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**PLASMA CORTICOSTERONE VARIATIONS ASSOCIATED WITH
STRESSOR CONTROLLABILITY, PRIOR AVERSIVE STIMULATION
AND INNATE DEFENSIVE RESPONSE STYLES**

by

Christopher R. Prince

**A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of
the requirements for the degree of**

**Doctor of Philosophy
Department of Psychology**

**Carleton University
Ottawa, Ontario
January, 1990**

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ABSTRACT

The immediate and proactive effects of controllable and uncontrollable stressors on plasma corticosterone concentrations were assessed in CD-1 mice. Mild stimulation (handling and exposure to a novel environment) provoked a small increase of plasma corticosterone concentrations, while more severe stressors (footshock, forced-swim or water-escape) provoked greater increases in corticosterone secretion. Irrespective of the stressor severity, however, adaptation of the corticoid response did not occur with repeated environmental insults. Moreover, the corticoid response was independent of shock controllability, regardless of whether (a) 60 or 360 shock trials were applied, (b) trials were massed (i.e., applied within a single session) or spaced over days, or (c) mastery over the response contingencies had been established. Likewise, the decay rate of the corticoid response was comparable after controllable and uncontrollable stressors.

In a water-escape task, plasma corticosterone secretion varied with stressor controllability, and the nature of the response required. In animals required to emit a contraprepared response (swimming to a nonilluminated arm of a Y-maze) the corticosterone increase was comparable to that of mice exposed to inescapable (yoked) swim. However, the corticoid secretion in mice required to emit a highly prepared response (swimming to an illuminated arm) was less pronounced than it was in their yoked partners. Curiously, the increased plasma corticosterone secretion appeared to be unrelated to the animal's discrimination performance, being apparent even when the response contingencies had been well established. Finally, exposure to either an acute or chronic stressor, applied in juvenile or adult mice, did not

proactively influence the corticoid response upon subsequent challenge with either swim or inescapable shock.

In addition to changes in plasma corticosterone concentrations, swim exposure increased central hypothalamic norepinephrine (NE) and dopamine (DA) utilization, as gauged by the accumulation of their respective metabolites, 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) and dihydroxyphenylacetic acid (DOPAC). Once again, however, escapable and inescapable swim yielded comparable increases of amine utilization. When animals were required to emit a contraprepared response NE levels declined relative to that of nonstressed mice or mice required to emit a prepared response. However, the more pronounced amine alterations may have been secondary to the efforts expended by the animal, rather than to stressor controllability.

It was hypothesized that the secretion of adrenocorticoids may be a fundamental adaptive response to environmental demands. As such, the contribution of psychological variables to the corticoid response may be limited. The preparedness of an animal to emit a behavioral response may influence plasma corticoid concentrations, but such an effect may be obviated if the stressful experience continues. Presumably, with protracted stressor exposure, irrespective of its controllability, corticoid secretion may be necessary to meet environmental demands.

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The proposition that exposure to stressful events may promote or exacerbate physical and psychological pathologies has gained increasingly greater acceptance in recent years. Exposure to aversive stimulation has been implicated not only in the etiology of physical illnesses, such as heart disease (Glass, 1977), ulcer formation (Pare & Glavin, 1986; Weiss 1971a, 1971b, 1971c), and immunologically related disorders (see Irwin & Anisman, 1984), including neoplasia (Sklar & Anisman, 1981), but also in the development of psychological disorders, such as depression (Akiskal & McKinney, 1975; Anisman & Zacharko, 1982) and schizophrenia (Rabkin, 1980).

Different views have been expressed concerning the contribution of aversive events to the induction of pathological outcomes. While some investigators have maintained that cognitive changes associated with stressor exposure may be responsible for pathologies, such as depression (Abramson, Garber, & Seligman, 1980; Beck, 1976), it is equally likely that neurochemical and hormonal variations induced by stressors are capable of provoking disease states (Anisman & Zacharko, 1982; Selye, 1976). Regardless of the theoretical view adopted, it is generally accepted that the ability of the organism to exert control over the impact of the aversive event largely determines whether or not pathology ensues (Anisman, Kokkinidis, & Sklar, 1981; Weiss, 1971a, 1971b, 1971c). In addition, several experiential and organismic factors may also contribute to the impact of stressors on physiological and emotional disturbances. For instance, the organism's prior stressor history, age, and environmental backdrop upon which the stressor is superimposed may all influence the magnitude of the behavioral and physiological changes which occur.

One of the major physiological consequences of stressor application is the secretion of glucocorticoids from the adrenal glands. On the one hand, it is thought that the action of elevated circulating levels of glucocorticoids on biochemical systems is to assist the organism to deal more effectively with an ongoing stressor (Selye, 1976). On the other hand, it has been proposed that elevated corticoid levels may limit the potential adverse effects of excessive physiological (e.g., immunological) changes elicited by stressors (Munck, Guyre, & Holbrook, 1984). While glucocorticoid action following exposure to acute stressors is primarily associated with changes in carbohydrate metabolism that increase the availability of substrates for utilization, elevated levels of circulating glucocorticoids or prolonged exposure to the steroid have also been implicated in physiological disturbances, such as myopathy, steroid diabetes, hypertension, immunosuppression, infertility, and inhibition of growth (see Melby, 1977). Thus, both limited corticosterone secretion as well as diminished rates of disappearance of the glucocorticoid after stressor exposure can be damaging to the organism.

Despite the numerous investigations that have been conducted assessing stressor effects on corticosterone, there still remain several fundamental questions that have not been fully resolved. Among other things, early reports (Davis, Porter, Livingstone, Herrman, MacFadden, & Levine, 1977; Hanson, Larson, & Snowdon, 1976; Weiss, 1971b; Weiss, Glazer, & Pohorecky, 1976) had suggested that control over a stressor may be an important factor in determining the magnitude of the corticoid response. Yet these effects are generally small, and indeed, there are ample data suggesting that corticoid secretion may be elicited by even exceptionally mild stressors (e.g., M.B. Hennessy & Levine, 1978). Moreover, there is reason to believe that stressor controllability may

actually not be all that important in determining serum corticoid levels (Maier, Ryan, Barksdale, & Kalin, 1986). Accordingly, one purpose of the present series of experiments was to determine whether exposure to controllable and uncontrollable stressors differentially affects corticoid concentrations in mice. In addition, the hypothesis was entertained that control over a stressor may not always be effective in limiting corticoid secretion. Indeed, it is conceivable that under some conditions stressor controllability may have adverse effects (e.g., in a conflict situation; Weiss, 1971a). Accordingly, in the present investigation, the corticosterone response was assessed in animals required to emit either highly prepared or contraprepared responses in situations where control over a stressor was possible.

A third factor that was assessed concerns the time course for the stressor-provoked corticoid alterations. Most investigators have typically been preoccupied with the magnitude of the corticoid response, when in fact, it may be more important to assess the time course of the effect. After all, if corticoid levels remain high for protracted periods this may have adverse consequences (viz. the immunosuppression engendered by corticoids; see Sapolsky & Donnelly, 1985). Thus, in the present investigation both the magnitude and the time course of the corticoid response were evaluated.

Finally, an attempt was made to determine whether stressful events proactively influence the corticoid response promoted by later stressor experiences. It has been demonstrated that brain amine changes may be subject to conditioning or sensitization processes, such that neutral cues associated with a stressor elicit neurochemical changes when the organism is reexposed to the stimuli at a later time. Whether the corticoid response

is governed by the same processes as central amines was assessed in the present investigation.

In the sections that follow, the stressor-induced alterations of the hypothalamic-pituitary-adrenal (HPA) system and the negative-feedback effects of corticosterone on the system will be described. This will be followed by a review of the response of various transmitter systems to controllable and uncontrollable stressor exposure, with particular attention devoted to those systems which are involved in regulating HPA activity. Finally, the behavioral consequences of exposure to controllable and uncontrollable stressors and the contribution of neurochemical systems and glucocorticoid function on behavior will be described.

Response to Stressors

When confronted with an aversive stimulus, several options may be available to the organism in order to eliminate or minimize the impact of the stressor. Bolles (1970) has argued that the behavioral response strategy emitted by the animal will depend upon both the organism's defensive repertoire and the requirements of the situation. Similarly, Anisman, Hamilton, and Zacharko (1984) have proposed that in problem-solving situations involving aversive stimulation the organism engages either in strategies which previously resulted in the termination of the stressor or in strategies which reflect innately prepared defensive response styles.

Accompanying these behaviors are a series of neurochemical and hormonal changes which may, among other things, facilitate the organism's ability to initiate and maintain appropriate defensive responses, and blunt the physical and psychological impact of the stressor. If the animals' behavioral coping attempts are successful, then the demands made on physiological mechanisms may be limited, and hence,

pathology will not occur. In the absence of effective behavioral coping strategies, however, greater emphasis is placed on physiological systems. It is thought that if these physiological systems are overly taxed, then pathology may ensue (see Anisman & Zacharko, 1986).

The Hypothalamic-Pituitary-Adrenal Axis

Following Selye's demonstration that the adrenal gland was involved in the organism's response to aversive physical stimulation (see Selye, 1976), numerous investigations assessed the role of the HPA axis in the response to physical and psychological stressors. Cells containing corticotrophin-releasing factor (CRF) are situated in the paraventricular nucleus of the hypothalamus and have axons projecting to the median eminence. Following neuronal stimulation, CRF is released from the median eminence and is carried by the portal blood system to the anterior pituitary where it stimulates the release of adrenocorticotrophic hormone (ACTH) from corticotroph cells. Circulating blood then carries ACTH to the adrenal glands where it stimulates the secretion of glucocorticoids (corticosterone in most animals, cortisol in primates, sheep and certain other species) from the adrenal cortex (Dunn & Kramarcy, 1984; Gann, Bereiter, Carlson, & Thrivikraman, 1985; Jones, 1978; Keller-Wood & Dallman, 1984; Sayers & Portanova, 1975; Weiner & Ganong, 1978).

Glucocorticoid Function. The prevailing view holds that following stressor exposure enhanced secretion of corticoids is capable of influencing a wide variety of physiological mechanisms which are necessary for adaptive physiological and behavioral functioning. It may be noted, however, that some investigators have argued that elevated levels of glucocorticoids may be unnecessary to confer resistance to stressors (e.g., Ingle, 1952; cited in Munck et al., 1984). In effect, basal concentrations of glucocorticoids are thought to be sufficient to activate

the appropriate physiological mechanisms after stressor exposure. Glucocorticoids, then, are thought to play a permissive role in response to stressors even though it is recognized that, under stressful conditions, animals both require and secrete elevated levels of glucocorticoids (Munck et al., 1984; Sayers & Portanova, 1975).

It has been demonstrated that, among other things, increased levels of glucocorticoids stimulate the formation of glucose from tissue protein (gluconeogenesis), suppress the synthesis of fat from carbohydrates, stimulate the induction of protein synthesis in liver, and mobilize fat by the lipolysis of stored triglycerides to free fatty acids. They also increase cardiovascular tone and inhibit growth and reproduction, as well as suppress immune and inflammatory responses (Munck et al., 1984). It is thought that these changes are necessary for successful adaptation to acute physical stressors. However, the finding that elevated glucocorticoid levels result in lymphocytosis and a general suppression of immunological activity (see Melby, 1977) is inconsistent with the view that elevated steroid levels serve an adaptive function in the organism's resistance to stressful conditions.

In an attempt to reconcile the apparently puzzling effects of glucocorticoids with the traditional view that elevated corticoids increase the organism's resistance to stressors, Munck et al. (1984) proposed that the function of stressor-induced increases of glucocorticoid levels is not to protect the organism against the stressor itself, but rather to militate against the excessive physiological reactions elicited by the stressor. These investigators noted that in almost every instance elevated glucocorticoid levels actually suppressed the synthesis, secretion, or action of components of physiological reactions that occurred in response to stressor exposure (e.g., insulin, endorphin, or lymphokine secretion). Thus,

it is conceivable that elevated, regulatory levels of glucocorticoids may function to turn-off the normal physiological reactions, thus preventing them from overshooting and threatening the physiological stability of the organism. It is assumed that the suppressive effects of glucocorticoids do not affect the activity of physiological systems until some time after the appropriate mechanisms have been activated (Munck et al., 1984). It follows, then, that the analysis of a corticoid-pathology relationship should not only focus on the magnitude of the corticoid response, but also on the rate of recovery of this response following stressor inception.

Feedback Mechanisms. Although the feedback mechanisms of the HPA axis are not fully understood, it is believed that activity of the HPA system is regulated by the negative-feedback effects of circulating corticosterone and ACTH. Under stressor conditions, neuronal stimulation associated with the aversive event is thought to be capable of overriding the normal, inhibitory influence of circulating glucocorticoids on HPA activity, thereby permitting the heightened corticoid concentrations typically observed following stressor exposure. Upon stressor termination, the stressor-induced increase of circulating corticoids is thought to exert its inhibitory influence on HPA activity and inhibit CRF and ACTH secretion (Keller-Wood & Dallman, 1984).

Considerable evidence indicates that plasma corticoids inhibit the release of CRF from the anterior hypothalamus and ACTH from the anterior pituitary (Dunn & Kramarcy, 1984; Jones, 1978). For instance, stressor-induced and basal CRF activity within the hypothalamic-median eminence region have been shown to be inhibited by the administration of corticosterone or dexamethasone (a potent synthetic glucocorticoid). When local injections or implants of corticosterone or dexamethasone were placed in the median eminence or anteromedial hypothalamus, activity of

the HPA axis was effectively suppressed (see Sayers & Portanova, 1975). It should also be mentioned that several studies have demonstrated that ACTH is extremely effective in inhibiting the release of hypothalamic CRF and suppressing HPA activity (see Keller-Wood & Dallman, 1984; Gann et al., 1985; Jones, 1978; Sayers & Portanova, 1975).

Based on studies which examined the inhibitory effects of corticosterone on stimulated CRF and ACTH release *in vivo* and *in vitro*, and on basal activity of the HPA axis in adrenalectomized rats, as well as from ACTH-secreting tumor cells, Keller-Wood and Dallman (1984) have proposed that the negative-feedback action of glucocorticoids on HPA activity may be divided into three major time frames: (a) a rate-sensitive, fast feedback loop, (b) a level-sensitive, intermediate feedback loop, and (c) a level-sensitive, slow feedback loop. It was proposed that these time frames are the consequence of independent mechanisms of corticosteroid action at feedback-sensitive sites in the pituitary and brain. The fast feedback loop involves rapid inhibition (within seconds to minutes) of CRF and ACTH secretion, as a result of an increase of glucocorticoid levels. This inhibitory effect of glucocorticoids on HPA activity, then, may depend on the rate of change of blood steroid levels. Although the mechanism of inhibition is not known, the rapidity of the corticoid suppression on HPA activity suggests a change in release, rather than synthesis of ACTH and CRF. The intermediate corticoid feedback effect appears after a relatively short duration of corticosterone exposure (from 2-10 hr) or after repeated elevations in circulating plasma corticoids. Intermediate feedback appears to affect the release of ACTH, as well as both the synthesis and release of CRF. In the slow feedback loop, which appears after protracted periods of medium to high plasma corticoid concentrations, glucocorticoids have been shown to suppress the synthesis of CRF and ACTH.

The fast and intermediate feedback loops, then, are thought to occur when plasma corticosterone levels increase in response to moderate or severe stressor conditions. It is believed that the fast, rate sensitive feedback determines the rate and magnitude of the ACTH and corticoid responses to aversive stimulation, whereas the intermediate feedback loop limits the response of the HPA axis to repeated stimulation within a moderate period of time (hours). Slow feedback, however, is thought to occur when elevated plasma corticosteroid levels persist for days (i.e., after chronic corticosteroid administration or in pathological conditions, such as Cushing's Disease), and appears to limit activity of the HPA system during prolonged stressor exposure (but see Keller-Wood & Dallman, 1984 for an extended discussion).

Corticoid Receptors and Binding. Glucocorticoid receptors may be found in virtually every nucleated cell type in the body (McEwen, De Kloet, & Rostene, 1986). It is thought that the glucocorticoids exert their negative-feedback effects on the HPA system by binding to relatively specific intracellular receptor proteins found in the cytoplasm of neurons, glia, and pituitary cells (McEwen, 1982; Meaney & Aitken, 1985; Sapolsky, Krey, & McEwen, 1986; Sapolsky & Meaney, 1986). The binding of corticosterone with the cytoplasmic receptor protein alters the structure of the hormone-receptor complex, thereby permitting it to pass into the nucleus of the cell. Once inside the nucleus, the hormone-receptor complex affects genomic events, such as protein synthesis, by binding to DNA or its associated proteins (McEwen, 1982; Meaney & Aitken, 1985; Muldoon, 1980). It may be noted that since the slow feedback effect of corticosterone is known to influence protein synthesis in hypothalamic-CRF containing cells, it would suggest that the effects of corticosteroids on

nuclear events may not necessarily be involved in the feedback regulation of HPA activity (Keller-Wood & Dallman, 1984).

Not all circulating corticosterone binds to unoccupied cytoplasmic receptor protein and influences genomic activity in the nucleus. Corticosterone also binds to protein in plasma, commonly referred to as corticosterone-binding globulin (CBG; Ballard, 1979), which prevents corticosterone from entering into the cytoplasm of the neuron. Thus, it appears that the physiological action of plasma corticoids is associated with unbound or free plasma steroid, since corticoids bound to plasma CBG complexes fail to enter the cell, and are therefore unable to influence genomic events (Ballard, 1979). It has been suggested that CBG binding plays an important regulatory function by controlling the amount of corticosterone available to the receptors. Hence, the inactivation of circulating corticoids by CBG complexes serves to buffer the action of corticosterone and helps to diminish the feedback signal of corticosterone (McEwen et al., 1986; Sapolsky & Meaney, 1986).

In addition to their peripheral actions, corticoids have been shown to bind to specific receptors in brain regions, such as the amygdala, hippocampus, septum, and frontal cortex (McEwen, Davis, Parsons, & Pfaff, 1979; Meaney & Aitken, 1985). Studies in adrenalectomized animals have demonstrated that regions of the limbic system have the greatest number of binding sites for ^3H -corticosterone. It seems likely that ACTH and corticosterone in these brain areas may influence the release of CRF from the hypothalamus (Dunn & Kramarcy, 1984; Seggie, Singhal, & Hrdina, 1980). Indeed, reductions of hippocampal corticosterone receptor sites, ordinarily evident following chronic administration of high doses of corticosterone, were found to limit the suppression of HPA activity

otherwise engendered by stressor-provoked corticoid secretion (Sapolsky et al., 1986).

In the rat brain, autoradiographic studies have shown that tracer amounts (~0.5 ug) of ^3H -corticosterone were retained in the cell nuclei of neurons in several brain regions, such as the amygdala, frontal cortex (Meaney & Aitken, 1985), cerebellum (Pavlik & Buresova, 1984), hypothalamus (McEwen, 1982), caudate putamen (Defiore & Turner, 1983), and pituitary (McEwen, 1982). In addition, particularly high concentrations were observed in the hippocampus and lateral nucleus of the septum (McEwen, 1982; Sapolsky et al., 1986; Warembourg, 1975). Subsequently, it was found that dexamethasone had a markedly different pattern of uptake in the brain than did corticosterone. Indeed, dexamethasone binding was found to be very high in the pituitary and very low in the hippocampus, whereas corticosterone binding, it will be recalled, was relatively low in the pituitary and high in the hippocampus (De Kloet, Wallach, & McEwen, 1975). The differential binding of corticosterone and dexamethasone to limbic brain structures, hypothalamus, and cortical brain regions *in vitro* suggested at least two different adrenal receptor subtypes in brain tissue (Veldhuis, Van Koppen, Van Ittersum, & De Kloet, 1982). More recently, it has been shown that corticosterone interacts with at least two subtypes of adrenal steroid receptors, and that corticosterone differentially occupies these receptors in brain tissue (Reul & De Kloet, 1986). For instance, corticosterone receptors predominate in the hippocampus and septum, whereas glucocorticoid receptors have a more widespread distribution and may be found in neurons and glial cells. The highest concentrations of glucocorticoid receptors, however, occur in the lateral septum, dentate gyrus of the hippocampus, and amygdala. Substantial amounts are also

found in the locus coeruleus and hypothalamic CRF-containing cell bodies, such as the paraventricular nucleus (see McEwen et al., 1986; and Reul & De Kloet, 1986).

The differential occupation of corticosterone receptors and glucocorticoid receptors by corticosterone, as well as the differential affinity of corticosterone for these receptor types (see De Kloet & Reul, 1987; and Reul & De Kloet, 1986), led to the suggestion that corticosterone may be involved in two fundamentally different aspects of brain function (McEwen et al., 1986; Reul & De Kloet, 1986). The corticosterone receptor system, which appears to be restricted to hippocampal and septal regions, is almost completely occupied (>90%) following immobilization stress at the trough (i.e., 0900 h) and peak (2000 h) of the circadian cycle, and after administration of graded doses of corticosterone to adrenalectomized rats. Hence, these investigators suggested that the action of corticosterone (via the corticosterone receptors) may be one of exerting a tonic, permissive effect on the septal-hippocampal complex. In contrast, marked glucocorticoid receptor occupation is only observed after stressor exposure, at the circadian peak, and following exogenously induced increases in plasma corticosterone concentrations. It is thought that the action of corticosterone on the glucocorticoid receptor system may be involved in feedback mechanisms following stressor exposure. Indeed, the abundance of glucocorticoid receptors in limbic regions, such as the lateral septum, central amygdala, and paraventricular nucleus, which is the site of CRF synthesis, as well as in the locus coeruleus, suggests that these regions may indeed be involved in the stressor-provoked corticoid feedback loop (De Kloet & Reul, 1987; Reul & De Kloet, 1986).

Neurochemical Variations Following Stressor Exposure

As indicated earlier, activity of the pituitary-adrenal axis is thought to be influenced by changes in brain amine activity. Before examining the role of biogenic amines on alterations of HPA activity, however, it is necessary to outline some of the neurochemical changes associated with stressor exposure.

It is generally believed that many of the neurochemical changes which occur after exposure to stressors are adaptive in nature. Application of an acute stressor is thought to result in the release of several neurotransmitters, including norepinephrine, dopamine, and serotonin (see Anisman, Kokkinidis, & Sklar, 1981; and Stone, 1983). Furthermore, the release of transmitters is accompanied by an increase in the rate of amine synthesis, thus resulting in steady state amine levels being maintained. In the absence of appropriate behavioral coping strategies, or if the stressor is sufficiently severe and protracted, release of the transmitter may exceed synthesis, resulting in a net depletion of neurotransmitter. It has been suggested that vulnerability to pathology may be increased under those stressor conditions which favor reductions of transmitter concentrations (Anisman, 1984; Anisman & Zacharko, 1982).

Norepinephrine. Numerous stressors, such as footshock, restraint, cold exposure, swim, and forced running, have been shown to alter brain norepinephrine (NE) levels (Anisman, Kokkinidis, & Sklar, 1981). Exposure to aversive stimulation increases the rate of utilization and synthesis of brain NE in several brain regions. Studies demonstrating increased disappearance of exogenously administered radioactively labelled norepinephrine (^3H -NE; Thierry, Blanc, & Glowinski, 1971), increased accumulation of the NE metabolite 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG; Glavin, 1985a, 1985b; Glavin et al., 1983; Iimori et al., 1982;

Nakagawa, Tanaka, Kohno, Noda, & Nagasaki, 1981; Tanaka et al., 1982; Tsuda et al., 1982), and increased disappearance rate of NE following the inhibition of biosynthetic enzymes necessary for NE production (see Anisman, 1978; and Stone, 1975) are consistent with the suggestion that stressors increase the rate of NE utilization. Similarly, increased activity of the biosynthetic enzymes, tyrosine hydroxylase (Oka, Ashiba, Kiss, & Nagatsu, 1982) and dopamine- β -hydroxylase (Daiguji, Okada, & Yamashita, 1982; Kobayashi, Palkovits, Kizer, Jacobowitz, & Kopin, 1976; Thierry, 1973), as well as increased ^3H -NE formed from ^3H -tyrosine (Thierry, 1973; Weiss, Glazer, Pohorecky, Brick, & Miller, 1975) following stressor exposure provide evidence for increased synthesis of brain NE.

Although the alterations of NE appear to be relatively widespread throughout the brain (Dunn & Kramarcy, 1984), the severity of the stressor may differentially affect NE variations in specific brain regions. For instance, reductions of NE concentrations and increases of MHPG levels may be readily obtained in the hypothalamus and amygdala after relatively moderate amounts of immobilization or footshock stress. In the hippocampus, thalamus, and cortex, however, depletions of NE become apparent only after relatively severe or prolonged exposure to a stressor (Nakagawa et al., 1981; Tanaka et al., 1982). Furthermore, it appears that the rate of recovery of brain NE varies not only in relation to stressor severity, but also varies from region to region. Following exposure to a relatively moderate stressor, reductions of NE in the hippocampus were found to return to control values more rapidly than depletions in the hypothalamus (Anisman, Irwin, Bowers, Ahluwalia, & Zacharko, 1987). After relatively intense stressor exposure, reductions of NE in the locus coeruleus have been shown to persist for as long as 72 hr, while reductions in the hypothalamus are apparent for only 48 hr (Weiss et al.,

1981). It appears, then, that it would not only be appropriate to evaluate stressor effects on amine concentrations, but also to determine the rate of recovery of neurochemical systems. Indeed, it is not unreasonable to assume that the inability of a neurochemical system to recover after a stressful encounter may render the organism more susceptible to pathology.

Psychological factors have also been reported to provoke changes in NE. For instance, Anisman and Sklar (1979) demonstrated that previous exposure to inescapable shock and then reexposure to limited amounts of footshock increased the release of hypothalamic and hippocampal NE and resulted in decreased amine levels in mice. Moreover, Cassens, Roffman, Kuruc, Orsulak, and Schildkraut (1980) showed that even environmental cues associated with a footshock stressor increased the accumulation of MHPG in rats previously exposed to the aversive stimulation. Similarly, Tsuda et al. (1986) reported increases in MHPG in the hypothalamus, amygdala, and locus coeruleus in rats re-introduced to the same environment in which they had received footshock 48 hr earlier. These findings demonstrate that neurochemical responses may be sensitized or conditioned to cues previously associated with stressors, and that it may be important to consider not only the immediate neurochemical impact of stressors, but also the potential proactive effects of such a treatment.

Several investigators have argued that variations of brain NE may be related to the ability of the organism to exert behavioral control over the stressor (Anisman, Kokkinidis, & Sklar, 1981; Stone, 1975; Weiss, Glazer, & Pohorecky, 1976; Weiss, Glazer, Pohorecky, Bailey, & Schneider, 1979; Weiss et al., 1981). For instance, these investigators reported that if animals were given the opportunity to escape from electric footshock, negligible changes in brain NE levels were observed. If, however, animals

were exposed to an equal amount of inescapable footshock (administered in a yoked paradigm), then reductions in NE content were observed in the hypothalamus, hippocampus, and cortex (Anisman, Pizzino, & Sklar, 1980; Weiss, Stone, & Harrell, 1970). Similarly, Swenson and Vogel (1983) demonstrated differences in brain NE levels in rats exposed to either escapable or inescapable footshock. While exposure to escapable footshock was without effect, the latter treatment condition resulted in marked reductions of hypothalamic NE concentrations, which were observed for as long as 30 min after stressor termination. However, these investigators also reported reductions in hippocampal NE immediately after a 60 min shock-escape training session, and no changes following exposure to inescapable footshock. These findings are in contrast to those typically observed by other investigators. Indeed, exposure to inescapable/uncontrollable footshock is thought to result in depletions of hippocampal NE, while exposure to escapable/controllable footshock is thought to have limited effects on amine concentrations (see Anisman, Kokkinidis, & Sklar, 1981; Stone 1975; and Weiss et al., 1979).

When the stressor is relatively severe and/or sufficiently protracted, amine depletions may be expected to occur regardless of whether or not the organism exerts control over the situation. Indeed, simply having control does not necessarily confer resistance to central amine depletions. Recent data indicate that as animals gain mastery over task requirements, amine utilization rates are attenuated and reductions of amine content are abrogated. Specifically, Tsuda and Tanaka (1985) found that after 3 or 6 hr in a free operant avoidance-escape procedure, both escapably and inescapably shocked rats exhibited increased concentrations of the NE metabolite MHPG in the hypothalamus, amygdala, thalamus, and cortex. These investigators also observed greater reductions

of hypothalamic NE in escapably shocked rats than in their yoked counterparts. Since the operant required to terminate shock (i.e., pulling a disk manipulandum) was difficult to acquire, it was argued that an extended test session would be necessary to observe reductions of NE content following exposure to inescapable shock. Indeed, following 21 hr of training, after which animals had mastered the requirements of the avoidance-escape procedure, MHPG levels in the hypothalamus, amygdala, and thalamus of rats receiving escapable shock were significantly lower than those in the inescapable treatment condition. However, no differences in brain NE levels were noted in animals exposed to either the escapable, inescapable, or nonshocked treatment condition.

It has also been shown that if animals receive prior avoidance or escape training (i.e., a controllable stressor), subsequent exposure to inescapable shock is ineffective in provoking NE depletions (Anisman, Irwin, Beauchamp, & Zacharko, 1983; Anisman et al., 1980; Tsuda & Tanaka, 1985). Moreover, the opportunity to engage in fighting (which may serve as a coping response) during shock exposure actually prevented the reductions of NE otherwise induced by the shock stressor (Stolk, Conner, Levine, & Barchas, 1974). Fighting has also been shown to prevent stressor-induced ulceration (Weiss, Pohorecky, Salman, & Gruenthal, 1976) and the enhanced tumor growth ordinarily associated with footshock (Sklar & Anisman, 1980). It would appear that prior training or engaging in effective coping response styles may prohibit the occurrence of NE depletions ordinarily observed after exposure to inescapable footshock.

Prolonged or repeated exposure to stressors has also been shown to attenuate NE depletions associated with an acute stressor. For example, Kvetnansky et al. (1976) reported that following exposure to 180 min of

restraint stress, the NE reductions ordinarily observed after 20 min of restraint were eliminated. In addition to changes in NE content, increased tyrosine hydroxylase and dopamine- β -hydroxylase activity were noted. Daily exposure to footshock (i.e., 15 sessions) has been shown to be as effective as prolonged stressor exposure in eliminating the NE depletions ordinarily observed after a single session of footshock (Anisman et al., 1987; Irwin, Ahluwalia, & Anisman, 1986; Weiss et al., 1975). Similarly, it was observed that following 21 days of oscillation stress concentrations of NE in the hypothalamus and hippocampus of rats were increased (Roth, Mefford, & Barchas, 1982).

The changes in brain NE content following exposure to repeated stressors may be attributable to several factors. For instance, compensatory increases in tyrosine hydroxylase and dopamine- β -hydroxylase activity are thought to be largely responsible for preventing the reduction of brain NE levels (Kramarcy, Delanoy, & Dunn, 1984; Kvetnansky et al., 1976; Stone & McCarty, 1983; Weiss et al., 1975). Moreover, it appears that the synthesis of NE persists at an enhanced rate even 24 hr after the termination of repeated stressor application (Anisman et al., 1987; Irwin, Ahluwalia, Zacharko, & Anisman, 1986; Roth et al., 1982). However, increases in NE utilization ordinarily observed immediately following repeated footshock are reduced, such that at the 24 hr interval MHPG accumulation is comparable to that of nonstressed animals. It was hypothesized that increases in amine synthesis, coupled with reductions of NE utilization, ensure the availability of adequate amine concentrations to deal with both current and impending stressor exposure (Anisman et al., 1987).

The compensatory increase of NE synthesis observed after chronic stressor application may be subject to the conditioning or sensitization

effects observed after acute stressor application. For instance, animals exposed to repeated inescapable footshock and subsequently reexposed to a limited amount of the stressor two weeks later exhibit elevations of NE concentrations. Since footshock reexposure also produces an elevation in MHPG accumulation in those animals previously exposed to chronic shock, the increased NE concentrations were thought to reflect increased rates of synthesis (Irwin, Ahluwalia, & Anisman, 1986).

It might be noted that chronic shock exposure has also been shown to reduce the uptake of ^3H -NE in rats (Weiss et al., 1975). These data suggest NE uptake mechanisms may be altered, thus prolonging the availability of the transmitter in the synaptic cleft.

Taken together, it appears that the augmented amine levels observed following exposure to chronic stressors reflect adaptive changes in NE synthesis. These alterations assure that adequate amine levels are available for the organism to deal not only with an existing stressor, but also with imminent stressors. In addition, following neurochemical adaptation to a particular stressor regimen, amine synthesis may be subject to a sensitization or conditioning process, thereby enhancing the organism's ability to deal with a subsequent stressor (Irwin, Ahluwalia, & Anisman, 1986).

The variations of NE induced by stressors are not limited to alterations in turnover and levels. Several investigators have reported subsensitivity of postsynaptic β -NE receptors (Cohen, Cohen, & McLellan, 1986; Nomura, Watanabe, Ukei, & Nakazawa, 1981; Stone, 1983; Stone & Platt, 1982; Stone, Slucky, Platt, & Trullas, 1985; U'Pritchard & Kvetnansky, 1980), and decreases in the activity of NE-sensitive cyclic adenosine 3',5'-monophosphate (cAMP; Stone, 1983, 1987; Stone et al., 1985; U'Pritchard & Kvetnansky, 1980), particularly in frontal cortex and

hypothalamus (Stone, 1983; Cohen et al., 1986). It appears, however, that alterations of cAMP formation in brain slices may be independent of β -NE receptor changes. Stone, Platt, Trullas, and Slucky (1984) found that unlike the transient decrease of β -NE receptors, exposure to repeated restraint stress provoked a down-regulation of cAMP in cortex which persisted over a 24 hr period.

The subsensitivity of the β -NE coupled adenylate cyclase system is also known to occur after repeated treatment with antidepressant agents (Sulser, 1982). Thus, it was suggested that changes in β -NE receptor activity may play an important role in adaptation to chronic emotional stress (Stone, 1983). However, it has been shown that antidepressant agents, as well as exposure to repeated footshock, are also responsible for the up-regulation of α_2 -NE receptor sites in the cortex and brain stem (Cohen et al., 1986; Vetulani, 1984). Indeed, the therapeutic efficacy of antidepressant agents may be due to alterations in α -NE receptors, as well as reductions in β -NE receptor sites.

Recently, Weiss and Simson (1985) have proposed that changes involving NE in the locus coeruleus may be associated with the up-regulation of α -adrenergic receptors and down-regulation of β -adrenergic receptor sites following chronic stressor exposure. It was suggested that following exposure to repeated stressors, NE concentrations are decreased in the locus coeruleus, thereby resulting in less stimulation of the adrenergic autoreceptor sites (i.e., α_2 -NE receptor) located on the cell bodies of these neurons. The diminished feedback on the availability of endogenous transmitter in the synaptic cleft, in turn, stimulates the synthesis and release of transmitter at terminal regions (e.g., hippocampus and cortex). It is the increased release of NE in terminal fields which is thought to be responsible for the induction of postsynaptic β -NE receptor

subsensitivity observed following chronic stressor application. These data are particularly interesting in that they not only account for the compensatory increases in amine synthesis and elevated NE concentrations in several brain regions, but also for the down-regulation of the β -NE coupled adenylate cyclase system. The therapeutic effects of antidepressant agents, then, are attributable to their ability to down-regulate postsynaptic receptor sites and eliminate the effects of stressors, but more importantly, these changes may be involved in alleviating the symptoms associated with affective disorders.

Dopamine. Various forms of aversive stimulation have been shown to influence dopamine (DA) neuronal activity. Unlike the widespread changes of brain NE, it appears that DA alterations are limited to discrete brain regions. For instance, following exposure to stressors DA reductions have been observed in the arcuate nucleus of the hypothalamus (Kobayashi et al., 1976; Kvetnansky et al., 1976), mesolimbic frontal cortex (Thierry, Tassin, Blanc, & Glowinski, 1976), and lateral septum (Saavedra, 1982). In addition, increased accumulation of the DA metabolite, 3,4 dihydroxyphenylacetic acid (DOPAC), has been observed in the hypothalamus, brain stem, mesolimbic frontal cortex, and nucleus accumbens (Blanc et al., 1980; Thierry et al., 1976; Roth et al., 1982), thus suggesting enhanced release of DA in these brain regions.

In assessing the effects of stressors on DA activity, it is important to distinguish between the various DA systems. Evidence has accumulated indicating that stressors may selectively affect DA in mesolimbic and mesocortical systems, but not in regions associated with the nigrostriatal system (Gilad, Gilad, & Rabey, 1986; Glowinski, Tassin, & Thierry, 1984; Lavielle et al., 1979; Saavedra, 1982; Thierry et al., 1976). For instance, after suppressing the synthesis of DA with the tyrosine hydroxylase

inhibitor, α -methyl-p-tyrosine (α -MpT), Thierry et al. (1976) observed that acute exposure to shock augmented the reductions of DA concentrations in the frontal cortex, and to a lesser extent in the nucleus accumbens, without affecting DA in the olfactory tubercles or the striatum. Similarly, Blanc et al. (1980) reported stressor-induced increases in the accumulation of DOPAC and an increase of the DOPAC/DA ratio (a more sensitive measure of utilization) in the frontal cortex and nucleus accumbens (see also Claustre, Rivy, Dennis, & Scatton, 1986) without provoking striatal changes. More recently, studies conducted in this laboratory (Anisman & Zacharko, 1986) revealed an increase of DOPAC accumulation and a decrease of DA content in the frontal cortex, while less pronounced changes were observed in the nucleus accumbens even though DOPAC accumulation was noticeably increased. In contrast, footshock failed to affect DOPAC or DA concentrations in the substantia nigra, striatum, and hypothalamus. Although changes in DOPAC or DA levels were not detected in the ventral tegmental area (site of origin of mesocortical projections), Deutch, Tam, and Roth (1985) demonstrated a selective increase of DOPAC levels in this brain region, but not in hypothalamic and striatal slices. While the responsivity of DA neurons in the nucleus accumbens may be limited, it seems likely that other stressors, such as immobilization, may be more effective in altering DA activity in this region (see Watanabe, 1984).

Although stressors appear to have little effect on DA regions comprising the nigrostriatal pathway, several studies have reported changes in nigrostriatal dopaminergic activity (see Beley, Beley, Rochette, & Bralet, 1976; Dunn & File, 1983; Keller, Zigmond, & Striker, 1981). These studies tended to employ stressors involving some aspect of cold exposure (e.g., exposure to 4°C, cold restraint, or cold water immersion). Thus, it is

possible that variations in nigrostriatal DA content may be attributable to thermoregulatory mechanisms, rather than to the effects of the stressor per se.

Paralleling the effects on NE alterations, cues previously associated with a stressor have been shown to increase the utilization of DA. For instance, Herman et al. (1982) found that in rats exposed to acute shock, DOPAC concentrations were increased in the anteromedial and sulcal frontal cortices, nucleus accumbens, olfactory tubercle, and amygdala. Reexposing the animals to the chamber where they had been shocked 24 hr earlier increased DOPAC accumulation in the anteromedial frontal cortex, but was without effect in other brain regions. Similarly, Claustre et al. (1986) observed an increase in DOPAC levels in the frontal cortex in rats exposed to an environment previously associated with footshock. Finally, Deutch et al. (1985) demonstrated that after reexposure to a stimulus (i.e., tone) that had been previously paired with footshock, increased DOPAC accumulation was observed in the ventral tegmental area. Thus, it appears that alterations of mesocortical DA concentrations may be subject to conditioning or sensitization factors, such that the DA alterations may become pronounced when the organism is reexposed to cues previously associated with the stressor.

Few studies have evaluated the effects of controllable and uncontrollable stressors on DA activity. Anisman and Zacharko (1986) evaluated the effects of escapable and inescapable shock on DA levels and turnover in the nucleus accumbens, but found that both treatments had similar effects. Cherek, Lane, Freeman, & Smith (1980) reported decreased affinity of DA receptor binding in the frontal cortex of rats exposed to 8 hr of uncontrollable shock over 4 days, while controllable shock was without effect. However, it is possible that the effects on DA receptor sensitivity

were related to the chronicity of the procedure, rather than an effect of control over footshock per se.

