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The Adaptive Nature of Stress-Induced Analgesia and Pituitary-Adrenal Factors: An Examination Employing a Concurrent Behavior Approach.

Zvi Harry Galina

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
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ABSTRACT

The Adaptive Nature of Stress-Induced Analgesia and Pituitary-Adrenal Factors: An Examination Employing a Concurrent Behavior Approach.

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Concordia University, 1985

Exposure to a range of intense stimuli will raise pain thresholds. This phenomenon was termed "stress-induced analgesia" (SIA). Despite efforts to identify the physiological substrates of SIA, little is known about the possible adaptive consequences of this response. Seven experiments are reported attempting to address the issue of the adaptive nature of SIA through an examination of behaviors occurring concurrently with SIA and the role that the pituitary-adrenal axis, in particular ACTH, play in these processes. The method used to explore these processes was to initially examine locomotion under conditions that lead to SIA and then observe the effects of a reduced pain threshold in avoidance paradigms. First, the effects of a short intense heat-stress applied to the paws of rats was assessed. This temporarily reduced activity in an open field. To ascertain pituitary-adrenal involvement, adrenalectomy and adrenal-medullectomy were performed before the heat-stress but they had no effect on locomotion. Hypophysectomy, however, prevented the reduction in locomotion and this reduction could be reinstated by

administration of ACTH-(4-10). Administration of naloxone or γ-MSH-(1-12) did not effect heat stress induced inactivity. The data support the notion that a short intense stressor can release ACTH which when acting at a specific ACTH receptor, can be responsible for a short term reduction in activity. Next we reported that this heat-stress induced temorary non-opiate mediated analgesia measured in a tail flick test. Finally, in two studies, the effects of analgesia induced by various intensities of heat were studied in avoidance paradigms. Heat-stress did not affect acquisition, perhaps due to heat-stress induced analgesia. Extinction of avoidance behavior was found to be affected by various intensities of heat-stress. The effects of this stressor on avoidance suggested that stress may have adaptive consequenses leading to improved performance capabilities during stress.

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I am indebted to my family for their love and support in the pursuit of my dream. And a Special Thanks to Brenda and Elisabeth who made it all worthwhile.

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Our understanding of the physiological substrates underlying the behavioral response to stress has greatly expanded over the last few decades. That stress has enormous consequenses on behavior is widely recognized. Yet, the functional advantages of stress to the instigation of appropriate physiological and behavioral responses is only now beginning to be understood. Very often, progress can be achieved through the concurrent analysis of data from two interrelated disiplines. Therefore it was the general intent of this thesis to study, analyse, and synthesize two areas of research. The examination of stress-induced analgesia (SIA) and the role of adrenocorticotrpin (ACTH) in performance are linked by a notion, common to both, that stress is one of the major contributing factors in the interpretation of the results.

The text is divided into several major sections. In the first section the concept of stress is briefly examined and an operational definition is offered. This is followed by successive reviews of the literature, first on stress-induced analgesia, and then on the role of ACTH in learning, memory and performance. Next, a set of experiments are described that attempted to elucidate the functional relationship between stress, analgesia and performance. This effort led to the generation of another set of experiments which attempted to join the two areas of study. Finally, an attempt was made to integrate the

results of the experiments and conclusions were suggested. It was argued that stress-induced analgesia is part of the animal's repertoire of behaviors that allow the integrity of the organism to maintained.

Stress defined operationally; response organization

Though the concept of stress in the biological sciences is not new (i.e. Cannon, 1914; Selye, 1946; see Mason, 1975a), there is still a serious debate as to the exact meaning of the term "stress" (Mason, 1975a,b). The debate stems from the fact that the word is very frequently used in common parlance; its usage seems to convey a message which is readily comprehended by most people. Yet, there are many words with somewhat different meanings that are commomly used synonymously with stress and which can lead to confusion. Scientific discipline demands that more precise and unambiguous definitions be used for hypothesis testing. Therefore the terms must be defined operationally (Lyons, 1965, p. 18).

There are three basic approaches to the definition and even the study of stress (Cox, 1978; Mason, 1975,b). These approaches are based on considerations stemming from the following factors: the characteristics of stress stimuli, the response to these stimuli, and an interaction between stimuli and the response to them. I will discuss these in turn.

A stimulus oriented definition of stress states that the stimulus properties of the environment place certain demands on the organism (Cox, 1978). These demands can be recognised since they are disruptive to the organism. Within this definition stress is treated as an independent variable. It is the search to identify the characteristics of the stimuli which predict disruption which is the primary focus of this line of research. Stimulus based definitions are characterized by concern with the causes of stress and not with the symptoms or results of stress.

On the other hand, a response-based definition of stress states that the nature of the organism's response to stimuli defines the presence or absence of stress (Cox, 1978). It is the measured reaction to certain stimuli that was in retrospect assessed as stressful. The pattern of responses in reaction to a stressor are what define stress. Within this definition stress is treated as a dependent variable.

The third approach, and perhaps most useful in research on stress effects in humans and animals, is a definition that is based on the interactions between the characteristics of the stimulus, the response that is elicited, and the ability of the organism to mediate between these two factors (Derogatis,1982). Within this definition stress is treated as a dynamic interaction and the researchers task is elucidation of the relationship between

the stimulus, response and the interaction of these two (Cox, 1978).

Stress as a continuous intervening variable

The view of stress that I have taken in this thesis incorporates these three commonly held definitions of stress, and in addition takes into consideration the organization of behavioral responses when under stress. In the view taken here, the organization of a behavioral response is governed by many interrelated factors. The most important are the interactions between the internal state of the organism and environmental circumstances at the time the behavior is expressed. There is constant change in the state of both the internal and external environments of the organism, therefore this interaction must be viewed as an ongoing dynamic process. It would follow, logically, that the behavior emitted by the organism at any time throughout its life would be a product of this interaction. The focus of this thesis is the determination of how the pattern and organization of "species-typical" defensive responses are modulated by stressful events in the immediate environment. These defensive responses are seen as a product of the interaction between the internal and external environments of the animal.

Unconditioned noxious stimuli will elicit responses which may be construed as automatic. However, if the

species-typical behavior results in a response that terminates or at least attenuates the intensity of the aversive stimuli, the probability of a reoccurence of this response is increased. Thus the relationship between stimuli in both the external and internal environment and the behavior of the organism can be defined by well established "laws" of learning. Nevertheless, during certain environmental conditions, particularly those of "acute stress", the usual organisation and pattern of particular behaviors may not follow the prescriptions of the previously mentioned "lawful relationship" but instead appear to be compromised by an ensuing disorganization of the behavior.

Disorganization, however, is not always the immediate consequence of stressful events. Moderate levels of acute stress may in fact facilitate or activate physiological and behavioral "coping" mechanisms and strategies and result in an improved response pattern. Continued stress may lead to a breakdown in normal coping mechanisms and result in disruption of the species-typical response patterns. Even acute stressful stimuli can have diverse effects on the organism. Some researchers have postulated that acute stress can have either positive or negative consequences. Under certain conditions stress may disrupt behavior, while under different circumstances it can have an integrating effect on performance and behavioral response patterns (i.e.

Hebb, 1955; Malmo, 1959; Selye, 1974; Yerkes & Dodson,1908).

Using these conceptualizations, that events in the environment can organize or disrupt responding, a useful operational definition of stress and, by implication, its synonyms, can emerge. Many words have been used synonymously with the term stress, though all have clearly different connotations (e.g. activation, arousal). An alternate way to view stress and its synonyms is to regard them as representations of different locations along a single hypothetical continuum.

This hypothetical continuum derives from the following conceptualization. According to Selye (1974) the term stress should be reserved for noxious stimuli that induce the same physiological syndrome. On the other hand, the term arousal which was often used in the same context was in fact describing concurrently, stimulus dimensions such as intensity, duration and frequency as well as psychological variables such as fear, and reaction to novelty and conflict (Hennessy & Levine, 1979). However, in general, stress was traditionally used to describe the status of the organism itself. Furthermore, arousal was traditionally viewed as an adaptive process, while stress has been viewed as a maladaptive, even pathological process. However, it is more likely that stress and arousal, in this traditional frame of reference, simply describe different positions on the same continuum. In this case they merely constitute terms used

to describe the response of the organism to different degrees, levels, or intensities of the same set of environmental stimuli impinging on the organism. Furthermore, arousal may describe the portion of the continuum where the response of the organism to the impinging stimuli is improved in its efficiency while stress describes a segment of the continuum where the response is disrupted and disintegrated. Therefore the working hypothesis taken here is that stress may be viewed as a position on an arousal continuum (i.e. too little or too much arousal), or that arousal constitutes a position on a stress continuum (i.e. a reaction to different intensities of noxious stimuli). Clearly, the choice would depend on whether one focuses on the stimulus continuum or the continuum representing the status of the organism.

This notion that stress can be viewed as a hypothetical position on a continuum of stress or arousal can be further developed by considering the view put forward by Malmo (1959). As with other researchers, Malmo prefered his own terminology because he felt that it would better represent his own conceptualizations. In the context of the present discussion, it is interesting to note that Malmo speculated that an appropriate level of environmental stimulation ("activation") would sensitize the organism. Such activation at any given point on the continuum would be a consequence of an interaction between environmental and

hormonal conditions. The hormonal conditions would not necessarlily provide the directional impetus for a particular behavior ("streering function") but would set up conditions to facilitate appropriate responding. In fact, Malmo delineated a specific experimental paradigm which included three levels of activation: low, moderate, and high. He went on to suggest that the expected corresponding performance levels of the organism under these three levels of activation would be low, optimal, and low; i.e. an inverted U-shaped curve. Stress, activation and arousal in this schema have meaning only as relative terms determined by their position on the inverted U-shaped curve. It is imperative to note that the characterization of a behavioral response as high or low in this context is only meaningful in relation to its position on the curve. What follows from this line of reasoning is that experiments which employ only one level of stimulus intensity cannot yield meaningful data. This framework seems to have particular merit and wherever feasible the experiments that follow used this conceptual approach.

By incorporating these notions into a conceptual theme it becomes possible to better measure defensive reactions in terms of an integrated response to a stimulus configuration. This response, however, is necessarily constrained by the parameters of the experiment. The performance in the absence of stress becomes the baseline against which the

behavioral response in all phases of the experiment is assessed. In this context any change in performance may be defined as the product of stress. For example, one of the adaptive responses of rodents to electric footshock is avoidance. If the stimulus configuration impinging on the organism alters this adaptive response then this interaction is considered "stressful". One must therefore conclude that despite the prevailing confusion in terminology, stress and arousal are by definition the intervening variables between environmental stimuli and the response. Figure 1 is a simplified graphic description of these notions.

The forgoing discussion leads to the conclusion that the operational definition of stress that was used in the present experiments was defined by the response of the organism to different configurations of a stimulus. These different configurations included the variables of intensity and duration which interacted with the physiological state of the organism to produce adaptive behavior.

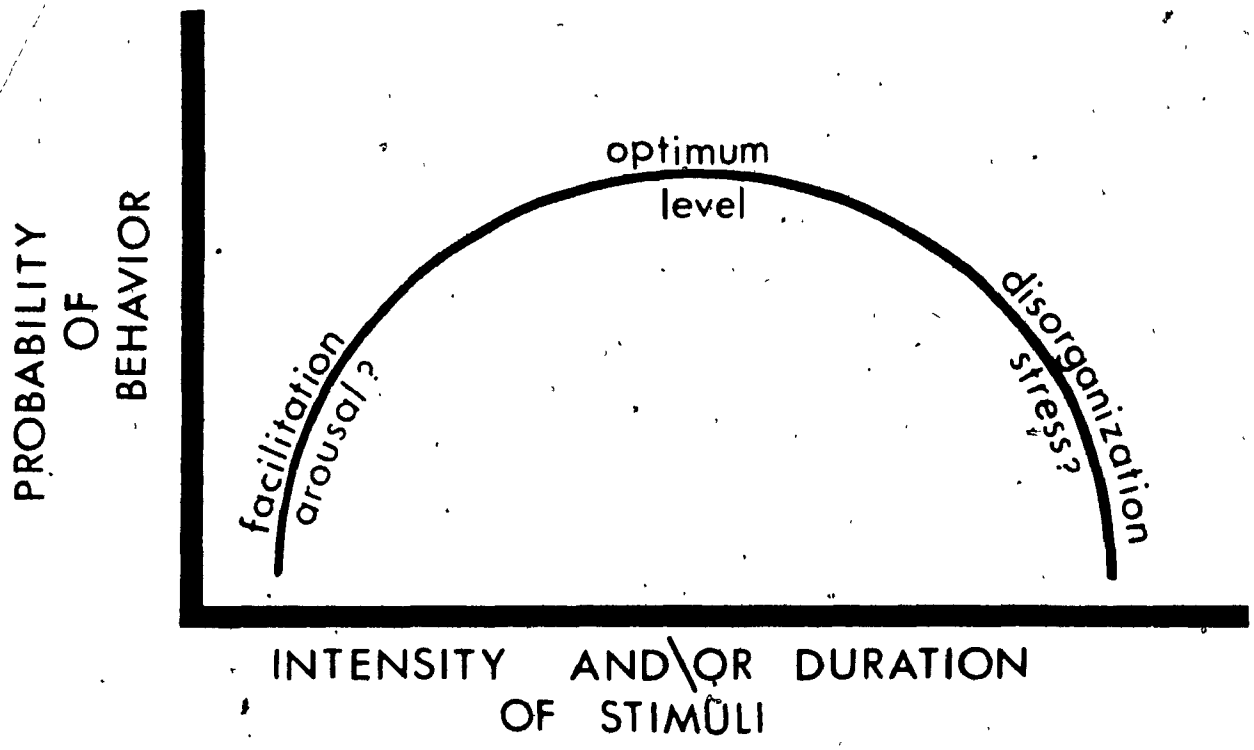

Fig. 1. A graphic presentation of a conceptual hypothesis concerning the term stress as a continuous variable. The probability of behavior is a function of the intensity or duration of stimuli. The stimulus variables will either facilitate, disrupt, or provide an optimum level of behavioral performance.

Stress-induced analgesia

Stress stimuli may have adaptive consequences

As stated above stress can be considered either as an adaptive or a disruptive phenomenon. One of the observable results of exposure to stress stimuli is a diminished reaction to pain. This decreased sensitivity was termed stress-induced analgesia (SIA). Appropriate and adaptive responding during the presence of noxious stimuli (i.e. avoidance, escape, freezing) may be disrupted or blocked due to accompanying pain. Within obvious limits, a diminished perception of pain would allow the organism to better focus its attention on a suitable response (Amir, Brown & Amit, 1980; Bodnar, Kelly, Brutus & Glusman, 1980a; Bolles & Fanselow, 1980). When viewed in these terms, SIA can be considered an adaptive process.

A large body of literature has been gathered documenting the physiological, and anatomical substrates of SIA. In addition; many of the variables that may be subsumed under the term stress have also been studied in terms of their capacity to modulate the perception of pain.

In 1976, three laboratories independently reported a phenomenon which has since been refered to as "stress-induced analgesia" (SIA) (Akil, Madden, Patrick & Barchas, 1976; Hayes, Bennet, Newlon & Mayer, 1976; Rosecrans & Chance, 1976). In the first series of experiments Akil and her colleagues (Akil et al., 1976;

Madden, Akil, Patrick & Barchas, 1977) demonstrated that exposure to inescapable footshock would lead to an elevated pain threshold. They also reported that following the same manipulation, stereo-specific binding of opiates to opiate receptors was reduced and the elevation in pain threshold could be partially blocked by the administration of naloxone, an opiate receptor antagonist.

In a second series of experiments, Rosecrans and Chance (1976; Chance, White, Krynock & Rosecrans, 1978; Chance, Krynock & Rosecrans, 1978) used inescapable footshock as a conditioning agent in a classical (Pavlovian) conditioning paradigm. The footshock was repeatedly paired with a neutral stimulus and this neutral stimulus eventually elicited elevations in pain threshold when presented alone. They also showed that the elevation in pain threshold was correlated with an inhibition of opiate binding and an increase in radio-labelled Leu-enkephalin binding in brain tissue.

In a third series of experiments Mayer and coworkers (Hayes et al., 1976; 1978a,b) used a number of different stimuli (i.e. footshock, centrifugal rotation) and reported that exposure to these stimuli would elicit elevations in pain threshold. Of particular interest was their demonstration that stress, as defined by adrenocortical activation, was a sufficient but not a necessary condition for the increase in pain threshold. They also reported that

they could not affect pain threshold increases with naloxone administration.

The diverse nature of the results reported above; i.e. increases in endogenous opioid binding, reversible, non-reversible and partially reversalble analgesia by opiate antagonists, and the demonstration that stress was not necessarily a contributing factor to the elevation in pain threshold, instigated a very fruitful search into the nature of "stress" induced analgesia.

In the sections that follow I will first describe the experimental evidence for stress-induced analgesia and then outline the nature of the physiological substrates and psychological mechanisms underlying this phenomenon. It is my contention that the interpretation of the results of these experiments, as well as others, must lead to the conclusion that SIA is one of a constellation of overlapping systems that may enable an organism to adapt to changing environmental circumstances. In addition, an attempt was made to synthesize the data and speculate on its possible role in the functional organization of behavior.

Parameters of Stress-induced Analgesia

One of the apparent paradoxes of SIA research is that the measures employed to infer analgesia are, of necessity, painful and aversive, while in most cases the analgesia inducing stimuli are themselves both painful and aversive.

This has led to the use, by some investigators, of analgesia testing procedures that others have used as stimuli to induce analgesia. For example, footshock has been used as a pain stimuli (Hayes et al., 1976; 1978a,b) and also as a pain measure (Bodnar, Kelly & Glusman, 1978). For this reason I will begin with a discussion of the factors that determine the analgesia inducing properties of the stimuli.

Induction of SIA: Intensity, Duration, and Temporal Aspects

There are many stimuli that, when applied to an organism, can lead to SIA. It has become apparent that different types of stimulus, or even the same stimuli under different conditions may change the nature of SIA and its underlying substrate.

The most frequently used procedures for the induction of SIA are those that employ inescapable footshock. It has been consistently reported that continuous (up to 3 min) inescapable shock (Akil et al., 1976; Hayes et al., 1976; Madden et al., 1977; Rosecrans & Chance, 1976) raises pain thresholds that are neither blocked by naloxone nor are cross tolerant to morphine (Lewis, Cannon & Liebeskind, 1980; Lewis, Sherman & Liebeskind, 1981; Ross & Randich, 1984). On the other hand, prolonged (5 min or more), intermittent shock (Amir & Amit, 1979b; Lewis, Cannon & Liebeskind, 1980; Lewis, Sherman & Liebeskind, 1981; Millan,

Gramsch, Przewlocki, Hollt & Herz, 1980), also induces analgesia that is in turn affected by naloxone and is morphine tolerant. Others have shown that keeping the intensity and duration of the shock constant (5sec, 1mA), while varying the total length of exposure also produced differential results in the induction of SIA (Grau, Hyson, Maier, Maddon & Barchas, 1981). Twenty min of shock (variable interval; mean = 1 min) induced analgesia that was insensitive to naloxone, longer periods of exposure to shock (60 amd 80 min) produced analgesia that was blocked by naloxone. However, a number of researchers have reported that shock durations of 5 or 10 minutes produced analgesia that was opiate mediated (Amir & Amit, 1979b; Millan et al., 1980) while others have shown that 30 seconds of exposure to shock produced analgesia that was naloxone reversible (Buckett, 1980). In an elegant parametric study (Terman, Shavit, Lewis, Cannon & Liebeskind, 1984) all three shock parameters were varied independently and the results indicated that interactions between intensity, duration, and time determined whether the substrate for the analgesia was opiate or non-opiate related.

