotor activity: Antagonism by naloxone. *Pharmacol Biochem Behav* **9:** 161–171, 1978.
9. Blair, R., Z. H. Galina, L. J. Holmes and Z. Amit. Stress-induced analgesia: A behavioral deficit or a change in pain responsiveness? *Behav Neural Biol* **34:** 152–158, 1982.
10. Bodnar, R. J., D. D. Kelly, M. Brutus and M. Glusman. Stress-induced analgesia: Neural and hormonal determinants. *Neurosci Biobehav Rev* **4:** 87–100, 1980.
11. Bodnar, R. J., M. Glusman, M. Brutus, A. Spiaggia and D. D. Kelly. Analgesia induced by cold-water stress: Attenuation following hypophysectomy. *Physiol Behav* **23:** 23–26, 1979.
12. Browne, R. G. and D. S. Segal. Behavioral activating effects of opiates and opioid peptides. *Biol Psychiatry* **15:** 77–86, 1980.
13. Browne, R. G., D. C. Derrington and D. S. Segal. Comparison of opiate- and opiate-peptide-induced immobility. *Life Sci* **24:** 933–942, 1979.
14. Cunningham, D. J., W. M. Benson and J. D. Hardy. Modification of the thermal radiation method for assessing antinociceptive activity in rats. *J Appl Physiol* **11:** 459–464, 1957.
15. Eddy, N. B., C. F. Touchberry and J. E. Lieberman. Synthetic analgesics: 1. Methadone isomer and derivatives. *J Pharmacol Exp Ther* **98:** 121–137, 1950.
16. Eddy, N. B. and D. Leimbach. Synthetic analgesics: 2. Dithienylbutenyl- and dithienalbutylamines. *J Pharmacol Exp Ther* **107:** 385–393, 1953.
17. File, S. E. ACTH but not corticosterone impairs habituation and reduces exploration. *Pharmacol Biochem Behav* **9:** 161–166, 1978.
18. Friedman, S. B., R. Ader, L. S. Grota and T. Larson. Plasma corticosterone response to parameters of electric shock stimulation in the rat. *Psychosom Med* **29:** 323–328, 1967.
19. Gann, D. C. Parameters of the stimulus initiating the adrenocortical response to hemorhage. *Ann NY Acad Sci* **156:** 740–755, 1969.
20. Glick, D., D. Von Redlich and S. Levine. Fluorometric determinations of corticosterone and cortisol in 0.02–0.05 milliliters of plasma or submilligram samples of adrenal tissue. *Endocrinology* **74:** 653–655, 1964.
21. Gold, P. E. and R. Van Buskirk. Enhancement and impairment of memory processes with post-trial injection of adrenocorticotropic hormone. *Behav Biol* **16:** 387–400, 1976.
22. Grau, J. W., R. L. Hyson, S. F. Maier, J. Madden and J. D. Barchas. Long-term stress-induced analgesia and activation of the opiate system. *Science* **213:** 1409–1411, 1981.
23. Guillemin, R., T. Vargo, J. Rossier, S. Minick, N. Ling, C. Rivier, W. Vale and F. Bloom. β-endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* **197:** 1367–1369, 1977.
24. Hardy, J. D., A. M. Stoll, D. Cunningham, W. M. Benson and L. Greene. Responses of the rat to thermal radiation. *Am J Physiol* **189:** 1–5, 1957.
25. Hebb, D. O. Drives and the C.N.S. (Conceptual Nervous System). *Psychol Rev* **62:** 243–254, 1955.
26. Hennessy, M. B. and S. Levine. Sensitive pituitary-adrenal responsiveness to varying intensities of psychological stimuli. *Physiol Behav* **21:** 295–297, 1978.
27. Hennessy, J. W., M. G. King. T. A. McClure and S. Levine. Uncertainty as defined by the contingency between environmental events, and the adrenocortical response of the rat to electric shock. *J Comp Physiol Psychol* **91:** 1447–1460, 1977.
28. Jacob, J. J. C. and K. Ramabadran. Role of opiate receptors and endogenous ligand in nociception. *Pharmacol Ther* **14:** 177–196, 1981.
29. Janicki, P. and J. Libich. Detection of antagonist activity for narcotic analgesics in mouse hot-plate test. *Pharmacol Biochem Behav* **10:** 623–626, 1979.
30. Kakihana, R. and J. A. Moore. Regional uptake of endogenous corticosterone by rat brain following stress. *Res Commun Chem Pathol Pharmacol* **21:** 213–222, 1978.
31. Kirk, R. E. *Experimental Design: Procedures for the behavioral Sciences*. Belmont, CA: Wadsworth Publishing Co., 1968.
32. Lewis, J. W., J. T. Cannon and J. C. Liebeskind. Opioid and and non-opioid mechanisms of stress analgesia. *Science* **208:** 623–625, 1980.
33. Maclennon, A. J., R. C. Drugan, R. L. Hyson, S. F. Maier, J. Madden and J. D. Barchas. Corticosterone: A critical factor in an opioid form of stress-induced analgesia. *Science* **215:** 1530–1532, 1982.
34. Mason, J. W. A review on psychoendocrine research on the pituitary-adrenocortical system. *Psychosom Med* **30:** 576–607, 1968.
35. Mason, J. W., C. T. Harwood and N. R. Rosenthal. Influence of some environmental factors on plasma and urinary 17-hydroxycorticosteriod in the Rhesus Monkey. *Am J Physiol* **190:** 429–433, 1957.
36. Matte, A. Biphasic dissociated effects of ACTH on motor activity, aggression and emotionality in mice. *Psychoneuroendocrinology* **4:** 21–26, 1979.
37. Moncola, F., F. G. Peron and R. I. Dorfman. The fluorometric determination of corticosterone in rat adrenal tissue and plasma. Effect of administering ACTH subcutaneously. *Endocrinology* **65:** 717–728, 1959.
38. O'Callahan, J. P. and S. G. Holtzman. Quantification of the analgesic activity of narcotic analgesics by a modified hot-plate procedure. *J Pharmacol Exp Ther* **192:** 497–505, 1975.
39. Pfister, H. P. and M. G. King. Adaptation of the glucocorticosterone response to novelty. *Physiol Behav* **17:** 43–46, 1976.
40. Pfister, H. P. The glucocorticosterone response to novelty as a psychological stressor. *Physiol Behav* **23:** 649–652, 1979.
41. Porsolt, R. D., G. Anton, N. Blavet and M. Jalfre. Behavioral dispair in rats: A new model sensitive to antidepressant treatments. *Eur J Pharmacol* **47:** 379–391, 1978.
42. Sandman, B. A., B. E. Beckwith and A. J. Kastin. Are learning and attention related to the sequence of amino acids in ACTH/MSH peptides. *Peptides* **1:** 277–280, 1980.
43. Sands, S. F. and A. A. Wright. Enhancement and disruption of retention performance by ACTH in a choice task. *Behav Neural Biol* **27:** 413–422, 1979.
44. Smotherman, W. P., J. W. Hennessy and S. Levine. Plasma corticosterone levels as an index of illness induced taste aversions. *Physiol Behav* **17:** 903–908, 1976.
45. Veith, J. L., C. A. Sandman, J. M. Walker, D. H. Coy and A. J. Kastin. Systemic administration of endorphins selectively alters open field behavior of rats. *Physiol Behav* **20:** 539–542, 1978.
46. Woolfe, G. and A. D. Macdonald. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). *J Pharmacol Exp Ther* **80:** 300–307, 1944.