As in the case of NE, following exposure to a chronic stressor, the reductions of DA levels in frontal cortex and nucleus accumbens observed after an acute stressor were eliminated. In fact, it has been reported that DA levels actually surpassed control values in the hypothalamus, neostriatum, and forebrain after repeated stressor application (Richardson, 1984). In contrast, Roth et al. (1982) found DA concentrations in several brain regions to be unaffected by repeated (21 day) oscillation stress. Finally, Kramarcy et al. (1984) noted that the increased accumulation of ^3H -DA in frontal cortex slices observed after acute footshock was eliminated after exposure to five days of footshock. In fact, DA accumulation was slightly reduced. Furthermore, Herman, Stinus, and Le Moal (1984) reported that exposure to repeated footshock increased the sensitivity of postsynaptic DA receptors. In particular, these investigators found that even though stressor-provoked elevations of DOPAC concentrations in the frontal cortex, nucleus accumbens, and amygdala returned to basal levels 24 hr after repeated stressor application, low doses of d-amphetamine effectively increased locomotor activity in chronically shocked rats relative to nonshocked controls. Enhanced locomotor activity after d-amphetamine administration was also observed, to a lesser extent, 24 hr after acute stressor exposure.

Serotonin. In addition to the NE and DA alterations, exposure to aversive stimulation has been shown to influence the activity of serotonin (5-HT) neurons (see reviews in Anisman, Kokkinidis, & Sklar, 1981; Dunn & Kramarcy, 1984; Zacharko & Anisman, 1984). In general, elevated levels of 5-HT have been reported after mild stressor application (De Souza & Van Loon, 1986; Welch & Welch, 1970), while reductions of this amine

become apparent only after relatively intense stressor administration (Telegdy & Vermes, 1976; Thierry, 1973). For instance, following relatively brief exposure (30 min) of restraint stress 5-HT levels have been reported to be increased in suprachiasmatic and ventromedial hypothalamic nuclei (Culman, Kvetnansky, Zeman, & Kiss, 1983; Kiss, Culman, Mitro, Kvetnansky, & Palkovits, 1980). After 3 hr of immobilization, however, decreases in 5-HT content were observed not only in hypothalamic nuclei, such as the median eminence and suprachiasmatic nucleus, but also in other brain regions, including the amygdala, hippocampus, dorsal raphe, and cingulate cortex (Palkovits, Brownstein, Kizer, Saavedra, & Kopin, 1976; but see Richardson, 1984; and Saito, Morita, Miyazaki, & Takagi, 1976).

Not surprisingly, stressors have been shown to increase the synthesis and utilization of 5-HT. Thierry, Fekete, and Glowinski (1968) found an increase in radioactively labelled 5-HT formed from ^3H -tryptophan in rat brain mesencephalon after prolonged exposure (3 hr) to footshock. In addition, Thierry (1973) reported that footshock facilitated the decline of 5-HT after the inhibition of tryptophan hydroxylase with p-chlorophenylalanine methylester (PCPA). Accumulation of the serotonergic metabolite, 5-hydroxyindolacetic acid (5-HIAA), has been shown to increase after exposure to a variety of stressors (see Anisman, 1978). For instance, Morgan, Rudeen, and Pfiel (1975) reported increased accumulation of 5-HIAA in the hypothalamus, brain stem, cerebral cortex, and striatum after immobilization stress. However, it will be noted that elevations of 5-HIAA content in the striatum were observed only after a considerably extended stressor session (i.e., 300 min).

Alterations in 5-HT levels appear to be dependent upon the brain region examined, as well as the time after stressor exposure at which the

brain is examined. For instance, in the dorsal raphe nucleus 5-HT reductions were observed 10 min after stressor exposure, but not at the 30 min time period. In the median eminence and medial forebrain bundle, however, less pronounced reductions of the indolamine were noted at the 10 min interval, and transmitter levels actually increased 30 minutes after stressor application (see Palkovits et al., 1976).

Several investigators have reported that following many independent stressor sessions, the elevations of 5-HT concentrations observed after acute stressor administration are minimized or eliminated (Richardson, 1984; but see Culman et al., 1983). While some studies have reported a within session adaptation of 5-HT alterations after protracted stressor exposure (e.g., Morgan et al., 1975), others have failed to observe a return to baseline levels of 5-HT concentrations even after a 24 hr exposure to a stressor (e.g., Saito et al., 1976).

The contribution of psychological factors to changes in 5-HT content has been examined by several investigators. While it appears that cues associated with a stressor are unable to provoke alterations of 5-HT (Herman et al., 1982), the ability to control the impact of the stressor has been shown to limit the 5-HT changes in some brain regions. Specifically, Petty and Sherman (1982) reported that the marked 5-HT reductions evident in the lateral septum and anterior cortex after exposure to yoked inescapable shock were absent following exposure to an equivalent amount of escapable shock. Similarly, Weiss et al. (1981) found that uncontrollable shock reduced 5-HT content in the locus coeruleus, whereas escapable shock did not significantly alter 5-HT concentrations. In the brain stem, however, 5-HT levels were markedly higher in rats exposed to avoidable/escapable shock than in animals receiving either yoked inescapable shock or no shock whatsoever. It was suggested that the

levels of 5-HT in the brain stem may reflect the organism's behavioral response (i.e., the act of escaping from shock), rather than the effects of the stressor per se (Weiss et al., 1981).

Acetylcholine. Acetylcholine (ACh) changes have been found to occur in response to stressors. While some investigators reported alterations in choline acetyltransferase (ChAT) activity following stressor exposure (Fatranska, Budai, Oprsalova, & Kvetnansky, 1987; Gottesfeld, Kvetnansky, Kopin, & Jacobowitz, 1978; Longoni, Mulas, Oderfeld-Novak, Pepeu, & Pepeu, 1976), others found stressors, such as immobilization, to be ineffective in altering ChAT activity (Gilad & McCarty, 1981).

Stressors have also been shown to reduce the utilization of ACh (Gabriel & Soliman, 1983; Costa, Tagliomonte, Brunello, & Cheney, 1980). For instance, Gabriel and Soliman (1983) demonstrated reduced activity of the degradative enzyme, acetylcholine esterase (AChE), after exposure to a combination of immobilization and cold. Similarly, Fatranska et al. (1987) reported lowered AChE activity in the hypothalamus after 24 hr of cold exposure (5°C). However, these investigators also reported increases of AChE activity in the hippocampus after 1 and 24 hr exposure to cold. These findings suggest that the impact of stressors on AChE activity may vary from region to region.

It may be noted that alterations of ACh activity appear to be influenced by the duration and severity of the stressor. Moreover, regional differences in brain ACh activity also exist following exposure to aversive stimulation (Takayama, Mizukawa, Ota, & Ogawa, 1987). For instance, Fatranska et al. (1987) reported significant decreases of ACh concentrations in the hypothalamus and dorsal hippocampus of rats exposed to 1 or 24 hr of cold. In addition, exposure to 20 min of forced swimming in water (18°C) also provoked reductions of ACh levels in the

hippocampus and cerebral cortex. Likewise, Costa et al. (1980) reported marked reductions of ACh activity in the hypothalamus after 1, 4, and 24 hr of cold exposure. However, exposure to immobilization alone was without effect on ACh turnover, as measured by ACh concentrations following the administration of the deuterium labelled precursor phosphorylcholine. In contrast to ACh changes in the hypothalamus, reductions of ACh turnover in the frontal cortex were evident only after 60 min, and not after 4 or 24 hr of cold exposure (Costa et al., 1980). Indeed, it would seem that changes in ACh activity were observed only when either protracted restraint procedures (24 hr) or a combination of immobilization plus cold exposure were employed.

Several investigators have described biphasic changes in ACh activity after stressor exposure. For instance, Zajackowska (1975) reported that following an initial decline in ACh activity, levels of the transmitter increased 40 min to 4 hours after stressor termination. Similarly, Cooper, Schmidt, and Barrett (1983) observed increases in dorsal hippocampal ACh activity 1 and 4 hr after shock avoidance training. Moreover, these investigators reported a temporal relationship between increases in ACh activity and deficits in avoidance performance which ordinarily occurs after limited training (i.e., Kamin effect). Indeed, some investigators have suggested that the initial decline of ACh turnover may be associated with behavioral excitation and that the subsequent increase in ACh turnover may reflect behavioral inhibition (see Anisman, 1975). However, it is not clear whether the biphasic changes in ACh activity are a direct result of stressor exposure or a compensatory reaction to the catecholamine changes typically observed after aversive stimulation (Anisman, 1975).

There is reason to believe that psychological factors (e.g., conditioning or stressor controllability) may be associated with the ACh

increase. For instance, Karczmar, Scudder, and Richardson (1973) found that rats exposed to uncontrollable shock exhibited increased levels of ACh. In a separate experiment, where rats received escapable shock ACh concentrations were unaffected. However, since no attempts were made to equate the amount of shock delivered in the two experiments, differences in ACh concentrations may not necessarily reflect factors associated with control. In yet another study, Cherek et al. (1980) exposed rats to either controllable or uncontrollable footshock over five consecutive days and observed that, in contrast to controllable shock, exposure to uncontrollable shock resulted in an increase of muscarinic receptor binding. Finally, although there are limited data concerning the possibility that stressor-provoked ACh changes are subject to conditioning/sensitization effects, Hingtgen, Smith, Shea, Aprison, and Gaff (1976) demonstrated that a stimulus previously paired with shock was capable of eliciting a rise in ACh concentrations in rats.

Gamma-Aminobutyric Acid. Relatively few studies have been conducted concerning the effects of aversive stimulation on gamma-aminobutyric acid (GABA). In general, enhanced GABA concentrations have been observed after exposure to stressors, such as repeated immobilization and footshock (e.g., Bliss & Zwanziger, 1966; and Chattopadhyay & Uniyal, 1975).

Studies assessing changes in GABA receptor binding and density have demonstrated that stressors, such as handling or footshock, selectively decreased the density of low affinity ^3H -GABA binding sites in cerebellum, frontal cortex, and caudate nucleus (Biggio, Concas, Mele, & Corda, 1987; Corda & Biggio, 1986; Costa, Guidotti, & Mao, 1975). It should be noted, however, that reductions in binding to low affinity receptor sites following exposure to footshock reported in these studies were observed

only if the animals had been handled repeatedly prior to aversive stimulation. Indeed, footshock failed to provoke changes in receptor binding in nonhandled animals (Figgio et al., 1981). Since acute handling was found to decrease while repeated handling was found to increase low affinity binding, it was argued that the effects of footshock on receptor binding were evident because of elevated GABA receptors in handling-habituated rats. Parenthetically, the recovery of the density of the low affinity receptor sites (approximately 90 min) following footshock stress roughly paralleled the time course of recovery of a behavior suppressed by punishment (i.e., number of licking periods) in a conflict test. Thus, it was suggested that the response suppression may have been related to GABA receptor density (see Corda & Biggio, 1986).

It is not clear whether alterations in GABA mechanisms are secondary to stimulation of benzodiazepine recognition sites which are coupled to low affinity GABA receptors or to changes in activity of other transmitter systems. Nevertheless, it would appear that GABAergic systems are sensitive to aversive stimulation and the contributions of this transmitter to stress reactions warrants further consideration.

Opiate Peptides. It has been demonstrated that in response to environmental stressors opiate peptides (e.g., endorphins) are secreted from the pituitary into the blood stream (Dunn & Kramarcy, 1984). It has also been shown that radioactively labelled β -endorphin may be transported into the brain after release from the pituitary (Mezey & Palkovits, 1982). Since it was believed that peptides released from the anterior pituitary was the main source of peptides entering the brain, much research was directed towards describing entry of pituitary peptides into the brain (see De Wied & Jolles, 1982). However, immunochemical evidence subsequently revealed that endorphins exist in

several regions of the brain, including the arcuate nucleus of the hypothalamus, thalamus, amygdala, septum, central grey area, and locus coeruleus (De Wied & Jolles, 1982). Unfortunately, conflicting reports exist concerning the effect of stressors on central endorphin activity. Stressors have been found to either increase whole brain concentrations of endorphin (Akil, Richardson, Barchas, & Li, 1978) or decrease hypothalamic content of β -endorphin (Vuolteenaho, Leppaluoto, & Mannisto, 1982) and leu-enkephalin (Rossier, Guilleman, & Bloom, 1978).

It appears that stressor-induced opioid changes may be influenced by the severity and duration of the aversive stimulation. For instance, Barta and Yashpal (1981) found increases in β -endorphin content in several brain regions, including the paraventricular nucleus of the hypothalamus, nucleus interstitialis, striae terminalis, central amygdaloid nucleus, and dorsal raphe nucleus, after exposure to either relatively mild (20°C, 3 min) or severe (4°C, 3 min) acute swim stress. After 21 days of swim exposure, however, the β -endorphin changes observed after mild swim stress were ameliorated. In contrast, following chronic exposure to the severe stressor condition the β -endorphin changes ordinarily provoked by the acute treatment persisted. Similarly, Madden, Akil, Patrick, and Barchas (1977) reported elevations of central opioid levels following a single exposure to electric footshock, but not after 13 consecutive days of stressor exposure.

As in the case of catecholaminergic systems, psychological factors are thought to influence opioid changes (Lewis, 1986). It has been shown that stressor-induced alterations of β -endorphin binding may be provoked by cues that had been associated with the stressor (Chance, White, Krynock, & Rosecrans, 1978; De Vries, Chance, Payne, & Rosecrans, 1979). Moreover, behavioral studies have led to the suggestion that the

activation of opioid systems may be dependent upon stressor controllability (Maier, Drugan, & Grau, 1982; Maier, Sherman, Lewis, Terman, & Liebeskind, 1983).

Contribution of Neurochemical Activity on HPA Functioning

Variations in pituitary-adrenal activity are thought to be influenced by brain amines. It has been demonstrated that corticoid activity is influenced by the administration of pharmacological agents which inhibit or potentiate central amine activity (e.g., Smythe, Bradshaw, & Vining, 1983). Additionally, it has been shown that changes in amine metabolism are correlated with variations in hormone secretion (e.g., De Souza & Van Loon, 1986).

Anatomical Considerations. Surgical destruction of aminergic neurons has been employed to identify structures or pathways affecting HPA activity. These studies have implicated hypothalamic and limbic nuclei in the regulation of the HPA axis. For instance, deafferentation of the medial basal hypothalamus, including the arcuate, ventromedial, and periventricular nuclei, as well as the median eminence, produced reductions of plasma corticosterone concentrations in stressed animals (Makara et al., 1986; Seto, Saito, Kaba, & Kawakami, 1983; Van De Kar, Kerteszi, Bethea, & Ganong, 1985). Discrete bilateral electrolytic lesions of the medial basal hypothalamic nuclei, including the paraventricular arcuate nucleus and ventromedial nucleus, attenuated the corticoid response elicited by immobilization in rabbits (Seto et al., 1983). In contrast, bilateral knife cuts of the preoptic area failed to alter basal plasma corticoid concentrations and the adrenocorticoid response to ether stress (Feldman & Conforti, 1987).

While it is generally accepted that the paraventricular nucleus is an important site for the regulation of the pituitary-adrenal axis (Makara,

1985), greatest attention has been devoted to CRF fibers in the median eminence. For instance, the disappearance of CRF fibers in the median eminence following anterolateral hypothalamic deafferentation is accompanied by reductions of pituitary-adrenal activity in response to a variety of stressors, such as ether exposure and surgery (Makara, 1985; Palkovits, Makara, & Stark, 1976; Tilders, Schipper, Lowry, & Vermes, 1982). Parenthetically, deafferentation of the medial basal hypothalamus, especially the paraventricular nucleus, not only diminished activity of the pituitary-adrenal axis, but also abolished the circadian pattern of corticoid secretion (Makara, 1985; Wilson & Critchlow, 1975). Likewise, bilateral lesions of the medial forebrain bundle and suprachiasmatic nuclei of the hypothalamus eliminated the circadian rise in plasma ACTH in rats (Cascio, Shinsaka, & Dallman, 1987; Gann, Allen-Rowlands, Bereiter, & Plotsky, 1984).

With respect to limbic structures, bilateral lesions of the hippocampus (McEwen, 1982), septum (Seggie et al., 1980), and central nucleus of the amygdala (Beaulieu, Di Paulo, Cote, & Barden, 1987) have been shown to influence ACTH and corticosterone secretion. While some brain regions exert a tonic influence on basal HPA activity, the impact of other brain regions become apparent only after the application of an environmental challenge. For instance, destruction of the hippocampus has been shown to elevate serum corticoid levels under both basal conditions and following stressor exposure (see McEwen, 1982). While septal lesions were ineffective in altering basal levels of corticosterone, this manipulation potentiated the corticoid response in animals exposed to aversive stimulation (Seggie et al., 1980; but see Nyakas, De Kloet, & Bohus, 1979). In contrast to the inhibitory influence of the hippocampus and septum on HPA activity, an excitatory role has been ascribed to the

central nucleus of the amygdala. Following bilateral electrolytic lesions of this region, the secretion of ACTH ordinarily induced by immobilization stress was significantly attenuated (Beaulieu et al., 1987; but see Seto et al., 1983).

Catecholamines. Although some investigators have argued that hypothalamic NE stimulates the activity of the HPA axis after stressor exposure (Smythe et al., 1983), it is generally accepted that NE in hypothalamic nuclei tonically inhibits CRF and ACTH release (see Dunn & Kramarcy, 1984; and Weiner & Ganong, 1978). For instance, NE depletion induced by 6-hydroxydopamine (6-OHDA) lesions of the paraventricular nucleus was shown to inhibit the stressor-provoked corticoid secretion (Feldman, Conforti, & Melamed, 1986). Moreover, agents that decrease NE and/or DA turnover or receptor activity or deplete brain catecholamines (e.g., α -MpT, FLA-63, chlorpromazine, phentolamine, 6-OHDA, and reserpine) result in large increases in ACTH secretion and plasma corticoids in rats (Al-Damluji, 1988; Ogren, Archer, Fuxe, & Eneroth, 1981). The increased ACTH secretion following reserpine administration may be partially antagonized by drugs that increase NE activity, such as monoamine oxidase (MAO) inhibitors and the reuptake blocker, imipramine (Depue & Kleiman, 1979).

It appears that the inhibitory effects of NE on HPA activity may be mediated by α_2 -NE receptors in the hypothalamus (Steiner & Grahame-Smith, 1980). For instance, the intraventricular administration of clonidine, an α_2 -NE receptor agonist, was found to inhibit ACTH secretion, whereas phenylephrine and methoxamine, which exert their effects primarily on α_1 -receptor sites, were found to be ineffective (Ganong, 1984). Moreover, the α -noradrenergic blocker, phenoxybenzamine, prevented the inhibition of ACTH induced by clonidine, while prazosine,

which is thought to block α_1 -receptor sites, was without effect on ACTH activity (Ganong, 1984). In contrast to the effects of agents that influence α_2 -receptors, treatments that affect β -adrenergic receptor sites have generally been found to be ineffective in altering corticoid levels (e.g., Kynch & Eisenberg, 1974).

Dopamine plays a role in affecting HPA activity, although its contribution is less prominent than that of NE (Steiner & Grahame-Smith, 1980; Weiner & Ganong, 1978). On the one hand, the administration of dopaminergic antagonists, such as haloperidol and thioproperazine, have been shown to produce an increase in pituitary-adrenal activity (Giraud, Lissitzky, Conte-Devold, Gillioz, & Oliver, 1980; Proulx-Ferland et al., 1983), thereby supporting an inhibitory role of DA in the HPA response. On the other hand, it has been proposed that DA activation may stimulate ACTH and corticosterone secretion (Jezova, Jurcovicova, Vigas, Murgas, & Labrie, 1985). For instance, it has been found that DA agonists, such as pergolide mesylate, elevated serum corticosterone concentrations in rats (Fuller & Snoddy, 1981). Similarly, it was reported that the administration of the DA agonist, apomorphine, increased plasma corticosterone levels in mice (Freeman & Thurmond, 1986). Moreover, some investigators have found that although dopaminergic manipulations did not alter corticosteroid levels, NE manipulations produced predictable changes in corticosterone (Ganong, Kramer, Reid, Boryczka, & Shackelford, 1976). Thus, the role of DA neurons on HPA functioning remains to be fully elucidated.

It has been suggested that peripheral catecholamines, released from the medulla of the adrenal gland in response to aversive stimulation, might also influence ACTH release from the pituitary. While it is thought that increased ACTH secretion induced by peripheral catecholamines

occurs only in rats (and not in dogs or humans; Weiner & Ganong, 1978), recent evidence suggests that these plasma catecholamines may not be involved in the activation of the HPA axis in rats after all. Makara et al. (1986) reported that after recovery from an anterolateral cut around the medial basal hypothalamus, rats exposed to immobilization showed an attenuated ACTH response, a normal rise in corticosterone, and an increase in plasma catecholamines. However, chronic pretreatment with guanethidine (an agent which prevents the release of plasma catecholamines), coupled with adrenal demedullation, did not alter the increase in plasma ACTH and corticosterone elicited by immobilization in lesioned animals. Moreover, under these conditions, prior treatment with phentolamine or propranolol failed to influence the rise in serum corticosterone and ACTH. Thus, these results suggest that plasma catecholamines may not be involved in the secretion of ACTH following immobilization in rats (see also Al-Damluji, 1988).

Serotonin. While several investigators have found serotonin to have an excitatory effect on HPA activity (see Jones, 1978), others have actually found it to exert an inhibitory effect (see Dunn & Kramarcy, 1984; Weiner & Ganong, 1978). For instance, pharmacological stimulation of 5-HT neurons with acute p-chloroamphetamine (PCA) has been shown to result in elevated serum corticosterone levels (see Weiner & Ganong, 1978; and Van De Kar et al., 1985). Administration of the serotonergic precursor, 5-hydroxytryptophan (5-HTP), has been reported to increase ACTH secretion, an effect which was potentiated by the 5-HT reuptake blocker fluoxetine (Fuller, Snoddy, & Molloy, 1976). Moreover, the administration of the serotonergic synthesis inhibitor PCPA blocked the PCA-induced secretion of corticosterone (Ganong, 1984). These findings argue strongly for a stimulatory role for 5-HT.

In contrast to studies supporting an excitatory role, Telegdy and Vermes (1976) found that PCPA actually enhanced plasma concentrations of ACTH and corticosterone, thereby indicating an inhibitory role for 5-HT neurons on HPA functioning. Consistent with this view, De Souza and Van Loon (1986) observed an inverse relationship between increased hypothalamic 5-HT activity and decreased corticoid response following application of immobilization stress.

Conflicting data also exist concerning the effects of neurotoxic lesions of 5-HT neurons. For example, the enhanced corticoid response ordinarily associated with immobilization stress was greatly exaggerated in rats treated intraventricularly with the 5-HT neurotoxin, 5,6-dihydroxytryptamine (5,6-DHT; Richardson, 1984). It was suggested that the enhanced corticoid response may be attributable to the development of serotonergic postsynaptic supersensitivity in 5,6-DHT treated rats. Hence, the stressor-induced release of 5-HT onto supersensitive receptors was thought to be responsible for the elevated corticoid concentrations. In contrast to the purported stimulatory effects of 5-HT on corticoid secretion, Feldman (1985) found that an injection of 5,7-DHT into the dorsal and median raphe nuclei resulted in depletions of 5-HT in the hypothalamus and a significant increase of plasma corticoid levels after exposure to sciatic nerve and light stimulation. This finding is consistent with an inhibitory effect of hypothalamic 5-HT on pituitary-adrenal activity. However, it is possible that changes in 5-HT receptor sensitivity in other brain regions may have contributed to elevated corticoid levels. Indeed, triangular knife cuts of the dorsal and median raphe nucleus failed to prevent corticoid secretion after PCA administration, thus indicating that these nuclei may not be involved in the regulation of HPA activity (Van De Kar, Wilkinson, Skrobik, Brownfield, & Ganong, 1982).

Acetylcholine and GABA. Although most studies have focused on the effects of NE and 5-HT on glucocorticoid activity, other transmitter systems have also been shown to influence HPA activity. For instance, acetylcholine (ACh) is thought to have an excitatory role in the regulation of the negative-feedback mechanism. Intraventricular administration of ACh was found to increase CRF secretion from the hypothalamus (Plotsky, Otta, & Sutton, 1987), and administration of the antimuscarinic, atropine, into the anterior hypothalamus suppressed CRF and ACTH secretion (Krieger & Krieger, 1970; Steiner & Grahame-Smith, 1980). In vitro experiments have also demonstrated the stimulatory effects of ACh on CRF-activity in hypothalamic synaptosomes (see Jones, 1978).

Although limited information is available concerning the effects of GABA, it is generally thought that it exerts an inhibitory influence on the HPA system. For instance, intraventricular administration of GABA was reported to inhibit CRF activity, while the pretreatment with GABAergic antagonists, picrotoxin and bicuculline, prevented the inhibition of CRF secretion induced by GABA (see Jones, 1978; and Plotsky et al., 1987).

Effects of Glucocorticoids on Neurochemical Activity

Catecholamines. It is generally accepted that glucocorticoids increase activity of central catecholamine mechanisms. The magnitude and the direction of these catecholaminergic changes, however, appear to vary with the glucocorticoid dose administered, as well as the specific brain region examined (see Dunn & Kramarcy, 1984; Weiner & Ganong, 1978).

The administration of high doses of glucocorticoids has been shown to increase the formation of both ^3H -norepinephrine and ^3H -dopamine formed from ^3H -tyrosine in whole mouse brain (Iuvone, Morasco, & Dunn, 1977). Rothschild et al. (1985) reported that dexamethasone administration increased DA levels in the hypothalamus and nucleus

accumbens, but not in frontal cortex or striatum of rats. In addition, Markey, Towle, and Sze (1980) found that repeated dexamethasone application (i.e., over a three day period) increased tyrosine hydroxylase activity in the pons and locus coeruleus, but not in the substantia nigra of 7-day-old mice. Although glucocorticoid treatment has no effect on tyrosine hydroxylase activity in adult mouse brain, it has been shown to elevate brain tyrosine levels (see McEwen et al., 1986). Inasmuch as glucocorticoid administration also elevates plasma concentrations of tyrosine, the amino acid necessary for the synthesis of catecholamines, the possibility was raised that the elevations in catecholamine activity associated with glucocorticoid treatment may be related to increased uptake of tyrosine, rather than to increased tyrosine hydroxylase activity per se (McEwen et al., 1986). However, given that tyrosine hydroxylase is the rate limiting step in this reaction (tyrosine is ordinarily present in supersaturated concentrations), this is an unlikely possibility.

Consistent with the effects of corticoids on central catecholamines, it was reported that adrenalectomy markedly reduced the activity of tyrosine hydroxylase in the median eminence, arcuate nucleus, and dorsomedial nucleus of the hypothalamus (Dunn, Gildersleeve, & Gray, 1978; Kizer et al., 1974). Similarly, Rastogi and Singhal (1978) found that adrenalectomy decreased catecholamine levels in the striatum, and selectively decreased NE concentrations in the hypothalamus and DA levels in the brain stem. Furthermore, these catecholamine variations were reversed by corticosterone administration. Adrenalectomy also appeared to provoke reductions in dopamine- β -hydroxylase activity in the hypothalamus and brain stem (see Weiner & Ganong, 1978). Again, large doses of corticosterone (over a two day period) effectively increased

hypothalamic dopamine- β -hydroxylase activity and restored steady state levels of this enzyme.

In contrast to those studies reporting reduced tyrosine hydroxylase and dopamine- β -hydroxylase activity after adrenalectomy, several investigators reported increases in catecholamine activity after such treatment (e.g., Fuxe et al., 1973; Javoy, Glowinski, & Kordon, 1968). Since variations in NE and DA activity after adrenalectomy have been shown to vary with the passage of time, it is possible that the aforementioned increases may be due to the short-term effects of adrenalectomy (McEwen et al., 1986). In fact, Versteeg, Van Zoest, and De Kloet (1984) found increases in NE turnover 1 hr after adrenalectomy, whereas 6 days after removal of the adrenal glands decreases of NE were noted in several hypothalamic nuclei, including the median eminence, arcuate nucleus, paraventricular nucleus, and dorsomedial nucleus.

It might be argued that increases in catecholamine activity induced by adrenalectomy may be a result of elevations in circulating ACTH which occur following such treatment. Support for this position comes from studies showing that a single injection of ACTH is capable of increasing NE concentrations in the locus coeruleus, as well as DA content in the arcuate nucleus of the rat (Fekete, Herman, Palkovits, & Stark, 1976). Additionally, Telegdy and Kovacs (1979) found increases of DA in striatum and mesencephalon 30 min after administering high doses of ACTH, whereas Delanoy, Kramarcy, and Dunn (1982) found intraventricular injections of ACTH to stimulate the synthesis of ^3H -DA formed from ^3H -tyrosine in slices of mouse frontal cortex.

The effects of hypophysectomy on catecholaminergic biosynthetic enzymes are less clear. For instance, Van Loon, De Souza, and Kim (1978) found that hypophysectomy was associated with an increase in tyrosine

hydroxylase activity in the hypothalamus, pons-medulla, cerebellum, and hippocampus, and that chronic treatment with ACTH attenuated the increase in tyrosine hydroxylase activity in the hypothalamus, but not in any other brain region examined. In another study, however, Van Loon (1976) reported decreased hypothalamic dopamine- β -hydroxylase activity in hypophysectomized rats, and that this effect could be reversed by low doses of ACTH or high doses of corticosterone. Parenthetically, the ACTH-induced increase of dopamine- β -hydroxylase activity was not attributable to the activation of corticosterone, as the dose of ACTH employed in this study was insufficient to provoke corticoid secretion from the adrenal glands.

Since stressors have been shown to alter the responsiveness of the NE-coupled adenylate cyclase (NE-cAMP) system in the brain (see Stone, 1983; Stone et al., 1984), attempts have been made to evaluate the effects of adrenalectomy and hypophysectomy on the sensitivity of the NE-cAMP system. It will be recalled that exposure to repeated stressor application reduced the sensitivity of the NE-coupled adenylate cyclase system. In contrast, it appears that both adrenalectomy and hypophysectomy increased the responsiveness of this system to NE stimulation in the rat frontal cortex (Mobley, Manier, & Sulser, 1983). However, this effect was restricted to the β -adrenergic receptors as the cAMP response was increased after stimulation with NE, but not after stimulation with the β -adrenergic receptor agonist, isoproterenol. Moreover, the adrenalectomy-induced enhancement of the cAMP response to NE was completely antagonized following three days of corticosterone administration.

In accordance with the data showing hypophysectomy-induced α -NE up-regulation, it has been reported that repeated ACTH application effectively attenuated the enhanced cAMP response (Stone, Platt, Herrera,

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& Kirk, 1986). Interestingly, this effect of ACTH on cAMP could be abolished by the administration of prazosine (an α_1 -receptor blocker). These reports indicate that corticosterone and ACTH effectively reduce the adenylate cyclase activity, and that this effect is mediated by the α -adrenergic component of the system (see also Stone, 1987). It should be noted, however, that some investigators have reported that glucocorticoids produce marked changes in β -adrenergic receptor density in the brain (Roberts & Bloom, 1981). Thus, the influence of glucocorticoids and ACTH on the β -adrenergic receptors should not be dismissed.

Serotonin. It appears that glucocorticoid administration increases central 5-HT activity in some brain regions (Rastogi & Singhal, 1978; Dunn & Kramarcy, 1984). For instance, Telegdy and Vermes (1976) reported that corticosterone increased 5-HT levels in the hypothalamus, amygdala, and mesencephalon of rats, but was ineffective in altering 5-HT content in hippocampus and septum. Rothschild et al. (1985) found that dexamethasone also increased hypothalamic 5-HT concentrations, but markedly decreased 5-HT and 5-HIAA levels in the frontal cortex (see also Wolkowitz et al., 1986). It appears, however, that corticosterone may have dose dependent effects on 5-HT content. Indeed, Kovacs, Telegdy, and Lissak (1976) found that while low doses of corticosterone, such as those employed by Telegdy and Vermes (1976), increased hypothalamic 5-HT concentrations, high doses of corticosterone had an opposite effect.

Consistent with the notion that corticoids increase 5-HT activity, several investigators demonstrated that glucocorticoid administration increased brain tryptophan hydroxylase activity (Azmitia & McEwen, 1974), while adrenalectomy decreased tryptophan hydroxylase (Azmitia & McEwen, 1974) and 5-hydroxytryptophan decarboxylase activity (Kizer, Palkovits, Kopin, Saavedra, & Brownstein, 1976). Moreover, adrenalectomy

attenuated the increase in tryptophan hydroxylase activity induced by either footshock, cold exposure, or ether stress (Azmitia & McEwen, 1974). Although ACTH administration has been shown to produce changes in 5-HT concentrations comparable to that induced by corticosterone (Kovacs et al., 1976), it seems unlikely that this hormone is directly responsible for regulation of serotonergic activity. Not only are the effects of ACTH on 5-HT neurons paralleled by corticoids, but they can be prevented by adrenalectomy (Kovacs et al., 1976).

In addition to the enzymatic changes, reductions of 5-HT turnover after adrenalectomy have been reported in several brain regions, including the hypothalamus, hippocampus, raphe, and brain stem (De Kloet et al., 1982; De Kloet, Versteeg, & Kovacs, 1983; Van Loon, Shum, & Sole, 1981). The decrease in 5-HT turnover in these brain regions was reversed by corticosterone administration, and was prevented by pretreatment with low doses of either corticosterone or dexamethasone prior to adrenalectomy (Van Loon et al., 1981). Dexamethasone, however, was ineffective in ameliorating the reduction of 5-HT activity in the dorsal hippocampus (De Kloet et al., 1982). While the data described thus far are consistent with the proposition that glucocorticoids influence 5-HT activity, contradictory results have been reported. In particular, Kizer et al. (1976) found that neither adrenalectomy nor repeated dexamethasone application provoked changes in tryptophan hydroxylase activity in hypothalamus, raphe nuclei, hippocampus, and amygdala. Unfortunately, the source for these divergent results is not immediately evident.

Acetylcholine and GABA. Limited data are available concerning the effects of glucocorticoids on ACh neurons. Gabriel and Soliman (1983) found that, like the combination of restraint and cold exposure, the administration of corticosterone provoked a decline of AChE activity. The

comparable effects of corticosterone and stressor exposure on AChE activity indicates that alterations of ACh functioning may be influenced by pituitary-adrenal activity. Indeed, Wood, Matthe-Sorensen, Cheney, and Costa (1978) reported that the intraventricular administration of ACTH increased ACh turnover rates in the hippocampus, but not in the cortex, striatum, or diencephalon. In contrast, however, neither adrenalectomy nor corticosterone administration altered cholinergic muscarinic receptor binding in the cerebral cortex, hippocampus, and hypothalamus of rats (Kendall, McEwen, & Enna, 1982).

The administration of corticosterone or ACTH has been shown to decrease GABA concentrations in the brain (Kendall et al., 1982; Leonard, 1974). Moreover, it appears that glutamic acid decarboxylase (GAD) activity is not influenced by the application of these hormones (Dunn et al., 1978). Adrenalectomy has been shown to increase GABA receptor binding in the corpus striatum and midbrain of rats (Kendall et al., 1982). However, increased receptor binding occurred at different times in these brain regions. While increases in receptor binding to ^3H -GABA occurred within three days in the midbrain, a delayed increase (14 days) was noted in the corpus striatum. Furthermore, prior administration of corticosterone was found to prevent adrenalectomy-induced increase in GABA receptor binding.

In contrast to adrenalectomy, hypophysectomy was found to decrease ^3H -GABA receptor binding in the midbrain. Interestingly, corticosterone was unable to reverse the decrease in GABA receptor binding, whereas the administration of ACTH for three days induced an increase in midbrain GABA receptor binding which was comparable to that observed after adrenalectomy (Kendall et al., 1982). These studies

indicate that ACTH itself may be fundamental in altering central GABAergic activity.

Behavioral Disturbances Associated with Stressor Exposure

A great deal of attention has focussed on the wide range of behavioral disturbances provoked by aversive stimulation. As in the case of the biochemical changes, several of the stressor-induced behavioral changes may be influenced by organismic (e.g., age or genetic) and experiential factors (e.g., prior stressor history or social housing conditions). Not surprisingly, the ability to exert control over the impact of the stressor has been found to play a prominent role in determining whether or not impairments in responding will be evident in tasks involving aversive motivation. Accordingly, the behavioral changes observed after exposure to uncontrollable stressors, as well as some of the neurochemical and hormonal variations accompanying these changes will be presented.

Although response decrements after uncontrollable stressor exposure have been shown in several behavioral paradigms, none has received as much attention as the escape deficit observed in avoidance/escape paradigms (see Anisman, Kokkinidis, & Sklar, 1981; Maier & Seligman, 1976; and Weiss, Glazer, & Pohorecky, 1976). In contrast to those animals which had the opportunity to avoid or escape from shock, animals that had been exposed to comparable amounts of uncontrollable or inescapable shock (delivered in a yoked procedure) subsequently exhibited retarded response latencies and failures to avoid or escape from aversive stimuli. Indeed, these animals appeared to accept shock passively, without apparent attempts to escape. If, however, animals had previously received escape training, then the behavioral interference ordinarily observed after inescapable shock was minimized.

These findings obviously implicate stressor controllability in determining subsequent behavioral deficits in paradigms involving aversive motivation.

Several views have been expressed to account for the behavioral disturbances induced by uncontrollable stressors. According to one view (learned helplessness hypothesis), animals exposed to inescapable shock learn that behavioral responding is independent of stressor termination. It is argued that as a consequence of learning that responses and outcomes are unrelated, the incentive to respond actively is diminished and animals cease all attempts to escape. Moreover, it is thought that the uncontrollability of the situation proactively interferes with the organism's ability to make response-outcome associations (see Maier & Seligman, 1976). In the analysis of clinical depression, Seligman and his associates (Abramson et al., 1980; Abramson, Seligman, & Teasdale, 1978) extended their formulations to include attributional changes induced by uncontrollable stressors. These investigators suggested that the individual's attribution of aversive events and expectancy concerning future performance impacts significantly upon the occurrence of the clinical condition (see Abramson et al., 1978). Several investigators have offered alternative hypotheses to account for the behavioral deficits induced by inescapable shock. According to the "learned competing response" hypothesis, during the inescapable shock session animals learn to adopt behaviors that are correlated with shock offset. These response styles may be incompatible with the response necessary in subsequent escape or avoidance tasks. In effect, the performance deficits observed after inescapable shock may be attributable to these unauthorized responses competing with the acquisition of the response necessary for

successful escape or avoidance (Black, 1977; Bracewell & Black, 1974; Glazer & Weiss, 1976a, 1976b).

While not necessarily at odds with the learned competing response position, a third hypothesis maintains that the stressor-induced behavioral impairments may be the result of nonassociative deficits in motor activation. For instance, Anisman, deCatanzaro, and Remington (1978) reported that immediately after shock onset, high levels of shock-elicited motor activity were evident. Following several seconds of shock, however, activity levels declined substantially. Among animals previously exposed to inescapable shock the decline of motor activity was exacerbated. Thus, it was suggested that disturbances of escape performance may be attributable to the particularly low levels of activity engendered by exposure to a shock of long duration. However, when escape is made possible soon after shock onset the high levels of activity elicited during the first few seconds of shock delivery would favor successful escape performance.

Consistent with this proposition, profound behavioral deficits were observed when animals were required to sustain active responding for relatively protracted periods in shuttle-escape tasks (Anisman, Remington, & Sklar, 1979). Delaying escape for several seconds after shock inception in a shuttle-escape task (Anisman, Remington, & Sklar, 1979) or increasing the height of the barrier separating the compartments of the shuttle box (Weiss & Glazer, 1975) enhanced the shuttle-escape deficits induced by inescapable footshock. In contrast, successful escape responding was observed when escape was made possible shortly after shock onset or when the requirements of the task were simplified (i.e., a nose-poke response to terminate the delivery of shock). Similarly, imposing an escape delay in a water-escape task or lowering the

temperature of the water (which increases energy expenditure) was found to impair escape performance in animals exposed to inescapable shock (Irwin, Suissa, & Anisman, 1980). Taken together, these findings indicate that stressor-induced response disturbances are more likely to be manifested as the motor demands of the task are increased.