Cold-water swim (CWS) stress has received considerable attention as a procedure that raises pain thresholds. Bodnar and his colleagues carried out a series of experiments that examined the parameters and the substrate of CWS analgesia (Bodnar, Kelly & Glusman, 1978). Following 3.5 min of

inescapable swimming in water at 2 C, a profound analgesia that was neither naloxone reversible nor cross tolerant with morphine was observed (Bodnar, Kelly, Steiner & Glusman, 1978f). A 3.5 min swim in water at 15 and 21 C was also found to raise pain thresholds, however, this analgesia was blocked by naloxone and found to be cross tolerant to morphine (Bodnar et al., 1978f; Chester & Chan, 1977; Christie, Chester & Bird, 1984). By altering the procedure from continuous CWS to intermittent CWS it was found that 3.5 min of CWS induced a non-opiate mediated analgesia (Bodnar et al, 1978f), while intermittent CWS (18, 10 sec exposures, 3/min) produced an opiate mediated analgesia (Girardot & Holloway, 1984a,b).

Glucoprivation has been found to induce a transient antinociceptive response in rats. Acute administration of 2-deoxy-D-glucose (2-DG), an antimetabolic glucose analogue, insulin, or acute food deprivation, increased pain thresholds in a number of tests in a dose dependent fashion (Bodnar et al., 1978a; Bodnar, Kelly, Mansour & Glusman, 1979b; Bodnar, Merrigan & Wallace, 1981). Chronic administration of 2-DG on the other hand reduced the 2-DG antinociceptive effects in pain tests indicating that adaptation to the pain inducing qualities developed but adaptation to the hyperphagic properties of the drug did not (Bodnar, Kelly Brutus, Mansour & Glusman, 1978c; Bodnar, Kelly, Brutus, Glusman, 1978b). The antinociceptive

response that was seen after food deprivation was antagonised by naloxone and in some circumstances was reported to summate with 2-DG induced analgesia (Bodnar et al., 1978c; McGivern, Berka, Bernston & Walker, 1979, McGivern & Bernston, 1980).

It has been suggested (Bodnar et al., 1978c) that 2-DG induces an antinociceptive response because it mimics a stressor, or is itself a stressor and not because of its glucoprivic effects. A number of differences between the glucoprivic and stressor effects of 2-DG have been documented; the time course of the effects differ substantially, tolerance to the hyperphagic and analgesic effects of the two manipulations develop differently, different doses are needed to induce the effects centrally, and the neurotransmitter and anatomical substrates are dissociable (Badillo-Martinez, Kirchgessner, Butler & Bodnar, 1984; Bodnar, Kramer, Simone, Kirchgessner & Scalisi, 1983; Bodnar, Merrigan & Wallace, 1981). The constellation of effects after 2-DG administration is similar to that seen with other stressors and thus may have the capacity to activate pain suppressive systems. Indeed, 2-DG and CWS analgesia exhibit cross tolerance to each other in that adaptation induced by chronic administration or application of either manipulation reduced the pain suppressive effects of the other (Bodnar et al., 1979b). In general, as with studies reporting on shock-induced

analgesia, the studies on the antinociceptive effects of administration of 2-DG, insulin, or food deprivation indicate that intensity (dose dependency) and duration (acute vs chronic) are important variables determinig the nature of SIA.

Several other stimuli have been reported to induce SIA, but they have not been studied in such detail as have footshock or CWS. Exposure to ambient temperatures of 40 C for one hour was necessary to observe elevations of pain threshold, while chronic exposure for eight days (1 hr/day) did not elevate pain thresholds (Kulkarni, 1980). An early demonstration of a "peculiar" type of "stress-induced analgesia" was shown in female rats (Komisaruk & Larson, 1971). The peculiarity of this demonstration stems from the fact that its aversive or even stressful nature is rather questionable. In this study, vaginal stimulation induced significant levels of analgesia that was attenuated by opiate receptor blockade and was attenuated in animals previously adapted to morphine or CWS (Bodnar & Komisaruk, 1984; Crowley et al., 1976; Crowley, Rodriguez-Sierra & Komisaruk, 1977a; 1977b; Hill & Ayliffe, 1981; Komisaruk & Wallman, 1977). Furthermore, a variety of other procedures and manipulations whose aversive or stressful nature was similarly questionable (e.g. cervical probing, Komisaruk & Larson, 1971; centrifugal rotation, Hayes et al., 1978; and body pinch, Amir, Brown, Amit & Ornstein, 1981) were all

found to induce changes in pain threshold. Taken together these studies seem to reveal a unique and seemingly paradoxical nature of SIA, i.e. that the "stressor" need not be severe, or even aversive in order to be effective in inducing analgesia. Indeed, this set of studies seem to raise a fundamental question as to what in fact constitutes a stressor, necesssary and sufficient to induce analgesia.

A review of the studies included in this section seems to illustrate the need to design experiments where multiple dimensions of the impinging stimuli are utilized in order for the full range and complexity of the factors governing the induction of SIA to be revealed.

Conditioning and Control factors in SIA

When an animal is confronted by a stressful situation that may potentially be threatening to its survival, the efficiency of its response aimed at extricating itself from the threat is of obvious importance. Therefore if an animal could draw upon its past experience with the same stressful, threatening situation to increase its efficiency in responding, it would be adaptive. Since analgesia can be a by-product of stress, it would follow that analgesia could form part of the animal's experience following a confrontation with stress. Since the diminished response to pain may increase the ability of the animal to respond efficiently to terminate the stressful situation, it would

seem highly adaptive if analgesia could be conditioned so that it could be readily induced by exposure to cues in the environment previously associated with the stressful situation, thereby maximizing the efficiency of the animal's defensive response. Naturally, the confirmation of such a process would suggest the presence of adaptive plasticity in the systems which underlie SIA.

Analgesia can be conditioned to the environment in which the animal received footshock. Conditioned analgesia induced by footshock can be achieved through the pairing of distinctive environments with the footshock and with the measurement of analgesia in the same environment (Chance, 1980; Chance, Krynock & Rosecrans, 1978; Devries, Chance, Payne & Rosecrans, 1979; Fanselow, 1984; Fanselow and Baakes, 1982; Hayes et al., 1978a; Gaiardi, Bartoletti, Gubellini, Bacchi & Babbini, 1983; Kinscheck, Watkins & Mayer, 1984; Oliverio & Castellano, 1982; Rosencrans, Hong & Tilson, 1982;).

The general procedure in these studies is the contiguous association of a distinctive environment with a stressor and the pain test. For example, a study carried out in Mayer's laboratory utilized the following procedure (Kinscheck, Watkins & Mayer, 1984). The animals received footshock (90 sec, 1.2mA)(UCS) which was paired with the grid or with a sound (CS) or both. Tail-flick inhibition was the measure of analgesia (UCR). The rats were tested for conditioned

analgesia on the fourth day, by being placed in the shock box (CS) exactly as in days one to three but with no shock delivered. Ninety seconds following placement in the shock box analgesia was measured (UCR). Using this and similar procedures it has been reported that on day four significant analgesia was observed, induced presumably by the shock-environment pairings. In a related experiment the conditioned analgesic response could be observed 24 hours following one pairing session of shock and environment on the previous day. In addition, it has been reported that this type of conditioned analgesia was naloxone reversible (i.p. or intrathecal) when administered before exposure to the CS and that it was cross tolerant to morphine (Hayes et al., 1978; Kinscheck, Watkins & Mayer, 1984; Watkins, Cobelli & Mayer, 1982; but see Chance, 1980; Chance, Krynock & Rosecrans, 1978). Furthermore, when naloxone was administered before exposure to the CS it prevented the appearance of conditioned analgesia. Watkins & Mayer (1982a), have suggested that the findings that naloxone can prevent but not reverse SIA may be interpreted to signify that once opiate systems in the spinal cord are activated, continued opiate release is unnecessary to keep the system active.

Other researchers have used a procedure analagous to that which produced a "learned helplessness" response which they termed "long-term stress-induced analgesia" (Jackson,

Maier & Coon, 1979). The term "learned helplessness" refers to a hypothesis that prior exposure to inescapable shocks interferes with later escape and avoidance learning because the animal learns that no response will terminate shock exposure (Seligman & Maier, 1967). In other words the animal learns that it has no control over the shock and thus does not respond when the shock becomes escapable. Using the same type of procedure it has been reported that 24 hours after exposure to eighty (1mA) inescapable, but not escapable shocks to the tails of rats, during a period of one hour, and subsequent exposure to a different shock apparatus with as little as five shocks of a lower intensity, which by itself does not produce analgesia (5 sec, .6mA), reinstated the analgesia induced by the shock on the previous day (Jackson, Maier & Coon, 1979; but see Mah, Suissa & Anisman, 1980; and Zacharcko & Anisman, 1984). It is important to note that this type of analgesia was seen only in the groups that received the subsequent brief shock procedure and only in those animals that did not have control over shock termination. The effect was seen only in animals preexposed to 80 shocks and not in rats preexposed to 20 or 40 shocks. The antinociception after preexposure to 80 shocks has been reported to be opiate mediated since naloxone or naltrexone can dose dependently block the effect when administered either before initial shock or before the reinstatement procedure (Maier, Davies, Grau, Jackson,

Morrison, Moye, Madden & Barchas, 1980). These authors also reported that a naltrexone reversible analgesia was observed 30 minute after the eighty shock-reinstatement procedure. However, when the animals were tested 1 min after termination of the eighty shocks the resultant analgesia was not naloxone reversible (Hyson, Ashcroft, Druggan, Grau & Maier, 1982; Maier, Drugan & Grau, 1982). Also, the ability to terminate shock determined whether the analgesia would be manifested or not when tested for analgesic responses 30 minutes, but not one minute later. That is to say that the analgesia that is observed one minute after the 80 shock procedure is evident whether the shocks were escapable or not. This may indicate that the "controlability" factor must have sufficient time to develop before it becomes manifest. In other words, the animal may need extended exposure to the shock in order to "learn" that is has no control over the shock (Maier, Drugan & Grau, 1982). This "learning" would then shift the substrate of analgesia to an opiate form which may in this instance be necessary for conditioning to occur.

Given the relationship between controlability of the stressor and "learning" it should be noted that tolerance (diminished analgesia) develops to the opiate mediated prolonged footshock-induced analgesia but not to the non-opiate brief footshock analgesia (Lewis, Sherman & Liebeskind, 1981). This may suggest that the opiate and

non-opiate systems, though both involved in antinociception, serve different functional purposes. Since the non-opiate analgesia induced by brief stressors does not readily adapt it may be the mechanism which is constantly available to the animal, while the opiate mediated form comes into play either when the pain is of "higher" intensity or as an outcome of conditioning processes. This is corroborated by the observations that opiate and non-opiate analgesias can occur in succession; brief non-opiate can be followed by prolonged (or more intense) opiate analgesia (Grau et al., 1981). Also, the two forms of analgesia are not cross tolerant (Terman, Lewis & Liebeskind, 1983) suggesting their independence.

Further testing carried out by Maier and his group (Maier, Ryan & Kurtz, 1984) determined that essentially the same results could be observed when shock parameters were substituted by the stress of a formalin injection. They also showed that a non-opioid form of analgesia induced by a few shocks can be converted to an opioid form by pretreating the rats with a painful formalin injection to the paws (Maier, Ryan & Kurtz, 1984). This once again seems to confirm that the duration and intensity of the stress are crucial for determining the underlying substrate for the observed analgesic reaction. The reinstatement procedure only seemed to work when the substrate was the opiate form which was usually found when the duration or intensity were

increased. This was supported by reports that a very short footshock stress which induced a non-naloxone reversible analgesia could not be used as a conditioning stimulus (Ross & Randich, 1984). In this section I have outlined how the stimulus parameters can determine the substrate; in the next section I will review the evidence pertaining to the substrates.

Physiological Substrates of SIA

It would seem clear from the previous sections that SIA is not a uniform homogeneous behavioral phenomenon. Even a cursory scrutiny seems to reveal several subtypes which are modulated by different mechanisms whose boundaries are quite unclear. It would follow then that an examination of the underlying substrates of SIA should also reveal considerable heterogeneity.

Neuropeptides and SIA

Opioids. The most plausible hypothesis concerning the physiological mechanism of SIA was that its mediation was a function of the release of endogenous opiates and that those in turn interacted with receptors in the CNS. Numerous observations tended to support this notion (see Basbaum & Fields, 1984). The fact that opiate receptors exist in the CNS and that endogenous ligands interact with these receptors is well established. These endogenous opioid

ligands are distributed in the CNS in a manner which for the most part parallels the distribution of the receptors. The sites of these receptors, in turn, have distinct overlap with sites known to play a role in the alleviation of pain (Basbaum & Fields, 1984). Furthermore, many studies have reported an increased release of endorphins, both peripherally and centrally, during stress (see Amir, Brown & Amit, 1980). These observations would tend to make the endogenous opioid system a logical candidate for the mediation of SIA.

To determine whether a particular behavior is mediated by the opioid system, a number of criteria are normally used. Naloxone blockade of the behavior or cross tolerance to morphine are the criteria most often used (Sawynok, Pinsky & Labella, 1979). Naloxone is a competitive antagonist at some opiate receptor sites. Thus, administration of naloxone should block or significantly reduce opioid-induced analgesia at those receptors. However naloxone antagonism may not be a sufficient condition since opiate receptors are not homogeneous. It is thought that a number of subtypes of receptors exist and naloxone has different affinities for each subtype of receptor. Increasing the dose of naloxone is thought to be sufficient to block all the opiate receptors but this may also lead to non-opiate receptor effects of naloxone which may be difficult to separate from its opiate receptor effects. Also

since the receptors are found along a number of critical points along known pain pathways, the systemic administration of naloxone tells little of the location of the relevant receptor. Finally, it is worth noting in this context that naloxone blocks the receptor but does not necessarily interfere with the release of endogenous opiates, therefore the time of administration of the antagonist should be considered when opiate mediation is inferred. Therefore more than one criterion should be used to infer opiate mediation (Duggan & Johnson, 1983; Sawynok, Pinsky & Labella, 1979).

Another method used to infer opiate mediation of a particular behavior is to induce cross-tolerance. In the present context cross-tolerance refers to a situation where morphine is administered to the animal over a number of days. After repeated administration of morphine its capacity to induce analgesia is diminished. When the diminished analgesic response to morphine becomes apparent, a stressor that is known to induce SIA is presented. If a diminished response to the analgesic effects of the stressor is also observed, indicating cross-tolerance between SIA and morphine, opiate mediation of SIA is inferred. If the stressor continues to induce analgesia at levels not different than that observed in controls one must deduce that it is mediated by mechanisms not sensitive to opioids.

Using these criteria, it seems that stressors of short

duration tend to induce a non-opioid analgesia while more protracted stressors induce an opioid mediated form of SIA. As stated above, footshock will induce either an opioid or non-opioid mediated form of analgesia. Acute exposure to continuous footshock for three minutes resulted in an analgesic response that was unaffected by naloxone or dexamethasone (Lewis, Cannon & Liebeskind, 1980). (Dexamethasone is a synthetic streroid that blocks the release of anterior pituitary endorphins). On the other hand, thirty minutes of intermittent footshock induced analgesia that was reduced by these manipulations (Lewis, Cannon & Liebeskind, 1980). It is also interesting to note that when the opiate receptor antagonist, naltrexone, was administered for 21 days, leading to a supersensitivity of the receptors, it could potentiate the analgesia induced by exposure to footshock thus further implicating opiate involvement (Amir & Amit, 1979b). Liebeskind and his associates have also determined that no cross-tolerance between the effects of the opioid and non-opioid form of footshock analgesia were found indicating a dissociation of the substrates (Terman, Lewis & Liebeskind, 1983). These researchers argued that (Lewis, Terman, Nelson & Liebeskind, 1984) their results supported the notion that SIA was not due to adaptation of sensory receptors, habituation of a central processes, or non-specific changes such as tissue damage, since brief stress analgesia can be instigated in a

rat already tolerant to prolonged footshock analgesia.

CWS analgesia could be mediated by both opioid and non-opioid mechanisms. As has been found with footshock induced analgesia, continuous CWS for 3.5 minutes induced a non-opiate mediated analgesia (Bodnar, Kelly, Spiaggia, Ehrenberg & Glusman, 1978d), while intermittent CWS was found to be opiate mediated (Girardot & Holloway, 1984). Subsequent tests have shown that rats that exhibited a greater analgesic response compared to controls were also the ones in which naloxone would reverse the analgesia induced by CWS. In addition, the longer the duration of exposure to the CWS the more likely it would be that naloxone could block the resultant analgesia (Bodnar & Sikorszky, 1983).

Interestingly, CWS (and insulin-induced analgesia) were attenuated by hypophysectomy (removal of the pituitary, one major source of endogenous opiates), while 2-DG induced analgesia was potentiated by the same manipulation (Bodnar et al., 1979b; Bodnar, Glusman, Brutus, Spiaggia & Kelly, 1979a). Thus animals that have been made tolerant to the analgesic effects of CWS would still respond to morphine, but this did not seem to be the case with rats tolerant to the effects of 2-DG (Bodnar et al., 1978f). Curiously, naloxone at various doses failed to affect 2-DG analgesia, yet 2-DG analgesia was found to summate with morphine to further increase pain thresholds. This observation suggests

that this particular stressor may in fact activate both opiate and non-opiate pain suppressive systems (Bodnar, Kelly & Glusman, 1979).

The substrate for immobilization (restraint) -induced analgesia seems also to be dependent upon opioid systems. Thirty or sixty minutes of restraint induced significant pain suppression that was blocked by the administration of naloxone (Amir & Amit, 1978; 1979a; Greenberg & O'Keefe, 1982; Kulkarni, 1980; but see Jorgensen, Fasmer, Berge, Tvieten & Hole, 1983).

Both major sources of peripheral endogenous opioids, the pituitary and the adrenals, have been implicated in SIA. Pituitary involvement was demonstrated by the observations that hypophysectomy will attenuate analgesia induced by 30 minutes of immobilization (Amir & Amit, 1979a), 3.5 minutes of CWS (Bodnar et al ., 1980a), intense footshock (Millan et al., 1980), and prolonged footshock (Lewis et al., 1984; MacLennan, Drugan, Hyson, Maier, Maddon & Barchas, 1982). On the other hand, conditioned analgesia (Chance, Krynock & Rosencrans, 1979; Chance, 1980; Watkins, Cobelli, Newsome & Mayer, 1982) and brief footshock analgesia (Watkins et al., 1982) were enhanced by pituitary removal suggesting that the endogenous opiates contained within this gland are necessary for the induction of these types of analgesia (Lewis et al., 1984). It is worth noting that mediation of SIA by brain opioid peptides was not necessarily ruled out by these

observations since brain peptides are distinct from peripheral pools (Krieger & Liotta, 1979) and would therefore still be available for the induction of analgesia. Yet, it should be emphasized that in most cases where analgesia is attenuated by hypophysectomy it is also attenuated by systemic naloxone, which should block the receptors throughout the body irrespective of the source of the endogenous opiate. This suggests that when hypophysectomy reduces the analgesia the pituitary and not the brain is the most likely source of release.

Adrenal medullary opioids have also been implicated in prolonged footshock-induced analgesia and short intense footshock since demedullation or denervation of the adrenal medulla reduced this form of analgesia (Lewis et al., 1982). These results were supported by the findings that adrenal medullary enkephalin content was reduced by prolonged footshock, but was unaffected by brief footshock (Lewis et al., 1984). Exactly at which sites peripheral opioids exert their effects is as yet unknown. However, peripheral peptides have been demonstrated to affect centrally mediated behavior after systemic injection (Kastin, Coy, Schally & Miller, 1978; Kastin, Zadina, Coy, Schally & Sandman, 1980) and to enter the CNS by various routes such as retrograde flow from the pituitary to the brain through the portal vessels or through permeation of the extracellular space (Mezey, Kivovics & Palkovits, 1979; Rappaport, Klee,

Pettigrew & Ohno, 1980; Rees, Verhoef, Witter, Gispen & de wied, 1980) thus supporting the notion that peripheral peptides can effect central processes.