The duration of the behavioral impairments observed following inescapable shock varies across species and with the stressor parameters employed. For instance, Maier and Seligman (1976) reported that in dogs the disruption of escape performance was absent within 72 hr of stressor termination, whereas in rodents, they may persist for weeks (Glazer & Weiss, 1976a, 1976b). In rodents, however, the time course of the motor deficits appears to be dependent upon the shock parameters employed. Although long duration shock of moderate intensity was found to induce long lasting escape deficits, these stressor parameters failed to provoke performance deficits when animals were tested shortly after stressor exposure. Interestingly, short duration shock (i.e., 2 sec) of high intensity produced only a transient performance deficit, such that the behavioral disturbances seen at 30 min after shock were absent 24 hr after such treatment (Glazer & Weiss, 1976a, 1976b). Comparable results were also obtained in mice (Anisman et al., 1978), in that immediately after long duration shock of low intensity mice exhibited proficient shuttle-escape performance, whereas 24-96 hr following stressor termination deficits in escape performance were evident. It was suggested that transient reductions in NE were responsible for the performance deficits observed shortly after exposure to high intensity shock of short duration. In contrast, the long lasting escape deficits provoked by long duration shock of moderate intensity were a consequence of learned competing motor

responses (acquired during the inescapable shock session) interfering with the response necessary for successful escape (Glazer & Weiss, 1976b).

While not denying a role for the development of instrumentally learned competing response tendencies, Anisman and his associates (Anisman & Sklar, 1979; Anisman, Kokkinidis, & Sklar, 1981; Zacharko & Anisman, 1984) proposed that alterations in neurochemical activity may account adequately for the behavioral disturbances observed following uncontrollable shock. In addition to the decline in NE, stressor-provoked alterations in DA and ACh were hypothesized to produce disturbances of response maintenance which interfered with escape (see Anisman, Kokkinidis, & Sklar, 1981). To account for the fact that the stressor-induced behavioral deficits are long lasting while the amine depletions are transient, Anisman and Sklar (1979) proposed that the long term interference of performance could be due to the sensitization of neurochemical mechanisms. In particular, it was demonstrated that NE reductions provoked by stressors could be readily reestablished upon subsequent reexposure to a limited amount of shock (Anisman & Sklar, 1979; see also Zacharko & Anisman, 1984). As indicated earlier, subsequent studies also reported that cues associated with shock will increase utilization of both NE and DA (Cassens et al., 1980; Herman et al., 1982). Hence, it was maintained that although adequate levels of amine are available when testing is conducted at long intervals after inescapable shock, the reinduction of NE or DA depletion during the first few shock trials is responsible for the emergence of behavioral disturbances (Anisman & Sklar, 1979).

While NE and DA (Kobayashi et al., 1976; Kvetmanský et al., 1976), as well as 5-HT, changes (see Anisman, Kokkinidis, & Sklar, 1981; Thierry, 1973) may induce the motor deficits and the behavioral disturbances

observed some time after stressor application, the absence of these deficits immediately or shortly after exposure to inescapable shock of moderate intensity is thought to be related to increased levels of arousal and anxiety. It is conceivable that the anxiety and behavioral arousal engendered by the aversive stimulation competes with the expression of learned competing motor response tendencies acquired during the inescapable shock session. In a series of experiments, Prince and Anisman (1984) demonstrated that the biphasic changes (i.e., initial excitation and the subsequent behavioral depression) observed with moderate intensity inescapable shock exposure are not limited to tasks involving shock escape, but are also evident in a forced-swim task. In particular, when placed in a water-filled tank immediately after shock application mice engaged in active swimming; however, 24 hr after stressor termination behavioral depression (i.e., floating or immobility) was evident. The motor excitation evident soon after stressor treatment appeared to be unrelated to stressor controllability, whereas the response depression evident at the 24 hr interval was evident only in mice that received inescapable shock. While the response depression may be subserved by catecholamine depletion, it was argued that the behavioral excitation observed immediately after stressor exposure may be due to anxiety engendered by the aversive stimulation. Consistent with such a proposition, the response invigoration induced by inescapable shock was effectively reduced by benzodiazepine treatment (Prince, Collins, & Anisman, 1986).

It appears that the behavioral arousal evident after exposure to moderate footshock may be overcome with the application of relatively intense uncontrollable shock (see Glazer & Weiss, 1976a, 1976b). It is likely that the catecholaminergic depletion evident after exposure to intense inescapable shock is sufficient to provoke profound deficits in

motor activity which mask the anxiety effects that would otherwise be elicited by the stressor. It is thought that alterations of NE activity in the locus coeruleus may be related to the stressor-induced behavioral depression (Weiss & Simson, 1985). As indicated earlier, it has been hypothesized that the role of the locus coeruleus and its projections to major brain regions (e.g., cerebellum, hippocampus, cortex) is to regulate the organism's vigilance to environmental stimuli and to initiate the selection of adaptive behavioral responses once a state of heightened arousal exists (Aston-Jones, 1985). Under moderately stressful conditions, activation of locus coeruleus neurons is thought to release NE in terminal regions which serves to heighten the organism's attention to the environmental stimulus. Following exposure to strong uncontrollable shock, however, Weiss and Simson (1985) suggested that NE concentrations are decreased in the locus coeruleus, thereby resulting in less stimulation of the adrenergic receptor sites (i.e., α_2 -NE receptors which inhibit locus coeruleus cells) located on the cell bodies of these neurons. The diminished stimulation of autoreceptors results in increased synthesis and release of transmitter at terminal regions (e.g., hippocampus and cortex). It is the increased release of NE in terminal fields which is thought to be responsible for the behavioral depression observed following exposure to intense inescapable shock. Moreover, these investigators have proposed that changes involving NE in the locus coeruleus may be associated with the up-regulation of α -adrenergic receptors and the induction of postsynaptic β -NE receptor subsensitivity observed following chronic stressor application (see Weiss & Simson, 1985 for extended discussion). These data are particularly interesting in that they not only account for the compensatory increases in amine synthesis and elevated NE concentrations in several brain regions, but also for the

down-regulation of the β -NE coupled adenylate cyclase system associated with a chronic stressor regimen (Stone, 1983).

In addition to the motoric consequences, it has been suggested that uncontrollable stressors result in the restriction of the range of defensive behaviors emitted by the organism. It is thought that following aversive stimulation the likelihood of emitting species specific defensive behaviors high in the organism's innate repertoire (or previously established responses) are increased (Bolles, 1970). For instance, the innate tendency of mice to approach the illuminated portion of a water-filled maze or arena was found to be enhanced by exposure to uncontrollable shock (Prince et al., 1986; Szostak & Anisman, 1985). It was suggested that the excitation or anxiety engendered by prior inescapable shock treatment resulted in mice persisting in emitting the perseverative response strategies ordinarily observed in these paradigms. In accordance with this view, it was demonstrated that the response perseveration in the water-filled arena was eliminated by treatment with diazepam (Prince et al., 1986).

Adopting highly prepared response styles may prove to be adaptive in some situations. For instance, the response excitation observed immediately following inescapable shock favors proficient escape performance when the response required is an active, simple response. In other situations, however, prepared defensive strategies may actually hinder the development of alternate behavioral responses which could eliminate or minimize the impact of the stressor (e.g., when the required response is a contraprepared one; Anisman et al., 1984; Szostak & Anisman, 1985). Although inescapable shock did not influence response-choice/positional discrimination performance, reversal of a previously acquired response (i.e., entering the arm to the right of the start arm on

any given trial in a Y-maze) was disrupted by prior inescapable shock. These animals persisted in maintaining a response style which previously proved successful (i.e., response perseveration).

While considerable data have accumulated demonstrating performance deficits in tasks involving aversive motivation, stressors have also been shown to disrupt performance in tasks involving dominance hierarchies, appetitive motivation, and intracranial self-stimulation. For instance, inescapable shock altered the dominance hierarchy in a food-competition paradigm. In an experiment in which rats were required to compete for food, Rapaport and Maier (1978) reported that controllably shocked animals spent significantly longer periods of time at the food source than their yoked counterparts. Moreover, when animals were tested individually, comparable latencies to approach the food source, as well as the quantity of food consumed, were observed in escapably shocked and inescapably shocked rats. Thus, it is unlikely that the lowered dominance of inescapably shocked rats was related to the behavioral changes observed.

In tasks involving appetitive motivation, exposure to uncontrollable shock has been shown to interfere not only with the acquisition of a lever press operant for food pellets, but also with the acquisition of an appetitive response-choice discrimination (i.e., reversal of a previously preferred response; Rosellini, 1978; Rosellini, DeCola, & Shapiro, 1982). Moreover, performance deficits in the response-choice discrimination task were attributable to the uncontrollability of the shock and not to shock exposure per se (Rosellini et al., 1982).

Motivational changes following uncontrollable stressor exposure have also been demonstrated in paradigms involving responding for electrical brain stimulation. These stressor-provoked changes in

responding for intracranial self-stimulation (ICSS) appear to be limited to discrete brain regions. In particular, mice that received uncontrollable shock exhibited marked reductions in ICSS response rates relative to nonshocked and escapably shocked mice (Zacharko, Bowers, Kokkinidis, & Anisman, 1983); however, these reductions were observed only in mice with electrodes implanted in mesolimbic DA regions (e.g., nucleus accumbens) or areas through which DA fibers traverse (e.g., medial forebrain bundle), and not in mice with electrodes implanted in the nigrostriatal DA system (e.g., substantia nigra). Since exposure to uncontrollable stressors provoked alterations of DA activity in the nucleus accumbens and ventral tegmental area (but not in the substantia nigra; Claustre et al., 1986; Deutch et al., 1986; Glowinski et al., 1984), it was suggested that stressors affect responding for rewarding ICSS owing to the DA variations (Zacharko et al., 1983).

Neurochemical Correlates. As indicated earlier, several investigators have attributed the stressor-provoked behavioral impairments to variations in central amine activity. Indeed, several lines of evidence are commensurate with this view. For instance, the behavioral disturbances in shuttle-escape performance, like the central depletions of NE, appear only after exposure to yoked uncontrollable shock and not after exposure to controllable shock (Anisman, Pizzino, & Sklar, 1980; Weiss et al., 1970). Similarly, Weiss et al. (1981) noted that the time course of the NE depletions in the locus coeruleus roughly paralleled the time course of the behavioral depression observed in a forced-swim task. Moreover, the elimination of the stressor-induced behavioral deficits after repeated stressor application coincided with the increased central NE levels and activity associated with a chronic stressor regimen (Irwin, Ahluwalia, & Anisman, 1986; Weiss et al., 1975; Weiss, Glazer, & Pohorecky, 1976). It

might be noted, however, that in addition to the alteration of NE turnover, chronic stressors also provoke β -NE receptor down-regulation (see Stone, 1983). In fact, Platt and Stone (1982) reported that reductions of the immobility response in a forced-swim task after chronic stressor exposure were associated with the development of β -NE receptor subsensitivity. Thus, it is possible that alterations in β -NE receptor sensitivity, rather than alterations of NE turnover may be responsible for the reinstatement of behavioral responding following repeated stressor application.

In addition to the data suggesting a relationship between central NE changes and the behavioral impairments observed following uncontrollable stressor exposure, there is evidence linking DA changes with stressor-provoked behavioral disturbances. As indicated earlier, alterations in responding for ICSS appear to be restricted to those brain regions which are associated with changes in DA activity after uncontrollable stressor exposure (i.e., nucleus accumbens and medial forebrain bundle, but not substantia nigra; Zacharko et al., 1983). Moreover, paralleling the finding that chronic stressor application eliminated reductions of DA levels in frontal cortex and nucleus accumbens ordinarily observed after an acute stressor (Kramarcy et al., 1984; Richardson, 1984), the disruption of self-stimulation responding induced by acute shock was absent after repeated exposure to stress (Zacharko & Anisman, 1989).

Consistent with the proposition that stressor-induced alterations in brain 5-HT influence behavioral responding, Sherman and Petty (1980) observed that the behavioral deficits provoked by inescapable shock exposure were antagonized by direct injection of 5-HT into the frontal cortex and septum of rats. Accordingly, these investigators maintained that decreased levels of 5-HT were responsible for the escape deficits

following uncontrollable exposure. Weiss et al. (1981) also reported a relationship between changes in serotonergic activity and behavioral depression observed in a forced-swim task. These investigators reported changes in serotonergic activity in the locus coeruleus which were similar to NE variations in this brain region. For instance, the reductions of central 5-HT, evidenced 90 min and 48 hr following inescapable stressor application, were eliminated at the 72-96 hr interval. Furthermore, these changes in 5-HT levels coincided with the time course of behavioral deficits observed in the forced-swim procedure. Although the correlation between 5-HT concentrations and behavioral depression failed to reach statistical significance, the importance of this transmitter in stressor-induced behavioral impairments should not be dismissed.

Pharmacological Manipulations. Support for the neurochemical position was derived from studies which assessed the effects of pharmacological agents on stressor-provoked behavioral impairments. While some investigators have provided evidence implicating alterations of NE activity, others have ascribed a role for DA, ACh, 5-HT, and GABA (Anisman, Irwin, Sklar, 1979; Anisman, Glazier, & Sklar, 1981; Petty, 1986; Sherman & Petty, 1980; Simson, Weiss, Ambrose, & Webster, 1986; Weiss, Glazer, & Pohorecky, 1976; Weiss et al., 1979). If alterations of central amine activity were responsible for stressor-induced performance deficits, then the administration of pharmacological agents that promote neurochemical changes which are similar to those provoked by inescapable shock exposure would be expected to influence shuttle-escape performance. Indeed, Weiss, Glazer, and Pohorecky (1976) reported that tetrabenazine, which reduces biogenic amine levels, provoked performance deficits in a shuttle task. Similarly, agents which reduce concentrations of NE (e.g., FLA-63) or both NE and DA (e.g., α -MPT)

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provoke shuttle-escape deficits only under those test conditions where inescapable shock elicits the behavioral impairments (Anisman, Irwin, & Sklar, 1979; Anisman, Remington, & Sklar, 1979). That is, when escape was made possible immediately after shock onset, these agents were ineffective in provoking behavioral disturbances. If, however, escape was delayed for 4 to 6 seconds after shock onset (i.e., gate delay manipulation), then severe behavioral deficits were induced by these compounds (Anisman, Remington, & Sklar, 1979). Furthermore, the administration of the neurotoxin 6-OHDA, as well as, dopamine receptor blockers, such as haloperidol and pimozide, provoked deficits in escape performance (Anisman, Remington, & Sklar, 1979; Weiss et al., 1979).

A second prediction which follows from the neurochemical hypothesis is that drug treatments which increased catecholamine activity should reduce or eliminate stressor provoked behavioral disturbances. In accordance with this prediction, it was demonstrated that agents which increase NE or both NE and DA activity eliminated the performance deficits induced by inescapable stressor exposure. For instance, agents which prevent the degradation of NE, such as the MAO inhibitor, pargyline, eliminated the response decrements observed after inescapable shock exposure (Simson et al., 1986). Likewise, the administration of the catecholamine precursor, l-DOPA, as well as the DA agonist, apomorphine, and the NE agonist, clonidine (in high doses), prevented the occurrence of the escape deficits, such that escape performance in drug treated animals was comparable to that of nonshocked controls (Anisman, Remington, & Sklar, 1979; Anisman, Suissa, & Sklar, 1980).

In addition to eliminating escape deficits induced by previously applied inescapable shock (i.e., a therapeutic drug effect), the administration of a drug prior to inescapable shock application may have

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prophylactic effects on subsequent escape responding. For instance, 1-DOPA, apomorphine, and clonidine, as well as pargyline, were found to prevent shuttle-escape deficits when injected prior to the inescapable shock session (Anisman & Sklar, 1979; Anisman, Suissa, & Sklar, 1980; Weiss et al., 1975). Moreover, the therapeutic effects of these agents were not attributable to either state dependent learning or carry-over effects of the drug treatments (Anisman, Suissa, & Sklar, 1980).

As in the case of catecholaminergic agents, cholinergic manipulations have been shown to influence escape performance following uncontrollable stressor exposure. For example, the cholinesterase inhibitor, physostigmine, disrupted escape performance in a motorically demanding shuttle-escape task, whereas the cholinergic (muscarinic) receptor blocker, scopolamine, eliminated the behavioral disturbance engendered by prior inescapable shock (Anisman, Remington, & Sklar, 1979; Anisman, Glazier, & Sklar, 1981). Moreover, the administration of the peripherally acting anticholinergic, methylscopolamine, failed to prevent the occurrence of the escape deficits provoked by physostigmine, suggesting that central ACh mechanisms influence behavioral responding after inescapable shock exposure.

Although both anticholinergics and catecholamine stimulants eliminated the behavioral deficits engendered by previously administered inescapable shock, the effects of these agents could be distinguished from one another. In particular, unlike the prophylactic effects of catecholaminergic stimulants (i.e., 1-DOPA, apomorphine, clonidine) on shuttle-escape performance, the administration of scopolamine prior to inescapable shock exposure was ineffective in preventing escape deficits (i.e., the drug did not act as a prophylactic). It was suggested that while catecholamine changes may be a necessary condition for the initial

provocation of the behavioral disturbance, cholinergic alterations may be involved in the maintenance of the response deficit (Anisman, Glazier, & Sklar, 1981).

Conflicting data exist concerning the effects of serotonergic agents on stressor-provoked behavioral disturbances. Although Sherman and Petty (1980) maintained that decreased levels of 5-HT were responsible for the escape deficits observed after uncontrollable shock exposure, others have reported that inhibition of tryptophan hydroxylase by systemic administration of PCPA eliminated the shock-induced disturbances in escape responding (Edwards, Johnson, Anderson, Turano, & Henn, 1986), whereas pretreatment with the serotonergic precursors, l-tryptophan and 5-HTP, produced deficits in the acquisition of shuttle-escape responding (Brown, Rosellini, Samuels, & Riley, 1982). In another experiment, Brown et al. (1982) reported that the escape deficits induced by 5-HT precursors were antagonized by the 5-HT depleting agent methysergide. In contrast to these findings, however, Anisman, Irwin, and Sklar (1979) failed to observe performance deficits with the serotonergic precursor 5-HTP. Moreover, Hamilton, Zacharko, and Anisman (1986) reported that the serotonin release induced by PCA eliminated the escape deficits induced by uncontrollable shock, whereas methysergide effectively induced decrements in shuttle-escape responding. It may be noted that, unlike the prophylactic effects of catecholamine stimulants on the escape deficits engendered by exposure to inescapable shock, the administration of PCA antagonized the behavioral deficits only if applied prior to shuttle-escape testing. Hence, it was suggested that while catecholaminergic alterations may be implicated in the provocation of the behavioral disturbance, changes in 5-HT activity may be involved in the expression of these escape deficits (Hamilton et al., 1986).

Pharmacological manipulation of GABA mechanisms has been shown to both prevent the occurrence of stressor-induced performance decrements or reverse these deficits once established. For instance, prior injection of GABA directly into the frontal cortex, hippocampus, or lateral geniculate body acted prophylactically and prevented the occurrence of the behavioral disturbance (Sherman & Petty, 1980). Moreover, the administration of GABA directly into either the hippocampus or lateral geniculate body effectively reversed the behavioral deficit induced by inescapable shock exposure. Since the antidepressant, desmethyylimipramine (DMI), applied to the same brain regions also eliminated the behavioral impairments, it was suggested that prevention of the behavioral disturbance by antidepressant agents may involve GABAergic mechanisms (Sherman & Petty, 1980; Petty, 1986).

Further evidence for the neurochemical hypothesis stems from studies evaluating the effects of clinically active agents to reverse or prevent the occurrence of the behavioral deficits following exposure to inescapable shock. For instance, Kametani, Nomura, and Shimizu (1983) reported that the acute administration of either tricyclic antidepressant agents (imipramine or DMI) or an atypical antidepressant (nomifensine) effectively reversed shuttle-escape deficits in rats previously exposed to inescapable shock. In contrast, Sherman, Sacquitne, and Petty (1982) reported that chronic, but not acute, administration of tricyclic antidepressants (e.g., DMI, amitriptyline), atypical antidepressants (iprindole or mianserin), or MAO inhibitors (iproniazid or pargyline) was effective in reversing the shock-induced behavioral depression.

In contrast to antidepressants, anxiolytics (e.g., diazepam, lorazepam, or chlordiazepoxide) were shown to be ineffective in altering the deficits in performance engendered by previously applied inescapable shock

(Sherman et al., 1982). It appears, however, that anxiolytic agents may have prophylactic effects on stressor-provoked behavioral impairments. In particular, Drugan, Ryan, Minor, and Maier (1984) reported that the administration of diazepam prior to inescapable shock prevented the occurrence of escape deficits when rats were tested 24 hr later. If the drug was administered prior to escape testing, instead of prior to inescapable shock training, then diazepam was ineffective in altering the escape deficits. It was suggested that anxiety may be a necessary precondition for the neurochemical changes that promote the occurrence of behavioral impairments. In effect, alleviating anxiety with diazepam may have prevented the neurochemical changes ordinarily engendered by inescapable shock exposure. However, once these neurochemical changes occur, treatment with the anxiolytic is ineffective in modifying escape performance.

It should be underscored that the ability of pharmacological agents to alter stressor-induced performance deficits may vary across strains of mice. For instance, studies conducted in this laboratory demonstrated that DMI effectively reduced shuttle escape latencies in A/J mice, but was without effect in either CD-1, Balb/cByJ or C57BL/6J strains (Shanks & Anisman, 1987). It is likely that the differential effects of antidepressant treatment may be attributable to stressor-induced variations in neurochemical activity in these strains of mice. Indeed, it is well established that in response to stressors strains of mice differ in their distribution, concentration, and turnover of central amines (Herve et al., 1979; Schmidt, Cooper, & Barrett, 1980; Wahlsen, 1982). For example, in strains of mice (i.e., Balb/cByJ, C57BL/6J, and DBA/2J) which differ in their neurochemical responsivity to uncontrollable stressors, strain dependent decrements in responding for ICSS in the nucleus accumbens

were observed following exposure to inescapable shock (Zacharko, Lalonde, Kasian, & Anisman, 1987). As previously demonstrated in CD-1 mice (see Zacharko, Bowers, & Anisman, 1984), ICSS response rates decreased in mice of the DBA/2 strain. In Balb/cByJ mice, however, responding for ICSS actually increased, whereas performance in C57BL/6J mice was unchanged by stressor application. Unlike the ameliorative effects of repeated treatment with DMI on responding for ICSS in CD-1 mice, repeated drug administration was without effect on performance in the DBA/2 strain of mice (Zacharko et al., 1987). It was provisionally hypothesized that existing strain differences in the capacity of neurochemical systems to deal with environmental stressors influence the ability of pharmacological agents to alleviate stressor-provoked behavioral impairments.

Corticoid Responses Following Stressor Exposure

As in the case of stressor-induced amine changes, exposure to aversive stimulation has been shown to modify circulating glucocorticoid levels. Marked increases in plasma corticosterone have been observed following exposure to a variety of stressors, including electric footshock, restraint, forced running, cold-water immersion, noise, and formalin injection, as well as placement in a novel environment (Armario, Castellanos, & Balasch, 1984; Kant, Mougey, Pennington, & Meyerhoff, 1983; Maickel & Odio, 1983; Natelson, Tapp, Adamus, Mittler, & Levin, 1981; Natelson et al., 1987; Paris et al., 1987). It appears that the magnitude and time course of these corticoid changes vary with a great number of factors related to the stressors themselves, as well as a host of experiential, environmental, and organismic variables.

Stressor Severity. It appears that corticoid variations are exquisitely sensitive to aversive stimuli. Whether glucocorticoid

responsiveness serves as a discriminative measure of environmental stressors, however, is dependent upon the characteristics of the stressor employed. For instance, exposure to relatively mild stressors, such as handling or placement in a novel environment, has been shown to result in moderate elevations of plasma corticosterone (Armario, Montero, & Balasch, 1986; M.B. Hennessy & Levine, 1978; Irwin, Ahluwalia, Zacharko, & Anisman, 1986; Misslin, Herzog, Koch, & Ropartz, 1982). Moreover, studies assessing changes in plasma corticosterone in response to various intensities across a range of relatively mild stressors have found the magnitude of the corticoid response to be a sensitive index of stressor severity (Armario et al., 1986; M.B. Hennessy & Levine, 1978; Holson, Scallet, Ali, Sullivan, & Gough, 1988). Indeed, mice exposed to different degrees of environmental change exhibited graded elevations in serum corticosterone levels (M.B. Hennessy & Levine, 1978).

In contrast to the moderate steroid elevations induced by relatively mild stressors, exposure to relatively intense stressors, such as immobilization, footshock, and cold, have been shown to produce marked elevations of plasma corticosterone (Kant, Meyerhoff, Bunnell, & Lenox, 1982; Maickel & Odio, 1983). However, studies assessing differences in plasma corticosterone levels following exposure to shock of various current intensities have found the range of the corticoid response to be limited (Kant et al., 1983; Natelson et al., 1981, 1987). Thus, it appears that elevations in plasma corticosterone may be a poor measure for distinguishing between relatively severe stressors.

Taken together, it appears that the corticoid response is particularly sensitive to environmental stimuli, such that stressors of mild to moderate severity differentially influence glucocorticoid secretion. Ceiling effects,

however, appear to prevent differentiation between moderate and severe stressors (Kant et al., 1983).

Time Course. While most investigators have focused on the magnitude of the corticoid response, relatively few studies have assessed the initial rise and subsequent attenuation of the plasma corticosterone changes during prolonged stressor exposure, and still fewer have assessed such temporal changes to evaluate different treatment effects. It is known that plasma corticosterone levels begin to increase within a few minutes of immobilization and peak about 15 to 30 min later (De Souza & Van Loon, 1982; Kvetnansky et al., 1976; Paris et al., 1987). While some investigators reported a slight decrease in plasma corticoid concentrations following peak values (Kant et al., 1983; Keim & Sigg, 1976; Murison, 1983; Tanaka et al., 1982), others have failed to detect an attenuation of the corticoid response with prolonged stressor application (see Maickel & Odio, 1983). Keim and Sigg (1976) reported that maximal concentrations of corticosterone were reached between 30 and 60 min of exposure to strong stressors, such as restraint or electric footshock, whereas mild stressor exposure (placement in a novel environment) resulted in a slower rise of the corticoid response, with peak concentrations occurring 120 min after stressor inception. While it is difficult to equate the severity of these stressors, these findings underscore the importance of the severity of the stressor in determining the time course of the corticoid response.

Not surprisingly, the time course for the recovery of the corticoid response after stressor termination may be dependent upon, among other things, the duration of the stressor employed. It has been demonstrated that following exposure to a relatively brief footshock stressor (e.g., 2 min of shock), corticosterone concentrations peaked 30 min later and returned to control values 60 to 90 minutes after stressor termination (Telegdy &

Vermes, 1976: see also Paris et al., 1987). Using a more severe and protracted stressor (approximately 90 min of unsignaled footshock presented on a variable time schedule (VI 60)), Maier et al. (1986) found corticosterone levels did not return to control values until 150 min after stressor termination.

Diurnal Patterns. In addition to factors, such as stressor severity and duration, it appears that the corticoid response may be dependent upon the phase of the diurnal cycle at which the stressor is applied. Seggie and Brown (1975) compared the effects of 5 seconds of handling and 3 minutes of placement in a novel environment on corticoid responses at the crest and trough of the diurnal adrenal cycle. The corticosterone response was found to vary as a function of the time of day and the nature of the stimulation. For instance, at the trough of the circadian cycle, corticosterone levels reached a peak 15 minutes after stressor application, and by 60 min rapidly declined to baseline levels in rats exposed to the novel environment stressor. The corticosterone response to handling also resulted in elevations 15 min after stressor termination, however, these elevations persisted for the duration of the 60 min observation period. Moreover, the magnitude of the corticoid response in handled rats was less than that of rats exposed to the novel environment. In contrast, at the crest of the circadian cycle, corticoid concentrations remained elevated for comparable periods regardless of the stressor. Evidently, it is necessary to consider not only the type of stressor employed, but also the context in which aversive stimulation takes place (Seggie & Brown, 1975).

Organismic and Experiential Factors. In the analysis of stressor-induced glucocorticoid changes, several investigators have evaluated the influence of organismic factors, such as the species, strain, and age of the organism, as well as experiential factors, such as the ability to cope with

aversive stimuli and the organism's prior stressor history. It appears that the corticoid response to stressor exposure may vary from species to species. For instance, Armario and Castellanos (1984) reported that following exposure to prolonged immobilization circulating levels of plasma corticosterone were lower in rats than in mice. However, differences in plasma corticoid secretion were not evident after 45 minutes of stressor exposure. Indeed, while both rats and mice exhibited similar elevations of plasma corticosterone, glucocorticoid levels in mice remained elevated, whereas in rats corticoids markedly declined from peak values after 5 hr of stressor application. Thus, differences in plasma corticoid secretion between these species were apparent only after prolonged restraint. These findings suggest that differences in the pattern of decline, or alternatively that differences in the effectiveness of the negative-feedback of corticosterone on HPA activity, may exist between species.

When assessing stressor-induced glucocorticoid changes, consideration must also be given to the strain employed. Keim and Sigg (1976), for example, reported a slightly faster rate of decay in circulating corticoid levels 30 minutes after the termination of restraint stress in Long Evans Hooded and Fisher rat strains than in Wistar and two Sprague-Dawley strains. Moreover, the magnitude of the corticoid response immediately after 30 minutes of restraint was similar in all strains. Thus, both intra-species and inter-species differences appear to be important in the evaluation of the corticoid response to stressors.

Ontogenic Changes. Ontogenic studies of the pituitary-adrenal axis in the newborn rat demonstrated biphasic alterations in response to stressors. Following a short period (approximately 2 days postnatally) of heightened responsivity to stressors, the corticoid response induced by

aversive stimuli is markedly reduced for the next 2 weeks of life (Sapolsky & Meaney, 1986; Walker, Perrin, Vale, & Rivier, 1986). During this period, it is likely that, among other things, lowered concentrations of hypothalamic CRF, decreased responsiveness of pituitary receptors to CRF stimulation, and exaggerated glucocorticoid negative-feedback on the pituitary contribute to decreased serum corticoid secretion following stressor application (see Sapolsky & Meaney, 1986). Since enhanced glucocorticoid activity is generally involved in inhibiting cell division, protein synthesis, and immunocompetence (Munck et al., 1984; Sapolsky & Meaney, 1986), it seems likely that this period of nonresponsiveness to stressor application serves an adaptive function for the developing organism by limiting the secretion of glucocorticoids (Sapolsky & Meaney, 1986). Although the precise duration of this period of nonresponsiveness is not known, it appears that the corticoid response to stressors is functioning as it does in adults by about the third week (21-25 days) in rats.

In assessing the rate of recovery of the corticoid response after exposure to various stressors, Goldman, Winget, Hollingshead, and Levine (1973) observed differences in the corticoid response in weanling (25-day-old) and adult (65-day-old) rats. In particular, after exposure to either 60 sec of footshock or 1.5 min of ether anesthesia, weanling rats exhibited a later peak (30 min) and a slower return to control values than their adult counterparts. These investigators attributed the hyposecretion of plasma corticoids in weanling rats to delayed maturation of the HPA axis.

Differences in the stressor-induced corticoid secretion also exist between adult (3-5 months) and aged (24-28 months) rats (see Sapolsky et al., 1986). Following exposure to 60 minutes of restraint, corticosterone

concentrations in young rats returned to control values within 60 min of stressor termination, whereas corticoid concentrations in aged rats remained elevated for as long as 24 hr after stressor termination (Sapolsky, Krey, McEwen, 1984).

While the hyposecretion of plasma corticoids in weanling rats has been attributed to delayed maturation of the negative-feedback of the HPA axis (Goldman et al., 1973), hypersecretion of glucocorticoids in aged rats is thought to be due to degenerative changes in the hippocampal region (Sapolsky et al., 1986). In particular, it has been demonstrated that excessive corticosterone promotes the down-regulation of the number of corticoid receptors in the hippocampus (see Sapolsky et al., 1986). It is thought that in adult organisms stressor-induced elevations of glucocorticoids lead to the loss of hippocampal receptors, but that this loss recovers after stressor termination. At later stages of the organism's maturation, however, the cumulative effect of stressor-induced corticoid secretion disrupts the recovery of hippocampal receptors, thereby leading to decreased sensitivity of glucocorticoid feedback mechanisms. Consequently, the capability of the hippocampus to exert its inhibitory feedback effects on HPA functioning is diminished and corticoid hypersecretion ensues. Ostensibly, continued corticoid secretion is thought to precipitate further down-regulation of receptors. This feed-forward cascading loop of receptor down-regulation and corticoid secretion persists until permanent loss of hippocampal neurons occur in aged organisms (Sapolsky et al., 1986). Thus, although aged rats showed no impairments in initiating adrenocortical secretion following stressor exposure, the ability to terminate glucocorticoid secretion following stressor termination was severely impaired.

Stressor Controllability. Several investigators have reported that when animals were given the opportunity to control the offset of aversive stimuli, relatively limited elevations of circulating glucocorticoids were observed (Hanson et al., 1976; Weiss et al., 1979). For instance, Coover, Ursin, and Levine (1973) reported that corticoid concentrations increased sharply during avoidance/escape training, but once the avoidance response was established plasma corticosterone concentrations decreased considerably from peak values.

Studies evaluating the effects of controllable and uncontrollable stressors on circulating corticoid levels have reported that glucocorticoid secretion may be differentially influenced by stressor controllability (Dess, Linwick, Patterson, Overmier, & Levine, 1983; Hanson et al., 1976; Swenson & Vogel, 1983; Tsuda & Tanaka, 1985). For instance, Weiss (1971b) reported that rats exposed to yoked inescapable shock exhibited higher plasma corticosterone concentrations than rats with the opportunity to avoid or escape from shock. Similarly, it was reported that following two sessions of a stress induction procedure in which a panel press response terminated shock delivery, dogs allowed to control shock offset exhibited lower plasma corticosterone levels than dogs with no control over shock, even though both groups were exposed to equal amounts of the stressor (i.e., a yoked procedure was employed; Dess et al., 1983). Moreover, unlike the influence of control on plasma corticoid secretion in the stress induction phase, controllability was not found to be important in determining the corticoid response to subsequent testing in a shuttle avoidance/escape task. In contrast to studies demonstrating only moderate increases in plasma corticosterone secretion after exposure to controllable footshock stressors, others have reported marked elevations in plasma glucocorticoids. Bassett and Cairncross (1975) reported that in

rats exposed to irregular signaled footshock where escape was possible (i.e., controllable) not only was the plasma corticoid elevation more pronounced than in rats exposed to regular unsignaled footshock where escape was not possible (i.e., uncontrollable), but there was also an indication of a biphasic response. That is, initially comparable elevations of plasma corticosterone were observed in animals exposed to controllable and uncontrollable footshock, but differences between treatments emerged over the course of the session.

Still other investigators have failed to observe any differences in plasma corticosterone levels after exposure to controllable and uncontrollable stressor regimens. For instance, Davis et al. (1977) found no differences in corticosterone concentrations between escapably and inescapably shocked rats (see also Maier et al., 1986). However, if animals were exposed repeatedly to the stressor regimen (15 sessions), then differences between the two groups emerged. While escapably shocked rats exhibited reductions of plasma corticosterone, inescapably shocked rats exhibited elevations in corticosterone secretion. Unfortunately, no attempt was made to equate for the amount of shock received between the two treatment conditions. Similarly, Tsuda and Tanaka (1985) did not observe differences in animals exposed to either a single session of avoidable/escapable shock (Sidman free operant avoidance-escape) or inescapable shock. Following 5 days of training, however, significant differences emerged between treatment conditions, such that escapably shocked rats exhibited considerably lower plasma corticosterone concentrations than yoked inescapably shocked animals. However, the difference in corticoid secretion was attributable to considerable elevations of plasma corticosterone after repeated application of

inescapable shock relative to that observed after only a single stressor session.

Prior Stressor Exposure. While it is clear that prior stressor exposure may proactively influence central catecholaminergic mechanisms, there is limited evidence to suggest that such a treatment proactively alters plasma corticosterone secretion. For instance, Murison and Isaksen (1982) reported that immobilization resulted in higher plasma corticosterone concentrations in rats previously exposed to yoked inescapable shock than in rats that received prior escapable shock. In contrast to these findings, however, Maier et al. (1986) failed to observe differences in the magnitude and recovery of the corticoid response when rats previously exposed to either escapable and inescapable shock were reexposed to a shuttle-escape task. Similarly, Dess et al. (1983) reported that controllability did not proactively influence corticoid responsivity when animals were tested in an avoidance-escape shuttle task.

While it appears that stressor controllability may not proactively influence the corticoid response, it has been suggested that reexposure to limited amounts of shock, as well as cues associated with prior inescapable shock may influence the magnitude of the corticoid response (Bunnell, Meyerhoff, & Kant, 1988; J.W. Hennessy & Levine, 1979; Levine, Madden, Conner, Moskal, & Anderson, 1973; Madden, Rollins, Anderson, Conner, & Levine, 1971; Tsuda et al., 1986). For instance, Madden et al. (1971) demonstrated that rats previously exposed to inescapable shock for 5 consecutive days exhibited greater plasma corticosterone elevations upon reexposure to mild shock exposure (1 sec of footshock) than control rats exposed to the limited shock trial for the first time. Moreover, Bunnell et al. (1988) reported that reexposure to the apparatus in which rats received inescapable footshock for 4 consecutive days resulted in marked

elevations in plasma corticosterone. Similarly, when testing was conducted in either an open field or passive avoidance task, greater corticoid concentrations were observed in rats previously exposed to several sessions of inescapable shock than in their nonshocked counterparts (Levine et al., 1973; Madden et al., 1971). In contrast to the aforementioned findings, Murison, Overmier, and Skoglund (1986) reported that reexposure to a water-restraint procedure resulted in lower plasma corticosterone levels in animals previously exposed to 4 sessions of water-restraint than in animals that received either no prior exposure or only one prior exposure. These investigators failed to detect any evidence for sensitization of the corticoid response to stressor application even after a single prior exposure to the water-restraint procedure. The reduction of the magnitude of the corticosterone response following repeated prior exposure to water-restraint is similar to the findings of Weiss et al. (1975) who reported an attenuation of the corticoid response following exposure to a chronic footshock regimen.

Predictability. It has been demonstrated repeatedly that rats prefer predictable (signaled) over unpredictable (unsignaled) footshock (see Abbott, Schoen, & Badia, 1984). In contrast, however, the effects of stressor predictability on the corticoid response has yet to be clearly established. For instance, while Weiss (1970) reported lower corticosterone values following exposure to predictable footshock, Bassett, Cairncross, and King (1973) found higher elevations of plasma corticosterone levels in rats that received predictable shock than in rats exposed to unpredictable shock. Still other investigators failed to detect differences between the effects of the two treatments (see Davis & Levine, 1982; and J.W. Hennessy, King, McClure, & Levine, 1977).

One factor which may contribute to the apparently conflicting results in studies evaluating the magnitude of the corticoid response is the time at which corticoid secretion is determined following stressor application. For instance, Davis and Levine (1982) reported that in animals that received predictable shock, the initial rise to peak values was followed by an attenuation of circulating levels of plasma corticosterone. Corticosterone values were higher in animals that received predictable shock, but by the end of the 30 minute session corticosterone levels were comparable to those of animals that received unpredictable shock early in the session. Unfortunately, these investigators did not evaluate the recovery of plasma corticoids to control values after stressor termination. Muir and Pfister (1987), however, examined the recovery pattern of corticosterone following predictable and unpredictable exposure to a novel environment. After 5 days of placement in an experimental apparatus, these investigators observed a more rapid return to baseline corticosterone levels in rats that received unpredictable exposure than in rats that received predictable exposure to the apparatus. However, this difference in recovery rate was attributable to differences in the magnitude of the corticoid response, rather than to differences in the temporal pattern of recovery.