Further support for the separation of opiate from non-opiate analgesias induced by stress were found when enzymatic degradation of opiates were prevented. Inhibition of the enzymatic degradation of endogenous opiates by thiorphan led to a potentiation of footshock- (1mA, ten min, Chipkin, Latranyi & Iorio, 1982) immobilization- (Greenberg & O'Keefe, 1982) and warm water swim-induced analgesia (O'Connor & Chipkin, 1984) all of which are known to be opiate mediated. In contrast, inhibition of endogenous opiate degradation by D-phenylalanine also reduced the non-opiate mediated CWS analgesia (Bodnar, Lattner & Wallace, 1980). These reports led some researchers to suggest that one pain system could in fact inhibit the other (Kirchgessner, Bodnar & Pasternak, 1982). They reasoned that having two or more systems activated at the same time would reduce the animal's capability to further respond to a second pain inducing stimuli. Instead, it would be more adaptive for the organism's functioning if the activation of one system would lead to the inhibition of the other. In this case, at least one system would always remain functional. This notion constitutes collateral inhibition and in fact a hypothetical neural circuitry for this type of collateral inhibition has been postulated (Akil & Watson,

1980). The existence of collateral inhibition was corroborated by the demonstrations that the opioid and non-opioid systems were not cross tolerant (Bodnar et al., 1980a; Terman, Lewis & Liebeskind, 1983).

A potentially important result concerning the opioid nature of SIA was described by Watkins and Mayer (1982b). These researchers found that when footshock was confined to either the front paws or the back paws, the subsequent analgesia was differentially mediated. Brief shock to the front paws induced elevations in pain threshold that were blocked by systemic naloxone administration or morphine tolerance. Brief shock to the back paws induced elevations in pain threshold that were resistant to naloxone or morphine tolerance. The importance of these observations was pointed out by Watkins and Mayer (1982a). They suggested that these data may parallel data obtained in studies of human pain. Mayer, Price and Rafii (1977) have found that naloxone could block acupuncture analgesia induced by stimulation of sites far removed from the original painful site. Chapman and Benedetti (1977), however, were unable to block acupuncture analgesia when the stimulation and the pain areas were in close proximity. The suggested parallel stems from the fact that front paw shock which is opiate mediated was far removed from the painful stimuli (radiant heat to the tail) while shock to the back paws which is non-opiate mediated was naturally much closer

to the tail.

To summarize, from the data described in the above section on the involvement of endogenous opiates in SIA it would seem that the higher the intensity and the longer the duration of the stress inducing stimuli, the more likely it is that naloxone will antagonize the analgesia induced by it. In the specific case of conditioned analgesia, it is obvious that conditioning requires (in most cases) the repetition of the stressful event thus altering intensity, or at least the duration of the stressor. This repetition and its necessary increase in the duration of the stress experience would tend to shift the resultant analgesia from non-opiate mediated to opiate mediated. This state of affairs seems to suggest that recruitment of the opiate substrate is important for the following two reasons. First, when the stress or pain is of severe intensity the two systems combine to provide greater relief (or the opiate component may inhibit the non-opiate one). Second, the opioid system may be recruited because it aids in conditioning by improving memory of the situation (Belluzzi & Stein, 1981; Kovacs & de Wied, 1981). This type of reasoning is in line with demonstrations that the conditioning of SIA is dependent on opiate mechanisms, whether the initial analgesia was opiate in nature or not (Fanselow, 1984; Fanselow & Baakes, 1982; Fanselow & Bolles, 1979; Hayes et al., 1978a; Oliverio & Castellano, 1982;

Watkins, Cobelli & Mayer, 1982; but see Chance & Rosencrans, 1979a,b,c). These implications are relevant to conditioning of analgesic responses and are testable. For example, a method to deplete endogenous opioids, preferably at specific locations, should reveal that classical conditioning of analgesia does not appear under circumstances in which the animal is depleted of opioids. This position allows, for example, a parallel response of avoidance or escape responding to be conditioned.

Vasopressin. In addition to the opiate peptides, other endogenous peptides have also been studied in the context of SIA. For example, the neuropeptide vasopressin has been implicated as one of the possible mediators of the non-opioid forms of analgesia (i.e. cold-water swim) primarily due to the work of Bodnar and his colleagues. The evidence stems from the following observations. Brattleboro rats, which are deficient in vasopressin, fail to exhibit analgesia after cold-water swim while normal animals exhibit this phenomenon (Bodnar, Zimmerman, Niliver, Mansour, Thomas, Kelly & Glusman, 1980; Bodnar, Wallace, Kordower, Niliver, Cort & Zimmerman, 1982a). Administration to rats of antisera to vasopressin, which prevents the action of vasopressin, will reduce pain thresholds in the tail flick test at high temperatures (Bodnar et al., 1982a; Bodnar, Niliver, Wallace, Badillo-Martinez & Zimmerman, 1984) while

central injections of vasopressin or longer lasting analogues will increase pain thresholds in some nociceptive tests (tail-flick) but does not seem to in others (flinch-jump) (Kordower, Sikorsky & Bodnar, 1982). In addition, vasopressin-induced analgesia is not blocked by the opiate antagonist naloxone, but seems to be mediated through its own binding sites due to the fact that the changes in pain threshold were eliminated by specific vasopressin antagonists (Kordower & Bodnar, 1984). It has also been suggested that vasopressin-induced analgesia was not mediated through the release of pituitary peptides since pretreatement with dexamethasone (which inhibited the release of ACTH and endorphins from the pituitary) potentiated vasopressin mediated analgesia (Kordower & Bodnar, 1984). This evidence tends to support a role for vasopressin in the mediation of some non-opioid forms of analgesia, in particular CWS, however, more work is needed for verification of its involvement in other types of SIA.

Neurotransmitters

Concurrent with studies which have linked peptides to the mediation of SIA there have been other studies which have suggested that neurotransmitters may also mediate some forms of this phenomenon. In this section a summary of neurotransmitter involvement in SIA is presented.

Serotonin. In general, it seems that the involvement of serotonin (5-HT) in SIA is determined by the duration of the stressor which is used to induce the SIA. Depletion of 5-HT before the induction of short duration stressors (Bodnar, Kordower, Wallace & Tamir, 1981; Buckett, 1981; Hutson, Tricklebank & Curzon, 1982; 1983; Jensen & Smith, 1982; Terman, Lewis & Liebeskind, 1982; Tricklebank, Hutson & Curzon, 1982; but see Snow, Tucker & Dewey, 1982) usually does not effect the subsequent analgesia (but see Hutson, Tricklebank & Curzon, 1984) but will reduce analgesia induced by longer duration stressors (Bhattacharya, Keshary & Sanyal; but see Coderre & Rollman, 1984). When only spinal levels of 5-HT are depleted only opiate-mediated SIA is affected (Watkins, Johannsessen, Kinscheck & Mayer, 1984). In addition, opioid-mediated analgesia was attenuated by intrathecal administration of the 5-HT antagonist BC-105. Conversly, selective depletion of 5-HT by the neurotoxin 5,7-dihydroxy-tryptamine (following the administration of desmethyl-imipramine, to protect norepinephrine levels) in the spinal cord does not affect the non-opioid form of SIA. These results are in line with other findings suggesting that 5-HT systems are in some way involved in opiate analgesia (Basbaum & Fields, 1978).

In a series of experiments conducted by Ogren, Archer and Johansson (1984) the effects of 5-HT manipulations on avoidance learning and nociception were examined. Using

various pharmacological manipulations to reduce 5-HT availability (p-chloroamphetamine, p-chlorophenylalanine) these authors suggested that manipulations of 5-HT neurons affect both learning and the nociceptive response. They did not observe, however, any relationship between these two classes of responses since increases or deceases in analgesia were not related to the acquisition or retention of an avoidance response. This seems to suggest that analgesia and learning may be mediated by at least two different pools of 5-HT neurons both of which are affected by the same stimuli that govern different reactions to stress.

Dopamine. Another transmitter that has been implicated in SIA is dopamine (DA). Manipulation of DA systems, no matter how severe, however, do not seem to totally block or augment SIA. This has raised the possibility that DA may serve in a modulatory role; increasing or decreasing SIA as a result of availability of DA within the CNS. This notion is supported by various studies of DA involvement in SIA (i.e. Snow, Tucker & Dewey, 1982). Pharmacological manipulations of dopaminergic neurons have revealed that the antagonists haloperidol or pimozide, or the neurotoxin 6-hydroxy-dopamine (with DMI) increased analgesic responses to stress (Bodnar & Nicotera, 1982; Curzon, Hutson & Tricklebank, 1981; Snow, Tucker & Dewey, 1982; Terman et

al., 1982; Tricklebank, Hutson & Curzon, 1984; but see Jensen & Smith, 1982). Administration of the agonists apomorphine and amphetamine decreased SIA (Curzon, Hutson & Tricklebank, 1981; Snow, Tucker & Dewey, 1982; Terman et al., 1982).

It is important to note that since DA manipulations do not exert large effects on SIA, other explanations concerning its role may be appropriate. In this vein, Bodnar and Nicotera (1982) reported that under the same conditions that induce SIA, haloperidol and chlorpromazine reduced activity levels below the levels of non-treated controls suggesting that the effects on analgesia may be secondary to the reduction in activity. The findings that reduction in activity levels of animals treated with drugs that manipulate the activity of DA systems points out the need for analysing a variety of behaviors in conjunction with analgesia. Others studies, that have analyzed the locomotor behavior under conditions that lead to SIA, have also reported that activity levels would be reduced following manipulations that also induce hot-plate analgesia (Galina, Sutherland & Amit, 1983). Furthermore, other studies have shown that restraint-induced analgesia was not accompanied by reductions in activity (Blair, Galina, Holmes & Amit, 1982) and this analgesia was decreased by treatment with haloperidol (Kulkarni, 1980). Therefore measurement of analgesia after stress manipulations that affect DA or other

neuronal transmission must test for simultaneous effects on performance capabilities.

Norepinephrine. The reports of the possible involvement of norepinephrine (NE) systems in SIA are not conclusive but are supported by the following observations. Systemic administration of clonidine (alpha-NE agonist) can potentiate CWS analgesia and animals made tolerant to the analgesic effects of clonidine exhibit reduced analgesic effects of CWS, footshock-induced analgesia and conditioned analgesia (Bodnar, Merigan & Sperber, 1983; Chance, 1983). In addition, other investigators have found that the systemic administration of clonidine reduced SIA and the antagonist, phenyoxybenzamine, prolonged the duration of SIA produced by short duration footshock (Snow, Tucker & Dewey, 1982), but not thirty minutes of restraint (Kulkarni, 1980). Intrathecal administration of 5,7-DHT which depletes both 5-HT and NE in the spinal cord attenuated front paw SIA. Back paw and conditioned analgesia, however, were unaffected by these depletions. To assess the relative importance of the two systems (NE vs 5-HT) in the spinal cord, phentolamine (NE antagonist) or BC-105 (5-HT antagonist) were injected intrathecally. Phentolamine significantly reduced SIA but not to the same degree as did BC-105. These results suggest that in the spinal cord 5-HT neuronal transmission may play a more prominent role (Watkins et

al., 1984a) than the NE systems. The NE system may play only a small or non-significant role since drastic depletions are necessary to produce results on SIA. The role of NE involvement in SIA is unclear at present and it would seem that more work is needed to identify the exact nature of NE involvement in the phenomenon.

Despite the considerable data accumulated concerning the pharmacological mechanisms of SIA, it is not possible at present to describe a comprehensive set of pharmacological processes mediating this phenomenon. Nevertheless, it is possible that this very difficulty may, in itself, aid in a further understanding of the biological substrates of SIA. I will attempt to consider this possibility in a later section, however; in order to aid the clarity of this discussion I will first consider the available data on the anatomical underpinnings of SIA.

Anatomical Substrates

The attempts to localize the anatomical sites that subserve SIA have focused on the differences and similarities between morphine (opiate) and stimulation-produced analgesia and their relation to SIA (Watkins and Mayer, 1982a). Given the limits of this thesis I will focus only on the experiments which have directly utilized "stressful" manipulations.

The final common pathway, for pain inhibitory systems

due to footshock (1.2mA, 90 sec) in the spinal cord, seems to be the dorsolateral funiculus (DLF). Hayes et al., (1978b) first demonstrated that complete spinal cord transection reduced the non-opiate footshock (to all four paws) and rotation-induced analgesia by as much as 80 per cent. They also demonstrated that bilateral lesions specific to the dorsolateral funiculus (DLF) of the spinal cord did not affect footshock-induced analgesia. This suggested that this particular form of SIA was mediated at a supraspinal level. Further work showed that the opioid form of footshock-induced analgesia was blocked by 1 ug of naloxone administered directly to the lumbosacral section of the cord and given before shock (but not after). This study pointed to an opioid link within the spinal cord (Watkins & Mayer, 1982b). DLF lesions reduced classically conditioned analgesia whether derived from conditioning of opioid or non-opioid footshock analgesia. Again as with the non-conditioned opioid form of SIA, conditioned analgesia was prevented but not reversed by administration of naloxone (lug) into the lumbosacral section of the cord (Watkins, Cobelli & Mayer, 1982)

Bilateral DLF lesions have been reported to block or attenuate both front paw (opiate), and hind paw (non-opiate) footshock-induced analgesia, as well as short term non-opiate (1-5 shocks to the tail, 1mA), and short term opiate (20-60 tail shocks, 1mA) analgesia. In addition,

long term opiate (80 tail shocks and reinstatement of shock 24 hrs. later) analgesia, were also attenuated by this manipulation, although a transient, though significant, analgesia immediatly after the shock was not affected by these lesions (Watkins, Cobelli & Mayer, 1982; Watkins, Drugan, Hyson, Moye, Ryan, Mayer & Maier, 1984). Furthermore, complete spinalectomy attenuated only the opiate form but not the non-opiate form of analgesia.

Further investigations revealed that ventral medullary lesions of the nucleus raphe alatus (NRA) reduced front paw and classically conditioned analgesia while lesions of the nucleus raphe magnus (NRM) or nucleus reticularis paragiganocellularis (PGC) did not (but see Chance, 1980). This suggests that the source of the neural connections for these forms of analgesia is the NRA since the NRA is formed from the NRM and the PGC (Watkins, Young, Kinscheck & Mayer, 1983). The non-opiate mediated hind paw footshock-induced analgesia was reduced but not abolished by NRA lesions. Centers more rostral than the NRA do not seem to be involved since lesions of the periaqueductal grey (PAG) or decerebration had little or no effect on the analgesia induced by either type of footshock (Watkins, Kinscheck & Mayer, 1983). However, classically conditioned footshock analgesia was potentiated by rostral PAG lesions, and attenuated by caudal and dorsolateral PAG lesions, although significant analgesia was still observed after dorsolateral

lesions (Kinscheck, Watkins & Mayer, 1984).

The non-opiate cold-water swim and the opiate 2-DG induced analgesias were attenuated by lesions of the ventral portion of the PAG, while only 2-DG analgesia was attenuated by caudal PAG lesions when measured by the liminal escape procedure but not the tail flick (Bodnar et al., 1980a).

In summary then, data attempting to delineate the neural circuits of the opiate and non-opiate systems of SIA, while incomplete, reveal the following picture; it seems to support the presence of a descending neural system, originating in the brainstem medullary area, which then passes through the DLF of the spinal cord. This neural circuit seems to mediate primarily opiate, and only partially, non-opiate footshock-induced analgesia.

The functional role of SIA

In order to survive in its natural habitat an animal must continuously make choices which determine its responses. Under certain circumstances, these "choices" are automatic. Noxious stimuli will elicit withdrawal responses away from threatening stimuli. However, in other circumstances, it may be vital for the animal to remain within the environment. It is probable that the animal came to be in the particular situation because it was motivated by such factors as food seeking, marking or maintainance of territorial boundaries. Alternatively, familiarization with

surrounding areas through exploratory behavior may have been the goal. These needs may bring the animal into contact with various unexpected and often aversive and even threatening stimuli. In order to fulfill the specific goal that motivated the organism to be in that situation, the animal must choose to stay or flee, perhaps to return at a more opportune time. As an example, consider the situation of an animal attacking its prey. In many instances the intended victim will inflict damage on the attacker. It is obvious that if the attacker flees, due to the pain of injury, then the attacker's goal would not be fulfilled. If however the pain or stress of injury could be diminished, this state of affairs could allow the animal to remain in the situation and fulfill its goals. Therefore, it would seem to follow logically that, within limits, diminution of pain could play a functional role in determining an animal's response and would therefore be advantageous.

In a paradigm that was designed to test, experimentally, situations such as those described above and which might be considered "natural", the behavior of animals was examined by placing animals in situations of "social conflict". In this paradigm an "intruder" mouse is placed in the home cage of another mouse. This situation elicits a number of typical behaviors in the intruder mouse that include threat of attack, actual attack and fighting. These almost invariably lead to defeat and submission in the same

intruder. "Defeated" animals display characteristic behaviors or postures (Miczek, Thompson & Shuster, 1982) allowing easy identification. It has been found that a raised pain threshold accompanied the submissive type of postures in defeated intruders. In addition, pain suppression was also observed in the attacker mice (Teskey, Kavaliers and Hirst 1984). This elevated pain threshold was naltrexone and naloxone reversable and tended to adapt over chronic exposure to the social conflict situation and the observed analgesia was cross-tolerant to morphine (Miczek, Thompson & Shuster, 1982; Teskey, Kavaliers and Hirst, 1984; Rodgers & Hendrie, 1984; Siegfried, Frischknecht & Waser, 1984). These results, that show that both the attacker and the resident mice are analgesic, lend support for the notion that was expressed above, that being analgesic in this type of situation would be advantageous. It would be advantageous to the intruder mouse since it could not flee this situation and would thus be subjected to further biting attacks. The reduced pain sensation may allow the mouse to assume the defeat posture thus increasing the chances that the biting will cease. In the case of the resident mouse the initial "boxing" and fighting may include biting. A diminished reaction to the pain of the bites may allow the resident to effectively deal with the intruder.

Other experiments have shown that repeated pinches to the scruff of the neck in mice is accompanied by cataleptic

and analgesic responses (Amir et al., 1981). Naloxone administration before pinching will block both responses, however naloxone administration after induction of catalepsy or analgesia will only block the analgesia suggesting some dissociation of the mechanisms underlying these behaviors. It is worth noting that pinching at the scruff of the neck is a behavior that is often seen in maternal situations where the mother picks up its offspring. In some situations the emerging catalepsy may serve to allow the mother to get her pup out of danger without undue loss of time or harm to the pup (Ornstein & Amir, 1981).

Perhaps of more significance to the present analysis are the observations of a cat attacking a mouse (Ornstein & Amir, 1981). Ornstein and Amir (1981) reported that a kitten would initially attempt to bite the back or the scruff of the neck of a mouse. These bites to the mouse led to immobility which outlasted the duration of the bites. Once the mouse became immobile the kitten lost interest in them. In fact the kitten always preferred to chase the mobile as opposed to the immobile or cataleptic mice (Ornstein & Amir, 1981). Since the catalepsy is accompanied by analgesia it may be surmised that this analgesia aids the mouse in remaining immobile. It may do so by dampening the pain and thereby lessening pain related behavior (e.g. turning of head to lick the wound) or this pain suppression would reduce the amount of vocalization (squeaking) in

response to the pain of the bites from the kitten. The suppression of these pain related behaviors may reduce the kittens interest in the mouse allowing either the mouse or the kitten to leave the encounter thus insuring further survival for the mouse.

The functional significance of SIA can also be gleaned from a concurrent analysis of a number of studies which were originally designed to investigate other aspects of SIA. For example, some studies were reported that the location of shock application on the body (e.g. front vs back paws) could determine if the resultant analgesia was opiate mediated or not (for details see opioid section, Watkins, et al., 1982). The significance of the specific body region where shock is applied can be derived from experiments on "aggression-induced analgesia". In that situation the rat takes on a "boxing" stance with its front paws out in a defensive manner. It would seem that in this situation the first line of defense is the front paws and they may therefore receive the most damage from the attacker. As just pointed out, the scruff of the neck also seems to be a an area that receives a lot of attention during an attack and thus may be a particularly sensitive area. Since these two areas are initially the most affected during encounters that are life threatening it would seem to follow that the analgesias that become manifest at these times would have features that would aid survival. This line of reasoning is

corroborated by the finding that these types of analgesias are opiate mediated. As was discussed above, mediation by opiate mechanisms is associated with more intense pain and conditioning of SIA. Therefore in these situations opiate systems act to decrease the pain at the time of the encounter and are concurrently involved in the conditioning and remembering of the encounter and in this way the animal may avoid these situations in the future. Thus, the functional advantage of SIA becomes clear when the region of the body that is shocked and the underlying substrate are analyzed and compared with studies that have examined "natural" situations where SIA is observed.

As with the naturalistic "intruder-aggression" studies some of the experiments reported in this thesis were designed to analyse the functional advantage of a reduced pain threshold. Before introducing these experiments, another area of research, which has been associated with stress and its effects on learning and performance must be reviewed.

Behavioral effects of ACTH

The pituitary gland of rats is the major source of a chain of peptides which comprise the hormone adrenocorticotropin (ACTH). This peptide hormone is most frequently associated with the physiological effects of

stress. ACTH is released into the systemic circulation from the pituitary in response to a variety of noxious stimuli such as, restraint or administration of foreign proteins (Krieger, 1980). A number of pathological phenomena such as hypertrophy and hyperfunction of the adrenal cortex, involution of the thymus and the lymph nodes and ulcerations in the stomach and intestines, result from the continued release of ACTH (Selye, 1950). Aside from the effects of continued exposure to stimuli that induce increased release of ACTH, ACTH functions to regulate the production and release of steroid hormones such as corticosterone. In addition, and in parallel to the endocrine effects, ACTH has also been found to have significant behavioral effects (see de Wied, 1977).

This section reviews the effects of ACTH in "learning" paradigms. The section is divided into several major areas. The first section examines ACTH from the perspective of its aversive effect. This is followed by sections that examine the added effects of such variables as dose, time, and route of administration.

Aside from the fact that both the effects of ACTH and the phenomena of SIA are studied in the context of stress, these two areas of study share other commonalities. It should be emphasized that the variables that seem to mediate SIA (e.g. intensity, duration) seem also to mediate the effects observed in the study of the role of ACTH in

avoidance paradigms.

Applezweig and Baudry (1955) and Murphy and Miller (1955) are credited as the first to study the behavioral effects of ACTH. These investigators found that systemically injected ACTH would delay the extinction of an avoidance response, while removal of the pituitary (hypophysectomy), the major source of ACTH retarded the acquisition of the response. Subsequently the impaired acquisition of avoidance behavior, produced by pituitary section or removal of the adrenals (adrenalectomy), was found to be restored by systemically injected ACTH (Applezwieg & Moeller, 1959; de Wied, 1964;1969), thus implicating ACTH in both the acquisition and extinction of avoidance behavior.

A significant advance in the study of the behavioral effects of ACTH occurred when de Wied (1969) reported that only a small portion of the full ACTH molecule was needed to induce behavioral effects. In these structure activity studies it was found that only seven of the thirty-nine amino acids which comprised the ACTH molecule were needed to correct the impairments induced by hypophysectomy (de Wied, 1969). Of importance was the finding that only seven of the ~~amino acid~~s positioned within the full ACTH molecule (ACTH-4-10), could induce behavioral effects but had only a minor and insignificant effect on steroidogenesis. The use of ACTH-(4-10) could now serve as a tool to study the behavioral effects of ACTH while at the same time ruling out

the effect of ACTH on the adrenals, since this was previously thought to be the major effect of ACTH. The endocrine effects could now be separated from the behavioral effects.

How does ACTH affect behavior?

Generally, it can be stated that in the intact animal ACTH seems to effect the acquisition and extinction of aversively motivated behavior. These effects can be explained in at least three different ways. In the first instance, ACTH effects in avoidance paradigms can be attributed to an increase in its release relative to the level of aversion. Thus, the intensity of aversive stimulation would be augmented by the increased release of the peptide which would raise the level of "fear". This hypothesis assumed that "fear" maintained the avoidance response (see Bohus & de Wied, 1966). Second, release of ACTH may increase perceptual/attentive factors, or third, ACTH may have a direct effect on memory (engram). However, since most of the studies on the effects of ACTH have been done in the context of aversive conditioning paradigms, the fear induction hypothesis has usually been invoked to explain the effects of the peptide on behavior in general.

The question of whether ACTH has aversive properties seems important when one considers the history of experimentation with this hormone. The discovery by Selye

(see Selye, 1950) of a substance later called ACTH occurred in animals that had been stressed in some way. Subsequently, explanations of the effects of ACTH in avoidance paradigms were dominated by assumptions that the peptide induced an "emotional reaction" such as fear or anxiety. ACTH was proposed to influence the level of "fear" that was induced in aversive situations thereby increasing anxiety. Increases or (decreases) in fearfullness and anxiety were, in turn, thought to be responsible for the changes in the effectiveness of conditioning. Since ACTH release was disinhibited in aversive paradigms it was assumed to be responsible for the motivational effect (see de Wied & Bohus, 1966; Sandman & Kastin, 1981).

De Wied and Bohus (1966) rejected the "fear and anxiety response" hypothesis and suggested, in turn, that the effects of the peptide occurred through enhancement of memory processes. Because of the increased "motivational state" of the organism that occurred after the release or administration of ACTH, the associations necessary for conditioning would be stronger. This would enhance retrieval of the memory of the previously experienced conditioned event. The increased motivational state would seem to be defined, in this context, as a change in the state of the organism so that when a number of choices are available, the behavior, approach or avoidance, would be directed by previous associations, the strength of which are modulated

by ACTH administration or release. Thus, the "motivational state" of the organism is an intervening variable between the strength of conditioning and the amount remembered.

Examination of the studies done by Rigter and colleagues (Rigter, van Riezen & de Wied, 1974; Rigter & van Riezen, 1975; Rigter & Popping, 1976) using step-through passive avoidance paradigms provide support for the enhancement of memory hypothesis. In those studies, retrograde amnesia of the avoidance response, induced by CO2- or electroconvulsive shock, was reversed by ACTH administration given before the "retrieval" test. However, when the peptide was given before acquisition training it had no effect. This finding was interpreted as support for the notion that the effect of ACTH was on short term memory processes thought to be involved in the retrieval of information from long term memory.

Further studies reported that in the intact rat ACTH can facilitate acquisition of one way active avoidance (Beatty, Beatty, Bowman & Gilchrist, 1970; Bohus & Endroczi, 1965; Bohus, Nyakas & Endroczi, 1968; Stratton & Kastin, 1974). It has also been found to affect passive avoidance behavior when administered before acquisition training or retention tests (Ader, Weijnen & Moleman, 1972; Levine & Jones, 1965; Martinez, Vasquez, Jensen, Soumireu-Mourat & McGaugh, 1979). When ACTH was administered after acquisition (post-training) it facilitated passive avoidance responding

measured 24 hours later (Gold & van Buskirk, 1976a,b; Flood, Jarvik, Bennet & Orme, 1976). ACTH also effected the extinction phase of passive avoidance such that administration during training or extinction produced a resistance to extinction (Murphy & Miller, 1955; de Wied, 1969). In these studies ACTH was administered at various times after acquisition criteria had been reached resulting in improved performance of the avoidance response. The fact that the peptide could affect behavior when it was administered before or after acquisition training led to the suggestion that it affected memory processes and not simply motor behavior.

Before describing the data collected in one- and two-way avoidance studies it may be instructive to review data collected from conditioned taste aversion (CTA) paradigms. These are different from shock avoidance paradigms in that an interoceptive cue is used as the conditioning agent.

In CTA experiments classical conditioning procedures are used to pair an aversive event with a specific taste. The administration of a toxin usually comprised the aversive event. The toxin used in the examination of the effects of ACTH on CTA has usually been lithium chloride (LiCl). Rigter and colleagues (Rigter, 1975; Rigter & Popping, 1976) initially reported that when ACTH-(4-10) was administered prior to extinction trials in a LiCl-induced CTA, it delayed

extinction of the aversion. Rats injected with ACTH persisted in avoiding long after saline treated controls had returned to baseline levels.

The investigation of ACTH effects on CTA were then extended by Levine and his coworkers. They were first able to replicate Rigters' (1975) results using the full ACTH molecule ((ACTH)-1-39) (Kendler, Hennessy, Smotherman & Levine, 1976). They also reported that dexamethasone (DEX), a synthetic corticosteriod which reduces ACTH release, would attenuate LiCl-induced CTA when administered prior to conditioning (Hennessy, Smotherman & Levine, 1976). DEX inhibited the release of ACTH and it was therefore suggested that since ACTH was not present at the time of conditioning, this could account for the attenuation of the CTA. At about the same time other investigators suggested that ACTH release may actually be responsible for aversion seen in these paradigms since all CTA producing drugs also result in the concomitant release of endogenous ACTH (Riley, Jacobs & LoLordo, 1976).

In an attempt to show that, in fact, ACTH-(4-10) was able to affect extinction behavior without steroid release, Smotherman and Levine (1980) measured the levels of corticosterone during the course of experimentation. They did not find a change in the release of corticosterone that would parallel the effect of ACTH-(4-10) on extinction behavior thus suggesting that it was ACTH and not

corticosterone that was responsible for the mediation of extinction behavior in these CTA experiments.

It seems reasonably clear then that ACTH is involved in the CTA to LiCl. Perhaps the pairing of the novel solution with the toxin and the resultant "poisoning" elicited the release of ACTH which then played a modulatory role in the conditioning process. It would follow then that administration of ACTH could lead to a resistance to extinction when compared to control animals (i.e. de wied, 1964; 1969). Since, there are no reports in the literature that ACTH will by itself induce a CTA, it is probable that the peptide itself is not aversive but only enhances the perceived aversiveness of the agents that it is paired with. In this way the enhanced aversion leads to a resistance to extinction. However the mechanism by which ACTH acts in this process was not determined by these studies.

Further insights into the nature of ACTH effects on avoidance paradigms can be gleaned through the examination of studies using appetitive motivated behaviors. If it was found that the effects of ACTH in appetitive paradigms resembled those found in aversion studies, this would necessitate a revision of the hypothesis that ACTH exerts its effects by enhancing aversion.

A number of experiments have used food motivated tasks in an attempt to examine the effects of ACTH. Stratton and Kastin (1974) have shown that the acquisition of a food

motivated maze performance was facilitated by ACTH. In another study using an operant task with water as the reward, it was also found that ACTH facilitated learning (Guth, Levine & Seward, 1971). These studies that have observed effects of ACTH in appetitive paradigms which are similar to those found in aversive paradigms point at a need to revise the "aversion" hypothesis.

When the results of the studies presented above are taken together it becomes difficult to defend the "enhancement of aversion" hypothesis. Aversive paradigms, whether maintained by operant or classical conditioning procedures, and appetitive motivation tasks using food or water reward are all affected by ACTH. It would seem therefore that the effects of this peptide in these various learning paradigms lies in its ability to alter the response to environmental stimuli in general and not specifically to aversive situations. Whether it does so through attentional, or motivational mechanisms is a question which remains unanswered by these studies.

Other data that support the notion that ACTH is not an "aversive agent" emanate from studies of self-stimulation behavior. In studies of the effects of ACTH on self-stimulation of various brain areas (medial forbrain bundle & medial septal area), it was found that ACTH increased the response rate from both brain areas only when the baseline response rate was low (Nyakas, Bohus & de Wied,

1980). Instead of reducing approach behavior, which would be expected if ACTH was an aversive agent, the peptide lowered the threshold for positive responding. This was interpreted as support for the notion that the peptide role is to amplify motivational processes in general (Nyakas, Bohus & de Wied, 1980).

A second hypothesis attempting to explain the behavioral actions of ACTH was proposed by Sandman and colleagues. Sandman and Kastin (1981) suggested that the effects of ACTH could be explained as the enhancement of perceptual/attentional processes. From this hypothesis one would predict that ACTH aids in filtering out irrelevant stimuli from the environment thus allowing more selective attention. The idea was based on studies where the animal must shift its attention from one dimension (i.e. white+) to another dimension (i.e. black-) of the task. The change from one dimension to another would necessitate that the subject pay attention to only relevant stimuli (Beckwith & Sandman, 1982; Sandman & Kastin, 1981). If the subject has a better perception of, or can attend to relevant stimuli while filtering out irrelevant stimuli then the effects of ACTH in learning paradigms may be due to attentional variables.

Sandman and Kastin (1981) argued that usage of the two-choice visual discrimination task would allow examination of attributes of selective attention (Sandman, Miller, Kastin, & Schally, 1972; Sandman, Alexander, &

Kastin, 1973; Sandman, Beckwith, Gittis & Kastin, 1974; Sandman, Beckwith, & Kastin, 1980). In this paradigm, rats were initially trained to go through a white door to avoid shock. After the rat acquired the response the task was reversed. On the reversal trials the rats had to run through the black door regardless of which side of the chamber it was located in the initial learning of the task. It was thought that two factors may have operated in this type of experiment. The first part was thought to measure the animal's ability to learn a new response (avoidance). The second part was thought to measure the animals ability to selectively attend to stimuli (Mackintosh, 1965; 1969). The selective attention aspect of the task can be explained as follows: The animal can learn only that the white door is the correct one (spatial localization) or it can learn that the correct response resides along the dimension of brightness (black or white). The rat would, therefore, need to selectively attend to the brightness dimension of the door rather than its location. Animals treated with ACTH needed significantly fewer trials to solve the reversal task but no effect of the peptide was found during acquisition. These data were interpreted as an indication that ACTH effects behavior in learning paradigms primarily through attentional processes (Sandman & Kastin, 1981). In addition, observations that the effects of administration of amphetamine, which has been associated with arousal

mechanisms, were opposite to the effects of ACTH in discrimination tasks (Beckwith, Sandman, Alexander, Gerald & Goldman, 1974) and in avoidance responding (Kovacs & de Wied, 1978) also tended to support the "attention" hypothesis.

Another avenue of exploration possibly yielding further evidence for the action of ACTH on perceptual/attentive processes can be found in the examination of its effects in habituation paradigms. Responses to new stimuli tend to habituate with repeated presentations. However, the administration of ACTH has been shown to retard this effect. Habituation of an orienting response, hole-board exploration and open field behavior was found to diminish after ACTH administration (Bohus & de Wied, 1981; File, 1978). These findings, however, could be interpreted as an effect of ACTH on the intensity of the aversion inherent in these situations. One must note that these are novel, hence, stressful environments and therefore the neophobia which normally accompanies exposure to these environments may be enhanced by ACTH with the resultant retardation of habituation. Alternatively, the novel cues in these environments may have remained salient after ACTH administration due to the putative effect of ACTH in enhancing perceptual/attentive factors.

The third hypothesis was concerned with the putative motivational aspects of ACTH. De Wied (1980) has recently

extended his earlier hypothesis of "memory effects" to include the involvement of motivational factors. Improved acquisition of avoidance behavior observed after ACTH administration may be interpreted in a number of ways. The fact that ACTH was effective in many different and seemingly unrelated paradigms may argue for an effect of ACTH on motivation. De Wied (1980) referred to this phenomenon as the ability of ACTH to "temporarily enhance the motivational influence of specific environmental cues". This may be further extended to incorporate temporary enhancements of memory, attention or goal-directed behavior. At this stage in the research, this notion may be the most appropriate, since the behavioral effects of ACTH are known to be dependent on activity in limbic structures long known to be involved in motivational processes (de Wied, 1977). Also, ACTH containing neurons have been found in the limbic system, with their soma in the hypothalamus and the terminals extending to various other limbic structures (Watson & Akil, 1980). The effect of ACTH on self-stimulation behavior can also be cited in this vain since the stimulating electrodes in this study were placed in limbic system structures (Bohus, Nyakas & Endroczi, 1968). Also in keeping with the notion of an interaction between ACTH and activity in the limbic system it is worth noting that the pattern of electical wave activity in the hippocampus and thalamus induced by stimulation of the

reticular formation was altered by ACTH (Urban & de Wied, 1975). This effect of the peptide resembled the effect of increasing the intensity of the stimulation. Increases in the motivational valence of environmental stimuli may provide the background for improved performance of "species specific" defensive responses. In addition, since the effects of ACTH were apparent in both active and passive avoidance tests, it would follow that performance factors played only a minimal part (de Wied, 1980). It would seem then that the enhancement of memory hypothesis can be better understood within the context of a motivational hypothesis. While the effects of ACTH may indeed be manifested by improved memory, the improved memory itself may be due to improvement in identification and perception of the stimuli.

The studies described in this section suggest that ACTH can influence behaviors that are maintained by both avoidance or approach, thus lending support to the notion that ACTH is itself not an aversive agent. On the basis of these studies one may assume that ACTH is neither toxic nor aversive (given the right dose), but rather that it functions to enhance the motivational properties of environmental variables.

Dose and Time Dependence of ACTH

As will become clear from the following discussion, the time of administration and the dose of ACTH administered,

are important variables when determining the effects of ACTH. Time and dose considerations seem important within the context of the present thesis because they suggest a parallel with the role of these variables in the induction of SIA, since as mentioned earlier the intensity and duration of stimuli were found to be critical determinants of SIA. The comparison naturally rests on the assumption that a dose of ACTH can be considered to be similar to stress intensity, while time of administration to the duration of exposure to stress stimuli.

Time of Administration

When the metabolic clearance rate of a single dose of ACTH in the CNS of the rat is determined, it is apparent that it disappears in a biphasic manner. After the initial "quick" phase (0-10 min) there is a second slower phase where ACTH is still detected with a half-life of forty to sixty minutes (Matsuyama, Ruehman-Weinhold, Johnson & Nelson, 1972; Normand & Lalonde, 1979). Thus ACTH is a fast acting compound of relatively short duration of action indicating that it may need to be administered close in time to the behavioral event that is modified.

It has been demonstrated that the time of administration of ACTH is critical in terms of the increased retention of some behaviors observed following the administration of this compound. However, before describing

these studies, an explanation of the terms retention and other memory variables seems in order.

As reviewed above, ACTH has been implicated in theories of memory retention in that its administration leads to a resistance to extinction (e.g. de Wied, 1964). The resistance to extinction seen after ACTH administration was interpreted as the manifestation of an enhanced learning of the original acquisition. Retention then, may depend upon the process of recall or retrieval and would necessarily be tied to consolidation and storage of information. Better consolidation and storage of information may improve the retrieval process. The effect of a drug on retention of information can be determined by examining the time of administration of the drug (McGaugh, 1961; 1966). Treatments given after the acquisition phase has been completed would, (theoretically), have effects on the consolidation of the experience and therefore upon the retention. The period between the time that acquisition criterion was attained and the time that the retention of the behavior was tested is the period of consolidation. The effects of post acquisition treatment of ACTH were very strongly tied to the time after training when this peptide was given. As might be expected, the shorter the interval between training and treatment, the larger the effect, be it enhancement or impairement of memory (Gold & van Buskirk, 1976b).

Latency to re-enter a side of a box previously associated with shock experience in a passive avoidance paradigm has been used as a measure of retention by a number of investigators. Gold and van Buskirk (1976b), using this paradigm, found that the effects of ACTH on this measure decreased as they increased the time between post-trial administration of ACTH and the aversive (shock) stimulus. This observation is consistent with a consolidation hypothesis; that is, given that consolidation of memory occurs within a specific period, then ACTH must be administered within the boundaries of this time period.

Evidence that ACTH released from the pituitary may be the physiological source of these ACTH effects was presented by Gold and his colleagues (Gold, Rose, Spanis, & Hankins 1977). They found that retention deficits observed after hypophysectomy were alleviated by post-training administration of ACTH.

Shuttlebox avoidance paradigms can also be used to measure retention of information. During the extinction phase of avoidance experiments, the animal must learn a new contingency, that the aversive stimulus is no longer present. The animal, however, is not initially aware of the new contingency and therefore usually continues to respond for a time, to the conditioned stimuli. This continued responding during extinction may be taken as a measure of retention (Riccio & Concannon, 1981; Spear, 1973). It

follows that the persistence of responding during the extinction phase of avoidance experiments after the administration of ACTH can be construed as an effect on retention. The conditioning studies reported in the present thesis have used this approach. It was surmised that the data collected in a one-way shuttle avoidance paradigm might reveal the effects of stress on acquisition and extinction of avoidance in addition to the effects of analgesia in the same paradigm. This notion is discussed in further sections.