Psychosocial Factors. Although housing conditions have been shown to influence changes in neurochemical activity (see Anisman & Sklar, 1979), it appears that this stressor does not influence the corticoid response. For example, Misslin et al. (1982) and Seggie, Brown, Campbell, and Grotta (1984) reported comparable basal levels of plasma corticosterone in individually-housed and group-housed animals. Moreover, aversive stimulation failed to influence corticosterone levels in animals maintained under these housing conditions.

In contrast to the limited influence of social housing conditions on glucocorticoid secretion, the opportunity to engage in fighting has been shown to alter the secretion of plasma corticosterone. For instance, it has been demonstrated that fighting in the absence of aversive stimulation stimulates glucocorticoid secretion in a variety of species (see Brain, 1980). However, it appears that diminished glucocorticoid secretion may be apparent in animals rated as being aggressive in situations not involving aversive stimulation. For example, in addition to observing attenuated corticosterone levels in animals subjected to shock-elicited fighting, Mormede et al. (1984) reported that in nonshocked rats those animals that were rated as being dominant or aggressive exhibited lower plasma corticoid concentrations than those animals rated as being submissive or subordinate.

As indicated earlier, fighting in response to inescapable shock has been shown to influence regional brain NE activity (Stolk et al., 1974). It was suggested that these changes were adaptive for the organism, and that fighting may be viewed as an effective coping response to stressor application. Similarly, several investigators demonstrated lower pituitary-adrenal activity in animals that were given the opportunity to engage in fighting (i.e., two animals placed in a shock chamber) compared to animals without such an opportunity (i.e., placed individually into the chamber; Mormede et al., 1984; Weinberg, Erskine, & Levine, 1980). Taken together, it would appear that diminished corticosterone secretion may be dependent upon the context or backdrop in which fighting occurred.

Chronicity. As in the case of central biogenic amines, chronic stressor application has been shown to influence plasma corticosterone levels. However, conflicting data exist concerning the effects of prolonged and repeated exposure to stressors on glucocorticoid secretion. In

assessing the effects of prolonged stressor application on the corticoid response, it appears that the nature of the stressor may be an important factor in altering corticosterone secretion. For instance, although prolonged exposure to stressors, such as restraint, cold, and placement in a novel environment, resulted in the attenuation of the corticoid response (see Keim & Sigg, 1976; Maickel & Odio, 1983; Tsuda & Tanaka, 1985), prolonged footshock exposure resulted in persistent elevations of circulating corticoids (Maickel & Odio, 1983). Since a discontinuous footshock regimen (as opposed to continuous application of immobilization or cold) was employed, it was suggested that the attenuation of the corticoid response may be influenced by continuous stressor application, rather than by the type of stressor per se (Maickel & Odio, 1983). This supposition, parenthetically, has also been applied to the presumed opioid changes associated with footshock. In particular, it was demonstrated that the opioid systems mediating stressor-induced analgesia may be differentially affected by continuous and intermittent footshock. For instance, the analgesia induced by high severity continuous footshock (4 min, 2.5 mA) is not modifiable by the opiate antagonist naloxone, whereas the analgesia associated with intermittent footshock (20 min, 2.5 mA) is eliminated by the opiate antagonist (Lewis, 1986; Terman & Liebeskind, 1986). Curiously, however, continuous footshock of low severity (1 min, 2.5 mA) may involve opioid peptides (see Terman & Liebeskind, 1986 for an extended discussion).

Studies assessing the effects of repeated stressor application on plasma corticoid secretion have yielded conflicting results. Some investigators observed reductions of high levels of circulating corticoids following repeated applications of immobilization, noise, water-restraint, or inescapable footshock (Armario et al., 1984; J.W. Hennessy & Levine,

1979; Jindra, Kvetnansky, Belova, & Sudakov, 1980; Keim & Sigg, 1976; Weiss et al., 1975). In other studies persistent elevations of plasma corticosterone were evident following exposure to chronic stressor regimens, such as restraint, footshock, forced running, and cold (M.B. Hennessy & Levine, 1978; Irwin, Ahluwalia, Zacharko, & Anisman, 1986; Kant et al., 1983, 1985; Pitman, Ottenweller, & Natelson, 1988; Richardson, 1984; Yahata, Murazumi, & Kuroshima, 1987). Moreover, when a cross-over design was employed, such that exposure to one chronic stressor was followed by a single session of another stressor, the elevated levels of plasma corticosterone persisted (Kant et al., 1985; see also Armario et al., 1986 and Levine et al., 1973).

Contrary to the reports of Weiss and his associates (Weiss et al., 1975, 1979) which demonstrated that reductions in corticoid secretion coincided with increased central NE activity after repeated footshock (15 sessions), other investigators found elevations in glucocorticoids even after the reductions of hypothalamic NE were eliminated with repeated inescapable shock application (Irwin, Ahluwalia, & Anisman, 1986). The dissociation of corticoid and central amine activity suggests that these variations may be mediated by different mechanisms or that they may reflect different attributes of the stressor (e.g., arousal or previous stressor history).

While the determination of factors associated with changes in plasma corticoid activity have yet to be elucidated, it is likely that there may be some adaptive value in the activation of glucocorticoids after chronic stressor application. It will be recalled that elevated levels of circulating corticoids were thought to suppress the action of certain physiological systems (e.g., immune system), thereby preventing excessive activity which could potentially have adverse consequences (e.g.,

autoimmune disorders; Munck et al., 1984). Under chronic stressor conditions, then, the corticoid response might be expected to remain elevated as long as other physiological processes threaten the organism's well being. If, however, physiological stability has been achieved after repeated stressor application, only then, would the corticoid response be expected to wane.

Summary

Although there is agreement that stressors will reliably increase glucocorticoid secretion, there is much less agreement concerning the variables which determine the magnitude of the glucocorticoid response. It is clear that to a limited extent glucocorticoid secretion is sensitive to environmental stimuli of varying severity (Armario et al., 1984; Kant et al., 1983; Paris et al., 1987). Stressors of mild to moderate severity appear to influence glucocorticoid secretion differentially (M.B. Hennessy & Levine, 1978), whereas moderate to severe stressors result in comparable increases in plasma corticosterone (Kant et al., 1983; Natelson et al., 1981, 1987). Most studies evaluating stressor-induced corticoid alterations, however, have been concerned with the magnitude of the response, and have not considered the persistence of this elevation. Inasmuch as elevations of corticoid levels for protracted periods are thought to have both adverse and positive consequences for the organism (e.g., immunosuppression enhancing vulnerability to pathogens, but limiting autoimmune disorders; Munck et al., 1984), it seems likely that the rate of decay of circulating corticoid levels after stressor exposure may be a particularly important consideration of treatment effects.

One factor which may be important in determining the secretion of plasma corticosterone is the organism's ability to exert control over the aversive stimulus (Weiss, 1971b; Weiss et al., 1979). However, while some

studies reported that glucocorticoid secretion may be influenced differentially by stressor controllability (Dess et al., 1983; Hanson et al., 1976; Swenson & Vogel, 1983; Tsuda & Tanaka, 1985), others failed to observe any differences in plasma corticosterone levels after exposure to controllable and uncontrollable stressor regimens (Maier et al., 1986; Murison & Isaksen, 1982). Stressor controllability, likewise, did not proactively influence the corticoid response to a subsequently applied stressor (Dess et al., 1983).

Analysis of the plasma corticoid response associated with acute and chronic stressors have also yielded inconsistent results. While some investigators have found chronic stressor exposure to be associated with an adaptation of the corticoid response (Armario et al., 1984; Jindra et al., 1980; Weiss et al., 1975), others have found no such effect (Irwin, Ahluwalia, Zacharko, & Anisman, 1986; Kant et al., 1983, 1985; Richardson, 1984). Similarly, although stressors have been shown to proactively influence plasma corticosterone levels upon reexposure to a stressor, the contribution of psychological factors (stressor controllability or mastery over the response contingencies) remains in doubt (cf. Maier et al., 1986 vs. Dess et al., 1983). Finally, it remains to be determined whether the response style required of the animal (i.e., the necessity of emitting prepared vs. contraprepared responses) influences plasma corticoid secretion. The purpose of the present series of experiments was to determine (a) whether exposure to controllable and uncontrollable stressors (footshock or water immersion) differentially affects plasma corticoid levels in mice, (b) the effects of stressor controllability on the time course of the corticoid alterations, (c) whether repeated exposure to a controllable (permitting mastery of the response contingencies) and an uncontrollable stressor differentially influences the corticoid response, (d)

the proactive effects of prior controllable and uncontrollable stressor exposure on the corticoid response promoted by later stressor experiences, and (e) corticoid variations as a function of whether mice in an aversive situation were required to emit prepared vs. contraprepared response in order to escape.

EXPERIMENT 1

Stressor Controllability and Time-Dependent Variations in Plasma Corticosterone

The ability to exert control over aversive events is thought to be fundamental in determining alterations in central amine activity. It has, likewise, been suggested that behavioral coping factors may also be important in altering the secretion of adrenal corticoids. However, differences between the effects of escapable and inescapable stressors on corticoid secretion, when evident, appear to be minimal (e.g., Davis et al., 1977; Tsuda & Tanaka, 1985; Weiss, Glazer, & Pohorecky, 1976). In these studies, corticoid concentrations were typically assessed immediately after stressor termination when circulating levels of the hormone are at their peak. However, when plasma corticoid concentrations were assessed some time after stressor termination, differences between controllable and uncontrollable shock exposure emerged (see Swenson & Vogel, 1983). Thus, decay of the corticoid response may be a more sensitive index of the consequences of stressor controllability. Maier et al. (1986), however, were unable to demonstrate differences in decay rates of corticosterone and ACTH between escapably and inescapably shocked rats. The reasons for these divergent findings is not immediately evident, although the stressor parameters may simply not have been the most appropriate to permit the expression of these corticoid differences. Experiment 1 was undertaken to determine the contribution of controllable and uncontrollable footshock to the magnitude of the corticoid response in mice, as well as to assess whether behavioral control differentially affects the rate of recovery of the corticoid response after stressor termination. It should be underscored that the stressor parameters for controllable and uncontrollable shock that were employed in Experiment 1 were

previously shown in this laboratory to differentially influence NE activity. It was thus considered that these parameters might likewise be appropriate for the detection of potential differences in corticoid reactivity. However, as will be seen in ensuing experiments other shock parameters were employed as well.

Method

Subjects

Ninety, naive, male CD-1 mice, 55-60 days of age and weighing 25-30 g, were procured from Charles River (Canada), St. Constance, Quebec. Upon arrival in the laboratory, mice were housed in groups of five in standard opaque polypropylene cages and maintained on a 12 hr day-night cycle with ad lib access to food and water. Mice served as experimental subjects following a 7-day acclimatization period to the laboratory environment. All stress procedures, testing, and the sacrificing of animals were conducted between 8:30 and 14:30 hr.

Apparatus

Escape Training. Escape training was conducted in three identical Plexiglas shuttle boxes, 26.4 x 9.0 x 15.5 cm. The floor of each box was composed of 0.32 cm stainless-steel rods spaced 1.0 cm apart (centre to centre). The rods were connected in series with neon bulbs. The end walls of each chamber were lined with thin stainless-steel plates, and connected in series with the grid floor. The roofs of the boxes consisted of 0.63 cm red Plexiglas lids which reduced the amount of light entering the chamber. Shock was delivered to the grid floor from a 3,000 V source, thereby providing relatively constant current. Two of the three boxes were connected to the shock source, the third box, which was located in a different room, was not connected to any shock source.

Each shuttle box was divided into two compartments by a stainless-steel gate. In the open gate position a 1.27 cm hurdle separated the two compartments, and a 7.0 x 7.7 cm space permitted access to the adjacent compartment. Thin stainless-steel plates, which lined the hurdle, were connected in series with the grid floor, thereby preventing a mouse from sitting on the hurdle to avoid shock. Situated 1.1 cm on either side of the partition were two infrared photodetector units, 1.0 and 4.0 cm above the grid floor. The photodetectors were wired such that if the beams on both sides were crossed simultaneously, which might occur when the mouse is halfway across the partition, the cells would not trigger. Only when the mouse crossed the beam in the shock compartment and broke the beam on the safe side, did the cells trigger. Additional photocells located at the rear of the compartment, 2.5 cm from the end walls and 1.0 cm above the grid floor, made certain that if the mouse jumped over the first set of photodetectors, the cells would be triggered. The shuttle boxes, which were maintained in sound attenuating chambers, were operated by a microcomputer system constructed at the Carleton University Science Workshops.

Procedure

Mice were assigned to one of three conditions. Mice of one group ($n = 30/\text{group}$) were placed individually in the shuttle boxes and received 360 escape trials. On each trial, shock was presented for 1.5 sec (150 μA , 60Hz, AC) after which the gate separating the two compartments opened, thus permitting escape. This procedure was employed to ensure that mice received approximately 2 sec of shock on each trial, a procedure which has previously been shown to produce reliable behavioral deficits in several paradigms, as well as alterations of NE levels and turnover (see Anisman et al., 1984). The maximum amount of shock presented on any

given trial was 24 sec, and the interval between each trial was 9 sec. In the event that mice exhibited an escape failure, the delay between shock onset and gate opening was shortened to 0.5 sec to facilitate escape. Mice of the second group were placed individually in the shuttle boxes and exposed to 360 shocks over which behavioral control was not possible (i.e., yoked condition). For these animals shock onset occurred at exactly the same time as it did for mice in the escapable group. Shock offset, however, occurred when their respective partners in the escape group successfully escaped from shock. Thus, both escapably and inescapably shocked mice received identical amounts of shock at exactly the same time, but only mice in the escapable condition could terminate shock by making the appropriate response. Mice in the third group served as the nonshocked control condition. Mice were placed individually in the shuttle boxes for periods that matched the escapable and inescapable shock groups, but shock was not delivered.

Following the shock treatment, independent groups of mice ($n = 6/\text{group}$) were decapitated either immediately, 15 min, 30 min, 60 min, or 180 min later. Trunk blood was collected, centrifuged, and the plasma samples stored at -70°C until plasma corticosterone concentrations were assessed fluorometrically using a modification of the method of Givner and Rochfort (1966; see Appendix A).

Results and Discussion

The mean amount of shock mice received on each trial of the shuttle-escape session was 2.65 ± 0.23 sec. Figure 1 shows the mean plasma corticosterone concentrations at various times after exposure to escapable shock, yoked inescapable shock, or no shock treatment. Analysis of variance revealed that corticosterone values varied as a function of

2

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No. 2)

Insert Figure 1 about here

Shock Treatment $\times$ Time interaction, $F(8, 68) = 6.51$, $p < .001$. Newman-Keuls multiple comparisons of the main effects comprising the interaction ($\alpha = .05$) confirmed that among nonshocked mice plasma corticosterone levels did not vary significantly over time following the initial treatment, although immediately after apparatus exposure corticoid levels were somewhat higher than at the 15-60 min intervals ($p < .10$). In contrast, in both shocked groups plasma corticosterone concentrations decayed with time, such that levels were significantly lower 30-180 min after shock than immediately after stressor exposure. Between-group comparisons at each time interval revealed that at the 0 min, 15 min, 30 min, and 60 min intervals, mice that received either escapable or yoked inescapable shock exhibited significantly higher plasma corticosterone concentrations than their nonshocked counterparts. The between-group differences were absent 180 min following stressor termination. These data are consistent with those of Maier et al. (1986) who failed to detect differences in plasma corticosterone concentrations in escapably and inescapably shocked mice. Indeed, as seen in Figure 1, similar decay rates occurred in both escapable and inescapable shock conditions. It is possible that the failure to detect differences in the corticoid response between escapably and inescapably shocked mice was due to the particular severity of the stressor leading to peak corticoid concentrations in these animals, hence precluding potential differences between the stressor conditions.

Figure 1

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice exposed to either 360 escapable shocks of 2 sec duration, yoked inescapable shocks, or no shock at various intervals after the last shock received.

EXPERIMENT 2

Stressor Controllability and

Time-dependent Variations Following Moderate Stressor Exposure

Given that the marked plasma corticosterone changes in escapably and yoked inescapably shocked animals may have prevented detection of between-group differences, the possibility was entertained that with a moderate degree of aversive stimulation (i.e., fewer shock trials), intermediate variations of corticosterone levels would be elicited and may thus have permitted detection of potential differences between animals that received escapable and inescapable footshock. Accordingly, another experiment was conducted using a smaller number of shock trials (60 rather than 360 escapable or inescapable shocks).

Method

Subjects, Apparatus, and Procedure

A total of 105 male CD-1 mice served as subjects in Experiment 2. The subject and apparatus particulars were the same as those described in Experiment 1.

The procedure of Experiment 2 was identical to Experiment 1, except that only 60 escape or yoked shock trials were delivered. Likewise, trunk blood was collected either immediately ($n = 7/\text{group}$), 15 min, 30 min, 60 min, or 180 min later. Following centrifugation, the plasma samples were stored at -70°C until plasma corticosterone concentrations were assessed fluorometrically.

Results and Discussion

Mice received an average of 5.45 sec ($\pm$ 0.67) of shock per trial. Figure 2 shows the time-dependent changes in mean plasma corticosterone concentrations following exposure to escapable, yoked inescapable, and no shock. As seen in Figure 2, the moderate stressor of

Insert Figure 2 about here

Experiment 2 produced a less dramatic rise in plasma corticosterone levels in shocked mice compared to those observed following more severe stressor application (cf. Figures 1 and 2). Analysis of variance revealed that plasma corticosterone concentrations varied as a function of Shock Treatment x Time interaction, $F(8, 85) = 2.09$, $p < .05$. Subsequent Newman-Keuls multiple comparisons of the main effects comprising the interaction ($\alpha = .05$) revealed that in nonshocked animals plasma corticosterone levels tended to decline over time following apparatus exposure (see Figure 2). Indeed, whereas in Experiment 1, this decline approached but did not reach statistical significance, in Experiment 2, plasma corticosterone levels immediately after exposure to the apparatus exceeded those observed 60 and 180 min following this experience. In both shocked groups, plasma corticosterone concentrations were increased by the stressor treatment, but this effect was evident only at the 15, 30, and 60 min time periods. Indeed, corticoid concentrations immediately after shock were comparable to those of the nonshocked animals. Interestingly, in Experiment 2, plasma corticosterone levels did not reach the magnitude they did in the preceding study, but the rate of decay tended to be slower than in

Figure 2

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice exposed to either 60 escapable shocks of 2 sec duration, yoked inescapable shocks, or no shock at various intervals after the last shock received.

Experiment 1. That is, despite the relatively small rise of corticosterone in the stressed groups at 30 min after stressor termination plasma corticoid values in Experiment 2 were comparable to those seen at this time in Experiment 1. Even though the maximal corticosterone response induced by the stressor treatments was approximately half that of Experiment 1, the escapable and inescapable treatments did not differentially influence steroid concentrations. Thus, the failure to detect effects of stressor controllability cannot be ascribed to the treatment being too intense, thereby precluding potential treatment effects from being detected.

It is likely that the limited increase in plasma corticosterone observed immediately after 60 shock trials may be due not only to the fewer number of shocks received, but also to the time following the initial shock presentation. Indeed, in contrast to Experiment 1, in which treatment was administered over 1.1 hr, in Experiment 2 treatment was administered over 11 min. Thus, it is possible that the delay of peak values in Experiment 2 may be attributable to the time required for the expression of the plasma corticoid response after stressor exposure.

Although a nonhandled control group was not included in this study, the time-dependent decay of plasma corticosterone concentrations suggests that the experience of being handled and placed in the apparatus was responsible for the elevated plasma corticosterone levels seen immediately after treatment. Moreover, on the assumption that handling, coupled with apparatus exposure, reflects a mild stressor relative to that of footshock, the time-dependent variations of plasma corticosterone levels suggest that (a) at the lower range, increments of stressor severity engender graded plasma corticosterone changes, and (b) the decay of the corticoid response following stressor termination varies as a function of mild vs. relatively severe stressors.

EXPERIMENT 3

Effects of Stressor Controllability on Plasma Corticosterone

Following Repeated Stressor Application

Based on the preceding experiments, it is apparent that exposure to either moderate or severe stressors increased circulating corticosterone levels (see also M.B. Hennessy & Levine, 1978) regardless of stressor controllability. Inasmuch as the corticoid response appears to be exquisitely sensitive to stressors, coupled with the fact that during the initial shock trials animals will not have learned about response-outcome relations, it really should not be altogether surprising that corticoid differences did not appear as a function of controllability. It is possible, however, that once mastery over the response contingencies are well established, the corticoid secretion associated with escapable shock may be minimized, and hence differences as a function of stressor controllability would be evident. Indeed, Tsuda and Tanaka (1985) found that after 5 days of escape training in a free operant avoidance-escape procedure, escapably shocked rats exhibited considerably lower plasma corticosterone than yoked inescapably shocked animals. To a large extent, however, this difference occurred as a result of a substantial increase of plasma corticosterone after 5 sessions of inescapable shock relative to that seen after only a single stressor session. Thus, it is not clear whether the difference between escapable and inescapable conditions stemmed from animals in the former group acquiring mastery over the task. Experiment 3 was undertaken to determine whether corticoid secretion could be differentially affected by controllability following 1, 3, and 5 days of repeated stressor application, and whether any such differences would result from a relative decline of corticoid enhancement with repeated escape training.

Method

Subjects and Apparatus

Fifty, naive, male CD-1 mice served as subjects in Experiment 3. The subject and apparatus particulars were identical to those of Experiment 1.

Procedure

On each of either 1, 3, or 5 days, mice ($n = 15/\text{group}$) were exposed either to 60 escapable shocks, 60 yoked inescapable shocks, or placement in the apparatus but not shocked. An additional group of mice ($n = 5$) were placed individually in home cages and left undisturbed on the shelf for 5 days until the time of decapitation.

Since peak plasma corticosterone concentrations were reached approximately 15 minutes after stressor termination in the previous experiment, it was assumed that changes in the corticoid response may be adequately represented at this time period. Thus, mice were sacrificed 15 min after the last shock received. Trunk blood was collected and centrifuged, and plasma samples frozen. The samples were stored at -70°C until plasma corticosterone concentrations were assessed fluorometrically.

Results and Discussion

Mice received an average of 4.73 (± 0.83), 3.77 (± 0.45), and 2.37 (± 0.19) sec of shock per trial on the first, third, and fifth day of escape training, respectively. Analysis of variance revealed that the shock treatment influenced plasma corticosterone concentrations, $F(2, 34) = 24.89$, $p < .001$ (see Figure 3). Once again, Newman-Keuls multiple

Insert Figure 3 about here

Figure 3

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice 15 minutes after exposure to either 1, 3, or 5 days of escapable (60 shocks of 2 sec duration), yoked inescapable, or no shock treatment. Mice not exposed to any treatment whatsoever yielded a mean ($\pm$ S.E.M.) plasma corticosterone value of 3.39 (0.43).

comparisons revealed that shocked animals exhibited comparable increases of plasma corticosterone regardless of whether or not control over shock offset was possible. Moreover, even though escape latencies on the last test day after 5 daily stressor applications were quite rapid, no differences in corticosterone secretion were observed in escapably and inescapably shocked mice. Likewise, there were no differences between groups as a function of the number of training sessions mice received.

The present finding that gaining mastery over response contingencies in a shuttle-escape paradigm failed to influence the corticoid response was at odds with the data reported by Tsuda and Tanaka (1985). It is likely that procedural differences may be responsible for the divergent results. Indeed, not only was the operant required (i.e., disk-pulling) to terminate shock in the Tsuda and Tanaka study more difficult for the animals to acquire than the shuttle-escape response in the present study, but also animals were subjected to a 3 hr test in a Sidman free operant procedure.

EXPERIMENT 4

Controllability, Sensitization, and Time-Dependent Changes in Plasma Corticosterone

As indicated earlier, it has been reported that the NE variations associated with stressors may be subject to conditioning or sensitization processes. In particular, reexposure to cues associated with the stressor or to a limited amount of aversive stimulation may lead to marked alterations of NE or DA turnover and levels (Anisman & Sklar, 1979; Cassens et al., 1980; Herman et al., 1982; Irwin, Ahluwalia, Zacharko, & Anisman, 1986). It has likewise been suggested that exposure to aversive stimulation may proactively influence the corticoid release associated with subsequent exposure to stressors (e.g., Dess et al., 1983; Weiss et al.,

1970). While the results of the preceding experiments indicated that controllable and uncontrollable stressors did not differentially influence corticosterone concentrations, the possibility was considered that a stressor experience would proactively influence the response to a subsequent mild stressor, and that such an effect would be dependent on stressor controllability.

Method

Subjects and Apparatus

A total of 160 naive, male CD-1 mice served as subjects for Experiment 4. The subject and shuttle box particulars were the same as those described in Experiment 1.

Procedure

Mice received either a single session of 360 escapable or inescapable shocks according to the procedure of Experiment 1. A third group of mice was exposed to the shuttle boxes, but shock was not delivered. Immediately following shock treatment, mice were housed individually and provided with food and water. Twenty-four hr afterward, mice in each group were subdivided ($n = 5/\text{group}$) and exposed either to 30 inescapable shocks of 2 sec duration or to no shock. Inescapable footshock was administered in four identical black Plexiglas chambers. The characteristics of these shock boxes were similar in all respects to the shuttle boxes, except that they measured 30 x 14 x 15 cm. The nature and circuitry of the shock source were also the same as those described in Experiment 1. A separate group of mice ($n = 5/\text{group}$) was housed individually at the outset of the study and left undisturbed. As in Experiment 1, mice were sacrificed by decapitation either immediately, 15 min, 30 min, 60 min, or 180 min after the last shock received. Trunk

blood was collected, centrifuged, and the plasma samples stored at -70°C until plasma corticosterone concentrations were assessed fluorometrically.

Results and Discussion

The mean amount of shock received per trial during the training session was $2.23 (\pm 0.17)$ sec. Unlike the proactive influence of aversive stimulation on central neurochemical changes, prior stressor exposure failed to alter the corticoid response to subsequent reexposure to limited amounts of shock. As seen in Figure 4, regardless of the initial treatment received (i.e., escapable, inescapable, or no shock) reexposure to a limited shock stressor (30 shocks of 2 sec duration) induced comparable plasma corticosterone elevations and decay rates. Likewise, mice that were not reexposed to the 30 shock trials did not differ as a function of the initial treatment administered.

Insert Figure 4 about here

Analysis of variance of plasma corticosterone levels yielded a Shock Reexposure $\times$ Time interaction, $F(4, 114) = 3.75$, $p < .01$. Subsequent Newman-Keuls multiple comparisons ($\alpha = .05$) confirmed that all mice exposed to 30 shocks 24 hr after initial treatment exhibited higher plasma corticosterone concentrations at 15 min and 30 min after stressor termination than nonshocked controls. As in Experiment 3, peak corticosterone values in shocked animals were reached early after stressor termination and returned to baseline levels at approximately 60 minutes. Moreover, as observed in Experiments 1 and 2, relative to a severe stressor, 30 shocks did not induce as marked an increase of plasma corticosterone concentration, and the rate of decay was more gradual. Pairwise comparisons between escapably and yoked inescapably shocked

Figure 4

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice exposed to either 30 shocks of 2 sec duration or no shock. Mice had previously been exposed to either escapable (360 shocks of 2 sec duration), yoked inescapable, or no shock treatment 24 hr earlier. In mice left undisturbed in their home cages a mean ($\pm$ S.E.M.) plasma corticosterone value equalled 1.66 (0.23).

PLASMA CORTICOSTERONE (ug/100 ml)

mice failed to reveal differences at any of the sampling intervals. Moreover, planned comparisons between handled (i.e., placement in apparatus only) and nonhandled mice revealed differences at the 0 min and 15 min sampling intervals after footshock termination, suggesting that the corticoid response was sensitive to the mild stressor exposure.

EXPERIMENT 5

Sensitization of Plasma Corticosterone Following

Repeated Exposure to Controllable and Uncontrollable Footshock

Irwin, Ahluwalia, and Anisman (1986) recently demonstrated long-term proactive effects of aversive stimulation in mice. In particular, it was demonstrated that among animals previously exposed to a chronic stressor regimen, reexposure to the stressor or to apparatus cues associated with the stressor provoked increased NE levels and turnover. The possibility was thus considered that the proactive effects of controllable and uncontrollable shock exposure on circulating plasma corticosterone levels may emerge following chronic exposure to stressors, and only after mice had acquired mastery over the response contingencies. Accordingly, another experiment was conducted to assess whether repeated escape training would alter the corticoid response upon reexposure to a relatively mild shock.

Method

Subjects and Apparatus

Fifty, naive, male CD-1 mice served as subjects in Experiment 5. The subject and apparatus particulars were identical to those of Experiment 1.

Procedure

As in Experiment 3, mice were assigned to one of four treatment conditions. Mice ($n = 15$) received either 60 escapable shocks, 60 yoked inescapable shocks, or no shock. An additional group of mice ($n = 5$) was

housed individually and left undisturbed on the shelf until the time of decapitation. Following 6 days of training, mice were housed individually for a period of 14 days after which they were exposed either to 30 inescapable shocks of 2 sec duration with 9 sec intertrial intervals, to the no-shock apparatus, or to no treatment whatsoever. Fifteen minutes after the last shock received, mice were decapitated, trunk blood collected and centrifuged. Plasma samples were frozen and stored at -70°C until plasma corticosterone concentrations were assessed fluorometrically.

Results and Discussion

Analysis of variance of the corticosterone values 14 days after repeated exposure to escapable, yoked inescapable, or no shock revealed only a significant effect of Shock Reexposure, $F(2, 35) = 107.60$, $p < .001$ (see Figure 5). Animals that received 30 shocks of 2 sec duration

Insert Figure 5 about here

exhibited higher corticosterone concentrations than nonshocked mice. Despite rapid escape latencies on the last test day ($\bar{x} = 2.36 \pm 0.13$), elevations of plasma corticosterone were independent of whether mice had previously received repeated escape training or repeated inescapable shock. Furthermore, as in the preceding studies the mild stressor of handling and apparatus exposure provoked a moderate increase of plasma corticosterone concentrations.

Within the parameters of the present studies, it appears that, unlike the differential effects of stressor controllability on brain catecholamines, the magnitude of the rise and the subsequent decay rates of plasma corticosterone concentrations are comparable irrespective of whether the stressor was controllable or uncontrollable. Moreover, it seems that

Figure 5

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice reexposed to either 30 shocks of 2 sec duration, the shock apparatus, or no treatment. Mice were initially exposed to 6 days of either escapable (60 shocks of 2 sec duration), yoked inescapable, or no shock 14 days earlier. Plasma corticosterone levels were determined 15 minutes after the last shock received. In mice left undisturbed in their home cages for the duration of the study a mean ($\pm$ S.E.M.) plasma corticosterone value equalled 1.32 (0.67).

controllable and uncontrollable stressors do not differentially influence corticosterone concentrations even after mice had gained mastery over the response contingencies. Finally, the organism's previous history, in terms of controllable versus uncontrollable footshock, does not differentially influence the corticoid response to subsequent aversive stimulation.

EXPERIMENT 6

Effect of Water Immersion on Plasma Corticosterone

Based on the preceding experiments, it appears that stressor controllability may not be fundamental in determining plasma corticoid levels (see also Maier et al., 1986). It is conceivable that the failure to observe changes in corticoid secretion following exposure to controllable and uncontrollable footshock were due to, among other things, the nature of the stressor itself. For instance, it is possible that controllability may be important when a continuous stressor is employed (i.e., where there is not repeated discrete onset and offset of the stimulus, such as with water immersion or restraint), and not when a discontinuous or intermittent stressor (e.g., footshock) is used. Alternatively, the differential effects of controllability on plasma corticoid secretion may become apparent only after the application of more naturalistic stressors, such as water immersion, and not after exposure to electric footshock. Accordingly, another series of experiments was undertaken to determine the effects of brief exposure to varying amounts of water immersion (escapable and inescapable) on circulating levels of plasma corticosterone. The initial study of this series assessed the effects of different durations of cold water immersion on plasma corticosterone.

Method

Subjects

A total of 28 naive, male CD-1 mice served as subjects in Experiment 6. The subject particulars were the same as those described in Experiment 1.

Apparatus

Four 4000 ml Pyrex beakers (height: 25 cm; diameter: 17 cm), filled with water (15°C) to a height of 17.0 cm, were placed side by side approximately 10 cm from one another. The beakers, which were housed in a separate room, were separated from one another by nontransparent panels which prevented mice from seeing one another.

Procedure

Mice were assigned to one of four treatment conditions ($n = 7/\text{group}$). Mice were placed individually in water-filled beakers for either 1, 2, or 4 trials of 2 min duration (1 min intervals between trials). It may be noted that this procedure was modified from the forced-swim procedure described by Porsolt, Bertin, and Jalfre (1977). The fourth group of mice was not exposed to water and served as control subjects. Immediately following stressor application, the animals were decapitated, trunk blood collected in chilled heparinized test tubes and centrifuged. The plasma samples were stored at -70°C until assayed for plasma corticosterone concentrations.

Results and Discussion

Figure 6 shows the magnitude of the plasma corticosterone response

Insert Figure 6 about here

Figure 6

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice exposed to forced-swim for either 0, 1, 2, or 4 trials of 2 min duration.

following exposure to varying amounts of swim. Analysis of variance indicated that plasma corticosterone concentrations varied with the number of swim trials, $F(3, 23) = 23.21$, $p < .001$. Newman-Keuls multiple comparisons ($\alpha = .05$) revealed significant plasma corticoid elevations in mice exposed to 4 min and 8 min of water immersion compared to control subjects. Moreover, significantly higher plasma corticosterone levels were observed after 8 min of forced-swim than after 4 min of such treatment. These findings suggest that changes in corticoid levels are sensitive to differences in the severity of cold water immersion (see also Holson et al., 1968), and thus may be useful in detecting the effects of psychological variables associated with the stressor experience.

EXPERIMENT 7

Time-Dependent Variations in Plasma Corticosterone

Following Water Immersion

The results of the preceding experiment indicated that marked elevations of circulating corticosterone concentrations may be provoked by the forced-swim procedure. Experiment 7 was undertaken to determine the time course of the recovery of the corticoid response following exposure to the water stressor.

Method

Subjects and Apparatus

Eighty, naive, male CD-1 mice served as subjects in Experiment 7. The subject and apparatus particulars were the same as those described in Experiments 1 and 6, respectively.

Procedure

Half the mice were placed individually in beakers filled with water and exposed to 4 trials of 2 min duration with 1 min intertrial intervals. The remaining mice were simply placed individually in polypropylene

holding cages and were not exposed to the swim stressor. Following this treatment, mice were returned to their home cages and either immediately, 15 min, 30 min, 60 min, or 180 min later mice were decapitated and trunk blood collected. Following centrifugation, plasma samples were stored at -70°C until determination of plasma corticosterone concentrations.

Results and Discussion

Figure 7 shows the magnitude and the rate of decay of plasma

Insert Figure 7 about here

corticosterone concentrations in stressed and nonstressed mice. Analysis of variance revealed that plasma corticosterone values varied as a function of Swim Treatment $\times$ Time interaction, $F(4, 66) = 9.39$, $p < .001$. Newman-Keuls multiple comparisons of the main effects comprising the interaction ($\alpha = .05$) revealed that among mice not exposed to the swim stressor plasma corticosterone levels did not vary significantly over time, although somewhat higher corticoid levels were observed at the 30 min interval than at any other time after handling and placement in polypropylene holding cages. In contrast, mice exposed to swim exhibited an increase in plasma corticosterone concentrations immediately after stressor termination, followed by the decay of the response over time. Corticoid levels were significantly lower 30-180 min after water immersion than immediately and 15 min after stressor exposure. Between-group comparisons at each time interval revealed that at 0 min, 15 min, and 60 min, mice that received forced-swim exhibited significantly higher plasma corticosterone concentrations than control animals. These differences were absent 180 min following stressor

Figure 7

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice at various intervals after either 4 swim trials of 2 min duration or no swim.

termination. Failure to detect between-group differences at the 30 min interval may be attributable to the elevation in corticoid levels in control animals (see Figure 7). Indeed, corticoid concentrations in control mice were as high as those in the swim treatment condition. In addition, it might be noted that the time-dependent rise and decay of plasma corticosterone concentrations observed in control animals suggests that the experience of being handled and placed in the apparatus (i.e., polypropylene cages) was responsible for elevations in plasma corticosterone levels seen 30 min after treatment. This finding is consistent with earlier studies which demonstrated an increase of plasma corticosterone levels following handling and placement in a novel environment (i.e., apparatus; see also Armario et al., 1986; and M.B. Hennessy & Levine, 1978).

EXPERIMENTS 8a - 8d

Long-Term Proactive Effects of Acute and Chronic Forced-Swim

Exposure on Plasma Corticosterone Concentrations

As indicated earlier, the catecholamine depletion ordinarily observed after exposure to inescapable shock may be reinduced when animals are reexposed either to a mild stressor (Anisman & Sklar, 1979) or to cues associated with the initial stressor (Cassens et al., 1980). It is thought that the corticoid response may also be subject to sensitization or conditioning processes in that prior exposure to aversive stimulation may proactively influence corticoid release in subsequent encounters with aversive stimuli (Bunnell et al., 1988; Dess et al., 1983; Weiss et al., 1970). Hence, the possibility was entertained that prior exposure to the forced-swim procedure may proactively influence the response to subsequent exposure involving limited amounts of stressor application. Accordingly, a series of experiments was conducted to evaluate the proactive effects of

both acute and repeated stressor application on plasma corticosterone levels.

A preliminary study (Experiment 8a) was undertaken to determine whether the enhanced plasma corticosterone response observed after a single session of water immersion was subject to an attenuation of the response with repeated forced-swim. Indeed, it has been shown that the enhanced plasma corticoid response is subject to within session adaptation following repeated exposure to continuous stressors, such as immobilization and cold (Odio & Maickel, 1985; Murison, 1983). Although there is substantial evidence to suggest that elevated corticoid levels persist even after exposure to 10 sessions of forced-running, restraint, and inescapable footshock (Kant et al., 1985; Irwin, Ahluwalia, Zacharko, & Anisman, 1986), it was of interest to determine whether such an effect also occurred with repeated daily exposure to the water stressor.

Experiments 8b and 8c were undertaken to evaluate the long-term proactive effects of acute and repeated water immersion on the plasma corticosterone response. In these experiments, mice that previously received forced-swim were exposed to a limited duration of water immersion. The possibility was also considered that prior experience of swim may proactively influence the corticoid response to novel stressor application (J.W. Hennessy & Levine, 1979; Levine et al., 1973). It was considered, as well, that prior stressor exposure may differentially influence the corticoid response depending upon the nature of the stressor to which animals were subsequently exposed (i.e., footshock or forced-swim). Experiment 8d, thus, assessed the proactive effects of water immersion on plasma corticosterone levels after subsequent exposure to a limited number of inescapable footshocks. In Experiments 8b, 8c, and 8d, the reexposure session was applied 30 days after the initial aversive

stressor regimen, thereby permitting the assessment of the relatively long-term proactive effects of water immersion.

Method

Subjects and Apparatus

Experiments 8a, 8b, 8c, and 8d involved 30, 42, 42, and 60 naive, male CD-1 mice, respectively. The subject and apparatus particulars were the same as those described in Experiments 1 and 6, respectively.

Procedure

In Experiment 8a, mice ($n = 6/\text{group}$) were exposed either to 1, 2, 3, or 4 days of swim testing. As in the previous experiments, each session involved placing mice individually in water-filled beakers (15°C) for 4 trials of 2 min duration with 1 min intertrial intervals. The remaining mice were not exposed to water and served as controls. Immediately after the last swim session, mice were decapitated and trunk blood collected. Following centrifugation, plasma samples were stored at -70°C until determination of plasma corticosterone concentrations.

In Experiment 8b, half the mice were exposed to 4 trials of forced-swim (i.e., 2 min duration with 1 min intertrial intervals), while the remaining half were simply placed in a holding cage. Following stressor exposure, mice were housed individually for 30 days before being reexposed to either no treatment, 1 min, or 3 min of swim (15°C ; $n = 7/\text{group}$).

In Experiment 8c, mice were exposed to exactly the same procedures as those described in Experiment 8b, except that half the mice were exposed repeatedly to 4 trials of forced-swim over a 4 day period, while the remaining mice were simply placed in holding cages for comparable amounts of time and were not subjected to the water stressor. As in Experiment 8b, after stressor application mice were housed

individually for a 30 day period before reexposure to either no treatment, 1, or 3 min of forced-swim ($n = 7/\text{group}$).

In Experiment 8d, half the mice were exposed repeatedly to 4 trials of forced-swim according to the procedures described in Experiment 8b, while the remaining half were simply placed in a holding cage. Following stressor exposure, mice were housed individually for a 30 day period. Mice then received either no treatment, reexposure to 3 min of water immersion (15°C), or 60 inescapable shocks (150 μA) of 2 sec duration with intertrial intervals of 9 sec ($n = 10/\text{group}$).

In Experiments 8b, 8c, and 8d, mice were sacrificed and their trunk blood collected immediately after the reexposure session. Following centrifugation, plasma samples were stored at -70°C until assayed for plasma corticosterone.

Results and Discussion

Figure 8 shows the mean plasma corticosterone concentrations following 0, 1, 2, 3, and 4 days of forced-swim in Experiment 8a. Analysis of variance of plasma corticosterone values revealed

Insert Figure 8 about here

significant differences between treatment conditions, $F(4, 25) = 9.66$, $p < .001$. Subsequent Newman-Keuls multiple comparisons ($\alpha = .05$) revealed that exposure to forced-swim provoked significant elevations of plasma corticosterone compared to the no swim treatment condition. In mice exposed to swim, however, comparable elevations of plasma corticoids were observed regardless of the number of swim sessions mice received. Hence, under the present stressor conditions no evidence of plasma

Figure 8

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice exposed to 4 trials of 2 min duration for either 1, 2, 3, or 4 days. Another group of mice was not exposed to water and served as control subjects (Experiment 8a).

corticoid adaptation was evident (see also Kant et al., 1985; Irwin, Ahluwalia, Zacharko, & Anisman, 1986).

Figure 9 depicts the plasma corticosterone concentrations of each group in Experiments 8b, 8c, and 8d. Analysis of variance of

Insert Figure 9 about here

plasma corticosterone concentrations in Experiment 8b indicated that prior exposure to a single swim session did not proactively influence plasma corticosterone concentrations upon reexposure to limited amounts of forced-swim ($F < 1$). However, plasma corticosterone secretion was found to vary as a function of the Reexposure Treatment, $F(2, 29) = 8.91$, $p = .001$. Newman-Keuls multiple comparisons ($\alpha = .05$) showed that mice exposed to 1 min of water immersion did not differ from control mice, but exhibited lower corticosterone levels than mice exposed to 3 min of forced-swim.

The analysis of variance of Experiment 8c also revealed that plasma corticosterone values varied as a function of the Reexposure treatment, $F(2, 35) = 13.29$, $p < .001$. Multiple comparisons ($\alpha = .05$) again showed that mice exposed to 3 min of water immersion exhibited higher plasma corticosterone levels than mice in the other two treatment conditions. The initial chronic swim treatment did not influence corticoid concentrations.

Consistent with the results of Experiments 8b and 8c, an analysis of variance of plasma corticosterone values in Experiment 8d yielded only a main effect of reexposure to limited amounts of aversive stimulation, $F(2, 51) = 45.24$, $p < .001$. Newman-Keuls multiple comparisons ($\alpha = .05$) revealed that each of the Reexposure treatment means differed significantly from each of the other treatment condition means. Once

Figure 9

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice initially exposed to either a single session of forced-swim (4 trials of 2 min duration; Experiment 8b), repeated sessions of forced-swim (Experiment 8c and 8d), or no swim, and then reexposed to either limited amounts of forced-swim or inescapable shock. The reexposure session was applied 30 days after the initial stressor regimen.

PLASMA CORTICOSTERONE (ug/100 ml)

again, neither main effects nor the interaction involving the initial Swim Treatment reached statistical significance. Taken together, the results of these experiments suggest that the initial stressor (forced-swim) does not proactively influence the plasma corticoid response to subsequent stressor exposure involving either water immersion or footshock. These findings, at first glance, appear to be at odds with those studies reporting that marked elevations in plasma corticosterone levels occurred in animals that were reexposed to cues associated with the initial stressor (Bunnell et al., 1988) or were exposed to a novel environment after prior stressor application (Levine et al., 1973). However, in these studies animals were reexposed to relatively mild stressors. Hence, it is conceivable that the severity of the stressors employed in the present investigation (i.e., water immersion and footshock) did not permit the expression of the corticoid response to limited amounts of stress.

EXPERIMENTS 9a - 9d

Long-Term Proactive Effects of Forced-Swim

Exposure on Plasma Corticosterone Concentrations Following

Stimulation in Early Life (21 Days of Age)

In the preceding experiment, prior experience of water immersion did not appear to affect the plasma corticoid response to subsequent stressor application, regardless of whether reexposure was to limited amounts of the same stressor or to a novel stressor (e.g., footshock). It has been shown that the long-term consequences of stressors on the plasma corticoid response occurred more reliably when the initial stressor was applied in early life (see J.W. Hennessy & Levine, 1979; and Sapolsky & Meaney, 1985). For instance, it was demonstrated that the adrenocorticoid response to aversive stimulation (i.e., vibratory stress) in adult rats varied as a function of handling applied during the first 21 days of life (Meaney,

Aitken, Bodnoff, Iny, & Sapolsky, 1985). It is not unreasonable, then, to expect that stressors encountered during early life might proactively influence corticosterone secretion upon subsequent exposures to aversive stimulation in adulthood. Hence, it was of interest to determine whether the corticoid changes associated with stressor reexposure were dependent upon the age of the organism at the time of the initial exposure to water immersion. Accordingly, a series of experiments was conducted to examine the consequences of reexposure to the forced-swim procedure in adult animals that had been previously exposed to the stressor at 21 days of age. These experiments were conducted to determine whether (1) prior experience of swim could proactively influence the corticoid response to subsequent stressor application in adulthood, and (2) these changes were comparable to those previously seen in adult animals. These experiments, then, roughly paralleled those described in the preceding study.

It might be noted that the nature of the stressor may be an important factor in eliciting the corticoid response following aversive stimulation in juvenile mice. For instance, repeated exposure to mild stressor application, such as handling, resulted in the attenuation of the corticoid response (Ader, Friedman, Grotta, & Schaefer, 1968), whereas repeated exposure to more intense stressors, such as footshock, failed to diminish the secretion of plasma corticosterone (Ader et al., 1968). Hence, a preliminary experiment (Experiment 9a) was conducted to determine the effects of acute and repeated water immersion on the corticoid response, and to compare these changes with those observed in adult animals.

Experiments 9b and 9c were conducted to evaluate the proactive effects of both acute and repeated stressor application on plasma corticosterone levels in juvenile mice. In addition, since several

investigators have reported that in adult animals the corticoid response to novel stressor application may be influenced by stimulation during early development (see Ader, 1970; and Meaney et al., 1985), Experiment 9d was conducted to determine whether prior experience of swim could proactively influence the corticoid response to subsequent exposure to limited amounts of inescapable footshock (i.e., novel stressor). As in Experiment 8, the reexposure session was applied 30 days after the initial aversive regimen.

Method

Subjects and Apparatus

Experiments 9a, 9b, 9c, and 9d involved 50, 42, 42, and 48 juvenile (i.e., 21 days of age), male CD-1 mice, respectively, raised in this laboratory. Initially, twenty pregnant female CD-1 mice were obtained from the Canadian Breeding Laboratories, LaPrairie, Quebec. The pregnant females were housed individually in standard polypropylene cages and permitted ad lib access to food and water. In order to minimize the impact of potential prenatal stressors (e.g., travelling) on corticoid secretion, the progeny of these females was not used for experimentation but was permitted to reproduce. Thus, it was the second filial generation which was used for experimentation. In Experiment 9a, juvenile mice were weaned 19 days after birth and male mice were swim tested two days later. In Experiments 9b, 9c, and 9d, mice were sexed two days after being weaned and the male mice were then housed in groups of 4 to 6 per cage and permitted ad lib access to food and water until the time of testing.

As in Experiment 6, four Pyrex beakers, measuring 25 cm in height and 17 cm in diameter, were filled with water (15°C; and 17 cm high) and used for swim testing. The beakers were placed approximately 10 cm

from each other and were separated by nontransparent panels to prevent mice from seeing one another.

Procedure

In Experiment 9a, mice ($n = 10/\text{group}$) were exposed to either 1, 2, 3, or 4 days of swim testing. Juvenile mice were placed individually in the water-filled beakers and exposed to 4 trials of 2 min duration with intertrial intervals of 1 min. It may be noted that preliminary studies indicated that these stressor parameters in juvenile mice clearly produced fatigue, but all appeared to have recuperated within 60-90 min. The remaining mice served as controls. Immediately after the last swim session, mice were decapitated and trunk blood collected for determination of serum corticosterone concentrations.

In Experiment 9b, half the juvenile (i.e., 21 days old) mice were exposed to a single session of 4 trials of forced-swim according to the procedures described in previous experiments. The remaining mice were placed in holding cages for comparable amounts of time and were not exposed to the water stressor. Following stressor application, mice were housed individually for 30 days before being reexposed to either 0, 1, or 3 min of swim (15°C ; $n = 7/\text{group}$).

Experiment 9c was identical to Experiment 9b, except that mice were exposed either to 4 trials of forced-swim on 4 consecutive days or to holding cages for comparable amounts of time. Following stressor exposure, mice were housed individually for 30 days before being reexposed to either 0, 1, or 3 min of swim (15°C ; $n = 7/\text{group}$).

In Experiment 9d, half the mice were exposed to 4 trials of forced-swim (i.e., 2 min duration with 1 min intertrial intervals) on 4 consecutive days, while the remaining half were simply placed in a holding cage. Following stressor exposure, mice were housed individually for 30 days.

Mice were then subdivided and reexposed either to 3 min of water immersion (15°C), or to 60 inescapable shocks (150 uA) of 2 sec duration with intertrial intervals of 9 sec, or to no treatment ($n = 8/\text{group}$).

Immediately after reexposure to swim or shock application, mice were sacrificed and trunk blood collected. Following centrifugation, plasma samples were stored at -70°C until assayed for plasma corticosterone.

Results and Discussion

Analysis of variance of plasma corticosterone values in Experiment 9a revealed that plasma corticosterone varied as a function of Swim Treatment, $F(4, 45) = 8.74$, $p < .001$. Newman-Keuls multiple comparisons ($\alpha = .05$) indicated that juvenile mice exposed to the swim condition did not differ from each other, but exhibited significantly higher plasma corticosterone concentrations than nonstressed control subjects (see Figure 10). As in adult mice, repeated exposure to water immersion did not

Insert Figure 10 about here

result in the attenuation of the corticoid response (cf. Figures 8 and 10). The rise in plasma corticosterone following repeated water immersion in juvenile mice is consistent with the Ader et al. (1968) study which employed a footshock stressor during early development. In addition, however, Ader et al. reported that when a mild stressor was employed, such as repeated handling, an attenuation of the plasma corticosterone response was observed. Hence, it is possible that the severity of the forced-swim parameters employed in the present investigation may have obfuscated detection of an adaptation effect after several days of testing.

Figure 10

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in juvenile mice exposed to 4 swim trials of 2 min duration for either 1, 2, 3, or 4 days. Another group of mice was not exposed to water and served as control subjects (Experiment 9a).

Figure 11 depicts the plasma corticosterone concentrations of each group of mice in Experiments 9b, 9c, and 9d.

Insert Figure 11 about here

Analysis of variance of plasma corticosterone concentrations of Experiment 9b yielded a significant effect of Reexposure Treatment, $F(2, 35) = 8.92$, $p = .001$. Newman-Keuls multiple comparisons ($\alpha = .05$) revealed that each Reexposure treatment mean differed significantly from each of the other treatment condition means. Although Prior Swim x Reexposure Treatment interaction did not reach statistical significance, Newman-Keuls multiple comparisons ($\alpha = .05$) were made on treatment means comprising this interaction, since a priori predictions had been made. These comparisons revealed that varying amounts of swim provoked a dose dependent increase in plasma corticosterone secretion in control mice. In contrast, reexposure to limited amounts of water immersion failed to influence the corticoid response in mice previously exposed to water immersion at 21 days of age. Between-group comparisons revealed that following reexposure to 3 min of water immersion significantly higher plasma corticosterone levels occurred in the control condition relative to mice with a prior history of stressor exposure.

The analysis of variance of Experiment 9c revealed that plasma corticosterone values varied as a function of reexposure to limited amounts of swim treatment, $F(2, 39) = 36.07$, $p < .001$. Once again, multiple comparisons ($\alpha = .05$) showed that mice reexposed to 3 min of water immersion exhibited higher plasma corticosterone levels than mice reexposed to 1 min of swim which, in turn, was higher than nonstressed

Figure 11

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice that were exposed, as juveniles, to either a single session of forced-swim (4 trials of 2 min duration; Experiment 9b), repeated sessions of forced-swim (Experiment 9c and 9d), or no swim. As adults, mice (50-55 days of age) were reexposed to either limited amounts of forced-swim or inescapable shock.

(ug/100 ml)

CORTICOSTERONE

PLASMA

mice. Comparisons of the means ($\alpha = .05$) comprising the Prior Swim Treatment $\times$ Reexposure interaction ($F < 1$) confirmed that plasma corticosterone concentrations increased significantly with the duration of forced-swim in both mice previously exposed to repeated water immersion and in nonstressed mice. Unlike the findings in Experiment 9b, no differences between treatment conditions were observed when between-group comparisons were conducted ($p > .10$). While it might appear from the results of the preceding study (Experiment 9b) that prior exposure to acute swim proactively attenuated the corticoid response to subsequent stressor exposure, no such diminution of the corticoid response was evident upon stressor reexposure in animals previously exposed to repeated forced-swim (Experiment 9c).

Analysis of variance of plasma corticosterone values in Experiment 9d yielded a significant effect of Reexposure Treatment, $F(2, 40) = 112.10$, $p < .001$, and Prior Swim, $F(1, 40) = 5.25$, $p < .05$. Subsequent Newman-Keuls multiple comparisons ($\alpha = .05$) of Reexposure revealed that each treatment condition differed significantly from each of the other treatment conditions. Exposure to inescapable shock provoked greater elevations of mean plasma corticosterone levels than exposure to forced-swim (see Fig. 11, Experiment 9d). It is clear, however, that prior stressor exposure failed to influence proactively the corticoid response to subsequent stressor exposure in adult mice. Moreover, unlike Experiment 9b, prior exposure to the swim stressor failed to influence the corticoid response to subsequent exposure to a limited amount of water immersion.

It appears that elevations of plasma corticosterone concentrations occurred in the present investigation regardless of the animal's prior stressor history. It might appear that these results are at odds with those studies which found an attenuation of the corticoid response upon

reexposure to a novel stressor (Ader, 1970; Sapolsky & Meaney, 1985). However, studies reporting proactive effects of stressors on the corticoid response have typically employed mild stressors, such as handling or placement in a novel environment (see Ader, 1970; Levine et al., 1973). It is possible that a sensitization or an adaptation effect might have been detected if a handling group was included in the present investigation. However, it will be recalled that no evidence of a sensitization was evident in adult animals that had been stressed and then reexposed to the apparatus (see Experiment 5).

EXPERIMENT 10

Catecholamine and Plasma Corticoid Variations Associated with Prepared and Contraprepared Defensive Responses

The forced-swim paradigm described by Porsolt et al. (1977) may be employed to assess the impact of experiential and organismic factors on the plasma corticoid response, however, it obviously does not lend itself to the assessment of stressor controllability. Accordingly, a modification of this procedure was employed (i.e., water-escape) to assess the contribution of stressor controllability to corticoid secretion. It has been reported that in a brightness discrimination task involving water-escape mice exhibited a tendency to approach an illuminated arm of a water-filled Y-maze, and tended to avoid the nonilluminated area (Corradini, Tombaugh, & Anisman, 1984). Indeed, the response of swimming to the illuminated arm of the maze is acquired readily, whereas numerous test sessions are necessary for animals to acquire the response of swimming to the nonilluminated arm (Szostak & Anisman, 1985). Accordingly, this paradigm not only permits evaluation of the neurochemical and endocrine response to an escapable stressor, but also permits assessment of the biochemical response under conditions where

animals are required to emit either a highly prepared (i.e., swim to light) or a contraprepared (i.e., swim to dark) response.

While it is clear that experiential and organismic factors influence central catecholamine concentrations, little is known about the contribution of innate defensive response styles to central catecholamine levels following exposure to aversive stimulation. Indeed, it might be expected that central catecholamine and plasma corticoid levels may vary with the defensive response required of the animal. For instance, it is not unreasonable to suggest that having to perform the contraprepared response, which may be more aversive than performing the prepared response of swim to light, may provoke more pronounced variations in central catecholamine and plasma corticoid levels than having to perform the highly prepared response. Accordingly, Experiment 10 was undertaken to assess the effects of escape involving highly prepared and contraprepared responding on brain catecholamines and plasma corticoids in a water-filled Y-maze.

Method

Subjects and Apparatus

A total of 45 naive, male CD-1 mice served as subjects for Experiment 11. The subject characteristics were the same as those described in Experiment 1.

Water Y-Maze. Water-escape training was conducted in a 30 cm high x 11.5 cm wide Y-maze constructed of 0.4 cm thick dark gray polyvinylchloride panels. The start arm of the maze was 15 cm in length and the two goal arms, which were separated from each other by a 30° angle, were 60.5 cm in length. The outer edge of each goal arm measured 44 cm, whereas the inside edge measured 38 cm. A movable ramp, placed at an angle of 26° from the floor of either of the goal arms,

permitted mice to escape from the water. The maze was filled with water (20°C) to a height of 22 cm. A guillotine door, situated at the choice point of the maze, was lowered once the mouse entered the choice area. This prevented the mouse from reentering the start arm. Each goal arm of the maze was illuminated by a light bulb (14 V. No. 1815) 6 cm from the end of each arm, and located on the ventral surface of the apparatus roof. A 0.4 cm thick red translucent Plexiglas roof, which permitted observation of the mouse, covered the V-shaped portion of the maze. Since water-escape trials were presented in a counterbalanced fashion over 10 trials, the light in each goal arm was alternated with a switch located on the dorsal surface of the apparatus roof. The location of the ramp was altered manually during intertrial intervals.

Procedure

Mice were assigned to one of three treatment conditions. One group of mice ($n = 15$) was required to swim to the illuminated arm, whereas a second group of mice was required to swim to the nonilluminated arm to escape from the water. Mice in the third group served as control subjects and were left undisturbed in their cages. On each trial, mice were placed individually in the start arm and required to swim the length of that arm, turn in a predetermined direction, and climb onto the ramp before being removed from the water. If a mouse failed to find the escape ramp within 60 sec, it was removed from the maze and placed in a dry holding cage for an intertrial interval of 90 sec. The position of the correct arm was varied from trial to trial according to a predetermined random sequence. Mice received 10 trials/day for either 1, 5, or 10 days. Immediately after the last trial on the final training day, mice were decapitated and the trunk blood collected and centrifuged. Plasma samples were stored at

-70°C until plasma corticosterone concentrations were assessed fluorometrically.

In addition to trunk blood collection, brains were removed and sectioned on an adjustable dissecting block (see Appendix B). With the ventral side up, the hypothalamus was removed rapidly by making an anterior cut at the optic chiasma, and a posterior cut at the mammillary bodies. Lateral cuts were made at the entrance of the optic tracts, thus separating the brain region from the temporal lobes (Anisman & Sklar, 1981). The excised brain was then cut into 250 μ m sections with razor blades and placed onto glass slides for freezing with dry ice. A petri dish filled with powdered dry ice served as a cold stage for sectioning. Using a stereomicroscope, the frontal cortex and nucleus accumbens were punched with hollow microdissection needles. The brain tissue pellets were placed in round-welled titer trays and frozen at -70°C. Catecholamine content was determined using high performance liquid chromatography (HPLC) with electrochemical detection using a modified version of Mefford (1981, see Appendix C).

Results and Discussion

Consistent with earlier reports, mice required to swim to the dark arm exhibited longer latencies to escape from the maze than mice required to swim to the illuminated arm. Figure 12 shows the mean latency to reach the

Insert Figure 12 about here

choice point and the mean latency to escape from water after 1, 5, and 10 days of testing. Analysis of variance of the latency to reach the choice point yielded a significant Swim Condition x Days interaction,

Figure 12

Mean ($\pm$ S.E.M.) latences (in sec) to swim to the choice point and the exit ramp, as well as the number of discrimination errors and errorless trials in mice required to emit either the highly prepared response of swim to light or the contraprepared response of swim to dark. Independent groups of mice received escape training for either 1, 5, or 10 days in a water-filled Y-maze (Experiment 10).

MEAN LATENCY IN SEC

MEAN NUMBER

DAYS OF TRAINING

$F(2, 82) = 4.96, p < .01$. Although the Swim Condition $\times$ Days interaction of the latencies to reach the exit ramp failed to reach statistical significance ($F(2, 82) = 2.94, p = .059$, Newman-Keuls multiple comparisons of the simple effects ($\alpha = .05$) were conducted, since a priori predictions had been made. These comparisons indicated that in mice required to swim to light latencies to reach the choice point and the exit ramp did not differ over the course of the study, with relatively rapid latencies being evident as early as the first test day. In contrast, however, in mice required to swim to dark significantly longer latencies were observed during the first training session than after 5 days of training. While the latencies to the choice point remained relatively stable thereafter, latencies to reach the exit ramp, particularly in mice required to swim to dark, increased dramatically between the fifth and tenth training session. In fact, while the difference in escape latencies in mice required to swim to light and mice required to swim to dark had disappeared by the fifth session, it had reappeared by the tenth session.

The measures of discrimination performance, namely the total number of discrimination errors made and the number of errorless trials, paralleled the escape latency data (see Fig. 12). In general, discrimination performance progressed less readily in animals required to swim to the nonilluminated arm than in mice required to swim to light. Analysis of variance of the total number of discrimination errors yielded main effects of Days and Swim Condition, $F(2, 82) = 3.57, p < .05$ and $F(1, 82) = 72.78, p < .001$, respectively. Analysis of variance of the number of errorless trials yielded a significant Swim Condition $\times$ Days interaction, $F(2, 82) = 3.54, p < .05$. Newman-Keuls multiple comparisons ($\alpha = .05$) were conducted between the means of the main effects comprising the Swim Condition $\times$ Days interaction. Multiple comparisons were also performed between the

means of the simple effects comprising the Swim Condition x Days interaction ($p < .10$) for the total number of discrimination errors, since a priori predictions had been made regarding this interaction. These comparisons confirmed that a greater number of discrimination errors occurred on each test day in mice required to perform the contraprepared response relative to mice required to swim to light. Among mice required to perform the prepared response of swimming to light, the total number of discrimination errors did not vary over the course of the study. In addition, significantly fewer errorless trials occurred on the fifth day of testing than during the initial test session. In mice required to swim to the nonilluminated arm, an improvement in discrimination performance also occurred between the first and fifth days of training. However, the total number of errors and number of errorless trials increased thereafter, such that performance was inferior on the tenth day of testing than on the fifth day.

The finding that performance after 10 days of training was inferior to that seen after 5 such sessions was somewhat unexpected. Accordingly, an additional analysis was conducted to determine the daily variations in behavior over the course of the study in mice tested for 10 sessions. Analysis of variance of the mean latency to reach the exit ramp in mice tested on 10 consecutive days yielded main effects of Swim Condition, $F(1, 27) = 20.79$, $p < .001$, and Days, $F(9, 243) = 4.38$, $p < .001$. Not surprisingly, mice performing the prepared response exhibited shorter escape latencies on each test day (see Figure 13).

Insert Figure 13 about here

Figure 13

Mean ($\pm$ S.E.M.) latencies (in sec) to swim to the choice point and the exit ramp, as well as the number of discrimination errors and errorless trials over 10 consecutive days. Mice were required to enter either the illuminated arm or the nonilluminated arm of the water-filled Y-maze (Experiment 10).

MEAN LATENCY IN SEC

MEAN NUMBER

DAYS OF TRAINING

However, between-days comparisons ($\alpha = .05$) indicated that after a rapid decrease in escape latencies, which occurred over the first 4 test days of testing, performance of mice in both swim conditions stabilized until the eighth day of testing. Thereafter, behavioral responding deteriorated steadily until the tenth session, particularly in mice required to swim to the nonilluminated arm (see Figure 13). Similarly, analysis of variance of the mean latency to reach the choice point yielded a comparable pattern of responding over 10 days, $F(9, 243) = 3.25$, $p < .001$. Analysis of variance of the measures of discrimination performance (i.e., total number of discrimination errors and the number of errorless trials) yielded Swim Condition $\times$ Days interactions, $F's(9, 243) = 2.01$, and 3.08 , $p < .05$, respectively. Multiple comparisons confirmed that discrimination performance improved over days in mice required to swim to light, but deteriorated in mice required to swim to dark. Throughout the course of training the performance of mice required to enter the illuminated arm was superior to that of mice required to enter the nonilluminated arm. The analysis of variance of escape failures, likewise, revealed a significant Swim Condition $\times$ Days interaction, $F(2, 243) = 4.89$, $p < .001$. Multiple comparisons confirmed that the number of escape failures increased significantly in mice required to enter the nonilluminated arm during the last three days of testing. The source for the deterioration is not known although several possibilities should be considered. Specifically, the escape ramp was submerged, and thus may have limited the reinforcing properties of a "safe-place access" that the ramp might have otherwise acquired (Crawford & Masterson, 1982). Moreover, it is conceivable that in an associatively and motorically demanding task, factors such as fatigue may have come to disrupt the response-outcome association, thereby leading to a deterioration of

performance. Indeed, as will be seen shortly, when the demands of the task were reduced, the deterioration of discrimination was not evident.

Figure 14 shows the mean ($\pm$ S.E.M.) plasma corticosterone concentrations after 1, 5, and 10 days of escape training. Analysis of

Insert Figure 14 about here

variance of plasma corticosterone values yielded a significant Swim Condition $\times$ Days interaction, $F(4, 108) = 2.56, p < .05$. Subsequent Newman-Keuls multiple comparisons ($\alpha = .05$) of the main effects comprising the interaction revealed that plasma corticosterone concentrations were comparable in nontreated mice on each day of testing. Among mice trained to emit the contraprepared response of entering the nonilluminated arm plasma corticosterone concentrations exceeded those of nonstressed mice, and did not vary as a function of the number of test days (see Figure 14). In mice trained to perform the highly prepared response of entering the illuminated arm plasma corticoid levels were likewise increased relative to nonstressed animals. However, a small, but significant, decline of plasma corticoid levels occurred between the first and fifth sessions. In fact, plasma corticosterone levels in mice required to swim to the illuminated arm were lower on the fifth day of testing than in mice required to swim to the nonilluminated arm.

In general, plasma corticoid changes were not found to be associated with behavioral performance. For instance, after 5 days of training the rise in plasma corticoid secretion was more pronounced in animals that were required to make the contraprepared response than in animals that were required to swim to light, even though escape latencies after 5 days of training were comparable in both conditions. It is interesting to note that

Figure 14

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice required to enter either the illuminated arm or the nonilluminated arm of the water-filled Y-maze after 1, 5, and 10 days of training. Additional groups of mice were not exposed to swim and served as control subjects (Experiment 10).

in spite of fluctuations in behavioral performance of mice exposed to the swim-escape task, plasma corticoid levels exhibited a steady decline over the course of the study. Even though performance of mice entering the illuminated arm was superior to the performance of mice entering the nonilluminated arm, only marginal differences in the corticoid response were observed on the first and last day of testing. Indeed, the circulating levels of plasma corticosterone over the course of the study seemed to be incongruent with the deterioration of discrimination performance, especially in mice required to make the contraprepared response.

In addition to changes in plasma corticosterone levels, determination of central catecholamine and metabolite concentrations were made in the hypothalamus, frontal cortex and nucleus accumbens (see Table 1). Several tissue samples were lost

Insert Table 1 about here

during the course of biochemical determinations, and hence the degrees of freedom for the analyses varied with the brain region examined. Analysis of variance of the MHPG values (expressed as the percent of control values) in the hypothalamus revealed significant main effects of Swim Condition, $F(2, 118) = 19.46$, $p < .001$, and Days, $F(2, 118) = 4.18$, $p < .05$. Newman-Keuls multiple comparisons ($\alpha = .05$) confirmed that MHPG accumulation was significantly greater in stressed mice than in nonstressed mice. Between-days comparisons revealed greater elevations in percent MHPG accumulation after 5 and 10 days swim testing than after a single training session. Although the Swim Condition $\times$ Days interaction failed to reach statistical significance ($F < 1$), multiple comparisons were conducted on the means comprising this interaction

TABLE 1

Mean ($\bar{X} \pm \text{S.E.M.}$) Concentration (expressed as a percentage of control) of Norepinephrine (NE), Dopamine (DA), 3-methoxy, 4-hydroxyphenylethylene Glycol (MHPG), and Dihydroxyphenylacetic Acid (DOPAC) (in ng/mg protein) in the Hypothalamus, Frontal Cortex, and Nucleus Accumbens After 1, 5, and 10 Days of Water-escape Training to either the Illuminated Arm (Light) or Nonilluminated Arm (Dark) of the Y-Maze.

Brain Region/ Amine	DAY 1				DAY 5				DAY 10			
	No Treatment	Swim to Light	Swim to Dark		No Treatment	Swim to Light	Swim to Dark		No Treatment	Swim to Light	Swim to Dark	
Hypothalamus												
NE	100.00 (6.89)	86.93 (5.37)	76.40 (6.16)		112.00 (7.49)	113.00 (6.82)	88.36 (3.91)		102.90 (6.91)	98.66 (5.25)	96.39 (5.82)	
MHPG	100.00 (30.61)	190.90 (19.88)	169.30 (13.58)		148.70 (24.36)	240.50 (25.27)	218.40 (18.17)		121.30 (13.88)	213.90 (22.66)	230.10 (21.31)	
DA	100.00 (6.29)	94.51 (7.96)	85.81 (8.66)		97.17 (8.95)	104.65 (9.56)	95.43 (9.07)		91.38 (5.99)	91.01 (7.00)	95.46 (7.52)	
DOPAC	100.13 (10.24)	130.95 (17.63)	120.64 (15.92)		91.40 (9.75)	118.94 (7.04)	107.37 (11.05)		95.10 (7.58)	115.00 (11.62)	123.43 (16.82)	
Frontal Cortex												
NE	100.00 (6.32)	100.90 (8.09)	100.80 (10.87)		100.20 (8.99)	97.95 (7.92)	101.20 (7.21)		102.80 (5.92)	93.08 (10.23)	108.20 (8.95)	
MHPG	100.00 (5.88)	133.20 (11.93)	147.40 (20.69)		109.90 (8.65)	133.30 (14.05)	149.00 (14.32)		95.44 (13.14)	127.50 (16.59)	143.20 (11.23)	
DA	100.00 (24.06)	97.77 (14.42)	90.50 (9.90)		95.78 (12.86)	102.50 (17.28)	97.84 (14.90)		94.77 (8.70)	81.11 (4.79)	82.15 (17.40)	
DOPAC	100.00 (3.83)	132.80 (31.45)	185.70 (19.21)		84.10 (10.06)	104.40 (11.84)	136.20 (11.84)		104.80 (22.76)	142.90 (22.67)	136.90 (20.76)	
Nucleus Accumbens												
NE	100.00 (6.59)	95.43 (8.21)	107.18 (12.14)		104.69 (10.75)	110.18 (15.66)	90.17 (13.71)		109.09 (13.01)	98.96 (9.02)	112.54 (10.02)	
MHPG	100.00 (6.59)	110.41 (13.45)	121.48 (26.43)		102.17 (14.84)	124.20 (18.45)	120.07 (15.87)		117.93 (18.45)	126.62 (18.71)	118.05 (18.74)	
DA	100.00 (13.44)	105.30 (18.84)	81.15 (18.77)		85.67 (8.25)	112.30 (28.54)	126.30 (21.61)		113.40 (20.88)	116.20 (23.71)	65.79 (9.18)	
DOPAC	100.00 (13.35)	118.80 (15.19)	90.91 (12.65)		116.70 (14.37)	107.10 (7.51)	129.80 (24.15)		86.26 (11.32)	117.60 (13.79)	90.05 (15.44)	

($\alpha = .05$). Between-group comparisons revealed that after 1 day of swim testing MHPG concentrations were elevated in mice exposed to both swim conditions relative to mice in the nonstressed condition. After 5 days of training, percent MHPG concentrations were significantly higher in mice required to swim to light compared to control mice, while the metabolite accumulation in mice required to swim to dark did not significantly exceed control values ($.05 > p < .10$). The lack of a significant effect appeared to stem from increased MHPG concentrations in control subjects at the 5 day interval, which might have been due to the fact that mice were housed in isolation (see Anisman & Sklar, 1981). Finally, following 10 days of escape training, MHPG levels in both swim conditions were significantly greater than those of nonstressed animals.

Analysis of variance of hypothalamic NE content also revealed significant main effects of Swim Condition, $F(2, 118) = 6.56, p < .01$, and Days, $F(2, 118) = 5.63, p < .01$. Newman-Keuls multiple comparisons ($\alpha = .05$) indicated that NE levels in mice required to swim to the nonilluminated arm were significantly lower than those of nonstressed animals or mice required to swim to light. Furthermore, it appeared that NE concentrations increased significantly between the first and last test session. Although the Swim Condition $\times$ Days interaction did not reach statistical significance ($p < .30$), it was predicted that animals required to emit the contraprepared response would exhibit greater reductions in central amine levels than animals required to emit the highly prepared response. Newman-Keuls multiple comparisons conducted on the simple effects comprising the interaction ($\alpha = .05$) confirmed that relative to nonstressed mice significant reductions occurred in hypothalamic NE concentrations following one and five days of swim testing in mice required to swim to dark. In contrast, only a small, nonsignificant decline

of NE was seen on the first session in mice that were required to swim to light. Moreover, the level of NE after 5 days of training was significantly lower in mice required to perform the contraprepared response than in mice required to perform the prepared response. After ten days of testing, however, NE concentrations in mice required to swim to dark did not differ from those of nonstressed animals or mice that had been required to swim to light.

Taken together, these data suggest that after a single session of water-escape training utilization of NE is increased, regardless of whether mice are required to emit the prepared or contraprepared response. The reduction of hypothalamic NE under these conditions possibly results from utilization exceeding the rate of synthesis. However, after several sessions of water-escape training, NE levels increased to control values in mice exposed to swim. Given that utilization rates were increased, regardless of the number of swim sessions mice received, it is likely that with repeated testing the increased utilization was accompanied by a compensatory increase in synthesis, thereby resulting in increased amine concentrations relative to that seen after a single swim session.

In contrast to variations in hypothalamic NE levels, concentrations of hypothalamic DA did not vary as a function of the stressor treatment. However, analysis of variance of hypothalamic DOPAC concentrations indicated that the metabolite was significantly influenced by Swim Condition, $F(2, 116) = 3.76$, $p < .05$. Multiple comparisons of DOPAC accumulation revealed that the metabolite was significantly elevated in stressed mice relative to nonstressed mice. Likewise, in the frontal cortex the stressor treatment did not appear to influence DA concentrations, although after 10 days of training DA concentrations were moderately reduced in stressed animals. The stressor treatment was found to

influence DOPAC accumulation in the frontal cortex, $F(2, 53) = 5.31$, $p < .01$, with both the swim to light and swim to dark treatments increasing DOPAC accumulation. The nature of the response required by mice, however, did not systematically influence DOPAC accumulation, although it is noteworthy that mice required to emit the contraprepared response exhibited an 85% increase of DOPAC accumulation after the first test session while only a 32% increase was evident in mice required to emit the prepared response. Finally, in the nucleus accumbens neither DA nor DOPAC was significantly affected by the stressor conditions ($F(4, 93) = 1.65$, $p < .20$, and $F < 1$, respectively). Such a finding is consistent with earlier reports showing that DA activity in the nucleus accumbens is less readily affected by stressors relative to mesocortical DA activity (Herve et al., 1982; Zacharko & Anisman, 1989). However, closer inspection of the data revealed that after a single session of swimming to dark, DA levels were reduced by 19%, levels increased as performance improved (i.e., after 5 days of training), and then fell by 35% of control values as performance deteriorated with 10 days of training. Such a pattern, albeit nonsignificant, is precisely what had been predicted. However, the reduced DA was not accompanied by increased accumulation of the metabolite DOPAC. This pattern of DA and DOPAC alterations was not spurious since a subsequent partial repetition of the study yielded comparable results. At any rate, the absence of the enhanced DOPAC accumulation may have been due to DOPAC being converted to homovanillic acid (HVA), which unfortunately was not measured in the present investigation. Indeed, relatively recent data collected in this laboratory revealed that within the nucleus accumbens HVA may be a better index of DA activity than DOPAC, while in other regions (e.g.,

mesocortex) DOPAC is the better index of DA utilization (Anisman & Zacharko, 1986).

EXPERIMENT 11

Catecholamine and Corticoid Variations Associated with Innate Defensive Responses in a Water-Escape Task

In the preceding experiment, escape was accomplished by mice finding the ramp which was submerged and hence hidden from view. Moreover, when the animal found the ramp and rested on it, the animal was still in water, and thus feedback for a correct response might have been less than it would have been had the ramp not been submerged. It is possible that these factors contributed to nonassociative disturbances (e.g., fatigue), which in turn, may have disrupted the acquisition of the escape response. Accordingly, an additional experiment was conducted to minimize these contaminating factors. To this end, the water level in the Y-maze was lowered (and thus the escape ramp was exposed) and the duration of each trial was reduced to minimize fatigue.

Method

Subjects, Apparatus and Procedure

A total of 33 naive, male CD-1 mice served as subjects in Experiment 11. The subject characteristics were the same as those described in Experiment 1. The water-filled Y-maze was the same as that described in Experiment 10, except that the water level was 15 cm high.

The water-escape training procedures were identical to those in Experiment 10, except that (a) the upper half of the escape ramp was above the level of the water, (b) mice ($n = 11/\text{group}$) received swim escape training for 10 days, and (c) each water-escape trial was a maximum of 30 sec after which time mice were removed from the maze and placed in a dry holding cage for an intertrial interval of 90 sec. A

third group of mice was not exposed to the water and was left undisturbed in their cages for the duration of the study.

As in Experiment 10, immediately after the experiment mice were decapitated and their brains removed for sectioning. Trunk blood was collected, centrifuged, and stored until plasma corticosterone concentrations were assessed fluorometrically.

Results and Discussion

Figure 15 shows the discrimination performance and response

Insert Figure 15 about here

latencies of mice required to enter into either the illuminated arm or the nonilluminated arm of the maze. Analysis of variance of the latency to reach the choice point and the exit ramp revealed significant Swim Condition $\times$ Days interactions, $F's(9, 180) = 3.03, p < .01$ and $5.60, p < .001$, respectively. Newman-Keuls multiple comparisons of the main effects comprising these interactions ($\alpha = .05$) indicated that in mice required to swim to light the latencies to the choice point and exit ramp did not differ over the course of the study, and relatively rapid escape latencies occurred as early as the first test day. In contrast, however, in mice required to swim to the nonilluminated arm longer latencies to the choice point were evident on the first 2 test days than in mice required to swim to light. Thereafter, performance improved and latencies were comparable to those of mice required to swim to light. In mice required to enter the nonilluminated arm a progressive decline in escape latencies was evident over the first 5 test days after which performance stabilized. Not surprisingly, longer latencies to the exit ramp occurred in mice required to emit the contraprepared response relative to mice required to swim to

Figure 15

Mean ($\pm$ S.E.M.) latencies to reach the choice point and the exit ramp, as well as the mean number of discrimination errors and errorless trials over 10 consecutive days. Mice were required to enter either the illuminated arm or the nonilluminated arm of the water-filled Y-maze (Experiment 11).

MEAN LATENCY IN SEC

MEAN NUMBER

DAYS OF TRAINING

light. However, the groups did not differ from one another on the seventh and tenth test day.