Dose response

When researchers examining the effects of ACTH have employed more than one dose of this peptide in their studies, many studies revealed a dose-response relation of the action of ACTH (Gold & van Buskirk, 1976a). As will be described below, the dose-response relationship of peripherally administered ACTH in a variety of behavioral paradigms does not seem to be a linear function but rather resembles a hyperbolic function (Gold & van Buskirk, 1976a,b). It should be recalled that Malmo (1959) predicted that the effects of hormones on behavior in general, would, when analyzed appropriatly, reveal a complex interaction resembling a U-shaped dose response curve.

Subcutaneously administered doses of ACTH can either augment or retard retention. The direction of the effect

has been found to be dependent upon the specific dose administered (Gold & van Buskirk, 1976a). Gold and van Buskirk (1976a) have found that low doses of ACTH administered immediatly after a passive avoidance task, enhanced retention when measured 24 hours later. A high dose produced retrograde amnesia. In general they found that the data describing ACTH effects on memory resembled a U-shaped dose response curve.

It has also been shown that shortening the chain of amino acids contained in the ACTH sequence in conjunction with an increase in the molecular weight of the reduced peptide (to compensate for the lowered weight) resulted in an inverted U-shaped curve when attentional processes as visual discrimination, reversal learning, (Sandman, Beckwith & Kastin, 1980) or memory processes were studied in shock avoidance tasks (Sands & Wright, 1979).

In addition it was found that the intensity of shock interacted with the effects of ACTH (Gold & van Buskirk, 1976a). A weak footshock coupled with a low or high dose of ACTH enhanced retention, while these same doses of ACTH impaired performance when coupled with a high footshock level.

Studies of self-stimulation behavior generated similar effects with doses of ACTH interacting with the current intensity that supported stimulation (Nyakas, Bohus & de Wied, 1980; described in "aversion").

It is likely, then, that the dose of administered ACTH interacts with stimulus induced release of ACTH and even summates with exogenous administration of the peptide to affect behavior. In general, in those studies that have used a range of doses, the interaction of ACTH and behavior can be reliably described as a U-shaped curve.

The analysis of the effects of ACTH from the perspective of dose level and duration of effects, reveals a number of commonalities between the studies of ACTH and the studies of SIA. The behavioral effects of ACTH and the induction of stress-induced analgesia are both dependent on intensity and duration of stimulation. Thus it may be fruitful for the understanding of the effects of stress on behavior to examine SIA within the same experimental paradigms that have been used to examined the effects of ACTH.

Can ACTH enter the CNS to affect behavior?

In most animal studies ACTH is injected intraperitoneally (i.p.) or subcutaneously (s.c.) yet it is assumed that the behavioral effects of ACTH stem from its central action. It would therefore be beneficial in this context to consider the differential routes of administration of ACTH. As stated earlier, evidence that ACTH effects were mediated through its action at the level of the central nervous system (CNS) were obtained by using

analogues of ACTH which were devoid of steroidogenic effects (de Wied, 1969; 1974) thus avoiding an effect mediated by adrenal steroid production. Also, administration of ACTH directly into the brain was found to result in such behavioral effects as grooming and avoidance of aversive situations (e.g. Ferrari, Gessa & Vargiu, 1963; van Wimersma Greidanus & de Wied, 1971).

Recently, data have been gathered which indicates that ACTH may be able to cross the blood-brain barrier or that in some circumstances it may circumvent it entirely (Mezey, Palkovits, de Kloet, Verhoef & de Wied, 1978; Oliver, Mical and Porter, 1977). Evidence has been presented which suggests that there may be a backflow of blood from the pituitary up toward the hypothalamus through the vascular channels of the pituitary stalk. Thus ACTH from the pituitary may be able to reach brain areas through this vascular system. Using radioimmunoassay techniques, Oliver, Mical and Porter (1977) were able to show that in animals with intact pituitary glands a significantly greater amount of plasma ACTH was found in the pituitary stalk as compared to hypophysectomized or anterior pituitary lobectomized animals. These observations suggested that some ACTH of anterior pituitary origin was flowing up to the brain. Subsequently, it has been shown that when a radioactive analogue of ACTH was injected directly into the pituitary, high levels of radioactivity were observed in the

hypothalamus. This in turn could be attenuated by pituitary stalk section (Mezey, Palkovits, de Kloet, Verhoef & de Wied, 1978). Other areas of the brain also displayed higher radioactivity and these authors (Mezey et al, 1978) suggested that this may have resulted from pituitary ACTH circulating through the cerebrospinal fluid.

In addition, the data gathered in experiments on electrically induced seizures which stimulate the secretion of ACTH have also tended to support the suggestion that ACTH of pituitary origin can reach the brain (Bergland, Blume, Hamilton, Monica & Paterson, 1980). In these studies the release of ACTH was electrically induced and then, at various intervals, the level of ACTH was measured in the brain. After electrical simulation, higher levels of ACTH were found in the brain (Bergland, et al., 1980). Anatomical studies using the technique of scanning electron microscopy provided evidence supporting the concept of retrograde flow. Short portal vessels have been identified which connect the anterior pituitary to the hypothalamus (Bergland & Page, 1978).

In another series of experiments tritiated ACTH analogues were administered into the pituitary and radioactivity was subsequently measured in various brain regions (Mezey & Palkovits, 1982). Uptake was determined in various areas by the punch technique which showed the presence of tritiated ACTH in the hypothalamus, septal area,

preoptic area, olfactory bulbs, medulla and spinal cord. In fact the labeled material was found in most brain areas but these areas had a higher level of uptake. Again, pituitary stalk transection reduced hypothalamic uptake suggesting a retrograde flow from the pituitary.

Taken together the evidence presented above strongly supports the notion that peripheral ACTH may enter CNS structures to influence behavior. It should also be noted that other peptides have also been shown to effect centrally mediated behavior (Kastin, Olson, Schally, & Coy, 1979). It follows then, that stimuli which induce the release of ACTH may be used to study the effects of this peptide under "normal" conditions. For example, hotplate exposure is known to release corticosterone (Galina, Sutherland & Amit, 1983), which is under the direct influence of ACTH release. It may be possible then to use the hotplate as a stimulus to study the effects of ACTH. Stimuli that induce the release of ACTH through natural mechanisms (i.e. stress) may be used to reveal the functional role of ACTH release. This notion is discussed more fully in the following section.

The present investigation

The experiments presented in this thesis were designed to assess the adaptive nature of SIA. The specific plan adopted was to examine the effects of various stimuli on

behavioral responses which normally occur simultaneously with analgesia. It was hypothesised that the adaptive nature of SIA would be best revealed and studied by analyzing these "concurrent behaviors". It seems logical that in testing situations other than specific tests of analgesia, concurrent behaviors may be governed by the same environmental characteristics inherent in those situations. If the analgesia normally observed in these situations is, in, fact a consequence of stress stimuli, its adaptive effects should be revealed by a facilitation of adaptive responding in the paradigm employing concurrent behaviors.

The initial studies examined the effects of a stressor on an unconditioned response such as locomotion, while later studies examined the effects of this same stressor on conditioned responding. In addition, involvement of the pituitary-adrenal axis in the mediation of the behavior induced by stress was also studied. Within each study the behavioral and physiological consequences of more than one level of stress stimuli were assessed. Thus, the more general hypothesis concerning the nature of the term "stress" and its role in adaptive responding could be evaluated. The goal of the present experiments was to analyse the nature of the analgesia produced in the context of conditioning studies.

Experiment 1

When rats are placed on a hotplate heated to varying temperatures, it was found that this experience would yield differential behavioral responses (Galina, Sutherland & Amit, 1983). The most reliable finding was that 57°C heat would significantly reduce the locomotor activity of rats when compared to activity of control animals placed on a plate at room temperature (21°) or other temperatures (47 and 52°). In that experiment, locomotor activity of rats was measured for thirty minutes but no attempt was made to determine at which point the reduction in behavior was manifested. Therefore in the present initial study the time course of the "stress-induced reduction in activity" was examined.

Method

Subjects. All animals in all the experiments reported below were male, albino Wistar rats obtained either from the rat colony facilities at the Rudolf Magnus Institute or Canadian Breeding Farms Laboratories. The animals were handled each day for 5 days before experimentation began. They were maintained on free access to standard lab chow and tap water. They weighed from 200-250 gms and were housed five to a cage under a 12 hour light-dark cycle. In the first experiment 68 animals were used. Number of animals per group follows designation of group membership which is

given in the method section. The number to the left of the hyphen denotes the time elapsed after stress, while the the number to the right denotes the temperature of the hotplate: (0min-21°) = 10; (0-57°) = 10; (5-21°) = 7; (5-57°) = 7; (10-21°) = 10; (10-57°) = 10; (30-21°) = 7; (30-57°) = 7.

Apparatus. The hotplate was constructed in the laboratory and consisted of an aluminum container filled with water through which a coil was looped. A circulating heat pump maintained the water at the desired temperature (57°C). The alluminum plate was placed above the circulating water. Another plate was maintained at room temperature (21-22°C) and was used as the control condition. A Plexiglass transparent tube (19.5 cm) was positioned over the plates and served to confine the animals to the plates. The activity boxes consisted of a transparent Plexiglass tube (19.5 cm) that was placed on a plastic board which was divided into four equal sections for the analysis of horizontal locomotion; a counter was activated each time the rat crossed from one section to another. Electronic stopwatches and counters were employed to time and record behaviors.

General Procedure. Animals were brought to the sound attenuated test rooms at least one hour before testing. Each animal was individually placed on either the 21° or 57° hotplate for thirty seconds. Immediatly after, (0 min), or

following a delay of 5, 10, or 30 min the rats were tested in the activity boxes. They were replaced in their holding cages during the delay but remained in the testing room. Five behaviors were recorded: horizontal locomotion, rearing, time spent sniffing, and length and frequency of grooming bouts.

A separate group of rats (n = 4) was run in the same manner as described above. The rats were tested to determine the effect that simple exposure to the activity box would have on their locomotor behavior. They were tested on a different day and thus were not included in the statistical analysis used to analyze the other groups.

Results

Thirty seconds of exposure to the hotplate stress induced a short term reduction in locomotor activity (Fig. 2). Analysis of variance revealed a significant effect of temperature ($F(1,60) = 22.5$, $p<0.001$) and a significant effect of time ($F(3,60) = 8.9$, $p < 0.001$), as well as a non-significant interaction. It would seem, therefore, that the short term reduction in activity was still evident when animals were tested 5 minutes after stress, but dissipated within 10 minutes. This was confirmed by post-hoc Newman-Keuls analysis ($p < 0.05$, Fig. 2).

Depicted in Figure 2 are the mean locomotor activity counts of a separate control group (no-stress) from the same

batch of animals. This group did not receive any exposure to any of the hotplates. They were simply removed from their cages and placed in the activity box for 5 minutes. A t-test revealed no difference between this group and the 21 controls.

The effects of heat-stress on grooming behavior were not significant. The data collected on the rearing and sniffing behavior resembled the locomotion data with respect to differences between groups. Therefore, in Figure 1, only the locomotion data for this experiment is displayed.

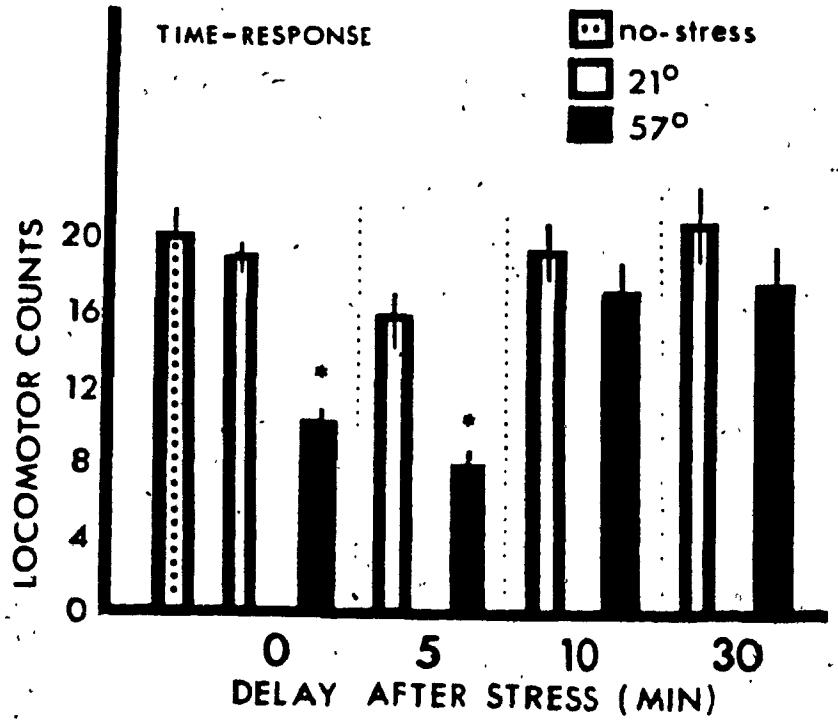

Fig. 2. Effect of heat-stress on locomotor activity after various time intervals. There was a significant effect of Time ($p<0.001$) and Temperature ($p<0.001$). Significant differences were found between 21° and 57°C only at 0 and 5 min post stress (Newman-Keuls, $p<0.05$).

Experiment 2

Since we had previously shown that heat-stress raises the plasma levels of corticosterone (Galina, Sutherland & Amit, 1983) indicating an increased release from the adrenal cortex and since others have detected opiate-like material in the adrenal medulla (R.V. Lewis, Stern, Rossier, Stein, & Udenfriend, 1979, R.V. Lewis, Stern, Kimora, Rossier, Stein, & Udenfriend, 1980; Shultzberg, Lundberg, Hokfelt, Terenius, Brandt, Elde & Goldstein, 1978; Viveros, Diliberto, Hazum, & Chang, 1979; Yang, Digiulio, Gratta, Hong, Majane & Costa, 1980). I repeated the previous experiment (Exp. 1) following surgical removal the adrenal medulla and the adrenal cortex. I reasoned that adrenalectomy and adrenal-medullectomy would reveal whether adrenal activity was involved in the mediation of the present results (Exp.2). In addition, as reviewed above, some forms of SIA may be mediated by endogenous opiates from the adrenal medulla (J.W. Lewis et al., 1982), therefore this experiment also served to document the similarities between the locomotor and analgesic effects of this short-duration stressor.

Method

Subjects. Sixty male, albino Wistar rats were obtained from the rat colony facilities at the Rudolf Magnus Institute. The animals were handled each day for 5 days

before experimentation began. They were maintained on free access to standard lab chow and tap water. They weighed from 200-250 gms and were housed five to a cage under a 12 hour light-dark cycle. Each cage contained two bottles; one with tap water, the other with a physiological saline solution to maintain normal sodium balance. There were ten animals per group.

Procedure. Bilateral adrenalectomy (ADX), adrenal-medullectomy (ADMX), or sham operations were performed under ether anesthesia one week before testing. The same experimental conditions existed as in Exp. 1 Briefly, the rats were brought to the sound attenuated test rooms at least one hour before testing. Each animal was individually placed on either the 21° or 57° hotplate for thirty seconds. Immediatly after (0 min) hotplate exposure, the rats locomotor activity in the activity boxes was recorded.

Results

The effects of the surgical manipulations on locomotor activity are depicted in Fig. 3. Analysis of variance (3 surgical treatments x 2 temperatures) revealed a significant treatment effect ($F(2,54) = 4.28$, $p<0.05$) and a significant effect of temperature ($F(1,54) = 41.34$, $p<0.0001$) and no interaction. Post hoc Newman-Keuls tests (Fig. 3) indicated that each group exposed to 57°C hotplate was significantly

different from its appropriate 21° control group. Also a significant difference was found between the 21° ADX and the 21° ADMX groups. None of the 57° groups differed from each other. Therefore, it would seem that these surgical manipulations did not affect the 57° heat stress-induced reduction in locomotor activity. Thus, the reduction in locomotion observed in this study did not seem to be mediated by adrenal hormones.

Adrenal medullary opioids have been implicated in the analgesia induced by three minutes of intense footshock (continuous 2.5 mA) since demedullation or denervation of the adrenal medulla reduced this form of analgesia (Lewis et al., 1982). These results did not parallel the results obtained when SIA was examined. However, since the duration and the nature of the two stressors are different (3 min. vs 30 sec.) the results are not directly comparable.

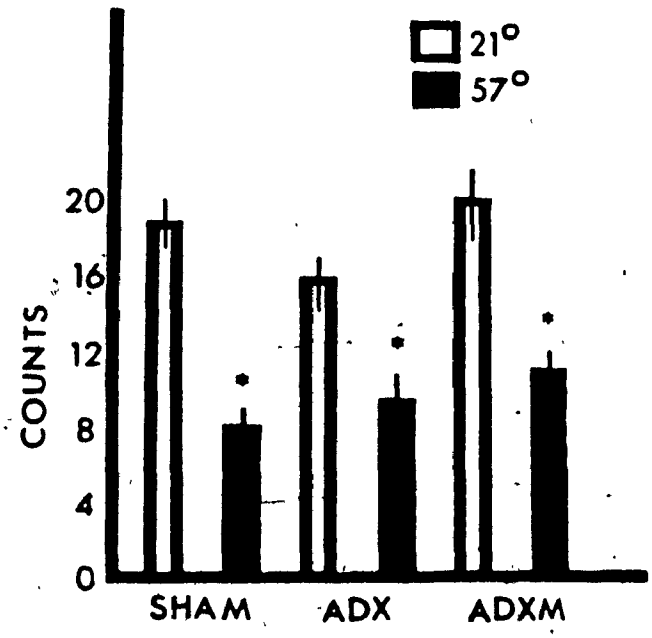

Fig. 3. Effect of adrenalectomy or adrenal-medullectomy or sham surgery on locomotor activity after heat-stress. Newman-keuls post hoc tests indicated that all 57° groups differed significantly from 21° groups ($p < 0.05$).

Experiment 3

Having ruled out the involvement of one component of the pituitary-adrenal axis, I then examined the possibility that a pituitary factor was involved in the mediation of the reduction in activity following heat-stress. The major source of endogenous ACTH is the pituitary, therefore removal of the pituitary could determine the involvement of this source of ACTH and other pituitary factors in the reduction in activity. Furthermore, since it had previously been established that exogenous administration of ACTH could affect motor activity (Amir, et al., 1980; File, 1978), this present study could provide an opportunity to verify these suggestions.

Hypophysectomy has also been shown to affect SIA in that it attenuated analgesia induced by 30 minutes of immobilization (Amir & Amit, 1979), 3.5 minutes of CWS (Bodnar et al ., 1979; 1980), intense footshock (Millan et al., 1980), and prolonged footshock (J.W. Lewis et al., 1984; MacLennan et al., 1982).

Since it has previously been shown that ACTH-(1-39) reduced activity when administered to animals before open field testing (Amir, Galina, Blair; Brown & Amit, 1980), it was decided to monitor the locomotor activity of a number of different groups to ascertain the effect of the administration of ACTH-(4-10). ACTH-(4-10) was used in this experiment since most of the behavioral actions of ACTH on

central mechanisms reside within this peptide sequence (Gispen et al., 1975). Also this sequence does not stimulate the adrenal cortex nor does it induce the syndrome of excessive grooming (Gispen & Isaacson, 1981; Gispen et al., 1975) which was found to be absent in Experiment 1.

Method

Subjects. Fifty-eight male, albino Wistar rats obtained from the rat colony facilities at the Rudolf Magnus Institute. The animals were handled each day for 5 days before experimentation began. They were maintained on free access to standard lab chow and tap water. They weighed from 200-250 gms and were housed five to a cage under a 12 hour light-dark cycle.