As in Experiment 10, two additional analyses were conducted to evaluate discrimination performance. Analysis of variance of the number of errorless trials revealed that mice required to swim to light made fewer errors than mice required to swim to dark, $F(1, 20) = 22.50$, $p < .001$. However, in both swim conditions discrimination performance improved over the course of the study, $F(9, 12) = 15.18$, $p < .001$. Analysis of variance of the total number of discrimination errors yielded a Swim Condition $\times$ Days interaction, $F(9, 180) = 2.33$, $p < .05$. Multiple comparisons of the simple effects ($\alpha = .05$) revealed that in mice required to swim to light the total number of discrimination errors did not vary over the course of the study. In contrast, in mice required to swim to dark a progressive decline in the number of discrimination errors was evident between the first 5 test days. Between-group comparisons indicated that mice required to enter the nonilluminated arm made more discrimination errors on each test day than mice required to swim to light. These data are consistent with the longer latencies to reach the exit ramp observed in animals required to swim to the dark arm.

Unlike the deterioration of escape performance seen in the final days of escape training in the previous experiment, improvement in discrimination performance and escape latencies occurred rapidly in the present study and was maintained over the 10 test days. It is interesting to note, however, that discrimination performance in mice required to emit the highly prepared response was comparable in the two experiments, thus suggesting that choice accuracy is not disrupted by increased task difficulty. In contrast, in mice required to emit the

contraprepared response discrimination performance was markedly affected in the more difficult task (cf. Figures 13 and 15).

The mean ($\pm$ S.E.M.) plasma corticosterone concentrations (in ug/100 ml) for the control mice, swim to light, and swim to dark treatment conditions were 3.10 (0.45), 13.28 (1.10), and 13.34 (1.04), respectively. Analysis of variance revealed that mean plasma corticosterone concentrations varied as a function of Swim Condition, $F(2, 29) = 38.05$, $p < .001$. Not surprisingly, multiple comparisons ($\alpha = .05$) of the means indicated that plasma corticosterone levels were significantly elevated in mice exposed to the swim treatment relative to nonstressed mice. As in the previous experiment, however, the plasma corticoid response failed to differentiate between prepared and contraprepared response styles following 10 days of water-escape training.

Table 2 depicts the percent of control mean ($\pm$ S.E.M.) values of NE

Insert Table 2 about here

and DA (in ng/mg protein), and their metabolites, MHPG and DOPAC, respectively, in various brain regions following 10 days of water-escape training. With the exception of the hypothalamus, catecholamine and metabolite concentrations were not significantly altered by the water-escape task within the frontal cortex and nucleus accumbens. Consistent with the results of Experiment 10, analysis of variance of hypothalamic MHPG and DOPAC concentrations indicated that the metabolites were significantly influenced by the Swim Condition $F(2, 30) = 19.20$, $p < .001$, and $F(2, 15) = 10.51$, $p < .001$, respectively. Newman-Keuls multiple comparisons ($\alpha = .05$) of hypothalamic MHPG values revealed that each treatment condition mean differed significantly from the other treatment

TABLE 2

Mean ($\pm$ S.E.M.) Concentration (expressed as a percentage of control) of Norepinephrine (NE), Dopamine (DA), 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG), and Dihydroxyphenylacetic acid (DOPAC) (in ng/mg protein) in Several Brain Regions Following 10 Days of Water Escape Training.

Brain Region/ Amine	No Treatment	Swim to Illuminated Arm	Swim to Nonilluminated Arm
Hypothalamus			
NE	100.00 (5.49)	92.57 (4.74)	106.84 (9.00)
MHPG	100.00 (8.68)	174.42 (13.68)	232.98 (20.78)
DA	100.03 (6.86)	97.62 (9.25)	109.74 (10.47)
DOPAC	100.00 (7.74)	159.40 (12.29)	185.13 (18.30)
Frontal Cortex			
NE	100.00 (6.42)	95.59 (4.97)	91.87 (5.39)
MHPG	100.07 (11.69)	95.52 (8.90)	95.35 (10.64)
DA	100.00 (15.76)	83.59 (14.14)	85.37 (11.14)
DOPAC	100.00 (15.21)	113.64 (16.37)	105.13 (14.99)
Nucleus Accumbens			
NE	100.03 (11.08)	100.14 (6.90)	95.51 (10.57)
MHPG	100.01 (15.96)	106.17 (10.80)	131.58 (16.33)
DA	100.00 (6.85)	92.66 (6.70)	100.01 (9.26)
DOPAC	100.00 (6.03)	91.94 (5.25)	107.78 (18.63)

condition means, such that the greatest accumulation of MHPG was observed in mice required to emit the contraprepared response. Multiple comparisons of DOPAC accumulation in the hypothalamus revealed that the metabolite was significantly elevated in stressed mice relative to nonstressed animals. While the metabolites of NE and DA were affected by the stressor conditions, the amines themselves were unaltered as a function of the swim conditions. It is possible that the enhanced utilization of the amines in the hypothalamus was met by increased synthesis, and thus NE and DA concentrations remained stable.

EXPERIMENT 12

Effects of Stressor Controllability on Catecholamine and Corticoid Variations in a Water-Escape Task

In the studies described thus far, animals were required to perform either prepared or contraprepared defensive responses to escape from the water maze. In these experiments, the escape response of swimming to light or dark provided animals with control over the amount of exposure to the water stressor. Since it is thought that exposure to uncontrollable stressors provoke more elevated levels of plasma corticosterone than controllable stressors (Weiss et al., 1979), it was of interest to determine whether differences in plasma corticoid secretion would emerge under conditions of inescapable and escapable water immersion. While the obvious prediction was that uncontrollable events would have greater effects than controllable stimulation, an alternative hypothesis was considered. Specifically, the proposition was entertained that having control over the stressor may actually be more aversive than not having control under conditions where animals are required to emit a contraprepared response. Thus, it is possible that the effects of controllability on central catecholamine levels and plasma corticoid

secretion may vary depending not only upon the nature of the task, but also upon the specific response required.

Method

Subjects and Apparatus

A total of 120 naive, male CD-1 mice served as subjects for Experiment 12. The subject and apparatus particulars were the same as those described in Experiments 1 and 10, respectively.

Procedure

Mice were assigned to one of the following treatment conditions. Using the procedures of Experiment 11, one group of mice was required to swim to the illuminated arm to escape from the water-Y-maze, while a second group of mice was required to swim to the nonilluminated arm of the maze to escape. A third group of mice was matched (yoked) with those mice that were required to swim to light to escape from the maze. For these animals, however, escape was not possible from the water-Y-maze. The guillotine was kept in place, thus separating the start arm from the rest of the maze. Mice were placed at the choice point (i.e., base) of the V-shaped segment of the maze and were permitted to swim freely. For this group of mice, the frequency and duration of light and dark presentations on each trial was dependent upon the pattern of responding of those mice that were required to emit the prepared response. A fourth group of mice was also exposed to this yoked procedure, however, these animals were exposed to the same pattern of responding of those mice required to emit the contraprepared response (i.e., swim to the nonilluminated arm). Mice in each group were subdivided ($n = 12/\text{group}$) and received either 1 or 5 days of water-escape training.

Two additional groups served as control subjects. Mice of one group were left undisturbed in their cages for a single day, whereas mice of the other group remained in their cages for 5 days.

Immediately after swim testing mice were decapitated, their brains removed, and placed for sectioning. Catecholamine content in various brain regions (described in Experiment 10 and including the caudate nucleus) were determined using HPLC with electrochemical detection. In addition, trunk blood were collected, centrifuged, and plasma samples stored at -70°C until plasma corticosterone concentrations assessed fluorometrically.

Results and Discussion

As in previous experiments, analyses were conducted to evaluate the escape and discrimination performance of animals trained to escape to the illuminated arm and nonilluminated arm of the maze (see Figure 16).

Insert Figure 16 about here

Analysis of variance of the latency to reach the choice point and exit ramp revealed longer latencies in mice required to swim to the dark than in mice required to swim to light, $F(1, 44) = 17.15$, $p < .001$ and 32.95 , $p < .001$, respectively. Not surprisingly, shorter latencies to reach the choice point and exit ramp were evident on the fifth test day than on the first test day, $F(1, 44) = 5.94$, $p < .05$ and 7.62 , $p < .01$, respectively.

The measures of discrimination performance paralleled the escape latency data. Analysis of variance of the total number of discrimination errors indicated that fewer errors were made by mice required to swim to light than by mice required to swim to dark, $F(1, 44) = 37.73$, $p < .001$. In addition, the total number of errors made decreased over the course of

Figure 16

Mean ($\pm$ S.E.M.) latencies to reach the choice point and the exit ramp, and the mean number of discrimination errors and errorless trials after 1 and 5 days of training. Mice required to enter either the illuminated arm or the nonilluminated arm of the water-filled Y-maze (Experiment 12).

MEAN LATENCY IN SEC

MEAN NUMBER

DAYS OF TRAINING

the study, $F(1, 44) = 15.62$, $p < .001$. Similarly, analysis of variance of the number of errorless trials revealed that in mice required to swim to light a greater number of errorless trials occurred relative to mice required to swim to dark, $F(1, 44) = 25.74$, $p < .001$. After 5 days of testing, an overall improvement in discrimination performance was observed in both swim conditions, $F(1, 44) = 11.94$, $p < .001$. Consistent with the results of Experiment 11, it appears that in mice required to perform the contraprepared response reliably poorer escape and discrimination performance was evident relative to mice required to swim to light, despite improvements in performance over the course of the study,

Figure 17 shows the mean ($\pm$ S.E.M.) plasma corticosterone

Insert Figure 17 about here

values in mice exposed to either escapable or yoked inescapable swim after 1 and 5 days of testing. A completely randomized factorial design ($2 \times 2 \times 2$) was employed to analyze plasma corticosterone values in swim treated animals, and Dunnett's t tests ($\alpha = .05$) were used to compare the effects of swim treatment to the nonstressed control groups. Analysis of variance of the mean plasma corticosterone levels yielded a significant Controllability $\times$ Days interaction, $F(1, 85) = 4.38$, $p < .05$. Newman-Keuls multiple comparisons ($\alpha = .05$) of the simple effects comprising this interaction indicated that after a single training session animals exposed to yoked inescapable swim exhibited significantly higher plasma corticosterone levels than animals that could escape. After 5 days of training, however, these differences were not evident. In addition, Dunnett's t statistic confirmed that greater elevations in plasma

Figure 17

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice required to enter either the illuminated (LIGHT-ESC) or the nonilluminated (DARK-ESC) arm of the water-filled Y-maze and in their yoked inescapable (i.e., LIGHT-YIES or DARK-YIES) partners. Mice received either 1 or 5 days of training. Additional groups of mice were not exposed to swim and served as control subjects (Experiment 12).

corticosterone concentration occurred in mice exposed to any of the swim conditions than in nonstressed animals.

Although the Swim Condition $\times$ Controllability $\times$ Days interaction failed to reach significance ($p < .10$), multiple comparisons were conducted on the simple effects comprising this interaction. These comparisons revealed that among mice required to swim to dark, no differences in corticoid secretion existed between escapable and yoked inescapable swim conditions over the course of the study. In contrast, however, mice required to swim to light exhibited significantly lower plasma corticosterone levels relative to their yoked counterparts following a single training session. After 5 days of testing, however, this difference was absent.

It appears that stressor controllability influenced the secretion of plasma corticosterone in the water-escape task. After a single session of escape training mice permitted to escape from the water-filled Y-maze exhibited lower plasma corticoid values than their yoked counterparts. These differences, however, were not observed following repeated exposure to the swim task. Moreover, the organism's innate defensive response style influenced the magnitude of the plasma corticoid response, such that the differential effects of controllable and uncontrollable swim were only evident in the swim to light condition. When mice were required to emit the contraprepared response of swimming to dark, plasma corticosterone levels did not differ from their yoked counterparts. Moreover, it appeared that the effects of the manipulation were evident only after the first swim session, thereafter corticoid levels increased in mice required to swim to light, possibly reflecting the cumulative impact of swim.

Table 3 shows the mean ($\pm$ S.E.M.) concentrations of NE and

Insert Table 3 about here

DA (in ng/mg protein), as well as MHPG and DOPAC, for each treatment group. Once again, several tissue samples were lost during the course of biochemical determinations, and hence the degrees of freedom for the analyses varied with the brain region examined. Although MHPG accumulation was not differentially influenced by the swim conditions ($F < 1$), comparisons of treatment means with the outside control group (Dunnett's tests; $\alpha = .05$) revealed significant elevations of the metabolite in all swim groups. Moreover, these elevations were evident after both 1 and 5 days of testing, thus suggesting that the utilization of NE was increased following exposure to water immersion.

Unlike the reductions of hypothalamic NE content observed after a single session of training in Experiment 10, analysis of variance of hypothalamic NE concentrations after 1 and 5 days of swim testing failed to reach statistical significance ($F < 1$). Inasmuch as the concentrations of the amine were unaltered in the hypothalamus, but MHPG accumulation had increased as a function of swim treatment, it is likely that amine synthesis was able to keep pace with utilization under these stressor conditions. Indeed, the minimization of task demands (i.e., reduced time trials and exposed exit ramp) in this experiment might have prevented the emergence of the hypothalamic NE depletion seen in Experiment 10. Interestingly, animals exposed to the yoked inescapable swim conditions also exhibited stable NE concentrations.

As in the case of MHPG, accumulation of hypothalamic DOPAC concentrations was not influenced by swim conditions ($F < 1$). However,

TABLE 3

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Mean ($\bar{X} \pm \text{S.E.M.}$) Concentration of Norepinephrine (NE), Dopamine (DA), 3-methoxy, 4-hydroxyphenylethylene Glycol (MHPG), and Dihydroxyphenylacetic Acid (DOPAC) (in ng/mg protein) in Several Brain Regions as a Function of Test Days, Swim Treatment and Controllability.

DAY 1					
Brain Region/ Amine	No Treatment	Swim to Illuminated Arm		Swim to Nonilluminated Arm	
		Escapable	Yoked Inescapable	Escapable	Yoked Inescapable
Hypothalamus					
NE	16.50 (0.12)	16.00 (0.76)	14.93 (0.76)	14.51 (0.72)	16.39 (0.72)
MHPG	0.81 (0.17)	2.00 (0.26)	1.94 (0.23)	2.08 (0.26)	1.92 (0.24)
DA	4.18 (0.49)	4.92 (0.68)	5.51 (0.34)	4.43 (0.35)	6.17 (0.19)
DOPAC	1.23 (0.32)	1.60 (0.28)	1.47 (0.31)	1.90 (0.32)	2.15 (0.29)
Frontal Cortex					
NE	6.65 (0.40)	7.58 (0.86)	6.26 (0.63)	8.46 (0.96)	6.92 (0.69)
MHPG	1.48 (0.28)	1.48 (0.33)	2.34 (0.49)	2.37 (0.46)	1.99 (0.48)
DA	1.20 (0.36)	1.61 (0.32)	1.87 (0.35)	2.84 (0.51)	2.16 (0.37)
DOPAC	1.40 (0.42)	1.95 (0.32)	2.13 (0.52)	1.61 (0.34)	1.72 (0.41)
Nucleus Accumbens					
NE	14.41 (0.97)	11.73 (1.38)	15.80 (2.51)	14.95 (1.39)	12.99 (1.28)
MHPG	2.19 (0.37)	2.17 (0.31)	2.70 (0.74)	2.17 (0.40)	1.77 (0.32)
DA	113.12 (10.61)	118.85 (5.70)	115.50 (9.37)	124.29 (10.77)	91.57 (12.24)
DOPAC	21.33 (2.46)	25.30 (3.45)	23.21 (3.84)	23.19 (1.77)	16.31 (1.65)
Caudate Nucleus					
NE	3.28 (0.56)	4.40 (0.41)	3.99 (0.68)	3.44 (0.53)	2.44 (0.26)
MHPG	0.66 (0.22)	0.68 (0.21)	0.71 (0.19)	0.93 (0.23)	0.90 (0.22)
DA	200.86 (15.27)	227.01 (12.35)	193.03 (17.72)	197.24 (4.67)	181.25 (13.50)
DOPAC	21.87 (1.93)	23.91 (2.26)	20.34 (2.56)	24.26 (1.92)	25.21 (1.67)

TABLE 3 (contd.)

Mean ($\bar{X} \pm \text{S.E.M.}$) Concentration of Norepinephrine (NE), Dopamine (DA), 3-methoxy, 4-hydroxyphenylethylene Glycol (MHPG), and Dihydroxyphenylacetic Acid (DOPAC) (in ng/mg protein) in Several Brain Regions as a Function of Test Days, Swim Treatment and Controllability.

DAY 5					
Brain Region/ Amine	No Treatment	Swim to Illuminated Arm		Swim to Nonilluminated Arm	
		Escapable	Yoked Inescapable	Escapable	Yoked Inescapable
Hypothalamus					
NE	17.44 (1.65)	15.24 (0.82)	15.40 (0.85)	15.20 (0.95)	16.12 (1.25)
MHPG	0.79 (0.16)	1.78 (0.22)	1.98 (0.31)	2.15 (0.18)	1.84 (0.16)
DA	4.29 (0.61)	5.31 (0.46)	5.04 (0.43)	4.51 (0.57)	5.27 (0.43)
DOPAC	0.98 (0.12)	1.91 (0.29)	1.70 (0.43)	1.61 (0.34)	1.81 (0.44)
Frontal Cortex					
NE	6.66 (0.54)	6.17 (0.74)	7.27 (0.74)	7.09 (0.46)	8.12 (0.99)
MHPG	1.92 (0.36)	1.75 (0.31)	2.25 (0.42)	2.08 (0.49)	2.26 (0.62)
DA	1.50 (0.35)	1.64 (0.24)	1.63 (0.38)	2.45 (0.38)	1.78 (0.17)
DOPAC	1.24 (0.28)	1.97 (0.29)	2.32 (0.33)	1.66 (0.36)	2.66 (0.42)
Nucleus Accumbens					
NE	12.72 (1.22)	18.44 (2.22)	17.60 (2.03)	16.73 (2.19)	12.90 (1.52)
MHPG	1.95 (0.22)	2.15 (0.52)	2.60 (0.36)	2.12 (0.28)	2.08 (0.36)
DA	112.88 (9.68)	123.34 (15.15)	128.85 (5.47)	122.28 (10.53)	113.40 (9.80)
DOPAC	20.41 (2.66)	20.18 (2.51)	25.63 (1.74)	21.09 (1.77)	21.54 (1.98)
Caudate Nucleus					
NE	2.96 (0.50)	3.84 (0.47)	4.67 (0.74)	3.48 (0.43)	2.95 (0.29)
MHPG	1.42 (0.32)	1.13 (0.23)	0.77 (0.24)	1.19 (0.32)	0.43 (0.15)
DA	206.53 (14.79)	210.58 (13.81)	208.93 (16.12)	197.66 (19.69)	173.42 (17.40)
DOPAC	20.39 (2.00)	20.02 (1.90)	25.22 (1.90)	19.15 (2.15)	22.70 (1.98)

comparisons made with the outside control group revealed that an elevation of DOPAC was evident in mice yoked to the swim to dark group. Moreover, DOPAC concentrations appeared to be somewhat elevated in all swim conditions relative to nonstressed controls. Analysis of variance of hypothalamic DA content revealed higher concentrations of the amine in animals exposed to yoked inescapable swim than in mice that received escapable swim, $F(1, 75) = 4.59$, $p < .05$. Dunnett's tests ($\alpha = .05$) indicated that relative to nonstressed animals DA levels were elevated in mice exposed to the inescapable swim conditions on the first day of testing.

Accumulation of DOPAC in the frontal cortex was unaffected by any of the swim conditions ($F < 1$). Although DOPAC levels in the yoked swim conditions appeared to exceed those of nonstressed animals, Dunnett's tests ($\alpha = .05$) revealed that this difference was only significant in mice tested for 5 days. It is possible that the increase in DOPAC accumulation may reflect the cumulative impact of inescapable swim.

Analysis of variance of DA content in the frontal cortex revealed that in mice required to swim to dark amine concentrations were higher than in animals required to swim to light, $F(1, 73) = 5.96$, $p < .05$. Surprisingly, in contrast to the reduction of DA concentration observed in mice required to approach the nonilluminated arm in Experiment 10, a single session of water-escape training in this experiment actually resulted in elevations of the amine in animals required to swim to dark, as well as in their yoked counterparts (Dunnett's tests; $\alpha = .05$). Similarly, after 5 days of training elevations in DA were observed in mice required to perform the contraprepared response of swim to dark relative to nonstressed mice. The source for these divergent results are not readily apparent at this time, although it should be underscored that the finding of increased mesocortical DA in the present investigation is inconsistent

with all other studies conducted in this laboratory, despite the fact that the augmentation appeared in each stressed group of the present study.

Although MHPG accumulation in the frontal cortex was not influenced by the swim conditions, analysis of variance of frontal cortex NE values yielded a significant Controllability x Days interaction, $F(1, 80) = 5.05$, $p < .05$. Newman-Keuls multiple comparisons of the main effects ($\alpha = .05$) revealed that mice permitted to escape exhibited higher NE levels than mice exposed to yoked inescapable swim on the first day of testing. These differences were absent after 5 days of training.

Within the nucleus accumbens, a reduction of DA relative to nonstressed animals was evident only in mice yoked to the swim to dark (one session) group (Dunnett's t ; $\alpha = .05$). Analysis of variance of DOPAC values yielded a significant Controllability x Days interaction, $F(1, 61) = 4.27$, $p < .05$. Newman-Keuls multiple comparisons revealed that in mice permitted to escape higher DOPAC values occurred after the first test day than in mice exposed to yoked inescapable swim. These differences were absent after the fifth training session. It might be noted that the differences in DOPAC concentrations between escapable and yoked inescapable groups were largely attributable to marked reductions of DOPAC in animals yoked to the swim to dark group. It will be recalled, however, that DOPAC is not the optimal index of DA utilization, and hence these data must be considered cautiously.

Finally, with respect to amine alterations in the caudate, the analysis revealed that mice required to swim to light exhibited greater NE and DA concentrations than mice required to swim in the dark, $F(1, 66) = 10.13$, $p < .01$, and $F(1, 72) = 3.91$, $p < .05$, respectively. Since the analysis of DOPAC concentrations approached statistical significance for the Controllability x Day interaction, $F(1, 67) = 3.78$, $p < .056$, multiple comparisons were

performed on the main effects comprising this interaction. These comparisons revealed higher DOPAC concentrations occurred after 5 days of testing in mice exposed to yoked inescapable swim than in their escapable counterparts.

GENERAL DISCUSSION

The results of the present investigation revealed that exposure to a stressor resulted in a marked increase in plasma corticosterone concentration. The increase persisted for approximately 1-3 hr, after which corticosterone concentrations approached control values. Although some investigators have demonstrated that the plasma corticosterone response does not distinguish between moderate and severe stressor conditions (see Kant et al., 1983; Natelson et al., 1981, 1987), the magnitude of this response in the present investigation appeared to vary with stressor severity. In particular, plasma corticosterone elevations were marked upon exposure to a relatively strong stressor (360 shocks), but were not as pronounced after lesser amounts of footshock (60 shocks). Additionally, the corticoid response was exquisitely sensitive to mild stressors, such as handling and exposure to a novel environment. These manipulations provoked small, but reliable, increases in plasma corticoid levels, which persisted for brief intervals (15-30 min). Taken together, the present results, like those of previous investigations (see Armario et al., 1986; Holson et al., 1988; and M.B. Hennessy & Levine, 1978), indicate that the magnitude and the duration of the corticoid response may be sensitive to graded levels of environmental change.

It might be noted that following exposure to 360 shocks, peak corticosterone concentrations were evident immediately upon stressor termination, whereas after a milder form of the stressor (60 shocks) the appearance of peak corticoid concentrations was delayed. Inasmuch as the former treatment was applied over a longer period, the delay of peak values in mice exposed to 60 shocks may be related to the time from initial presentation, rather than from stressor termination. Indeed, such a delay in peak corticosterone values has also been reported following

presentation of stressors of brief duration (see De Souza & Van Loon, 1982; and Paris et al., 1987).

Despite the apparent sensitivity of the plasma corticosterone response, it did not appear that controllable and uncontrollable footshock differentially influenced the secretion of the steroid. Both treatments engendered comparable effects, regardless of whether 60 or 360 trials were used, or whether the trials were massed (i.e., applied within a single session) or spaced over days. Indeed, differential effects were not obtained with respect to either the magnitude of the corticosterone response or the time course for this response (i.e., the rate of decay of the stressor-provoked corticoid increase). Moreover, the magnitude of the corticoid response was not diminished even when animals had established mastery over the response contingencies (i.e., after several days of escape training). These findings extend the previous results of Maier et al. (1986), which revealed that the ability to control stressor termination does not influence the secretion of plasma corticosterone. The present data support the null hypothesis, and thus, should be considered cautiously. Yet, it should be underscored that the same parameters of controllable and uncontrollable footshock, applied under essentially identical conditions in the same strain of mouse, have previously been shown in this laboratory to differentially influence hypothalamic and hippocampal NE turnover and levels (Anisman et al., 1980; Irwin, Ahluwalia, Zacharko, & Anisman, 1986), shuttle-escape performance, and responding for ICSS (see Anisman, Kokkinidis, & Sklar, 1981; Anisman & Zacharko, 1986; Zacharko & Anisman, 1989). Thus, it is not likely that the failure to detect corticoid variations attributable to stressor controllability stems from the specific stressor or task parameters employed.

The finding that controllable and uncontrollable stressors did not differentially influence plasma corticoid concentrations is at odds with earlier reports in rats (Weiss, 1971b), dogs (Dess et al., 1983), and rhesus monkeys (Hanson et al., 1976). The source for between laboratory differences is not readily discernable given the numerous procedural differences between these studies. Nevertheless, it should be emphasized that in the report of Weiss (1971b) both controllable and uncontrollable shock increased corticoid concentrations appreciably, and while the corticoid increase induced by the latter treatment exceeded that of the former, the difference (e.g., in terms of percentage) between the two conditions was actually quite small. Indeed, in light of the failure of Maier et al. (1986) to detect corticoid variations associated with stressor controllability (see also Murison & Isaksen, 1982), coupled with the results of the present investigation, it is not unlikely that the findings of Weiss (1971b) may well have been spurious. However, in a study involving rats, marked corticoid elevations were noted after inescapable shock relative to those provoked by escapable/avoidable shock (Herrmann et al., 1984). These investigators noted that the magnitude of the corticosterone increase exceeded that of previous experiments in that laboratory. It was suggested by these investigators that the particularly high plasma corticosterone levels may have been due to (a) the use of female rats, and/or (b) the extended training animals received. In the remaining reports, which showed differences between controllable and uncontrollable stressors (i.e., Dess et al., 1983; and Hanson et al., 1976), dogs and rhesus monkeys were used, respectively. Thus, it is conceivable that the failure to detect an effect of stressor controllability in the present investigation may be species related. Indeed, unlike the diurnal variations of plasma corticosterone typically seen in other species, such an effect was

not evident in mice (Shanks, Griffiths, Zalcman, Zacharko, & Anisman, 1989, submitted). In effect, the corticoid response in the mouse may not be characteristic of that seen in other species.

In contrast to reports which suggested that corticoid activity is attenuated following the establishment of an escape/avoidance response (Coover et al., 1973; Davis et al., 1977; Dess et al., 1983; Tsuda & Tanaka, 1985), repeated training in the present investigation failed to alter the corticoid response in escapably shocked mice. Indeed, although rapid latencies to escape were observed after 5 days of escape training, plasma corticosterone concentrations were comparable in escapably and inescapably shocked mice. It is possible that with further stressor exposure the differential effects of stressor controllability on the plasma corticoid response might have emerged (e.g., Herrmann et al., 1984; and Tsuda & Tanaka, 1985). For instance, using active avoidance and escape paradigms, respectively, Coover et al. (1973) and Davis et al. (1977) showed that following 15 training sessions plasma corticoid concentrations decreased considerably from peak values. However, in these studies, as in the present investigation, animals were successful in acquiring the response contingencies by the fifth session, but the secretion of plasma corticosterone did not reflect the accompanying behavioral changes until much later in training (see Davis et al., 1977). Since the present series of experiments did not evaluate the corticoid response following 15 sessions of training, it is not known whether differences in corticoid values would have emerged following a more extended training regimen.

Despite the fact that stressor controllability did not appear to influence corticosterone secretion differentially under a variety of test conditions, the possibility was entertained that because the stressor (i.e., footshock) employed was an "unnatural one", defined here as one that

animals would not encounter in the wild, potential effects of controllability may have been obfuscated. While the entire laboratory environment may, of course, be considered unnatural, and hence might not permit the expression of potential consequences associated with stressor controllability, an additional set of studies was undertaken to evaluate the impact of forced-swim and swim escape on corticoid secretion. Not surprisingly, forced-swim was found to increase corticoid secretion, as was swim escape. Of particular interest was the finding of the present investigation that in the water-escape task plasma corticosterone concentrations varied as a function of controllability, but such an effect was dependent upon the specific response required of the animal. In particular, in animals required to emit the contraprepared response of swimming to dark, the plasma corticosterone increase was comparable to that of the yoked mice. In contrast, in animals required to perform the highly prepared response of swimming to light lower plasma corticosterone concentrations were evident relative to their yoked inescapable partners. Such an effect was evident only on the first day of swim testing. Curiously, the differential effect of controllability on corticoid secretion was absent by the fifth day of testing. It is conceivable that the cumulative impact of swim on the secretion of plasma corticosterone may have obscured the differential effects of stressor controllability after repeated testing. Alternatively, the increase in corticosterone secretion observed after the fifth water-escape session in mice required to perform the prepared response may have been due to the sensitization of mechanisms provoking the corticoid response (see J.W. Hennessy et al., 1977; J.W. Hennessy & Levine, 1979). The latter hypothesis, however, is unlikely given that 1, 5, or 10 days of water-

escape training yielded comparable increases of plasma corticosterone. In effect, there was no evidence of a sensitization effect occurring.

In all swim escape experiments conducted in this laboratory, plasma corticosterone concentrations were comparable after repeated swim testing, regardless of whether mice were required to perform the prepared or contraprepared response. This was the case even when the tasks were motorically taxing or associatively difficult (e.g., under conditions in which the exit ramp was submerged, and hence hidden from view). Moreover, plasma corticoid variations did not appear to be related to discrimination performance in the water-escape task. Even though profound differences in response latencies to the exit ramp and in discrimination errors existed between mice emitting the prepared and the contraprepared response (i.e., swim to light vs. swim to dark), these behaviors were not accompanied by parallel variations of corticosterone concentrations. Indeed, after 10 days of training, mice required to swim to light exhibited few discrimination errors, but still demonstrated plasma corticosterone levels which were comparable to those of mice required to swim to dark. Thus, it appears that gaining mastery over task requirements was not particularly important in determining the plasma corticosterone response in the swim-escape paradigm.

Although repeated stressor exposure did not result in the adaptation of the plasma corticosterone response in the present investigation, it has been reported that when a mild stressor (e.g., handling, weighing, and isotonic saline injections) was applied twice daily the extent of the plasma corticoid response was attenuated (Barrett & Stockham, 1965). It is thought that the negative feedback of plasma corticosterone on the central nervous system and anterior pituitary may counteract ACTH secretion. Thus, when stressors are administered in close temporal proximity the

enhanced adrenal corticoid activity that might otherwise have occurred would have been prevented (J.W. Hennessy & Levine, 1979). In accordance with the results of Barrett and Stockham (1965), it was demonstrated that when the interval between restraint stress was 30-60 min apart (but not 90 min), the plasma corticosterone response was attenuated. However, this hyporesponsivity of the corticoid response was not associated with altered ACTH secretion (De Souza & Van Loon, 1982). These investigators suggested that factors other than circulating plasma corticosterone levels may be involved in stressor-induced inhibition of the corticoid response. It was considered that either the influence of bound corticosterone to brain receptors or an endogenous opioid peptide, such as met-enkephalin, secreted in response to stressor application might contribute to the attenuation of the corticoid response to restraint (De Souza & Van Loon, 1982). Like De Souza and Van Loon (1982), Dallman and Jones (1973) demonstrated a decline in the elevated plasma corticosterone concentrations over the course of a 4 hr restraint session. These investigators demonstrated that although corticosterone or ACTH administration inhibited an acute stressor-induced corticoid response. This effect was not provoked by a previous stressor applied 1-24 hr earlier. Thus, these investigators suggested while corticosterone release does inhibit ACTH secretion, it can also induce a protracted period of hyperresponsiveness in either the central nervous system or anterior pituitary. In effect, it appears that although adaptation of the corticoid response may occur when stressor sessions are applied at close temporal intervals, such an effect may result from factors unrelated to negative-feedback of corticosterone on hypothalamic-pituitary activity. Moreover, there may be occasions wherein repeated exposure to a stressor may result in hyper- rather than hyporesponsiveness of pituitary-adrenal

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activity, thus leading to a sensitization, rather than an inhibition of the corticoid response. Unfortunately, the factors which determine the corticoid response have yet to be determined. Regardless of the mechanisms, it is possible that the absence of adaptation in the present investigation may be related to the fact that stressors were applied at widely spaced intervals.

In addition to assessing the effects of stressor controllability and chronicity, the present investigation also determined the effect of prior stressor exposure on the corticoid response elicited by a subsequent stressor experience. This evaluation was of particular importance, since it has been suggested that some of the long term behavioral consequences of stressors may be due to the sensitization or conditioning of physiological processes (Anisman & Sklar, 1979; Zacharko & Anisman, 1984). It will be recalled that although the central neurochemical consequences of stressors are fairly transient, persisting for only a few hours (Glazer & Weiss, 1976b), exposure to uncontrollable stressors may disrupt performance as long as weeks or months afterwards. To account for these disturbances on the basis of stressor-provoked neurochemical alterations, it was demonstrated that among animals exposed to uncontrollable footshock, subsequent reexposure to a limited amount of shock produced a marked reduction of NE concentrations (Anisman & Sklar, 1979). Moreover, it has been demonstrated that reexposure to cues previously associated with the stressor may influence NE and DA turnover (Cassens et al., 1980; Herman et al., 1982). In contrast to these findings, in the present investigation a stressor experience did not influence the plasma corticoid response to a subsequently applied stressor or to cues that had been associated with the stressor. These results are consistent with other reports which failed to demonstrate proactive effects of stressor exposure on the subsequent

corticoid response to an environmental insult (see J.W. Hennessy & Levine, 1979; and Jindra et al., 1980). Similarly, using a conditioned emotional response (CER) paradigm, it has been shown that no evidence of conditioning of the corticoid response was demonstrated, even though behavioral indications of conditioned fear were evident (J.W. Hennessy et al., 1977; see also Garcia-Marquez & Armario, 1987).

It is noteworthy that, in contrast to the present study, some investigators have reported that reexposure to cues associated with the initial stressor or subsequent exposure to a novel environment influenced plasma corticosterone concentrations (Bunnell et al., 1988; Levine et al., 1973; Murison & Isaksen, 1982). Although reexposure to apparatus cues provoked a moderate rise in plasma corticoid concentrations in the present investigation, this was the case irrespective of the initial treatment mice received. This is somewhat surprising since the corticoid response appears to be particularly sensitive to mild stressor application, such as handling and apparatus exposure. It would not have been unreasonable to expect that apparatus cues associated with a stressor would have influenced plasma corticoid secretion. Indeed, as indicated earlier, it has been demonstrated that such a manipulation effectively provoked increased central NE (Anisman & Sklar, 1979; Cassens et al., 1980; Tsuda et al., 1986) and DA utilization (Claustre et al., 1986; Herman et al., 1982), as well as variations of endogenous opioid activity (Chance et al., 1978; De Vries et al., 1979). In fact, there is evidence indicating that the corticoid response may be classically conditioned in aversive paradigms (see Ader, 1976; and Coover, Hart, & Frey, 1986).

It has been reported that olfactory cues may be essential in determining the behavioral disturbance associated with inescapable shock (Minor & LoLordo, 1984). For instance, impairments in shuttle-escape

performance occurred more readily when both preshock and test chambers contained the odor of previously shocked conspecifics. It was thus considered that olfactory stimuli associated with footshock might also be fundamental to the expression of enhanced corticosterone secretion upon reexposure to the shock apparatus. In most of the studies of the present investigation, shocked animals were reexposed to an apparatus which was identical to that in which shock was initially delivered, but the odor associated with the initial treatment was absent. Accordingly, an additional study (see Appendix D) was undertaken to assess the contribution of olfactory cues to the corticoid response. These data revealed that inescapably shocked mice that were reexposed to chambers in which inescapable footshock was delivered (i.e., had not been washed and fecal matter not removed, hence permitting odors to persist), exhibited elevations of plasma corticosterone that were comparable to those of shocked animals which were reexposed to an "odorless" apparatus (i.e., one that had fecal matter removed and had been washed with alcohol and water). Thus, it appeared that elevations in plasma corticosterone were not related to olfactory cues previously associated with shock delivery. Evidently, odor cues were not responsible for the absence of an enhanced corticoid response upon reexposure to the stressor or to cues associated with the stressor. Whether contextual cues served as specific discriminative stimuli which promoted the secretion of plasma corticosterone remains to be demonstrated (see Pitman, Ottenweller, & Natelson, 1986).

Inasmuch as stressor controllability appeared to play a role in determining the plasma corticosterone response in a swim paradigm, it was thought that proactive effects of a stressor might likewise be evident when such a stressor was employed. Once again, however, the organism's

prior stressor experience, whether it included exposure to acute or chronic water immersion, did not proactively influence the corticoid response to limited exposure to either swim or inescapable shock in adult mice. In addition, the inability to demonstrate long-term proactive effects of water immersion was not age-dependent. For instance, neither acute nor repeated water immersion in juvenile mice (i.e., 21 days of age) influenced the plasma corticosterone response to a stressor subsequently applied in adulthood.

Several investigators have shown that exposure to stressors in early life may have long-term proactive effects on the plasma corticoid response (Ader, 1970; Halmeyer, Denenberg, & Zarrow, 1967; Meaney et al., 1985). In particular, aversive stimulation during the preweaning or postweaning developmental period reduced, rather than increased, the magnitude of the plasma corticoid response when stressors, such as handling, placement in a novel environment, or vibratory stress, were presented in adulthood. Moreover, animals exposed to mild stimulation (i.e., handling) in early life exhibited a more rapid return to basal plasma corticosterone levels than control (nonhandled) animals (Ader, 1970; Meaney et al., 1985). In contrast to these reports, it is clear from the results of the present investigation that prior exposure to water immersion did not influence the corticoid response to subsequent encounters with stressors in adulthood.

Unlike the graded corticoid response to varying amounts of forced-swim seen in adult mice, in juveniles limited elevations in plasma corticosterone occurred following exposure to different periods of swim exposure. The apparent nonresponsivity to stressor manipulation observed in juvenile mice is consistent with reports which have identified a period of hyposecretion of plasma corticoids in rats (see Sapolsky &

Meaney, 1985). However, unlike the studies conducted with rats where an adult-like corticoid response occurs after approximately two weeks of life (see Sapolsky & Meaney, 1985; Rivier & Vale, 1987), our preliminary findings in mice indicated that stressor exposure provoked limited increases in plasma corticoid levels three weeks postnatally. It is possible that a species-related delay in the development of the corticoid response accounts for these effects in mice. Similar delays in the development of central neurotransmitter systems have, in fact, been reported in mice as well (Remington, 1977).

Irrespective of the mechanism, it is conceivable that application of a stressor during the hyporesponsive period may be ineffective in altering subsequent plasma corticoid secretion in response to a mild stressor in adulthood. It should be underscored, however, that the hyporesponsive period, in as far as the corticoid response is concerned, may be dependent on the backdrop upon which a stressor is imposed. Studies conducted in this laboratory (Prince & Zalcman, 1988, unpublished data, see Appendix E) revealed that in mice that had been weaned, sexed at 19 days of age, and housed in a new cage plasma corticosterone levels were significantly elevated relative to similarly treated mice that were not transferred to a new cage (thus allowing odors of the siblings and maternal parent to remain). Evidently, the corticoid response may be increased in juvenile mice, but this effect appears to be stressor-specific.