Procedure. Transauricular hypophysectomy (HPX) or sham operations were performed one week before testing (N = 33). Four groups were run; the group code refers to the type of surgery and to the temperature of the hotplate: sham/21°, sham/57°, HPX/21 , and HPX/57°. An additional four groups (N = 25) of HPX or sham animals were administered ACTH-(4-10) (s.c.) one hour before testing. The testing procedure was identical to that described in EXP. 2. Briefly, the rats were brought to the sound attenuated test rooms at least one hour before testing. Each animal was individually placed on either the 21° or 57° hotplate for thirty seconds. Immediately after (0 min) hotplate

exposure, the rats' locomotor activity in the activity boxes was recorded. All hypophysectomies were verified by macroscopic examination of the sella turcica. The number of animals per group was as follows: 21-sham, 57-sham and 57-HPX consisted of eight animals each; 21-HPX had nine. Sham-saline, sham-ACTH-(4-10) and HPX-saline consisted of six animals each; HPX-ACTH-(4-10) contained seven animals.

Results

Figure 4 illustrates the data of the HPX and ACTH-(4-10) groups. There was no significant effect of HPX or temperature, but there was a significant interaction (ANOVA; Surgery x Temperature ($F(1,29) = 14.4$, $p<0.001$). Newman-Keuls ($p<0.05$) post-hoc tests indicated that the 21° sham and the 57° sham groups were significantly different (see first panel on left in Fig. 4). This finding would suggest that the original effect of the stress was present despite the sham operation. In addition, groups 57° sham and 57° HPX were significantly different, as were groups 21° HPX and 57° HPX. There were no other significant group differences. Since 57° sham and 57° HPX were different and there was no difference between 21° sham and 57° HPX this would suggest that the present results are consistent with the observation that HPX prevented or masked the reduction in activity which was manifest when the severe stressor was presented.

Also depicted in Fig. 4 are the data from the additional groups that were run under the same conditions (see panel on right side of Fig. 4). All these groups received only the 57° stress. Sham-saline, sham-ACTH-(4-10), and HPX-ACTH-(4-10) were not significantly different from each other (t-test, $p>0.05$). HPX-saline group was significantly different from all the rest (t-test, all $p<0.05$ after correction for multiple t-tests). The HPX-saline group did not exhibit the reduction in locomotion seen in the other groups exposed to 57°. Therefore it seems that ACTH-(4-10) can reinstate the reduction in activity which was prevented by HPX. These data are compatible with the view that ACTH from pituitary sources at least partially mediates the reduction in locomotor behavior following stress.

Concerning the previously discussed similarities between locomotion and SIA, the present results indicate that the mediation of the locomotor effects, like the reduction of SIA, may be mediated by pituitary factors. However, the reduction in locomotion observed in this experiment was mediated by ACTH while there have been no reports suggesting that ACTH can mediate SIA. These results suggest that there is a dissociation between pituitary involvement in SIA and locomotor behavior.

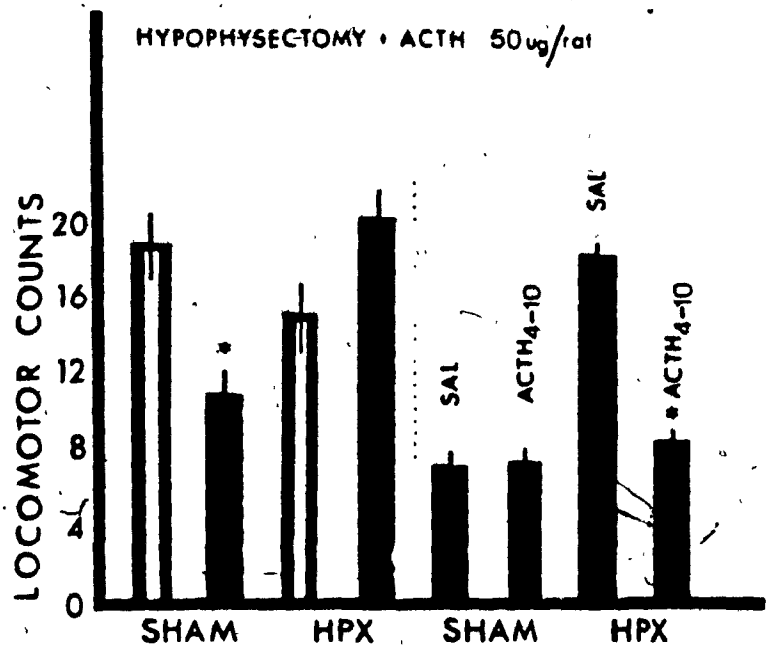

Fig. 4. Effect of hypophysectomy (HPX) on locomotor activity after heat-stress. There was a significant Interaction (Temp x HPX, p 0.001) and Newman-Keuls indicated that sham and 57° was significantly different from the other groups ($p<0.05$). ACTH-(4-10) administered s.c. 1 hr before testing to sham or HPX animals reduced activity compared to HPX and saline group (reinstated the reduction in activity in HPX animals) (T-test, $p<0.05$). See Fig.1 for legend.

Experiment 4

Having established that ACTH could influence locomotor behavior after stress in HPX animals I decided to investigate the role that ACTH could have in intact animals using the same experimental paradigm as in the earlier experiments in this thesis.

The ACTH molecule is part of a larger molecule, termed, pro-opiomelanocortin known to be the common precursor to a number of peptides (Krieger & Liotta, 1979). Pro-opiomelanocortin is a molecule of both hypothalamic and pituitary origin. Cleavage of the molecule into smaller chains (Nakanishi, Inouve, Kita, Nakamura, Chang, Cohen & Numa, 1979) yields sequences, such as ACTH and alpha melanocyte stimulating hormone (y-MSH), that have both biochemical and behavioral effects that are often antagonistic. For example, y-MSH and ACTH were found to have opposite effects on avoidance behavior (van Ree, Bohus, Csontos, Gispen, Greven, Niijkamp, Opmeer, de Rotte, Wimersma Greidanus, Witter & de Wied, 1981a,b) y-MSH can attenuate ACTH-induced excessive grooming behavior (van Ree, et al., 1981), on the other hand both y-MSH and ACTH can induce withdrawal signs in opiate naive animals. Since y-MSH has been suggested to have agonist as well as antagonist actions in relation to ACTH-induced behaviors (Plomp & van Ree, 1978; van Ree, et al., 1981a,b), I reasoned that administration of y-MSH would aid in the

elucidation of the mechanism underlying the action of ACTH.

Method

Subjects. Seventy-five male, albino Wistar rats obtained from the rat colony facilities at the Rudolf Magnus Institute and were handled each day for 5 days before experimentation began. They were maintained on free access to standard lab chow and tap water. They weighed from 200-250 gms and were housed five to a cage under a 12 hour light-dark cycle.

Procedure. The procedure was identical to that in Exp. 2 except that ACTH-(4-10) was administered (sc) to intact animals one hour before testing began. The doses of ACTH-(4-10) were: 0, 0.4, 2, 10, or 50 ug/rat. One hour following injection the rats were brought to the sound attenuated test rooms at least one hour before testing. Each animal was individually placed on either the 21° or 57° hot-plate for thirty seconds. Immediatly after, (0 min), the rats were tested in the activity boxes. Each dose of ACTH was paired with the two temperatures (21° and 57°). There were six animals per group except for those receiving 0.4 ug of ACTH-(4-10) which consisted of five subjects.

Gamma-MSH (y-MSH) was administered to two groups of rats. The dose of y-MSH-(1-12-OH) was 50 ug/rat given one hour before testing. There were 10 rats in the group that received y-MSH and then exposure to 21° hot-plate and 5

animals in the group that received γ-MSH and exposure to 57° hot-plate.

Results

Figure 5 depicts the data obtained from the various control and experimental groups employed in this study (see both panels on left side of Fig. 5). ANOVA (Temperature x ACTH treatment) revealed a significant effect of Temperature ($F,(2,38) = 42.3$, $p<0.001$) and a significant interaction between Temperature and ACTH ($F,(2,38) = 7.6$, $p<0.001$). There were no differences between the saline and ACTH-(4-10) groups in the no-stress group (taken from home cage to activity box with no intervening stress). When saline was administered to the 21° group it had no effect on locomotion, however when ACTH-(4-10) was administered at a dose of 50 ug/rat, a reduction of activity levels of the animals, almost to the same level as the group that received the 57° stress and saline administration, was observed. That is, when ACTH was administered to the group that received no intervening stress before placement into the activity boxes, it had no effect. However, when it was administered to the group that was exposed to the room temperature hot-plate (21°) it significantly reduced activity. (All other doses had no effect). Thus it seems that ACTH-(4-10) alone, at this dose, is not a sufficient condition, but that it must be combined with environmental factors (e.g. novelty stress

associated with exposure to the 21° hot-plate) to exhibit the temporary akinesia. Other studies also reported that the administration of the full ACTH-(1-39) peptide at comparable doses to that applied here also lead to a reduction in activity when combined with stress novelty-stress (Amir, Galina, Blair, Brown & Amit, 1980) indicating that the effect generalizes to the larger peptide sequence.

There was no effect of γ-MSH in the present study, (see far right panel of Fig. 5), which suggests that within the context of the present experiment the effects of ACTH on locomotor activity operated through mechanisms which were dissociable from those involved in the effects of γ-MSH.

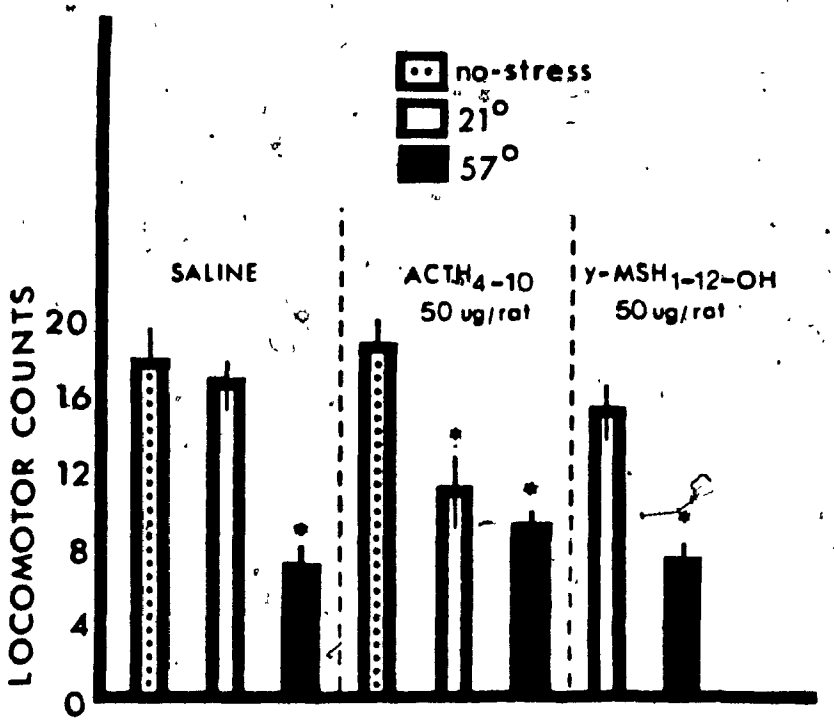

Fig. 5. Effect of ACTH-(4-10) and γ-MSH on intact animals given s.c. 1 hr before testing. ACTH-(4-10) significantly reduced activity ($p<0.05$) in 21° animals. γ-MSH was ineffective in modulating the stress-reduced activity.

Experiment 5

ACTH-like peptides were reported to bind and compete for occupation of opiate receptor sites in CNS tissue as determined by pharmacological assays (Akil, Hewlitt, Barchas & Li, 1980; Plomp & van Ree, 1978; Terenius, 1976; Terenius, Gispen & de Wied, 1975) as well as behavioral studies (Amir et al., 1980; Belcher, Smock & Fields, 1982; Bertolini, Poggioli & Ferrari, 1979; Gispen, Wiegant, Greven & de Wied, 1975; Smock & Fields, 1981). Since the results presented here suggested that ACTH was a candidate for mediating the reduction in locomotor behavior, an analysis of the effect of an opiate antagonist, naloxone, was undertaken. If this reduction was mediated through a naloxone sensitive receptor then naloxone administration should attenuate or block ACTH-induced behavior. Since naloxone and naltrexone, at some doses have been shown to reduce activity on their own (Amir et al., 1980; Arnsten & Segal, 1979; Katz, 1979; Walker, Bernston, Paulucci & Champney, 1981) a wide range of doses were used in the present experiment.

Method

Subjects. Male, albino Wistar rats were obtained from the rat colony facilities at the Rudolf Magnus Institute and were handled each day for 5 days before experimentation began. They were maintained on free access to standard lab chow and tap water. They weighed from 200-250 gms and were

housed five to a cage under a 12-hour light-dark cycle.

Procedure. Immediatly after exposure to one of the hot-plates (21 and 57°) each group of rats was injected with one of three doses of naloxone (i.p.), saline (vehicle), or received no injection. Five minutes later the rats were placed in the activity boxes for five minutes where their locomotor behavior was recorded. The doses of naloxone were: 0.0, 0.1, 0.25, and 1.0 mg/kg. Each dose of naloxone was combined with 21° hot-plate and with the 57° hot-plate. Each group contained five animals except the groups that were exposed to 57° and then injected with 0.25 mg naloxone and those exposed to the 21° hot-plate and injected with saline (0.0) which had four each. Each rat was tested in only one condition.

Results

Figure 6 represents the data collected from the naloxone experiment. Analysis of variance indicated that there was a significant effect of temperature ($F(4,47) = 18.6$, $p<0.001$, of treatment (naloxone) ($F(4,47) = 17.7$, $p<0.001$) and an interaction between treatment and temperature ($F(4,47) = 4.4$, $p<0.01$). Naloxone at doses of 0.25 and 1.0 mg/kg mimicked the reduction in activity seen after exposure to the 57° hot-plate even in the 21° groups, however the 0.1 mg/kg dose which had no effect on the 21° group was also without effect on the reduction in activity induced by

exposure to 57°.

In a separate study the effects of naltrexone (0.0, 0.1, 1.0, or 10 mg/kg), given two hours before hot-plate stress were examined (Galina, Sutherland & Amit, 1982, unpublished). The results were similar to those obtained with naloxone. Since the opiate antagonists naloxone and naltrexone were unable to attenuate or block the reduction in activity one can assume that the ACTH mediated reduction in activity was not functioning as an agonist at opiate receptors in the present studies. Therefore, it can be concluded that the reduction in activity was not mediated by endogenous opioids interacting at a naloxone or naltrexone sensitive site.

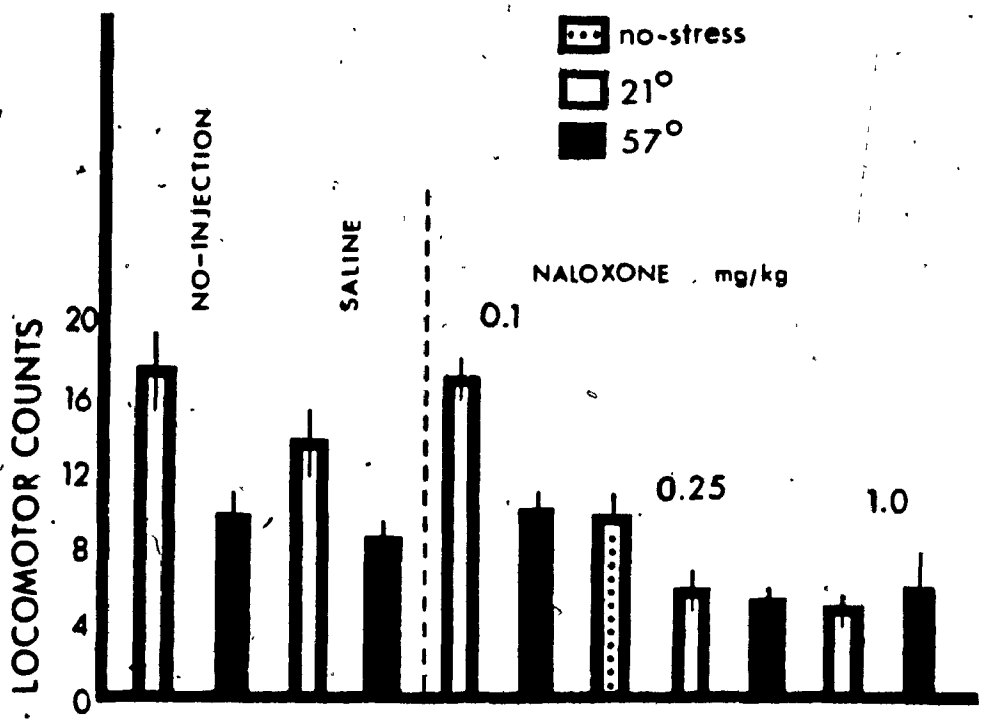

Fig. 6. Effect of various doses of naloxone (i.p.) immediatly after stress on heat-stress induced inactivity. There was a significant effect of Temperature ($p<0.001$) and Naloxone ($p<0.001$) and Temp x Naloxone ($p<0.05$). Newman-Keuls post-hoc tests indicated that naloxone (0.1 mg/kg) was not different than the control that did not receive an injection of saline and 21° group.

Discussion

The previous five experiments were designed to assess the physiological and behavioral parameters of the effects of exposure to the stress of a hot-plate heated to various temperatures. In summary, it was found that heat-stress of 30 seconds duration on a hot-plate heated to 57°C would temporarily reduce activity levels of rats when compared to non-stressed control groups. In the course of experimentation a number of putative mediators of this phenomenon were ruled out. Neither adrenalectomy, adrenal-medullectomy, naloxone administration, nor injection of γ-MSH were able to effect the reduction observed after stress. HPX, however, did abolish or mask the reduction in locomotor activity and this reduction in activity could be reinstated by administration of ACTH-(4-10) (50 ug/rat). Not only did the (4-10) analog reduce activity but the full molecule, at 50 ug and even higher doses also reduced activity (Amir, et al., 1980; Galina, Sutherland & Amir, 1983), which indicates that these behavioral effects may reside in the (4-10) sequence. The possibility should be noted that after a peripheral injection of a dose of 50 ug and the subsequent flow through the systemic circulation, only a small dose (perhaps of physiological level) would reach the CNS. In fact it has been reported that when an analog of ACTH was administered subcutaneously, brain uptake was in the order of 10-(-5) to 10-(-4) times the administered dose (Verhoef & Witter, 1976).

Behavioral support for the presence of an ACTH receptor in the CNS

An unexpected dividend obtained from analysis of the present experiments was that interpretation of the data may support the notion of the existence of a specific ACTH receptor in the central nervous system (CNS). Attempts to characterize a corticotropin (ACTH) receptor in the brain have been fraught with difficulties. The prevailing speculation is that ACTH does bind with high affinity to brain tissue, but that the paucity of such binding sites makes it a difficult class of receptors to isolate (de Kloet & de Wied, 1980; Watson & Akil, 1981; de Wied, 1977; Witter; 1980). It would seem that at present a pharmacological demonstration of such receptors must await newer technologies. Nevertheless, behavioral evidence for the existence of such receptors has emerged.

For example, many novel and/or stressful situations were found to induce a syndrome of excessive grooming in the rat (see Gispen & Isaacson, 1981). This novelty-induced grooming was blocked by anti-ACTH serum administered centrally (Dunn, Green & Isaacson, 1979). ACTH-induced excessive grooming occurred after central administration of the peptide (Gispen et al., 1975) and was attenuated by pre-administration of opiate antagonists (Dunn, Green &

Isaacson, 1979; Gispen et al., 1975). Since ACTH-induced excessive grooming was attenuated by opiate antagonists it seemed reasonable to ascribe these effects to an interaction between ACTH and opiate receptor sites. These behavioral observations along with some pharmacological and biochemical data that reported low binding capability of ACTH to brain tissue (Gispen, Buitelaar, Wiegant, Terenius & de Wied, 1976; Terenius, 1976; Terenius, Gispen & de Wied, 1975) were consistent with this hypothesis. However, new evidence has accumulated which challenged the notion that the opiate receptor is the natural site of the central actions of ACTH. It was recently shown that some peptides which lack opiate receptor affinity can nonetheless induce the syndrome of excessive grooming (Aloyo, Zwiers & Gispen, 1982).