In addition to changes in plasma corticosterone concentrations, the present investigation also determined the contribution of innate defensive response styles to central catecholamine alterations following aversive stimulation. It will be recalled that there are considerable data suggesting that exposure to uncontrollable, but not controllable, stressors provoke reliable reductions of NE in the hypothalamus, hippocampus, cortex, and

locus coeruleus (Anisman et al., 1980; Irwin, Ahluwalia, Anisman, 1986; Weiss et al., 1970, 1981). In the present investigation, the increased accumulation of the catecholamine metabolites, MHPG and DOPAC, following exposure to water immersion is consistent with the suggestion that amine utilization increased under these stressor conditions. However, in contrast to the findings employing a shock procedure, in the present investigation where water immersion was employed, stressor controllability did not influence central NE levels. That is, when presented with the opportunity to escape mice exhibited hypothalamic NE levels comparable to those of animals that were not permitted to escape (yoked inescapable) from the water-filled maze. Variations in DA content also failed to differentiate between escapable and inescapable swim treatments. Inasmuch as amine levels remained unchanged, and metabolite concentrations increased, it is likely that synthesis could keep pace with utilization. It is conceivable that the stressor parameters employed in the present investigation were not sufficiently severe (i.e., the minimization of task demands) to provoke the reductions in hypothalamic NE levels typically observed after inescapable footshock.

It was expected that central catecholamine levels might vary with the defensive response required of the animal, such that having to perform a contraprepared response (i.e., swimming to dark) in a water-escape task might provoke more pronounced variations in central catecholamine levels than having to perform a highly prepared response (i.e., swimming to light). Under relatively stressful conditions (i.e., longer test trials and submerged ramp), it was confirmed that animals required to swim to the nonilluminated arm of the water-filled Y-maze exhibited significantly lower hypothalamic NE levels than nonstressed mice or mice required to swim to light. Inasmuch as the swim to dark response was

associated with a greater number of errors and longer escape latencies relative to the swim to light response, the enhanced NE alterations may have been secondary to the effort expended, rather than to the psychological aspects of emitting the contraprepared response.

Consistent with earlier reports, the NE reduction ordinarily associated with an acute stressor was absent after repeated stressor application (Irwin, Ahluwalia, Anisman, 1986; Roth et al., 1982). In contrast to the relatively rapid NE adaptation associated with repeated swimming to an illuminated arm, mice required to swim to dark exhibited a slower recovery in NE levels, such that control NE values were evident only after 10 days of testing. In particular, a transient reduction of hypothalamic NE concentrations (i.e., 13.1%) was observed immediately after a single session of escape training in mice required to approach the illuminated arm. In these animals NE concentrations reached control values after 5 stress sessions. More pronounced reductions of hypothalamic NE concentrations were observed following one and five days of swim testing in mice required to swim to dark (i.e., 23.6%) and reached control values after 10 sessions of swim. Hence, it appears that after a single session of water-escape training utilization of NE is increased, regardless of whether mice were required to emit the prepared or contraprepared response. The reduction of hypothalamic NE under these conditions probably results from utilization exceeding the rate of synthesis. It is likely that with repeated testing the increased utilization was accompanied by a compensatory increase in synthesis in both swim treatment conditions, but this effect occurs more readily when a less strenuous response is required of the animal.

Although stressors have been shown to influence mesocortical and mesolimbic DA turnover and concentrations, such effects were not

particularly marked in the present investigation. It is noteworthy, however, that there was some indication of DA activity paralleling behavioral changes evident in the swim paradigm. In particular, DA concentrations and DOPAC accumulation were affected after a single stressor session, approached control values as performance improved (i.e., after 5 sessions), and then were appreciably altered once again as performance in the swim task deteriorated (i.e., after 10 sessions). Inasmuch as this was the case regardless of the response required of the animal and the actual escape latencies displayed, it is unlikely that the variations of DA activity were simply due to the motoric demands of the test situation. However, given that stressor controllability did not appear to influence DA activity, it is unclear which psychological attributes of the test situation may have been responsible for the altered DA activity.

Summary

It is clear from the present investigation that stressors induce graded variations of adrenal corticoid secretion. Indeed, the corticoid response is an exquisitely sensitive one being elicited by mild stressors, such as handling and placement in a novel environment. Moreover, moderate elevations of plasma corticosterone concentrations occurred following exposure to a limited number of footshock trials and relatively marked increases of corticoid levels were apparent with a greater number of shock presentations.

The fact that the corticoid response is not affected by factors such as stressor controllability was somewhat surprising, given the apparent sensitivity of this response. Yet, if it is assumed that the corticoid secretion is a fundamental response to stressful events and thus essential for the organism's well being, it might be disadvantageous for such a response to be determined by factors such as the psychological dimension

of controllability. After all, when an organism is initially exposed to a stressor it is not immediately apparent to the animal whether the stressor is controllable or uncontrollable. It is only with repeated trials that the animal learns whether offset of the aversive event is dependent upon its responses. Indeed, if one function of the corticoid response is to prevent excessive activation of other systems, hence preventing physiological damage (e.g., preventing an excessive immune response; see Munck et al., 1984), then it would be expected that the corticoid response would be activated in response to stressor application, regardless of factors such as controllability.

It must be underscored, however, that the plasma corticosterone response may not be restricted to aversive stimulation, but may also be part of a preparatory response which enables the organism to engage in adaptive coping behaviors at an appropriate time (Herrmann et al., 1984). Indeed, there is considerable evidence that the presence of a predictor of an aversive stimulus results in significant corticoid elevations (see Abbott et al., 1984). Likewise, it has been shown that pretest levels of plasma corticosterone were higher in animals exposed to 20 sessions of avoidable shock (Sidman free operant avoidance) than in animals exposed to yoked inescapable shock. However, posttest analysis of plasma corticosterone concentrations in exceptionally well trained animals revealed opposite results to the pretest analysis, in that plasma corticoid concentrations were higher in yoked animals than in the avoidable condition (Herrmann et al., 1984). Hence, it was suggested that the elevated corticoid response represented the focusing of resources that prepare the animal to deal effectively with a physical stressor (J.W. Hennessy et al., 1977; Herrmann et al., 1984). In the present investigation, however, reexposure to apparatus cues in animals given several sessions of controllable and

uncontrollable shock resulted in only a marginal rise in plasma corticosterone. As indicated earlier, it is conceivable that more extended training would have resulted in an anticipatory elevation of plasma corticoid secretion.

Although it might be expected that the adrenocorticoid response would diminish with repeated exposure to a stressor, such an effect was not observed. Indeed, this was the case irrespective of whether repeated stressor application involved handling, footshock, or water immersion. Again, these data attest to the notion that the corticoid response is a relatively "primitive" one, and is engendered by any form of stress whether acutely or chronically applied. Parenthetically, in contrast to the corticoid secretion, the immunological response elicited by a stressor is subject to an adaptation effect with repeated application of the environmental insult (Irwin & Anisman, 1984), and as such appears to be independent of the corticoid response. Accordingly, it would appear that while the corticoid response might be important in preventing excessive stimulation of some physiological mechanisms, immunological responsivity, as suggested by Munck et al. (1984), does not appear to be one of these.

Finally, it should be underscored that some of the diverse results reported in the literature, as well as some of the discrepancies between the present results and earlier findings may stem from the species employed (i.e., mice vs. rats). It is not unreasonable to suggest that species-differences exist in the pituitary-adrenal response to stressors. For instance, while it has been shown that the corticosterone response in rats diminishes from peak values following prolonged stressor exposure, the response in mice showed no signs of declining after 5 hr of immobilization stress (Armario & Castellanos, 1984). Moreover, the data of

the present investigation suggest that in mice the hyporesponsive adrenocorticotrophic response is evident 3 weeks postnatally, whereas other investigators have shown an adult-like corticoid response approximately two weeks after birth in rats (Sapolsky & Meaney, 1985). In addition, data collected in this laboratory (Shanks et al., 1989, submitted) suggest that diurnal variations of corticoid activity that are common in rats, are not evident in mice. It seems likely, then, that some manipulations which induce differential effects of stressor-provoked alterations in plasma corticosterone in rats may not have comparable effects in mice. Whether the effectiveness of the negative-feedback of plasma corticosterone on the hypothalamic-pituitary axis differs in both species warrants further consideration. In light of the fact that pituitary sensitivity to CRF, glucocorticoid receptor number, and corticosterone clearance rates may vary between lines of rats (Walker, Rivest, Meaney, & Aubert, 1989, in press), it would not be surprising to find still greater differences between species.

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APPENDIX A

Assay for Plasma Corticosterone Concentrations

Frozen plasma samples were allowed to thaw and were then added to dichloromethane (methyl chloride; Baker analyzed for spectrophotometry). After centrifugation at 3000 r.p.m. and the removal of the aqueous layer, the methyl chloride extract was washed with 0.1 N solution of sodium hydroxide for the removal of neutral lipids (e.g., sterols) and phenolic compounds (e.g., estrogens). Next, the methyl chloride extract was combined with an ethanol-sulphuric acid reagent, centrifuged and allowed to stand for 45 min to allow plasma corticoids to react with the reagent. Corticosterone standards were evaporated under nitrogen and water added. The extraction for the standards was identical to the procedure for plasma.

For the determination of corticosterone concentrations, a 1 ml aliquot of each sulphuric acid extract was read in a spectrofluorometer with activation and emission wavelengths set at 470 nm and 515 nm, respectively. Plasma corticosterone concentrations were determined by the ratio of the sample fluorescence units to standard fluorescence units, and multiplying by a correction factor of 500 to give the final concentration in terms of ug corticosterone /100 ml plasma.

APPENDIX B

Microdissection of the Mouse Brain

Brain regions were sectioned on a dissecting block fashioned from 0.50 mm thick aluminum templates and separated by 0.25 mm thick brass plates. The 0.50 mm slots created by the brass plates in the block served as guides for single-edged razor blades. The brain sections were teased from the blades onto glass slides and frozen on dry ice. A petri dish filled with powdered dry ice served as a cold stage for sectioning and was situated under a stereomicroscope (American Optical), which was illuminated by a fiber optic source (American Optical Illuminator; Model 11-80). Under low magnification, the frontal cortex, nucleus accumbens, and caudate nucleus (in Experiment 12 only) were punched with hollow microdissection needles, which ranged in diameter from 22 gauge to 16 gauge. In addition, the entire hypothalamus was dissected as described by Anisman and Sklar (1981). The punched sections were placed in round-welled titer trays and frozen at -70°C until the determination of catecholamine concentrations and their metabolites (i.e., NE, MHPG, DA, and DOPAC) using HPLC with electrochemical detection (see Appendix C).

APPENDIX C

High Performance Liquid Chromatography with Electrochemical Detection for Catecholamines and Metabolites

The concentrations of tissue catecholamines and metabolites (i.e., NE, MHPG, DA, and DOPAC) were determined following extraction of the compounds into monochloroacetic acid, separation by high-performance liquid chromatography (HPLC), and detection using a coulometric electrochemical detector system. This detector system produces an initial oxidation of the sample passing through the separating column, followed by reduction of the compounds of interest. Use of this method (i.e., Lowery method) provided considerable sensitivity and selectivity, since many of the compounds which interfere with the detection of catecholamines and their metabolites (e.g., ascorbic acid) are unable to undergo the reduction process (see Mefford, 1981).

Frozen tissue was homogenized in 0.3 M monochloroacetic acid containing 0.1 mM disodium EDTA as an antioxidant. Denatured protein was removed by centrifugation, following which the samples were filtered through 0.45 μ m regenerated cellulose filters using centrifugal microfiltration units (BAS, W. LaFayette, Ind.) to remove particulate matter.

An aliquot of each sample was injected onto a CSC-S ODS2 separating column (15 x 0.45 μ m; 5 μ m pore size; Chromatography Sciences Co., Montreal, Que.), which was protected by a Brownlee RP-18 Spheri-5 guard column (3 x 0.45 cm; Brownlee Labs., Santa Clara, Ca), in an HPLC system (Waters 6000A pump; Waters Associates, Bedford, Mass.) equipped with an electrochemical detector (Coulochem 5100A detector; ESA, Bedford, Mass.). The detector was connected to three coulometric cells in series. The mobile phase used for the separation of catecholamines and their

metabolites was comprised of 50 mM dihydrogen sodium phosphate with 0.5 mM disodium EDTA and 0.3 mM sodium octyl sulphonate (adjusted to a pH 3.55 with glacial acetic acid) to which 12 ml/l acetonitrile was added. The solution was filtered and degassed prior to use by passing it under vacuum through a cellulose acetate filter (0.22 μ m pore size). The mobile phase passed through a Waters WISP 710B autoinjector which controlled the injection of samples onto the separating column. The flow of mobile phase through the system was maintained at a rate of 1.8 ml/min.

The guard cell on the electrochemical detection system was maintained at a potential of +0.5 V versus a Ag/AgCl reference electrode. This cell was used to oxidise all components in the column effluent. The first analytic cell was maintained at -0.01 V to screen out potentially interfering compounds, whereas the second analytical (recording) electrode was held at -0.45 V. The output from the detector was plotted and measured using a Hewlett-Packard HP-3390A plotting integrator (Hewlett-Packard, Palo Alto, Ca.). Identification and quantification of the compounds of interest in the samples was achieved by comparing the retention times and peak areas generated to those resulting from injection of known quantities of pure substances.

APPENDIX D

The Role of Olfactory Cues in the Plasma Corticosterone Response

Several investigators have reported that reexposure to cues associated with the initial stressor effectively provoked increased central NE (Anisman & Sklar, 1979; Cassens et al., 1980; Tsuda et al., 1986) and DA utilization (Claustre et al., 1986; Herman et al., 1982). Likewise, reexposure to cues associated with the initial stressor or subsequent exposure to a novel environment has been shown to influence plasma corticosterone concentrations (Bunnell et al., 1988; Levine et al., 1973; Murison & Isaksen, 1982).

In the present investigation (see Experiments 4 and 5), reexposure to apparatus cues provoked a moderate rise in plasma corticoid concentrations. However, this was the case regardless of the initial treatment mice received. In light of studies demonstrating the influence of olfactory cues in determining the behavioral disturbance associated with inescapable shock (see Minor & LoLordo, 1984), it was considered that olfactory stimuli associated with footshock might have enhanced corticosterone secretion upon reexposure to the shock apparatus. Indeed, in most of the studies of the present investigation, shocked animals were reexposed to an apparatus which was identical to that in which shock was initially delivered, but the odor associated with the initial treatment was absent. Accordingly, an additional study was undertaken to assess the contribution of odor cues to the corticoid response.

A total of 18 naive, CD-1 male mice served as subjects. The subject and apparatus particulars were the same as those described in Experiment 1. Mice ($n = 12$) received 360 inescapable shocks according to the procedure of Experiment 1. Immediately following shock treatment, mice were housed individually and provided with food and water. Half of the

shock boxes were then cleaned with water and alcohol to remove residual odors, while the remaining half were not. Twenty-four hr afterward, mice were reexposed to the shock boxes for 6 min, but no shock was delivered. Half the mice were returned to the shock boxes that were cleaned (i.e., "odorless"), while the other half were placed in the uncleared boxes (presumably odors associated with the initial shock session were present in these boxes). A separate group of mice ($n = 6/\text{group}$) was housed individually at the outset of the study and left undisturbed. Mice were sacrificed by decapitation 15 min after reexposure to the shock apparatus. Trunk blood was collected, centrifuged, and the plasma samples were stored at -70°C until plasma corticosterone concentrations were assessed fluorometrically.

The mean ($\pm$ S.E.M.) plasma corticosterone concentrations of mice exposed to odor cues, to shock boxes in which odor cues were absent, and to no treatment whatsoever were 13.14 (± 1.66), 10.74 (± 1.40), and 5.73 (± 0.72), respectively. Analysis of variance of mean plasma corticosterone concentrations yielded a significant treatment effect, $F(2, 11) = 11.01$, $p < .001$. Newman-Keuls multiple comparisons ($\alpha = .05$) revealed that stressed mice exhibited higher levels of plasma corticosterone than nonstressed mice. However, inescapably shocked mice that were reexposed to the shock boxes in which footshock was originally delivered exhibited elevations of plasma corticosterone which were comparable to those of shocked animals which were reexposed to an "odorless" apparatus. Thus, elevations in plasma corticosterone were not related to odor cues previously associated with shock delivery, thus suggesting that odor cues were not responsible for the absence of an enhanced corticoid response upon reexposure to the stressor or cues associated with the stressor.

APPENDIX E

Effects of Maternal Deprivation and Placement in a Novel Environment
on Plasma Corticosterone Concentrations in Juvenile Mice

As indicated earlier, it has been shown that the application of a stressor during the hyporesponsive period may be ineffective in provoking marked elevations of plasma corticosterone (Sapolsky & Meaney, 1986). Indeed, in the present investigation modest (but significant) elevations of plasma corticosterone occurred in 21-day-old mice exposed to acute and repeated exposure to the forced-swim procedure (see Experiment 9a). It was of interest to determine whether the modest elevations of plasma corticosterone would also occur under conditions of relatively mild stressor application, such as weaning and placement in new home cages. Accordingly, an experiment was conducted to assess the impact of maternal deprivation (weaning) and placement in a novel environment on plasma corticosterone secretion in juvenile mice.

A total of 32 naive CD-1 mice served as subjects. The subject particulars were the same as those described in Experiment 9a. Mice were assigned to one of four groups. One group of mice ($n = 8/\text{group}$) was weaned and sexed on the 19th postnatal day and transferred to new home cages with their litter mates. A second group of mice was also weaned and sexed, however, these animals were replaced in home cages in which odors of their siblings and maternal parent were present. A third group of mice was weaned, but not sexed, and returned to their home cages. The remaining mice were left undisturbed and were permitted to remain with their maternal parent. On the 21st day, mice in each group were sacrificed by decapitation. Trunk blood was collected, centrifuged, and the plasma samples stored at -70°C until assayed for plasma corticosterone.

Analysis of variance of plasma corticosterone values yielded a significant effect of the Treatment condition, $F(3, 26) = 3.57$, $p < .05$. Newman-Keuls multiple comparisons ($\alpha = .05$) indicated that plasma corticosterone levels were significantly elevated in mice that had been weaned, sexed at 19 days of age, and maintained in a new cage relative to mice in the other conditions (see Figure 18). In mice that were similarly

Insert Figure 18 about here

treated, but were not placed in a new cage, plasma corticosterone levels were comparable to mice that were weaned (but not sexed), as well as to mice that were left undisturbed. Thus, it appears that the absence of odors of siblings and maternal parent provoked elevations of plasma corticosterone in juvenile mice. It is likely, then, that the corticoid response during the hyporesponsive period may be dependent on the backdrop upon which a stressor is imposed. Moreover, it appears that the enhanced corticoid response in juvenile mice may be stressor specific (Prince & Zalcman, 1988, unpublished data).

Figure 18

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in juvenile mice as a function of weaning, sexing, and placement in a new home cage (Appendix E).

APPENDIX F

Analysis of Variance Tables

ANOVA Table 1 (Expt. 1)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice exposed to either 360 escapable shocks of 2 sec duration, yoked inescapable shock, or no shock at various intervals after the last shock received.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Shock Treatment(S)	2	2144.21	1072.12	39.73***	p<.001
Time after Treatment(T)	4	3891.89	972.97	36.06***	p<.001
S x T	8	1404.81	175.60	6.51***	p<.001
Within error	68	1834.86	26.98		

*p<.05

**p<.01

***p<.001

ANOVA Table 2 (Expt. 2)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice exposed to either 60 escapable shocks of 2 sec duration, yoked inescapable shock, or no shock at various intervals after the last shock received.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Shock(S)	2	469.42	234.71	16.76***	p<.001
Time after Treatment(T)	4	1714.81	428.70	30.62***	p<.001
S x T	8	233.98	29.25	2.09*	p<.05
Within error	85	1190.07	14.00		

ANOVA Table 3 (Expt. 3)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice 15 minutes after exposure to either 1, 3, or 5 days of escapable (60 shocks of 2 sec duration), yoked inescapable, or no shock treatment.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Shock(S)	2	2297.42	139.71	24.89***	p<.001
Days of Treatment	2	29.99	15.00	2.67	p=.084
S x D	4	10.32	2.58	0.46	p=.765
Within error	34	190.85	5.61		

ANOVA Table 4 (Expt. 4)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice reexposed to either 30 shocks of 2 sec duration or no shock. Mice were initially exposed to either escapable (360 shocks of 2 sec duration), yoked inescapable shock, or no shock treatment 24 hr earlier.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Shock (S)	2	2.81	1.41	0.27	p=.765
Reexposure Treatment (R)	1	118.24	118.24	22.60***	p<.001
Time after Treatment (T)	4	765.94	191.48	36.60***	p<.001
S x R	2	5.65	2.82	0.54	p=.584
S x T	8	30.89	3.86	0.74	p=.658
R x T	4	78.50	19.63	3.75**	p<.01
S x R x T	8	68.65	8.58	1.64	p=.121
Within error	114	596.45	5.23		

ANOVA Table 5 (Expt. 5)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice reexposed to either 30 shocks of 2 sec duration, the shock apparatus, or no treatment. Mice were initially exposed to 6 days of either escapable (60 shocks of 2 sec duration), yoked inescapable, or no shock 14 days earlier. Plasma corticosterone levels were determined 15 minutes after the last shock received.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Shock (S)	2	0.50	0.25	0.13	p=.880
Reexposure (R)	2	420.07	210.04	107.60***	p<.001
S x R	4	12.76	3.19	1.63	p=.188
Within error	35	68.32	1.95		

ANOVA Table 6 (Expt. 6)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in young adult mice, 51-55 days old, following varying amounts of water immersion.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Trials	3	2041.51	680.50	23.21***	p<.001
Within error	23	674.41	29.32		

ANOVA Table 7 (Expt. 7)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in young adult mice at various time intervals following exposure to 4 swim trials of 2 min duration.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	850.65	850.65	59.32***	p<.001
Time after Treatment (T)	4	587.36	146.84	10.24***	p<.001
S x T	4	538.57	134.64	9.39***	p<.001
Within error	66	946.43	14.34		

ANOVA Table 8a (Expt. 8a)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in young adult mice following repeated water immersion.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days	4	2104.61	526.15	9.66***	p<.001
Within error	25	1362.16	54.49		

ANOVA Table 8b (Expt. 8b)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice exposed to a single session of forced swim and subsequently reexposed either to 3 min or 1 min of water immersion, or to no treatment whatsoever 30 days later.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	7.57	7.57	0.84	p=.368
Reexposure (R)	2	161.23	80.61	8.91***	p<.001
S x R	2	0.95	0.48	0.05	p=.949
Within error	29	262.40	9.05		

ANOVA Table 8c (Expt. 8c)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice exposed to repeated water immersion (4 days) and subsequently reexposed either to 3 min or 1 min of water immersion, or to no treatment whatsoever 30 days later.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	1.82	1.82	0.22	p=.646
Reexposure (R)	2	223.91	111.96	13.29***	p<.001
S x R	2	2.46	1.23	0.15	p=.865
Within error	35	295.95	8.43		

ANOVA Table 8d (Expt. 8d)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice exposed to repeated water immersion (4 days) and subsequently reexposed either to 3 min of water immersion, 60 shock of 2 sec duration (150 uA), or no treatment whatsoever 30 days later.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	9.17	9.17	1.39	p=.245
Reexposure (R)	2	598.81	299.40	45.24***	p<.001
S x R	2	2.88	1.44	0.22	p=.805
Within error	51	337.50	6.62		

ANOVA Table 9a (Expt. 9a)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in juvenile mice following repeated water immersion.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days	4	142.25	35.56	8.74***	p<.001
Within error	45	183.22	4.07		

ANOVA Table 9b (Expt. 9b)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in juvenile mice exposed to a single session of forced swim and subsequently reexposed to amounts of water immersion 30 days later.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	48.29	48.29	2.81	p=.098
Reexposure (R)	2	306.10	153.05	8.92***	p<.001
S x R	2	92.30	46.15	2.69	p=.080
Within error	35	600.37	17.15		

ANOVA Table 9c (Expt. 9c)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in juvenile mice previously exposed to repeated swim immersion (4 days) and subsequently reexposed to varying amounts of water immersion 30 days later.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>E</u>	<u>Sig</u>
Swim (S)	1	13.74	13.74	3.30	p=.073
Reexposure (R)	2	300.03	150.02	36.07***	p<.001
S x R	2	3.22	1.61	0.38	p=.680
Within error	39	162.21	4.16		

ANOVA Table 9d (Expt. 9d)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in juvenile mice exposed to repeated water immersion (4 days) and subsequently reexposed either to 3 min of water immersion, 60 shock of 2 sec duration (150 uA), or no treatment whatsoever 30 days later.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	23.73	23.73	5.25*	p<.05
Reexposure (R)	2	1013.05	506.52	112.10***	p<.001
S x R	2	0.74	0.38	0.08	p=.930
Within error	40	180.74	4.52		

ANOVA Table 10a (Expt. 10)

Analysis of variance of mean latencies to reach the choice point after 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	171.14	85.57	1.75	p=.187
Swim Condition (S)	1	860.74	860.74	17.62***	p<.001
D x S	2	242.43	242.43	4.96**	p<.01
Within error	82	4006.42	48.86		

ANOVA Table 10b (Expt. 10)

Analysis of variance of mean latencies to reach the exit ramp after 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	3613.87	1806.94	11.08***	p<.001
Swim Condition (S)	1	6491.40	6491.40	39.80***	p<.001
D x S	2	959.13	479.57	2.94	p=.059
Within error	82	13375.04	163.11		

ANOVA Table 10c (Expt. 10)

Analysis of variance of the total number of discrimination errors after 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	166.20	83.10	3.57*	p<.05
Swim Condition (S)	1	1695.13	1695.13	72.78***	p<.001
D x S	2	118.35	59.18	2.54	p=.085
Within error	82	1909.98	23.29		

ANOVA Table 10d (Expt. 10)

Analysis of variance of the number of errorless trials after 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	48.19	24.10	5.37**	p<.01
Swim Condition (S)	1	412.98	412.98	92.00***	p<.001
D x S	2	31.75	15.88	3.54*	p<.05
Within error	82	368.07	4.49		

ANOVA Table 10e (Expt. 10)

Analysis of variance of mean latencies to reach the choice point over the course of the study in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze for 10 sessions.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	338.08	338.08	1.55	p=.224
Within error	27	5902.49	218.61		
<u>Within</u>					
Days (D)	9	1017.63	113.07	3.25***	p<.001
S x D	9	298.51	33.17	0.95	p=.480
D x Within error	243	8455.59	34.80		

ANOVA Table 10f (Expt. 10)

Analysis of variance of mean latencies to reach the exit ramp over the course of the study in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze for 10 sessions.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	13414.58	13414.58	20.79***	p<.001
Within error	27	17421.12	645.22		
<u>Within</u>					
Days (D)	9	3118.75	346.53	4.38***	p<.001
S x D	9	678.26	75.36	0.95	p=.480
D x Within error	243	19224.01	79.11		

ANOVA Table 10g (Expt. 10)

Analysis of variance of the total number of discrimination errors over the course of the study in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze for 10 sessions.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	338.08	338.08	1.55	p=.224
Within error	27	5902.49	218.61		
<u>Within</u>					
Days (D)	9	192.37	21.38	1.36	p=.208
S x D	9	284.32	31.59	2.01*	p<.05
D x Within error	243	3826.51	15.75		

ANOVA Table 10h (Expt. 10)

Analysis of variance of the number of errorless trials over the course of the study in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze for 10 sessions.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	1624.02	1624.02	96.43***	p<.001
Within error	27	454.72	16.84		
<u>Within</u>					
Days (D)	9	25.26	2.81	1.00	p=.438
S x D	9	77.61	8.62	3.08**	p<.01
D x Within error	243	680.47	2.80		

ANOVA Table 10i (Expt. 10)

Analysis of variance of the mean number of escape failures over the course of the study in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze for 10 sessions.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	76.12	76.12	15.52***	p<.001
Within error	27	132.41	4.90		
<u>Within</u>					
Days (D)	9	84.38	9.38	7.09***	p<.001
S x D	9	58.15	6.46	4.89***	p<.001
D x Within error	243	321.40	1.32		

ANOVA Table 10j (Expt. 10)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice required to approach either the illuminated arm or the nonilluminated arm of the water-filled Y-maze after 1, 5, and 10 days of testing.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	55.70	27.85	1.68	p=.192
Swim Condition (S)	2	6846.86	3423.43	206.14***	p<.001
D x S	4	177.64	44.41	2.67*	p<.05
Within error	108	1793.55	16.61		

ANOVA Table 10k (Expt. 10)

Analysis of variance of percent of control values of hypothalamic norepinephrine (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	6090.31	3045.15	5.63**	p<.01
Swim Condition (S)	2	7097.98	3548.99	6.56**	p<.01
D x S	4	2600.03	650.01	1.20	p=.314
Within error	118	63794.03	540.63		

Mean differences necessary for $p < .05$ (see Table 1).

<u>Days</u>	<u>Swim Condition</u>
$r_2 = 10.02$	$r_2 = 10.01$
$r_3 = 12.02$	$r_3 = 12.01$

ANOVA Table 101 (Expt. 10)

Analysis of variance of percent of control values of hypothalamic 3-methoxy,4-hydroxyphenylethylene glycol (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	45226.30	22613.20	4.18*	p<.05
Swim Condition (S)	2	210500.00	105250.00	19.46***	p<.001
D x S	4	14458.70	3614.68	0.67	p=.615
Within error		118638263.39	5409.01		

Mean differences necessary for $p < .05$ (Table 1).

<u>Days</u>	<u>Swim Condition</u>
r ₂ = 31.68	r ₂ = 31.66
r ₃ = 38.02	r ₃ = 37.99

ANOVA Table 10m (Expt. 10)

Analysis of variance of percent of control values of hypothalamic dopamine (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	974.45	487.22	0.58	p=.560
Swim Condition (S)	2	478.54	239.27	0.29	p=.752
D x S	4	1722.47	430.62	0.52	p=.725
Within error	110	91888.30	835.35		

ANOVA Table 10n (Expt. 10)

Analysis of variance of percent of control values of hypothalamic dihydroxyphenylacetic acid (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	2630.42	1315.21	0.62	p=.541
Swim Condition (S)	2	15972.01	7986.01	3.76*	p<.05
D x S	4	1779.81	444.95	0.21	p=.933
Within error	116246654.85	2126.34			

Mean differences necessary for $p < .05$ (see Table 1).

Swim Condition

$$r_2 = 20.01$$

$$r_3 = 24.01$$

ANOVA Table 10o (Expt. 10)

Analysis of variance of percent of control values of frontal cortex norepinephrine (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	50.30	25.15	0.03	p=.972
Swim Condition (S)	2	720.57	360.28	0.41	p=.667
D x S	4	802.16	200.54	0.23	p=.923
Within error	107	94927.86	887.18		

ANOVA Table 10p (Expt. 10)

Analysis of variance of percent of control values of frontal cortex 3-methoxy, 4-hydroxyphenylethylene glycol (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	1432.86	716.43	0.33	p=.717
Swim Condition (S)	2	37314.80	18657.40	8.68***	p<.001
D x S	4	463.89	115.97	0.05	p=.995
Within error	101217168.82	2150.19			

Mean differences necessary for $p < .05$.

Swim Condition

$$r_2 = 21.47$$

$$r_3 = 25.77$$

ANOVA Table 10q (Expt. 10)

Analysis of variance of percent of control values of frontal cortex dopamine (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	2321.91	1160.95	0.71	p=.494
Swim Condition (S)	2	576.62	288.31	0.18	p=.838
D x S	4	893.71	223.45	0.14	p=.968
Within error	62	101025.67	1629.45		

ANOVA Table 10r (Expt. 10)

Analysis of variance of percent of control values of frontal cortex dihydroxyphenylacetic acid (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	9716.36	4858.18	1.66	p=.201
Swim Condition (S)	2	31177.80	15588.90	5.31**	p<.01
D x S	4	7927.85	1981.96	0.68	p=.612
Within error	53	155502.57	2934.01		

Mean differences necessary for $p < .05$ (see Table 1).

Swim Condition

$$r_2 = 33.54$$

$$r_3 = 40.24$$

ANOVA Table 10s (Expt. 10)

Analysis of variance of percent of control values of nucleus accumbens norepinephrine (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	713.11	356.55	0.25	p=.781
Swim Condition (S)	2	149.94	74.97	0.05	p=.949
D x S	4	3591.02	897.75	0.62	p=.646
Within error	89	127991.85	1438.11		

ANOVA Table 10t (Expt. 10)

Analysis of variance of percent of control values of nucleus accumbens 3-methoxy, 4-hydroxyphenylethylene glycol (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	1206.12	603.06	0.22	p=.806
Swim Condition (S)	2	2908.02	1454.01	0.52	p=.596
D x S	4	1589.27	397.32	0.14	p=.966
Within error	63	175443.22	2784.81		

ANOVA Table 10u (Expt. 10)

Analysis of variance of percent of control values of nucleus accumbens dopamine (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	2692.44	1346.22	0.34	p=.712
Swim Condition (S)	2	6672.43	3336.21	0.84	p=.433
D x S	4	26128.00	6532.01	1.65	p=.167
Within error	93	367315.15	3949.63		

ANOVA Table 10v (Expt. 10)

Analysis of variance of percent of control values of nucleus accumbens dihydroxyphenylacetic acid (ng/mg protein) following 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Days of Testing (D)	2	7457.34	3728.67	1.30	p=.278
Swim Condition (S)	2	3548.59	1774.29	0.62	p=.541
D x S	4	9988.35	2497.09	0.87	p=.486
Within error	99	284515.30	2873.89		

ANOVA Table 11a (Expt. 11)

Analysis of variance of mean latencies (sec) to reach the choice point over 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y maze. The height of the water was reduced, such that half the escape ramp was above the level of the water.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	112.61	112.61	6.23*	p<.05
Within error	20	261.54	18.08		
<u>Within</u>					
Days	9	235.56	26.17	7.64***	p<.001
D x S	9	93.58	1.04	3.03**	p<.01
D x Within error	180	616.68	3.43		

ANOVA Table 11b (Expt. 11)

Analysis of variance of mean latencies (sec) to reach the exit ramp over 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y maze.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	995.56	995.56	18.42***	p<.001
Within error	20	1081.18	54.06		
<u>Within</u>					
Days	9	1072.78	119.20	13.93***	p<.001
D x S	9	430.88	47.88	5.60***	p<.001
D x Within error	180	1539.76	8.55		

ANOVA Table 11c (Expt. 11)

Analysis of variance of the total number of discrimination errors over 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y maze.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	442.47	442.47	27.67***	p<.001
Within error	20	319.76	15.99		
<u>Within</u>					
Days	9	447.62	49.74	13.51***	p<.001
D x S	9	77.16	8.57	2.33*	p<.05
D x Within error	180	662.42	3.68		

ANOVA Table 11d (Expt. 11)

Analysis of variance of the number of errorless trials over 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y maze.

<u>Between</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition (S)	1	226.04	226.04	22.50***	p<.001
Within error	20	200.91	10.05		
<u>Within</u>					
Days	9	279.19	31.02	15.18***	p<.001
D x S	9	30.10	3.34	1.64	p=.108
D x Within error	180	367.82	2.04		

ANOVA Table 11e (Expt. 11)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice required to swim either to the illuminated arm or to the nonilluminated arm of the water-filled Y-maze for 10 days.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition	2	716.49	35.83	38.05***	p<.001
Within error	29	27.30	9.41		

Mean differences necessary for $p < .05$.

Plasma Corticosterone

$$r_2 = 2.72$$

$$r_3 = 3.28$$

ANOVA Table 11f (Expt. 11)

Analysis of variance of percent of control values (ng/mg protein) of hypothalamic norepinephrine (NE) and 3-methoxy,4-hydroxyphenylethylene glycol (MHPG) following 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

NE

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition	2	1121.59	560.79	1.14	p=.332
Within error	30	14703.73	490.12		

MHPG

Swim Condition	2	97722.75	48861.37	19.20***	p<.001
Within error	30	76364.88	2545.50		

Mean differences necessary for $p < .05$ for MHPG (see Table 2).

MHPG

$$r_2 = 43.96$$

$$r_3 = 53.09$$

ANOVA Table 11g (Expt. 11)

Analysis of variance of percent of control values (ng/mg protein) of hypothalamic dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) after 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

DA

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition	2	792.61	396.30	0.49	p=.616
Within error	28	22487.30	803.12		

DOPAC

Swim Condition	2	42050.70	21025.35	10.51***	p<.001
Within error	30	60035.14	2001.17		

Mean differences necessary for $p < .05$ for DOPAC (see Table 2).