Another area which recently received some attention was the study of the change in pain responses after central administration of ACTH-related peptides. ACTH was shown to induce analgesia in a dose dependent fashion which was not affected by naloxone administration nor morphine-induced tolerance (Walker, Bernston, Sandman, Kastin & Akil, 1981). This again supported the idea that ACTH-induced behaviors may be mediated at a site different from the opiate receptor.

The data collected in the previous five experiments support the notion that a short intense stressor can release ACTH and that this ACTH can be responsible for mediating a

reduction in activity for a short time period. In addition, the fact that ACTH-(4-10) does not have any steroidogenic properties and induced a reinstatement of the behavior in HPX animals leads to the suggestion that these effects were of central origin. Furthermore, since naloxone was not capable of altering the behavior, the suggestion was that ACTH in this paradigm acts at a receptor site distinct from the naloxone sensitive receptor. This site may in fact be an ACTH specific receptor.

Therefore, it is conceivable that ACTH acting at its own receptor, can induce a transient reduction in activity in animals which have been exposed to stress. Within an evolutionary framework the advantage of this state of affairs would be that it would seem to allow the organism time to recuperate from the physiological effects of the stressor and help it mobilize for further action. ACTH alone, at the doses tested, does not seem to be a sufficient condition to induce the reduction in locomotor activity but must be combined with environmental stimuli in order to observe the reduction in activity. This implies that tonic levels of the hormone are involved in endocrine balance and that under conditions of stress, the greater release of ACTH elicited by environmental factors can cause a transient reduction in activity. This is supported by recent data which showed that 57 °C stress increased significantly the plasma levels of ACTH-like immunoreactivty reduced the

hypothalamic levels of this immunoreactivity and increased the levels in the rest of the brain (Galina, in preparation). Also data previously collected on corticosterone release by this same stressor supports this notion since corticosterone is known to be under the control of ACTH secretion (Galina, Sutherland & Amit, 1983). These observations agree with the motivational and attentional role attributed to ACTH-related peptides (Sandman & Kastin, 1981; de Wied, 1977; 1980).

Finally, because of the high dose needed for ACTH to act as an agonist or antagonist at opioid receptor sites, a more parsimonious explanation for the site of the effects of ACTH requires the postulation that many of the effects of ACTH formerly ascribed to interaction with an opiate receptor should in fact be ascribed to an action at a specific ACTH receptor. ACTH-(1-39) has exhibited the greatest affinity to the opiate receptor while ACTH-(4-10) in some preparations will not bind to these receptors even at high concentrations (Akil et al., 1980; Plomp & van Ree, 1978; Stengard-Pederson & Larson, 1981; Terenius, 1976; Terenius, Gispen & de Wied, 1975). It should be emphasized that ACTH-(4-10) has no steroidogenic properties and that its effects are restricted to central sites (see de Wied, 1977; 1980). Also, it may be suggested that naloxone and naltrexone can act at this putative receptor as a partial agonist or antagonist depending on dose. In line with this

reasoning, naltrexone will affect ACTH-induced behavior in a dose dependent fashion (Amir et al., 1980). Also there is evidence to suggest that naloxone can activate corticosterone release from the adrenals (Eisenberg, 1980). Naloxone may then be capable of interacting with ACTH receptors on the surface of the adrenals to initiate corticogenesis. Therefore it may also have the capacity to bind to ACTH receptors in brain tissue.

Experiment 6

In the previous five experiments the effects of exposure to the hot-plate stressor were examined in a number of different ways. It was found that the intensity of heat would determine the direction of effects of this stressor on locomotor activity in an open field. These results are in line with a previous demonstration that midrange levels of heat stress (47°or 52°C) increased, while the higher levels depressed exploratory behavior relative to a control group (21°) (Galina, Sutherland & Amit, 1983). It was also found that the reduction in activity lasted five minutes and that it had returned to baseline by ten minutes. In addition, it was also demonstrated that removal of the pituitary would prevent the reduction in locomotion seen after exposure to the 57° C hot-plate and that this behavior could be reinstated by administration of ACTH, thus implicating ACTH of pituitary origin in the mediation of this behavior.

In the following experiment the same heat stressor was used as a stimulus in an attempt to examine whether changes in pain threshold would also be evident in addition to the changes in locomotor behavior seen in the previous experiments. Since, SIA is known to be dependent on stimulus parameters such as intensity and since it is known that locomotor activity and activation of the pituitary adrenal axis (i.e. corticosterone) can also be a function of the intensity of stress stimuli, four different intensities

of heat were used in the present experiment. Furthermore, since some forms of analgesia were shown to be dependent on activity in opiate receptor systems, naltrexone, an opiate antagonist was also used. Naltrexone blockade of the resultant analgesia would indicate involvement of opiate receptor systems in the mediation of the analgesia.

Method

Subjects. Sixty-four male wistar rats (n=8) obtained from the Canadian Breeding Farms Laboratories. The rats were handled each day for 5 days before experimentation began. They were maintained on free access to standard lab chow and tap water. They weighed 200-250 gms and were singly housed in stainless steel cages under a 12 hour light-dark cycle.

Apparatus. The hot-plates were similar to those described in Experiment 1 except that in this case the aluminum hot-plates were heated by a Plexiglass box filled with distilled water situated immediatly underneath the plates. The water was heated and circulated by a Haake (model E2) water pump and heater.

In order to measure analgesia in a water tail-flick test the animals had to be lightly restrained so that the only movement in response to the hot water would be a tail flick. A plastic baby bottle (Evenflo, 8 oz) that had the bottom cut out was used. The rats readily entered the

bottle at the end that was cut and crawled to the tapered end (the side to which the nipple would normally be attached) where their heads would protrude. This provided an unobtrusive restraining device that provided easy access to the tail.

Procedure. For seven days before experimentation began the rats was placed for 30 seconds into the "baby bottle" apparatus in order to acclimatize them to the procedure thus avoiding additional stress on the day of experimentation.

The procedure consisted of placing the rats on one of four hot-plates for 30 seconds. In this experiment, each hot-plate was set at a different temperature (21, 47, 52, or 57°C). Two hours before exposure to the hot-plates the rats were injected with either saline or naltrexone (10 mg/kg/ml). The rats were brought to the test room and were individually kept in small wooden holding cages for 30 minutes in order to habituate them to the noise of the hot-plate apparatus. The rats were then placed on one of the hot-plates for 30 seconds. After 30 seconds the animals were removed and placed in a plastic baby bottle. Analgesia was measured by the tail-flick method. A hot-water bath was heated to 45° C into which five cm of the tail was submerged. The latency to flick the tail out of the water was recorded. In order to avoid damage to the tail, a 30 second cutoff was established. Any rat that did not exhibit a clearly definable tail-flick during the 30 seconds of

immersion in the water was replaced in the holding cage to be tested at the next interval. Latency to tail-flick was measured at 0, 5, 10, 15, 30, 60, 120 minutes and 24 hours after exposure to the hot-plate. Thus, there were eight time periods, four hot-plate temperatures and eight animals per group.

Results

Mean (±S.E.M) latencies to withdraw the tail from the hot-water bath are shown in Figure 8. A three-way ANOVA (Temp x Naltrexone x Time) revealed a significant effect of temperature, ($F(3,52)=8.78$, $p<0.001$, Time, ($F(7,456)=3.20$, $p<0.001$, and an interaction between temperature and time ($F(21,456)=3.72$, $p<0.001$. The main effect of naltrexone was not significant. Post-hoc Tukey tests indicated that exposure to 57°C induced a transient (15 min duration) analgesia as compared to exposure to 21°C hotplate. The significant interaction effect seems to result from the slow decrease over time of the analgesia exhibited by the 57° group and the increase in analgesia exhibited over time by the 21 control group. This suggested that the tail-flick procedure itself can induce analgesia when the rat is repeatedly exposed to the testing procedure. The groups that were exposed to the 47 and 52° hot-plates, however, did not exhibit analgesia.

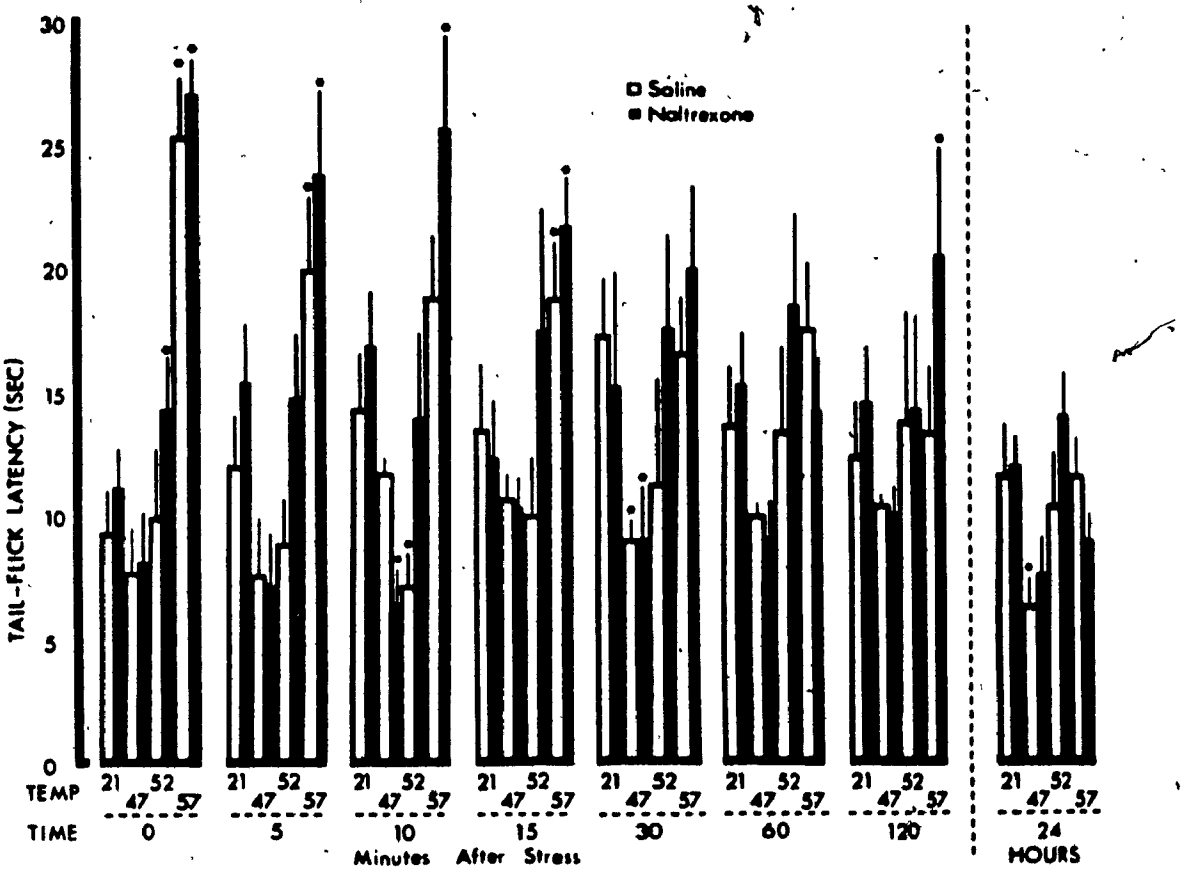

Fig. 7. Effect of various levels of heat-stress on induction of analgesia in a tail flick test. There was significant effect of exposure to 57° that was not reversed by naltrexone administration.

Discussion

The results of this experiment constitute a further demonstration that a short duration stressor induced a transient though significant analgesia that was dependent upon stress intensity. As would be predicted from previous research on SIA induced by short duration stressors, naltrexone did not effect the present analgesia (i.e. Terman, et al., 1984).

The results of this experiment provide the framework within which a component of the general hypothesis under discussion in this thesis can be tested. This hypothesis states that SIA is part of the animal's defensive system and would serve to aid the animal when its integrity was being challenged. If the analgesia present when the animal was stressed served an adaptive purpose then this would be evident in a situation designed to study it. The next two experiments addressed this particular hypothesis by presenting the same stressor to the animals before (or after) a threatening situation.

Experiment 7

The one way shuttle avoidance paradigm is a common procedure used in learning experiments with rats. Avoidance responding can be viewed as an integrated defensive reaction that would be functionally useful in natural settings. Rats will usually acquire this response within twenty trials when the shock intensity is set at .5mA. Since each trial in the avoidance procedure about to be described lasts less than one minute, the time needed to acquire the avoidance response is therefore well within the time frame of the analgesia induced by heat-stress. Thus, if the rat is exposed to the stress stimulus immediatly before training, an assessment of the effects of a raised pain threshold on avoidance acquisition could be carried out.

During the extinction phase of avoidance experiments the rat must learn a new contingency; that the aversive stimulus is no longer present. The rat is not initially aware of this new situation and may continue to respond to conditioned stimuli. This continued responding may be taken as a measure of retention (Riccio & Concannon, 1981; Spear, 1973). As Spear (1973) has suggested, the physiological changes brought about by stimuli that precede avoidance testing become part of the stimulus complex. It follows from this line of reasoning that in the present experiment stress-induced analgesia was part of the stimulus complex

for some of the groups.

This experiment could also verify some of the notions proposed in the Introduction. It should be recalled that Malmo (1959) advocated the use of more than one level of the "activating" stimulus. Since different levels of heat-stress induce different background hormonal conditions (i.e. level of corticosterone; Galina, Sutherland & Amit, 1983); the interaction between the hormonal state (corticosterone level) and the environment (avoidance training) could be tested. As a result of exposure to different intensities of stimuli some of the groups would be sensitized by preexposure to stress and this may facilitate adaptive responding. This sensitization may be manifested by improved attentional and motivational factors which may lead to improved responding as measured against baseline control level of responding.

Given the notions just expressed, in this experiment several groups of rats were exposed to different levels of heat-stress immediatly before acquisition of avoidance. In this way it was possible to determine the effect of a raised pain threshold on performance of an avoidance response (Exp.7a). In addition, an experiment (Exp. 7b) was performed in which the heat-stress was given immediatly after acquisition in order to rule out simple effects of the stress on performance.

Another component of the stress response, the release

of ACTH and its subsequent effects on behavior in avoidance situations could also be studied within the context of the present experiments. As discussed earlier, ACTH can modulate an animal's response in avoidance learning situations that may depend on the dose of ACTH or the intensity of the aversive stimuli. Thus, this experimental design allowed the study of the effects of a raised pain threshold in a paradigm similar to those used to assess the effects of ACTH on performance of learned responses.

Method

Subjects. Thirty-one male Wistar rats were used in Exp. 7a and twenty-eight were used in Exp. 7b. They were obtained from the Canadian Breeding Farms Laboratories and were handled each day for 5 days before experimentation began. They were maintained on free access to standard lab chow and tap water. They weighed from 200-250 gms and were singly housed in stainless steel cages under a 12 hour light-dark cycle.

Apparatus

The hot-plates were the same as those described in Experiment 6. A standard shuttlebox (Lafayette Instruments, # 85013) was used for the one-way avoidance training.

Procedure. The basic paradigm consisted of stressing the rats on a hot-plate before (Exp. 7a) or after Experiment 7b) shuttlebox avoidance training. Four heat-stress groups

were used: home cage control, 21°C hot-plate, (room temperature), 47°C hot-plate, 57°C hot-plate.

Immediatly after 30 seconds of exposure to one of the hot-plates each rat was placed on one side of the shuttlebox. This immediatly triggered a relay which started the timing sequences. The home cage control group was brought directly to the shuttlebox. Rats in this group did not receive any exposure to the hot plates. A one-way shock avoidance procedure was used. Rats were placed on one side of the chamber which was separated from the other by a electrically controlled door. Ten seconds after placement in the box a tone (CS) was sounded for 5 seconds. At the same time as the tone started, the door separating the two chambers was opened. A period of shock of ten second duration (UCS) followed the tone presentation (constant current 0.5mA scrambled). If the rat crossed to the other side of the chamber during these ten seconds the shock was terminated and the door was shut behind it (escape response). If the rat shuttled to the other side of the chamber before the shock began (CR), the shock was not turned on which signified that a shock was successfully avoided. A successful avoidance or escape was followed by a ten second wait on the safe side before the animal was again placed on the "shock" side. If the rat failed to shuttle during the tone or shock period the door closed and the procedure was repeated. Acquisition criterion was five

consecutive avoidances.

Twenty-four hours after the acquisition phase the animals were brought back to the testing room and the extinction procedure began. The exact same sequence of events outlined above for the acquisition phase was repeated except that the rats were brought to the shuttlebox without intervening stress (no hot-plate) and there was no shock given to the animals. In this phase the animal had to "learn" the new contingency; that the aversive consequences were no longer present. The criterion behavior was five consecutive non-avoidances.

The two phases of Experiment 7 differed with respect to the time that the hot-plate procedure was given. In Experiment 7a the rats were exposed to the hot-plates immediatly before the acquisition phase of shuttlebox training. In Experiment 7b the rats were exposed to the hot-plates immediatly after they had performed the five consecutive avoidances necessary to achieve the acquisition criterion.

Results

Figure 8 illustrates the data collected in Experiment 7a when the hot-plate stress was given immediatly before avoidance acquisition training. There was no effect of hot-plate stress on acquisition performance (Kruskal-Wallace, $p>.05$). All groups performed the

avoidance response to criterion within 15 trials. There was a tendency for the groups exposed to 52 and 57° hot-plate to require more trials, however this was not statistically significant. There was a significant effect of stress during the extinction phase of the present experiment (Kruskall-Wallace; $X^2(3, N=31)=11.48$, $p<.01$). Thus, analysis of differences between groups revealed that the 57° group performed significantly less trials than the home cage control group (post hoc Mann-Whitney $U=10$, $p<0.05$ two tailed).

Figure 9 illustrates the data collected from Experiment 7b in which the rats were subjected to the hot-plate stress immediatly after acquisition criterion had been established. There was no effect of heat stress on acquisition of avoidance since at this point in the experiment the groups were not exposed to any heat stress treatment (Kruskal-Wallace, $p>0.05$). There was a significant effect of the heat stress on extinction performance (Kruskall-Wallace; $X^2(3, N=28)=20.1$, $p<0.001$). A significant effect was found for extinction performance between the groups exposed to 47°and 57°(post hoc Mann-Whitney $U=7$, $p<0.05$). No other comparisons were significantly different.

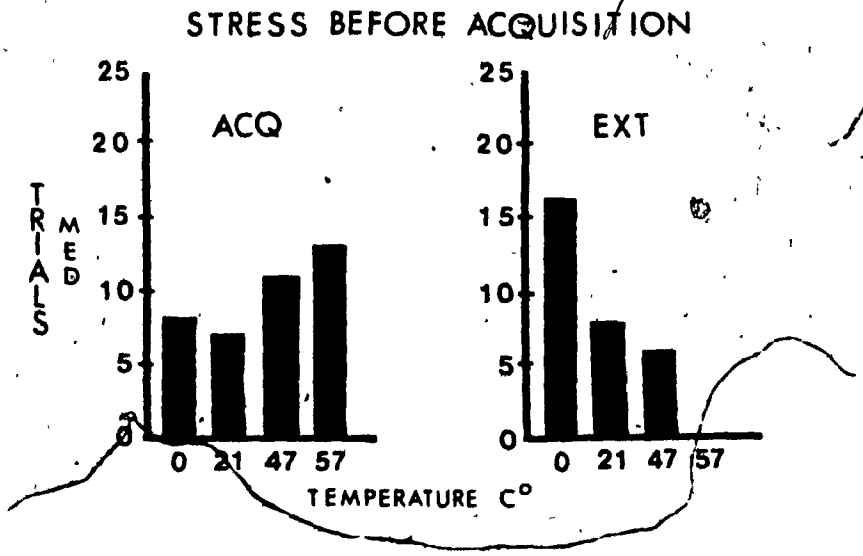

Fig. 8. Acquisition and extinction of one-way avoidance when heat-stress was applied immediatly before acquisition training. There was no difference in acquisition. A significant difference was found in extinction between groups (Kruskall-Wallis, $p<0,05$). Group 57° was significantly different from all other groups (Mann-Whitney U, $p<0.05$). (0 = home cage control)

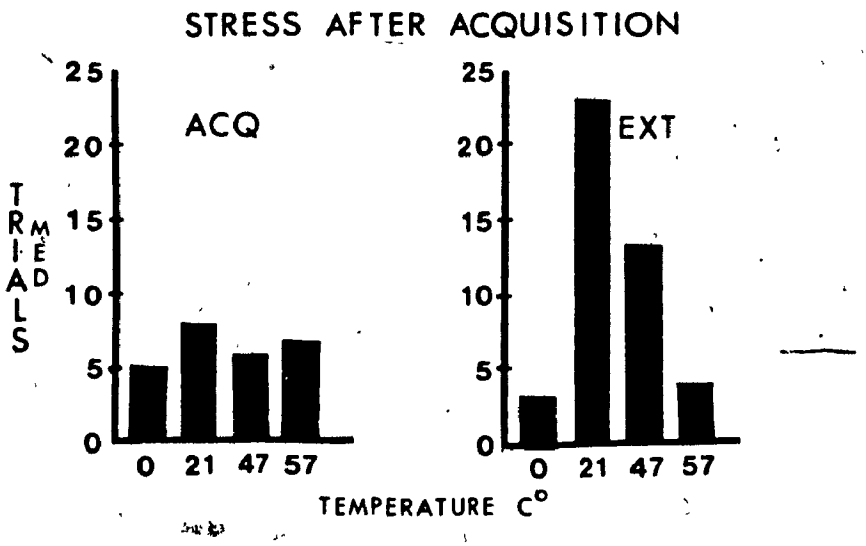

Fig. 9. Acquisition and extinction of one-way avoidance when heat-stress was applied immediatly after acquisition training. There was no difference in acquisition. Significant differences were found in extinction (Kruskall-Wallis, $p<0.001$). Groups 47° and 57° were significantly different (Mann-Whitney U, $p<0.05$).

Discussion

Acquisition of avoidance was unaffected by any of the stress stimuli. There were no differences in the number of trials necessary to acquire the avoidance response among the groups. A raised pain threshold, therefore, does not appear to affect acquisition of avoidance performance in this paradigm. In line with the previously stated notion, it is possible to interpret the lack of significant differences between the 57°C group and the other groups as indicative of the presence of an operating adaptive mechanism. The reduction in pain allowed this group to function in a manner indistinguishable from controls thus enabling the animals, despite the experience of stress, to escape from a noxious stimuli.

While no differences in avoidance acquisition was found between the various groups, exposure of the groups to extinction trials 24 hours later revealed significant differences in the number of trials required to extinguish the avoidance response. There was a significant relationship between the intensity of the stimuli and the rate of extinction (Fig. 8). The results illustrated in Figure 8 suggest that higher stress levels prior to avoidance training were associated with faster extinction.

While it is possible that these results may simply reflect differences in the ability of the differentially treated rats to perform the avoidance response, this is

unlikely, since no differences were found in the number of trials to acquire the avoidance response. In addition, the differences in extinction reflect differences of non-avoidance which are naturally not dependent on ability to perform a motor response.

An argument can be put forward that since the rat's feet have been stimulated on the hot-plate and then subsequently re-stimulated in the shuttlebox shock chambers, that the results may be confounded by increased sensitivity or conversely habituation to the aversive stimuli applied to the feet. In this case, the effects may not be related to physiological release of some endogenous agent but to performance deficits or changes in sensitivity distinct from true elevation of pain threshold or, in fact from an induction of analgesia. A number of observations negate this argument. First, the results indicate that the low (21^{o}) and high (57^{o}) temperatures affect the extinction of an avoidance response in the same manner which is different than the effect of the middle temperatures. This argues strongly against an interpretation in terms of a performance deficit. Second, an experiment which measured locomotor activity 24 hours after exposure to the same intensities of hot-plate as the present experiments found no significant differences in locomotor behavior between groups that had been exposed to different temperatures (Galina, Sutherland & Amit, submitted). This suggests that the differential

effects seen in extinction phase were not due to performance difficulties during the extinction phase.

It would appear then that the "memory" for the acquired behavior was differentially affected by exposure to different intensities of the heat stimuli whether the stimuli were applied before or after acquisition. These data, however, do not provide adequate evidence for an effect on memory processes per se, since the results of extinction training may be a reflection of alterations in sensory systems and not a direct effect on the memory trace itself (Gold & Zornetzer, 1983).

When animals were undergoing extinction trials, re-exposure to the shuttlebox was a necessary part of the stimulus complex. However, the heat stimuli which were also part of the stimulus comlex were not re-administered before the extinction phase, therefore only part of the stimulus complex was present. Nevertheless, this re-exposure to part of the stimulus complex may have been sufficient to trigger the physiological changes associated with the previous day's experience. In other words, the animal that was exposed to 57^{o} C heat stress before the acquisition phase may have undergone the acquisition training in a different state than the animals in the other groups. It is probable that the total sensory experience of the shuttlebox was therefore different for these animals. Upon re-exposure to the avoidance apparatus this altered experience may have

accounted for the faster extinction rates observed in these animals.

This question of altered motoric or sensory effects can also be addressed by examining the data of Experiment 7b in which the stressor was applied after acquisition criteria had been attained. Under these conditions, changes in performance during extinction 24 hours following avoidance training could be interpreted as attributable to an effect of the stressor on memory and not a motoric deficit. Since the heat stimuli came after acquisition it could not be expected to affect the original acquisition. Similar to the results observed in Experiment 7a, it was found that different intensities of heat led to differences in extinction. This time the differences were not linear but curved. An inverted U-shaped curve depicting the relationship between stimulus intensity and extinction can be seen in Figure 9. Groups that were exposed to 21 or 47°C exhibited enhanced retention compared to the home cage control group. The groups exposed to 57° , however, were indistinguishable from the home cage control group, suggesting that the effects on extinction were not due to motoric deficits.

Exposure to heat-stress should release endogenous ACTH which may then lead to changes in avoidance behavior that resemble the effects of exogenously administered ACTH. The results of the previous two experiments tend to confirm this

notion, since in fact, the hot-plate stress used was found to resemble the effects of exogenous administration of ACTH. This hypothesis is supported by the data of Experiment 7a in which no effect of the heat stress on acquisition performance was observed, but significant effects of heat stress on extinction performance were found. It should be recalled that exogenous administration of ACTH does not usually lead to changes in acquisition performance but does lead to changes in extinction of conditioned responding (e.g. de Wied, 1980).

Alternatively, the differential effects of heat stress observed on extinction may be due to increased release of corticosterone. Though active avoidance responding was unaffected by administration of corticosterone (Bohus & Lissak, 1968), corticosterone has been reported to facilitate the extinction of active avoidance responses (Bohus & Lissak, 1968; Kovacs et al., 1976; de Wied, 1967). Since the heat stressors used in the present studies were known to affect corticosterone release in an intensity dependent manner (Galina, Sutherland & Amit, 1983), it is possible that some of the facilitation of extinction observed in these experiments may be due to the increased release of corticosterone and not to a direct effect of ACTH. In order to determine which hormone affects avoidance behavior, experiments that include surgical techniques such as adrenalectomy and direct administration of corticosterone

within the context of the present paradigm are needed.

In summary then, the experiments using an avoidance paradigm revealed that intensity of the heat stress used did not effect the acquisition of an avoidance response but did effect the subsequent retention of this avoidance response. When applied before acquisition training, the intensity of the stimuli was found to affect the extinction and therefore retention of the response in a linear fashion; as the intensity was increased there was less retention. However, when applied after acquisition had been established, the intensity of the heat stress effected the retention of the avoidance response in a manner that resembled an inverted U-shaped curve.

GENERAL DISCUSSION

The experiments reported in this thesis were designed to elucidate the adaptive nature of stress-induced analgesia (SIA). The diverse behavioral and physiological parameters of SIA were reviewed and it was suggested that examination of behavior that occurs concurrently with analgesia may aid in the understanding of the functional nature of SIA. Moreover, it was also suggested that since the effects of the hormone ACTH and the the occurrence of analgesia are usually examined in aversive and "stressful" paradigms, an examination of the behavior in these aversive paradigms would lead to a better understanding of the behavioral effects of ACTH and the functional nature of SIA.

In the initial studies a stressor that was easily modified along the dimension of intensity was examined. It was found that heat stress induced by brief exposure to hot-plates heated to various temperatures induced a transient reduction in locomotor activity observed in an open field. These changes in locomotor activity in an open field after exposure to various intensities of heat stress were suggested to be mediated by a release of ACTH from the pituitary gland. ACTH was suggested to mediate this phenomenon since hypophysectomy, which removed the major source of ACTH, was found to prevent the stress-induced reduction in locomotion and administration of ACTH to hypophysectomized rats reinstated the reduction in

locomotion. In other experiments reported here it was found that neither adrenalectomy nor adrenal-medullectomy affected the reduction in locomotion thus ruling out the effects of ACTH on corticosterone production in the adrenal cortex and endogenous opioids from the adrenal medulla in the mediation of this behavior. In an attempt to ascertain the type of receptor interaction through which ACTH was operating, naloxone and γ-MSH were administered but were found not to effect heat-stress induced reduction in locomotion. It was also pointed out that the results of the first four experiments could be interpreted as behavioral evidence of a specific ACTH receptor in the central nervous system.

Subsequent experiments presented here reported that the same heat stress that affected locomotion could also induce a temporary analgesia when measured in a tail-flick test. The findings that heat stress could release ACTH and could also induce a temorary analgesia, led to the examination of the effects of this stressor in avoidance learning situations. It was found that the acquisition of avoidance responding was unnaffected by heat stimuli, however, the absence of differences were taken to suggest that an adaptive mechanism was operating to restore "normal functioning" in this avoidance situation. In addition, it was reported that retention, as measured by rate of extinction of avoidance responding, was differentially effected by the stressor. The differential results on

extinction performance were dependent on the intensity of the heat stimuli.

Overall, in terms of the proposed adaptive nature of SIA, the results of the experiments reported here indicate that the groups receiving the 57°C heat stimulus exhibited three different responses. These responses seemed to be dependent on the type of testing procedure used to measure the behavioral reaction to the stressor. These testing conditions, then, can be viewed as reflective of varying environmental circumstances. When the animal was placed in a novel environment where increased locomotion was not a necessary and adaptive component of the situation, the unconditioned response was one of reduced locomotion. Concomitantly, in a different paradigm rats exhibited longer latencies to tail withdrawal in a tail flick test indicating the presence of analgesia. Yet, when placed in a position where movement was the necessary and adaptive response (shuttle) the animals revealed that they can learn and perform the response as well as controls. Of particular interest was the finding that although acquisition was not disrupted by exposure to heat stimuli, the "memory" of the appropriate response was altered (as measured by latency to extinction). This, at first scrutiny, would not seem to be an adaptive response. If the animal encounters the same stimuli again, "remembering" the previous encounter should facilitate current avoidance. However, a better explanation

may be that since no shock stimuli are present during extinction sessions, the recognition of this fact, as reflected by the rapid acquisition, was in fact adaptive. The animal's normal pattern of responding in the avoidance paradigm during extinction was to continue avoiding, as if the shock was still present. The animals that did not receive stress before acquisition have been conditioned to avoid when exposed to some of the stimuli that predict "danger". The animals receiving the heat stimuli before acquisition exhibited progressively faster extinction as the heat intensity was increased. This indicates that even though these animals were also conditioned to avoid, they were not able to retain or retrieve this information 24 hours later.

Locomotor behavior, performance of an acquired avoidance response 24 hours later, and pituitary adrenal activity were all affected by the intensity of heat stimuli. This pattern can in many cases be reflected as a U-shaped curve. This pattern of behavior that is elicited by the various stimuli in the environment tends to interact with the internal physiological conditions that prevail at the time of testing to produce adaptive behavior. Changes in the background hormonal (and other) conditions were affected by manipulating the intensity of stimuli, such that the same stimuli could induce different behaviors within the same environment. Given these results, it may be appropriate to

consider the type of stimuli that induce stress as the product of intensity and duration of stimuli combined with environmental conditions that would elicit defensive behaviors. Changing the level of stimulation will determine at what point on the stress continuum the particular behavior will be located.

The stress continuum

In the Introduction to this thesis I suggested that stress should be viewed on a continuum that would be best represented by an inverted U-shaped curve. At some hypothetical locations on the curve, "stress" could prove to be adaptive. Such was the case in the active avoidance paradigm where the rate of acquisition of avoidance by the group receiving the higher intensity stress before avoidance training was not different than controls. I suggested that this is possible because of the parallel activation of pain suppressive systems. The pain suppressive system acts in this situation as the variable that corrects or helps maintain adaptive responding. Interpretation of the data of Experiment 7 suggest that stress-induced analgesia can function as an adaptive mechanism that may facilitate responding in avoidance learning paradigms and by implication improve the nature of the animals' defensive response in noxious or dangerous situations.

I interpreted these results to suggest that though

considerable pain was inflicted on the rats that had been exposed to the 57°C hot-plate, the significant pain-reduction induced by exposure to the hot-plate stress immediatly before avoidance sessions allowed the animal to learn and perform the required response of shuttling to avoid shock without the risk that the pain would debilitate the animals' response. While I did not observe differences in the rate of acquisition of the avoidance response immediatly after the painful stimuli, when I exposed the groups to extinction 24 hours later, significant differences were observed in the rate of extinction. The groups that were exposed to the 57 C hot-plate on the previous day performed the required response of "non-avoidance" significantly faster than did the other groups. It would seem then that, in this case, the functional benefit of analgesia seen after stress would allow the animal to escape faster in natural settings (if an escape route were present) and that 24 hours following such an exposure these same animals were able to learn a new adaptive response significantly faster than those rats not exposed to the hot-plate at a temperature that induced analgesia.

The rat's behavior suggested that pain inhibition may enable it to learn appropriate behavioral responses despite the presence of "stress". In order to act appropriately and perhaps even survive after exposure to severe stress the rat must focus its attention on escaping the threat to its

integrity and not on dealing with the accompanying pain. It is my contention that the data presented above constitute support for the notion that SIA is an adaptive mechanism which tends to facilitate appropriate responding in a threatening situation. In a situation of social conflict or in other potentially aversive situations, the animals' behavior may be modulated by the short duration (i.e. 10-30 minutes) pain inhibition which would aid in the production of appropriate defensive behavior.

The review of the literature presented in this thesis must leads one to contend that stress, when defined as a stimulus complex which elicits defensive reactions essential to maintain the integrity of the organism, is a necessary component of stress-induced analgesia.

Stress analgesia is adaptive

It seems clear from the preceeding discussion that the stimuli described in the various sections of this dissertation share one common behavioral characteristic. They have all been reported to induce analgesic responses regardless of the particular behavioral response pattern observed whether that response was a flick of the tail, vocalizations, or the pressing of a lever to terminate electric shock.

It is my contention that this analgesic response, regardless of the manner in which it was manifested, is

adaptive within the context of the testing situation and, by implication, within the real life situations that the testing situation represents. It results in the facilitation of the animals' ability to perform, or conversely to inhibit a motor response, the goal of which is the elimination of the aversive stimulus. This in turn aids the animal in maintaining its integrity in a potentially threatening environment. When seen in this light the analgesic response should be classified as goal directed and as part of a "species specific defense reaction" (Bolles, 1967; Bolles & Fanselow, 1980) which by definition involves activity of higher central nervous system integrative processes. Therefore, stress as inferred from the activation of a behavioral response that would maintain the integrity of the organism, is necessary for the induction of SIA in animals.

Support for this notion comes from a number of observations summarized below. First, the stressful component of a stimulus which triggers the defensive reaction does not necessarily need to be generated by pain. Clearly, it would be difficult to assert that centrifugal rotation, vaginal stimulation, or immobilization are painful in the same sense as intense footshock or exposure to hot-plate. It is more likely that the stress component of these manipulations stem from such variables as novelty or lack of controlability.

Second, the induction of SIA seems to be dependent on "cognitive processes" since footshock analgesia cannot be induced in anesthetized animals (Hayes et al., 1978a; Jensen & Smith, 1981). Furthermore, in some cases minor tranquilizers have been reported to reduce analgesia induced by stress or pharmacological manipulation (Bodnar et al., 1980c; Doi & Sawa, 1980; Wuster, Duka & Herz, 1980; but see Chance, 1980; Hayes et al., 1978a; Kinscheck, Watkins & Mayer, 1984). The effects of the minor tranquilizers does not seem to be related to the muscle relaxing properties since muscle relaxants had no effect on SIA (Doi & Sawa, 1980).

Third, when the stressful aspects of an analgesia inducing situation (e.g. novelty) were parcelled out the analgesia was diminished (see Bardo & Hughes, 1979; Sherman, 1979; Tiffany, Petrie, Baker, & Dahl, 1983). This notion is highlighted by observations that repeated presentations of footshock, CWS, 2-DG, and other stressors lead to tolerance or adaptation, resulting in a diminished analgesic response.

Fourth, in view of the fact that the same stimuli may or may not induce analgesia depending on such factors as intensity, duration, and controlability, one must conclude that it is not the specific nature of the stimulus that is the determining factor in the induction of analgesia, but the context in which the animal perceives it which is the major determining factor. Thus, for example, if one changes

the intensity of the stimulus applied to an animal previously adapted to a different intensity of the same stimulus, the analgesia will reappear. Furthermore, the very same manipulations will induce analgesia under one set of circumstances but can also lead to hyperalgesia in other circumstances (Simone & Bodnar, 1981; 1983; Vidal & Jacob, 1982; Vidal, Sandeau & Jacob, 1984). Along the same vein, it has been suggested that the induction of SIA (hyperstimulation analgesia) in humans is largely dependent on factors such as placebo, suggestion, and level of control rather than the specific nature of the aversive stimulus (see Melzack and Wall, 1982, pg. 332-355).

It seems clear from the summary above, that the emergence of SIA depends on the interplay among many variables. This wide diversity of variables that induce analgesia may explain the involvement of the many, seemingly disparate physiological substrates associated with the phenomenon of SIA. Given the complexity of the behavioral responses elicited by the various paradigms used to study SIA, it is inconceivable that all of these responses can be governed by the same substrates. It seems almost self evident that in addition to the mandatory activation of pain pathways, other mechanisms must also be selectively activated. Thus, for example, forced swimming in cold or warm water may activate thermoregulatory mechanisms while shock to the paws or tail may activate anti-inflamatory

processes.

Furthermore, support for the notion that the widely disparate substrates of the various analgesias are necessary for invoking other concurrent responses comes from the studies reported in this thesis. The release of hormones that effect learning and performance suggests that not only would pain pathways be activated in response to the perceived or actual threat but other mechanisms should be activated in parallel. In Experiment 7a, along with reduced pain threshold it is likely that ACTH or corticosterone was released to modulate behavioral reactions to threat or perceived threat in the environment.

The final common pathway for some of these analgesias has been postulated to be the DLF of the spinal cord while for others a humoral component seems of primary significance (Watkins & Mayer, 1982a). Activation or deactivation of these anatomical or humoral pathways, perhaps through a gating mechanism (Melzack & Wall, 1965), leads to pain suppression as measured in conventional pain tests. However, given the notion that SIA is a critical component in an adaptive behavioral response to a variety of threatening stimuli and situations, I must conclude that a number of "higher" mechanisms must interact with the spinal or humoral mechanisms to produce the adaptive responses necessary for coping with the stressful situation.

The experiments in this thesis attempted to elucidate

the adaptive nature of stress-induced analgesia through examination of concurrent behaviors. It was suggested that, within the framework of the testing situation the concommitant activation of physiological systems, such as the pituitary-adrenal axis and subsequent release of ACTH, could modulate "memory" processes. The putative central nervous system effects of ACTH could alter the motivational valence, such that the preferance and persistence of adaptive responding would be facilitated. It was also suggested that stress-induced analgesia was of short duration because this form of analgesia served to facilitate responding to threatening stimuli. In this vein, stress-induced analgesia is but one of a constellation of behaviors, that with concommitant physiological responses, have evolved to facilitate appropriate responding to environmental change.

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