DOPAC

$$r_2 = 38.98$$

$$r_3 = 47.07$$

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ANOVA Table 11h (Expt. 11)

Analysis of variance of percent of control values (ng/mg protein) of frontal cortex norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) after 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

NE

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition	2	327.33	163.67	0.51	p=.607
Within error	28	9030.68	322.52		

MHPG

Swim Condition	2	141.82	70.91	0.06	p=.939
Within error	27	30454.05	1127.93		

ANOVA Table 11i (Expt. 11)

Analysis of variance of percent of control values (ng/mg protein) of frontal cortex dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) after 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

DA

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition	2	1182.59	591.30	0.39	p=.680
Within error	20	30040.66	1502.03		

DOPAC

Swim Condition	2	943.71	471.85	0.19	p=.828
Within error	27	67131.00	2486.33		

ANOVA Table 11j (Expt. 11)

Analysis of variance of percent of control values (ng/mg protein) of nucleus accumbens norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) after 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

NE

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition	2	120.58	60.29	0.07	p=.932
Within error	22	18894.45	858.84		

MHPG

Swim Condition	2	5337.39	2668.69	1.31	p=.288
Within error	24	48863.33	2035.97		

ANOVA Table 11k (Expt. 11)

Analysis of variance of percent of control values (ng/mg protein) of nucleus accumbens dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) after 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

DA

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim Condition	2	310.60	155.30	0.32	p=.729
Within error	22	10660.48	484.57		

DOPAC

Swim Condition	2	1190.99	595.49	0.48	p=.622
Within error	24	29486.32	1228.60		

ANOVA Table 12a (Expt. 12)

Analysis of variance of mean latencies (sec) to reach the choice point after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of a water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Day (D)	1	45.83	45.83	5.94*	p<.05
Swim Condition (S)	1	132.34	132.34	17.15***	p<.001
D x S	1	12.51	12.51	1.62	p=.210
Within error	44	339.55	7.72		

ANOVA Table 12b (Expt. 12)

Analysis of variance of mean latencies (sec) to reach the exit ramp after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm in a water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Day (D)	1	162.81	162.81	7.62**	p<.01
Swim Condition (S)	1	703.80	703.80	32.95***	p<.001
D x S	1	16.33	16.33	0.76	p=.387
Within error	44	939.85	21.36		

ANOVA Table 12c (Expt. 12)

Analysis of variance of the total number of discrimination errors after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm in a water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Day (D)	1	88.02	88.02	15.62	p<.001
Swim Condition (S)	1	212.52	212.52	37.72***	p<.001
D x S	1	6.02	6.02	1.07	p=.307
Within error	44	247.92	5.64		

ANOVA Table 12d (Expt. 12)

Analysis of variance of the number of errorless trials after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm in a water-filled Y-maze.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Day (D)	1	46.02	46.02	11.94***	p<.001
Swim Condition (S)	1	99.19	99.19	25.74***	p<.001
D x S	1	1.69	1.69	0.44	p=.512
Within error	44	169.58	3.85		

ANOVA Table 12e (Expt. 12)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) after 1 and 5 days in mice required to swim to the illuminated arm or to the nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	10.18	10.18	0.22	p=.641
Day(D)	1	10.10	10.10	0.22	p=.642
Control(C)	1	8.08	8.08	0.17	p=.678
S x D	1	109.14	109.14	2.35	p=.129
S x C	1	41.09	41.09	0.88	p=.350
D x C	1	203.43	203.43	4.38*	p<.05
S x D x C	1	16.52	16.52	0.36	p=.553
Within error	85	3949.12	46.46		

ANOVA Table 12f (Expt. 12)

Analysis of variance of mean concentrations of hypothalamic norepinephrine (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	0.54	0.54	0.07	p=.798
Day(D)	1	0.02	0.02	0.00	p=.962
Control(C)	1	4.97	4.79	0.60	p=.440
S x D	1	0.72	0.72	0.09	p=.769
S x C	1	18.49	18.49	2.24	p=.138
D x C	1	0.09	0.09	0.01	p=.919
S x D x C	1	6.44	6.44	0.78	p=.380
Within error	80	659.80	8.25		

Dunnett's t Statistic (see Table 3)

Critical value for two-tailed $p < .05$ (i.e., $t_{.975}$)

One Day of Training

Five Days of Training

$t_{crit(10)} = \pm 2.97$

$t_{crit(11)} = \pm 2.92$

ANOVA Table 12g (Expt. 12)

Analysis of variance of mean concentrations of hypothalamic 3-methoxy,4-hydroxy-phenylethylene glycol (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	0.12	0.12	0.20	p=.659
Day (D)	1	0.05	0.05	0.08	p=.781
Control (C)	1	0.15	0.15	0.25	p=.620
S x D	1	0.04	0.04	0.07	p=.794
S x C	1	0.54	0.54	0.86	p=.355
D x C	1	0.01	0.01	0.02	p=.877
S x D x C	1	0.25	0.25	0.40	p=.527
Within error	80	49.60	0.62		

Dunnett's t Statistic (see Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit}(10) = \pm 2.97$

Five Days of Training

$t_{crit}(10) = \pm 2.97$

ANOVA Table 12h (Expt. 12)

Analysis of variance of mean concentrations of hypothalamic dopamine (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	0.22	0.22	0.10	p=.755
Day (D)	1	1.07	1.07	0.48	p=.492
Control (C)	1	1.03	1.03	4.59*	p<.05
S x D	1	0.71	0.71	0.31	p=.577
S x C	1	6.12	6.12	2.72	p=.103
D x C	1	4.41	4.41	1.96	p=.166
S x D x C	1	0.02	0.02	0.01	p=.929
Within error	75	168.78	2.25		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit}(10) = \pm 2.97$

Five Days of Training

$t_{crit}(10) = \pm 2.97$

ANOVA Table 12i (Expt. 12)

Analysis of variance of mean concentrations of hypothalamic dihydroxyphenylacetic acid (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	0.81	0.81	0.67	p=.414
Day (D)	1	0.01	0.01	0.01	p=.921
Control (C)	1	0.02	0.02	0.01	p=.904
S x D	1	1.81	1.81	1.50	p=.225
S x C	1	0.83	0.83	0.69	p=.410
D x C	1	0.03	0.03	0.02	p=.880
S x D x C	1	0.01	0.01	0.01	p=.984
Within error	77	92.96	1.21		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit}(10) = \pm 2.97$

Five Days of Training

$t_{crit}(10) = \pm 2.97$

ANOVA Table 12j (Expt. 12)

Analysis of variance of mean concentrations of frontal cortex norepinephrine (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	15.02	15.02	2.24	p=.139
Day (D)	1	0.43	0.43	0.06	p=.802
Control (C)	1	0.72	0.72	0.11	p=.745
S x D	1	0.81	0.81	0.01	p=.913
S x C	1	0.12	0.12	0.02	p=.895
D x C	1	33.93	33.03	5.05	p<.05
S x D x C	1	0.34	0.34	0.01	p=.944
Within error	80	536.97	6.71		

Mean differences necessary for $p < .05$ (see Table 3)

Between Days and Controllability

$$r_2 = 1.21$$

Dunnett's t Statistic

Critical value for two-tailed $p < .05$.

One Day of Training

Five Days of Training

$$t_{crit(11)} = \pm 2.92$$

$$t_{crit(10)} = \pm 2.97$$

ANOVA Table 12k (Expt. 12)

Analysis of variance of mean concentrations of frontal cortex 3-methoxy, 4-hydroxy-phenylethylene glycol (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	0.87	0.87	0.38	p=.540
Day (D)	1	0.13	0.13	0.01	p=.940
Control (C)	1	1.88	1.88	0.82	p=.367
S x D	1	0.08	0.08	0.03	p=.855
S x C	1	2.84	2.84	1.24	p=.269
D x C	1	0.09	0.09	0.04	p=.846
S x D x C	1	1.18	1.18	0.52	p=.475
Within error	74	169.43	2.29		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit}(10) = \pm 2.97$

Five Days of Training

$t_{crit}(10) = \pm 2.97$

ANOVA Table 121 (Expt. 12)

Analysis of variance of mean concentrations of frontal cortex dopamine (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	7.65	7.65	5.96*	p<.05
Day (D)	1	1.27	1.27	0.99	p=.324
Control (C)	1	1.50	1.50	1.17	p=.283
S x D	1	0.39	0.39	0.31	p=.581
S x C	1	3.30	3.30	2.57	p=.113
D x C	1	0.10	0.10	0.08	p=.778
S x D x C	1	0.11	0.11	0.09	p=.769
Within error	73	93.66	1.28		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit}(10) = \pm 2.97$

Five Days of Training

$t_{crit}(9) = \pm 3.04$

ANOVA Table 12m (Expt. 12)

Analysis of variance of mean concentrations of frontal cortex dihydroxyphenylacetic acid (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	0.67	0.67	0.48	p=.491
Day (D)	1	1.70	1.70	1.21	p=.274
Control (C)	1	3.21	3.21	2.29	p=.135
S x D	1	0.77	0.77	0.55	p=.462
S x C	1	0.44	0.44	0.31	p=.579
D x C	1	1.36	1.36	0.97	p=.329
S x D x C	1	0.61	0.61	0.44	p=.511
Within error	71	99.61	1.40		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit(9)} = \pm 3.04$

Five Days of Training

$t_{crit(9)} = \pm 3.04$

ANOVA Table 12n (Expt. 12)

Analysis of variance of mean concentrations of nucleus accumbens norepinephrine (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	40.37	40.37	1.25	p=.267
Day (D)	1	116.76	116.76	3.62	p=.062
Control (C)	1	7.42	7.42	0.23	p=.633
S x D	1	52.20	52.20	1.62	p=.208
S x C	1	90.98	90.98	2.82	p=.098
D x C	1	51.68	51.68	1.60	p=.210
S x D x C	1	10.42	10.42	0.32	p=.572
Within error	65	2096.60	32.26		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit}(9) = \pm 3.04$

Five Days of Training

$t_{crit}(9) = \pm 3.04$

ANOVA Table 12o (Expt. 12)

Analysis of variance of mean concentrations of nucleus accumbens 3-methoxy, 4-hydroxyphenylethylene glycol (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	2.01	2.01	1.62	p=.209
Day (D)	1	0.02	0.02	0.02	p=.902
Control (C)	1	0.26	0.26	0.21	p=.652
S x D	1	0.13	0.13	0.10	p=.752
S x C	1	1.88	1.88	1.51	p=.225
D x C	1	0.07	0.07	0.06	p=.808
S x D x C	1	0.17	0.17	0.14	p=.711
Within error	53	65.87	1.24		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit(7)} = \pm 3.24$

Five Days of Training

$t_{crit(7)} = \pm 3.24$

ANOVA Table 12p (Expt. 12)

Analysis of variance of mean concentrations of nucleus accumbens dopamine (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	1319.34	1319.34	1.28	p=.262
Day (D)	1	1526.40	1526.40	1.48	p=.228
Control (C)	1	1676.24	1676.24	1.63	p=.207
S x D	1	4.23	4.23	0.01	p=.949
S x C	1	2064.03	2064.03	2.01	p=.162
D x C	1	1151.83	1151.83	1.12	p=.294
S x D x C	1	241.47	241.47	0.23	p=.630
Within error	62	63797.51	1028.99		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit(7)} = \pm 3.24$

Five Days of Training

$t_{crit(8)} = \pm 3.13$

ANOVA Table 12q (Expt. 12)

Analysis of variance of mean concentrations of nucleus accumbens dihydroxy-phenylacetic acid (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	154.34	154.34	2.86	p=.096
Day (D)	1	0.22	0.22	0.01	p=.949
Control (C)	1	9.64	9.64	0.18	p=.674
S x D	1	35.16	35.16	0.65	p=.423
S x C	1	101.05	101.05	1.87	p=.176
D x C	1	230.10	230.10	4.27*	p<.05
S x D x C	1	0.03	0.03	0.01	p=.982
Within error	61	3289.27	53.92		

Mean differences necessary for $p < .05$ (see Table 3).

Between Days and Controllability

$$r_2 = 5.04$$

Dunnett's t Statistic

Critical value for two-tailed $p < .05$.

One Day of Training

$$t_{crit(9)} = \pm 3.04$$

Five Days of Training

$$t_{crit(8)} = \pm 3.13$$

ANOVA Table 12r (Expt. 12)

Analysis of variance of mean concentrations of caudate nucleus norepinephrine (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	25.48	25.48	10.13**	p<.01
Day (D)	1	0.22	0.22	0.09	p=.766
Control (C)	1	1.07	1.07	0.43	p=.516
S x D	1	0.09	0.09	0.04	p=.849
S x C	1	3.38	3.38	1.34	p=.251
D x C	1	3.90	3.90	1.55	p=.217
S x D x C	1	0.33	0.33	0.13	p=.718
Within error	66	165.99	2.52		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit}(9) = \pm 3.04$

Five Days of Training

$t_{crit}(8) = \pm 3.13$

ANOVA Table 12s (Expt. 12)

Analysis of variance of mean concentrations of caudate nucleus 3-methoxy, 4-hydroxy-phenylethylene glycol (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	0.03	0.03	0.06	p=.806
Day (D)	1	0.12	0.12	0.23	p=.632
Control (C)	1	1.48	1.48	3.00	p=.088
S x D	1	0.60	0.60	1.20	p=.277
S x C	1	0.24	0.24	0.48	p=.491
D x C	1	1.46	1.46	2.95	p=.091
S x D x C	1	0.13	0.13	0.27	p=.606
Within error	67	33.25	0.49		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit}(9) = \pm 3.04$

Five Days of Training

$t_{crit}(9) = \pm 3.04$

ANOVA Table 12t (Expt. 12)

Analysis of variance of mean concentrations of caudate nucleus dopamine (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	8962.84	18962.84	3.91	p=.052
Day (D)	1	207.56	207.56	0.09	p=.764
Control (C)	1	8118.82	8118.82	3.54	p=.064
S x D	1	4.38	4.38	0.01	p=.965
S x C	1	0.20	0.20	0.01	p=.993
D x C	1	451.76	451.76	0.20	p=.659
S x D x C	1	1570.65	1570.65	0.69	p=.411
Within error	72	165085.42	2292.85		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

Five Days of Training

$t_{crit}(10) = \pm 2.97$

$t_{crit}(10) = \pm 2.97$

ANOVA Table 12u (Expt. 12)

Analysis of variance of mean concentrations of nucleus accumbens dihydroxy-phenylacetic acid (ng/mg protein) after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of the water-filled Y-maze and in their yoked partners.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Swim (S)	1	3.87	3.87	0.10	p=.755
Day (D)	1	50.62	50.62	1.29	p=.261
Control (C)	1	43.37	43.37	1.10	p=.298
S x D	1	85.49	85.49	2.17	p=.145
S x C	1	9.41	9.41	0.24	p=.627
D x C	1	148.94	148.94	3.78*	p=.056
S x D x C	1	43.87	43.87	1.11	p=.295
Within error	67	2638.13	39.38		

Dunnett's t Statistic (Table 3)

Critical value for two-tailed $p < .05$.

One Day of Training

$t_{crit(9)} = \pm 3.04$

Five Days of Training

$t_{crit(9)} = \pm 3.04$

ANOVA Table 13 (Appendix D)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in juvenile mice as a function weaning, sexing, and placement in a new home cage.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Treatment	3	237.32	79.11	3.57*	p<.05
Within error	26	576.51	22.17		

ANOVA Table 14 (Appendix E)

Analysis of variance of plasma corticosterone concentrations (ug/100 ml) in mice reexposed either to shock boxes in which odor cues were absent or to shock boxes which contained odor cues associated with the initial shock delivery. Mice were initially exposed to 360 inescapable shocks 24 hr earlier. A separate group of mice was not exposed to any shock whatsoever.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>Sig</u>
Treatment	2	143.68	71.84	11.01***	p<.001
Within error	117	1.76	6.52		

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APPENDIX G

Tables

Table 1 (Expt. 1)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice exposed to either 360 escapable shocks of 2 sec duration, yoked inescapable shocks, or no shock at various intervals after the last shock received.

<u>Shock Treatment</u>	<u>Time after Shock Treatment (min)</u>				
	<u>0</u>	<u>15</u>	<u>30</u>	<u>60</u>	<u>180</u>
No Shock	12.36 (2.14)	5.62 (0.67)	5.85 (0.44)	5.17 (1.67)	8.70 (1.30)
Escapable	30.47 (3.96)	25.97 (2.42)	15.92 (1.87)	13.47 (1.81)	5.70 (1.41)
Yoked Inescapable	34.96 (3.49)	24.80 (2.49)	17.56 (2.64)	11.46 (1.30)	4.97 (0.85)

Mean differences necessary for $p < .05$.

Between Shock Treatment and Time Interval

$$r_2 = 6.27$$

$$r_3 = 7.74$$

$$r_4 = 8.29$$

$$r_5 = 8.82$$

Table 2 (Expt. 2)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice exposed to either 60 escapable shocks of 2 sec duration, yoked inescapable shocks, or no shock at various intervals after the last shock received.

<u>Shock Treatment</u>	<u>Time after Shock Treatment (min)</u>				
	<u>0</u>	<u>15</u>	<u>30</u>	<u>60</u>	<u>180</u>
No Shock	11.87 (1.66)	7.89 (1.87)	7.72 (0.94)	3.28 (0.56)	2.78 (0.54)
Escapable	14.14 (1.61)	15.23 (1.85)	14.96 (1.98)	7.47 (2.05)	3.80 (0.80)
Yoked Inescapable	12.78 (1.30)	16.55 (1.48)	16.71 (1.27)	7.41 (1.22)	3.83 (1.03)

Mean differences necessary for $p < .05$.

Between Shock Treatment and Time Interval

$$r_2 = 4.12$$

$$r_3 = 4.94$$

$$r_4 = 5.37$$

$$r_5 = 5.75$$

Table 3 (Expt. 3)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice 15 minutes after exposure to either 1, 3, or 5 days of escapable (60 shocks of 2 sec duration), yoked inescapable, or no shock treatment. Mice not exposed to any treatment whatsoever yielded a mean ($\pm$ S.E.M.) plasma corticosterone value of 3.39 (0.43)

<u>Shock Treatment</u>	<u>Days of Training</u>		
	<u>one</u>	<u>three</u>	<u>five</u>
No Shock	5.60 (0.60)	5.43 (0.63)	4.69 (0.56)
Escapable	1.11 (0.57)	11.95 (1.50)	10.03 (1.04)
Yoked Inescapable	11.78 (1.34)	11.37 (1.54)	8.53 (0.96)

Mean differences necessary for $p < .05$.

Shock Treatment

$$r_2 = 3.13$$

$$r_3 = 3.77$$

Table 4 (Expt. 4)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice reexposed to either 30 shocks of 2 sec duration or no shock. Mice were initially exposed to either escapable (360 shocks of 2 sec duration), yoked inescapable, or no shock treatment 24 hr earlier. Mice left undisturbed in their home cages yielded a mean ($\pm$ S.E.M.) plasma corticosterone value of 1.66 (0.23).

<u>Reexposure Treatment</u>	<u>Time after Shock Treatment (min)</u>				
<u>No Shock</u>	<u>0</u>	<u>15</u>	<u>30</u>	<u>60</u>	<u>180</u>
No Shock	6.19 (0.37)	6.26 (1.18)	4.04 (0.61)	3.76 (0.47)	2.84 (0.83)
Escapable	6.42 (1.07)	6.69 (1.11)	6.15 (1.36)	2.59 (0.48)	2.62 (0.85)
Yoked Inescapable	7.83 (0.71)	6.86 (0.71)	6.66 (1.35)	3.32 (0.67)	1.58 (0.37)

30 x 2

No Shock	6.59 (0.71)	10.40 (1.13)	9.97 (1.23)	3.63 (0.48)	2.74 (0.46)
Escapable	8.17 (1.11)	9.73 (1.09)	10.38 (1.93)	5.50 (0.78)	1.46 (0.24)
Yoked Inescapable	7.76 (1.08)	11.18 (1.98)	6.20 (2.49)	4.77 (0.83)	2.62 (0.61)

Mean differences necessary for $p < .05$ for the simple main effects of first-order interactions.

Between Shock Reexposure and Time Interval

$$r_2 = 1.69$$

$$r_3 = 2.03$$

$$r_4 = 2.22$$

$$r_5 = 2.37$$

Table 5 (Expt. 5)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice reexposed to either 30 shocks of 2 sec duration, the shock apparatus, or no treatment. Mice were initially exposed to 6 days of either escapable (60 shocks of 2 sec duration), yoked inescapable, or no shock 14 days earlier. Plasma corticosterone levels were determined 15 minutes after the last shock received. Mice left undisturbed in their cage for the duration of the study yielded a mean ($\pm$ S.E.M.) plasma corticosterone value of 1.32 (0.67).

<u>Shock Treatment</u>	<u>Reexposure</u>		
	<u>30 x 2</u>	<u>Apparatus Only</u>	<u>No Treatment</u>
No Shock	8.37 (0.63)	3.88 (0.74)	1.17 (0.29)
Escapable	9.77 (1.10)	2.76 (0.19)	1.26 (0.36)
Yoked Inescapable	8.29 (0.73)	4.06 (0.60)	1.87 (0.37)

Mean differences necessary for $p < .05$.

Reexposure

$$r_2 = 1.05$$

$$r_3 = 1.27$$

Table 6 (Expt. 6)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in young adult mice, 51-55 days old, following varying amounts of water immersion.

<u>Number of Trials</u>			
0	1	2	4
4.76 (1.18)	10.11 (3.19)	15.29 (1.25)	28.25 (2.03)

Mean differences necessary for $p < .05$

$$r_2 = 6.10$$

$$r_3 = 7.37$$

$$r_4 = 8.15$$

Table 7 (Expt. 7)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice at various time intervals following exposure to either 4 swim trials of 2 min duration or no swim.

<u>Swim Treatment</u>	<u>Time after Water Immersion (min)</u>				
	<u>0</u>	<u>15</u>	<u>30</u>	<u>60</u>	<u>180</u>
No Swim	2.96 (0.93)	6.68 (1.73)	8.30 (1.66)	6.21 (1.28)	3.66 (0.79)
Swim	18.27 (1.31)	16.74 (0.81)	11.49 (1.77)	11.57 (2.10)	3.60 (0.97)

Mean differences necessary for $p < .05$.

Between Swim Treatment and Time Interval

$$r_2 = 3.93$$

$$r_3 = 4.73$$

$$r_4 = 5.20$$

$$r_5 = 5.53$$

Table 8a (Expt. 8a)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ng/100 ml) in young adult mice following repeated water immersion.

<u>Number of Days of Swim (4 x 2 min)</u>				
<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
5.35 (2.47)	28.87 (2.42)	26.55 (3.75)	22.38 (2.47)	24.57 (3.65)

Mean differences necessary for $p < .05$.

$$\begin{aligned} r_2 &= 8.80 \\ r_3 &= 10.64 \\ r_4 &= 11.75 \\ r_5 &= 12.57 \end{aligned}$$

Table 8b (Expt. 8b)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice exposed to a single session of forced swim and subsequently reexposed to varying amounts of water immersion 30 days later.

<u>Swim Pretreatment</u>	<u>Swim Reexposure</u>		
	<u>No Swim</u>	<u>1 min</u>	<u>3 min</u>
No Swim	2.68 (0.66)	4.10 (0.86)	8.14 (2.12)
Swim (4 x 2)	4.08 (0.50)	4.93 (0.75)	8.74 (0.94)

Mean differences necessary for $p < .05$.

Swim Reexposure

$$r_2 = 2.55$$

$$r_3 = 3.08$$

Table 8c (Expt. 8c)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice previously exposed to repeated forced swim (4 days) and subsequently reexposed to varying amounts of water immersion 30 days later.

<u>Swim Pretreatment</u>	<u>Swim Reexposure</u>		
	<u>No Swim</u>	<u>1 min</u>	<u>3 min</u>
No Swim	2.48 (0.54)	3.11 (0.26)	7.21 (1.11)
Chronic Swim (4 x 2)	2.32 (1.19)	3.47 (0.53)	8.26 (2.03)

Mean differences necessary for $p < .05$.

Swim Reexposure

$$r_2 = 2.26$$

$$r_3 = 2.72$$

Table 8d (Expt. 8d)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice previously exposed to repeated water immersion (4 days) and subsequently reexposed 30 days later to either 3 min of water immersion, 60 shocks of 2 sec duration (150 uA), or no treatment whatsoever.

<u>Reexposure</u>			
<u>Swim Pretreatment</u>	<u>No Treatment</u>	<u>Swim</u>	<u>Shock</u>
No Swim	2.73 (0.35)	7.23 (0.86)	10.11 (1.04)
Chronic Swim (4 x 2)	2.90 (0.31)	8.34 (1.02)	11.24 (1.06)

Mean differences necessary for $p < .05$.

Reexposure

$$r_2 = 1.68$$

$$r_3 = 2.02$$

Table 9a (Expt. 9a)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in juvenile mice following repeated water immersion.

<u>Number of Days of Swim (4 x 2 min)</u>				
<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
4.28 (0.53)	8.55 (0.55)	9.07 (0.68)	7.41 (0.60)	8.02 (0.79)

Mean differences necessary for $p < .05$.

$$r_2 = 1.83$$

$$r_3 = 2.20$$

$$r_4 = 2.42$$

$$r_5 = 2.58$$

Table 9b (Expt. 9b)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in juvenile mice exposed to a single session of forced swim and subsequently reexposed to varying amounts of water immersion 30 days later.

<u>Swim Pretreatment</u>	<u>Swim Reexposure</u>		
	<u>No Swim</u>	<u>1 min</u>	<u>3 min</u>
No Swim	4.80 (0.98)	8.42 (2.53)	15.03 (1.47)
Swim (4 x 2)	6.06 (1.28)	6.69 (0.97)	8.97 (1.69)

Mean differences necessary for $p < .05$.

Swim Reexposure

$$r_2 = 3.22$$

$$r_3 = 3.88$$

Table 9c (Expt. 9c)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in juvenile mice previously exposed to repeated forced swim (4 days) and subsequently reexposed to varying amounts of water immersion 30 days later.

<u>Swim Pretreatment</u>	<u>Swim Reexposure</u>		
	<u>No Swim</u>	<u>1 min</u>	<u>3 min</u>
No Swim	4.19 (0.52)	7.19 (0.89)	11.01 (0.79)
Chronic Swim (4 x 2)	3.13 (0.48)	6.70 (0.94)	9.19 (0.53)

Mean differences necessary for $p < .05$.

Swim Reexposure

$$r_2 = 1.52$$

$$r_3 = 1.82$$

Table 9d (Expt. 9d)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in juvenile mice previously exposed to repeated water immersion (4 days) and subsequently reexposed 30 days later to either 3 min of water immersion, 60 shocks of 2 sec duration (150 uA), or no treatment whatsoever.

<u>Swim Pretreatment</u>	<u>Reexposure</u>		
	<u>No Treatment</u>	<u>Swim</u>	<u>Shock</u>
No Swim	2.81 (0.62)	6.96 (0.85)	14.48 (0.76)
Chronic Swim (4 x 2)	1.59 (0.17)	5.67 (1.13)	12.68 (0.82)

Mean differences necessary for $p < .05$.

Reexposure

$$r_2 = 1.55$$

$$r_3 = 1.87$$

Table 10a (Expt. 10)

Mean ($\pm$ S.E.M.) latencies to reach the choice point after 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm in a water-filled Y-maze.

	<u>Days of Training</u>		
	<u>1</u>	<u>5</u>	<u>10</u>
Illuminated Arm	7.35 (0.54)	3.64 (0.14)	9.31 (1.34)
Nonilluminated Arm	16.44 (1.26)	4.84 (0.30)	7.44 (0.15)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$$r_2 = 5.17$$

$$r_3 = 6.21$$

Table 10b (Expt. 10)

Mean ($\pm$ S.E.M.) latencies to reach the exit ramp after 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

	<u>Days of Training</u>		
	<u>1</u>	<u>5</u>	<u>10</u>
Illuminated Arm	12.06 (1.11)	5.70 (0.30)	12.66 (3.90)
Nonilluminated Arm	34.80 (3.41)	13.59 (2.09)	33.59 (6.00)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$$r_2 = 9.30$$

$$r_3 = 11.08$$

Table 10c (Expt. 10)

Mean ($\pm$ S.E.M.) number of total discrimination errors after 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm of the water-filled Y-maze.

	<u>Days of Training</u>		
	<u>1</u>	<u>5</u>	<u>10</u>
Illuminated Arm	4.27 (0.61)	1.36 (0.39)	0.53 (0.17)
Nonilluminated Arm	11.87 (0.90)	8.07 (1.43)	12.57 (2.57)

Mean differences necessary for $p < .05$.

Days of Training

$$r_2 = 2.57$$

$$r_3 = 3.12$$

Table 10d (Expt. 10)

Mean ($\pm$ S.E.M.) number of errorless trials after 1, 5, and 10 days in mice required to swim either to the illuminated or nonilluminated arm in the water-filled Y-maze.

	<u>Days of Training</u>		
	<u>1</u>	<u>5</u>	<u>10</u>
Illuminated Arm	6.60 (0.46)	8.86 (0.38)	8.73 (0.53)
Nonilluminated Arm	3.53 (0.48)	4.87 (0.59)	2.79 (0.81)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$$r_2 = 1.60$$

$$r_3 = 1.93$$

Table 10e (Expt. 10)

Mean ($\pm$ S.E.M.) latencies to approach the choice point over the course of the study in mice required to approach either the illuminated arm or non-illuminated arm of a water-filled Y-maze for 10 sessions.

<u>Swim Condition</u>		
<u>Days</u>	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	16.33 (0.44)	12.52 (2.78)
2	4.29 (0.37)	8.58 (1.34)
3	4.05 (0.26)	5.63 (0.69)
4	3.74 (0.18)	6.54 (1.02)
5	3.85 (0.26)	6.42 (1.17)
6	4.76 (0.73)	7.14 (1.83)
7	5.54 (1.02)	6.79 (1.98)
8	9.14 (3.14)	10.96 (3.39)
9	8.79 (3.06)	9.38 (2.88)
10	9.29 (3.15)	7.44 (1.85)

Mean differences necessary for $p < .05$.

Days

$r_2 = 3.03$ $r_7 = 4.57$
 $r_3 = 3.63$ $r_8 = 4.70$
 $r_4 = 3.98$ $r_9 = 4.81$
 $r_5 = 4.23$ $r_{10} = 4.90$
 $r_6 = 4.42$

Table 10f (Expt. 10)

Mean ($\pm$ S.E.M.) latencies to reach the exit ramp over the course of the study in mice required to approach either the illuminated arm or nonilluminated arm of a water-filled Y-maze for 10 sessions.

<u>Days</u>	<u>Swim Condition</u>	
	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	10.91 (2.45)	26.29 (2.45)
2	7.17 (0.72)	20.27 (2.98)
3	6.90 (0.38)	17.21 (2.28)
4	7.32 (0.64)	15.59 (2.17)
5	6.39 (0.61)	21.04 (3.24)
6	7.19 (0.95)	20.04 (3.36)
7	8.07 (1.36)	20.13 (4.23)
8	11.27 (3.43)	26.72 (5.11)
9	10.69 (3.19)	24.78 (5.12)
10	11.68 (3.54)	31.93 (6.28)

Mean differences necessary for $p < .05$.

Days

$r_2 = 4.58$ $r_7 = 6.89$
 $r_3 = 5.47$ $r_8 = 7.09$
 $r_4 = 6.00$ $r_9 = 7.25$
 $r_5 = 6.38$ $r_{10} = 7.38$
 $r_6 = 6.66$

Table 10g (Expt. 10)

Mean ($\pm$ S.E.M.) number of discrimination errors over the course of the study in mice required to approach either the illuminated arm or nonilluminated arm of the water-filled Y-maze for 10 sessions.

<u>Days</u>	<u>Swim Condition</u>	
	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	4.27 (0.69)	11.86 (1.61)
2	2.13 (0.39)	8.86 (1.26)
3	1.93 (0.43)	9.36 (1.33)
4	2.60 (0.57)	7.07 (0.89)
5	1.07 (0.36)	10.00 (1.59)
6	1.33 (0.36)	10.57 (1.71)
7	1.07 (0.30)	10.29 (1.84)
8	1.28 (0.42)	10.79 (1.86)
9	0.67 (0.22)	10.93 (2.27)
10	0.53 (0.17)	12.57 (2.57)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$r_2 = 2.89$	$r_7 = 4.35$
$r_3 = 3.45$	$r_8 = 4.47$
$r_4 = 3.79$	$r_9 = 4.58$
$r_5 = 4.02$	$r_{10} = 4.66$
$r_6 = 4.2$	

Table 10h (Expt. 10)

Mean ($\pm$ S.E.M.) number of errorless trials over the course of the study in mice required to approach either the illuminated arm or nonilluminated arm of the water-filled Y-maze for 10 sessions.

<u>Days</u>	<u>Swim Condition</u>	
	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	6.87 (0.41)	4.07 (0.53)
2	8.13 (0.34)	4.43 (0.53)
3	8.53 (0.26)	4.21 (0.45)
4	8.07 (0.35)	4.64 (0.48)
5	9.00 (0.33)	3.71 (0.70)
6	8.93 (0.28)	3.64 (0.77)
7	9.00 (0.26)	3.57 (0.82)
8	8.67 (0.47)	3.14 (0.81)
9	9.07 (0.32)	3.43 (0.81)
10	8.73 (0.53)	2.79 (0.81)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$r_2 = 1.22$ $r_7 = 1.83$
 $r_3 = 1.46$ $r_8 = 1.89$
 $r_4 = 1.60$ $r_9 = 1.93$
 $r_5 = 1.70$ $r_{10} = 1.97$
 $r_6 = 1.77$

Table 10i (Expt. 10)

Mean ($\pm$ S.E.M.) number of escape failures over the course of the study in mice required to approach either the illuminated arm or nonilluminated arm of the water-filled Y maze for 10 sessions.

<u>Days</u>	<u>Swim Condition</u>	
	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	0.00 (0.00)	1.00 (0.23)
2	0.00 (0.00)	0.36 (0.20)
3	0.00 (0.00)	0.50 (0.23)
4	0.00 (0.00)	0.36 (0.25)
5	0.00 (0.00)	0.50 (0.23)
6	0.00 (0.00)	0.29 (0.16)
7	0.00 (0.00)	1.14 (0.65)
8	0.20 (0.20)	1.21 (0.55)
9	0.00 (0.00)	1.71 (0.62)
10	0.33 (0.33)	3.71 (0.97)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$r_2 = 0.84$ $r_7 = 1.25$
 $r_3 = 1.00$ $r_8 = 1.30$
 $r_4 = 1.10$ $r_9 = 1.33$
 $r_5 = 1.17$ $r_{10} = 1.35$
 $r_6 = 1.22$

Table 10j (Expt. 10)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations in mice required to approach either the illuminated arm or the nonilluminated arm of the water-filled Y-maze after 1, 5, and 10 days of testing.

<u>Swim</u> <u>Condition</u>	<u>Days of Training</u>		
	<u>1</u>	<u>5</u>	<u>10</u>
No Treatment	3.77 (0.44)	6.36 (0.83)	5.26 (0.76)
Illuminated Arm	22.08 (0.77)	17.97 (1.06)	18.98 (1.27)
Nonilluminated Arm	24.48 (1.44)	22.23 (1.65)	21.30 (1.44)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$$r_2 = 3.17$$

$$r_3 = 3.80$$

Table 11a (Expt. 11)

Mean ($\pm$ S.E.M.) latencies to approach the choice point over 10 days in mice required to approach either the illuminated arm or nonilluminated arm of a water-filled Y-maze.

<u>Days</u>	<u>Swim Condition</u>	
	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	6.33 (0.44)	12.52 (2.78)
2	4.29 (0.37)	8.58 (1.34)
3	4.05 (0.26)	5.63 (0.69)
4	3.74 (0.18)	6.54 (1.02)
5	3.85 (0.26)	6.42 (1.17)
6	4.76 (0.73)	7.14 (1.83)
7	5.54 (1.02)	6.79 (1.98)
8	9.14 (3.14)	10.96 (3.39)
9	8.79 (3.06)	9.38 (2.88)
10	9.29 (3.15)	7.44 (1.85)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$r_2 = 1.55$	$r_7 = 2.34$
$r_3 = 1.85$	$r_8 = 2.40$
$r_4 = 2.03$	$r_9 = 2.46$
$r_5 = 2.16$	$r_{10} = 2.50$
$r_6 = 2.26$	

Table 11b (Expt. 11)

Mean ($\pm$ S.E.M.) latencies to reach the exit ramp over 10 days in mice required to approach either the illuminated arm or nonilluminated arm of a water-filled Y-maze.

<u>Days</u>	<u>Swim Condition</u>	
	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	10.91 (2.45)	26.29 (2.45)
2	7.17 (0.72)	20.27 (2.98)
3	6.90 (0.38)	17.21 (2.28)
4	7.32 (0.64)	15.59 (2.17)
5	6.39 (0.61)	21.04 (3.24)
6	7.19 (0.95)	20.04 (3.36)
7	8.07 (1.36)	20.13 (4.23)
8	11.27 (3.43)	26.72 (5.11)
9	10.69 (3.19)	24.78 (5.12)
10	11.68 (3.54)	31.93 (6.28)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$$r_2 = 2.44 \quad r_7 = 3.67$$

$$r_3 = 2.91 \quad r_8 = 3.77$$

$$r_4 = 3.19 \quad r_9 = 3.86$$

$$r_5 = 3.40 \quad r_{10} = 3.93$$

$$r_6 = 3.55$$

Table 11c (Expt. 11)

Mean ($\pm$ S.E.M.) number of discrimination errors over 10 days in mice required to approach either the illuminated arm or nonilluminated arm of the water-filled Y-maze.

<u>Days</u>	<u>Swim Condition</u>	
	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	4.27 (0.69)	11.86 (1.61)
2	2.13 (0.39)	8.86 (1.26)
3	1.93 (0.43)	9.36 (1.33)
4	2.60 (0.57)	7.07 (0.89)
5	1.07 (0.36)	11.00 (1.59)
6	1.33 (0.36)	11.57 (1.71)
7	1.07 (0.30)	11.29 (1.84)
8	1.28 (0.42)	11.79 (1.86)
9	0.67 (0.22)	11.93 (2.27)
10	0.53 (0.17)	12.57 (2.57)

Mean differences necessary for $p < .05$.

Between Days and Swim Condition

$r_2 = 1.60$ $r_7 = 2.42$
 $r_3 = 1.92$ $r_8 = 2.49$
 $r_4 = 2.11$ $r_9 = 2.55$
 $r_5 = 2.24$ $r_{10} = 2.59$
 $r_6 = 2.34$

Table 11d (Expt. 11)

Mean ($\pm$ S.E.M.) number of errorless trials over 10 days in mice required to approach either the illuminated arm or nonilluminated arm of the water-filled Y-maze.

<u>Days</u>	<u>Swim Condition</u>	
	<u>Illuminated Arm</u>	<u>Nonilluminated Arm</u>
1	6.87 (0.41)	4.07 (0.53)
2	8.13 (0.34)	4.43 (0.53)
3	8.53 (0.26)	4.21 (0.45)
4	8.07 (0.35)	4.64 (0.48)
5	9.00 (0.33)	3.71 (0.70)
6	8.93 (0.28)	3.64 (0.77)
7	9.00 (0.26)	3.57 (0.82)
8	8.67 (0.47)	3.14 (0.81)
9	9.07 (0.32)	3.43 (0.81)
10	8.73 (0.53)	2.79 (0.81)

Mean differences necessary for $p < .05$.

Days

$r_2 = 0.84$ $r_7 = 1.29$
 $r_3 = 1.03$ $r_8 = 1.33$
 $r_4 = 1.13$ $r_9 = 1.36$
 $r_5 = 1.20$ $r_{10} = 1.39$
 $r_6 = 1.25$

Table 12a (Expt. 12)

Mean ($\pm$ S.E.M.) latencies (sec) to reach the choice point after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm of a water-filled Y-maze.

	<u>Days of Training</u>	
	<u>one</u>	<u>five</u>
Illuminated Arm	4.96 (0.32)	4.03 (0.39)
Nonilluminated Arm	9.30 (1.12)	6.33 (1.03)

Table 12b (Expt. 12)

Mean ($\pm$ S.E.M.) latencies (sec) to reach the exit ramp after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm in a water-filled Y-maze.

	<u>Days of Training</u>	
	<u>one</u>	<u>five</u>
Illuminated Arm	7.94 (0.69)	5.43 (0.44)
Nonilluminated Arm	16.77 (1.20)	11.92 (2.24)

Table 12c (Expt. 12)

Mean ($\pm$ S.E.M.) number of discrimination errors after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm in a water-filled Y-maze.

	<u>Days of Training</u>	
	<u>one</u>	<u>five</u>
Illuminated Arm	3.00 (0.55)	1.00 (0.41)
Nonilluminated Arm	7.92 (0.66)	4.50 (0.99)

Table 12d (Expt. 12)

Mean ($\pm$ S.E.M.) number of errorless trials after 1 and 5 days in mice required to swim to the illuminated or nonilluminated arm in a water-filled Y-maze.

	<u>Days of Training</u>	
	<u>one</u>	<u>five</u>
Illuminated Arm	7.42 (0.42)	9.00 (0.41)
Nonilluminated Arm	4.17 (0.46)	6.50 (0.86)

Table 12e (Expt. 12)

Mean ($\pm$ S.E.M) plasma corticosterone concentrations (ug/100 ml) after 1 and 5 days in mice required to swim to the illuminated arm or to the nonilluminated arm of the water-filled Y-maze and in their yoked partners.

	<u>Days of Training</u>	
	<u>one</u>	<u>five</u>
<u>Illuminated Arm</u>		
Escapable	11.59 (1.36)	18.22 (2.48)
Yoked Inescapable	17.31 (2.20)	16.34 (1.94)
<u>Nonilluminated Arm</u>		
Escapable	16.59 (2.16)	17.20 (1.99)
Yoked Inescapable	17.97 (1.84)	14.34 (1.81)
<u>No Treatment</u>	3.00 (0.39)	2.43 (0.36)

Mean differences necessary for $p < .05$.

Between Days and Controllability

$$r_2 = 3.96$$

Dunnett's t Statistic for comparing all means with nonstressed controls.

Critical value for two-tailed $p < .05$ (i.e., $t_{.975}(12)$).

$$t_{crit} = \pm 2.88$$

Table 13 (Appendix D)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in juvenile mice as a function weaning, sexing, and placement in a new home cage.

<u>No Treatment</u>	<u>Weaned Home Cage</u>	<u>Weaned Sexed Home Cage</u>	<u>Weaned Sexed New Cage</u>
8.90 (1.80)	7.52 (1.42)	9.48 (2.05)	14.66 (1.68)

Mean differences necessary for $p < .05$.

$$r_2 = 5.02$$

$$r_3 = 6.06$$

$$r_4 = 6.69$$

Table 14 (Appendix E)

Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (ug/100 ml) in mice reexposed either to shock boxes in which odor cues were absent or to shock boxes which contained odor cues associated with the initial shock delivery. Mice were initially exposed to 360 inescapable shocks 24 hr earlier. A separate group of mice was not exposed to any shock whatsoever.

Reexposure to Shock Apparatus

<u>No Treatment</u>	<u>Odorless Box</u>	<u>Odorized Box</u>
5.73 (0.72)	10.74 (1.40)	13.14 (1.66)

Mean differences necessary for $p < .05$.

$$r_2 = 3.74$$

$$r_3 = 4.60$$

END

18/02-91

FIN